MONICA KAY GRANDISON
Pharmacokinetic Evaluation and Protein Binding of Suramin
(Under the direction of F. DOUGLAS BOUDINOT)

Suramin is a novel anti-cancer drug currently being investigated as a treatment for brain cancer. The objective of this study was to characterize the pharmacokinetics and intersubject variability of suramin in patients with brain cancer. Plasma samples were collected from 16 patients and data was analyzed using nonlinear mixed effects modeling. The data were best described by a two compartment model, with elimination from the central compartment with gender and body surface area (BSA) as covariates. Volume of distribution was estimated to be 4.16 L/hr/m^2 ($\pm$ 0.31) in male patients and in 4.16 L/hr/m^2 ($\pm$0.23) females in the first compartment. Volume of distribution in the second compartment was 21.2 ($\pm$ 2.26) in males and 12.6 ($\pm$ 3.8) in females. Elimination was from the central compartment. The results of this study showed that the pharmacokinetic parameters of suramin in brain patients were similar to those observed in studies examining use of suramin in prostate cancer. The study also showed that using a model that included gender as a covariate decreased the amount of inter- and intra-subject variability.

Plasma protein binding studies were performed utilizing equilibrium dialysis to fully characterize the *in vitro* binding of suramin to human serum albumin, α_{-1}-acid glycoprotein, and human plasma serum over wide range of drug concentrations. Suramin binds to two classes of binding sites on albumin, a high affinity saturable site and a low-affinity nonsaturable site (N_1=3.5, K_1=1.8 x 10^4 M^{-1}, N_2K_2=3.7 x 10^3 M^{-1}). Suramin binds to a single low-affinity nonsaturable site on α_1-acid glycoprotein (N_3K_3=1.5 x 10^5 M^{-1}). The fraction of suramin bound to plasma proteins predicted from the *in vitro* binding to human serum albumin and α_1-acid glycoprotein was identical to that observed in human plasma (95% $\pm$ 0.015). Thus, the plasma protein binding of suramin can be accounted for by the binding to these two proteins.

INDEX WORDS: Suramin, Clinical Pharmacokinetics, Nonlinear Mixed Effects

Modeling, Plasma Protein Binding

PHARMACOKINETIC EVALUATION

AND PROTEIN BINDING OF SURAMIN

by

MONICA KAY GRANDISON

B.S., Stillman College, 1995

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in

Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2001

PHARMACOKINETIC EVALUATION

AND PROTEIN BINDING OF SURAMIN

by

MONICA KAY GRANDISON

B.S., Stillman College, 1995

Approved:

Major Professor: F. Douglas Boudinot

Committee: Stuart Feldman
Daniel Hall
Philip Greenspan
James Price

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
December 2001

DEDICATION

This work is dedicated to

Grand, Mom, and Lana

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Development of Suramin as an Anticancer Drug

Suramin is a symmetrical polysulfonated naphthylurea (naphthylamine derivative of urea) originally used as an anti-parasitic agent. Serendipitous discoveries, continuing experimentation, and political and economic intrigue have characterized the development of suramin as an anti-cancer drug. Suramin was synthesized by Bayer & Co. in Germany as a result of innovations made by Paul Erhlich in the first decade of the twentieth century [1]. Erhlich theorized that a drug's specificity resulted from the relationship of its three-dimensional structure and its cellular receptor. During this period, Erhlich also made advances in the areas of drug screening and preclinical drug evaluation. Parasitic diseases were one of the therapeutic areas to which these ideas were applied. Erhlich was particularly interested in trypanosomiasis [1,4].

Trypanosomiasis refers to any disease caused by the presence of parasitic protozoans of the genus *Trypanosomia*. This genus of protozoans moves by means of a long trailing flagellum and a thin wavy membrane which project from the body surface. Trypanosomes undergo part of their development in the blood of a vertebrate host. The remaining stages occur in invertebrate hosts, which can transmit the parasites back to vertebrates. The two most prevalent forms of trypanosomiasis are Chagas' disease (South American trypanosomiasis) which is

caused by a bite from the reduviid bug and sleeping sickness (African trypanosomiasis) which is caused by the bite of a tsetse fly [3].

Erhlich found that the sulfonated cotton dye, trypan red, had significant activity against trypanosomiasis. Unfortunately this agent stained the treated mice red. This finding lead to a search for a compound that had the activity of trypan red but did not have the undesirable side effects [1,4]. Screening of a vast number of compounds led to the discovery of trypan blue [5] and afridol violet [6]. Like trypan red, these dyes also stained the treated animals. This observation led to the search for a compound that possessed the activity of the sulfonated dyes but did not stain animals treated in preclinical tests. After screening thousands of compounds, the search ended two years after the death of Erhlich with the discovery of suramin [7] (Figure 1.1).

Suramin is colorless because it possesses amide linkages instead of the azo linkages that are responsible for the color of the sulfonated dyes [8]. Suramin has a large molecular weight (1429 Dalton) and is anionic in nature because of the sulfonic acid groups [9-11]. Many of suramin's biologic and pharmacological properties are attributed to these acidic groups and their spatial orientation [9-11]. Synthesis of suramin analogues later showed that altering the structure in any way hindered anti-parasitic activity [11].

Although suramin was synthesized around 1916, information about its structure and synthesis was hidden in Bayer patent literature. It has been alleged that after World War I, the German government attempted to barter the compound toward the return of certain colonies (those located in Central Africa and plagued by infestation of livestock by sleeping sickness). Therefore, to reveal the structure of suramin, would have invited additional economic and political strife in post war Germany [1].   The structure was finally revealed in 1924 by Fourneau, after the development of twenty-five possible isomers [9].

Suramin was initially tested in a small group of patients in Europe (1921-1922) [12]. This study was followed by clinical trials in Africa in the early twenties by Kleine and Fischer [8]. African trypanosomiasis (sleeping sickness) is caused by *T. brucei gambiense* in West and Central Africa and by *T. brucei rhodesiense* in East Africa. Metacyclic forms inoculated by flies transform into trypomastigotes that multiply by binary fission and spread to the blood stream and lymph about one week after being bitten by the tsetse fly. Trypomastigotes multiply until specific antibodies are produced by the host and sharply reduce the parasitic levels. Some parasites escape immune destruction by altering their surface antigens and starting a new multiplication cycle if the disease is not treated. The cycle of multiplication and lysis repeats for many months. During the final stages of the disease, trypanosomes are found in the interstitial fluid of many organs, especially the central nervous system [3]. It was found that suramin was an effective prophylactic treatment for the disease. It was also observed that suramin was effective in the early stages of systemic trypanosomiasis. The compound, however, could not effectively penetrate the central nervous system and therefore is not useful against advanced stage sleeping sickness [13].

The success of suramin as a treatment for sleeping sickness lead to additional clinical investigations in which suramin was tested as a treatment for other parasitic disorders. Van Hoof conducted the most important of these studies in 1947 in Zaire in patients with onchocerciasis. The parasitic worm *Onchocerca volvulus* causes onchocerciasis [13]. Various species of the black fly, in which the worm undergoes part of its development, transmit the infective larvae into man. The adult worms found in fibrous nodules within the connective tissue beneath the skin cause the disease. Van Hoof observed that suramin was an effective treatment for patients simultaneously infected with sleeping sickness and onchocerciasis (river blindness).

The effectiveness of suramin as an anti-parasitic agent stimulated interest in sulfonated polyanionic compounds as possible treatment agents in other areas such as anti-viral activity and anti-neoplastic activity.

Proposed Mechanism for Anti-Parasitic Activity

Suramin's mechanism of action has yet to be explained. Several theories have been proposed to explain the anti-parasitic activity of suramin. A number of literature reviews have been published on the topic and to discuss all of the proposed theories goes beyond the scope of this chapter. Two hypotheses, however, are of particular interest and will be discussed in more detail.

Suramin is believed to inhibit a number of trypanosome enzymes in the range of 1 to 100 μM. Fairlamb et al observed that levels of suramin increase in a cumulative fashion within the trypanosome and this accumulation is associated with an increase of oxygen consumption. This finding validated an earlier hypothesis that suramin inhibits glycolysis in vivo by acting against glycerol-3-phosphate oxidase and NAD+ -dependent glycerol-3-phosphate dehydrogense. These investigators noted that the inhibition of oxygen consumption was directly correlated with exposure to suramin in vivo and the dose of suramin administered suggesting that suramin was taken up by endocytosis as a complex bound to plasma proteins. After entering the cell, the endocytic vesicles combine with lyosomes to form secondary lyosomes in which the suramin-plasma protein complex is degraded, and as a result, suramin is released into the cytoplasm. Suramin then inhibits ATP inhibiting the formation of glycerol-3-phosphate dehydrogenase which is dependent on NAD+ and glycerol-3-phosphate oxidase. The accumulation of suramin in the cell results in the decreased generation of ATP and the decreased rate of uptake of more drug from the plasma.   The result

of this decreased generation of ATP is the inhibition of metabolic function and results in the death of the organism[8].

Another suggested mechanism of action by which suramin exerts pharmacological activity is the inhibition of reverse transcriptase [15,17,19] and other nuclear enzymes. Suramin has been shown to inhibit DNA polymerases α and γ, DNA primase, terminal deoxynucleotidyl transferase (TdT), RNA polymerase and DNA topoisomerase II [14-18] in vitro at IC_{50} concentrations of $1 - 10$ µg/ mL. Trypsin , urease , several kinin-forming enzymes , and complement system enzymes are extracellular enzymes inhibited by suramin. Suramin's inhibition of fibrin-fibrinogen conversion by thrombin results in an anti-coagulant effect in vitro [16,25,26].

Suramin as an Antiviral Agent

DeClerq reported that at low concentrations, suramin (0.07-7 µM) could inhibit the reverse transcriptase activity associated with RNA tumor viruses [19]. After the onset of AIDS epidemic, the observation that suramin inhibits this enzyme triggered interest in suramin as a possible treatment for AIDS. Suramin inhibited the cytopathic activity of HIV in vitro at doses that are clinically achievable in humans [19-20]. Because of the extensive clinical data available for suramin, preclinical studies were not pursued [21-23].

The National Cancer Institute initiated two studies to examine the validity of suramin as a treatment of HIV. In the first study, ten patients with AIDS or HIV infection, were administered a 1 gram bolus injections of suramin weekly over a period of six weeks. Although four of the patients showed a reduction in levels of viral reverse transcriptase, no clinical or immunological improvement was noted [22].

In the second study, twelve patients with AIDS and a history of B-cell lymphoma were administered weekly bolus doses (1 g) of suramin. This was followed by weekly maintenance doses (500 mg). Three patients showed a reduction in viral load but like the previous study none of the patients showed immunological improvement. An unforeseen finding of this study was that one patient who had both Kaposi's sarcoma and stage IV small cleaved cell lymphoma showed complete and durable regression of both malignancies [21].

Collins et al characterized the pharmacokinetics of suramin in four HIV infected patients. Suramin (200 mg) was administered intravenously as a loading dose on day 1 and 1 gram of the compound was administered intravenously on days 3, 7, 14, 21, 28 and 35. The results of this study showed that in this patient group, the total body clearance of suramin was less than 0.5 mL/min and renal clearance was 0.30 mL/min. The volume of distribution of suramin was 4 L and the extent of protein binding was 99.7%. The terminal half-life of the compound was 50 days [23,97].

Suramin as an Anti-Tumor Drug

The anti-tumor activity of suramin was first noted in murine lymphosarcoma[25,26]. This was followed by years of inconsistent findings and waning interest in the anti-neoplastic properties of this compound. It was not until the anecdotal findings in the NCI HIV investigations that interest in this drug was renewed.

Stein studied the effectiveness of suramin as an anti-cancer drug in fifteen patients with metastatic cancer (adrenocortical carcinoma, renal adrenocortical carcinoma and HIV induced lymphoma). Adrenal carcinoma was selected as a disease model because suramin toxicity. Patient one was initially administered an intravenous bolus dose of 850 mg/m^2/wk, and the dose was escalated to 1200

mg/m^2/wk. The pharmacokinetic profile of suramin observed in this patient, and the assumption that concentration levels had to be maintained between 250 to 300 µg/mL to be effective therapeutically, lead Stein to increase the weekly bolus dosage to 1.4 g/m^2 with a 350 mg/m^2/d maintenance dose in the remaining patients. The outcome of this study demonstrated that suramin was active against metastatic cancer [24].

The findings in the metastatic cancer study lead to numerous investigations using suramin as a possible treatment for solid tumors. Suramin reportedly inhibited the growth of prostate cancer cell lines, breast cancer cell lines, and ovarian cell lines in vitro.

Proposed Mechanisms for Anti Tumor Activity

The anti-tumor activity of suramin has not been fully described. Anti-neoplastic activity ,like anti-parasitic activity has been attributed to inhibition of nuclear enzymes and lysosomal enzymes. A number of other mechanisms have been proposed to explain suramin's mechanism of action.

Suramin has been shown to inhibit the binding of growth factors to their receptors. The compound also has been shown to be responsible for the dissociation of these bonds. Suramin binds to platelet derived growth factor (PDGF) [28,30], epidermal growth factor (EGF) , basic fibroblast growth factor (FGF) , and transforming growth factor B (TGFB) . Suramin antagonizes the ability of EGF, FGF, and TGFB factors to stimulate the growth of tumor cells in tissue culture [28-31]. The binding of suramin inhibits the biological activity of PDGF to its receptor [29]. The compound's inhibitive activity occurred at concentrations between 50-300 µg/mL .

Suramin appears to have the ability to influence differentiation of adenocarcinoma, neuroblastoma and glioma cells. At 100 µg/mL, in vitro,

suramin induced the differentiation of human colic adrenocarcinoma cells into "enterocyte-like" cells [31]. Suramin also partially induced differentiation in rat glioma cells and mouse neuroblastoma cells [34-35].

Suramin also appears to have anti-angiogenesis activity. Prolonged exposure to suramin has been shown to effect glycosaminoglycan metabolism in patients. As a consequence, levels of circulating heparan and dermate sulfate are elevated [35-36].

Pharmacokinetic Profile of Suramin

Suramin is only minimally absorbed from the intestine when administered orally [36]. It was also observed that when suramin was administered intramuscularly or subcutaneously it caused irritation and pain [36]. The compound is generally infused intravenously infused over an hour and dispensed for a prolonged period (typically longer than a month) [37]. This drug is almost exclusively eliminated by renal excretion [23]. Total body clearance values of suramin were low (0.41ml/min) with little patient variability in clearance (coefficient of variation, 15%) observed [23]. The majority (80%) of free suramin elimination was via glomerular filtration[23]. Protein binding of suramin is greater than 99% [26,38-40]. This drug also has a narrow therapeutic range of 200-300 µg/mL [41-42].

Toxicity of Suramin

In a retrospective study performed by La Rocca et al, neurological toxicities were observed at concentrations above 350 µg/mL [41]. Neurological toxicities were also observed in the adrenal carcinoma study performed by Stein [24]. The patients display three types of neurotoxicity. In those patients with

protracted exposure to suramin blood concentrations greater than 350 μg/mL, an acute demyelinating peripheral neuropathy similar to acute Guillain-Barré syndrome. In patients with blood concentrations maintained between 200-300 μg/mL, a stocking glove paraesthesis was observed. Patients that received suramin treatment for more than one month had presentations of muscle weakness similar to that of a myopathy. Markers that indicate muscle weakness were absent, but alterations in nerve conduction were noted. In patients with acute demyelinating peripheral neuropathy and stocking glove paraesthesis, toxic symptoms improved 6-12 months after suramin therapy was discontinued. The symptoms in patients with muscle weakness improved gradually 4 to 9 months after suramin administration was ceased [24].

Feuillan et al reported that suramin interfered with the normal adrenal cortex in man and in a number of different animal models [44]. Suramin accumulates in the adrenal gland and this was believed to play a significant role in its toxicity. The suppression of adrenal function by suramin requires the concomitant dosage of hydrocortisone or prednisone.

In clinical studies that examined suramin as a possible treatment for prostate cancer, researchers could not conclusively determine if anti-tumor activity could be attributed to suramin or hydrocortisone since hydrocortisone has anti-neoplastic activity [46,47]. Small et al, performed a phase III study to compare the decline in prostate-specific antigen (PSA) in patients who were treated with suramin and hydrocortisone and those patients who received a placebo and hydrocortisone. Patients who received suramin experienced a greater decline in PSA, longer time to disease progression, and longer duration of response than those patients who received a placebo. Small concluded that the combination of suramin and hydrocortisone were well tolerated and provided a delay in disease progression in patients with symptomatic hormone refractory prostate cancer [47].

Renal injury is another toxic effect of the administration of suramin. Suramin causes 25-50% decrease in creatinine clearance in most patients. Renal injury is generally presented as proteinuria but a case of acute renal failure has been reported [48-49]. The patient's condition gradually improves after suramin is discontinued.

In addition to neurotoxicity, adrenal cortical failure and renal impairment, other toxic effects of suramin include anti-coagulation, bacterial infections [51-55], lymphocytopenia [54], thrombocytopenia, and vortex keratopathy[56].

The combination of the pharmacokinetic profile of suramin and possibility of severe toxicities made suramin an ideal candidate for pharmacokinetic optimization via individualized drug dosage regimens.

Individualized Drug Dosages Regimens

Masson and Zamboni present four criteria that must be met to justify pharmacokinetic optimization through individualized dosage. The drug must have 1) a narrow therapeutic range, 2) a large degree of inter-patient and intra-patient variability, 3) the monitoring of toxicity and pharmacological effect is difficult and 4) a relationship between toxicity and drug exposure exists [57]. The initial studies examining the anti-tumor properties of suramin met this criteria.

Individualized dosage regimens are composed of distinct but complementary parts that allow the use of measured drug concentrations to achieve a desired endpoint. The method of altering the dosing regimen to achieve the desired concentrations can be approached in the context of adaptive control [58-60,63]. The process of adaptive control for applied pharmacokinetics begins with the selection of the initial dosage based on therapeutic goals determined by known drug concentration-clinical response relationships and the clinical status of the patient and a pharmacokinetic model relating patient characteristics to

pharmacokinetic parameters . Revision of the dosing regimen involves adjusting the parameter estimates for the pharmacokinetic model based on measured drug concentrations in relation to the therapeutic goals and the patient's clinical response [60].

Control strategies in drug therapy are commonly present in empirical form in clinical practice and are often defined to as nomograms [61,62]. Nomographs are open loop control algorithms because these rules are based on a priori assumptions for drug disposition [60]. There are a number of clinical examples of open loop feedback control. Dosages may be adjusted for body size for pediatric patients [61] or may be modified for creatinine clearance in patients with renal failure [62]. Open loop feedback control refers to open loop control algorithms that are adapted for the individual . These algorithms include measured responses such as drug concentrations or blood pressure.

Suramin has been administered to patients using adaptive control with feedback. In an effort to control the plasma concentrations of suramin, a course of treatment, developed using adaptive control with feedback, was administered to patients. The objective of this study was not only to prevent drug levels associated with polyneuropathies (>350 μg/mL) but also to address issues associated with the optimization of suramin treatment. This study examined the estimation of pharmacokinetic parameters using two and three compartment models. The investigators concluded that the three compartment model best described the pharmacokinetics of suramin. The authors also stated that the low systemic clearance of suramin and the number of parameters needed to describe the three-compartment model suggested that a Bayesian approach would be an appropriate means to estimate individual parameters [63].

The pharmacokinetic applications of the Bayesian method are performed intuitively by clinicians [64]. Therapy is initialed in a patient using population

typical parameter values adjusted to take into account patient specific information such as weight and serum creatinine levels. Patient drug concentrations are taken at predetermined times and compared to expected drug concentrations. Individual parameter estimates for individual patients are calculated based on the expected and measured drug concentrations and their variability . Variability in expected drug concentrations is based on average parameter values and variability in the patient population. Expected variability in measured concentrations is attributed to random sources of variability such as measurement [64-65,68].

In terms of Bayes theorem:

$$\text{prob } (P|C) = (\text{prob } (P) * \text{prob}(C|P))/(\text{prob } (C))$$

In this equation, prob (P|C) is the probability distribution of the patient's pharmacokinetic parameters (P) taking into account 1) measured drug concentrations (C), 2) the probability of the patients parameters within the assumed population distribution ((prob (P)), 3) the probability of observed concentrations in terms of the pharmacokinetic model (prob(C|P)) and 4) the probability distribution of the measured concentrations (prob (C)) [60].

If the prior distribution of the population parameter is normal, the likelihood function given by of Bayes Theorem is:

$$OBJ_{Bayes} = \sum_{j=1}^{P} \frac{\left(Pj - \hat{Pj}\right)^2}{\sigma_{Pj}^2} + \sum_{i=1}^{n} \frac{\left(Ci - \hat{Ci}\right)^2}{\sigma_i^2}$$

Pj and P^j denote the population and the estimation of individual's j's pharmacokinetic parameters. The standard deviation of the population parameters is denoted by σ_{pj}. Ci and C^i are the observed and predicted concentrations and σi denotes standard deviations from the error model.   When the Bayes objective function is minimized, individual parameters are estimated. These estimates take into account measured and predicted drug concentrations as well as measurement error and variability of parameters in the population [64].

Clinicians examined the utilization of Bayesian methods with adaptive control with feedback in patients with hormone refractionary prostate cancer [67]. It was observed that although Bayesian methods allowed precise control of plasma concentrations and allowed a range of concentrations to be examined, it was time consuming, expensive and labor intensive.

In addition to adaptive control and Bayesian estimation, pharmacokinetically based dosing methods have also been used to improve the dosing of suramin. When a pharmacokinetically based regimens is utilized, pharmacokinetic performance is based on the accuracy and the precision of drug concentration prediction. Mean error (ME), mean absolute error (MAE) and root mean squared prediction error (RMSE) are used to assess the accuracy and precision of predicted drug concentrations [66]. Regimens that used pharmacokinetically based dosage of suramin have been evaluated [66,94,98,99]

As stated previously, adaptive control with feedback is generally intuitively used in the clinical setting but to implement this process in a routine fashion, formal structures must be implemented. The ultimate goal of adaptive control is to improve the precision of drug therapy. To accomplish this, adaptive control algorithms include a structural model, a variance model, a population model, and a model to characterize the relationship between the patient characteristics and the population model [60].

1)   A structural model refers to the pharmacokinetic model. Drug concentrations in the individual subjects can be described as follows:

$y_{ij} = f(\Phi_j, x_{ij}) + \varepsilon_{ij}$

y_{ij} is the ith concentration in the jth individual, Φ_j is the individual pharmacokinetic parameters such as volume of distribution and clearance for individual j, x_{ij} include dose and time information. The function f is

defined as the solutions of the differential equations that described the concentration time profile of an administered drug.

2) A variance model refers to the residual error (predicted - observed) or the random variability of data. This is the intraindividual error. The error model is a rearrangement of the structural model.

$\varepsilon_{ij} = y_{ij} - f(\Phi_j, x_{ij})$

ε_{ij} is the "noise" created by errors such assay variability, doses, times and incorrect structural model.

3) A population model reflects the variability that arises in between patients. To illustrate this intersubject variability the pharmacokinetic parameters must be described in terms of the population.

$\Phi_j = \theta + \eta_j$

In this equation, θ are the population average parameters. The difference of the individual parameters from the population is defined as η_j.

4) A model that describes the relationship between patient characteristics and the pharmacokinetic model parameters.

Population Pharmacokinetics

Population pharmacokinetics are determined by two methods, the traditional two-stage method and nonlinear mixed effects modeling. In stage 1 of the two-stage method, experimental studies are performed in a small group of individuals [60]. Each patient's data is then analyzed using nonlinear regression or noncompartmental methods to obtain individual estimates of pharmacokinetic parameters. Stage 2A includes a summary of those parameters obtained by calculating the mean and the standard deviation. These parameter estimates are taken to be the population typical values and description of the variability of the population. Stage 2B establishes the relationship between individual patient

characteristics and the estimated pharmacokinetic parameters using regression methods [120, 124, 127,133].

There are a number of advantages to using the two-stage method. First, nonlinear regression analysis can be performed using a number of widely available computer programs such as WINNONLIN or ADAPT. Second, in the presence of sufficient clinical data, this method calculates statistically precise individual parameter estimates. Stage 2 does an adequate job at predicting the population parameters, but these estimates tend to be upwardly biased [60].

Since the two-stage method requires extensive clinical data, two-stage estimation is often inadequate in predicting individual parameters. Since a tremendous amount of data is needed, it is often impossible to get these data from patients. Therefore, the data gathered from healthy patients would not be variable enough to characterize a population of patients undergoing therapy. The two-stage is also inadequate in predicting individual parameter estimates when data are sparse [60].

Mixed effects modeling allows the calculation of population parameters and individual-specific predictions in a single step. Extended least squares regression is used to estimate fixed effects and predict random effects. Fixed effects include the population typical values and regression coefficients that describe the effects of the individual patient characteristics and other covariates on the population typical values. These patient characteristics may include attributes such as age, weight, height and sex, physio-pathological characteristics such as renal and hepatic impairment and other patient specific traits such as concurrent drug therapy, smoking and alcohol intake. The random effects account for the interpatient variability [120-124,127].

The strength of nonlinear mixed effects model lie in the ability to accommodate sparse patient data and generate patient specific predictors

parameters that can be used to forecast clinical pharmacokinetics. The disadvantage of using nonlinear mixed effects modeling is that the theoretical statistics required are not widely known among pharmacokineticists [60].

Suramin and the Treatment of Brain Cancer

Brain cancer includes primary brain tumor (those tumors that occur solely in the brain and the intracranial cavity) and metastatic brain tumors (tumors that originate systemically before developing in the brain). Primary brain tumors account for 2% of newly diagnosed malignancies. This corresponds to 17,000 to 20,000 new patients in the USA each year. Primary brain tumors include meningiomas, glioblastomas, astrocytoma, pituitary tumors, nerve sheath tumors, anaplastic astrocytoma and lymphoma and oligodendrogliomas [110]

Metastatic brain tumors occur in 10-30% of adult cancer patients (about 100,000 to 150,000 new patients). Fifteen to 20% of patients with metastatic brain tumors have tumors that are solitary and can be removed surgically. However, these patients have a low median survival rate. Those patients whose only available treatment option is whole brain irradiation have a median survival rate of six to eight months. The cancers that are most likely to metastasize to the brain are breast cancer and lung cancer [110].

Brain tumors are usually treated by surgery, radiation therapy and chemotherapy. Chemotherapeutic agents used to treat brain cancer include carmustine, lomustine, numustine, 1-(2-Chloroethyl)-1-nitroso-3-(2-hydrooxyethyl)urea and 1-(2-Chloroethyl)-3-(2,6-dioxy-3-piperidyl)urea. These compounds are unionized and are either water soluble or lipophilic. Despite an aggressive treatment course, survival of the patients with malignant primary brain tumors and metastatic brain tumors is poor [110,116].

The treatment of brain cancer is usually made difficult because of the nature of the brain and the nature of brain tumors. The blood brain barrier (BBB) is a sheet of endothelial cells on a basement membrane. Fused membranes called tight junctions connect these cells. The blood brain barrier acts as an effective barrier to the free exchange of components of the vascular system with neuronal cells. Drugs that are hydrophilic and/or ionic in nature rarely penetrate the blood brain barrier. Mannitol is usually administered before chemotherapeutic activity to temporarily disrupt the BBB [129].

The findings concerning the anti-tumor and anti-angiogenesis activity of suramin made this a potential treatment for primary and metastatic brain tumors. A number of in vitro studies have been performed to examine the effectiveness of suramin in the treatment of brain cancer [110]. Suramin inhibited the glioma cell proliferation in a dose dependent manner in C6, 9L, T98G, A-172, U-118 and U-138 cell lines. Suramin treatment of cultured glioma cells resulted in changes in the cell cycle distribution. In the U-118, T98G and C6 cell lines, there was a significant decrease in the percentage of cells in the S-phase at saturated concentrations (500 µg/mL) [70].

Suramin has also been studied in vivo in rodents. In one study, Wistar-Furth rats were implanted with a C6 glioma cell suspension and administered suramin (10 mg/kg to 200 mg/kg) by intraperitoneal injections on alternate days. A Kaplan-Meier survival curve showed that rats administered suramin survived an average of 8.9 days while rats not administered suramin survived 16.4 days. The decrease in the survival rate in treated rats was attributed to intracerebral hemorrhage [32].

To examine the effect of suramin on glioma and endothelial cell kinetics, animals with the C6 implant were injected with BUdR and fluorodeoxyuridine. Fluorodeoxyuridine is a competitive inhibitor of thymidine uptake. The tumor

cell and the endothelial cell-labeling index were inhibited by suramin in a dose dependent manner. The effect of suramin on the tumor vascular density, mean tumor volume, brain water content, organ (liver, kidney and spleen) weight and total body weight were also examined in the C6 implanted rats. Results showed that there was no difference between the treated and the untreated groups [129].

Suramin is anionic in nature and does not typically cross the blood brain barrier. Without audioradiography, it can not be determined if suramin actually penetrated the barrier. Structural changes in the membrane of endothelial cells suggested that suramin can pass through the more permeable capillaries at the perimeter of the tumor. Despite the observation that some amount of suramin passed through the blood brain barrier, it has been noted that drug delivery to vascularized organs such as the brain is complicated by factors such as capillary transport, interstitial pressure and ischemic areas. These findings led the researchers to conclude that incomplete drug penetration was an explanation for no observed differences in the study groups [129].

Bernsen examined the effect of suramin on tumor growth, vascularity, and oxygenation on a human cell glioma (E106) xenografted in a nude mouse model. Tumors were allowed to proliferate for approximately three weeks after implantation into nude mice. Group I included animals with tumors that were treated with suramin (20 mg/kg, three times a week) immediately after the three-week growth period and controls that were not administered any treatment. To examine the effect that suramin would have on the tumor growth of well established tumors, the animals in Group II received therapy two weeks after Group I began. The controls in Group II did not receive any treatment [70].

Suramin significantly suppressed the growth of tumors in animals that received the drug not only at the initial growth phase (Group I) but also in those animals with established tumors (Group II). Both groups displayed an increased

vascular density but no change was noted in vascular area. This finding suggested that the newly formed tumor vessel structures were more numerous because they were smaller in size than those vessel structures in the control groups. The vessel structures in the treatment groups were also more homogeneously distributed than those structures in the control groups. Animals treated with suramin also showed a significant reduction in hypoxia [70].

Suramin was studied as a treatment for brain cancer in vitro and in vivo by Olsen using a 9L gliosarcoma cell line implanted in 334 Fisher rats. Suramin was shown to inhibit cell proliferation at concentrations of 100 to 200 ug/mL. To determine the efficacy of suramin in tumor-bearing animals, rats were injected intraperitoneally with 7 mg/kg of suramin. The study showed that the average survival rate of 24.7 day was not significantly different from that rate of control animals that had not received suramin. Therefore, suramin was not efficacious in that animal model [111].

Olsen also investigated the delivery of suramin to different organs. He found that when suramin is measured after a single intraperitoneal or intravenous dose, "concentration…actually increased over time in the organ [kidney]". This confirmed previous findings that renal clearance was the prominent elimination pathway for suramin. The heart and the lungs also had detectable drug concentrations after administration, but these amounts were no where near the concentrations observed in the kidney. The brain, muscle and liver were observed to have only negligible concentrations at all time points observed. To determine if delivery to the brain, muscle, and liver were dose-dependent, daily intraperitoneal doses were administered to rats. After multiple dosing, concentrations rose in muscle and the liver, but remained very low in the brain. Suramin was shown not to reach brain tumors in great quantities. No significant amount of suramin was found in the contralateral hemisphere of the brain. In an

effort to determine if the manner in which the compound did not reach the tumor was dose dependent, a higher daily dose was given to animals. This study showed that the higher dose administered decreased the survival time of the animals.

A final study was performed to determine if the delivery method was responsible for the low suramin concentrations found in the brain. Suramin was injected directly into the brain. The rats with tumors either died immediately without awakening from anesthesia or shortly thereafter because of intracerebral or intraventricular hemorrhage. The lack of efficacy of suramin in this animal model was attributed the inability of suramin to penetrate the blood brain barrier.

Despite the less than optimal findings of the in vivo studies, suramin has been studied as a treatment for high-grade gliomas in human adults. Grossman, et al investigated the toxicity, efficacy and pharmacology of suramin as a treatment for gliomas in adults [92]. This study was performed because of the limited activity of standard chemotherapeutic agents used to treat this condition. The principal concern of researchers in administering suramin to patients with high-grade gliomas was related to the coagulopathy and neurotoxicity of this drug that had been observed in the animal studies. Patients with gliomas were previously reported as bleeding spontaneously into their brain tumors. These patients also had a high incidence of thromboembolic disease. It was also stated that brain tumors, surgery, and associated brain edema resulted in neurological deficits in these patients. Another concern was the effect that the presence of cytochrome P-450 inducing anticonvulsants would have on the pharmacological activity of suramin.

Suramin was administered intravenously in twelve patients with high-grade gliomas (anaplastic oligodendroglioma, anaplastic astrocytoma, and high-grade astrocytoma or glioblastoma multiforme). All patients had received previous irradiation therapy. Patients varied in the number of previous

chemotherapeutic regimens received. Four patients had received two or more treatments. Seven patients had received one course of anticancer treatment. Only one patient had not received any treatment. After being administered suramin, the patients displayed no CNS bleeding and no coagulopathy. Those toxicities that were observed (neutopenia, leukopenia, increased creatinine levels, diarrhea, nausea and constipation) were mild and reversible. The conclusions of this study were that suramin was well tolerated in patients with recurrent high-grade gliomas and toxicity was modest and reversible. Suramin was also shown to be effective in 25% (3 patients) in the study group. These patients had late chemical stabilization or their MRI scans improved markedly and lived for more than 400 days after initial suramin treatment. [92]

The Protein Binding of Suramin

Plasma protein binding of drugs, particularly for highly bound drugs like suramin, may have significant clinical implications. The plasma protein binding of suramin has been characterized to some extent [38,39,71,87,104,117,131]. The compound is highly bound to plasma proteins (>99%), binding predominately to human serum albumin. Studies assessing the influence of pH on the binding of suramin to albumin have demonstrated decreased binding with increasing pH. This was attributed to conformational changes in albumin between pHs 6-9 (N-B transition). The pH dependent changes in the electrostatic interaction of the negatively charged suramin with net negative charge on the albumin molecule are also believed to be, in small part, responsible for the pH dependent binding of suramin to albumin. This interaction decreases with increasing pH. The effect of the pH on the binding of suramin has also been attributed to location on albumin to which suramin binds. Positively charged histidine residues are part of the salt

bridges between suramin and albumin. It is believed that the deprotonation of these histidine residues may also be responsible for decreased binding at high pH.

Age-Related Changes in Protein Binding

As stated in the previous section, suramin is a highly bound drug. Plasma protein binding is particularly important consideration when examining the disposition of an extensively bound drug such as suramin. Plasma protein binding is a function of the drug and protein concentrations, the strength of the drug protein interaction, and the number of binding sites. A number of factors including disease state and age can alter protein concentrations. For example, albumin levels are greater in the young than they are in the elderly. An understanding of the relationship between drug concentrations, the physiology of aging, disease and their effects on the pharmacokinetics and pharmacodynamics of selected drugs is necessary for effective therapeutic monitoring.

In summary, suramin is an old compound that has been studied extensively. Despite the extensive work done with this compound, its mechanism of action has not been fully elucidated. The theories attempting to explain the manner in which it exerts pharmacological activity have lead to suramin being used as a possible treatment for a number of indications. One of the recent indications suramin has been tested as a possible treatment is brain cancer. The pharmacokinetics of this treatment in brain cancer patients has yet to be described. The plasma protein binding of suramin has also been examined, but neither the possible binding with α_1-glycoprotein has been studied nor the effect of drug concentration has been investigated. Literature reviews and various articles [69,72,74,78,80,89,90,91,95,100,104,107,113,114,119,134] have extensively described the importance plasma protein binding and the effect of age and disease state on

protein concentrations but the implications for alterations caused the aging process has yet to be examined.

