OPTIMIZATION OF CANCER CHEMOTHERAPY: LOCAL DELIVERY OF PACLITAXEL AND PHARMACOKINETICS OF SURAMIN

DISSERTATION

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ABSTRACT

Cancer remains a major public health problem in the United States and in other developed countries. One in four deaths in the United States is caused by cancer. Although our knowledge in tumor biology has exploded during the last decades, it has not been accordingly translated into new substantially better anticancer drugs. It has been reported that only 12 among a total of 209 investigational approaches or drugs showed statistically significant benefit from January 1996 to October 2002. Besides the heterogeneity of tumors and the redundant signaling pathways for the tumor to evade the drug’s toxic effect, drug delivery and dosing regimen compose two major limitations for anticancer drug efficacy. This dissertation is focused on these two aspects to optimize cancer chemosensitization.

An ideal formulation for chemotherapy would be able to deliver high concentration of drug to the tumor while sparing other organs. This formulation would especially be preferred in the treatment of prostate cancer. Prostate cancer is the most common malignancy in man, with estimated 230,110 new cancer cases and 29,900 deaths in 2004. Over the past decade, the development of diagnosis techniques has resulted in a stage migration. A retrospective study shows from 1992 to 1998, 82% of newly diagnosed cases were localized. Current treatment options for localized prostate cancer are accompanied by side effects such as impotence and incontinence. These side effects can compromise a patient’s quality of life. The work presented in this
dissertation reflects a multidisciplinary approach towards the development and study of regional prostate cancer chemotherapy. Formulation studies (Chapter 2) were performed using biodegradable polymers. A Poly(lactide-co-glycolide) cylindrical matrix containing paclitaxel was developed for localized prostate cancer chemotherapy. The macro-porous PLGA matrix was used to increase paclitaxel release. The formulation was evaluated in PC-3 tumor bearing mice (Chapter 3). Results showed the formulation could inhibit tumor growth, while paclitaxel penetration was limited. And host toxicity was monitored by body weight change. No long-term toxicity was observed.

Traditionally, the Maximum Tolerated Dose paradigm has been employed to treat cancer patients. However, our lab has found an unconventional dose-response relationships of a grow factor inhibitor, suramin. The preclinical in vitro and in vivo studies showed that suramin reversed FGF-induced chemoresistance in a narrow therapeutic range from ~10-50 µM, with antagonism occurring above this concentration. Hence, a good understanding of the pharmacokinetics of suramin is important for the successful performance of preclinical studies in rodents, and clinical studies in animal and human patients. We studied the interspecies pharmacokinetics of suramin in mice, rats, dogs, and humans (Chapter 4) The parameters of interest were correlated across species as a function of bodyweight using the allometric equation. The steady-state volume of distribution (Vdss), total body clearance (CL), product of clearance and Maximum Life Potential (CL*MLP), and Area Under the Curve (AUC) correlated well across species. The dienetichron plot, an allometric scaling plot, which transforms clock time and absolute physical parameters into forms that become species
invariant, was used to predict the suramin concentration profile in human patients and the simulation was validated using data from our phase I study in Non-Small Cell Lung Cancer patients. A Phase I trial evaluated the effectiveness of low dose suramin in pet dogs with naturally occurring tumors (Chapter 5). Twenty-one dogs with late stage carcinomas, soft tissue sarcomas, or lymphoma were treated with suramin in combination with doxorubicin. The suramin pharmacokinetic data were analyzed using a nonlinear mixed-effect model (NONMEM) with a one-compartment model in order to identify the target suramin dose. An empirical equation to calculate dosage for target suramin concentration was developed from the data of first fourteen patients and validated by the later seven dogs. For a better understanding of suramin pharmacodynamics, tissue distribution of low-dose suramin was studied on s.c. tumor-bearing mice (Chapter 6). Suramin was found to accumulate in tissues at various levels. The highest concentration level was observed in kidneys and the lowest in brains. The tissue PK of suramin might be determined by both a shallow compartment and a deep compartment. A local physiologically based pharmacokinetic model was proposed for the tumors.
DEDICATION

Dedicated to my loving parents, parents in-law
& My dearest husband
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CHAPTER 1

INTRODUCTION

Cancer remains a major public health problem in the United States and in other developed countries. A total of 1,368,030 new cancer cases and 563,700 deaths are expected in the United States in 2004 (American Cancer Society, 2004). One in four deaths in the United States is caused by cancer. From 1975 to 2000, there was no significant decrease in cancer death rates, although much effort and money have been put in cancer research area. On the other hand, our knowledge in tumor biology has exploded during the last decades thanks to the investment. Unfortunately, it has not been accordingly translated into new substantially better anticancer drugs. Based on the search of the databases Medline and CANCERLIT from January 1996 to October 2002, and hand-search of 13,392 abstracts from the 2001 and 2002 proceedings of five major cancer conferences, a total of 209 drugs or approaches, categorized into eight groups by their mechanisms, were identified as in the clinical trials. For 28 drugs/approaches survival data were available from randomized controlled trials. Statistically significant benefit was observed for only 12 (Nygren and Larsson, 2003). A major problem in the development of anticancer drugs seems to be the challenging step from promising findings in preclinical models to the outcome of clinical trials. How to translate data from bench to bed is crucial for the clinical outcomes. In this
dissertation, we focus on approaches to relate the preclinical findings in the animal models to human patients, thereby to optimize the cancer chemotherapy. Chapters 2 and 3 focus on the optimization for localized prostate cancer. Chapter 4, 5 and 6 focus on optimization of the dose regimen for suramin, a chemosensitizer, targeting at both localized disease and metastatic disease. This chapter covers background information for prostate gland and prostate cancer, drug delivery carrier, and paclitaxel, followed by the discovery of suramin as a chemosensitizer, interspecies scaling and population pharmacokinetic analysis. In the end, an overview of the whole dissertation is provided.

Prostate cancer is the most common malignancy in man. Over the past decade, substantial improvements in diagnosis and staging of the disease have been made with the combined use of digital rectal examination, measurement of serum PSA levels, and transrectal ultrasound. Earlier diagnosis of prostate cancer has resulted in a stage migration, with an increased proportion of men diagnosed with organ-confined disease (Gleave et al., 1996; Stanford et al., 1996). Significant morbidity can result from local tumor progression, including bladder outlet and ureteral obstruction, pain, and bleeding. The current treatment options for localized prostate cancer include radical prostatectomy, radiotherapy and cryotherapy. These options are associated with low but significant morbidities, such as urinary incontinence, impotence, cystitis, and proctitis etc. Androgen ablation therapy is used with significant success. However, tumor recurrence after androgen ablation is common and the recurrent tumors are often hormone refractory. Therefore, effective nonsurgical treatments that
can eradicate the localized tumors in the prostate represent an attractive alternative treatment. A biodegradable implant at the site of the action, which releases the drug locally at a controlled rate for a decided period of time would provide high local concentrations and also minimize the potential of side effects and toxicity (Domb et al., 1993; Friend and Pangburn, 1987; Poznansky and Juliano, 1984). Paclitaxel has shown clinical efficacy against ovarian cancer, breast cancer, head and neck cancers and non-small cell lung cancer (Horwitz, 1992a; Rowinsky and Donehower, 1995; Spencer and Faulds, 1994). Although effective in preclinical model, systemic chemotherapy for localized prostate cancer is not recommended, because only a very small portion of drug could reach the prostate (Chen, 1997). Using paclitaxel cylinder implantation to treat localized prostate cancer can deliver high concentration of drug to the tumor, while sparing normal organs. This approach is to optimize the drug delivery, thereby enhancing treatment efficacy.

1.1 The prostate gland

The prostate gland is the largest male accessory gland in human. It resembles a walnut in shape, surrounds the urethera and locates immediately inferior to the neck of the urinary bladder and anterior to the rectum as shown in Figure 1.1. Approximately 20 - 70 tubuloaveolar glands comprise the whole organ and these glands converge into 16 – 32 ducts that connect to the prostatic urethra (Kovi, 1989). The major function of the prostate in human is to aid in reproduction and it accomplishes this by the secretion of a prostatic fluid (Guyton, 1991). This fluid is a thin, milky fluid that contains, among other things, zinc ions, calcium, clotting
enzymes, and a profibrinolysin. The components of this fluid help to create an optimal motility environment for sperm (Guyton, 1991). Two prostate specific enzymes that are secreted by the secretory epithelial cells into the lumina of the prostatic ducts are prostatic acid phosphatase (PAP) and prostate specific antigen (PSA). PSA is a single-chain glycoprotein protease which liquefies semen after ejaculation and is released into the lumina of the prostatic acini.

The human prostate gland is divided histologically into 4 distinct zones (Figure 1.2), including (a) the anterior fibromuscular zone, which occupies the anterior surface of the prostate, is nonglandular and comprised of smooth muscle cells and fibrous tissue (Rifkin, 1997), (b) the transition zone accounts for 5% of the glandular volume and is the location for 20% of prostate cancer incidences, but is the most frequent site of benign prostatic hyperplasia, (c) the central zone constitutes between 20 - 25% of the glandular volume and accounts for 5 - 10% of prostate cancer incidences, and (d) the peripheral zone, the largest of the zones, which represents 70 - 75% of the total glandular volume and is the most frequent (>70%) site of prostate cancer (Greene et al., 1995; Oesterling et al., 1997)

1.2 Overview of prostate cancer

1.2.1 Epidemiology of prostate cancer

Prostate cancer represents a growing public health problem in the United States and Europe. It is the most commonly occurring cancer among American men, accounting for nearly 33% of all newly diagnosed cancer cases, and is the second leading cause of death by cancer. The benefit of survival from currently available
treatment is minimum. The American Cancer Society estimated that 230,110 new cases and 29,900 prostate cancer-related deaths occurred in the United States in 2004 (American Cancer Society, 2004). The incidence of prostate cancer increases with age. More than 70% of all prostate cancers are diagnosed in men over age 65. Black Americans have the highest prostate cancer incidence rates in the world. The disease is common in North America and Northwestern Europe and is rare in Asia. International studies suggest that dietary fat may be a risk factor.

The survival of patients diagnosed with prostate cancer is heavily influenced by the stage at the time of diagnosis. Eighty-three percent of all prostate cancers are discovered in the local and regional stages. The 5-year relative survival rate for patients whose tumors are diagnosed at this stage is 100%. Relative survival is the preferred method for analyzing the survival of cancer patients in population-based studies. Relative survival compares the observed survival for a group of cancer patients with the survival of members of the general population who have the same characteristics, such as, age, gender, and state of residence, as the cancer patients. For example, men with a relative survival from distant prostate cancer are 34% as likely to live another 5 years as are men of the same age who live in the same state. Over the past 20 years, the 5-year survival rate for all stages combined has increased from 67% to 98%. Relative survival after a diagnosis of prostate cancer continues to decline with longer follow-up. According to the most recent data, relative 10-year survival is 84%, and 15-year survival is 56% (American Cancer Society, 2004). Once the disease is metastasized, the median survival time is 2.5 years (Meyers et al., 1989). Therefore, treating prostate cancer at the localized stage is more and more demanded by the
patients. This has warranted the development of an effective treatment option for the localized disease.

1.2.2 Staging of prostate cancer

There are several staging systems and one of the most commonly used system in the United States is the TNM classification system which gives three key pieces of information: (1) T refers to the Tumor. There are actually two types of T classifications for prostate cancer. The clinical stage is based on digital rectal exam, needle biopsy and transrectal ultra-sound findings. The pathologic stage is based on pathologic examination of the entire prostate gland, both seminal vesicles and, in some cases, nearby lymph nodes after their removal during surgery; (2) N describes how far the cancer has spread to nearby lymph Nodes; (3) M shows whether the cancer has spread (Metastasized) to other organs of the body. Table 1 summarizes the criteria for using this system of tumor classification. Another system of tumor classification is based on the architectural growth pattern of the tumor and is known as the Gleason grading system (Ro et al., 1997). Under this system a lower number such as 2-4 indicates a more differentiated tumor with architecture resembling the normal prostatic grandular tissue. A higher number such as 8-10 means the cancer cells are the least differentiated, and are likely to grow more quickly. Scores of 5-7 are considered “in between”. The stage of the cancer and grading are the most important factors when choosing a treatment option and predicting the survival rate (Kirby et al., 2001).
1.2.3 Current treatment of prostate cancer

1.2.3.1 Therapies for localized prostate cancer

The treatment of prostate cancer varies depending on the stage and grade of the disease. Surgery, radiotherapy, cryotherapy, and watchful waiting are recommended for localized tumors. Androgen ablation therapy and systemic chemotherapy are mainly used for metastatic disease. The heavily debated controversy is whether surgery or watchful waiting is the preferred treatment of localized disease, especially for the well and moderately differentiated tumors.