The objectives of the current studies were to:

a) characterize the clinical pharmacokinetics of suramin in brain cancer patients,

b) characterize the plasma protein binding of suramin,

c) characterize the plasma protein binding of a potential cardioactive agent and

d) illustrate the clinical implications of age-related changes in plasma protein binding.

REFERENCES

1. Dressel J, Oesper RE. The Discovery of Germanin by Oskar Dressel and Richard Kothe. J Chem Edu. 1961; 38: 620

2. Gibson DW. Histopathological studies on suramin toxicity in a chimpanzee. Tropenmed Parasit. 1977; 28:387-405

3. The Merck Manual of Diagnosis and Therapy. Accessed November 12, 2001. www.merck.com/pubs/mmanual/section13/chapter161/161a.htm

4. Ehrlich P, Shiga K. Berl Klin Wochenschr. 1904; 41: 329

5. Mesnil F, Nicolle M. Ann Inst Pasteur, Paris. 1906; 20: 513

6. Findlay GM, Recent Advances in Chemotherapy, 3rd ed. Churchhill London. 1950; 129

7. Steck EA. Sodium suramin. In The Chemotherapy of Protozoan Diseases. Divison of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D.C. 1972; 2(3): 11.31 – 11.40

8. Voog TE, Vansterkenburg ELM, Wilting J, et al. Recent Research on the Biological Activity of Suramin. Pharmacol Rev 1993; 45(2): 177-203

9. Fourneau E, Trefouel J, Trefouel J, et al. Recherches de chimiothérapie dans la série du 205 Bayer. Urées des acides-aminobenzoyl amino naphtaléniques. Ann Inst Pasteur 1924; 38:81-114

10. Bauer H, Becker J. Untersuchunger über Verbindungen aus dem Gebiete des "Germaniums" (Bayer 205). Arb St Inst Exp Ther Frankf A M 1928; 21:10-25

11. Adams A, Ashley JN, Bader H. A Search for New Trypanocides. Part III. Some Analogues of Suramin. J Chem Soc 1956; 3739-3744

12.   Fischer AH. Future Prospects the chemotherapy of human trypanosomiasis: Novel Approaches in the Treatment of Trypanosomiasis. Trans R Soc Trop Med Hyg 1990; 84: 613-617

13.   Hawking F. Suramin: with special reference to onchocerciasis. Adv Pharmacol Chemother 1978; 15:289

14.   Jindal HK, Anderson CW, Davis RG. Suramin affects DNA synthesis in HeLa cells by inhibition of DNA polymerases. Cancer Res 1990; 50: 7754

15.   Ono K, Nalene H, Fukushima M. Differential inhibition of various deoxyribonucleic acid polymerases by suramin. Eur J Biochem 1988; 172: 349

16.   Spigelman Z, Dowers A, Kennedy S, et al. Antiproliferative Effects of Suramin on Lymphoid Cells. Cancer Res 1987; 47: 4694

17.   Waring MJ. The effects of antimicrobial agents on ribonucleic acid polymerase. Mol Pharmacol 1965; 1:1

18.   Bojanowski K, Lelievre S, Markovits J, Couprie J Jacquemin-Sablon A, et al. Suramin is an inhibitor of DNA topoisomerase II in vitro and in chinese hamster sarcoma cells. Proc Natl Acad Sci USA 1992; 89: 3025

19.   De Clerq E. Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor virus. Cancer Lett 1979; 8:9

20.   Mitsuya H, Popovic M, Yarchoan R. Suramin protection of T cells in vitro against infectivity and cytopathic effect of HTLV-III. Science 1984; 226: 172

21.   Broder S, Collins JM, Markham PD, et al. Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS related complex: Clinical pharmacology and suppression of virus replication in vivo. Lancet 1985; 2: 627-630

22.   Levine A, Gill PS, Cohen J. Suramin antiviral therapy in AIDS: Clinical, immunological, and virologic results. Annu Intern Med 1986; 105: 32-37

23.   Collins JM, Klecker RW, Yarchoan R, et al. Clinical Pharmacokinetics of Suramin in Patients With HTLV-III/LAV Infection. J Clin Pharmacol 1986; 26: 22-26

24.   Stein CA, La Rocca RV, Thomas R. Suramin: An Anticancer Drug With A Unique Mechanism of Action. J Clin Oncol 1989; 7(4): 499-508

25.   Regelson W. The Biological Activity of Polyanions: Past History and New Prospectives. J Polymer Sci 1979; 483-538

26.   Regelson W. The Antimitotic Activity of Polyanions. Adv In Chemother 1968; 3:303

27.   Coffey RJ, Leof EB, Shipley GD. Suramin inhibition of growth factor receptor binding and mitogenity in AKR-2b cells. J Cell Physiol 1987; 132:143

28.   Hosang M. Suramin binds to platelet-derived growth factor and inhibits its biological activity. J Cell Biochem 1985; 29:265

29.   Huang JS. Bovine-brain derived growth factor. Purification and characterization of its interaction with responsive cells. J Biol Chem 1986; 29: 265

30.   Williams LT, Tremble PM, Lavin MF, et al. Platelet-derived growth factor receptors form a high affinity state in membrane preparations. J Biol Chem 1984; 259: 5287

31.   Fantini J, Rognoni JB, Roccabianca M, et al. Suramin inhibits cell growth and glycolytic activity and triggers differentiation in human colic adrenocarcinoma cell clone HT29-D4. J Biol Chem 1989; 264: 10282

32.   Fantini J, Guo X-J, Marvaldi J, et al. Suramin inhibits proliferation of rat glioma cells and alters N-CAM cell surface expression. Int J Cancer 1990; 45: 554

33.   Hensey CE, Boscoboinik D, Azzi A. Suramin, an anti-cancer drug, inhibits protein kinase C and induces differentiation in neuroblastoma cell clone NB2A. FEBS Lett 1989; 258: 156

34.   Jamis-Dow CA, Weiss GH, Merino MJ, et al. Suramin selectively localizes to vascular endothelial cells: a possible basis for the antiangiogensis activity of suramin. Proc Am Assoc Cancer Res 1991; 32: 495

35.   McDonald K, Horne I Stein CA, et al. Circulating glycosaminoglycan antcoagulants associated with suramin treatment. Blood 1988; 71: 273

36.   Myers C, Cooper M, Ransom M, et al. "Antitumor Activity of Polyanions" (1997) in Cancer Medicine ed 4. Holland JF ed.

37.   Beijnen JH, van Gijn R, Horenblas S, Underberg WJ. Chemical stability of suramin in commonly infusion fluids. DICP 1990; 24:1056

38.   Vansterkenburg ELM, Wilting J, and Jannsen LHM. Influence of pH on the binding of suramin to human serum albumin. Biochem Pharmacol 1989; 38(18): 3029-3035

39.   Bos OJM, Vansterkenburg ELM, Boon JPCI, et al. Location and characterization of the suramin binding sites of human serum albumin. *Biochem Pharmacol* 1990; 40(7): 1595-1599

40.   Muller WE and Wollert U. Spectroscopic studies on the complex formation of suramin with bovine and human serum albumin. *Biochimica et Biophysica Acta* 1976; 427: 465-480

41.   La Rocca RV, Meer J, Gilliatt RW, et al. Suramin-induced polyneuropathy. Neurology 1990; 40:954-60

42.   Stein CA. Suramin: A novel neoplastic agent with multiple potential mechanisms of action. Cancer Res 1993; 53: 2239-2248

43.   Berns E, Schuurmans Z, Bolt J, et al. Anti-proliferative effects of suramin on androgen responsive tumor cells. Eur J Cancer 1990; 26: 470-474

44.   Feuillan P, Raffeld M, Stein CA, et al. Effects of suramin on the function and structure of the adrenal cortex in the cynomlgus monkey. J Clin Endocrinol Metab 1987; 65: 153

45.   Tannock I, Gospodarowicz M, Meakin W, et al. Treatment of metastatic prostate cancer with low dose prednisone: Evaluation of pain and quality of life as pragmatic indices of response. J Clin Oncol 1993; 11: 1566-1572

46.   Kelly WK, Scher HI, Mazumdar M, et al. Suramin and hydrocortisone: Determining drug efficacy in androgen-independent prostate cancer. J Clin Oncol 1995; 13: 2214-2222

47.   Small EJ, Meyer M, Marshall ME, et al. Suramin Therapy for Patients With Symptomatic Hormone-Refractiory Prostate Cancer: Results of a Randomized Phase III Trial Comparing Suramin Plus Hydrocortisone to Placebo Plus Hydrocortisone. J Clin Oncology 2000; 18:144-1450

48.   Smith A, Harbour D, Liebmann J, et al. Acute Renal Failure in a Patient Receiving Treatment with Suramin

49.   Figg WD, Cooper MR, Thibault A, et al. Acute renal toxicity associated with suramin in the treatment of prostate cancer. Cancer 1994; 74: 1612-4

50.   Hart PD, Young MR, Jordan MM, et al. Chemical inhibitors of phagosome-lysosmoe fusion in cultured macrophages also inhibit saltatory lysosomal movements. A combined microscopic and computer study. J Exp Med 1983; 158: 477

51. Heyneman RA. Inhibition by suramin of the NADPH oxidase from horse polymorphonuclear leukocytes. Vet Res Commun 1987; 11:149

52. Sipka S, Danko K, Nagy P, et al. Effects of suramin on phagocytes in vitro. Ann Hematol 1991; 63: 45

53. Toshkov A, Neychev H, Dimov V. Suramin increases the nonspecific anti-bacterial through macrophage activation. Acta Microbiol Bulg. 1986; 19:13

54. Warr GA, and Jakab GJ. Lung macrophage defense responses suramin induced lysosomonal dysfunction. Exp Mol Pathol 1983; 38:193

55. Mills GB, Zhang N, May C, et al. Suramin prevents binding to interleukin 2 to its cell surface receptor: A possible mechanism for immunosuppression. Cancer Res 1990; 50: 3036

56. Holland EJ, Stein CA, Palestine AG, et al. Suramin keratopathy. Am J Opthalmol 1988; 106: 216

57. Masson E, Zamboni WC. Pharmacokinetic Optimisation of Cancer Chemotherapy: Effects on Outcomes. Clin Pharmacokinetics 1997; 32(4): 324-343

58. Vozeh S, Steimer JL. Feedback control methods for drug optimization. Clin Pharmacokin 1985; 10: 457-76

59. Jelliffe RW. Open-loop feedback control of serum drug concentrations: pharmacokinetic approaches to drug therapy. Medical Instr. 1983: 17:267

60. Peck CC, D'Argenio DZ, Rodman JH. Analysis of Pharmacokinetic Data for Individualizing Drug Dosage Regimens. In Applied Pharmacokinetics.

61. Crom WR, et al. Pharmacokinetics of anticancer drugs in children. Clin Pharmacokinet 1987; 12:168

62. Hull J, Sarubbi F. Gentamicin serum concentrations: pharmacokinetic predictions. Ann Intern Med 1976; 85: 183-89

63. Cooper MR, Liberman R, La Rocca RV, et al. Adaptive control with Feedback Strategies for Suramin Dosing. Clin Pharmacol Ther 1992; 52:11-23

64. Schumscher GE, Barr JT. Bayesian approaches in pharmacokinetic decision making. Clin Pharm 1984; 3:525-529

65. Sheiner LB, Beal SL. Some suggestions for measuring predicative performance. J Pharmacokinet Biopharm 1981; 9: 503-12

66. Reyno LM. Egorin MJ. Eisenberger MA. Development and Validation of a Pharmacokinetically Based Fixed Dosing Scheme for Suramin. J Clin Oncol 1995; 13(9): 2187-2195

67. Eisenberger MA, Sinibaldi VJ, Reyno LM, et al. Phase I and Clinical Evaluation of a Pharmacologically Guided Regimen of Suramin in Patients With Hormone Refractory Prostate Cancer. J Clin Oncol 1995; 13(9): 2174-2186

68. Amisaki T, Eguchi S. A Comparsion of Methods for Estimating Individual Pharmacokinetic Parameters. J Pharm Bioph. 1999; 27(1): 103-121

69. Behm HL, Wagner JG. Free fraction change with differing volume ratios in equilibrium dialysis: cases of non-linear drug-protein binding. J Pharm Pharmacol. 1981; 33:283-289

70. Bersen HJJA, Rijken PFJW, Peters JPW, et al. Suramin treatment of human glioma xenografts; effects of tumor vasculature and oxygenation status. J Neuro Oncol 1999; 44: 129-136

71.   Bos OJM, Vansterkenburg ELM, Boon JPC, et al. Location and Characterization of the Suramin Binding Sites of Human Serum Albumin. Biochem Pharmacol 1990; 40(7): 1595-1599

72.   Boudinot FD, Homon CA, Jusko WJ, et al. Protein Binding of Oxazepam and Glucuronide Conjugates to Human Albumin. Bio Pharm 1985; 34: 2115-2121

73.   Boudinot FD, Jusko WJ. Fluid Shifts and Other Factors Affecting Plasma Proein Binding of Prednisolone by Equilibrium Dialysis. J Pharm Sci 1984; 73(6); 774-780

74.   Boudinot FD, Jusko WJ. Plasma Binding Interaction of Prednisone and Prednisolone. J Steroid Biochem 1984; 21(3): 337-339

75.   Bowers WF, Fulton S, Thompson J. Ultrafiltration vs. Equilibrium Dialysis for Determination of Free Fraction. Clin Pharmacokinetics 1984; 9(Suppl. 1): 49-60

76.   Boxenbaum HG. Riegelman S, Elashoff RM. Statistical Estimations in Pharmacokinetics. 1974; J Pharm Biol 2(2): 123-148

77.   Braddock PS, HU DE, Fan PD, et al. A structure-activity of antagonism of the growth factor and angiogenic activity of basic fibroblast growth factor by suramin and related polyanions. Br J Cancer 1994; 69: 890-898

78.   Briggs CJ, Hubbard JW, Savage C. Improved Procedure for the Determination of Protein Binding by Conventional Equilibrium Dialysis. J Pharm Sci 1983; 72(8): 918-920

79.   Clark JW, Chabner BA. Suramin and Prostate Cancer: Where Do We Go From Here? J Clin Oncol 1995; 13(9): 2155-2157

80.   Clausen J, Bickel MH. Prediction of Drug Distribution in the Distributional Dialysis and In Vivo from Binding to Tissues and Blood. J Pharm Sci 1993; 82(4): 345-349

81.	Constantopoulos G, Rees S, Cragg BG, et, al. Experimental animal model for mucopolysaccharidosis: Suramin-Induced Glycosaminoglycan and spingolipid accumulation in the rat. Proc Natl Acad Sci USA; 1980; 77(6): 3700-3704

82.	D'Argenio DZ and Schumitzky A. A Program Package For Simulation and Parameter Estimation in Pharmacokinetic Systems. 1979; Comp Prog in Bio 9: 115-134

83.	D'Argenio DZ, Schumitzky A, Wolf W. Simulation of linear compartment models with application to nuclear medicine kinetic modeling. 1988; 27:47-54

84.	D'Argenio DZ. Incorporating Prior Parameter Uncertainty in the Design of Sampling Schedules for Pharmacokinetic Parameter Estimation Experiments. 1990; Math Bio 99:105-118

85.	D'Argenio DZ. Optimal Sampling Times for Pharmacokinetic Experiments. J Pharm Biop 1981; 9(6): 739-756

86.	Dawson NA, Figg WD, Cooper MR, et al. Phase II Trial of Suramin, Leuprolide and Flutamide in Previously Untreated Metastatic Prostate Cancer. J Clin Oncol 1997; 15(4): 1470-1477

87.	Driscoll DL, Steinkampf RW, Paradiso LJ, et al. Lack of Effect of Corticosteroids and Tamoxifen on Suramin Protein Binding and *In Vitro* Activity. Eur J Cancer 1996; 32A(2): 311-315

88.	Eisenberger MA, Reyno LM, Jodrell DI, et al. Suramin, an Active Drug For Prostate Cancer: Interim Observations in a Phase I Trial. J Nat Can Ins 1993; 85(8): 611-621

89.	Gibaldi M, Koup JR. Pharmacokinetic Concepts-Drug Binding, Apparent Volume of Distribution and Clearance. Eur J Clin Pharmacol 1981; 20: 299-305

90.   Grandison MK, Boudinot FD. Age-Related Changes in Protein Binding of Drugs: Implications for Therapy. Clin Pharmacokinetics 2000; 38(3):271-290

91.   Greenblatt DJ, Sellers EM, Koch-Weser J. Importance of Protein Binding for the Interpretation of Serum or Plasma Concentrations. J Clin Pharmacol 1982; 22:259-263

92.   Grossman SA, Phuphanich S, Lesser G, et al. Toxicity, Efficacy and Pharmacology of Suramin in Adults With Recurrent High-Grade gliomas. J Clin Oncol 2001; 19(13): 3260-3266

93.   Hussain M, Fisher EI, Petrylak DP, et al. Androgen Deprivation and Four Coursed of Fixed-Schedule Suramin Treatment in Patients With Newly Diagnosed Metastatic Prostate Cancer: A Southwest Oncology Group Study. J Clin Oncol 2000; 18:1043-1049

94.   Jodrell DI, Reyno LM, Sridhara R, et al. Suramin: Development of a Population Pharmacokinetic Model and Its Use With Intermittent Short Infusions to Control Plasma Drug Concentration in Patients With Prostate Cancer. J Clin Oncol 1994; 12(1): 166-175

95.   Jusko WJ, Gretch M. Plasma and Tissue Protein Binding of Drugs in Pharmacokinetics. Drug Metabolism Reviews; 1976; 5(1): 43-140

96.   Kassask M, Nickel P. Stability of Suramin in Aqueous Solution; Possible Implications for the Search for Suramin Metabolites in Patients. Arch Pharm Pharm Med Chem 1996; 329: 225-228

97.   Klecker RW, Collins JM. Quantification of Suramin By Reverse-Phase Ion-Pairing High-Performance Liquid Chromatography. J Liq Chromat 1985; 8(9): 1685-11696

98.   Kobayashi K, Vokes EE, Vogelzang NJ, et al. Phase I Study of Suramin Given by Intermittent Infusion Without Adaptive Control in Patients With Advanced Cancer. J Clin Oncol 1995; 13(9): 2196-2207

99.   Kobyashi K, Vokes EE, Vogelzang NJ. Phase I Study of Suramin Administered by Intermittent Infusion Without Adaptive Control to Cancer Patients: Update of Two Expanded Dose Levels Near the Maximally Tolerated Dose. J Clin Oncol 1996; 14(9): 2622-2624

100. Kurz H. Trunk H, Weitz B. Evaluation of Methods to Determine Protein Binding of Drugs. Drug Res 1977; 27(7): 1373-1380

101. La Rocca RV, Stein CA, Myers CE. Suramin: Prototype of A New Generation of Antitumor Compounds. Cancer Cells 1990; 2(4): 106-115

102. Larsen AK. Suramin: An Anti-Cancer drug with Unique Biological Effects. Cancer Chemother Pharmacol 1993; 32:96-98

103. Lockwood GF, Wagner JG. Plasma Volume Changes As A Result Of Equilibrium Dialysis. J Pharm Pharmacol 1983, 35:387-388

104. Lopez RL, Peters GJ, van Loenen AC.,et al. The Effect Of Schedule, Protein Binding and Growth Factors on the Activity of Suramin. Int J Cancer 1992; 51:921-926

105. Mallet A. A Maximum Likelihood Estimation Method For Random Coefficient Regression Models. Biometrika; 1986; 73(3): 645-656

106. Motzer RJ, Nanus DM, O'Moore P. Phase II of Suramin in Patients with Advanced Renal Cell Carcinoma: Treatment Results, Pharmacokinetics and Tumor Growth Factor Expression. Cancer Res 1992; 52: 5775-5779

107. Muller WE, Wollert U. Influence of Various Drugs on the Binding of L-Trytophan to Human Serum Albumin. Res Comm in Chem Path Pharmacol 1975; 10(3): 565-568

108. Muller WE, Wollert U. Spectroscopic Studies on the Complex formation of Suramin With Bovine and Human Serum Albumin. Biochimica et Biophysica Acta 1976; 427: 465-480

109. Myers C, Cooper M, Stein C, et al. Suramin: A Novel Growth Factor Antagonist With Activity in Hormone –Refractory Metastatic Prostate Cancer. J Clin Oncol 1992; 10(6): 881-889

110. Newton HB. Novel Chemotherapeutic Agents for the Treatment of Brain Cancer. Exp Opin Invest Drugs 2000; 9(12): 2815-2829

111. Olson JJ, Polk DM, Reisner A. The Efficacy and Distribution of Suramin in the Treatment of 9L Gliosarcoma. Neurosurgery 1994; 34(2): 297-308

112. Ong ST, Vogelzang NJ. Chemotherapy in Malignant Pleural Mesothelioma: A Review. J Clin Oncol 1996; 14(3): 1007-1017

113. Oosterhuis B, van Boxtel CJ. Kinetics of Drug Effects in Man. Ther Drug Monit 1988; 10: 121-132

114. Pike E, Skuterud B, Kierulf P. Binding and Displacement of Basic, acidic and Neutral Drugs in Normal and Orosomucoid-deficient Plasma. Clin Pharmacokinetics 1981; 6: 367-374

115. Pinedo HM, Rijswijk REN. Suramin Awakes? J Clin Oncol 1992; 10(6): 875-877

116. Plate KH, Risau W. Angiogenesis in Malignant Gliomas. Glia 1995; 15::339-347

117. Roboz J, Deng L, Ma L, Holland JF. Investigation of Suramin-Albumin Binding by Electrospray Mass Spectrometry. Rapid Commun Mass Spectrom 1998; 12: 1319-1322

118. Rosen PJ, Mendoza EF, Landaw EM, et al. Suramin in Hormone-Refractory Metastatic Prostate Cancer: A Drug With Limited Efficacy. J Clin Oncol 1996; 14(5): 1626-1636

119. Schentag JJ, Bellow CH. Tissue-Directed Pharmacokinetics. Amer J Med 1991; 91(suppl 3A): 3A-5S-3A-10S

120. Sheiner LB, Rosenburg B, Marathe VV. Estimation of Population Characteristics of Pharmacokinetic Parameters from Routine Clinical Data. J Pharm Bio 1977; 5(5): 445-479

121. Sheiner LB. Analysis of Pharmacokinetic Data Using Parametric Models.I. Regression Models. 1984; J Pharm Bio 12(1): 93-117

122. Sheiner LB. Analysis of Pharmacokinetic Data Using Parametric Models.II. Point Estimates of An Individuals's Parameters 1985; J Pharm Biol 13(5): 515-540

123. Sheiner LB. Analysis of Pharmacokinetic Data Using Parametric Models.III. Hypothesis Tests and Confidence Intervals 1986; J Pharm Biol 14(5): 539-555

124. Sheiner LB. The Population Approach to Pharmacokinetic Data Analysis: Rationale and Standard Data Analysis Methods. Drug Met Rev 1984; 15(1&2): 153-171

125. Small EJ, Meyer M, Marshall E, et al. Suramin Therapy for Patients With Symptomatic Hormone-Refractory Prostate Cancer: Results of a Randomized Phase III Trial comparing Suramin Plus Hydrocortisone to Placebo Plus Hydrocortisone. J Clin Oncol 2000; 18: 1440-1450

126. Sridhara R, Eisenberger, Sinibaldi VJ, et al. Evaluation of Prostate-Specific Antigen as a Surrogate Marker for Reponse of Hormone-Refractory Prostate cancer to Suramin Therapy. J Clin Oncol 1995; 13(12): 2944-2953

127. Steimer J, Mallet A, Golmard J, et al. Alternative Approaches to Estimation of Population Pharmacokinetic Parameters: Comparsion with

the Nonlinear Mixed Effect Model. Drug Met Rev 1984; 15(1&2): 265-292

128. Supko JG, Malspies L. A Rapid Isocratic HPLC Assay of Suramin (NSC 34936) in Human Plasma J Liq Chromat 1990; 13(4): 727-741

129. Takano S, Gately S, Engelhard H, et al. Suramin inhibits glioma cell proliferation in in vitro and in the brain. J Neuro Oncol 1994; 21: 189-201

130. Van Rijswijk REN, van Loenen AC, Wagstaff J. Suramin: Rapid Loading and Weekly Maintenance Regimens for Cancer Patients. J Clin Oncol 1992; 10(11): 1788-1794

131. Vansterkenburg ELM, Wilting J, Jannsen LH. Influence of pH on The Binding of Suramin To Human Serum Albumin Biochem Pharmacol 1989; 38(18): 3029-3035

132. Weiss GR, Burris HA, Eckhardt SG, et al. New Anticancer agents. Cancer Chemoth and Biol Res Mod 1997; 17:178-194

133. Whiting B, Kelman AW, Grevel J. Population Pharmacokinetics: Theory and Clinical Application. Clin Pharmacokinetics 1986; 11: 387-401

134. Wright JD, Boutinot FD. Measurement and Analysis of Unbound Drug Concentrations. Clin Pharmacokinet 1996; 30(6): 445-462
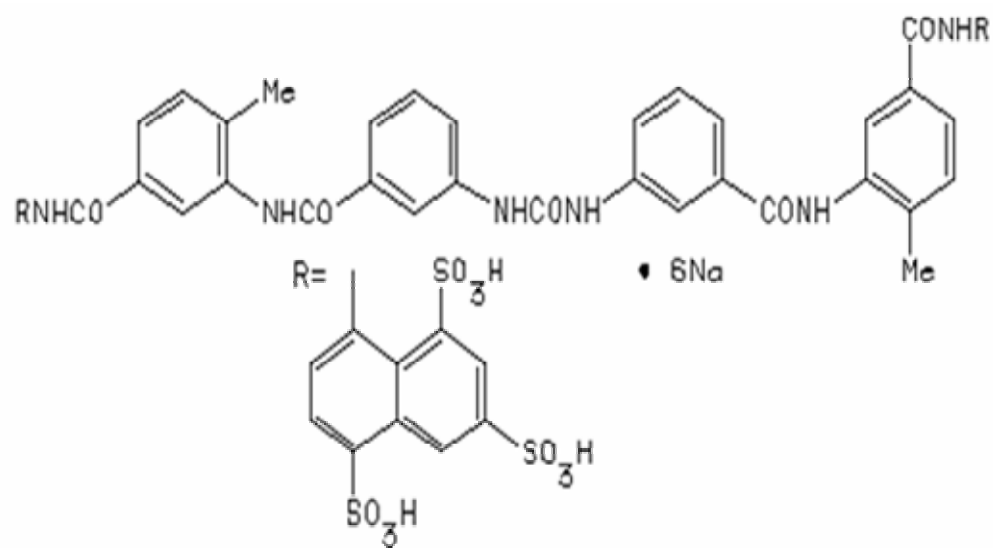
Figure 1.1. Chemical Structure of Suramin

CHAPTER 2

CLINICAL PHARMACOKINETICS OF SURAMIN

IN PATIENTS WITH BRAIN CANCER[1]

ABSTRACT

Suramin is a novel anti-cancer drug currently being investigated as a treatment for brain cancer. The objective of this study was to characterize the pharmacokinetics and intersubject variability of suramin in patients with brain cancer. Plasma samples were collected from 16 patients and data was analyzed using nonlinear mixed effects modeling. The data were best described by a two compartment model, with elimination from the central compartment with gender and body surface area (BSA) as covariates. Volume of distribution was estimated to be 4.16 L/hr/m^2 ($\pm$ 0.31) in male patients and in 4.16 L/hr/m^2 ($\pm$0.23) females in the first compartment. Volume of distribution in the second compartment was 21.2 ($\pm$ 2.26) in males and 12.6 ($\pm$ 3.8) in females. Elimination was from the central compartment. The results of this study showed that the pharmacokinetic parameters of suramin in brain patients were similar to those observed in studies examining use of suramin in prostate cancer. The study also showed that using a model that included gender as a covariate decreased the amount of inter- and intra-subject variability.

Suramin is a symmetric polysulfonated naphthylurea that was developed as a treatment for the parasitic condition trypanosomiasis[1]. Suramin was also used as an anti-filerial agent in the treatment of onchocerciasis[2]. Because suramin inhibits reverse transcriptase it has been investigated as a treatment for human immunodeficiency virus (HIV) infection[3-7]. Although clinical data showed that suramin was not an effective treatment for HIV, it was observed that an HIV infected patient who had Kaposi's sarcoma and small-cleaved cell lymphoma showed regression of both malignancies. As a consequence of this unexpected finding, suramin has been studied as an anti-tumor agent for a number of neoplastic conditions[8] including hormone-refractory prostate cancer [9-15] and renal cell carcinoma [16-17].

Suramin has also been investigated as a treatment of brain cancer. In vitro, suramin inhibited cell proliferation in a dose dependent manner in C6, 9L [18], T98G, A-172, U-118 and U-138 and other cell lines. Suramin has also been studied in rodents implanted with glioma cells [19-23]. Tumor bearing rats that had been administered suramin had a shorter survival rate than those rats not injected with suramin. This observation was attributed to intracerebral hemorrhage. Suramin did not improve either tumor vascular density or mean tumor size. Only negligible amounts of suramin penetrated the brain. This was expected because of the physiochemical nature of the drug. Suramin is highly hydrophilic and anionic in nature, and drugs with these properties generally can not penetrate the blood brain barrier. Suramin's lack of effectiveness as an anti-tumor agent was attributed to incomplete penetration of the brain or inadequacy of the drug delivery system. Suramin, however, was shown to penetrate the endothelial cells. It was suggested that suramin passed through the more permeable capillaries at the perimeter of the tumor.

While the results of the preclinical in vivo studies showed that suramin's ability to inhibit glioma cell proliferation in vitro could not be extrapolated to animal models, clinical studies in brain cancer patients were pursued because of the lack of any effective

chemotherapeutic agent against this disease[24]. Suramin was administered to twelve patients with varied gliomas to examine toxicity, efficacy and pharmacological activity in this patient population. Patient dosage was based on a dosing scheme used in clinical studies that examined suramin as a treatment of prostate cancer.

Suramin was shown to be well tolerated in these patients and toxicities that did arise were mild and reversible. Suramin improved or stabilized the gliomas present in three patients, and these individuals lived more than 400 days after beginning suramin therapy. While no pharmacokinetics results were presented, the authors stated that the typical pharmacokinetic parameters calculated for the brain cancer patient population was similar to those seen in the various prostate cancer studies.

The objective of this paper was to characterize the pharmacokinetics and intersubject variability of suramin in brain cancer patients using nonlinear mixed effects modeling.

MATERIALS AND METHODS

Patient Population

The clinical study was conducted at Emory University. Patients were administered suramin according to the scheme used in the previously reported prostate cancer study (Table 2.1). Data from the 16 patients with a total of 519 concentrations were obtained retrospectively from medical records and routine drug monitoring at the Emory University Hospital for population analysis. Suramin concentrations were measured at the beginning of the dose (peak concentrations) and before the next dose (trough concentrations). Washout data was obtained for several patients. Patient demographics and clinical data are presented in Table 2.2.

Pharmacokinetic Modeling

The population data analysis was performed using nonlinear mixed effects model (NONMEM version 5.1, NONMEM Project Group, University of California at San Francisco, CA). The first order estimation method with the POSTHOC option was used to predict the individual parameter estimates from the population values. The concentration time course of suramin was initially analyzed using two- and three-compartment models. Data was analyzed using both models because suramin has been reported as either a two compartment or a three compartment model drug. The pharmacokinetic parameters used by the two-compartment model (ADVAN 3, TRANS4 subroutines from the PREDP library) were as follows: clearance from the central compartment (CL), volume of distribution of the central compartment (V1), volume of distribution of the peripheral compartment (V2) and the distributional clearance between the central and peripheral compartment, (Q). The pharmacokinetic parameters used by the three compartment model (ADVAN 5) were as follows: clearance from the central compartment (CL), volume of distribution of the central compartment (V1), volume of distribution of the first peripheral compartment (V2), volume of distribution of the second peripheral compartment (V3) and the distributional clearance between the compartments (Q1 and Q2). These models are illustrated in Figure 1.

For the two compartment model:

$CL = \Theta_1$

$V1 = \Theta_2$

$Q = \Theta_3$

$V2 = \Theta_4$

For the three compartment model:

$CL = \Theta_1$

$V1 = \Theta_2$

$$Q1 = \Theta_3$$

$$V2 = \Theta_4$$

$$Q2 = \Theta_5$$

$$V3 = \Theta_6$$

Structural Model.

Diagnostics plots and the Akaike Information Criterion (AIC)[25-26] was used to determine if suramin concentrations as a function of time in this study were best described by an two-compartment or three-compartment model. The AIC value is calculated from the equation:

$$AIC = l_A\text{-}l_B + 2\ (p_A - p_B)$$

where l_A is the minimum objective function of the two-compartment model, (A) l_B denotes the minimum objective function of the three-compartment model, and p_A and p_B are the number of parameters in the two- and three-compartment model, respectively. If the calculated value is less than zero, then the full model (A) is used, whereas if the calculated valued is greater than zero, the reduced model (B) is used.

The structural model was fit using the simplest model likely to fit data. Each parameter was assigned a single theta and the inter-patient variability (eta) was determined for volume of distribution. The intra-patient variability was determined in terms of a simple additive model ($y = F + \varepsilon$).

Intermediate Model

The purpose of the intermediate model was to refine the characterization of the intra- and inter-patient variability. This was performed by using an additive, proportional or a combination of additive and proportional model to describe the residual error in concentration estimates and the variability in parameter estimates in between patients.

Inter-patient Variability:

Additive Model

$$\Theta = \Theta_N + \eta_N$$

Proportional Model

$$\Theta = \Theta_N * (1 + \eta_N)$$

Intra-patient Variability:

Additive Model

$$Y = F + \varepsilon_1$$

Proportional Model

$$Y = F * (1 + \varepsilon_1)$$

Additive and Proportional Model

$$Y = F * (1 + \varepsilon_1) + \varepsilon_2$$

Final Model

The final model population pharmacokinetic model in cancer relates the continuous variable body surface area (BSA) and the categorical variable, gender to the mean population parameter estimates. The final model was determined by analyzing data by adding BSA and gender to each parameter of the selected structural model in a stepwise fashion.

RESULTS

Structural Model

Figure 2.1 shows Model A (two compartment) and Model B (three compartment) with first-order elimination from the central compartment. Model A used four parameters to describe the elimination and distribution of suramin whereas Model B used six parameters to describe suramin's pharmacokinetics. The minimum objective function

for Model A was 4262 and the minimum objective function for Model B was 4181.   The

AIC calculated for the comparison of the two models was 77. The AIC value was greater

than zero suggesting that the three compartment model would be the appropriate model to

describe the data. The diagnostic plots however, showed that while the three

compartment model accurately predicted higher concentrations, it underestimated lower

concentrations. When the diagnostic plots of the two compartment model were

examined, it was observed that the two compartment predicted lower concentrations more

accurately than the three compartment model. The two compartment model was selected

as the best structural model to describe data. Figures 2.2A and 2.2B illustrate predicted

concentrations as a function of observed concentrations for Models A and B.

Intermediate Model

The intermediate model included a structural model and parameters to describe

the residual error (ε) and the inter-patient variability (η). Figure 2.3A depicts the

predicted values as a function of the observed values using the intermediate model. The

model that best fit the data used a proportional error to describe variability of the

clearance parameters and an additive model to describe the variability of the volume of

distribution in the central and peripheral compartment. Figure 2.3B depicts the weighted

residuals as a function of the predicted variables in the structural and intermediate

models. The residual error was best described by a proportional model. It was observed

that prediction of concentrations was improved when the interpatient variability was

described accurately.

Final Model

The final model includes the patient variables BSA and gender in the model used

to predicted suramin concentrations. Table 2.3 shows the relationship between the

minimum objective function and the variables included in the final model. The estimates

generated using this model include the mean parameter estimate, the influence of gender

and BSA on these estimates, and the intra- and inter- patient variability. Table 2.4 is a

listing of the parameter estimates. Figure 2.4A depicts predicted concentrations as a

function of observed concentrations. Figure 2.4B shows the relationship between

individual predictions and observed predictions. Figure 2.4C shows the weighted

residuals as a function of the predicted values. Figure 2.5 is representative of a patient

that received one course of treatment of suramin.

 DISCUSSION

The objective of this paper was to characterize the pharmacokinetics and

intersubject variability of suramin in brain cancer patients using nonlinear mixed effects

modeling.   The number of patients needed to definitively calculate population parameter

estimates has not been established. Generally with a population of sixteen patients the

two-stage iterative method is used. NONMEM allowed the calculation of population

typical values and population variability values without a large number of observations.

The final model used to describe the pharmacokinetics of suramin in brain cancer

patients included a two-compartment structural model, an additive and proportional

model to describe the variability in-between patients, and a proportional model to

describe the residual error.   In studies performed by Jodrell[26] and Cooper[27], a three

compartment model was used describe the data. In studies to improve the administration

of suramin using adaptive control and a Bayesian algorithm, researchers noted that three

compartment model did a better job in fitting the washout data in patients. The two

compartment model actually underestimated actual plasma concentrations at the final

stages of treatment. Despite this findings, it was stated that although the three

compartment model did a better job of predicting washout concentrations, there was no

difference in the time that suramin remained at therapeutic concentrations and the three

compartment model did not enhance the ability to control dosing. This was attributed to

the fact that the two compartment model and the three compartment model had similar

values for volume of distribution from the central compartment and that elimination occurs in a linear fashion from the central compartment.

Although the Akaike Information Criterion was larger for the two compartment model than the three compartment, the two compartment model was selected as the structural model. This selection was based on the observation that the two compartment model actually predicted lower concentrations and wash out data more accurately that three compartment model in this instance. The finding that two compartment model best fit the data in this study might be due to the limited number of subjects or the limited amount of plasma concentrations taken from the plasma compartment.

In this study the values estimated for the clearance were comparable to previous findings. The clearance from the central compartment was slow (0.020 L/hr/ m^2 in the male patients and 0.017 L/hr/ m^2 in the female patients). In the study performed by Jodrell et al, the clearance from the central compartment was 0.013 L/hr/ m^2 (C.V 46%). The volume of distribution in the central compartment (4.16 L/m^2 in males and 4.17 L/m^2) is similar to that predicted by Jodrell (3.5 L/m^2). This an interesting finding because suramin is highly bound to plasma proteins and is expected only to distribute in plasma water. The volume of plasma water in a healthy 70 kg human is 0.04 L/kg. In all studies the volume of distribution values are higher than the normal volume of plasma water. This finding suggests that the free fraction of suramin diffuses out of the plasma water into the interstitial fluid and body water. The volume of distribution (21.2 L/ m^2 in males and 12.6 L/ m^2 in females) in the peripheral compartment is also high in relation to the degree of protein binding. The volume of distribution values suggest that there is binding of suramin to tissue.

Suramin is a large molecular weight polyanionic compound and the findings presented in this study are comparable to those found in studies investigating the two possible treatments for HIV, the polyoxometalates and the dextran sulfates.

Polyoxometalates are condensed compounds that consist of insoluable minerals. These compounds are generally used as catalysts because of their strong acidity, oxidative power and their crystalline structure. The anti-viral activity of this group of compounds has been linked to their ability to prevent the binding or fusion of infected lymphocytes to uninfected cells. Pharmacokinetic studies examining both total drug concentration and unbound concentrations have shown that the plasma protein binding of these compounds are nonlinear. As a consequence the pharmacokinetics of polyoxometalates are concentration dependent. Like suramin, the polyoxometalates have a large volume of distribution and extremely long half-life. It has been proposed that the extended elimination rate may be due to the slow systemic clearance of the drug. It has also been observed that the polyoxometalates may be renally secreted or reabsorbed depending on the structure of the compound.

In this study, the ratio of the total clearance of suramin and normal levels of inulin (7.5 L/hr) was less than 1, suggesting that suramin undergoes renal reabsorption. This may include accumulation in the renal tubules or binding to the cell membrane.

The objective of this paper was to characterize the pharmacokinetics of suramin using nonlinear mixed effects modeling. The results of this study showed that the pharmacokinetic parameters of suramin in brain patients were similar those observed in studies examining use of suramin in prostate cancer. The study also showed that using a model that included gender as a covariate decreased the amount of inter- and intra-subject variability.

REFERENCES

1.   Dressel J, Oesper RE. The Discovery of Germanin by Oskar Dressal and Richard Kothe. J Chem Edu 1961; 38: 620

2.   Hawking F. Suramin: with special reference to onchocerciasis. Adv Pharmacol Chemotherapy 1978; 15: 289

3.   De Clerq E. Suramin: A potent inhibitor of reverse transcriptase of RNA tumor viruses. Cancer Lett 1979; 8: 9

4.   Mitsuya H, Popovic M, Yarchoan R, et al. Suramin protection of T cells in vitro against infectivity and cytopathic effect of HTLV-III. Science 1984; 226:172

5.   Broder S, Collins JM, Markham PD, et al. Effects of suramin on HTLV-III/LAV infection presenting as Kaposi's sarcoma or AIDS related complex: Clinical pharmacology and suppression of virus replication in vivo. Lancet 1985; 2: 627

6.   Levine A, Gill PS, Cohen J, et al. Suramin antiviral therapy in AIDS: Clinical immunology, and virologic results. Annu Intern Med 1986; 105: 32

7.   Collins JM, Klecker RW, Yarchoan R. Clinical pharmacokinetics of suramin in patients with HTLV-III/LAV infection. J Clin Pharmacol 1986; 26:22

8.   Stein CA, La Rocca RV, Thomas R. Suramin: an anti-cancer drug with a unique mechanism of action. J Clin Oncol 1989; 7:499

9.   Small EJ, Meyer M, Marshall ME. Suramin therapy for patients with symptomatic hormone-refractory prostate cancer: Results of a randomized phase III trial comparing suramin plus hydrocortisone to a placebo plus hydrocortisone. J Clin Oncol 2000; 18: 1440

10.   Dawson NA, Cooper MR, Figg WD, et al. Antitumor activity of suramin in hormone refractory prostate cancer controlling for hydrocortisone treatment and flutamide withdrawal a potentially cofounding variables. Cancer 1995; 76: 453

11.   Garcia-Schurmann JM, Schurlze H, Haupt G, et al. Suramin treatment in hormone-and chemotherapy –refreactory prostate cancer. Urology 1999; 53: 535

12.   Eisenberger MA, Reyno LM, Jodrell DI, et al. Suramin, an active drug for prostate cancer: Interim observations in a phase I trial. J Natl Cancer Inst 1993; 85: 611

13.   Hussain M, Fisher EI, Petrylak DP, et al. Androgen deprivation and four courses of fixed-schedule suramin treatment in patients with newly diagnosed metastatic prostate cancer: A Southwest Oncology Group study. J Clin Oncol 2000; 18: 1043

14.   La Rocca Rv, Cooper MR, Uhrich M, et al. Use of suramin in treatment of prostatic carcinoma refractory to conventional hormonal manipulation. Urol Clin North Am 1991; 18: 123

15.   Myers CE, Cooper MR, La Rocca RV, Suramin: A novel growth factor antagonist with activity in hormone-refractory metastatic prostatic cancer. J Clin Oncol 1992; 10: 881

16.   Motzer RJ, Nanus DM, O'Moore P, et al. Phase II Trial of Suramin in Patients with Advanced Renal Cell Carcinoma: Treatment Results, Pharmacokinetics and Tumor Growth Factor Expression. Cancer Res 1992; 52: 5775

17.   Dreicer R, Smith DC, Williams RD, et al. Phase II trial of suramin in patients with metastatic renal cell carcinoma. Invest New Drug 1999; 17: 183

18.   Takano S, Gately S, Engelhard H, et al. Suramin inhibits glioma cell proliferation in vitro and in the brain. Journal of Neuro-Oncology 1994; 21: 189

19.   Olsen JJ, Polk DM, Reisner A. The efficacy and distribution of suramin in the treatment of the 9L gliosarcoma. Neurosurgery 1994; 34: 297

20.   Coomber BL. Suramin C6 glioma induced angiogensis in vitro. J Cell Biochem 1995; 58: 199

21.   Ramirez LH, Julieron M, Bonnay M, et al. Stimulation of tumor growth in vitro and in vivo by suramin on the VX2 model. Invest New Drugs 1995; 13: 51

22.   Kim KJ, Li B, Winer J, et al. Inhibition of VEGF-induced angiogenesis suppresses tumor growth in vivo. Nature 1993; 362: 841

23.   Taki T, Ohnishi T, Arita N, et al. Anti-proliferative effects of TNP-470 on human malignant glioma in vivo: potent inhibition of angiogenesis. J Neuro-Oncol 1994; 19: 251

24.   Grossman SA, Phuphanich S, Lesser G, et al. Toxicity, Efficacy and Pharmacology of Suramin in Adults With Recurrent High-Grade Gliomas. J Clin Oncol 2001; 19(13): 3260

25.   Akaike H. A New Look at the Statistical Model Identification. IEEE Trans Automat Control 1974; 19: 716-723

26.   Yamaoka K, Nakagawa Y, Uno T. Application of Akaike's Information Criterion (AIC) in the evaluation of Linear Pharmacokinetic Equations. J Pharmacokin Biopharm 1978; 6: 165-175

27.   Ni Lan, Boudinot FD, Boudinot SG, et al. Pharmacokinetics of Antiviral Polyoxometalates in Rats. Antimicr Agents Chem 1994; 38:504-510

Table 2.1 Suramin Dosing Schedule

Treatment Day	Suramin Dose Cycle 1 (mg/m^2)	Suramin Dose Cycle 2 (mg/m^2)
1	1100	750
2	400	400
3	300	300
4	250	250
5	200	200
8	275	275
11	275	275
15	275	275
19	275	275
22	275	275
29	275	-
36	275	-
43	275	-
50	275	-
57	275	-
64	275	-
71	275	-
78	275	-

Table 2.2 Demographic data of patient population.

Characteristic	Mean ± SD
Number of Patients	16
Gender (M/F; %)	62.5/ 37.5
Weight (lbs)	174.01 ± 25.10
BSA (m^2)	1.911 ± 0.164
Number of Suramin Infusion	15.31 ± 6.5
Number of Suramin Samples	33.19 ± 17.36

A

I.V. RATE

V1 **Central** **Compartment**	**V2** **Tissue** **Compartment**

CLd

CLt

B

I.V. RATE

V2 **Tissue** **Compartment**	**V1** **Central** **Compartment**	**V3** **Tissue** **Compartment**

CLd12   CLd13

CLt
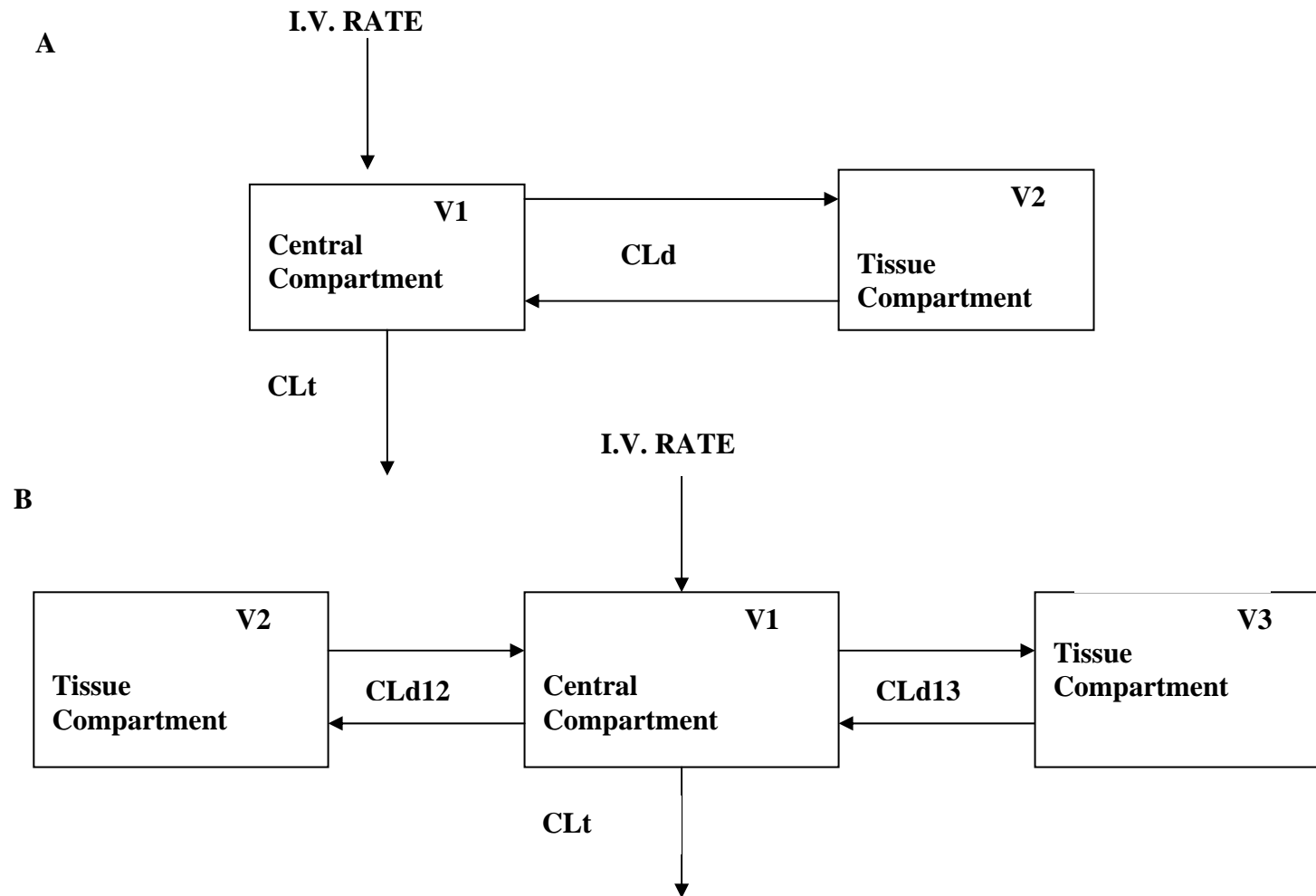
Figure 2.1 Open linear (A) 2- and (B) 3- compartment structural models.
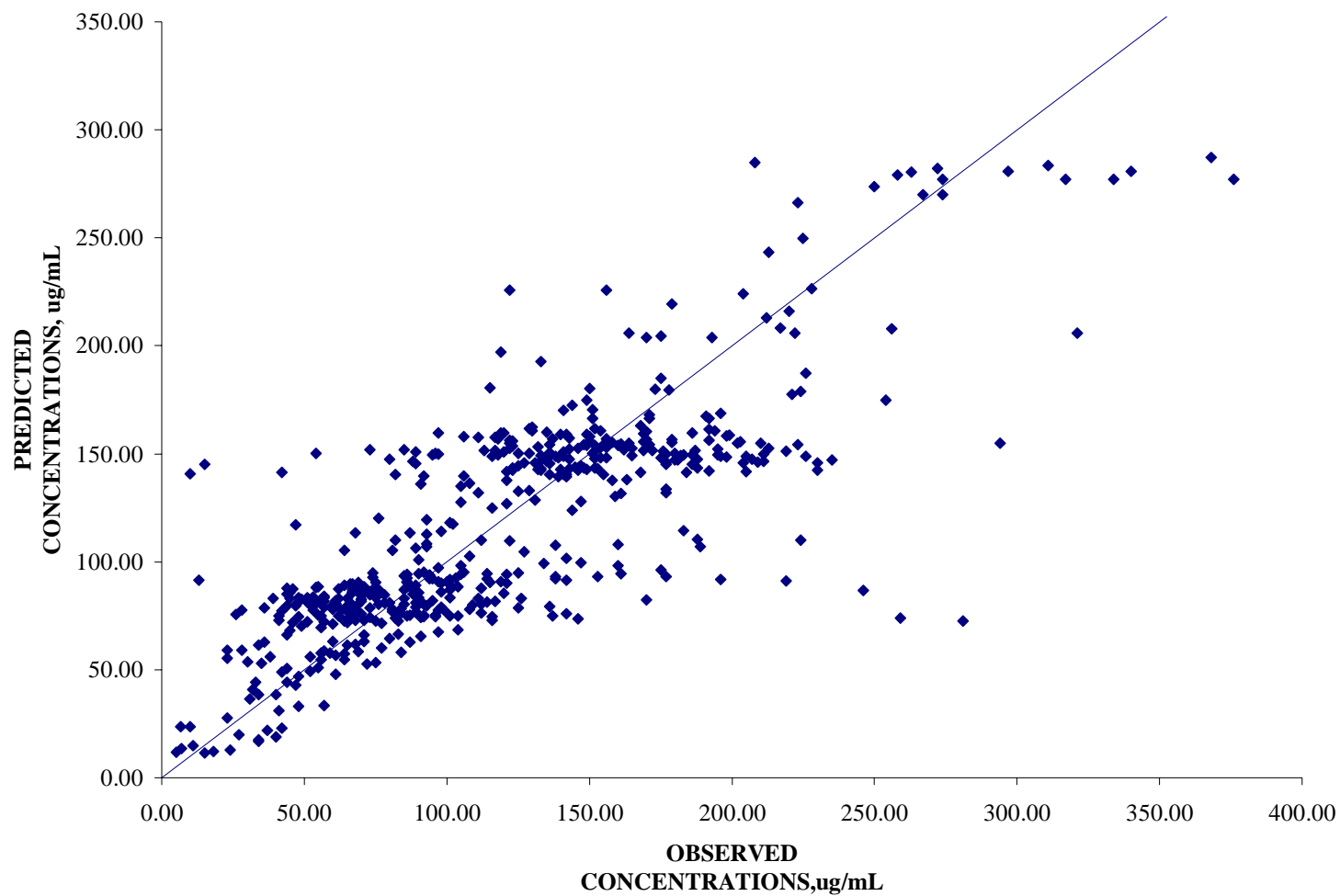
Figure 2.2A Predicted Concentrations as a function of Observed Concentrations with the Two Compartment Model
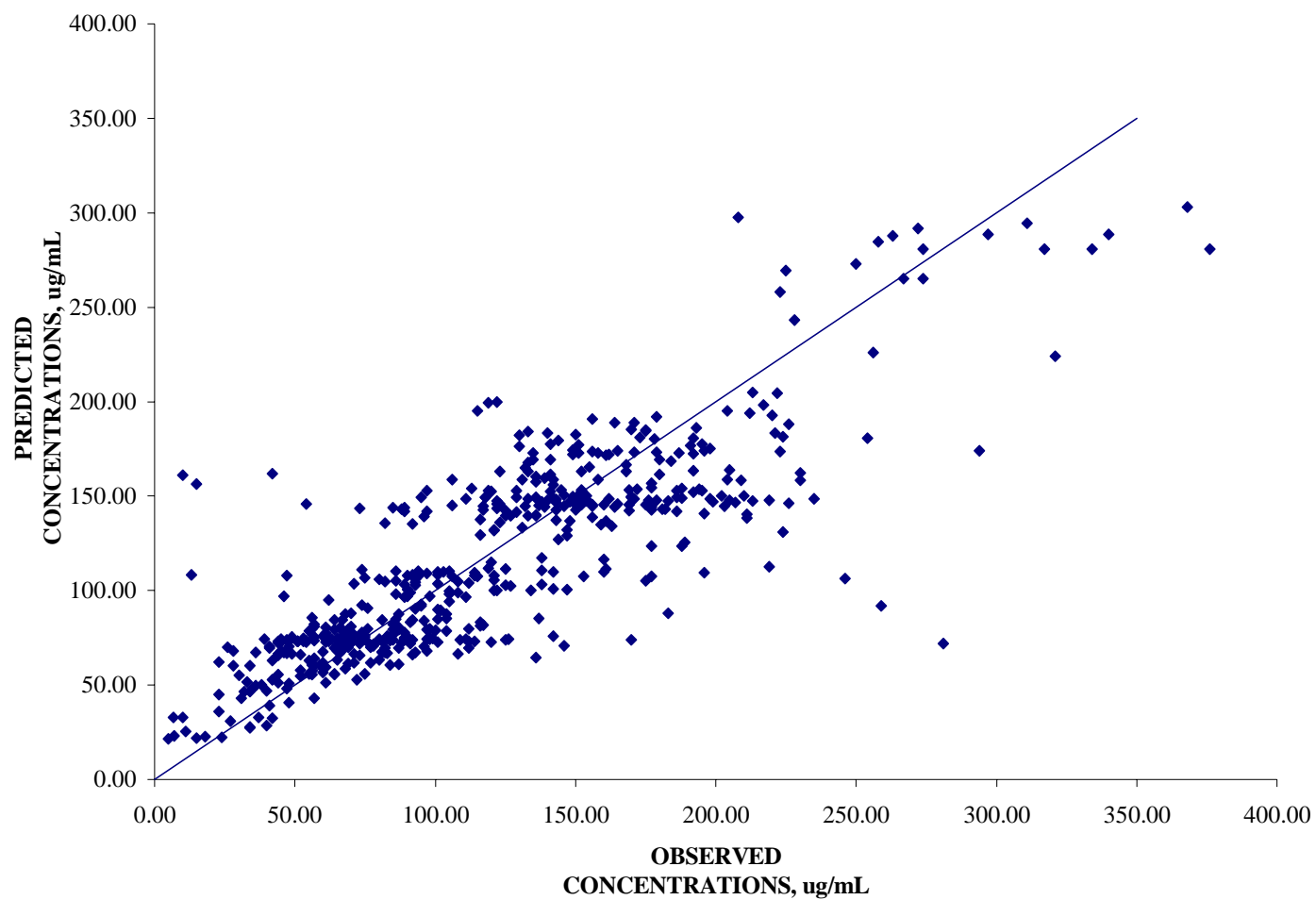
Figure 2.2B. Predicted Concentrations as a function of Observed Concentrations with the Three Compartment Model
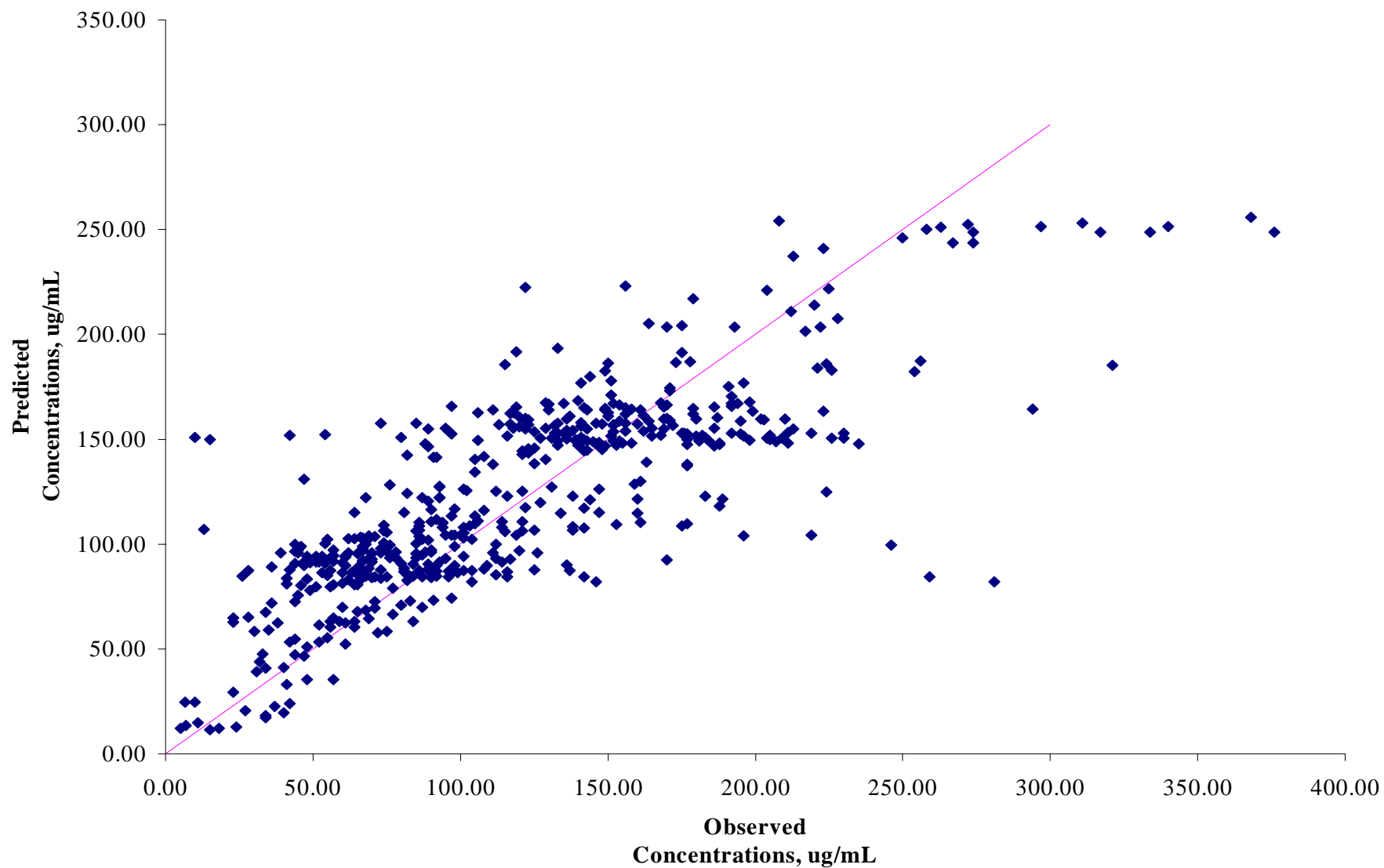
Figure 2.3A. The Predicted Concentrations Obtained Using The Intermediate Model As A Function of the Observed Concentrations

Figure 2.3B. Weighted Residuals as a Function of the Predicted Values

Figure 2.4A. Predicted Concentrations obtained in the Final Model as a function of Observed Concentrations
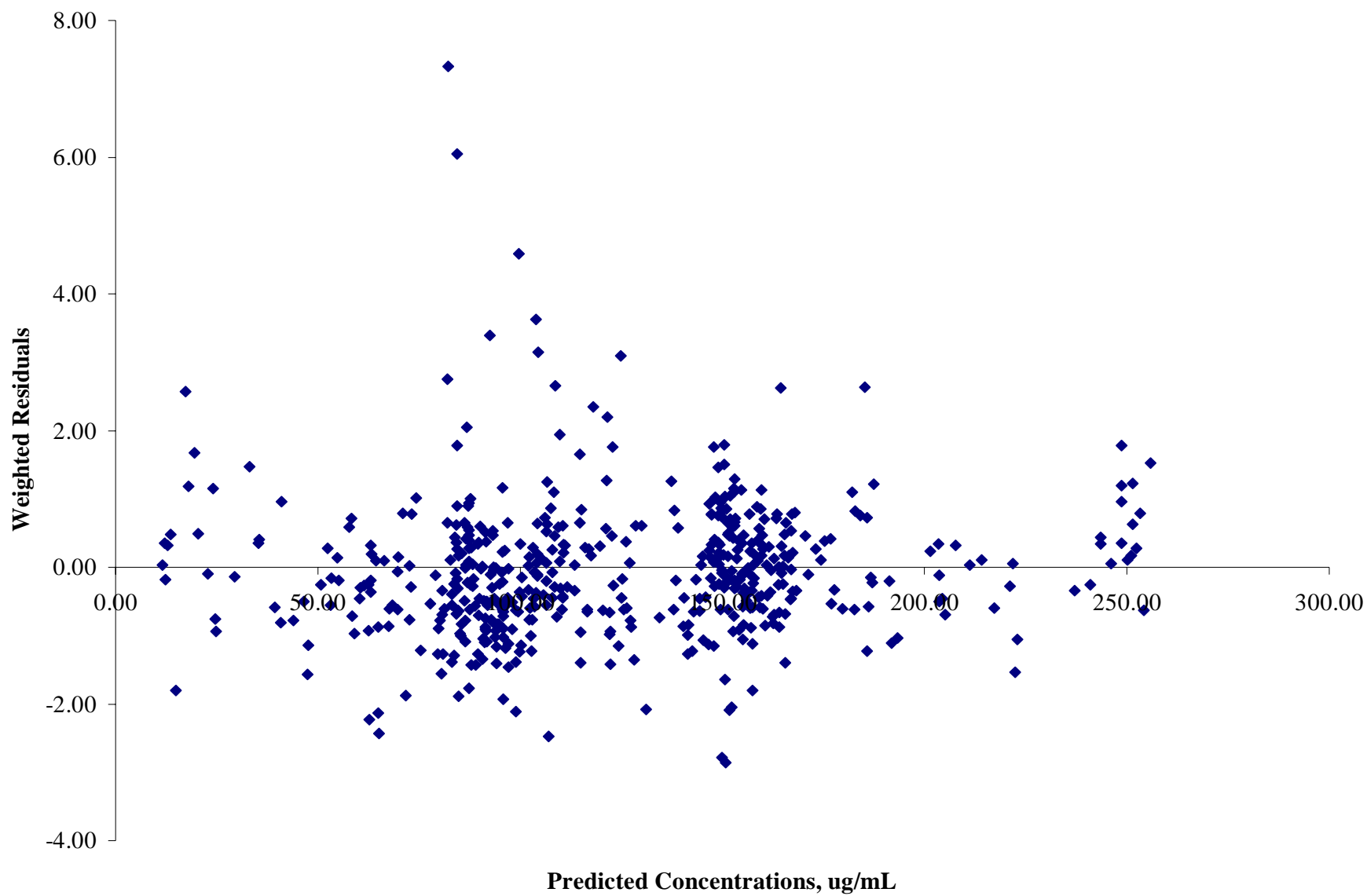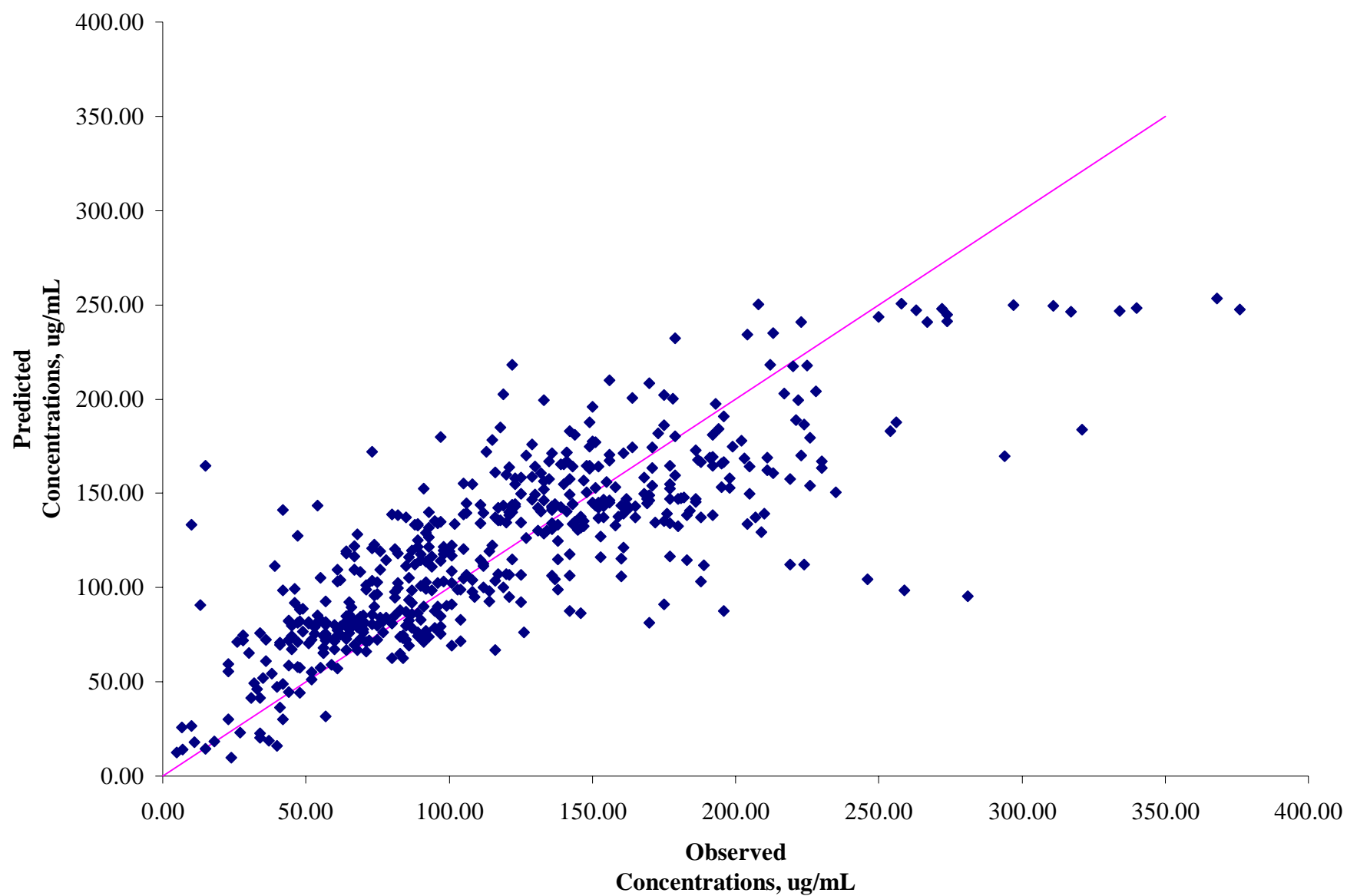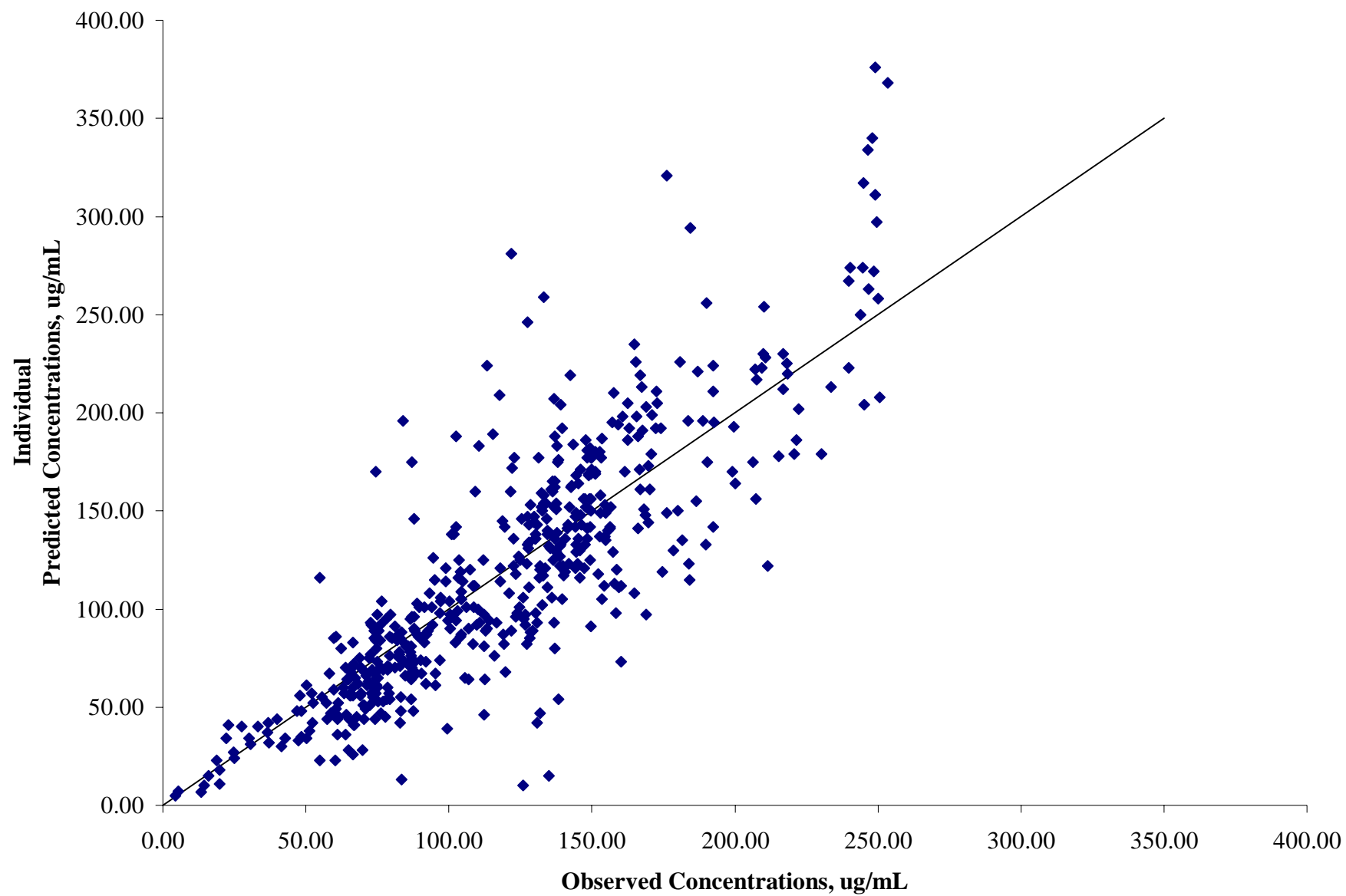
Figure 2.4B. Individual Predicted Concentrations as a function of Observed Concentrations
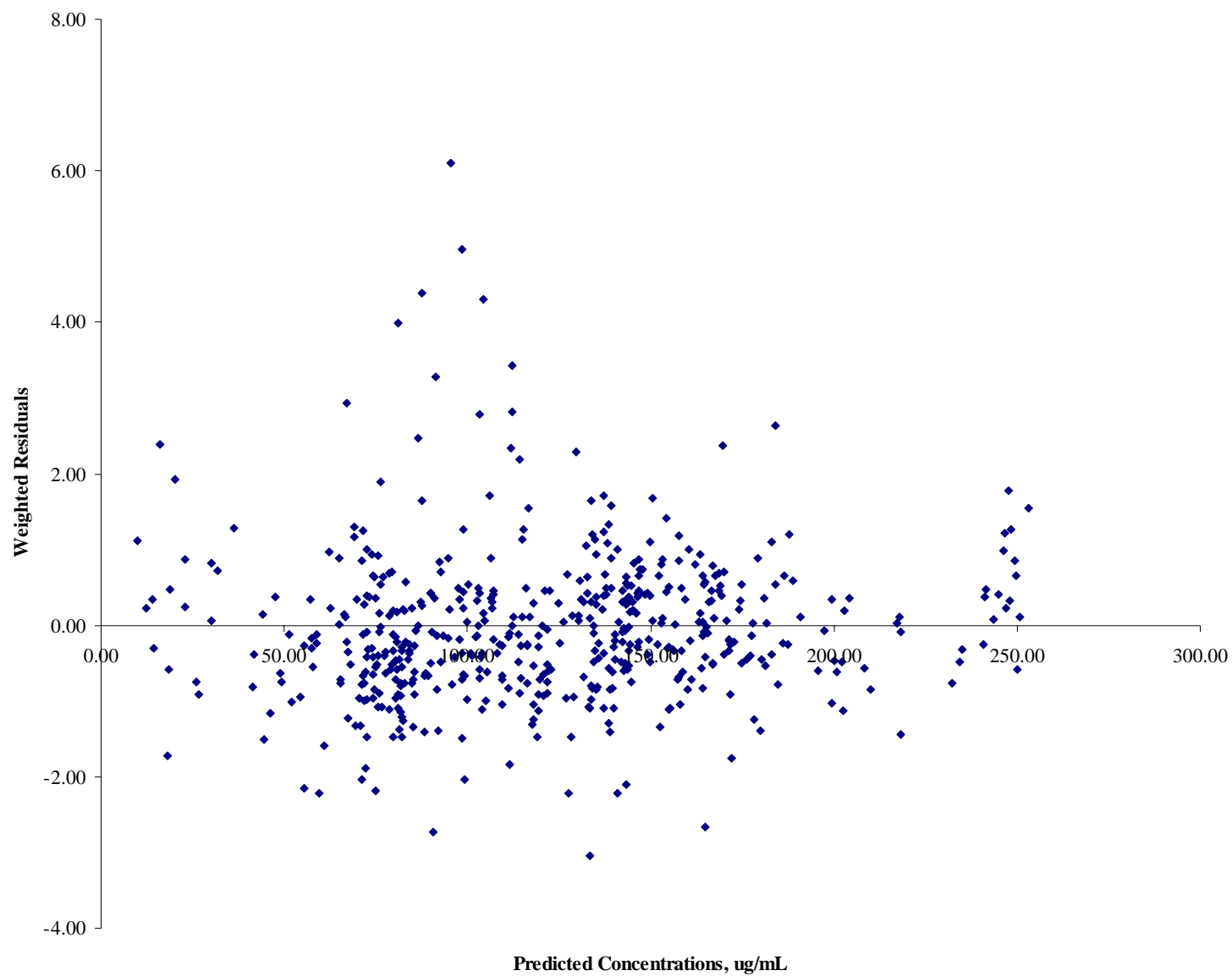
Figure 2.4C. Weighted Residuals as a Function of Predicted Concentrations