Radical prostatectomy was regarded the treatment of choice for early prostate cancer until recently (Hanks et al., 1993). In a radical prostatectomy, the entire gland plus some of the surrounding tissues are removed. While this procedure does offer the potential for tumor eradication, the treatment-related mortality is substantial, reaching as high as 1% and increasing to >2% for patients over 75 year of age (Murphy et al., 1994). Complete incontinence was found in 4% and stress incontinence in 15% of patients, and erectile potency was maintained in only 28% of patients. These significant morbidities compromise the quality of life. The 5-year survival of patients with clinically localized disease treated by surgery alone varies from 85 to 94% while 10-year survival ranges from 70 to 75% (Gibbons et al., 1984). These survival rates approximate the expected survival of an age-matched control population without surgery. Another factor disfavoring surgery is its high cost.

Radiotherapy, including brachytherapy and external beam irradiation, is most commonly and effectively used in patients with localized disease. Complications of
radiation include cystitis, proctitis, urinary incontinence and impotence. Brachytherapy results in a lower incidence of incontinence and impotence than external beam radiation (Badalament and Drago, 1991).

In cryotherapy prostate cells are frozen with a metal probe that is placed under ultrasonic guidance. Freezing the cells can cause damage to the surrounding nerves and tissues leading to impotence and incontinence (Perrotte et al., 1999; Pisters et al., 1999). It has been used as an alternative to radical prostatectomy, and also as a salvage therapy for locally recurrent prostate cancer after prostatectomy. Methods were explored to protect the rectal wall and urethral during treatments (Cytron et al., 2003; Onik et al., 1993). Higher rates of impotence were reported than other therapies (Hummel et al., 2003). Watchful waiting is equally effective as surgery or radiotherapy in producing a high 10-year metastasis-free (80-90%) and disease specific (84-97%) survival in patients with localized and low grade tumors (Gleason score of <7) (Beck et al., 1994; Fleming et al., 1993). The current consensus is that watchful waiting can be recommended for patients with low grade, small tumors (< 0.5 ml) or with <10 years of life expectancy, and that treatment selection should be individualized. Some patients may opt for a better quality of life and therefore watchful waiting, whereas others may prefer an opportunity to maximize survival and therefore opt for surgery or radiotherapy (Mettlin et al., 1993).

1.2.3.2 Therapies for metastatic prostate cancer

Hormonal therapy has been the first choice of treatment for advanced prostate cancer. Since 1941, androgen ablation therapy, either by surgical castration or by
medical castration, has been the main approach of hormonal therapy for advanced prostate cancer. The majority (70%-80%) of patients achieve symptomatic relief from initial hormone therapy. Hormonal therapy produces tumor shrinkage but does not eradicate tumors. Androgen insensitivity develops in nearly all patients with advanced prostate cancer.

The final approach for advanced hormone-refractory prostate cancer patients is to use systemic chemotherapy. So far, chemotherapy has been shown to confer adequate palliation but no overall survival benefit in prospective randomized controlled trials, and the only chemotherapy drugs approved by the US Food and Drug Administration are mitoxantrone and estramustine (Gilligan and Kantoff, 2002). Systemic administration of anticancer agents commonly results in cytotoxic side effects such as myelosuppression, nausea, and hair loss, and rarely produce complete responses (Assikis and Simons, 2004).

1.3 Regional cancer chemotherapy

The major goal of regional administration of drugs to tumor-bearing organs is to achieve high drug Concentration x Time in tumors while sparing the host tissues from drug toxicity. Examples of successful regional chemotherapy include intravesical treatment of superficial bladder cancer (Chai et al., 1994; Wientjes et al., 1996), topical treatment of skin cancer (Terwogt et al., 1999), intraperitoneal treatment of advanced ovarian cancer (McClay and Howell, 1990; Recio et al., 1998), and intrathecal treatment of brain cancer (Sandor et al., 1998). Each organ has
different physiological properties that affect drug distribution and the tissue targeting advantage.

The objective of intra-prostatic chemotherapy is to treat organ-confined prostate cancer and prevent local recurrence. Using intra-prostatic chemotherapy, the concentration in the prostate will be greatly increased as has been shown from preliminary studies from our lab (Zheng et al., 2001).

Intra-prostatic doxorubicin infusion studies have been performed in our lab (Zheng et al., 2001). Using this regional approach, high local drug concentrations could be achieved. However, the drug distribution in the prostate after an intra-prostatic infusion of 280 µg doxorubicin over 150 minutes was uneven, with high concentrations (1000 µg/g) localized in the prostatic lobule in which the drug was infused, and lower concentrations (<0.6 µg/g) in the other parts of the prostate. Fibromuscular stroma formed a barrier to drug distribution. The average tissue concentration was 3.2 µg/g and the peak plasma concentration was 6 ng/ml. These studies demonstrate the feasibility of using a regional approach to increase local concentrations while minimizing systemic exposure, while the efficacy might be limited by drug distribution pattern of intra-prostastatic infusion. It warrants the development of a more effective local chemotherapy for localized prostate cancer.

Regional chemotherapy can potentially lessen impotence and incontinence incidence compared with prostatectomy, brachytherapy and cryotherapy. In addition, drug molecules because of their small size can freely diffuse throughout the organ if the treatment sites are well mapped. Hence, chemotherapy has a greater potential of
ridding of hard-to-reach tumor cells. Furthermore, cryotherapy and surgery does not offer tumor cell selectivity, whereas anticancer drugs may have higher tumor selectivity.

1.4 Biodegradable drug delivery systems for intra-prostatic implant.

The controlled release of drugs from polymers has developed into an extremely important area of drug delivery. The great flexibility and general low cost of polymer materials have led to their wide-spread use in controlled release devices. Poly(lactide-co-glycolide) (PLGA) is the most common polymeric carriers. Its general molecule structure is shown in Figure 1.3. The polyesters are prepared by ring-opening polymerization. The degradation occurs through hydrolysis and their degradation products do not elicit undesirable tissue, blood, or inflammatory responses (Piskin, 1995; Shive and Anderson, 1997). PLGA is reported as having suitable characteristics such as strength, flexibility and hydrophobicity for use in drug delivery applications and has been approve by FDA for human use.