Table 2.3. Minimum Objective Function (MOF) as a function of model parameters

	Parameters	Minimum Objective Function
Structural Model	θ_{CLT} $\theta_{TVV1} = \theta_{V1} + \eta_V$ θ_Q θ_{V2} $y = F + \varepsilon$	4262
Intermediate Model	$\theta_{CLT} = \theta_{TVCLT} + \eta_{CLT}$ $\theta_{V1} = \theta_{TVV1} * (1 + \eta_{V1})$ $\theta_Q = \theta_{TVQ} + \eta_Q$ $\theta_{V2} = \theta_{TVV2} * (1 + \eta_{V2})$ $y = F * (1 + \varepsilon)$	4235
Final Model	$\theta_{TVCL} = (\theta_{TVCLM} * Gender) + (\theta_{TVCLF} * (1-Gender))$ $\theta_{CLT} = \theta_{TVCLT} + \eta_{CLT}$ $\theta_{TVV1} = (\theta_{TVV1M} * Gender) + (\theta_{TVV1F} * (1-Gender))$ $\theta_{V1} = \theta_{TVV1} * (1 + \eta_{V1})$ $\theta_{TVQ} = (\theta_{TVQM} * Gender) + (\theta_{TVQF} * (1-Gender))$ $\theta_Q = \theta_{TVQ} + \eta_Q$ $\theta_{TVV2} = (\theta_{TVV2M} * Gender) + (\theta_{TVV2F} * (1-Gender))$ $\theta_{V2} = \theta_{TVV2} * (1 + \eta_{V2})$ $y = F * (1 + \varepsilon)$	4210

Table 2.4. Pharamcokinetic Parameter Estimates (Mean (SD))

Parameter	Estimate (SD)
$CL_{T\ Male}$, L/hr/ m^2	0.020 (0.001)
$CL_{T\ Female}$, L/hr/ m^2	0.017 (0.014)
$V_{1\ Male}$, L/m^2	4.160 (0.31)
$V_{1\ Female}$, L/m^2	4.170 (0.23)
$CL_{D\ Male}$, L/hr/ m^2	0.150 (0.02)
$CL_{D\ Female}$, L/hr/ m^2	0.170 (0.016)
$V_{2\ Male}$, L/m^2	21.2 (2.26)
$V_{2\ Female}$, L/m^2	12.6 (3.8)
η_{CLT}, L/hr/ m^2	6.59×10^{-6}
η_{V1} (%)	1.46×10^{-5}
η_{CLD}, $L/hr/m^2$	1.53×10^{-4}
η_{V2} (%)	1.18×10^{-1}
σ_1	8.41×10^{-2}
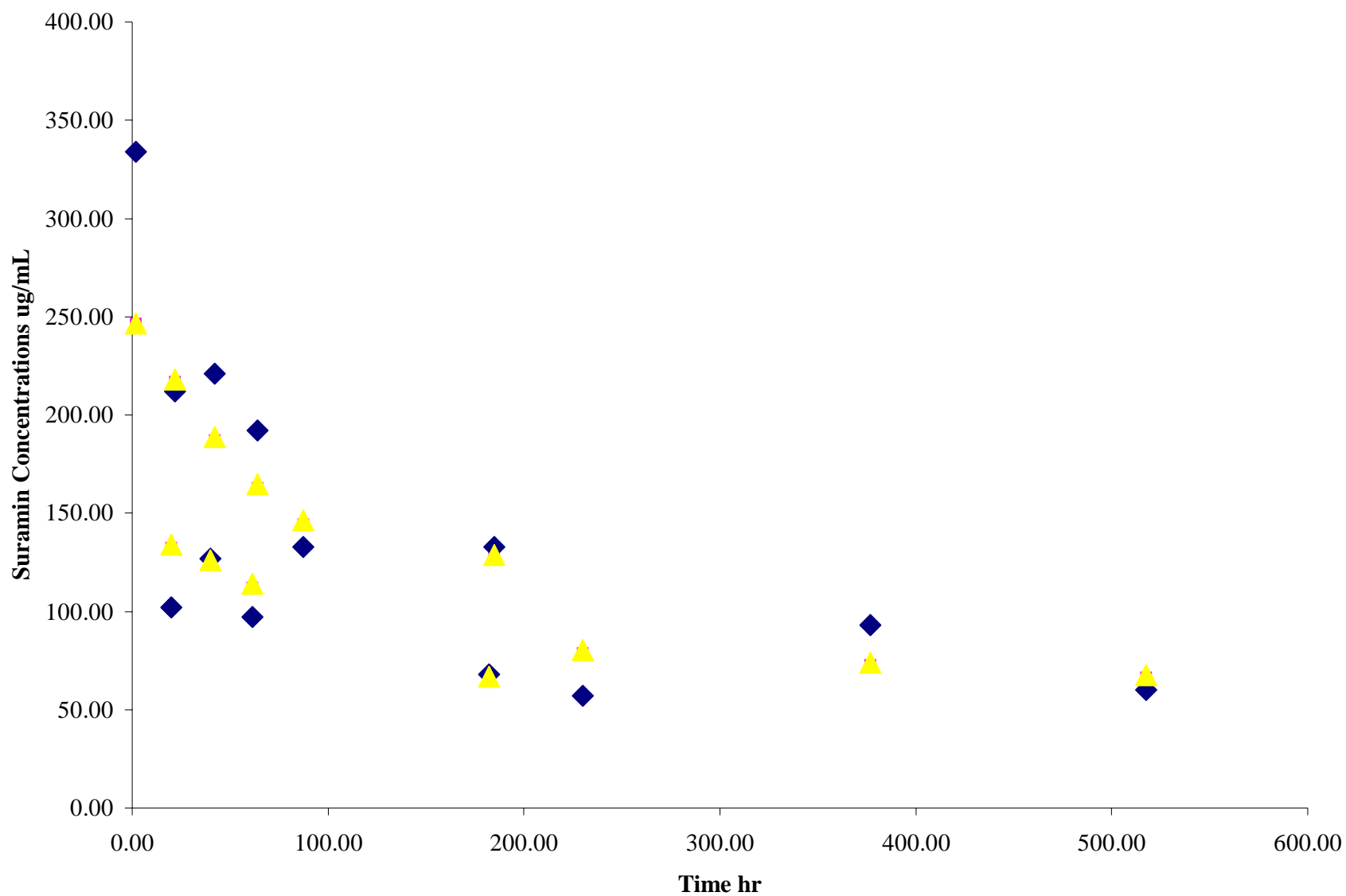
Figure 2.5. Representative Time Concentration Profile of a Patient That received One Course of Treatment The diamond symbol (◊) respresents the observed concentrations . The triangles (▲) represent the population estimates.

CHAPTER 3

PROTEIN BINDING OF SURAMIN TO HUMAN SERUM ALBUMIN AND

ALPHA $_1$ - ACID GLYCOPROTEIN[1]

ABSTRACT

Plasma protein binding studies were performed utilizing equilibrium dialysis to fully characterize the *in vitro* binding of suramin to human serum albumin, α_1-acid glycoprotein, and human plasma serum over wide range of drug concentrations. Suramin binds to albumin to two classes of binding sites, a high affinity saturable site and a low-affinity nonsaturable site (N_1=3.5, K_1=1.8 x 10^4 M^{-1}, N_2K_2=3.7 x 10^3 M^{-1}). Suramin binds to a single low-affinity nonsaturable site on α_1-acid glycoprotein (N_3K_3=1.5 x 10^5 M^{-1}). The fraction of suramin bound to plasma proteins predicted from the *in vitro* binding to human serum albumin and α_1-acid glycoprotein was identical to that observed in human plasma (95% $\pm$ 0.015). Thus, the plasma protein binding of suramin can be accounted for by the binding to these two proteins.

Keywords: suramin; plasma protein binding; albumin; α_1-acid glycoprotein

INTRODUCTION

Plasma protein binding of drugs is often of significant clinical importance, particularly for drugs that are highly bound. The pharmacokinetics of a drug are in part dependent on the extent of protein binding. Increases in volume of distribution values are often associated with decreased plasma protein binding. Hepatic clearance of drugs with low extraction ratios is inversely correlated to the extent of plasma protein binding. Renally cleared drugs may also be affected by plasma protein binding. Further, it is generally believed that only unbound drug can interact with receptor sites and stimulate pharmacological action, therefore the plasma protein binding also affects the efficacy and potential toxicity of a therapeutic agent.[1]

A number of factors influence the degree of plasma protein binding. These factors include the physical-chemical properties and concentration of the drug; the nature and concentration of protein available for binding; the affinity of the drug for the protein; the presence of other drugs; and the pathophysiological condition of the patient. Patients with liver disease [2,5] and renal disease [2-5] have been observed as having altered protein binding because of changes in plasma protein levels. During these disease states, albumin levels decrease and α_1-acid glycoprotein levels increase.[1]

Suramin is an organic polyanionic napthylurea used to treat to a number of conditions. This drug was originally used as an anti-parasitic agent for the treatment of trypanosomiaisis (sleeping sickness) and later onchocerciasis.[6] During the last twenty years, suramin has been investigated as a treatment for a number of other disease states including HIV (human immunodeficiency virus) [11], hormone-refractory prostatic cancer[7] and renal cell carcinoma.[8] Suramin has a narrow therapeutic index with effective concentrations between 200-300 ug/mL.[9] Severe neurotoxicity occurs at drug concentrations greater than 350 µg/mL.[10]

The plasma protein binding of suramin has been characterized to some extent. The compound is highly bound to plasma proteins (>99%)[11], binding predominately to

human serum albumin.[12] Studies assessing the influence of pH on the binding of suramin to albumin have demonstrated decreased binding with increasing pH. This was attributed to conformational changes in albumin between pHs 6-9 (N-B transition). The pH dependent changes in the electrostatic interaction of the negatively charged suramin with net negative charge on the albumin molecule are also believed to be, in small part responsible for the pH dependent binding of suramin to albumin. This interaction decreases with increasing pH.[12] The effect of pH on the binding of suramin has also been attributed to location on albumin to which suramin binds. The sites to which suramin binds on albumin are located on the top and bottom of the albumin structure. Positively charged histidine residues are part of the salt bridges between suramin and albumin. It is believed that the deprotonation of these histidine residues may also be responsible for decreased binding at high pH.[13]

The purpose of this study was to further characterize the plasma protein binding of suramin. Plasma protein studies were performed utilizing equilibrium dialysis to investigate the *in vitro* binding of suramin to human serum albumin, α_1-acid glycoprotein, and human plasma serum over wide range of drug concentrations.

MATERIALS AND METHODS

Albumin and α_1- *Acid Glycoprotein*. Human serum albumin (HSA), α_1-acid glycoprotein (AAG), and suramin sodium (MW 1429.2) were obtained from Sigma Chemical Co. (St. Louis, MO). Adult human plasma was obtained from normal volunteers. The serum albumin concentration used in the studies was 6.15×10^{-4} M (40 g/L) and the α_1- acid glycoprotein concentration was 1.61×10^{-5} M (0.7 g/L).

Tritiated suramin was obtained from Moravek Biochemical, Inc. (Brea, CA). Liquid scintillation fluid, ScintiVerse, was purchased from Fisher Scientific (Springfield, NJ). All other chemicals of reagent graded were purchased from Fisher Scientific (Springfield, NJ).

Protein Binding. Suramin sodium was added to HSA, AAG, and human plasma to yield concentrations ranging from 1.610×10^{-8} to 0.035 M, 2.10×10^{-8} to 0.007 M and 7.00×10^{-7} to 0.0035 M, respectively. Plexiglas dialysis cells, checked to insure that no leakage occurred, were used. Spectrapor II (Spectrum Medical Industries, Los Angeles, CA) dialysis membrane with a molecular cutoff of 12,000 to 14,000 was used for the protein binding experiments. Serum, HSA, or AAG (0.8 mL), with trace amounts of tritiated suramin added, was dialyzed against an equal volume of isotonic sodium phosphate buffer, pH 7.4, in a shaking water bath for 16 h. Preliminary studies showed that this was the time necessary to reach equilibrium. Post-dialysis serum and buffer volumes were measured and binding results were corrected for fluid shifts. [14] Aliquots (0.5 mL) of plasma and buffer were added to scintillation cocktail (5 mL) and DPMs were determined by liquid scintillation counting (Beckman, LS 6500). Experiments were done in triplicate.

Data Analysis. The Akaike's Information Criterion [5] and lack of systemic deviations around fitted curves were used to select model equations to fit the data. The protein binding of suramin to HSA was characterized by:

$$D_B = [(N_1 K_1 P_{HSA} D_F)/(1 + K_1 D_F)] + N_2 K_2 P_{HSA} D_F$$

where D_B, D_F, and P_{HSA} are the molar concentrations of bound drug, unbound drug, and HSA respectively; N is the number of binding sites per molecule; and K is the equilibrium association constant. Subscripts 1 and 2 refer to the first and second classes of binding sites, respectively. The protein binding parameters N_1, K_1, and $N_2 K_2$ were estimated by PCNONLIN nonlinear least squares regression.[16]

The protein binding of suramin to AAG was characterized by:

$$D_B = N_3 K_3 P_{AAG} D_F$$

where D_B, D_F, and P_{AAG} are the molar concentrations of bound drug, unbound drug, and AAG respectively. The protein binding parameters N_3K_3 were estimated by nonlinear least squares regression.

Simulations were performed using the binding parameters generated for HSA and AAG to predict the fraction bound of suramin to human plasma proteins using the equation:

$$D_B=[(N_1K_1P_{HSA}D_F)/(1+K_1D_F)] + N_2K_2P_{HSA}D_F + N_3K_3P_{AAG}D_F$$

The predicted protein binding based on HSA and AAG were compared to measured binding in human plasma.

RESULTS AND DISCUSSION

The plasma protein binding of suramin has been examined previously. Collins showed that suramin was 99.7% bound to plasma protein.[11] Since suramin is a highly bound drug, it is imperative that the binding of this drug be fully characterized. Vansterkenburg examined the influence of pH on the protein binding at an albumin concentration of 6.0×10^{-5} M (10-fold lower than normal albumin concentrations) and a varying suramin concentrations to yield suramin to albumin ratios of 0.00 to 3.00 M (257.26 ug/mL). The values for the number of binding sites and association constants correlated with pH. At a pH of 6.0, two saturable, non-cooperative classes of binding sites were observed. Class I included two high affinity binding sites (N=2.0, K=1.4 x 10^6) and class II had one low affinity site ((N=1.0, K=1.3 x 10^5). At the physiological pH (7.4), two classes of sites were present, but with lower affinities. The affinity constant (K) for class I and class II were 5.0 x 10^5 and 5.6 x 10^4, respectively. At pH 9.0, only one class of binding sites were present (N=2.0, K=2.0 x 10^5). The decreased binding with increased pH was attributed to the influence of the N-B transition of albumin.

Another study [17] examined the binding of suramin to human serum albumin and bovine serum albumin using gel filtration and circular dichroism. Binding was assessed at an albumin concentration of 0.45 g/dL (6.50 x 10^{-5}uM) while suramin concentrations ranged from 33 to 393 uM. In this evaluation, N_1 was 2.3 and K_1 was 0.93 x 10^6 M^{-1} for class I and N_2 was 4 and $K_2$was 0.63 x 10^5 M^{-1} for class II in human serum albumin. In bovine serum albumin, N_1 was 1.4 and K_1 was 2.9 x 10^6 M^{-1} for class I while N_2 was 5 and K_2 was 0.24 x 10^5 for the second class of binding sites.

Concentrations of suramin bound to human serum albumin as a function of the concentration of unbound suramin are shown in Figure 3.1. Suramin bound to albumin to two classes of binding sites, a high affinity saturable site and a low-affinity nonsaturable site. Suramin binding to human serum albumin ranged from 98.0% ($\pm$ 0.0003) at low suramin concentrations to 72% ($\pm$0.0015) at a high suramin concentration of 0.035 M. The protein binding parameters for suramin binding to HSA are presented in Table 3.1.

The results presented currently differ somewhat from what had been observed previously. The current studies were performed using normal physiological albumin and α_1- acid glycoprotein concentrations. Furthermore, suramin concentrations were studied over a wider range than in previous studies. In the experiments assessing the binding of suramin to human serum albumin, the concentration range of suramin was 1.61 x 10^{-8} to 0.035 M. In the α_1-acid glycoprotein binding studies, the concentration range for suramin was 2.10 x 10^{-8} to 0.007 M. At physiological pH, it was observed that suramin did indeed bind to albumin to two classes of binding sites. In contrast to what was observed previously, one class was saturable while the other was nonsaturable. Using nonlinear regression, the number of binding sites (N) for class I (N_1) was determined to be 3.5 and K_1 was 1.8 x 10^4. The protein binding to class II was nonsaturable, therefore N_2 and K_2 could not be separated, however N_2K_2 was determined to be 3.78 x 10^3. The affinity constants for the two classes of sites on albumin are lower than those previously reported. These discrepancies are likely due to the different albumin concentrations used

in the studies. The present study was the only investigation that assessed suramin binding at normal physiological albumin concentrations.

The protein binding of suramin to α_1-acid glycoprotein has not been previously reported. Figure 3.2 illustrates the binding of suramin to α_1-acid glycoprotein. The average fractional binding of suramin to α_1-acid glycoprotein was 63% ($\pm$0.009). Suramin bound to a single low-affinity nonsaturable site to this protein. The protein binding parameters N_3K_3 for suramin binding to AAG was 1.5×10^5 M$^-$1 (Table 3.1).

Taking into account binding to both albumin and α_1-acid glycoprotein, an additive equation was developed to predict the fraction of suramin bound in human plasma.   The predicted cumulative binding to albumin and α_1-acid glycoprotein compared to experimentally measured binding to human plasma proteins is depicted in Figure 3.3. There was good agreement between predicted and observed values. This shows that suramin binding to human plasma proteins (95% $\pm$ 0.015) over the entire concentration studied is due to contributions to both albumin and α_1-acid glycoprotein.

In conclusion, suramin binds to a saturable and nonsaturable site on albumin and to a nonsaturable site on α_1-acid glycoprotein. The human plasma protein binding of suramin can be accounted for by the binding to these two proteins.

REFERENCES

1.   L Shargel and ABC Yu. <u>Applied Biopharmaceutics and Pharmacokinetics</u>. Norwalk,Connecticut: Appleton & Lange, 91-107 (1993)

2.   H Jensen, N Rossing, SB Andersen, et al. Albumin metabolism in nephrotic syndrome in adults. *Clin Sci* **33**, 445-57 (1967)

3.   G Arredondo, R Martinez-Jorda, R Calvo, et al. Protein binding of itraconazole and fluconazole in patients with chronic renal failure. *Int J Clin Pharmacol Ther* **32**,361-4 (1994)

4.   MP Vasson, JC Baguet, MR Arveiller, et al. Serum and urinary alpha one acid glycoprotein in chronic renal failure and hemodialysis. *Nephron* **65**, 299-303 (1993)

5.   GM Pacifici, A Viani, G Taddeuci-Brunelli, et al. Effects of development, aging and renal and hepatic insufficiency as well as hemodialysis on the plasma concentrations of albumin and alpha one glycoprotein: implications for binding of drugs. *Ther Drug Monit* **8**, 259-63 (1986)

6.   F Hawking. Suramin: with special reference to onchocerciasis. *Adv Pharmacol Chemotherapy* **15**, 282-322 (1978)

7.   C Myers, M Cooper, C Stein, et al. Suramin: A novel growth factor antagonist with activity in hormone refractory metastic prostate cancer. *J Clin Oncol* **10,** 881-889 (1992)

8.   RJ Motzer, DM Nanus, P O'Moore, et al. Phase II trial in patients with advanced renal cell carcinoma: treatment results, pharmacokinetics and tumor growth factor expression. *Cancer Res* **52**, 5775-5779 (1992)

9.   CA Stein, RV LaRocca, R Thomas, et al. Suramin: An Anticancer Drug with a Unique Mechanism of Action. *J Clin Oncol* **7(4)**,499-508 (1989)

10.   RV LaRocca, J Meer, RW Gilliatt, et al. Suramin-induced polyneuropathy. *Neurology* **40**, 954-960 (1990)

11.   Collins JM, Klecker Jr RW, Yarchoan R, et al. Clinical Pharmacokinetics of Suramin in Patients With HTLV-III/LAV Infection. *J Clin Pharmacol* **26,** 22-26 (1986)

12.   ELM Vansterkenburg, J Wilting, and LHM Jannsen. Influence of pH on the binding of suramin to human serum albumin. *Biochem Pharmacol* **38(18),** 3029-3035 (1989)

13.   OJM Bos, ELM Vansterkenburg, JPCI Boon, et al. Location and characterization of the suramin binding sites of human serum albumin. *Biochem Pharmacol* **40(7)**,1595-1599 (1990)

14.   FD Boudinot and WJ Jusko. Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. *J Pharm Sci* **73**,774-780 (1984)

15.   A Akaike. Posterior Probabilities for Choosing A Regression Model. *Ann Int Math   Stat* **30A,** 9-14 (1978)

16. CM Metzler, GL Elfring and AJ McEwen. NONLIN, a computer program for nonlinear least-squares regression. *Biometrics* **30**, 562-563 (1974)

17. WE Muller and U Wollert. Spectroscopic studies on the complex formation of suramin with bovine and human serum albumin. *Biochimica et Biophysica Acta* **427,** 465-480 (1976)

Table 3.1. Mean (95% CI) protein binding parameters for the binding of suramin to human serum albumin and α_1-acid glycoprotein

Protein Parameter	Suramin
Human Serum Albumin	
N_1	3.5 (0.69 - 6.4)
K_1, M^{-1} x 10^4	1.8 (0.022 - 3.5)
N_2K_2, M^{-1} x 10^3	3.7 (1.6 - 5.9)
α_1-Acid Glycoprotein	
N_3K_3, M^{-1} x 10^5	1.5 (1.3 - 1.6)

Legend

Figure 3.1. Suramin bound concentrations (•) as a function of suramin free concentrations. The solid line (─) represents the nonlinear least-squares regression fitting of the data. This graph depicts the binding of suramin to human serum albumin with one saturable and one nonsaturable site.

Figure 3.2. Suramin bound concentrations (•) as a function of suramin free concentrations. The solid line (─) represents the nonlinear least-squares regression fitting of the data. This graph depicts the binding of suramin to α_1-acid glycoprotein with one nonsaturable site.

Figure 3.3. The fraction suramin bound (•) to human plasma as a function of total suramin concentrations. The solid line (─) represents the stimulation of the fraction of suramin bound to plasma calculated using the additive model.
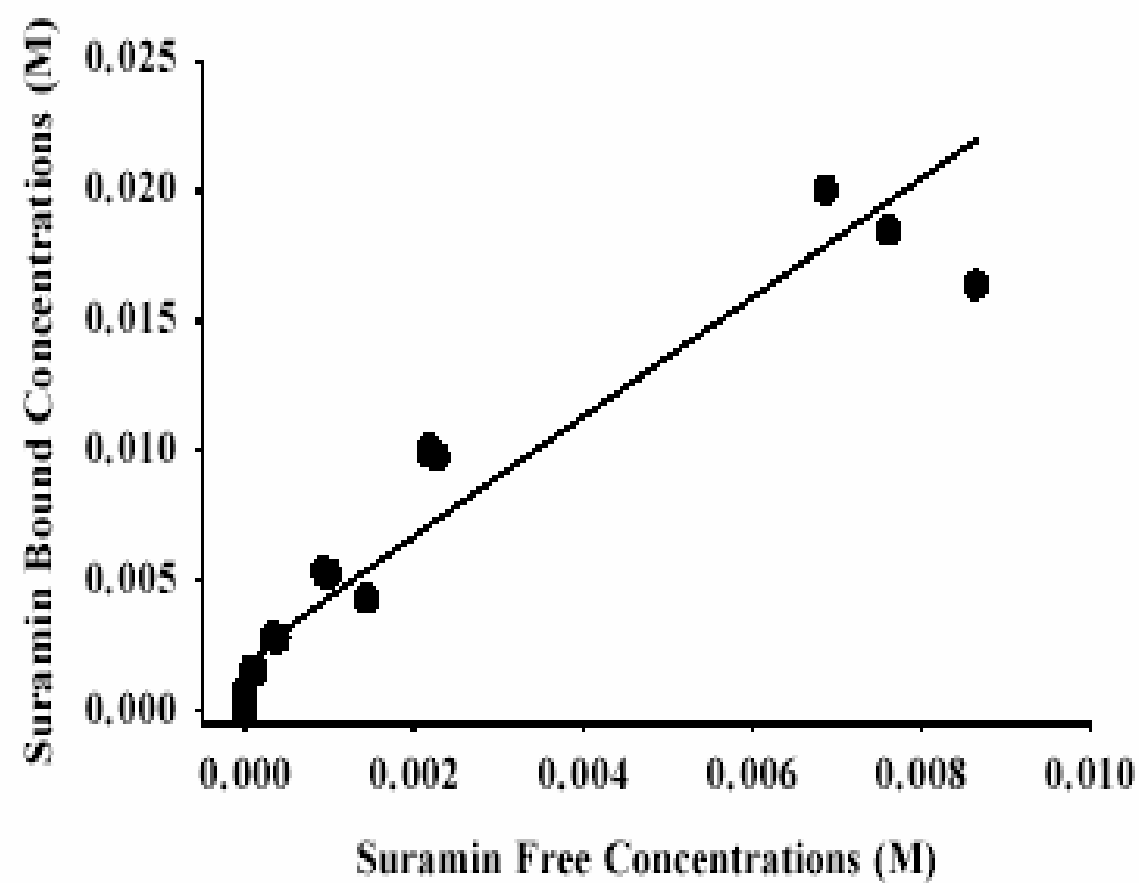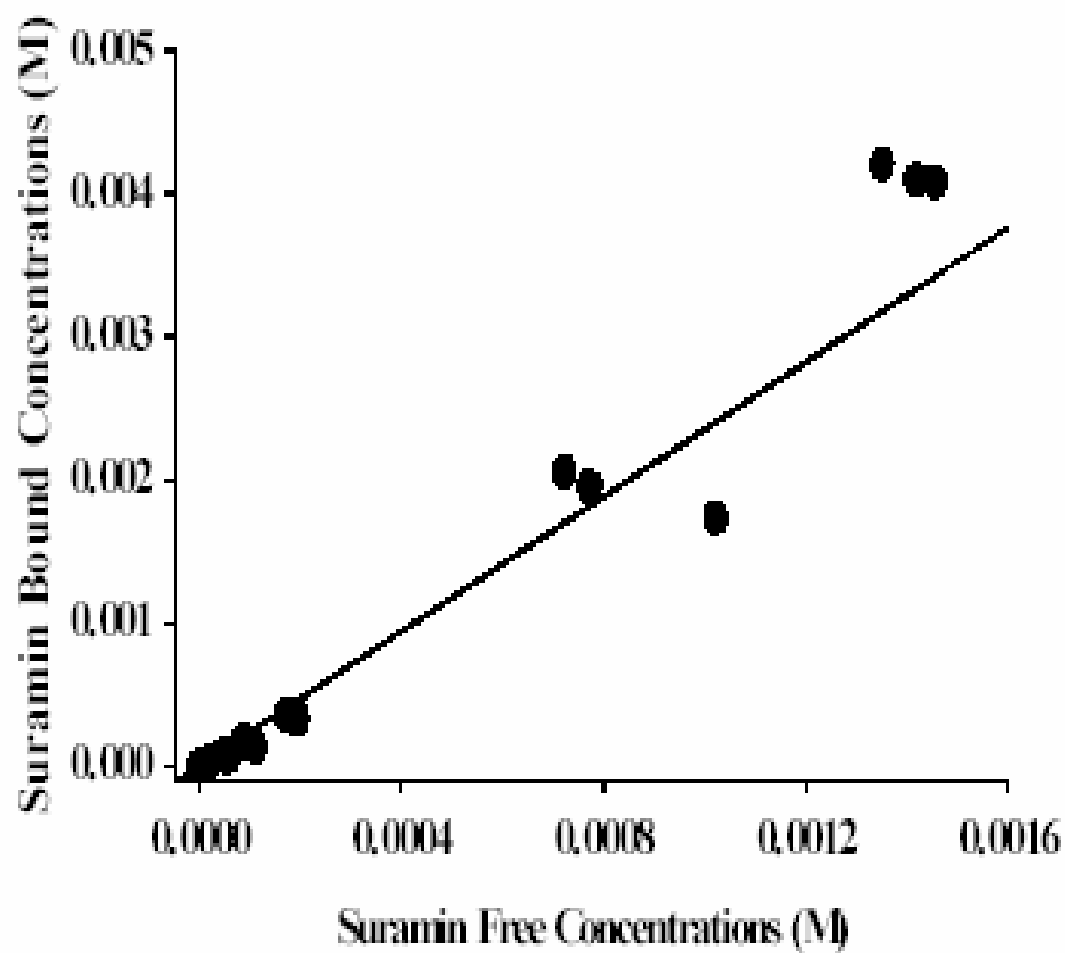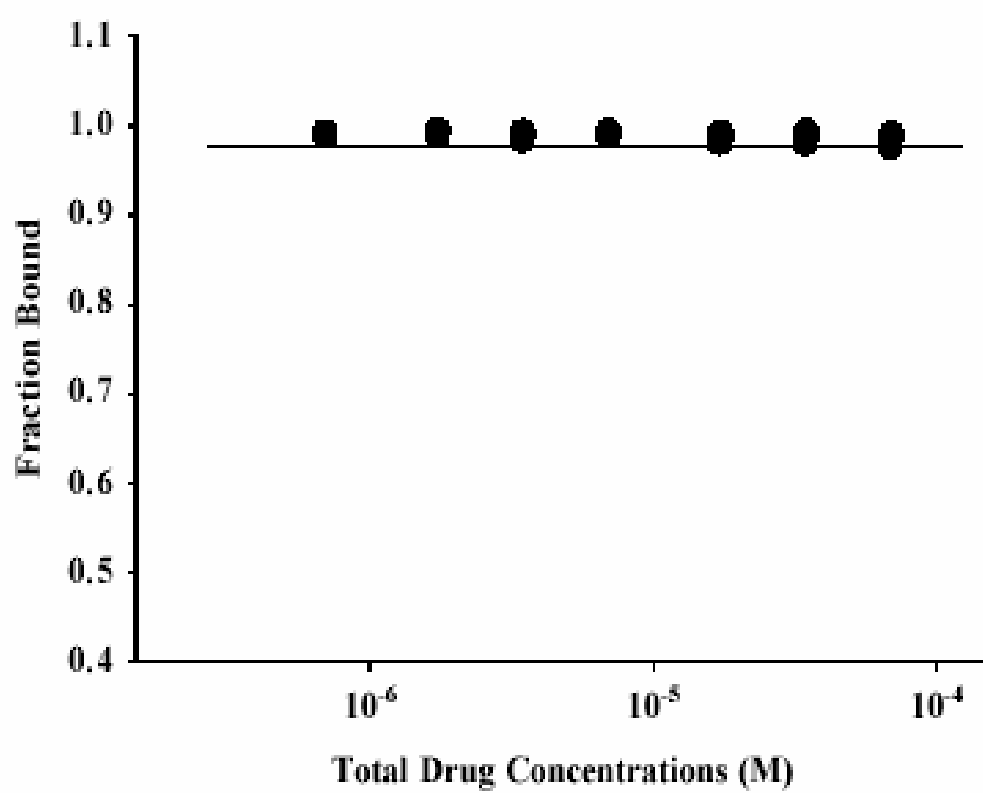
CHAPTER 4

PLASMA PROTEIN BINDING OF DRUG B,

A CARDIOACTIVE DRUG[1]

---

[1] Grandison, MK and FD. Boudinot. To be submitted to Pharmaceutical Research.

SUMMARY

Plasma protein binding may have a significant influence on the pharmacokinetics and pharmacokinetics of a novel drug entity. Plasma protein binding may influence the elimination, distribution and amount of free drug available for interaction with receptors. Drug B is a newly synthesized compound currently being investigated as an agent against cardiovascular disease. The plasma protein binding of this drug was evaluated using equilibrium dialysis and carbon-14 labeled Drug B. At equilibrium the percent of labeled Drug B bound by human plasma proteins was 95.0 ± 1.4 (mean $\pm$ SD) over the period of 16 to 24 h. Binding of Drug B to human plasma proteins was independent of drug concentration over the concentration range of 20 to 10,000 ng/mL. The percent bound was 95.6 ± 1.3 % and the percent free was 4.4 ± 1.3%. The percent of Drug B bound to rat, dog, and monkey plasma proteins was 91.6 ± 1.0 %, 93.5 ± 0.6 %, and 94.6 ± 0.6 %, respectively. The binding of Drug B to albumin was independent of concentration over the concentration of 20 to 10,000 ng/mL. The average percent of Drug B bound to albumin was 94.9 ± 0.3%. The binding of Drug B to α_1- acid glycoprotein was negligible (0.6 ± 1.2).

INTRODUCTION

Plasma protein binding is a major determinant of drug action, and thus has significant implications in drug therapy. Drug pharmacokinetics are determined, in part, by plasma protein binding. Pharmacologic activity, as well as drug toxicity, are generally assumed to be correlated with unbound drug concentrations in plasma. When drug distribution is governed by passive diffusion, an equilibrium exists between the unbound drug concentrations at the site of action and in plasma. Alterations in either plasma protein binding or tissue protein binding will be reflected in unbound drug concentrations. Further, changes in the plasma protein binding of drugs may result in clinical outcomes that require adjustment of dosage regimens.

The objective of this study was to examine the effect of drug concentrations on the plasma protein binding of Drug B. The extent of protein binding in human plasma was determined over a range of concentrations in order to access the linearity of the binding. In addition to studying the extent of protein binding, the binding of Drug B to human serum albumin and human serum α_1-acid glycoprotein was determined. The extent of binding was also assessed in rat, dog and monkey plasma.

MATERIALS AND METHODS

Chemicals. Radiolabelled Drug B ($[^{14}C]$ Drug B) and Drug B (specific activity 171.9 mCi/mmol, Biodynamics Research Limited, Cardiff, United Kingdom) with purity > 97% were provided by Cocensys, Inc. (Irvine,CA). Aliquots (20 µL) of ($[^{14}C]$ Drug B) were purified (<99%) by collecting high performance liquid chromatography (HPLC) eluant fractions corresponding to the retention time of Drug B and used without further characterization (1). Purified ($[^{14}C]$ Drug B) was aliqouted (3 mL)and stored at -20° C. Drug B was weighted on a Cahn 28 Automatic Electrobalance (Cerritos, CA) with accuracy to 0.01 mg and dissolved in the appropriate protein solution. Drug B

concentrations of 20, 50, 100, 200, 500, 750, 1000, 1500, 2000, 5000, and 10000 ng/mL were prepared by serial dilution with protein solutions.

Human plasma (heparin) was obtained from healthy volunteers and stored at -20° C. Rat (Sprague-Dawley) and dog (Beagle) plasma were obtained from Harlan Bioproducts for Science (Indianapolis, IN) and stored frozen at -20° C . Rhesus monkey plasma (heparin) was obtained from Yerkes Regional Primate Research Center (Emory University, Atlanta, Georgia) and stored frozen at -20° C. Human serum albumin (HSA)and human serum α_1-acid glycoprotein (AAG) were obtained from Sigma Chemical Co (St Louis, MO). Protein Solutions were prepared by dissolving known amounts of lyophilized protein in isotonic sodium phosphate buffer, pH 7.4 and used the day they were prepared. Protein concentrations were: 40 g/L human serum albumin and 0.7 g/L α1-acid glycoprotein. These are typical plasma protein concentrations found in healthy humans (2).

 Liquid scintillation fluid, ScintiVerse, was purchased from Fisher Scientific (Springfield, NJ).

Protein Binding Studies. Equilibrium dialysis was utilized to characterize the protein binding of Drug B. Experiments were performed at 37 ° C using Plexiglas dialysis cells and Spectro/Por (Spectrum Medical Industries, Los Angeles, CA) dialysis membrane (12,000 molecular weight cutoff) prepared for use according to the manufacturer's instructions (3). Aliquots of [^{14}C] Drug B were thawed and methanol was evaporated under a stream of nitrogen gas at ambient temperature. Isotonic sodium phosphate buffer was used to reconstitute the labeled drug. The solution was mixed and 0.5 mL aliquots were counted to determine the total amount of radioactivity in each cell.

Plasma or protein solutions (0.8 mL) containing unlabeled drug ("plasma side") were dialyzed against an equal volume of isotonic phosphate buffer containing [^{14}C] Drug B ("buffer side"). The cells were shaken at 40 shakes per minute in a water bath at 37°C for 16 hours, the time required to achieve equilibrium. The samples were collected

by use of 1 mL Hamilton syringes. Post-dialysis plasma side and buffer side volumes were measured and recorded. Aliquots (0.5 mL) of plasma and buffer samples were placed into 7mL scintillation vials, scintillation fluid was added and the samples were counted in a liquid scintillation counter (Tri Carb). All protein binding determinations were done in triplicate. The time required to achieve equilibrium was determined in preliminary experiments using an Drug B concentrations of 2000 ng/mL. Triplicate samples were collected at 4 h, 6, 8 h, 12 h, 16 h, and 24 h.

Human plasma. The extent of binding of Drug B to human plasma was determined at drug concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 0.75, 1, 1.5, 2.0, 5.0, and 10 mg/mL.

Rat, Dog and Rhesus Monkey Plasma. The protein binding of Drug B was measured in rat, dog, and monkey plasma at drug concentrations of 0.25, 0.75 and 1.5 mg/mL.

Human Serum Albumin and α_1 -Acid Glycoprotein. The protein binding of Drug B to human serum albumin (HSA) (40 g/L) was determined at drug concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 0.75, 1, 1.5, 2.0, 5.0, and 10 mg/mL.   The protein binding of Drug B to α_1 -acid glycoprotein (0.7 g/L) was determined at drug concentrations of 0.02, 0.05, 0.1, 0.2 0.5, 0.75 and 1 mg/mL.

Data analysis. The following equation was used to determined the extent of protein binding of Drug B to plasma and plasma proteins (4):

$$F_B = \frac{(DPM_{\text{plasma side}} - DPM_{\text{buffer side}}) \times (V_{pe}/V_{pi})}{(DPM_{\text{plasma side}} - DPM_{\text{buffer side}}) \times (V_{pe}/V_{pi}) + DPM_{\text{buffer side}}}$$

$DPM_{\text{plasma side}}$ and $DPM_{\text{buffer side}}$ are the disintegrations per minute after equilibrium in the plasma and buffer side, respectively. V_{pe} is the post dialysis volume of the plasma

side at equilibrium and V_{pi} is the initial plasma volume of 0.8 mL. In the absence of fluid shifts, the volume terms were excluded from the calculations.

The effects of drug concentration on protein binding of Drug B was assessed by one way analysis of variance (5).

RESULTS AND DISCUSSION

The time course of equilibration of Drug B in human plasma is shown in Figure 4.1. The data is listed in Table 4.1. Equilibrium was achieved by 16 hr. At equilibrium the percent of Drug B bound by human serum albumin was 95 ± 1.7 % (mean $\pm$ SD) over the time period of 16 to 24 h.

Human Plasma. The extent of binding of Drug B to human plasma over a concentration range of 0.02 to 10 mg/mL is shown in Figure 4.2. The data is listed in Table 4.2. There is no statistically significant differences in fraction of Drug B bound to human plasma proteins between concentrations. Therefore, binding of Drug B to human plasma proteins was independent of drug concentration over the concentration range studied. The percent of Drug B bound to plasma over this range averaged 95.2 ± 1.9 % (mean $\pm$ SD). The percent of Drug B free was 4.7 ± 1.9 % (mean $\pm$ SD).

Rat, Dog and Rhesus Monkey Plasma. The extent of Drug B binding to rat plasma is shown as a function of total drug concentrations in Figure 4.3. The data is listed in Table 4.3. At concentrations 0.25, 0.75 and 1.5 mg/mL the average percent of Drug B bound to rat plasma proteins was 91.5 (± 0.01) %. The percent of Drug B free was 8.4 (± 0.01)%. The binding of rat plasma to plasma proteins was independent of drug concentration over the concentration range of 0.25 to 1.5 mg/mL.

The extent of Drug B binding to dog plasma is depicted in Figure 4.4. The data is listed in Table 4.4. The Drug B was 93.5 (± 0.6) % bound to dog plasma proteins over the concentration range of 0.25 to 1.5 mg/mL. The percent of drug free was 6.5 (± 0.6).

There is no significant difference in the binding of Drug B to dog plasma proteins between drug concentrations. Binding to dog plasma was independent of drug concentration over this concentration range

The extent of Drug B binding monkey plasma is illustrated in Figure 4.5. Data is listed in Table 4.5.   Drug B was 94.5 ± 0.54% bound to plasma proteins. The percent of free fraction was 5.4 ± 0.54 %. There were no statistically significant differences in fraction bound values between drug concentrations, therefore binding of Drug B to monkey plasma proteins was independent of drug concentrations over this the concentration range of 0.25 to 1.5 mg/mL.

Human Serum Albumin. The extent of Drug B binding to human serum albumin (40 g/L) over a concentration range of 0.02 to 10 mg/mL is illustrated in Figure 4.6. The data is listed in Table 4.6. There were no statistically significant differences in the binding of Drug B to human serum albumin between concentrations. The average percent of Drug B bound to human serum albumin was 94.9 ± 0.3 % (mean ± SD) and percent free was 5.1 ± .3%. Binding to human serum albumin was independent of drug concentration over a concentration range of 0.02 to 10 mg/mL.

α_1-*Acid Glycoprotein*. The extent of binding of Drug B to α_1-acid glycoprotein is illustrated in Figure 4.7. The data is listed in Table 4.7. Binding to α_1-acid glycoprotein was essentially zero over the concentration range of 0.02 to 10 mg/mL. The percent bound to Drug B was 0.6 ± 1.2 % (mean ± SD) and the percent free of Drug B was 99.4 ± 1.2 %. The binding of Drug B to α_1-acid glycoprotein was not measured at higher drugs concentrations due to limited solubility.

CONCLUSIONS

Plasma protein binding of drug B to rat plasma proteins, dog plasma proteins, and monkey plasma proteins was 91.6%, 93.5% and 94.6%, respectively. Drug B is approximately 95.6 % bound to human plasma proteins and 94.9% bound to human

serum albumin. The extent of binding of Drug B to α_1-acid glycoprotein was essentially zero (0.6%). Albumin is the primary plasma protein responsible for the binding of Drug B. Binding is linear over the Drug B concentration of 0.02 to 10 mg/mL.

REFERENCES

1. CoCensys Report #2862-DA005, Determination of Co 102862 in Dog Plasma by HPLC-UV.

2. Diem K and Lenter C (Eds). Scientific Tables, Geigy Pharmaceuticals, 7th ed., Ardsley, NY, 1975.

3. Wright J.D., Boudinot F.D. and Ujhelyi M.R., Measurement and Analysis of Unbound Drug Concentrations. Clin Pharmacokinetics, 30: 445-462, 1996.

4. Boudinot FD. And Jusko WJ. Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. J Pharm Sci, 73: 774-780, 1984.

5. Bolton, S. , Pharmaceutical Statistics: Practical and Clinical Applications, 2nd ed., Marcel Dekker, Inc, New York, NY 1990.
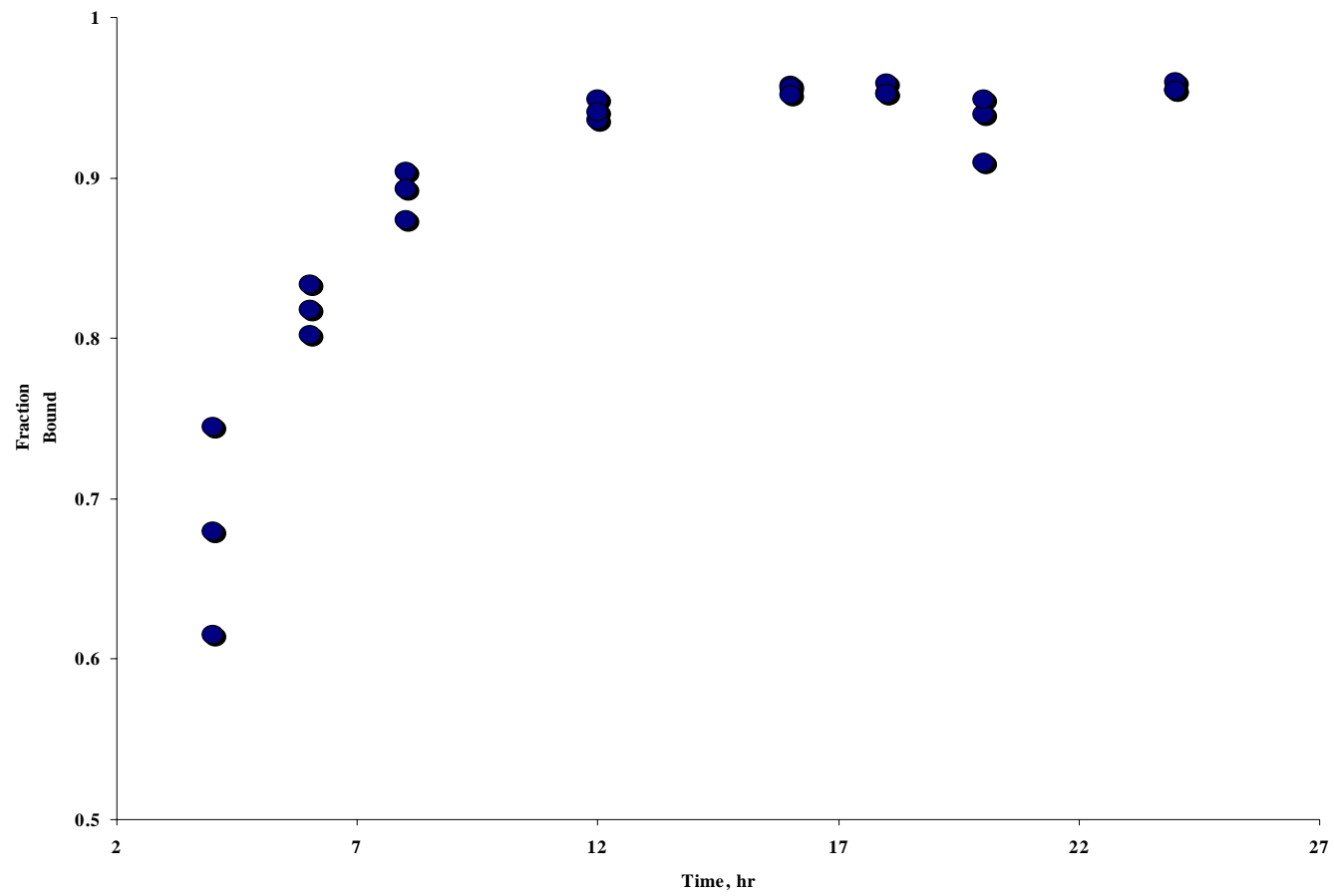
Figure 4.1. Time to equilibrium of Drug B in human plasma.

Figure 4. 2. Extent of binding of Drug B to human plasma proteins.
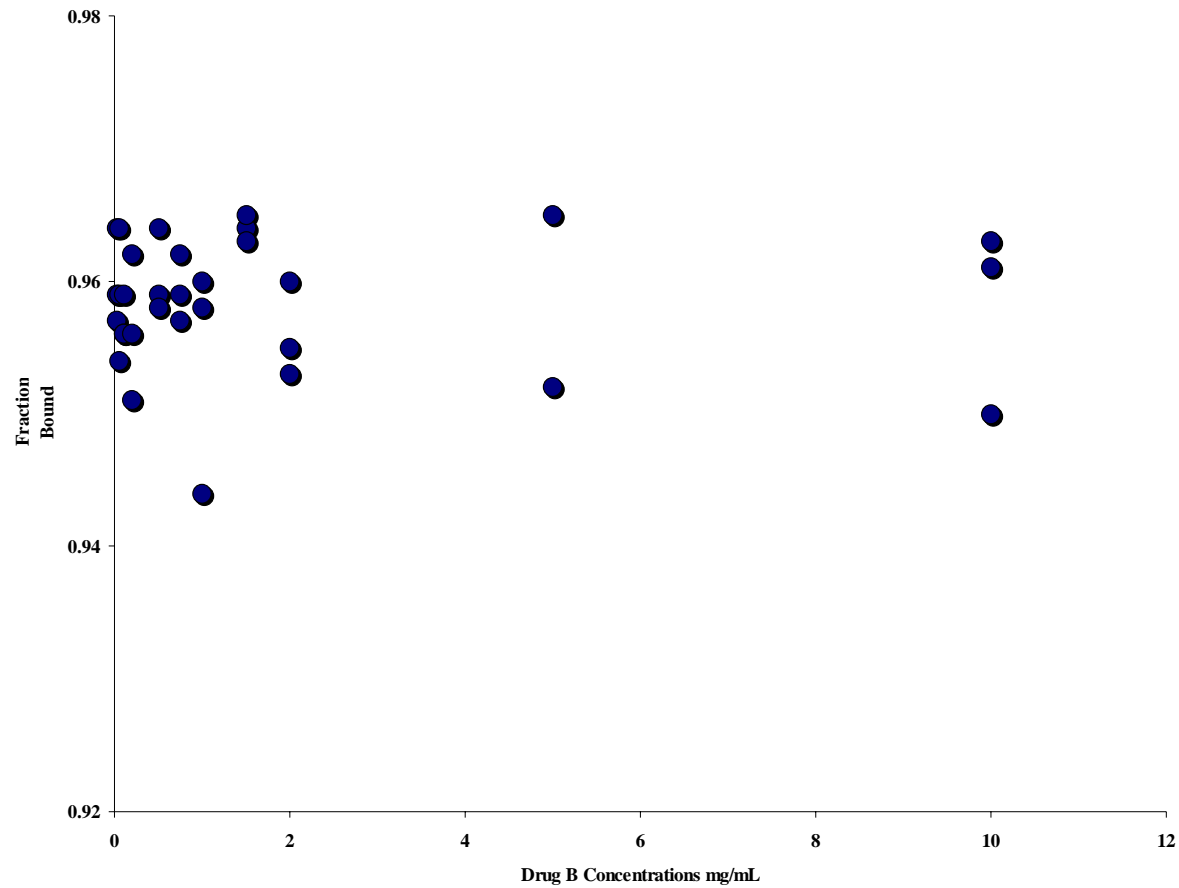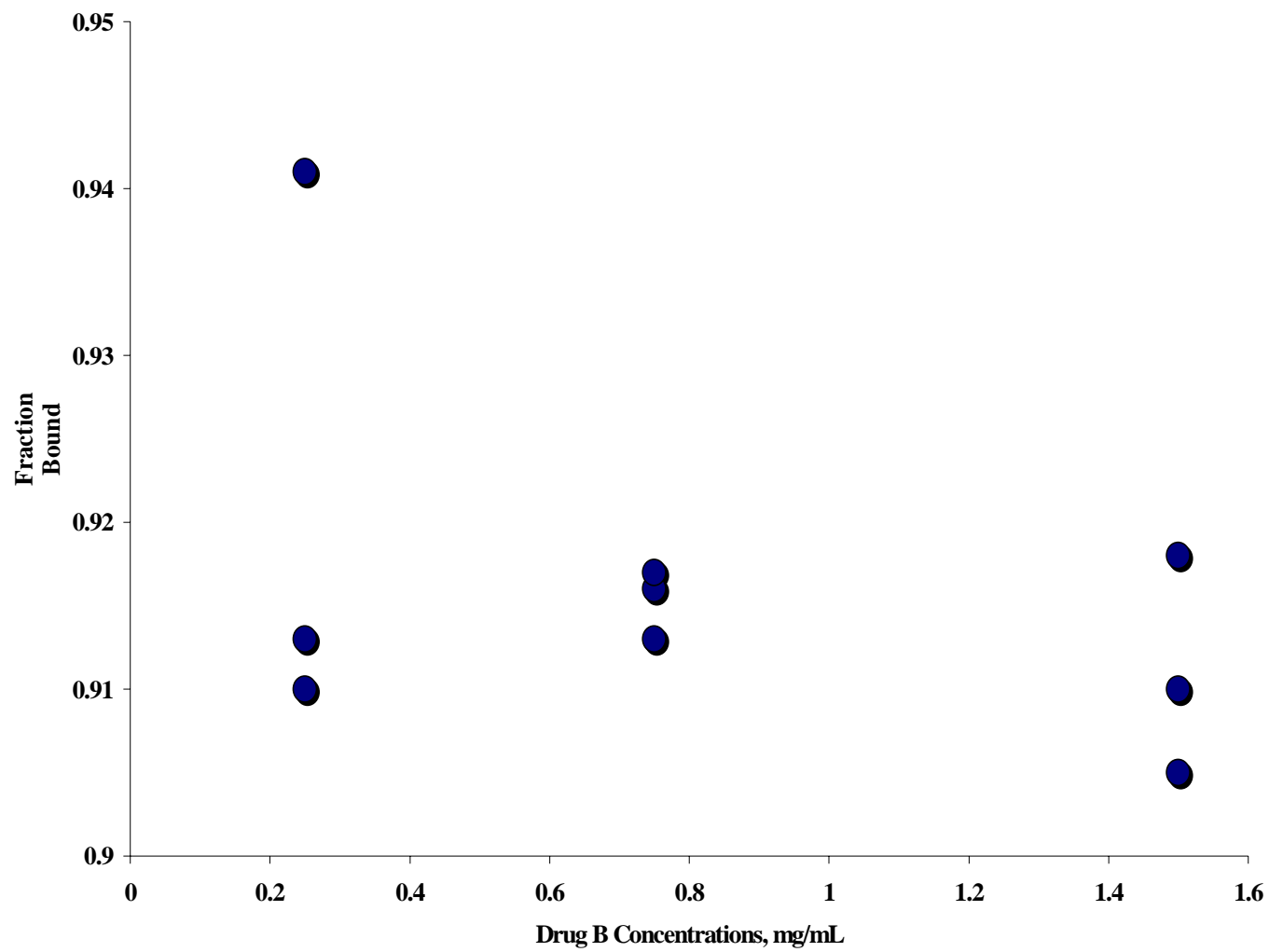
Figure 4. 3. Extent of binding of Drug B to rat plasma proteins.
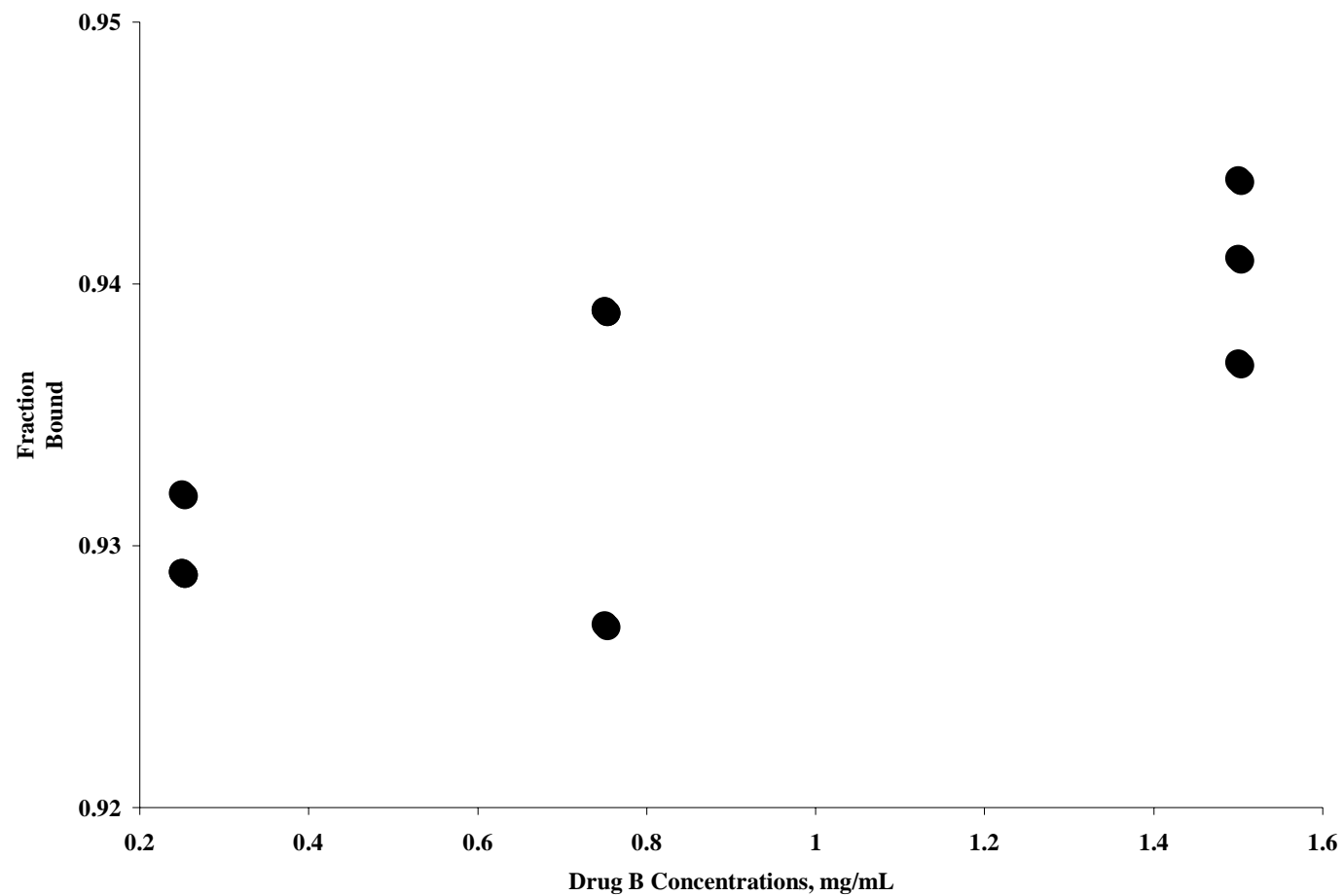
Figure 4.4. Extent of binding of Drug B to dog plasma proteins
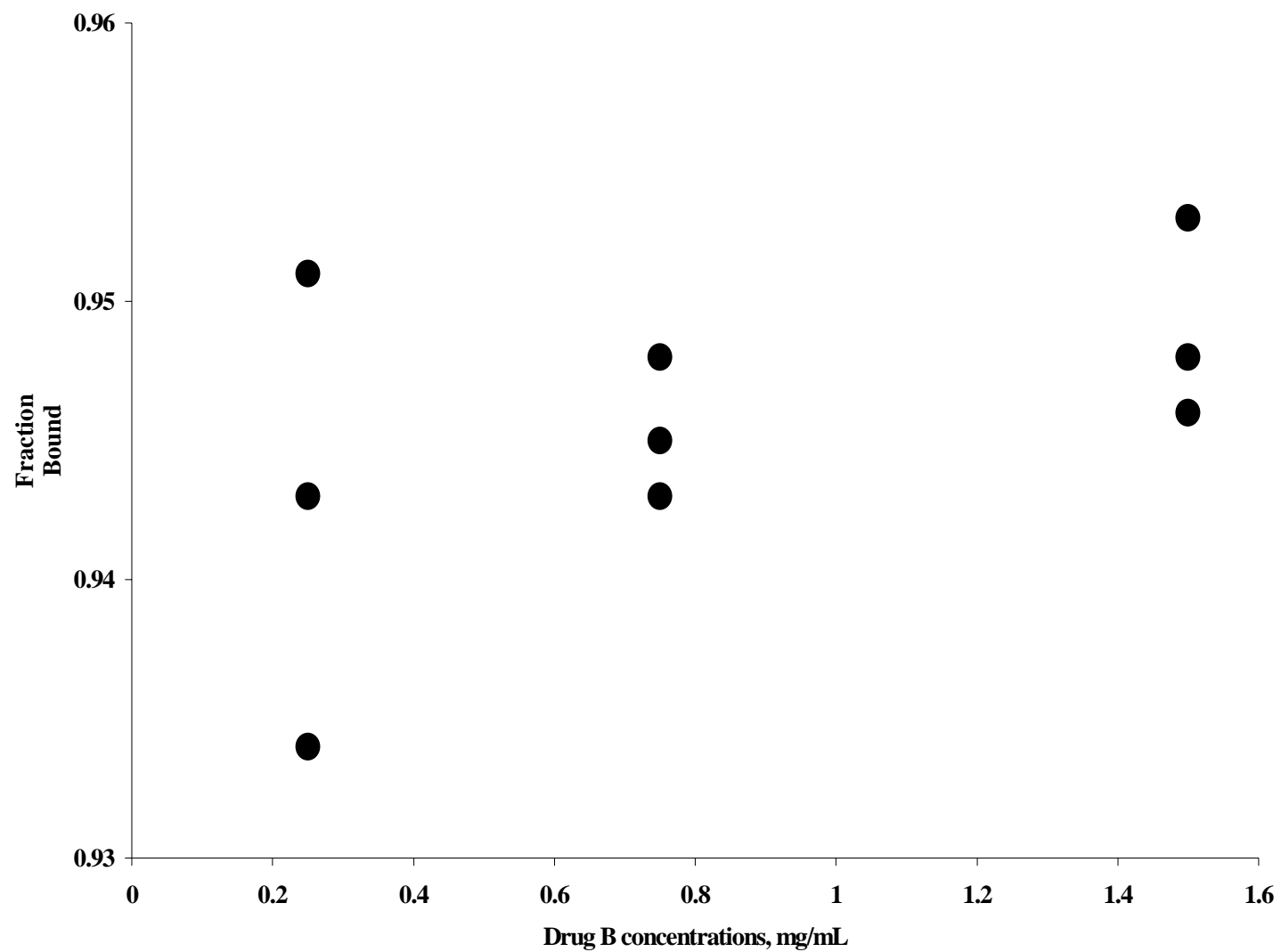
Figure 4.5. Extent of binding to Drug B to rhesus monkey plasma proteins.

Figure 4.6. Extent of binding of Drug B to Human Albumin
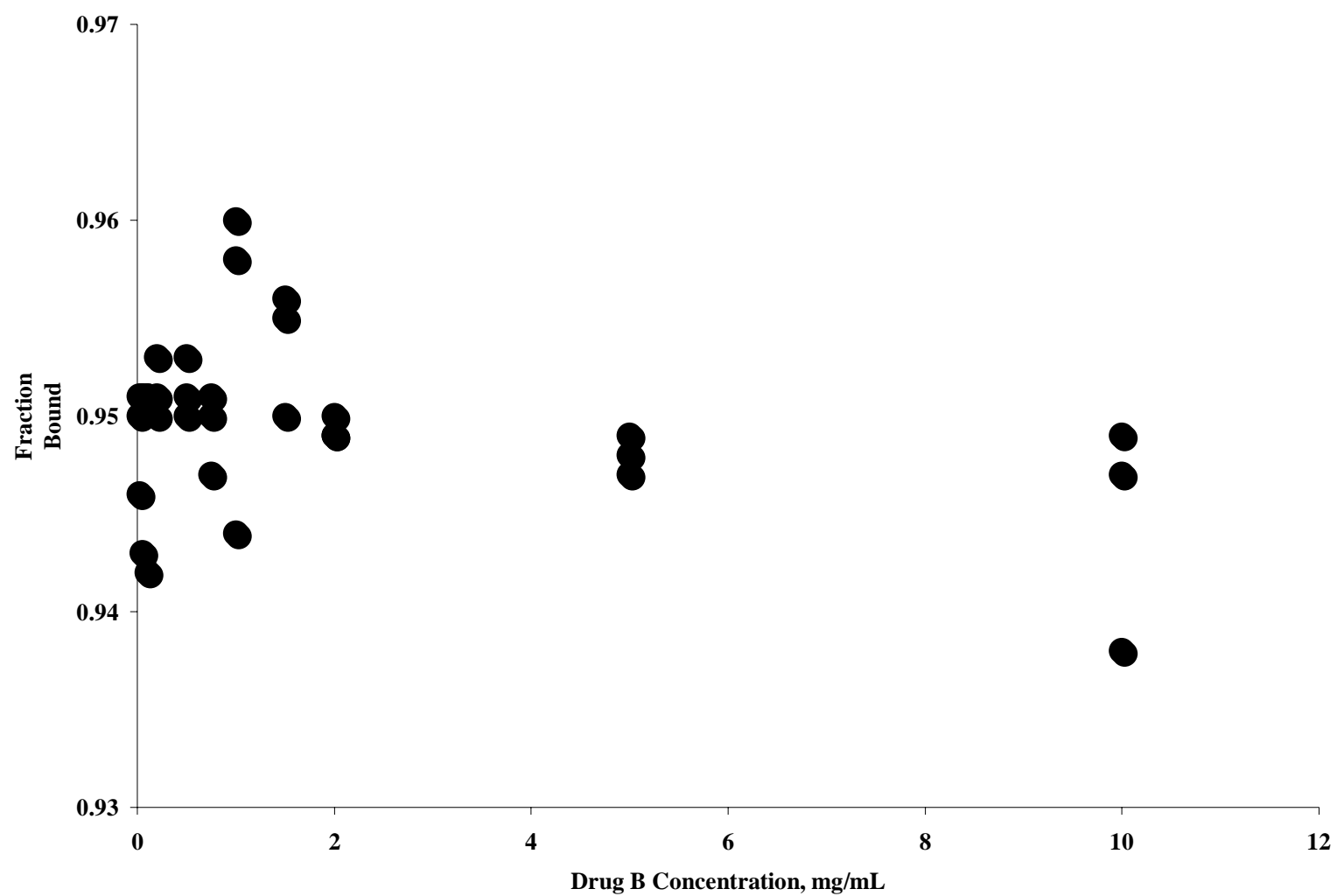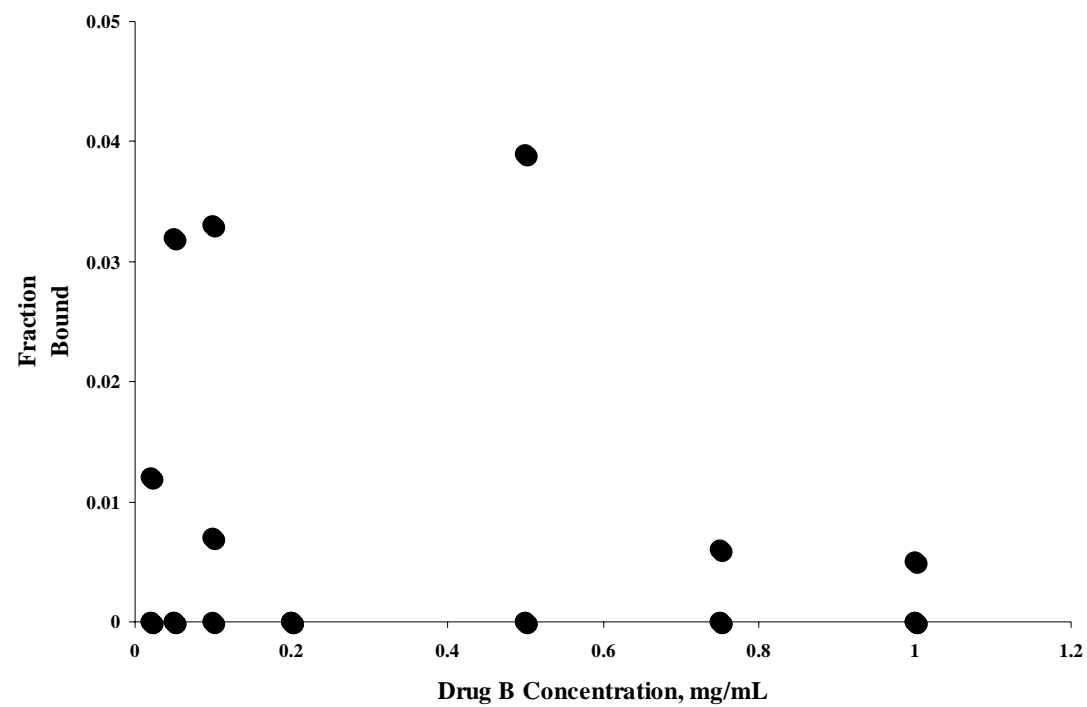
Figure 4.7. Extent of binding of Drug B to α₁ acid glycoprotein

Table 4.1. Time Course for Equilibration of Drug B in Human Plasma

Time (h)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
4	0.745	0.255		
4	0.615	0.385		
4	0.680	0.320	0.680	0.320
			(0.065)	(0.065)
6	0.834	0.166		
6	0.802	0.198		
6	0.818	0.182	0.818	0.182
			(0.016)	(0.016)
8	0.874	0.126		
8	0.904	0.096		
8	0.893	0.107	0.890	0.110
			(0.015)	(0.015)
12	0.936	0.064		
12	0.949	0.051		
12	0.941	0.059	0.942	0.058
			(0.0066)	(0.0066)
16	0.958	0.042		
16	0.956	0.044		
16	0.952	0.048	0.955	0.045

			(0.0031)	(0.0031)
18	0.959	0.041		
18	0.953	0.047		
18	0.953	0.047	0.955	0.045
			(0.0035)	(0.0035)
20	0.940	0.060		
20	0.949	0.051		
20	0.910	0.090	0.933	0.067
			(0.020)	(0.020)
24	0.955	0.045		
24	0.960	0.040		
24	0.955	0.040	0.957	0.043
			(0.0029)	(0.0029)

Table 4.2. Binding of Drug B to Human Plasma Proteins

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
0.020	0.964	0.036		
0.020	0.957	0.043		
0.020	0.959	0.041	0.960	0.040
			(0.0036)	(0.0036)
0.050	0.964	0.036		
0.050	0.954	0.046		
0.050	0.959	0.041	0.959	0.041
			(0.0050)	(0.0050)
0.100	0.956	0.044		
0.100	0.959	0.041		
0.100	0.956	0.044	0.957	0.043
			(0.0017)	(0.0017)
0.200	0.956	0.044		
0.200	0.962	0.038		
0.200	0.951	0.049	0.956	0.044
			(0.0055)	(0.0055)
0.500	0.959	0.041		
0.500	0.964	0.036		
0.500	0.958	0.042	0.960	0.040
			(0.0032)	(0.0032)

0.750	0.957	0.043		
0.750	0.962	0.038		
0.750	0.959	0.041	0.959	0.041
			(0.0025)	(0.0025)
1.00	0.944	0.056		
1.00	0.958	0.042		
1.00	0.960	0.040	0.954	0.046
			(0.0087)	(0.0087)
1.50	0.964	0.036		
1.50	0.963	0.037		
1.50	0.965	0.035	0.964	0.036
			(0.0010)	(0.0010)
2.00	0.960	0.040		
2.00	0.953	0.047		
2.00	0.955	0.045	0.956	0.044
			(0.0036)	(0.0036)
5.00	0.952	0.048		
5.00	0.965	0.035		
5.00	0.887	0.113	0.935	0.065
			(0.0042)	(0.0042)
10.00	0.950	0.050		
10.00	0.961	0.039		

10.00	0.963	0.037	0.958	0.042
			(0.0070)	(0.0070)
Overall			0.956	0.044
			(0.013)	(0.013)

Table 4.3. Binding of Drug B to Rat Plasma Proteins.