Currently, there are some implantable drug delivery formulations available commercially, such as Zoladex® for prostate cancer, Gliadel® for brain tumor. Zoladex is subdermal implant, using PLGA as matrix. The 1-mm diameter cylinder preloaded in a special use syringe is implanted subcutaneously in the upper abdomen. Gliadel wafer implant is approximately 1.45 cm in diameter, and 1 mm thick. It is designed to deliver the carmustine directly into the surgical cavity created when a brain tumor is resected.
Earlier studies conducted in our laboratory demonstrated the feasibility of encapsulating doxorubicin into a biodegradable PLGA matrix (Ortiz, 1998). Doxorubicin free base was successfully encapsulated into PLGA microspheres ranging in diameter from 30-60 µm. The drug delivery system was tested in healthy dog by suspending the microspheres in aqueous solution and injected into the prostate. It was found that regardless of the suspension’s volume, a portion of the dosing solution was always squeezed out of the prostate once the needle was removed. This challenge led to the decision to change the drug delivery system from microspheres to implantable milli-cylinders (Ortiz, 2002). The doxorubicin cylinders were prepared by solvent extrusion method. Doxorubicin cylinders were implanted into prostates under ultrasonic guidance and harvested at varying time points. Using laser confocal microscopy and image analysis, it was determined that doxorubicin distribution was localized within the implanted lobules. Doxorubicin tissue concentrations within the implanted lobule ranged from 31 – 585 µg/g, while plasma and lymph node concentrations were below the HPLC detection limit. Histopathological examination indicated that the implants produced localized tissue necrosis. Necrosis was limited to the implanted lobules and was not evident until 24 hours. The implants produced a mild inflammatory response lasting approximately one week. Twenty eight days after implantation, there was no evidence of tissue necrosis. There was no damage caused to neither the prostatic urethra, nor the peri-prostatic neurovascular bundles at any time. The efficacy of these implants was proven in dog patients diagnosed with spontaneously arising tonsillar and prostate
tumors. Although the feasibility has been proven, but the cytotoxicity of doxorubicin entails careful operation during the implantation procedure to avoid the contamination of doxorubicin in the peritoneal cavity. To circumvent this disadvantage and also for reasons described in Section 1.6, we started to develop similar formulation for paclitaxel and tested the formulation both in vitro and in vivo.

1.5 Degradation of PLGA

The process of degradation here describes the chain scission process during which polymer chains are cleaved to form oligomers and finally to form monomers. It can be measured by the loss of molecular weight. Hydrolysis is the major way of degradation for polyesters. Biodegradation of aliphatic polyesters such as PLGA is dependant on the structural and compositional properties of the polymer matrix. PLGA degrades predominately by bulk erosion in two steps (DeLuca et al., 1993; Lewis, 1990). The first step is a decrease in molecular weight caused by random hydrolytic cleavage of the ester linkage. The second step is the onset of weight loss and a change in the rate of chain scission (Lewis, 1990). This weight loss is caused by the fact that during hydrolysis the polymer becomes more hydrophilic due to the formation of new chemical species. The polymer then becomes soluble in the aqueous fluid, diffuses out of the matrix, and the result is mass loss. The rate at which this degradation occurs is primarily governed by the initial copolymer ratio, and polymer molecular weight (Scholes et al., 1997). The copolymer ratio reflects the structural composition of the matrix as well as its hydrophobicity. Amorphous regions within the matrix allow for easier water penetration and therefore degrade at a
faster rate than the more hydrophobic crystalline regions. The copolymer ratio of 50/50 PLGA has been shown to have the shortest degradation half life due to its high amorphous content (Miller et al., 1977). Degradation rates have been shown to increase with decreasing polymer molecular weight and chain lengths (Wang et al., 1990).

1.6 Paclitaxel (Taxol®)

Paclitaxel is a diterpenoid anticancer drug isolated from the stem bark of the western yew, taxus brevifolia (Wani et al., 1971). Paclitaxel is one of the most important anti-cancer drugs developed in the last two decades. It has shown impressive activity against many human solid tumors, i.e., ovarian, head and neck, bladder, breast and lung cancers (Rowinsky and Donehower, 1993). Its chemical structure is a taxane ring with a side chain at position C-13 (Figure 1.4). The C-13 side chain is essential for the biological activity of paclitaxel (Horwitz, 1992b). Paclitaxel enhances tubulin polymerization, promotes microtubule assembly and stabilizes microtubule dynamics, and thereby inhibits cell proliferation and induces apoptosis (Jordan et al., 1996; Schiff and Horwitz, 1981).

The paclitaxel formulation approved by the Food and Drug Administration for human use (Taxol®) employs 50% Cremophor (polyethoxylated castor oil) and 50% anhydrous ethanol to solubilize the drug at 6 mg/ml concentration. The major dose-limiting systemic adverse effects of paclitaxel are myelosuppression and peripheral neuropathy. Mucositis is another non-hematological dose-limiting toxicity. Cremophore is clinically related with serious hypersensitivity which is limiting the
use of Taxol®. Mild toxicities include reversible complete alopecia, symptoms of bradycardia, and ventricular tachycardia (Francis et al., 1995).

Paclitaxel is a good choice for local drug delivery for prostate cancer because (a) of the demonstrated clinical activity of paclitaxel against systemic prostatic cancer (Hudes et al., 1997), (b) the highly binding of paclitaxel to macromolecules may result in high prostatic tissue concentrations, (c) it is hydrophobic agent ($K_{oct:water} > 99$) which may provide higher tissue penetration than hydrophilic drug, and (d) the well defined pharmacology of paclitaxel facilitates the development of the PBPK model and provide the guidelines for dosage selection.

Chapters 4, 5 and 6 describe studies to optimize the dosing regimen of a chemosensitizer, suramin. Suramin has been traditionally used as an anti-parasitic drug and been tested as an anti-cancer drug. The effective concentration for these purpose are over 100 µM and associated with severe toxicity. Our lab discovered that suramin has broad-spectrum drug-resistance-reversal effect, in a concentration dependent manner. The effective concentration range is 10-50 µM in in vitro condition and in animal models. To achieve this target concentration range, interspecies scaling was employed to optimize the suramin dosing regimen in humans in Chapter 4. Chapter 5 describes how an empirical equation was developed based on population pharmacokinetic analysis. To obtain more information on the pharmacodynamics of suramin, the drug distribution was studied in tumor-bearing mice and a local physiological based pharmacokinetic model was developed for the
tumor compartment in Chapter 6. Some background information of suramin, interspecies scaling and population pharmacokinetic analysis were provided in the following sections.