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
.25	0.910	0.090		
.25	0.913	0.087		
.25	0.941	0.059		
			0.921	0.079
			(0.017)	(0.017)
0.75	0.916	0.0842		
0.75	0.917	0.0829		
0.75	0.913	0.0866		
			0.915	0.085
			(0.0021)	(0.0021)
1.5	0.918	0.082		
1.5	0.910	0.090		
1.5	0.905	0.095		
			0.911	0.089
			(0.066)	(0.066)
Overall			0.916	0.084
			(0.010)	(0.010)

Table 4.4. Binding of Drug B to Dog Plasma Proteins.

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
0.25	0.932	0.068		
0.25	0.929	0.071		
0.25	0.929	0.071		
			0.930	0.070
			(0.0017)	(0.0017)
0.75	0.939	0.061		
0.75	0.939	0.061		
0.75	0.927	0.073		
			0.935	0.065
			(0.0069)	(0.0069)
1.5	0.941	0.059		
1.5	0.937	0.063		
1.5	0.944	0.056		
			0.941	0.059
			(0.0061)	(0.0061)
Overall			0.946	0.054
			(0.0055)	(0.0055)

Table 4.5. Binding of Drug B to Rhesus Monkey Plasma

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
0.25	0.934	0.066		
0.25	0.943	0.057		
0.25	0.951	0.049		
			0.943	0.057
			(0.0085)	(0.0085)
0.75	0.948	0.052		
0.75	0.943	0.057		
0.75	0.945	0.055		
			0.945	0.055
			(0.0025)	(0.0025)
1.5	0.948	0.052		
1.5	0.946	0.054		
1.5	0.953	0.047		
			0.949	0.051
			(0.0036)	(0.0036)
Overall			0.946	0.054
			(0.0055)	(0.0055)

Table 4.6. Binding of Drug B to Human Serum Albumin

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
0.020	0.946	0.054		
0.020	0.951	0.049		
0.020	0.950	0.050	0.949	0.051
			(0.0026)	(0.0026)
0.050	0.943	0.057		
0.050	0.951	0.049		
0.050	0.951	0.049	0.948	0.052
			(0.0046)	(0.0046)
0.10	0.951	0.049		
0.10	0.951	0.049		
0.10	0.942	0.058	0.948	0.052
			(0.0052)	(0.0052)
0.20	0.953	0.047		
0.20	0.950	0.050		
0.20	0.951	0.049	0.951	0.049
			(0.0015)	(0.0015)
0.50	0.953	0.047		
0.50	0.950	0.050		
0.50	0.951	0.049	0.951	0.049
			(0.0015)	(0.0015)

0.750	0.951	0.049		
0.750	0.950	0.050		
0.750	0.947	0.053	0.949	0.051
			(0.0021)	(0.0021)
1.00	0.944	0.056		
1.00	0.958	0.042		
1.00	0.960	0.040	0.950	0.050
			(0.0012)	(0.0012)
1.50	0.950	0.050		
1.50	0.955	0.045		
1.50	0.956	0.044	0.954	0.046
			(0.0032)	(0.0032)
2.00	0.949	0.051		
2.00	0.949	0.051		
2.00	0.950	0.050	0.949	0.052
			(0.00058)	(0.00058)
5.00	0.949	0.051		
5.00	0.947	0.053		
5.00	0.948	0.052	0.948	0.052
			(0.00010)	(0.00010)
10.00	0.947	0.053		
10.00	0.938	0.062		

10.00	0.949	0.051	0.945	0.055
			(0.0059)	(0.0059)
Overall			0.949	0.051
			(0.0034)	(0.0034)

Table 4.7. Binding of Drug B to α_1-Acid Glycoprotein

Drug B (mg/mL)	F_B	F_U	F_B Mean (SD)	F_U Mean (SD)
0.020	0.012	0.988		
0.020	0.0	1.0		
0.020	0.0	1.0	0.004	0.996
			(0.0069)	(0.0069)
0.050	0.0	1.0		
0.050	0.0	1.0		
0.050	0.032	1.0	0.011	0.989
			(0.018)	(0.018)
0.100	0.007	0.993		
0.100	0.033	0.969		
0.100	0.0	1.0	0.013	0.987
			(0.0017)	(0.0017)
0.200	0.0	1.0		
0.200	0.0	1.0		
0.200	0.0	1.0	0.0	1.0
			(0.0)	(0.0)
0.500	0.0	1.0		
0.500	0.0	1.0		
0.500	0.039	0.963	0.013	0.987
			(0.0023)	(0.0023)

0.750	0.0	1.0		
0.750	0.006	0.995		
0.750	0.0	1.0	0.002	0.998
			(0.0035)	(0.0035)
1.000	0.0	1.0		
1.000	0.0	1.0		
1.000	0.005	1.0	0.002	0.998
			(0.0029)	(0.0029)
Overall			0.006	0.994
			(0.012)	(0.012)

CHAPTER 5

AGE-RELATED CHANGES IN PROTEIN BINDING OF DRUGS:

IMPLICATIONS FOR THERAPY[1]

Summary

Plasma protein binding of drugs, particularly for drugs that are highly bound, may have significant clinical implications. While protein binding is a major determinant of drug action, it is only one of a myriad of factors that influence drug disposition. Plasma protein binding is a function of drug and protein concentrations, the affinity constant for the drug-protein interaction and the number of protein binding sites per class of binding sites. Albumin is generally decreased in the elderly while α_1-acid glycoprotein concentrations are not altered by age per se. Age-related changes in protein binding are usually not clinically important in drug therapy. Alterations in plasma protein binding that occur in the elderly are generally not attributed to age, rather physiological and pathophysiological changes or disease states, which may occur more frequently in the elderly, most often account for altered protein binding. Age-related physiological changes such as decreased renal function, decreased hepatic function and decreased cardiac output generally produce more clinically significant alterations in drug disposition than do alterations in drug plasma protein binding. An understanding of the inter-relationships between drug concentrations, protein binding, the physiology of aging, disease, pharmacokinetics and pharmacodynamics is necessary for effective therapeutic monitoring. Monitoring of unbound drug concentrations simplifies these relationships and provides the fundamental information needed for dosage regimen development and adjustment. Drug therapy in the elderly should be individualized taking into account all of these factors.

Plasma protein binding is a major determinant of drug action, and thus has significant implications in drug therapy. Drug pharmacokinetics are determined, in part, by plasma protein binding. Pharmacologic activity, as well as drug toxicity, are generally assumed to be correlated with unbound drug concentrations in plasma. When drug distribution is governed by passive diffusion, an equilibrium exists between the unbound drug concentrations at the site of action and in plasma. Alterations in either plasma protein binding or tissue protein binding will be reflected in unbound drug concentrations. Further, changes in the plasma protein binding of drugs may result in clinical outcomes that require adjustment of dosage regimens.

Measurement of total drug concentration does not provide important information concerning the unbound drug in plasma which is available for distribution, elimination, and pharmacologic action.[1] Generally, it is believed that unbound drug concentrations are available for pharmacodynamic response.[2,4] Unbound drug at the site of action can associate with drug receptors eliciting the desired therapeutic effect or a toxicologic reaction. Clinically, it is usually not possible to measure drug concentrations at the site of action, however, unbound drug concentrations in plasma are assumed to reflect drug concentrations at the effect site.[2-3] This assumption is based on passive diffusion of unbound drug resulting in an equilibrium between unbound drug in plasma and at the effect site. Currently, established therapeutic ranges for drug monitoring for most drugs are reported in terms of total drug concentration. This is acceptable in many cases, however, unbound drug concentrations often provide a better assessment for therapeutic drug monitoring [1], particularly when plasma protein binding is high or dependent on drug concentrations, in disease states which are known to alter drug binding to plasma proteins, and in the elderly where age related alterations in protein composition and concentration may alter drug protein binding. Multiple disease states and concomitant drug therapies further complicate interpretation of total drug concentrations. Thus, unbound drug concentrations may be more appropriate for therapeutic monitoring. Unbound drug concentration has been shown to be a

better predictor of therapeutic response than total drug concentration for phenytoin and some highly protein bound antibiotics.[5-8] Thus, a clear understanding of drug protein binding and pharmacokinetics, and their role in drug action are paramount to the optimization of drug therapy.

The protein binding of a drug may vary widely between patients making determination of unbound concentrations pivotal for individualizing patient drug therapy. Numerous factors can significantly alter the plasma protein binding of drugs.[9-43] Physiological factors such as gender and nutritional status may affect plasma protein binding of drugs. The plasma protein binding of many drugs including propranolol, salicylate, diazepam, valproic acid and sulfisoxazole is reduced during pregnancy.[17-20] Pediatric patients, particularly neonates, have also been shown to have lower protein binding than adults.[12-18,21] Disease states such as renal disease, liver disease, thyroid disease, diabetes mellitus, acute myocardial infarction, arthritis, Crohn's disease, cancer and burns can alter the binding characteristics of drugs.[9,11,22-36] The plasma protein binding of phenytoin is decreased in renal dysfunction, liver disease, cancer and burns.[5,6,37-39] The unbound fraction of diazepam in plasma is increased in renal failure, burns, liver disease and diabetes.[36,40-42] The protein binding of propranolol is decreased in liver disease and hyperthyroidism, but increased in hypothyroidism, renal dysfunction, inflammatory disease, cancer, and acute myocardial infarction.[9-43] Therefore, determination of unbound drug concentrations in many clinical conditions may be essential for the individualization of drug dosage regimens in pharmaceutical care. The purpose of this review is to examine factors that may alter drug protein binding in aging, and to discuss the implications of changes in protein binding on drug therapy in the elderly.

1. Principles of Plasma Protein Binding

1.1 Measurement of Unbound Drug Concentrations

Currently, therapeutic drug monitoring is, for the most part, based on total plasma drug concentrations. For drugs that are not highly bound to plasma proteins, the use of total

drug concentrations is generally adequate for drug monitoring. However, therapeutic monitoring of unbound drug concentrations is clinically relevant for highly bound drugs or drugs which exhibit concentration dependent binding. The determination of unbound drug requires an additional procedure to separate unbound drug from bound drug. More importantly, for highly bound drugs, assay sensitivity using standard analytical techniques is often inadequate for measuring unbound drug concentrations. Indeed, determining plasma protein binding of highly bound drugs often requires the use of radioisotopes to achieve the needed sensitivity levels. Recent developments in analytical methodologies including analytical mass spectroscopy, however, are equipping laboratories with techniques capable of measuring very low drug concentrations. These procedures for measuring drug concentrations are also becoming more readily available to clinical laboratories.

Methodologies for quantitating plasma protein binding of drugs include equilibrium dialysis, ultrafiltration, ultracentrifugation, microdialysis, dynamic dialysis, gel filtration, electrophoresis, and spectrophotometry. Comprehensive reviews of these methods have been previously published.[44-47] Equilibrium dialysis and ultrafiltration are used most frequently for measuring plasma protein binding while microdialysis is a relatively new technique used primarily in research.

Equilibrium dialysis is one of the oldest and most established of the techniques used to measure plasma protein binding. This method employs a plexiglas or teflon cell consisting of two reservoirs separated by a semi-permeable dialysis membrane. Plasma, placed in one of the reservoirs, is dialyzed against a physiological buffer, pH 7.4, placed in the other reservoir. Cells are incubated in a water bath at physiologic temperature (37°C). During dialysis unbound drug diffuses across the membrane eventually reaching an equilibrium between unbound drug concentrations in the plasma and buffer compartments. Once equilibrium is reached, the post-dialysis drug concentrations in the plasma and buffer reservoirs are measured. The drug concentration in the buffer is equal to unbound drug concentration, C_U, while the drug concentration in the plasma reservoir

is equal to the total drug concentration, C_T. The bound drug concentration, C_B, is calculated as the difference between total and unbound drug concentrations. The fraction of drug unbound, F_U, is equal to the ratio of unbound to total drug concentrations (C_U/C_T) while the fraction bound, F_B, is equal to the ratio of bound to total drug concentration (C_B/C_T). Also as evident from these equations, $F_B = 1 - F_U$.

Since measurements are made at equilibrium, this method provides an accurate assessment of drug plasma protein binding. However, special dialysis cells are needed and the procedure is relatively time consuming. Further, during dialysis, the drug concentration in plasma changes from its initial value as free drug diffuses across the membrane to the buffer. This is particularly important for drugs which exhibit concentration dependent binding.

In the clinical laboratory, ultrafiltration is more frequently used for measuring drug protein binding. Plasma is placed in a commercially available ultrafiltration unit consisting of two reservoirs separated by a filter. The filter allows ultrafiltrate and lower molecular weight compounds to pass through, while larger molecular weight molecules, such as plasma proteins, are retained. The ultrafiltrate is forced through the filter by either negative pressure (centrifugation) or positive pressure (N_2 gas or syringe). As the plasma sample is filtered, the ultrafiltrate which emerges in the bottom reservoir contains unbound drug concentrations. Similar to equilibrium dialysis, this method gives a direct measurement of total and unbound drug concentrations. Protein bound concentrations are calculated by difference and fractional binding is calculated as described above.

Ultrafiltration is rapid, efficient, and simple. A fundamental advantage of this method is that it does not require the use of a buffer for protein binding measurements. It can also be applied to different types of biological matrices including tissue homogenates. A disadvantage of ultrafiltration is that the protein concentration in the plasma sample is concentrated as plasma water is filtered. Typically, only 10 - 15% of plasma volume should be collected to maintain an appropriate protein concentration in the upper reservoir. Similar

to equilibrium dialysis, the plasma sample must be maintained at physiologic pH and temperature to mimic physiological conditions. The availability of relatively inexpensive commercially made ultrafiltration devices that can be used with standard laboratory equipment has greatly enhanced the ability of clinical laboratories to determine unbound drug concentrations in patient samples.

Microdialysis allows for the *in vivo* measurement of unbound drug concentrations in plasma, tissues, and other biological fluids such as cerebral spinal fluid. For this technique, a microdialysis probe containing a dialysis membrane is implanted in a blood vessel, fluid containing space, or tissue space. Dialysate buffer is pumped at low flow rates through the probe, and unbound drug in the blood or tissue fluid diffuses across the membrane into the probe. Unbound drug concentrations are measured in the dialysate. Microdialysis measures only unbound drug concentrations, thus total drug or bound drug concentrations are not determined by this method. To determine total drug concentration (C_T), plasma samples must be collected and analyzed separately. Bound drug concentrations can then be calculated from C_T - C_U.

Since an equilibrium is not achieved, it is essential that drug recovery across the microdialysis membrane is quantitated. An *in vitro* method accomplishes this by placing the microdialysis probe in a buffer containing a known concentration of drug. The drug concentration of the dialysate after perfusing the solution can be determined and used to calculate recovery. Alternatively, recovery can be estimated *in vivo* by adding a compound with similar physicochemical characteristics to the dialysate buffer. The loss of the marker compound from the dialysate buffer reflects drug recovery. This calculation of recovery assumes that the recovery across the membrane will be identical regardless of the direction of the drug diffusing across the membrane. Drug recovery across the microdialysis membrane is typically low and thus can be a limitation of this method. Furthermore, since only small volumes of dialysate buffer are collected, highly sensitive analytical methods are needed to measure unbound drug concentrations,

$$C_B = \sum_{i=1}^{m} \frac{n_i \, P_{Ti} \, K_{Ai} \, C_U}{1 + K_{Ai} \, C_U}$$

particularly for drugs which are highly protein bound.

The primary advantage of using microdialysis is that unbound drug concentrations are measured directly *in vivo*. Although microdialysis is not practical for widespread use in clinical settings, this method has been used clinically to measure drug concentrations in muscle, plasma and subcutaneous tissue.[48] Tissue concentrations may better reflect the pharmacological response. For example, following standard doses, plasma concentrations of azithromycin are lower than bacteria minimum inhibitory concentrations. Tissue concentrations of azithromycin, however, are above the bacteria minimum inhibitory concentration resulting in successful antimicrobial therapy. [49-56]

1.2 Protein Binding Parameters

The characterization of protein binding, including binding parameters, is useful for the prediction of unbound drug concentrations. Initial drug dosage regimens can be designed to achieve desired unbound drug levels based on a patient's protein concentration and known binding parameters. Dosage regimens should be further optimized based on subsequent determinations of unbound plasma drug concentrations.

Plasma protein binding is a function of protein concentration, drug concentration, the equilibrium association constant (K_A) for the drug-protein interaction and the number of protein binding sites per class of binding sites (n).[4,51] Drug-protein association can be represented by a mass balance scheme:

$$C_U + P_U \rightleftharpoons C_B$$
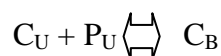
where P_U is the unbound protein concentration (protein that has no drug bound to it) and C_U and C_B are concentrations of unbound and bound drug, respectively. Derivation of the second-order equation for the drug-protein interactions yields equation 1 which describes bound drug concentrations as a function of unbound drug concentrations to *i* classes of binding sites:

where K_A is defined as $[C_B]/[C_U][P_U]$, $[P_T]$ is the total concentration of the binding protein. The fraction of drug unbound (f_U) is equal to $C_U/(C_U + C_B)$. For one class of binding sites, fraction unbound can be described by:

$$f_U = \frac{1 + K_A C_U}{1 + K_A C_U + n K_A P_T}$$

The equilibrium association constant provides an indication of the affinity or the strength of the drug-protein association. Highly protein bound drugs (90 - 99.9%) typically have K_A values ranging from 10^5 to 10^7 M^{-1}, while drugs with low to moderate protein binding have K_A values ranging from 10^2 to 10^4 M^{-1}. [52] The number of binding sites provides an indication of how many drug molecules can associate with a single class of binding sites. The binding capacity, determined from the product $n \times P_T$, is the maximum concentration of drug molecules that can associate with a protein binding site, or the total concentration of binding sites. Protein binding sites with lower binding capacities may become saturated, resulting in non-linear binding, more readily that those with larger binding capacities. The number of classes of sites is also significant. Separate binding sites may be on the same protein molecule, or binding may involve separate types of proteins. Hydrocortisone and prednisolone bind to corticosteroid binding globulin and albumin.[53,54] Imipramine, propranolol and quinidine bind to albumin, α_1-acid glycoprotein and lipoproteins.[9,55] Classes of binding sites are generally identified by distinct equilibrium association constants. For example, one class may be high affinity binding site with an equilibrium association constant of 10^6 M and the other site may be a low affinity binding site with K_A of 10^3 M. Similarly, one binding site may be saturable while other binding sites may have a high capacity.

Figures 1 depicts the fraction of drug unbound as a function of drug concentration and protein concentration for a drug binding to a single class of binding sites with a high

equilibrium association constant ($K_A = 10^6$ M^{-1}). Figure 2 shows the fraction of drug

unbound as a function of drug concentration and protein concentration for a drug binding

to a single class of binding sites with a low K_A of 10^3 M^{-1}. Comparison of Figures 1 and

2 illustrates the difference in fractional binding between proteins with different K_A

values. For the protein with a high K_A value (10^6 M^{-1}), the fraction unbound ranges from

less that 1% at low drug concentrations to nearly 100% at high drug concentrations.

Protein binding is lower for the protein with the low K_A value (10^3 M^{-1}) with the unbound

fraction ranging from 30% to almost 100%. For both cases, binding is linear at lower

drug concentrations, however as drug concentrations increase, binding sites became

saturated and fractional binding decreases. Clinically the changes in protein binding

associated with drug concentration are more important for drugs with higher equilibrium

association constants. Protein binding is also dependent on protein concentration. Figure

1 depicts the fraction unbound as a function of drug concentration and protein

concentration for a drug binding to a single class of binding sites with a K_A of 10^6 M^{-1}.

At very low drug concentrations, the fraction of drug unbound is very low and dependent

on protein concentration in a non-linear manner (Figure 3A). At a drug concentration of

10^{-6} M, the fraction unbound doubles from 0.2% at a protein concentration of 0.001 M to

0.4% at a protein concentration on 0.0005 M. At a lower protein concentration of 0.0001

M, the fraction unbound increases ten-fold to 2%. Thus, for highly bound drugs,

alternations in protein concentration can produce clinically significant changes in the

fraction of a drug unbound. At moderate drug concentrations, a decreased protein

concentration also results in a decrease in fractional binding (Figure 3B). At a drug

concentration of 0.0005 M, the fraction of drug unbound increases from 33% to 83% as

protein concentration decreases from 0.001 M to 0.0001 M. However, since fractional

binding at moderate drug concentrations is also moderate, changes in protein binding

due to decreased protein concentration are usually not clinically significant. At very high

drug concentrations, binding sites are saturated and the fraction unbound approaches

100%, and thus protein binding is independent of protein concentration.

Figure 2 shows the fraction unbound as a function of drug concentration and protein concentration for a drug with a K_A of 10^3 M^{-1}. At all drug concentrations, binding is moderate to low. At a drug concentration of 10^{-6} M, fraction unbound increases from 33% at a protein concentration of 0.001 M to 48% at a protein concentration of 0.0001 M (Figure 3C). At moderate drug concentrations (10^{-3} M), the fraction unbound increases from 50% to 65% as protein concentration deceases from 0.001 M to 0.0001 M (Figure 3D). Thus, at low to moderate drug concentrations, the degree of protein binding changes only 30% over a ten-fold difference in protein concentration. At high drug concentrations binding sites are saturated and the fraction unbound approaches 100%. Overall, for moderately bound drugs, changes in protein binding due to changes in protein concentration are usually not clinically important.

1.3 Binding Proteins

It is germane to consider the factors that affect protein binding and consequently unbound drug concentrations. Factors that affect protein binding include the drug concentration, the nature and concentration of the protein to which it is bound, the affinity of protein for the drug, the pathophysiological condition of the patient, and possible drug interactions. [4,51-52,56] Drugs may bind to albumin, α_1-acid glycoprotein, α, β, and γ globulins, lipoproteins or erythrocytes.[4,51-52,56] The plasma protein binding of drugs is typically reversible with drug-protein binding associations generally due to hydrophobic forces or ionic interactions.[58-59] Hydrophobic forces are typically weak resulting in a low to moderate degree of drug protein binding. Ionic interactions often result in a higher degree of binding.

Albumin is a large protein (MW_ 67,000 dalton) which distributes in plasma and interstitial fluid.[60] This protein maintains colloid osmotic pressure in the vascular system and transports fatty acids and bilirubin. [61] Drugs may associate with albumin by hydrophobic or ionic forces.[58-59] Neutral drugs and some basic drugs bind to albumin by

hydrophobic binding forces, and since albumin possesses a net cationic charge, anionic drugs bind avidly to this protein with electrostatic bonds.[58-60] Albumin also has specific binding sites.[58,62-63] The warfarin binding site binds drugs such as phenylbutazone, sulfoamide, phenytoin, and valproic acid. The benzodiazepine site is where probenecid, semisyntheic-penicillins and medium chain fatty acids are bound. Other binding sites include the bilirubin binding site, the digitoxin binding site, and the fatty acid binding site. Drug binding to these specific binding sites is typically characterized by a high affinity association and may be saturable over therapeutic drug concentrations. Albumin has a typical concentration of about 3.5-5.0 g/dL (0.0005 - 0.0075 M)[60]. Alterations in albumin concentration are likely due to altered synthesis or shifts in levels from the intravascular to extravascular space. Hypoalbuminemia is the most common age related alteration with albumin.

Cationic drugs bind primarily to the globulin, α_1-acid glycoprotein (orosomucoid).[64] The biological role of α_1-acid glycoprotein is undefined but it is known to be an acute phase reactant, meaning that levels of this protein rise in instances of physiological trauma or stress. The molecular weight of α_1-acid glycoprotein is 42,000 dalton and normal blood concentrations of the protein range from 40 to 100 mg/dL (9.5×10^{-6} - 2.5×10^{-5} M). Many basic drugs including propranolol, lidocaine, impramine, carbamazepine and verapamil bind with high affinity to α_1-acid glycoprotein.[56] However, since plasma concentrations of this protein are relatively low compared to albumin and other major plasma proteins, drug binding can be saturable over the range of therapeutic drug concentrations. Thus, α_1-acid glycoprotein is often referred to as a high affinity - low capacity protein. Albumin, on the other hand, is a high capacity protein.

Alpha, beta, and gamma globulins may be responsible for the binding of certain endogenous substance such as corticosteroids.[56] Lipoproteins (MW varied between 200,000-10,000,000 dalton) are large macromolecular complexes of lipids and

proteins.[65] Lipoproteins are divided into four different groups: chylomicrons, very low density lipoproteins, low density lipoproteins and high density lipoproteins. Neutral and basic lipophilic drugs such as quinidine, amtriptylline and diltiazem bind to these proteins.[56] Drug-lipoprotein interactions are generally weak resulting in a low degree of binding. Lipoproteins are responsible for the binding of drugs when other proteins becomes saturated.[53] Erythrocytes may also bind to endogenous and exogenous compounds.[56] The role of these proteins is lesser than that of albumin and α_1-acid glycoprotein. It is important to note that drugs may bind to more than one plasma protein. Examples of drugs which bind

to albumin, α_1-acid glycoprotein, and lipoproteins are propranolol, bupivicaine, and tricyclic antidepressants.[9,55]

Changes in plasma proteins contribute to the reduction of protein binding in the elderly .[66-68] A number of pathophysiological conditions including renal disease, hepatic dysfunction, acute myocardial infarction, neoplasms, arthritis, and Crohn's disease will alter proteins and drug protein binding .[9-43] Serum albumin concentrations are approximately 19% lower in the elderly than in young adults because of reduced renal function and the diminished capacity of the liver to synthesize proteins.[69] Drugs that are primarily bound to red blood cells also exhibit similar binding characteristics in the elderly. Decreased binding may occur in those who have a restricted diet and those on concomitant medication.[67] The mechanism of these alterations can not be limited to a single source. Endogenous displacers have been reported to compete for the protein-drug binding site and this competition may alter with age and disease. It has been speculated that bilirubin competes for binding sites. Another possibility for changes in protein binding may lay in the drug interactions that occur when one drug displaces another.[70]

Age *per se* does not play a role in changing levels of α_1-acid glycoprotein.[66-68] Rather, alterations in α_1-acid glycoprotein concentrations seem to be a function of disease state. Plasma levels of α_1-acid glycoprotein increase during acute myocardial infarction,

burns, cancer, inflammatory disease, surgery, and trauma .[9-44] This increase in protein leads to increase in binding of basic drugs. Severe liver disease including cirrhosis and nephrotic syndrome, on the other hand, lead to decreased plasma concentrations of α_1-acid glycoprotein.[9]

Another significant factor contributing to a change in drug distribution may be contributed to the change in body composition that occurs in the elderly. As individuals age there is an increase in the percentage of adipose tissue in the body. This elevation of fat tissues increases the volume of distribution of lipophilic drugs and disposition in the tissues and decreases the volume of distribution in hydrophilic drug. The tissue binding of the lipophilic compound decreases and the protein binding of the hydrophilic compound increases.[68]

2. Effects of Protein Binding on Drug Pharmacokinetics

Protein binding is one of a myriad of factors that influence drug disposition. The effects of protein binding on drug pharmacokinetics are well defined and have been the subject of previous reviews.[68,71-77] Drug disposition is defined simply by drug distribution and clearance. The relationships between the physicochemical properties of a drug, plasma protein binding, tissue binding, drug distribution, drug clearance and pharmacokinetics, however, are complex. Thus, the effects of protein binding on drug disposition are dependent on the overall pharmacokinetic characteristics of the drug, however generalizations on protein binding effects may be made.

The cornerstone of clinical pharmacokinetics is the equation:[78]

$$C_{SS} = \frac{F * Dose}{\tau * CL}$$

where C_{SS} is the steady-state total (bound + unbound) drug concentration, F is bioavailability, Dose is the maintenance dose of the drug, τ is the dosing interval, and CL is the systemic clearance of the drug. The physiologic variables controlling steady-state drug

concentrations are bioavailability and clearance, whereas Dose and τ can be adjusted by the clinician. Renal excretion and hepatic metabolism are the predominate routes of drug elimination. Drug clearance (CL) is a function of blood flow to the clearing organs, kidney (Q_{RP}) and liver (Q_H), and the efficiency, defined as extraction ratio (ER), of the clearing organ to clear the drug from blood (CL = Q × ER). The influence that plasma protein binding exerts on clearance is dependent on the inherent ability of the clearance organs to extract drug from plasma or blood. Renal clearance is the net result of glomerular filtration, active tubular secretion and tubular reabsorption. Only unbound drug in plasma is filtered by the glomerulus. The effect of protein binding on active tubular secretion depends on the affinity of the transport mechanism for the drug. If the extraction ratio for active tubular secretion is high, tubular secretion is independent of protein binding. However, for drugs with a low extraction ratio for active tubular secretion, the degree of tubular secretion is proportional to the fraction of drug unbound in plasma. The extent of tubular reabsorption is inversely related to unbound plasma drug concentrations. Higher unbound plasma drug concentrations result in a lower concentration gradient between urine and plasma, and thus, tubular reabsorption will be decreased. Thus, an increase in the fraction of drug unbound in plasma will lead to an increase in glomerular filtration, an increase or no change in active tubular secretion and a decrease in passive tubular reabsorption. Overall, this results in an increased renal clearance. However, alterations in renal clearance due to age related changes in protein binding are usually minor and not clinically significant. Furthermore, renal function and renal blood flow generally decrease with age. These physiological factors are clinically more important that protein binding.[76]

For hepatic clearance ($CL_H = Q_H \times ER$), extraction ratio is a function of hepatic blood flow, intrinsic clearance of unbound drug (CL_I^*) and the fraction unbound (f_U) in plasma of the drug such that:

$$CL_H = \frac{Q_H * f_U * CL_I^*}{Q_H + f_U * CL_I^*}$$

Figure 4 illustrates the effects of intrinsic clearance of unbound drug and fraction unbound on hepatic clearance. Hepatic clearance for high clearance drugs is limited by hepatic blood ($CL_H \cong Q_H$) and is independent of protein binding. Since hepatic clearance is not influenced by protein binding, steady-state total drug concentrations will not be affected by age-related alterations in protein binding. On the other hand, for low clearance drugs, hepatic clearance is limited by intrinsic clearance ($CL_H \cong f_U \times CL_I^*$) and, thus, is dependent on the degree of plasma protein binding. Hepatic clearance and steady-state drug concentrations for moderate clearance drugs are affected to varying degrees by protein binding. The effects of intrinsic clearance and fraction unbound on steady-state total drug concentrations is shown in figure 5.

Where the use of unbound drug concentrations ($C_{u,\,ss}$) provide a better assessment for therapeutic monitoring, equation 1 can be modified to:

$$C_{u,SS} = \frac{F * Dose}{\tau * CL / f_U}$$

The effects of intrinsic clearance and fraction unbound on steady-state unbound drug concentrations are also shown in figure 5. For high clearance drugs, clearance is not affected by protein binding. However, $C_{u,\,SS}$ is related to the fraction unbound and an increase in unbound fraction results in increased steady-state unbound drug concentrations. On the other hand, for low clearance drugs, clearance is dependent on protein binding. However, since protein binding induced changes in clearance are offset by an equal change in fraction unbound according to equation 5, steady-state unbound drug concentrations are not affected by alterations in protein binding. For moderate clearance drugs, steady-state unbound drug concentrations are affected to varying degrees by protein binding.

For low clearance drugs, an increase in the fraction of drug unbound in plasma will result in lower steady-state total drug concentrations, however steady-state unbound drug

concentrations will not change. In most cases, dosage adjustments are not needed. For high clearance drugs, an increase in the fraction of drug unbound in plasma will not alter steady-state total drug concentrations, however steady-state unbound drug concentrations will increase. Clinically, this is significant only if the drug is highly bound to plasma proteins. Thus, dosage adjustments may be needed for highly bound, high clearance drugs such as propranolol and nortriptyline.

While the discussion of drug clearance thus far has been focused on either hepatic or renal clearance, systemic clearance of a drug involves both renal and hepatic clearances ($CL = CL_H + CL_R$) in most cases. Thus, assessment of age-related changes in protein binding requires knowledge of the fraction of drug excreted and metabolized and how changes in protein binding affect each of these routes of elimination. Overall, the therapeutic implications of age related changes in plasma protein binding appear to be relatively minor. Age related alterations in hepatic function and blood flow are more important clinically. Following multiple dosing, steady-state maximum and minimum drugs concentrations are, in part, a function of volume of distribution. The distribution of a drug is highly dependent on the physicochemical characteristics of the drug as well as protein binding. Hydrophilic drugs are generally confined to blood and interstitial fluid, thus changes in plasma protein binding will not produce major alterations in the distribution of hydrophilic drugs. Lipophilic compounds more readily cross biological membranes, and therefore, have larger volumes of distribution. Drug distribution is a function of plasma protein binding and tissue protein binding. Highly plasma protein bound drugs, such as warfarin, valproic acid, and the penicillins, typically have relatively low volumes of distribution as their strong association with plasma proteins confines them to vascular spaces.[2] Conversely, drugs that are largely unbound in plasma are generally available for distribution out of the vascular system. However, the volume of distribution is also affected by the magnitude of drug binding by tissue proteins.[52] Several drugs, such as amiodarone, digoxin, and tricyclic antidepressants, although

highly bound to plasma proteins, are bound with greater affinity to tissue proteins resulting in large volumes of distribution.[2] Drugs characterized by large volumes of distribution are extensively bound to tissue proteins. Indeed, tissue binding often plays a more important role in drug distribution than does plasma protein binding. Age related changes in body composition, then play a more significant role in age related volume of distribution changes than plasma protein binding.[66-67]

It is important to assess therapeutic effects and toxicities as well as drug pharmacokinetics. If it is assumed that drug distribution occurs by passive diffusion of unbound drug, then an equilibrium exists between unbound drug in plasma and at the effect site. Drug concentrations at the effect site receptor, thus, are reflected by unbound drug concentrations in plasma. Therefore, monitoring of unbound drug concentrations may provide a better assessment of therapeutic response, than do total drug concentrations. For example, unbound drug concentrations have been reported to be a better predictor of therapeutic response than total drug concentration for phenytoin.[5-6] The plasma protein binding of phenytoin is often decreased in patients with hypoalbuminemia and renal disease. Phenytoin binding may also be decreased by displacement by concomitantly administered drugs. The elderly often show an increase incidence of central nervous system side effects, which appear to be associated with a decreased plasma protein binding of the drug, than do younger adults.[79] Dosage adjustments of phenytoin are often needed in these clinical situations where protein binding of the drug is decreased. Therefore, in clinical situations were plasma protein binding may be altered, total drug concentrations must be interpreted with caution.

3. Age-Related Changes in Protein Binding

Table 1 summarizes clinical studies that have investigated drug plasma protein binding in the elderly. Generally, plasma protein binding of drugs remains unchanged or decreases with age. However, alterations in plasma protein binding that occur with aging

may not be attributed exclusively to old age. Usually there are no significant changes in plasma protein binding except in the presence of pathophysiological changes. Renal and hepatic dysfunction most often account for altered protein binding. Unfortunately, assessment of alterations in plasma protein binding were not always determined in studies examining the effects of age on drug pharmacokinetics.

3.1 Therapeutic Implications

Comparisons made between pharmacokinetics studies in the elderly and young adults are often complicated. It is difficult to recruit a significant number of healthy individuals over the age of fifty years for comparison of pharmacokinetic profiles with healthy young individuals. Due to patient physiological variability and concomitant medication, it is difficult to determine whether or not altered pharmacokinetics is due to altered protein binding or pathophysiological changes. Indeed, even when protein binding is determined, it is difficult to ascertain whether any changes in binding are related to aging or disease states.

There are a few instances where age related changes in plasma protein binding may be clinically significant. Results of several studies with the non-steroidal anti-inflammatory agent naproxen demonstrate the difficulty in assessing the clinical effects of protein binding in the elderly. Naproxen, a weakly acidic compound, is bound extensively (>99.7%) to albumin in healthy young volunteers.[125] The protein binding of naproxen is also concentration dependent. Naproxen is eliminated primarily by hepatic metabolism, and is considered to have a low intrinsic clearance. Two studies, one using healthy elderly subjects and one using patients with rheumatoid arthritis or osteoarthritis, showed that the plasma protein binding of naproxen was decreased in the elderly resulting in a greater than two-fold increase in the fraction of naproxen unbound in plasma.[125,160] In both studies, serum albumin concentration was decreased in the elderly patients. These results are consistent with the simulations presented in Figure 1. As naproxen has a high affinity for binding to albumin and plasma concentrations of

naproxen are relatively low, the fraction of unbound naproxen is highly dependent on protein concentration. Another study in patients with osteoarthritis, however, suggested that naproxen binding was altered only in elderly female patients.[161] Since naproxen has a low intrinsic clearance, the age-related increase in unbound fraction is predicted to cause an increase in hepatic clearance according to figure 4. As shown in Figure 5, this should result in a decrease in steady-state total drug concentrations, but produce no changes in steady-state unbound naproxen concentrations. However, the elderly patients studied also showed a decrease in intrinsic clearance.[125] The increase in the unbound fraction and decrease in intrinsic clearance in the elderly offset each other and total drug concentrations in the elderly were similar to those seen in young subjects. Unbound concentrations, however, were higher in the elderly patients. Based on unbound naproxen concentrations, it was suggested that the dose of naproxen be reduced in the elderly. A later study, however suggested that there was no correlation between unbound naproxen concentrations and efficacy or adverse events.[161]

Pharmacokinetic investigations of naproxen in young and aged rats have also been conducted. These laboratory studies yielded virtually identical age-related differences in the disposition of naproxen as was seen in clinical studies.[162] A pharmacodynamic study in young and old rats demonstrated that the pharmacodynamic response of naproxen, determined by thromboxane B2 inhibition as a means of assessing cyclooxygenase inhibition, was virtually identical in both age groups of rats.[162] The elevation in unbound naproxen concentrations in the aged rats, was offset by a decreased receptor sensitivity. This example illustrates the complexity of drug monitoring in the elderly.

For most drugs age-related changes in protein binding in itself are usually not clinically significant to the pharmacokinetics and pharmacodynamics of therapeutic agents. Varied protein binding in the elderly is one of many factors that may lead to altered pharmacokinetics and consequently different dosing regiments in the elderly.

Other age-related factors appear to have greater therapeutic implications. Disease states which can significantly alter pharmacokinetics, should be considered when determining the dosage of drugs in the elderly. For example, alprazolam exhibited no difference in protein binding when pharmacokinetics studies were performed in the elderly (Table I). In the presence of renal failure, however, the fraction of free drug significantly increased.[85] Batanopride exhibited decreased protein binding in the presence of renal failure.[87] The antipsychotic drug risperidone did not exhibit altered protein binding in the elderly except in the presence of renal failure.[142] The protein binding of propranolol, a highly cleared drug, was not affected by age in men, but intrinsic clearance was significantly decreased.[134-135,138] There may also be gender-specific differences in plasma protein binding. Propranolol exhibited an increased fraction unbound in the S-enantiomer in older women, but not in men. Stereospecific age related changes in plasma protein binding have also been reported. The nonsteroidal anti-inflammatory, ibuprofen has decreased binding of the S-enantiomer. Similarly, the change in protein binding of propranolol in elderly women was only noted for the S-enantiomer.

Smoking, alcohol use, concomitant drugs, over the counter drugs, genetic and sex differences should also be taken in account.[66] Drug interactions, adverse drug reactions and poor compliance also affect the pharmacodynamics and pharmacokinetics in the elderly.[67] The concurrent use of other drugs, alcohol and tobacco may lead to adverse drug reactions and drug interactions. The frequency of adverse drug reactions increases with age and with the concurrent use of additional drugs.[67] Compliance issues are prevalent in elderly patients. These individuals usually have impaired sight and hearing making it difficult to first hear the doctor or pharmacist's instructions and later to distinguish between the medications taken at the same time. Impaired memory often prevents the patients from taking the medication when needed. Prescribing health care workers should make allowances for these during consulting and prescribing.[67]

When prescribing medication in the elderly, clinicians should take all of these factors

into account. It should be recognized that aging is not a uniform process therefore it is not possible to ascribe to a uniform method of dosing in the elderly. Dosing regimens should be individualized and frequent clinical assessment of the patient is needed.[65]

4. Conclusion

Plasma protein binding is a major determinant of drug action, particularly for drugs that are highly bound. Changes in protein binding can have clinical implications. Therapeutic monitoring of total drug concentrations often does not provide adequate information for the design of optimal dosing regimens. In aging, protein binding may be altered, however, physiological changes and pathophysiological disorders also occur. These changes usually have greater clinical significance than changes in drug plasma protein binding. A firm understanding of the inter-relationships between drug concentrations, protein binding, the physiology of aging, disease, pharmacokinetics and pharmacodynamics is necessary for effective therapeutic monitoring. Therapeutic monitoring of unbound drug concentrations simplifies these relationships and provides the fundamental information needed for dosage regimen development and adjustment. Clearly, drug therapy should be individualized taking into account all of these factors.

References

1. Greenblatt DJ, Sellers EM, Koch-Weser J. Importance of protein binding for the interpretation of serum of plasma drug concentrations. J Clin Pharmacol 1982 May-Jun; 22(5-6): 259-63

2. Ariëns EJ. Drug levels in the target tissue and effect.   Clin Pharmacol Ther 1974 Jul; 16(1): 155-75

3. Oosterhuis B, van Boxtel CJ. Kinetics of drug effects in man. Ther Drug Monit 1988; 10(2): 121-32

4. Wright JD, Boudinot FD, Ujhelyi MR. Measurement and analysis of unbound drug concentrations. Clin Pharmacokinet 1996 Jun; 30(6): 445-62

5. Blum MR, Riegelman S, Becker CE. Altered protein binding of diphenylhydantoin in uremic plasma. N Engl J Med 1972 Jan; 286(2): 109

6. Odar-Cederlof I, Lunde P, Sjogvist F. Abnormal pharmacokinetics of phenytoin in a patient with uremia. Lancet 1970 Oct; 2(7677): 831-2

7. Gibaldi M, Koup JR. Pharmacokinetic concepts-drug binding, apparent volume of distribution and clearance. Eur J Clin Pharmacol 1981; 20(4): 299-305

8. Kunin CM, Craig WA, Kornguth M, Monson R. Influence of protein binding on the pharmacologic activity of antibiotics. Ann NY Acad Sci 1973 Nov; 226: 214-24

9. Piafsky KM. Disease-induced changes in the plasma binding of basic drugs. Clin Pharmacokinet 1980 May-June; 5(3): 246-62

10. Kishino S, Nomura A, Di ZS,et al. Changes in the binding capacity of alpha-1-acid glycoprotein in patients with renal insufficiency. Ther Drug Monit 1995 Oct ; 17(5): 449-53

11. Kremer JMH, Wilting J, Janssen LHM. Drug binding of human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev 1988 March; 40(1): 1-47

12. Caswell M, Pike LA, Bull BS, et al. Effect of patient age on tests of acute-phase response. Arch Pathol Lab Med 1993 Sep; 117(9): 906-10

13. Crook MA, Treloar A, Haq M, et al. Serum total sialic acid and other acute phase proteins in elderly subjects. Eur J Clin Chem Clin Biochem 1994 Oct; 32 (10): 745-47

14. Lalonde RL, Tenero DM, Burlew BS, et al. Effects of age on the protein binding and disposition of propranolol stereoisomers. Clin Pharmacol Ther 1990 Apr; 47 (4): 447-55

15. Veering BT, Burm AG, Souverijn JH, et al. The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein. Br J Clin Pharmacol 1990 Feb; 29(2): 201-6

16. Ecoffey C. Pharmacokinetics of local anesthetics in children. Cah Anesthesiol 1992; 40(7): 533-6

17. Seta N, Tissot B, Forestier F, et al. Changes in alpha 1-acid glycoprotein serum concentrations and glycoforms in the developing human fetus. Clin Chim Acta 1991 Dec; 203 (2-3): 167-75

18. Bardy AH, Hiilesmaa VK, Teramo K, et al. Protein binding of antiepileptic drugs during pregnancy, labor and puerperium. Ther Drug Monit 1990 Jan; 12 (1): 40-6

19. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. Clin Pharmacokinet 1990 Jan; 18 (1): 20-36

20. Maher JE, Goldenberg RL, Tamura T, et al. Albumin levels in pregnancy: a hypothesis--decreased levels of albumin are related to increased levels of alpha-fetoprotein. Early Hum Dev 1993 Oct ; 34 (3): 209-215

21. Meistelman C, Benhamou D, Barre J, et al. Effects of age on plasma protein binding of sufentanil. Anesthesiology 1990 Mar; 72(3): 470-3

22. Jensen H, Rossing N, Andersen SB, et al. Albumin metabolism in the nephrotic syndrome in adults. Clin Sci 1967 Dec; 33(3): 445-457

23. Vasson MP, Baguet JC, Arveiller MR, et al. Serum and urinary alpha-1 acid glycoprotein in chronic renal failure. Nephron 1993; 65(2): 299-303

24. Movin-Osswald G, Boelaert J, Hammarlund-Udenaes M, et al . The pharmacokinetics of remoxipride and metabolites in patients with various degrees of renal function. Br J Clin Pharmacol 1993 Jun; 35(6): 615-22

25. Vasson MP, Paul JL, Couderc R, et al. Serum alpha-1 acid glycoprotein in chronic renal failure and hemodialysis. Int J Artif Organs 1991 Feb; 14 (2): 92-6

26. Pacifici GM, Viani A, Taddeuci-Brunelli G, et al. Effects of development, aging, and renal and hepatic insufficiency as well as hemodialysis on the plasma concentrations of albumin and α_1-acid glycoprotein: implications for binding of drugs. Ther Drug Monit 1986; 8(3): 259-63

27. Garcia-Morillas M, Gil-Extremera B, Caracuel-Ruiz MD. Differential effects of hepatic cirrhosis on the plasma protein binding of drugs. Int J Clin Pharmacol Res 1984; 4(5): 327-33

28. Hallen J, Laurell CB. Plasma protein pattern in cirrhosis of the liver. Scand J Clin Lab Invest Suppl 1972; 124: 97-103

29. Kindmark CO, Laurell CB. Sequential changes of the plasma protein pattern in inoculation hepatitis. Scand J Clin Lab Invest Suppl 1972; 124: 105-15

30. Joelsson B, Alwmark A, Gullstrand P, et al. Serum proteins in liver cirrhosis: effects of shunt surgery. J Clin Chem Clin Biochem 1987 Dec; 25(12): 865-68

31. Biou D, Chanton P, Konan D, et al. Microheterogeneity of the carbohydrate moiety of human α_1- acid glycoprotein in two benign liver diseases: Alcoholic cirrhosis and acute hepatitis. Clin Chim Acta Dec 1989; 186 (1): 59-66

32. Falleti E, Pirisi M, Fabris C, et al. Increase of serum alpha 1-acid glycoprotein despite the decline of liver synthetic function in cirrhotics with hepatocellular carcinoma. Eur J Clin Chem Clin Biochem 1993 Jul; 31(7): 407-11

33. Viani A, Rizzo G, Carrai M, Pacifici GM. Interindividual variability in the concentrations of albumin and alpha-1-acid glycoprotein in patients with renal or liver disease, newborns and healthy subjects: implications for binding of drugs. Int J Clin Pharmacol Ther Toxicol 1992 Apr; 30(4): 128-33

34. Piafsky KM, Borgä O, Odar-Cederlöf I, et al. Increased plasma protein binding of propranolol and chloropromazine mediated by disease induced elevations of plasma α_1-acid glycoprotein. N Engl J Med 1978 Dec; 299 (26): 1435-39

35. Bloedow DC, Hansrough JF, Hardin T, et al. Postburn serum drug binding and serum concentrations. J Clin Pharmacol 1986 Feb; 26(2): 147-51

36. Martyn JAJ, Abernethy DR, Greenblatt DJ. Plasma protein binding of drugs after severe burn injury. Clin Pharmacol Ther 1984 Apr; 35(4):535-9

37. Tozer TN. Implication of altered plasma protein binding in disease states. In: Benet LZ et al.,eds. Pharmacokinetic Basis for Drug Treatment. New York: Raven Press; 1984

38. Hooper WD, Bochner F, Eadie MJ, et al. Plasma protein binding of diphenylhydantoin. Effects of sex hormones, renal, and hepatic disease. Clin Pharmacol Ther 1974 Mar; 15:276-82

39. Wallace S, Brodie MJ. Decreased drug binding in serum from patients with chronic hepatic disease. Eur J Clin Pharmacol. 1976 Mar; 9(5-6): 429-32

40. Riuz-Cabello F, Erill S. Abnormal serum protein binding of acidic drugs in diabetes mellitus. Clin Pharmacol Ther 1984; 36: 691

41. Grossman SH, Davis D, Kitchell BB, et al. Diazepam and lidocaine plasma protein binding in renal disease. Clin Pharmacol Ther 1982 Mar; 31(3): 350-7

42. Thiessen JJ, Sellers EM, Denbeigh P,et al. Plasma protein binding of diazepam and tolbutamide in chronic alcoholics. J Clin Pharmacol 1976 Jul; 16(7): 345-51

43. Feely J, Stevenson IH, Crooks J. Altered plasma protein binding of drugs in thyroid disease. Clin Pharmacokinet 1981 Jul-Aug; 6(4):298-305

44. Kurz H, Trunk H, Wetrz B. Evaluation of methods to determine protein binding of drugs. Arzneimittelforschung 1977 Jul; 27(7):1373-80

45. Chigell CU. Protein Binding. In Garnett Er, Hirtz JL, editors. Drug Fate and Metabolism. Vol 1. New York: Marcel Dekker, 1977: 187-228

46. Bowers WF, Fulton S, Thompson J. Ultrafiltration *vs*. Equilibrium dialyis for determination of free fraction. Clin Pharmacokinet 1984 Jan; 9 (1 Suppl.): 49-60

47. Pacifici GM, Viana A. Methods of determining plasma and tissue binding of drugs: pharmacokinetic consequences. Clin Pharmacokinet 1992 Dec; 23(6):449-68

48. Muller M, Scmid R, Georgopoulos A, et al. Application of Microdialysis to clinical pharmacokinetics in humans. Clin Pharmacol Ther 1995 Apr; 57(4):371-80

49. Schentag JJ, Ballow CH. Tissue directed pharmacokinetics. Am J Med 1991 Sep 12; 91 (3A):5-11

50. Craig WA, Welling PG. Protein binding of antimicrobials: clinical pharmacokinetic and therapeutic implications. Clin Pharmacokinet 1977 Jul-Aug; 2(4):252-268

51. Vallner JJ. Binding of drugs by albumin and plasma protein. J Pharm Sci 1977Apr; 66(4): 447-463

52. Jusko WJ, Gretch M. Plasma and tissue protein binding of drugs in pharmacokinetics. Drug Metab Rev 1976; 5(1): 43-140

53. Sanderberg, AA, Slaunwhite WR, Antoniades HN. The binding of steroids and steroid conjugates to human plasma proteins. Recent Progr Horm Res 1957; 13: 209-67

54. Ballard PL. Delivery and transport of glucocorticoids to target cells. Monogr Endocrinol1979; 12: 25-48

55. Lemaire, M,et al. Lipoprotein binding of drugs. In: Reidenberg MM, Erill S, eds. Drug-Protein Binding. New York: Praeger Publishers; 1986; 201-19.

56. MacKichan J. Influence of Protein Binding and Use of Unbound (Free) Drug Concentrations. In: Evans WE, Schentag JJ, Jusko WJ, eds. Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring. Washington: Applied Therapeutics, Inc, 1993

57. Pike E, Skuterud B, Kierulf P, et al. The relative importance of albumin, lipoproteins and orosomucoid for drug serum binding. Clin Pharmacokinet 1984; 9(1 Suppl.): 84-101

58. Kragh-Hansen U. Molecular aspects of ligand binding to serum albumin. Pharmacol Rev 1981 Mar; 33(1): 17-53

59. Klotz IM, Physicochemical aspects of drug-protein interactions: a general perspective. Ann N Y Acad Sci 1973 Nov 26; 226: 18-35

60. Peters T. Serum Albumin. In: Putnam FW, Editor. The Plasma Proteins: Structure, Function and Genetic Control. New York: Academic Press, 1975:133-187

61. Veering BTH, Burm GL, Souverijn HM, et al. The effect of serum concentrations of albumin and alpha-1-acid glycoprotein. Br J Clin Pharmacol 1990 Feb; 29(2): 201-6

62. Iwatsuru M, Nishigori H, Maruyama K. The classification of drugs on the basis of the drug-binding site on human serum albumin. Chem Pharm Bull 1982 Dec; 30 (2): 4489-93

63. Sudlow G, Birkett DJ, Wade DN. The characterization of two specific drug binding sites on human serum albumin. Mol Pharmacol 1975 Nov; 11(6): 824-32

64. Schmid K. α-1-acid glycoprotein. In Putnam FW,editor. The Plasma Proteins: Structure, Function and Genetic Control. New York: Academic Press, 1975:183-228

65. Scanu AM, Edelstein C, Keim P. Serum lipoproteins. In Putnam FW, Editor. The Plasma Proteins: Structure, Function and Genetic Control. New York: Academic Press, 1975: 317-391

66.   Pucino F, Beck CL, Seifert RL, et al. Pharmacogeriatics. Pharmacotheraphy 1985 Nov-Dec; 5(6): 314-26

67.   Cohen JL. Pharmacokinetic changes in aging. Am J Medicine 1986 May 16; 80 (5A Suppl.): 31-8

68.   Dawling S, Crome P. Clinical pharmacokinetic considerations in the elderly: an update.Clin Pharmacokinet 1989 Oct; 17(4): 236-63

69.   Greenblatt DJ. Reduced serum albumin concentration in the elderly: a report from the Boston Collaborative Drug Surveillance Program. J Am Geriatr Soc 1979 Jan; 27(1):20-2

70.   Leslie C, Scott PJW, Caird FI. Principles alterations to drug kinetics and dynamics in the elderly. Med Lab Sci. 1992 Dec; 49(4): 319-25

71.   Rowland M. Protein binding and drug clearance. Clin Pharmacokinet 1984 Jan; 9(1 Suppl.): 10-7

72.   Smallwood RH, Mihaly GW, Smallwood RA, et al. Effect of a protein change on unbound and total plasma concentrations for drugs of intermediate hepatic extraction. J Pharmacokinet Biopharm 1988 Oct; 16(5): 529-42

73.   Huang J, Øie S. Hepatic elimination of drugs with concentration-dependent serum protein binding.   J Pharmacokinet Biopharm 1984 Feb; 12(1): 67-81

74.   Faed EM. Protein binding of drugs in plasma, interstital fluid and tissues: effect on pharmacokinetics. Eur J Clin Pharmacol 1981; 21(1): 77-81

75.   McNamara PJ, Levy G, Gibaldi M. Effect of plasma protein and tissue binding on the time course of drug concentration in plasma. J Pharmacokinet Biopharm 1979; 7(2): 195-206

76.   Wilkinson GR. Plasma and tissue binding considerations in drug disposition. Drug Metab Rev 1983; 14(3): 427-465

77.   Wallace SM,Verbeeck RK. Plasma protein binding of drugs in the elderly. Clinical pharmacokinetics in the elderly. Clin Pharmacokinet 1987 Jan; 12(1): 41-7

78.   Wagner JG, Northam JI, Alway CD, et al. Blood levels at the equilibrium state after multiple dosing. Nature. 1965 Sep 18; 207(3): 1301-2

79.   Kutt, H, et al, Diphenylhydantoin metabolism, blood levels and toxicity, Arch Neurol, 11: 642-648, 1964.

80.   Piquette-Miller M, Foster RT, Kappagoda CT, et al.   Effect of aging on the pharmacokinetics of acebutolol enantiomers. J Clin Pharmacol 1992 Feb; 32(2): 148-56

81.   Chapron DJ, Sweeney KR, Feig PU, Kramer PA. Influence of advanced age on the disposition of acetazolamide. Br J Clin Pharmacol 1985 Mar; 19(3): 363-71

82.   Gomolin IH and Chapron DJ. Elucidating the relationship between acetazolamide plasma protein binding and renal clearance using an albumin infusion. J Clin Pharmacol 1992   Nov; 32(11): 1028-32

83.   Lemmens HJM, Burn AGL, Bouill JG, et al. Pharmacodynamics of Alfentanil: The role of plasma protein binding. Anesthesiology 1992 Jan; 76(1): 65-70

84.   Belpaire FM, and Bogaert MG.   Binding of alfentanil to human α_1 - acid glycoprotein,   albumin and serum. Int J Clin Pharmacol Ther Tox 1991 Mar; 29(3): 96-102

85.   Greenblatt DJ, Divoll M, Abernethy DR, et al. Alprazolam kinetics in the elderly: relation to antipyrine disposition. Arch Gen Psychiatry 1983 Mar; 40(3) : 287-290

86.   Schulz P, Turner-Tamiyasu K, Smith G, et al. Amitriptyline disposition in young and elderly normal men. Clin Pharmacol Ther 1983 Mar; 33(3):360-6

87.   St. Peter JV, Brady ME, Foote EF, et al. The disposition and protein binding of batanopride and its metabolites in subjects with renal impairment. Eur J Clin Pharmacol 1993; 45(1):59-63

88.   Kaiser G, Ackermann R, Durnin CJ, et al. Pharmacokinetics and pharmacodynamics of the ace inhibitor benazepril hydrochloride in the elderly. Eur J Clin Pharmacol 1990; 38(4):379-85

89.   MacDonald NJ, Sioufi A, Howie CA, et al.   The effects of age on the pharmacokinetics and pharmacodynamics of single oral doses of benazepril and enalapril. Br J Clin Pharmacol 1993 Sep; 36(3): 205-9

90.   Ho PC, Bourne DWA, Triggs EJ, et al. Comparsion of plasma levels of canrenone and metabolites after base hydrolysis in young and elderly subjects following single and multiple doses of spirononlactone. Eur J Clin Pharmacol 27: 435-439, 1984

91.   Blanchard J. Protein binding of caffeine in young and elderly males. J Pharm Sci 1982 Dec; 71(12): 1415-8

92.   Karki SD, Bentley DW, Luzier A, et al. Disposition of intramuscular cefonicid in elderly patients. J Am Geriatr Soc 1993 Aug; 41(8): 808-810

93.   Dudley MN, Shyu WC, Nightingale CH. Effect of saturable serum protein binding on the pharmacokinetics of unbound cefonicid in humans. Antimicrob Agents Chemother 1986 Oct; 30(4):565-9

94.   Luderer JR, Patel IH, Durkin J,.et al. Age and ceftriaxone kinetics. Clin Pharmacol Ther 1984 Jan; 35(1): 19-25

95.   Roberts RK, Wilkerson GR, Branch RA, et al. Effect of age and parenchymal liver disease on the disposition and elimination of chlorodiazepoxide. Gastroenterology 1978 Sep; 75(3): 479-85

96.   Verbeeck RK, Cardinal JA, Wallace SM. Effect of age and sex on the plasma binding of acidic and basic drugs. Eur J Clin Pharmacol 1984; 27(1): 91-7

97.   Nation RL, Vine J, Triggs EJ, et al. Plasma levels of chlormethiazole and two metabolites after oral adminstration to young and aged subjects. Eur J Clin Pharmacol 1977 Oct 14; 12(2): 137-145

98.   Walker O, Birkett DJ, Alvan G, et al. Characterization of chloroquine plasma protein binding in man. Br J Clin Pharmacol 1983 Mar; 15(3): 375-7

99.   Augustijns P and Verbeerke N. Stereoselective pharmacokinetics properties of chloroquine and de-ethyl chloroquine in humans. Clin Pharmacokinet 1993 Mar; 24(3): 259-269

100.   Albertioni F and Heronren L. Protein binding of 2-chloro-2'-deoxyadenosine (cladribine) in healthy subjects and in patients with leukaemia. Eur J Clin Pharmacol 1994; 46(6): 563-4

101.   Abernathy DR, Greenblatt DJ, Shader RI. Imipramine and desipramine disposition in the elderly. J Pharmacol Exp Ther 1985 Jan; 232(1):183-8

102.   Allen MD, Greenblatt DJ. Comparative protein binding of diazepam and desmethydiazepam. J Clin Pharmacol 1981 May-June; 21(5-6):219-223

103.   Shader RI, Greenblatt DJ, Cirraulo DA, Divoll M Harmatz JS, et al. Effect of age and   sex on disposition of desmethyldiazepam formed from its precursor clorazepate.   Psychopharmacology 1981; 75(2): 193-197

104.   Viani A, Rizzo G, Carrai M, et al. The effect of aging on plasma albumin and plasma protein binding of diazepam, salicyclic acid, and digitoxin in healthy subjects and patients with renal impairment. Br J Clin Pharmacol 1992 Mar; 33(3):299-304

105.   Greenblatt DJ, Allen MD, Harmatz JS, Shader RI.   Diazepam disposition determinants. Clin Pharmacol Ther 1980; 27 :302-312

106.   Verbeeck RK, De Schepper PJ. Influence of chronic renal failure and hemodialysis on diflunisal plasma protein binding. Clin Pharmacol Ther 1980; 27: 628-635

107.   Bonde J, Pedersen LE, Bødtker S, et al. The influence of age and smoking on the elimination of disopyramide. B J Clin Pharmacol 1985 Nov; 20(5): 453-58

108.   Ohnishi A, Mihara M, Kamakura H, et al. Comparsion of the pharmacokinetics of E2020, a new compound for alzheimer's disease, in healthy young and elderly subjects. J Clin Pharmacol 1993 Nov; 33(11): 1086-91

109.   Scatina J, Hicks D, Kraml M, et al . Etodalac kinetics in the elderly. Clin Pharmacol Ther 1986 May; 39(5): 550-53

110. Carlos R, Calvo R, Erill S. Plasma protein binding of etomidate in different age groups and in patients with chronic respiratory insufficiency. Int J Clin Pharmacol Ther Toxicol 1981 Apr; 19(4): 171-174

111. Greenblatt DJ, Divoll M, Harmatz JS, et al. Kinetics and clinical effects of flurazepam in young and elderly noninsomniacs. Clin Pharmacol Ther 1981 Oct; 30(4): 475-486

112. Andreasen F, Hansen U, Husted SE, et al. The pharmacokinetics of frusemide are influenced by age. Br J Clin Pharmacol 1983 Oct; 16(4): 391-397

113. Rowell FJ, Hui SM, Fairburn AF, Eccleston D. Total and free serum haloperiol levels in schizophrenic patients and the effect of age, thioridazine and fatty acids on haloperidol serum protein binding in vitro. Br J Clin Pharmacol 1981 Apr; 11(4): 377-382

114. Rudy AC, Knight PM, Brater DC,et al. Enantioselective disposition of ibuprofen on elderly persons with and without renal impairment. J Pharmacol Exp Ther 1995 Apr; 273(1): 88-93

115. Cusack B, O'Malley K, Lavan J, Noel J, Kelly JG. Protein binding and disposition of lignocaine in the elderly. Eur J Clin Pharmacol 1985; 29(3): 323-9

116. Divoll M, Greenblatt DJ. Effect of age and sex on lorazepam protein binding. J Pharm Pharmacol 1982 Feb; 34 (2): 122-123

117. Norbury HM, Franklin RA, Graham DF. Pharmacokinetics of the new analgesic, meptazinol after oral and intravenous administration to volunteers. Eur J Clin Pharmacol 1983; 25(1): 77-80

118. Norbury HM, Franklin RA, Grahma DF, et al. Pharmacokinetics of meptazinol after single and multiple oral administration to elderly patients. Eur J Clin Pharmacol 1984; 27: 223-226

119. Mather LE, Tucker GT, Pflug AE, et al. Meperidine kinetics in man. Intravenous injection in surgical patients and volunteers Clin Pharmacol Ther 1975 Jan; 17(1): 21-30

120. Holmberg L, Odar-Cederlöft I, Nilsson JLG, Ehrnebo M, Boréus LO. Pethidine binding to blood cells and plasma proteins in old and young subjects. Eur J Clin Pharmacol 1982; 22(2): 457-461

121. Edno L, Bressolle F, Gomeni R, et al. Total and free methotrexate pharmacokinetics in rheumatoid arthritis patients. Ther Drug Monit 1996; 18:128-134

122. Rigby JW, Scott AK, Hawksworth GM, Petrie JC. A comparsion of atenolol, metoprolol, oxprenolol and propranolol in elderly hypertensive and young subjects. Br J Clin Pharmacol 1985 Oct; 20(4): 327-31

123. Greenblatt DJ, Abernathy DR, Locniskar A, Harmatz JS, Limjuco RA, et al. Effect of age, gender, and obesity on midazolam kinetics. Anesthesiology 1984 Jul; 61(1): 27-45

124. Cook CS, Schoenhard GL, Karim A. Effect of salicylic acid on the plasma protein binding and the misoprostol acid. J Pharm Sci 1994 Jun; 83(6): 883-6

125. Upton RA, Williams RL, Kelly J, et al. Naproxen pharmacokinetics in the elderly. Br J Clin Pharmacol 1984; 18: 207-14

126. Sugioka N, Koyama H, Kawakubo M, et al. Age-dependent Alteration of the Serum-unbound Fraction of Nicardipine, a Calicum-channel Blocker, in Man. J Pharm Pharmacol 1996 Dec; 48(12) :1327-31

127. Jochemsen R, Van Beusekom Br, Spoelstra P,et al. Effect of age and liver cirrhosis on the pharmacokinetics of nitrazepam. Br J Clin Pharmacol 1983 Mar; 15(3): 295-302

128. Greenblatt DJ, Abernathy DR, Locniskar A, et al. Age, sex and nitrazepam kinetics: relation to antipyrine disposition. Clin Pharmacol Ther 1985 Dec; 38 (6): 697-703

129. Greenblatt DJ, Matlis R, Scavone JM, Blyden GT, Harmatz JS, et al. Oxaprozin kinetics in the elderly. Br J Clin Pharmacol 1985 Jan; 19(1):373-378

130. Karim A, Noveck R, McMahon G, et al. Oxaprozin and piroxicam, nonsteriodal anti-inflammatory drugs with long half-lives: effect of protein binding differences on steady state pharmacokinetics. J Clin Pharmacol 1997 Apr; 37(4): 267-78

131. Bender AD, Post A, Meier JP, et al. Plasma protein binding of drugs as a function of age. J Pharm Sci 1975 Oct; 64(10): 1711-3

132. Patterson M, Heazelwood R, Smithhurst B,et al . Plasma protein binding of phenytoin in the aged: in vivo studies. Br J Clin Pharmacol 1982 Mar; 13(3):423-5

133. Richardson CJ, Blocka KLN, Ross SG, et al. Effect of age and sex on piroxicam disposition. Clin Pharmacol Ther 1985 Jan; 37(1): 13-8

134. Trovik TS, Jaeger R, Jorde R, et al. Plasma protein binding of catecholamines, prazosin and propranolol in diabetes mellitus. Eur J Clin Pharmacol 1992; 43(3):265-268

135. Andros E, Detmar-Hanna D, Suteparuk S, et al. The effect of aging on the pharmacokinetics and pharmacodynamics of prazosin. Eur J Clin Pharmacol 1996; 50: 41-46

136. Tenero DM, Bottorff MB, Burlew BS. Altered beta-adrenergic sensitivity and protein binding to 1-propranolol in the elderly. J Cardiovasc Pharmacol 1990 Nov; 16(5) 702-7

137. Gilmore DA, Gal J, Gerber JG, et al. Age and gender influence the stereoselective pharmacokinetics of propranolol. J Pharmacol Exp Ther 1992 Jun; 261(3): 1181-6

138. Bendayan R, Pieper JA, Stewart RB,et al. Influence of age on serum protein binding of propranolol. Eur J Clin Pharmacol 1984; 26 (2): 251-254

139. Colagelo P et al. Stereoselective binding of propranolol in the elderly. Clin Pharmacol Ther 1989; 27:519-22

140. Barber HE, Hawksworth GM, Petrie JC,et al. Pharmacokinetics of atenolol and propranolol in young and elderly subjects. Br J Clin Pharmacol 1981; 11: 118P-119P,

141. Ochs HR, Greenblatt DJ, Woo E, et al. Reduced quinidine clearance in elderly persons. Am J Cardiol 1978 Sep; 42(3):481-485

142. Snoeck E, Van Peer A, Sack M, et al. Influence of age, renal and liver impairment on the pharmacokinetics of risperidone in man. Psychopharmacology 1995; 122:223-229

143. Roberts MS, Rumble RH, Wanwimolruk S, et al. Pharmacokinetics of aspirin and salicylate in elderly subjects and in patients with alcoholic liver disease. Eur J Clin Pharmacol 1983; 25(2): 253-261

144. Lesko LJ, Narang PK, Yeager L, et al. Salicylate protein binding in serum from young and elderly subjects as measured by diafiltration. Eur J Clin Pharmacol 1985; 28(3):339-345

145. Carr RA, Pasutto FM, Lewanczuk RZ ,et al. Protein binding of sotalol enantiomers in young and elderly human and rat serum using ultrafiltration. Biopharm Drug Dispos. 1995 Nov; 16(8): 705-712.