1.7 Suramin

Suramin, an organic polyanion with six sulfonic groups, is a polysulfonated naphthylurea (Figure 1.5). Suramin has been commonly used for the treatment for parasitic diseases such as African trypanosomiasis and Onchocerciasis since the 1920’s (Hawking, 1978). Since 1980’s, suramin has received attention as a possible antiretroviral agent based on its ability to inhibit the reverse transcriptases of several retroviruses including human immunodeficiency virus (DeClercq, 1979; Levine et al., 1986; Mitsuya et al., 1984). Unfortunately, results of multi-institutional trials sponsored by the National Cancer Institute failed to demonstrate efficacy against the disease (Cheson et al., 1987).

As an antineoplastic agent, suramin has shown preclinical activity against prostate, breast, ovarian, adrenal, non-small cell lung cancers and malignant melanoma (Eisenberger and Reyno, 1994). In the clinical trials, significant antitumor activity was only observed in hormone-refractory prostate cancer patients. Although suramin has a number of biological actions, the precise mechanism of its antineoplastic activity is unknown. It inhibits the binding of several tumor growth factors to their respective receptors, including platelet derived growth factor (Hosang, 1985); epidermal growth factor (Coffey et al., 1987; Fujiuchi et al., 1997); vascular endothelial growth factor (Waltenberger et al., 1996); transforming growth factor β
(Wade et al., 1992); insulin-like growth factor 1 (Minniti et al., 1992; Pollak and Richard, 1990); and fibroblastic growth factors (FGF) (Basile and Holzwarth, 1994; Bernardini et al., 1993; Pesenti et al., 1992; Pienta et al., 1991). Suramin also inhibits the kinase activity of the protein kinase C beta 1 and other protein kinase C isoforms (Khaled et al., 1995).

Multiple clinical studies have evaluated suramin as a cytotoxic agent, where suramin was given alone or in combination with other chemotherapeutic agents. In these earlier studies, suramin was given at its maximum tolerated steady state concentrations of 100-200 µM (equivalent to approximately 150-300 µg/ml). Suramin has no antitumor activity at plasma concentration less than 100 µM and is associated with severe toxicity including polyneuropathy, adrenal insufficiency, skin and appendages alterations, coagulopathy, and renal insufficiency at plasma concentration above 200 µM (Arlt et al., 1994; Figg et al., 1994; Holland et al., 1988; Horne, III et al., 1992; Katz et al., 1995; Kobayashi K et al., 1996; LaRocca et al., 1990; May and Allolio, 1991).

In contrast to the high concentrations of suramin required for its antiproliferative activity, inhibition of some growth factors to their receptors occurs at substantially lower concentrations. For example, basic FGF (bFGF), a growth factor that has been implicated in neovascularization and tumor growth (Danesi et al., 1993; Pesenti et al., 1992) is inhibited at suramin concentrations below 50 µM (Song et al., 2000). Au and collaborators recently reported that elevated levels of acid FGF (aFGF) and bFGF in solid and metastatic tumors confers broad spectrum resistance to
chemotherapy drugs with diverse structures and mechanisms of action, and that low concentrations/doses of suramin that are devoid of antitumor activity (or toxicity) reverse the FGF-induced chemoresistance in vitro (Song et al., 2000; Zhang et al., 2001). In addition, in mice with well established lung metastases, low doses of suramin (10 mg/kg, twice weekly x 3 weeks) yielding plasma concentrations of between 10 to 20 µM, enhanced the antitumor effect of paclitaxel; the combination resulted in tumor eradication in 42% of animals compared to zero in paclitaxel alone group, a 9-fold greater reduction of the density of nonapoptotic cells and a 30% increase in the apoptotic cell fraction (Song et al., 2001). Suramin is currently under investigation in a Phase II trial as an enhancer of the antitumor effect of a standard treatment regimen for non-small cell lung cancer (NSCLC).

Several studies have discussed the processes involved in the clearance of suramin. A study in patients with acquired immunodeficiency syndrome showed that suramin is essentially unmetabolized, mainly eliminated by renal clearance, and suggested glomerular filtration as the mechanism of suramin clearance by the kidney (Collins et al., 1986). A subsequent study in prostate cancer patients found that the average total body clearance of suramin was about 2-fold higher than the average creatinine clearance and thereby suggested tubular secretion as a second mechanism of renal clearance (Cooper et al., 1992).

1.8 Interspecies scaling

In pharmacokinetics, the physiological time scale and parameters are usually obtained by transforming clock time and absolute physical parameters into forms that
become species invariant. This concept was first applied by Dedrick et al. to methotrexate in his elementary Dedrick plot, in which both time and drug plasma concentration were transformed based on allometric exponents for clearance and steady state volume of distribution, in respect to body weight (Bischoff et al., 1970). One of the limitations in the elementary Dedrick plot, which requires the exponent of allometric equation to be unity, was lifted in the complex Dedrick plot developed by Boxenbaum etc. (Boxenbaum and Ronfeld, 1983) and thereby broadened the application of interspecies scaling. However, there was still a big portion of drugs, for which a lack of good allometric relationship across species existed. It was found that lifespan of species is correlated with metabolic rate and drug disposition (Denckla, 1975). The product of CL and maximum lifespan potential (MLP) has a better allometric relationship than CL alone in the correlation with body weight, suggesting the disposition and elimination of suramin to be dependant on MLP. By adjusting the clock time with MLP, the dienetichron plot gave a better prediction of suramin concentration profile in human than the complex Dedrick plot for some drugs. Altogether, the elementary Dedrick plot, the complex Dedrick plot and the dienetichron plot provide useful tools in pharmacokinetics and toxikinetics, to predict the drug behavior in humans based on results in animal models

1.9 Population pharmacokinetic analysis

Population pharmacokinetic analysis provides estimates of the main PK parameters (fixed effects) with the evaluation of the their variability on the investigated population (random effects) together with the possibility to evaluate the sources of such a variability (second-stage models relating variability to some
explanatory covariables). Furthermore, mixed-effects modeling can account for random interoccasion parameter variability. This modeling approach makes it possible to account for PK parameters time dependence by partitioning the parameter dispersion according to variance components associated with intra- and intersubject variability.

1.10 Overview of the dissertation

The remaining chapters in this dissertation were organized according to their study subjects. Each chapter starts with an introduction that defines the research problem and the study objectives. Next follows the materials and methods, results, discussion, and conclusion of each chapter. Tables and figures are attached at the end of each chapter. The references are appended at the end of this dissertation. The remaining paragraphs of this chapter summarize the work presented in this dissertation.