146. Varoquaux O, LaJoie D, Gobart C, et al. Pharmacokinetics of the trimethoprim-sulphamethoxazole combination in the elderly. Br J Clin Pharmacol 1985 Dec; 20(6): 575-581

147. Caldwell JR, Kirby DS, Gardner MJ,et al. The effects of age and gender on the pharmocokinetics of tenidap sodium in patients with rheumatoid arthritis and osteoarthritis. Br J Clin Pharmacol 1995; 39(1 Suppl): 3S-9S.

148. Shin SG, Juan D, Rammohan M. Theophylline pharmacokinetics in normal elderly subjects. Clin Pharmacol Ther 1988 Nov; 44 (5): 522-30

149. Antal EJ, Kramer PA, Mercik SA, et al. Theophylline pharmacokinetics in advanced age. Br J Clin Pharmcol 1981; 12(5) 637-45

150. Lesko LJ, Tabor KJ, Johnson BF. Theophylline serum protein binding in obstructive airways disease. Clin Pharmacol Ther 1981 Jun; 29(6): 776-781

151. Nilsen OG, Storhaug A, Dale O. Binding of tiaprofenic acid to human sera and isolated serum proteins. Arzneimettelforschung 1985; 35(6); 984-7

152. Greenblatt DJ, Divoll M, Abernathy DR, et al. Reduced clearance of triazolam in old age: relation to antipyrine oxidizing capacity. Br J Clin Pharmacol 1983 Mar; 15(3): 303- 309

153. Perucca E, Grimaldi R, Gotti G, et al. Pharmacokinetics of valproic acid in the elderly.Br J Clin Pharmacol 1984 Jun; 17(6): 665-669

154. Bauer LA, Davis R, Wilensky A, et al. Valproic acid clearance: unbound fraction and diurnal variation in young and elderly adults. Clin Pharmacol Ther 1985 Jun; 37(6): 697-700

155. Cutler NR, Narang PK, Lesko LJ,et al. Vancomycin disposition: the importance of age.Clin Pharmacol Ther 1984; 36: 803-809

156. Wood DM, Ford JM, Roberts CJC. Variability in the plasma protein binding of velnacrine: a potential agent for Alzheimer's disease. Eur J Clin Pharmacol 1996; 50: 115-119

157. Schwartz JB, Capili H, Daugherty J. Aging of women alters s-verapamil pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 1994; 509-517

158. Shepherd AMM, Hewick DS, Moreland TA, Stevenson IH. Age as a determinant of sensitivity to warfarin. Br J Clin Pharmacol 1977 Jun; 4(3): 315-320

159. Braeckman RA, Granneman GR, Locke CS, et al. The pharmacokinetics of zileuton in healthy young and elderly volunteers. Clin Pharmacokinet 1995; 29: 42-48

160. Van Den Ouweland FA, Jansen PAF, Tan Y, et al. Pharmacokinetics of high-dosage naproxen in elderly patients. Int J Clin Pharmacol Ther Toxicol 1988; 26(3): 143- 147

161. Hundal Ø, Rugstad HE, Husby G. Naproxen free plasma concentrations and unbound fractions in patients with osteoarthritis: relation to age, sex, efficacy and adverse events. Ther Drug Monit 1991; 13: 478-484

162. Satterwhite JH, Boudinot FD. Age- and dose-dependent naproxen disposition in Fischer 334 rats. J Gerontol 1991; 46(6): B222-227

163. Satterwhite JH, Boudinot FD. Effects of age on the pharmacodynamics of naproxen in the rat. Exp Gerontol 1995; 30(5):505-515

Drug	Drug Classification	Indication In Study	Fraction Unbound in the Young	Fraction Unbound in the Elderly	Change in Binding	Reference
Acebutolol	Cardiovascular	Healthy	R -0.840 S -0.860	R-0.930 S-0.930	↔	80
Acetazolamide	Diuretic	Healthy	0.041	0.069	↑	81
		Glaucoma		0.098		82
Alfentanil	Analgesic	Gastrointestinal Surgery	0.093	0.093	↔	83

		Various	0.05-0.11	0.05-0.12	↔	84
Alprazolam	Central Nervous System	Healthy	0.286 M 0.293 F	0.306 M 0.289 F	↔ ↔	85
Amitriptyline	Central Nervous System	Healthy	0.052	0.044	↔	86
Batanopride	Anti- Emetic	Healthy Renal impairment		0.046 0.035		87
Benazepril	Cardiovascular	Healthy		0.053		88
Benazeprilat	Cardiovascular	Healthy	0.083	0.106	↑	89
Canrenone	Diuretic	Healthy	0.050	0.060	↔	90
Caffeine	Stimulant	Healthy	0.646	0.650	↔	91

Cefonicid	Anti-Infective	Bacterial pneumonia		0.090		92
		Healthy	0.020-0.176			93
Ceftriaxone	Anti-Infective	Healthy	0.136-0.114	0.146-0.157	↑	94
Chlodriazepoxide	Hypnotic, sedative	Cirrhosis Acute Viral Hepatits	0.350	0.035	↔	95
Chloropromazine	Anti-psychotic	Healthy Male Healthy Female			↓ ↔	96
Chloromethiazole	Hypnotic,	Healthy	0.308	0.403	↑	97

	Sedative					
Chloroquine	Anti-malarial	Healthy	0.390	0.390	↔	98
		Rheumatoid Arthritis	0.360	0.360	↔	
		Rheumatoid Arthritis	0.422	0.422	↔	99
Cladribine	Anti-neoplastic	Healthy	0.789		↔	100
		Hematological Malignancies		0.750		
Desipramine	Anti-depressant	Healthy Male	0.243	0.263	↔	101
		Healthy Female	0.269	0.247		
Desmethyldiazepam	Hynotic and	Healthy Male	0.0296	0.0293	↔	102
	Sedative	Healthy Female	0.0267	0.0309		
		Healthy Male	0.0257	0.0326	↑	103

					↔	
		Healthy Female	0.0342	0.0308		
Diazepam	Hypnotic,	Healthy	0.023	0.047	↑	
	Sedative	Renal Impairment	0.042	0.044	↔	104
		Haemodialysis	0.046	0.054	↑	
		Healthy Male	0.0125	0.0172	↑	105
		Healthy Female	0.0134	0.0166	↔	
Diflunisal	Non-steroid anti-	Healthy	0.0012	0.0019	↑	106
	inflammatory					
Digitoxin	Cardiovascular	Healthy	0.041	0.045	↔	104
		Renal Impairment	0.046	0.047	↔	
		Haemodialysis	0.052	0.059	↔	
Disopryramide	Antiarrhythmic	Healthy	0.300			107

		Patients		0.030	↔	
Donepezil	Anti-infective	Healthy	0.074			108
		Hypertension		0.061	↔	
Enalaprilat	Cardiovascular	Healthy	0.746	0.864	↑	89
Etodolac	Anti-inflammatory	Healthy	RS - 0.970			109
		Osteoarthritis		RS - 1.02	↔	
Etomidate	Hypotic and Sedative	Healthy	0.249	0.439	↑	110
Fluazepam	Hypotic and Sedative	Healthy	0.031	0.037	↑	111
Frusemide	Diuretic	Healthy	0.140	0.140	↔	112

Haloperidol	Anti-psychotic	Healthy	0.095	0.085	↔	113
Ibuprofen	Anti-inflammatory	Healthy	S - 0.0059	S -0 .0078	↑	114
			R- 0.0039	R - 0.0040	↔	
		Renal Impairment		S - 0.0086	↑	
				R - 0.0042	↔	
Imipramine	Anti-depressant	Healthy Male	0.145	0.144	↔	101
		Healthy Female	0.136	0.133		
Lidocaine	Antiarrhythmic	Healthy	0.519	0.305	↓	115
Lorazepam	Hypnotic, Sedative	Healthy	0.107	0.115	↑	116
Meptazinol	Narcotic Analgesic	Healthy	0.729	0.662	↓	117,118

Merperidine	Analgesic				↑	119
		Healthy	0.480	0.450	↔	120
Methotrexate	Anti-inflammatory	Rheumatoid Arthritis	0.430-0.800	0.430-0.800	↔	121
Metoprolol	β Blocker	Healthy	0.850		↔	122
		Mild Hypertension		0.850		
Midazolam	Hypnotic, Sedative	Healthy Male	0.9626	0.9650	↔	123
		Healthy Female	0.9624	0.9630		
Misoprostol Acid	Gastrointestinal And Liver	Healthy	0.130 - 0.177	0.122 - 0.179	↔	124
Naproxen	Anti-inflammatory	Healthy	0.00084-0.0023	0.0017-0.0051	↑	125

Nicardipine	Cardiovascular	Healthy	0.005-0.017	0.005-0.041	↑	126
Nitrazepam	Hypnotic, Sedative	Healthy	0.130	0.139	↑	127
		Healthy Male	0.178	0.189	↔	128
		Healthy Female	0.179	0.190		
Oxaprozin	Anti-inflammatory	Healthy			↔	129
		Healthy	0.001			130
		Osteoarthritis		0.0012	↔	
Oxprenolol	β-Blockers	Healthy	0.190		↔	122
		Mild Hypertension		0.160		

Phenobaritone	Hypnotic, Sedative	Healthy	0.582	0.582	↔	131
Phenytoin	Anticonvulsant	Healthy	0.176	0.164	↔	131
			0.111	0.125	↑	132
Piroxicam	Analgesic	Healthy	0.0078			130
		Osteoarthritis		0.0072	↔	
		Males	0.0069	0.0077	↔	133
		Females	0.0079	0.0066	↔	
Prazosin	Cardiovascular	Healthy	0.089		↓	134
		Type I diabetes mellitus	0.099			
					↓	
		Type II diabetes mellitus		0.096		
		Healthy	0.065	0.066	↔	135

Propranolol	β Blocker	Healthy	0.122		↓	134
		Type I diabetes mellitus	0.143		↔	
		Type II diabetes mellitus		0.133		
		Healthy	0.147	0.121	↔	136
		Healthy	R - 0.180	R - 0.186	↔	137
			S - 0.117	S - 0.121	↔	
		Healthy	0.059	0.079	↔	138
		Healthy	0.159	0.148	↔	139
		Healthy	0.133	0.113	↔	14
		Healthy	0.120	0.100	↔	140
Quinidine	Anti-arrythmic	Healthy	0.246	0.282	↔	141
Risperidone	Anit-psychotic	Healthy	0.159	0.141	↔	142
Salicyclic Acid	Anti-	Healthy	0.196	0.309	↑	104

	inflammatory	Renal Impairment	0.260	0.293	↔	
		Haemodialysis	0.342	0.363	↔	
		Healthy	0.075	0.093	↔	143
		Healthy	0.090	0.250	↑	144
Sotalol	Cardiovascular	Healthy	S-0.958-0.964	S-0.935-0.954	↔	145
			R- 0.958-0962	R-0.937-0.955	↔	
Sulphamethoxazole	Anti-Bacterial	Urinary Infection	0.398	0.399	↔	146
		Respiratory Infection				
Tenidap Sodium	Anti-inflammatory	Rhematoid Arthritis	0.240	0.310	↔	147
		Osteoarthritis	0.270	0.240	↔	

Theophylline	Respiratory	Healthy	0.620	0.780	↑	148
		Healthy	0.310	0.380	↑	149
		Asthmatics	0.357	0.430	↑	150
Tiaprofenic Acid	Anti-inflammatory	Healthy	0.010	0.010	↔	151
Triazolam	Hypnotic, Sedative	Healthy Male	0.213	0.247	↑	152
		Healthy Female	0.229	0.228	↔	
Trimethoprim	Anti-bacterial	Urinary Infection Respiratory Infection	0.500	0.509	↔	146
Valproic Acid	Anti-convulsant	Healthy	0.066	0.095	↑	153
		Healthy	0.061	0.097	↑	154

Vanomycin	Anti-infective	Healthy	0.480	0.440	↔	155
Velnacine	Cardiovascular	Healthy	0.452	0.481	↔	156
Verapamil	Cardiovascular	Healthy Females	0.090	0.100	↔	157
Warfarin	Anti-coagulant	Healthy	0.014	0.015	↔	158
Zileuton	Respiratory	Healthy Males	0.071	0.067	↔	159
		Healthy Females	0.074	0.730	↔	

Figure Legends

Figure 5.1:   Three dimensional plot showing the relationship between the fraction unbound as a function of protein concentration and free drug concentration for a drug with a equilibrium association, K_A, value of 10^6 M^{-1}.

Figure 5.2:   Three dimensional plot showing the relationship between the fraction unbound as a function of protein concentration and free drug concentration for a drug with a equilibrium association, K_A, value of 10^3 M^{-1}.

Figure 5.3:   Fraction of drug unbound as a function of protein concentration for a drug with a high equilibrium association constant of 10^6 M at a (A) low drug concentration (10^{-6} M) and a (B) moderate drug concentration (10^{-3} M) and for a drug with a low equilibrium association constant of 10^3 M at a (C) low drug concentration (10^{-6} M) and a (D) moderate drug concentration (10^{-3} M).

Figure 5.4:   Three dimensional plot showing the relationship between hepatic clearance as a function of fraction unbound and intrinsic clearance. A hepatic blood flow of 1.5 L/min was used for the calculations.

Figure 5.5:   Three dimensional plot showing the relationship between free steady state concentrations and total steady state concentrations as a function of intrinsic clearance and fraction unbound. A zero-order infusion rate of 0.8 mg/h and a hepatic blood flow of 1.5 L/min was used for the calculations.
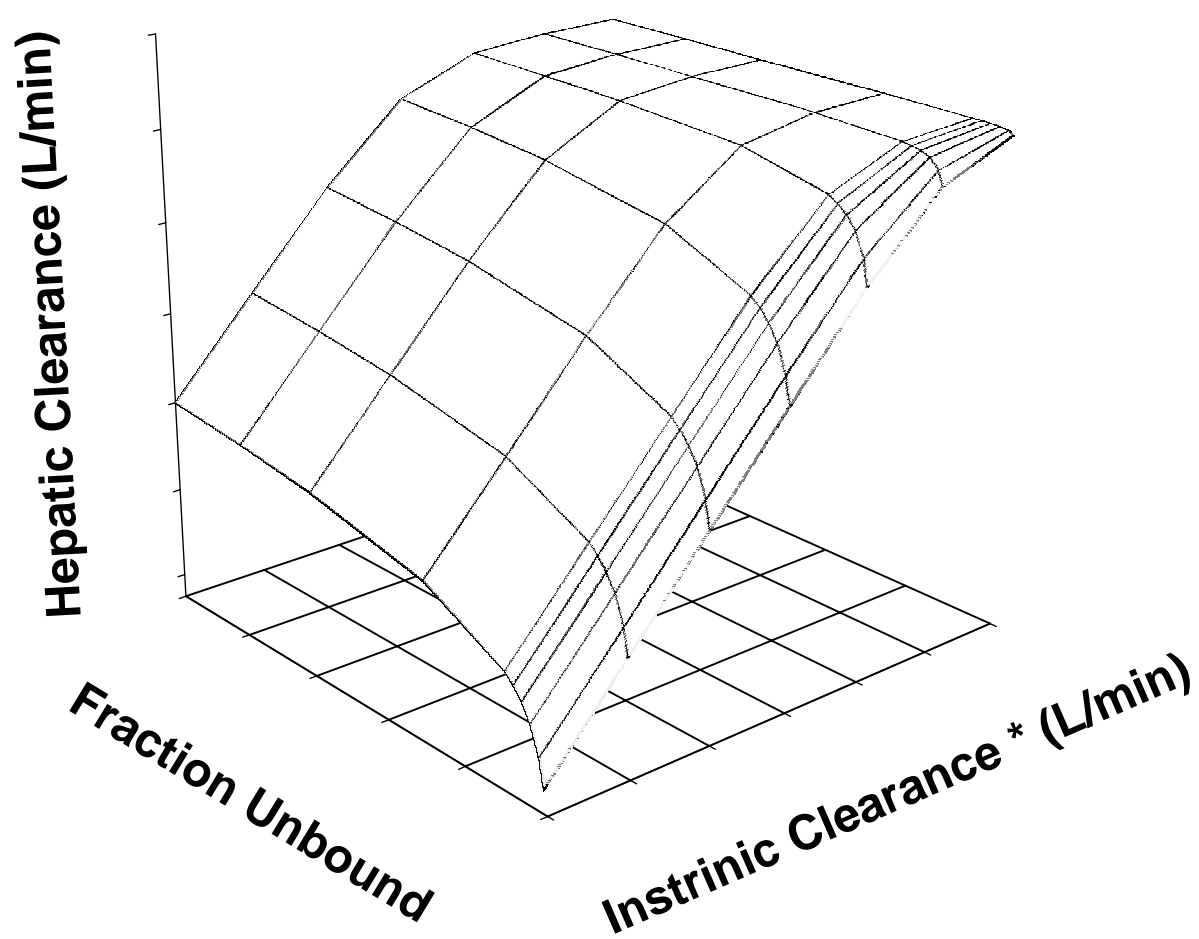
Figure 5.1.

Figure 5.2.

Protein Concentrations
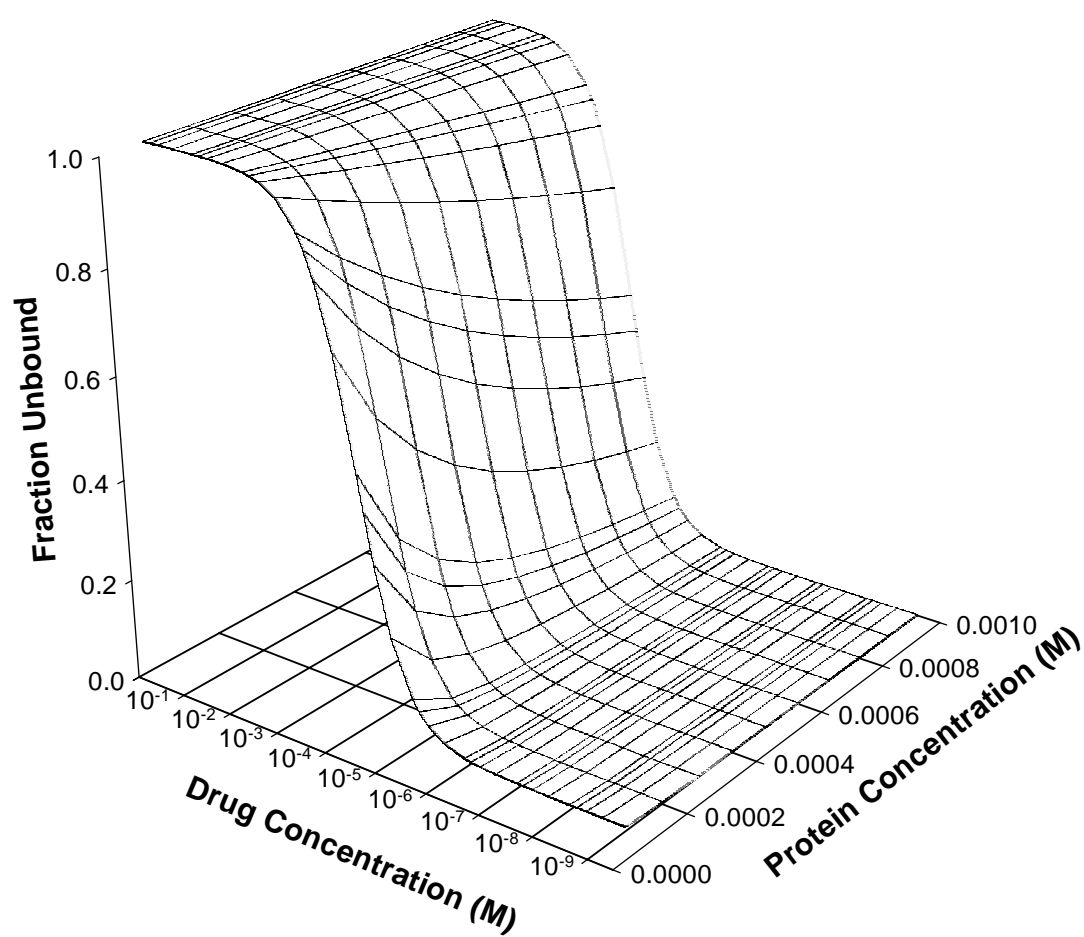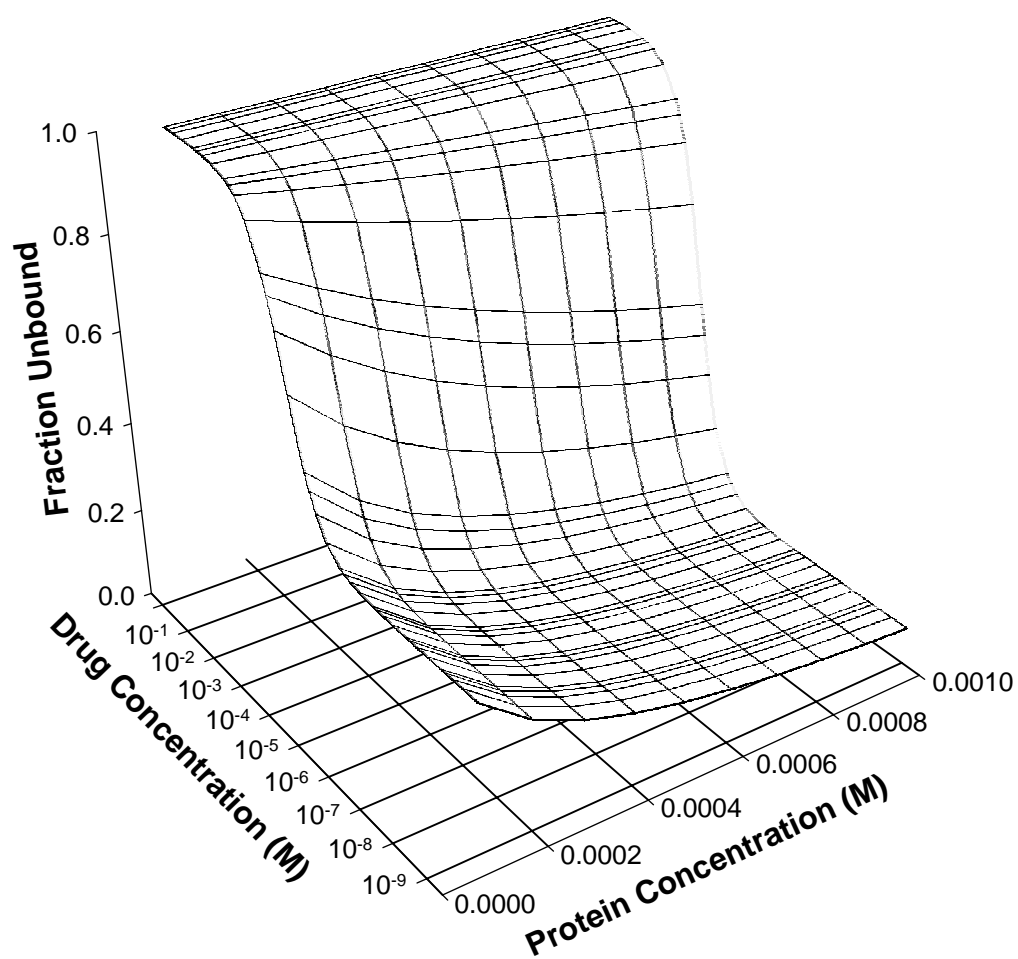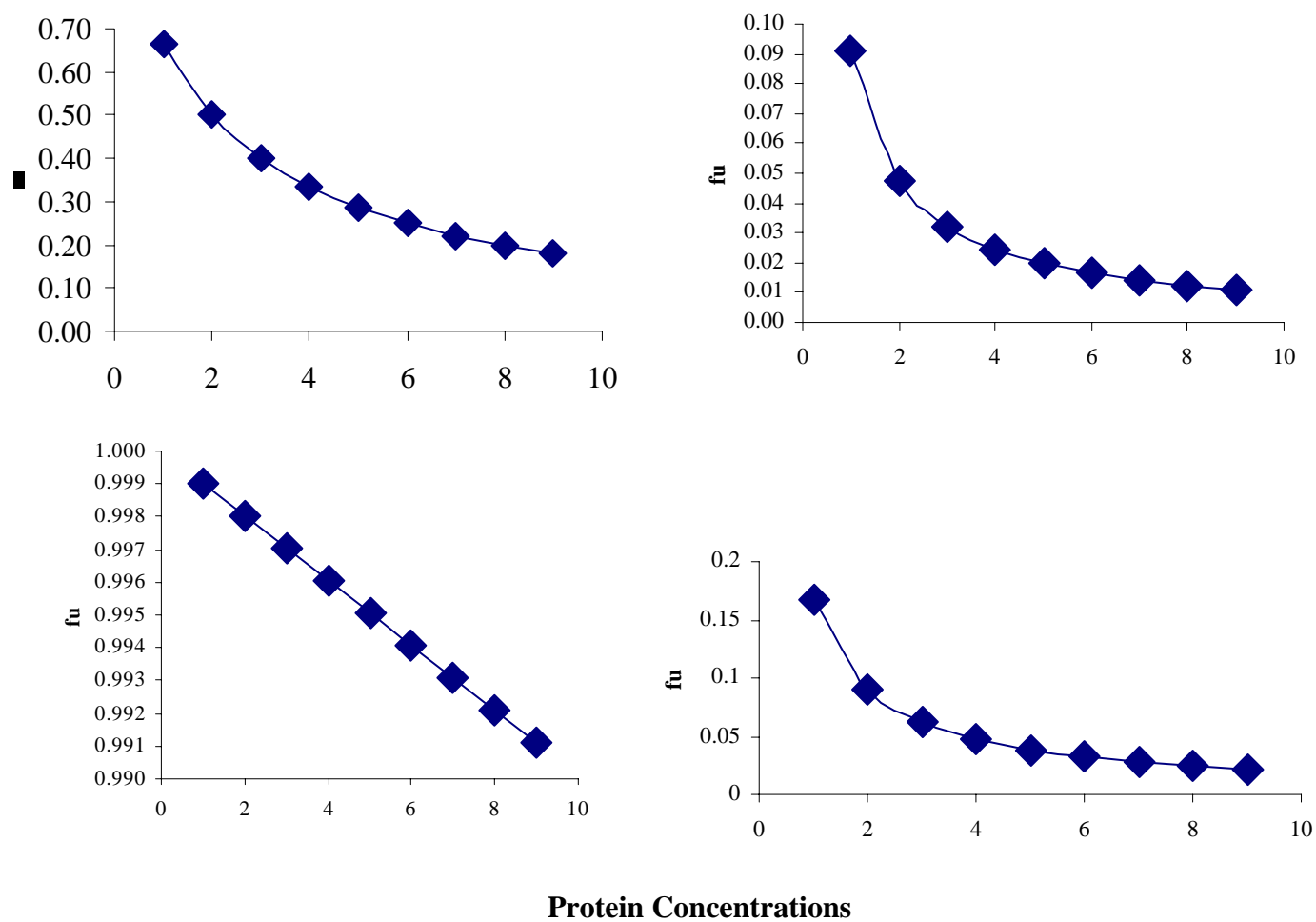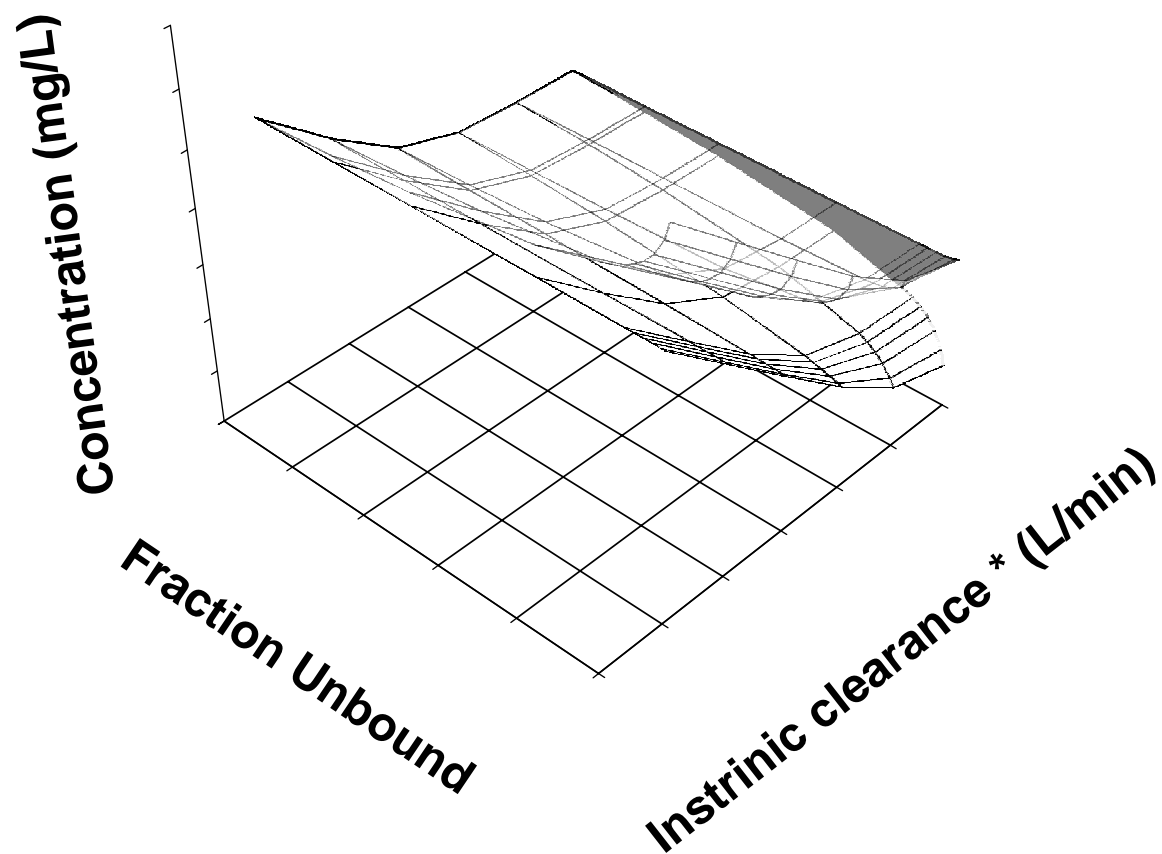
Figure 5.3.

Figure 5.4

Figure 5.5

CHAPTER 6

CONCLUSIONS

The novel anti-tumor agent suramin was evaluated as a treatment for brain cancer. The protein binding of suramin and a novel cardioactive agent was investigated. The influence of age-related alterations in protein binding on the therapeutics of drug was also investigated.

1.   **Clinical Pharmacokinetics of Suramin in Brain Cancer Patients**

Suramin is a symmetric polysulfonated naphthylurea that was developed as a treatment for the parasitic condition trypanosomiasis. Suramin was also used as an anti-filerial agent in the treatment of onchocerciasis. Because suramin inhibits reverse transcriptase it has been investigated as a treatment for human immunodeficiency virus (HIV) infection   Although clinical data showed that suramin was not an effective treatment for HIV, it was observed that an HIV infected patient who had Kaposi's sarcoma and small-cleaved cell lymphoma showed regression of both malignancies. As a consequence of this unexpected finding, suramin has been studied as an anti-tumor agent for a number of neoplastic conditions including hormone-refractory prostate cancer and renal cell carcinoma. In the study to determine if the pharmacokinetic parameters were similar in brain cancer those parameters found in prostate cancer and renal cell carcinoma, the following findings were made. The data was best described by a two compartment model, with elimination from the central compartment with gender as a covariates. Volume of distribution was estimated to be 4.16 L/hr/m^2 (0.31) in male patients and in 4.16 L/hr/m^2 (0.23) females in the first compartment. Volume of distribution in the second compartment was 21.2 (2.26) in males and 12.6 (3.8) in females. Elimination was from the central compartment . The results of this study

showed that the pharmacokinetic parameters of suramin in brain patients was similar

those observed in studies examining use of suramin in prostate cancer. The

study also showed that using a model that included gender as a covariate decreased the

amount of inter- and intra-subject variability.

2.   **Protein Binding of Suramin to Albumin and α_1-acid glycoprotein**

The purpose of this study was to further characterize the plasma protein

binding of suramin. Plasma protein studies were performed utilizing equilibrium dialysis

to investigate the *in vitro* binding of suramin to human serum albumin, α_1-acid

glycoprotein, and human plasma serum over wide range of drug concentrations. Suramin

binds to albumin to two classes of binding sites, a high affinity saturable site and a low-

affinity nonsaturable site (N_1=3.5, K_1=1.8 x 10^4 M^{-1}, N_2K_2=3.7 x 10^3 M^{-1}). Suramin binds

to a single low-affinity nonsaturable site on α_1-acid glycoprotein (N_3K_3=1.5 x 10^5 M^{-1}).

The fraction of suramin bound to plasma proteins predicted from the *in vitro* binding to

human serum albumin and α_1-acid glycoprotein was identical to that observed in human

plasma (95% $\pm$ 0.015). Thus, the plasma protein binding of suramin can be accounted for

by the binding to these two proteins.

3.   **The Protein Binding of Drug B**

Drug B is a newly synthesized compound currently being investigated as an agent

against cardiovascular disease. The plasma protein binding of this drug was evaluated

using equilibrium dialysis and carbon-14 labeled Drug B. At equilibrium the percent of

labeled Drug B bound by human plasma proteins was 95.0 $\pm$ 1.4 (mean $\pm$ SD) over the

period of 16 to 24 h. Binding of Drug B to human plasma proteins was independent of

drug concentration over the concentration range of 20 to 10,000 ng/mL. The percent

bound was 95.6 $\pm$ 1.3 % and the percent free was 4.4 $\pm$ 1.3%. The percent of Drug B

bound to rat, dog, and monkey plasma proteins was 91.6 $\pm$ 1.0 %, 93.5 $\pm$ 0.6 %, and 94.6

$\pm$ 0.6 %, respectively. The binding of Drug B to albumin was independent of

concentration over the concentration of 20 to 10,000 ng/mL. The average percent of Drug B bound to albumin was $94.9 \pm 0.3\%$. The binding of Drug B to α_1- acid glycoprotein was negligible (0.6 ± 1.2).

4. **Age Related Changes in Plasma Protein Binding**

In an effort to understand the effect that the aging process has on the protein binding and clinical outcomes. An extensively review was undertaken to illustrate the potential differences in pharmacokinetics due increases in age. Alterations in plasma protein binding that occur in the elderly are generally not attributed to age, rather physiological and pathophysiological changes or disease states, which may occur more frequently in the elderly, most often account for altered protein binding. Age-related physiological changes such as decreased renal function, decreased hepatic function and decreased cardiac output generally produce more clinically significant alterations in drug disposition than do alterations in drug plasma protein binding. An understanding of the inter-relationships between drug concentrations, protein binding, the physiology of aging, disease, pharmacokinetics and pharmacodynamics is necessary for effective therapeutic monitoring. Monitoring of unbound drug concentrations simplifies these relationships and provides the fundamental information needed for dosage regimen development and adjustment. Drug therapy in the elderly should be individualized taking into account all of these factors.

In conclusion, the extensive protein binding of suramin affects pharmacokinetics of suramin by extending it's half-life, impairing elimination, and limiting the amount free for interaction with therapeutic sites.