The overall objective of the work presented in this thesis was to optimize cancer chemotherapy. Chapters 2 and 3 contribute scientifically to the field of regional cancer chemotherapy, with a specific focus on prostate cancer. Chapters 4, 5 and 6 contribute to the different aspects of pharmacokinetics, including interspecies scaling, population pharmacokinetics, and tissue distribution. The reader will find that the work presented in this thesis spans many areas within the pharmaceutical sciences. The major conclusions and contributions of the research in this dissertation are as follows: (a) The establishment of paclitaxel loaded biodegradable implants that have desired in vitro release profile; (b) the efficacy of the drug delivery system was tested in PC-3 tumor bearing mice. The drug loaded cylinders inhibited tumor growth, while
the system exposure is negligible; (c) establishment of allometric relationship across four species for low-dose suramin; (d) development and validation of empirical equations for suramin dosing regimen in dog cancer patients; (5) study of tissue distribution of low-dose suramin and development of local PKPB model.

Chapter 2 describes the preparation and in vitro characterization of biodegradable implants containing paclitaxel. A solvent extrusion method was used to encapsulate paclitaxel into PLGA matrices. The cylinders have a diameter of 1.0-1.2 mm and can be placed by injection through a trocar needle. The release of paclitaxel from poly(lactide-co-glycolide) (PLGA) matrix is slow due to its low water solubility. Anionic surfactants to solubilize the drug, or lactose to induce porosity and surface area in the matrix was used to increasing the release rate. The release rate was increased by 10 fold with incorporation of 5% lactose and using lower molecular weight PLGA, yielding a release of 2% of loaded drug over 10 days. Incorporation of fatty acid, isopropyl myristate or diethylphthalate, did not increase further release rate significantly. To further increase release, preparations with 50, 75, and 83% lactose were tested, which released 6.3, 19.7, and 23.4% in 20 days. The cross-section was examined by SEM. Once having characterized the drug delivery system in vitro, the next logical step was to study distribution of paclitaxel from the implants in vivo.

Chapter 3 describes the in vivo studies to test the efficacy of paclitaxel-loaded cylinders. Drug penetration was evaluated in vivo, after implantation in subcutaneous PC3 tumors in immunodeficient mice. HPLC analysis showed that in 11 days, tissue concentration declined from \(23 \pm 5 \, \mu\text{g/g}\) at 0-2 mm from the cylinder, to \(0.7 \pm 0.1\)
"µg/g at 2-5 mm, to <0.02 µg/g at distances >5 mm from the cylinder. The concentration in plasma was below detection limit. Histological evaluation showed extensive necrosis extending to ~3 mm from the cylinder, with increased apoptosis up to ~4 mm from the cylinder. The paclitaxel-loaded cylinders inhibit tumor growth on tumor bearing mice model. Incorporation of IPM enhanced the efficacy greatly. The mechanism is not clear. Systemic pretreatment with Taxol showed enhancement of the efficacy, yet not as obvious as the effect of IPM. The results show efficacy of this administration form, which is limited to a highly localized distribution area.

Chapter 4 studies the pharmacokinetics of suramin in plasma of mice, rats, dogs, and humans. The interspecies pharmacokinetic scaling between these species was reported. Animals received doses of 6-10 mg/kg, and human patients received 240 mg/m². The parameters of interest were correlated across species as a function of body weight using allometric equations. The steady-state volume of distribution (Vdss), plasma clearance (CL), product of CL and maximum life potential (MLP), and area under the plasma concentration-time curve (AUC) correlated well across species. The dienetichron plot was used to predict the suramin concentration profile in human patients and the simulation was validated by our Phase I study.

Chapter 5 describes population pharmacokinetic analysis of suramin in dogs, in order to identify the target suramin dose, in combination with chemotherapy. Twenty-one dogs with late stage carcinomas, soft tissue sarcomas, or lymphoma were treated with suramin in combination with doxorubicin. Suramin was given at a fixed dose of 6.75 mg/kg as a 20-min infusion, and doxorubicin was given 3 hr later as 20-
30 min infusion at a dose of 30 mg/m² (or 1 mg/kg for body weight below 15 kg). The suramin pharmacokinetic data were analyzed using a nonlinear mixed-effect model (NONMEM) with a one-compartment model. An empirical equation to calculate dosage for target suramin concentration was developed from the data of first fourteen patients and validated by the later seven dogs. The physiological parameters evaluated were body surface area (BSA), gender, and concentrations of plasma protein, serum creatinine, albumin and bicarbonate. Through a stepwise build-up, a full model was developed, including BSA, gender, and serum creatinine concentration. Incorporation of the remaining covariates (concentrations of plasma protein, serum creatinine, albumin and bicarbonate) into the model did not significantly improve the model performance. A later model refinement resulted in a final model, which only includes BSA and gender. These results led to a BSA-based suramin dose calculation method for individual patients, thereby eliminating the need of real-time pharmacokinetic monitoring for the use of low dose suramin as a chemosensitizer.

Chapter 6 describes organ-specific bio-distribution of low-dose suramin. Nude mice implanted subcutaneously with HT-29 colon tumors received suramin by tail vein, at a dose of 10 mg/kg. The plasma concentration–time profile showed a biphasic decline. Suramin was found to accumulate in tissues at various levels. The highest concentration level was observed in kidneys and the lowest in brains. The tissue pharmacokinetics of suramin might be determined by a shallow compartment and a
deep compartment. A local physiologically-based pharmacokinetic model was proposed for the tumors.

The final chapter summarizes the contributions of this dissertation research and discusses future investigations.
<table>
<thead>
<tr>
<th>Primary tumor (T)</th>
<th>T1 Microscopic</th>
<th>a) Three foci or less</th>
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<tr>
<td></td>
<td></td>
<td>b) More than three foci</td>
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<tr>
<td>T2 Palpable</td>
<td>a) Less than 1.5 cm in diameter</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) More than 1.5 cm in diameter</td>
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<tr>
<td>T3 Palpable extending beyond capsule</td>
<td>a) Cancer extends into the periprostatic tissues</td>
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<tr>
<td></td>
<td></td>
<td>b) Cancer extends into the periprostatic tissues and is larger than 6cm</td>
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<td>T4</td>
<td>Tumor fixed or involving neighboring structures</td>
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<td>Nodal involvement (N)</td>
<td>N0</td>
<td>No involvement of regional lymph nodes</td>
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<td></td>
<td>N1</td>
<td>Cancer has spread to one node</td>
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<tr>
<td></td>
<td>N2</td>
<td>Cancer has spread to more than one node</td>
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<tr>
<td>Distant Metastasis (M)</td>
<td>M0</td>
<td>No known metastasis</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>Distant metastasis present</td>
</tr>
</tbody>
</table>

**Table 1.1  Clinical staging of prostate cancer.** The TNM system stages prostate cancer based on the involvement of the primary tumor, lymph nodes, and any known metastases.
Figure 1.1  Prostate anatomy. The prostate gland resembles a walnut in shape, surrounds the urethera and locates immediately inferior to the neck of the urinary bladder and anterior to the rectum. The image was reproduced from Krongrad et al, 1993.
Figure 1.2  A cross-sectional view of a human prostate. The prostate is comprised of 4 histologically distinct zones: the anterior fibromuscular stroma zone, the central zone, the transition zone, and the peripheral zone. The image was reproduced from Krongrad et al, 1993.
Figure 1.3  Molecular structure of PLGA.
Figure 1.4 The chemical structure of paclitaxel.

Paclitaxel, MW: 854
Figure 1.5  The chemical structure of suramin.
CHAPTER 2
IMPLANTABLE MINI-CYLINDERS FOR REGIONAL PaCLITAXEL DELIVERY TO PROSTATE

2.1 Introduction

Prostate cancer is the most common malignancy in man. The American Cancer Society estimated that 230,110 new cases and 29,900 prostate cancer-related deaths occurred in the United States in 2004 (American Cancer Society, 2004). Over the past decade, substantial improvements in diagnosis and staging of the disease have been made with the combined use of digital rectal examination, measurement of serum PSA levels, and transrectal ultrasound. Earlier diagnosis of prostate cancer has resulted in a stage migration, with an increased proportion of men diagnosed with organ-confined disease (Gleave et al., 1996; Stanford et al., 1996). Eighty-three percent of all prostate cancers are discovered in the local and regional stages (American Cancer Society, 2004).

The natural history of localized prostate cancer is variable but is generally one of slow local progression with later development of regional and distant metastasis (Foley and Thompson, 2000; Gleave et al., 1996). More than two-thirds of patients will suffer local or systemic progression, and more than half of patients with prostate cancer will die from their disease within 10 years (Chodak et al., 1994). Significant morbidity
can result from local tumor progression, including bladder outlet and ureteral obstruction, pain, and bleeding.

The current treatment options for localized prostate cancer include radical prostatectomy, radiotherapy and cryotherapy. These options are associated with low but significant morbidities, such as urinary incontinence, impotence, cystitis, and proctitis etc. Androgen ablation therapy is used with significant success. However, tumor recurrence after androgen ablation is common and the recurrent tumors are often hormone refractory. Therefore, effective non-surgical treatments that can eradicate the localized tumors in the prostate represent an attractive alternative treatment. A biodegradable implant at the site of the action, which releases the drug locally at a controlled rate for a decided period of time would provide high local concentrations and also minimize the potential of side effects and toxicity (Domb et al., 1993; Friend and Pangburn, 1987; Poznansky and Juliano, 1984). This approach has been used successfully to deliver BCNU into the brain from a polyanhydride disk for the post-surgical treatment of 9L liosarcoma (Tamargo et al., 1993). The approach also reduces hospitalization-related cost, as the biodegradable implant will dissolve completely obviating the need for further surgery.

The anatomical location of the prostate gland is amenable to direct local injection with drug delivery device. Brachytherapy for localized prostate cancer is now performed by transperineal placement of radioactive seed implants under transrectal ultrasound guidance.

Paclitaxel has shown clinical efficacy against ovarian cancer, breast cancer, head and neck cancers and non-small cell lung cancer (Horwitz, 1992; Rowinsky and
Donehower, 1995; Spencer and Faulds, 1994). Paclitaxel enhances tubulin polymerization, promotes microtubule assembly and stabilizes microtubule dynamics, and thereby inhibits cell proliferation and induces apoptosis (Jordan et al., 1996; Schiff and Horwitz, 1981). Paclitaxel is a good choice for local drug delivery for prostate cancer because (a) of its demonstrated clinical activity of Paclitaxel against systemic prostatic cancer (Hudes et al., 1997), (b) the highly binding of Paclitaxel to macromolecules may result in high prostatic tissue concentrations, (c) it is a hydrophobic agent which may provide higher tissue penetration than a hydrophilic drug.

To enable prolonged regional paclitaxel administration to localized tumors, we developed a biodegradable delivery device with a long cylindrical shape. The long shape is preferred over a preparation of microspheres, which often escape in retrograde direction along the injection needle tract. In addition, the long shape should enhance drug distribution throughout the target tissue. The present study described the development of paclitaxel loaded mini-cylinders in Poly(lactide-co-glycolide) (PLGA) matrix. The morphology of the cylinders before and after release was studied. Several methods were investigated to increase release rate.

2.2 Materials And Methods

2.2.1 Chemicals and reagents

Poly(DL-lactide-co-glycolide) (PLGA), with a 50:50 LA:GA ratio and an inherent viscosity of 0.63 dl/g (measured in hexafluoroisopropanol), was purchased from Birmingham Polymers (Birmingham, AL, USA). Paclitaxel was purchased from
Hande (Yunnan, China), Tween 20, Tween 80, isopropyl myristate (IPM), β-lactose, diethylphthalate (DEP) from Sigma Chemical Co. (St. Louis, MO) and Matrigel was from BD biosciences (Bedford, MA). Other chemicals and reagents for HPLC in highest grades were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Cephalomannine, an internal standard of paclitaxel for HPLC assay, was a gift from NIH. All chemicals were used as received.

2.2.2 Preparation of paclitaxel-loaded PLGA cylinders.

Paclitaxel-loaded PLGA cylinders were prepared using the previously described solvent extrusion and fluid energy micronization (FEM) methods with the following modification. Briefly, PLGA and paclitaxel were dissolved in acetone. IPM, if needed, was also dissolved to form uniform solution. A designated amount of lactose was added last, if necessary. The composition of the materials used to formulate the cylinders was listed in Table 2.1. After stirring to evaporate the extra solvent and homogenize the mixture, the paste was loaded into a 5-ml syringe and slowly extruded into the silicone tubing (Manostat, New York, NY) with 0.78 mm (1/32”) I.D. to form a cylindrical monolithic device. The extruded product was dried in the hood at room temperature for 24 hours, then in a vacuum over at 45 °C for another 24 h before testing.
2.2.3 Characterization of Paclitaxel-loaded PLGA cylinders

The morphology of the PLGA cylinder was studied using a scanning electron microscope (SEM, Philips XL-30). Samples were coated with gold under vacuum using a 200 µA conductance current. The voltage was set to 10 KV for SEM analysis.

2.2.4 Determination of drug loading

Five mg of cylinders were weighed and dissolved in 10 ml acetonitrile. After centrifugation, a 100 µl aliquot was mixed with 100 µl of the internal standard cephalomannine. The sample solvent was dried under air stream, reconstituted with 100 µl acetonitrile and vortexed for 30 sec. 100 µl deionized water was then added and vortexed for another 30 sec. The mixture was sonicated for 30 sec, followed by 30 sec of vortexing and centrifugation for 10 min at 3000 rpm. The supernatant was transferred and analyzed by HPLC.

2.2.5 Release of paclitaxel for PLGA cylinder

The release medium composed of PBS, pH 7.4, and 0.02% w/v Tween 80. The nonionic surfactant was used to minimize the adsorption of paclitaxel onto the walls of the release vessel, and to reduce the polymer/water interfacial tension and stabilize paclitaxel. Five mg of implant was suspended in 5 ml release medium, in a 15 ml polypropylene tube. Release medium was withdrawn at preset time points, and fresh medium was added. One ml of aliquot was used to analyze the paclitaxel concentration. Briefly, 100 µl of internal standard cephalomannine was added. The mixture was extracted with 3 ml ethyl acetate. After centrifugation at 3000 rpm for 10 min, the
supernatant was withdrawn and repeat the extraction one more time. The supernatant was combined and dried under air stream. The residue was reconstituted with 100 µl of acetonitrile and was analyzed by HPLC.

At the end of the release study, the eroded cylinder was retrieved, freeze-dried, and dissolved in acetonitrile to detect the residual drug content as described above. Sum of the amount of drug released and the amount of drug remained in the cylinders was compared to the initial drug loading to determine the mass balance. For each formulation, 3 cylinders were measured.

2.2.6 HPLC analysis of paclitaxel

The HPLC stationary phase consisted of a clean-up column (Novapak C_{8}, 75×3.9 mm ID, 4 µm particle size from Waters Associated, Milford, MA) and an analytical column (Bakerbond C_{18}, 250×4.6 mm ID, 5 µm particle size from J.D. Baker, Phillipsburg, NJ). Samples were injected into the clean-up column and eluted with clean-up mobile phase consisting of 37.5% acetonitrile in water at a flow rate of 1 ml/min. Concurrently, the analytical mobile phase consisting of 49% acetonitrile was directed through the analytical column at a flow rate of 1.2 ml/min. The fraction form 8-15 min containing paclitaxel and cephalomannine was transferred from the clean-up column into the analytical column. The limit of sensitivity for paclitaxel was 10 ng/ml.

2.2.7 Data analysis

The nonlinear regressions, ANOVA for repeated measurements and other statistical tests were conducted by SAS (SAS Institute Inc., Cary, NC).
2.3 Results

2.3.1 Characterization of paclitaxel – loaded cylinders

Two types of cylinders were developed (Table 2.1). One type used PLGA as the major component of the solid matrix (i.e., preparations A through D). These cylinders showed solid surface before and after release (referred to as solid cylinders). The second type used lactose as the major ingredient (i.e. preparations E though H). Figures 2.1 shows scanning electron micrograph (SEM) of the solid and porous cylinders. Solid cylinders showed no microscopic channels before and after release, whereas the porous cylinders showed channels before and after release. After lactose diffused out, PLGA had fiber-like structure and an interconnected channel network was formed inside the cylinder, which facilitates paclitaxel to diffuse out. Figure 2.2 shows the sequential morphological changes before release, at 24 hr, 5 day and 10 day after release for preparation F. Preliminary study showed the diffusion network was already visible at 1 hr.

2.3.2 Release of paclitaxel from PLGA cylinders

2.3.2.1 The effect of lactose

The release profiles with different lactose loadings are shown in Figure 2.3. The release rates consisted two phases for all the 6 Preparations, i.e. the initial-burst phase and the zero-order-rate phase. With a lactose loading of 5%, Preparation C did not increase the release rate, compared to Preparation A (p=0.97, ANOVA for repeated measurements). A 37.5% increase was observed when 50% of lactose was loaded in Preparation D, compared to A. Given an implant of 8 mg, the amount of paclitaxel
released into the medium would be 2.6, 2.5 and 3.5 µg in two weeks, for Preparations A, C, and D, respectively. When the lactose loading was increased to 2-6 fold of PLGA, release rates were increased drastically. In the first 12 hours, 2.2%, 5.4% and 9.4% of the loaded drug was released for Preparations E, F and G, respectively. Using linear regression to analyze the release rate for the later zero-order-rate phase, i.e., after 6 days, the release rate for these 3 formulations would be 0.08%, 0.20% and 0.29% per day. For a standard cylinder weighing 8 mg, the released drug would be as much as 8.8, 22 and 38 µg in the first 12 hours, and 0.32, 0.76, and 1.16 µg/day at later zero-order-rate phase, for Preparations E, F, and G, respectively. The relationship between release rate and lactose loading was explored, as seen in Figure 2.6. The data were fitted with exponential trend lines, expressed as Rate= a · e^{k · Loading}. For the initial-burst phase, a equals 0.00439 and k equals 0.104 (r^2= 0.99), while for the zero-order phase, a equals 0.00141 and k equals 0.0497 (r^2= 0.97).

2.3.2.2 The effect of IPM

Incorporation of IPM increased the release rate for both solid cylinders and porous cylinders, as shown in Figure 2.4. In the solid cylinder, addition of 20% of IPM increased the release percentage from 0.65% to 1.28% in two weeks, comparing Preparation B to Preparation A. In porous cylinder, the release percentage was increased from 17.4% to 21.5% when IPM was added in the Preparation H, compared to Preparation G. The ratio of IPM to PLGA in Preparation H was kept the same as in Preparation B.

2.3.2.2 The effect of gamma sterilization
To study if gamma sterilization, one common method to sterilize polymer devices, has effect on the release rate, the release profiles were compared before and after gamma sterilization, using a dose of 25 kGy gamma irradiation. After the irradiation, 1% less drug was released in three weeks, representing a 5% difference (Figure 2.5). ANOVA test for repeated measurements showed no significant difference between the release profiles before and after gamma sterilization (p=0.47)

2.4 Discussion

2.4.1 Mechanisms of paclitaxel release from PLGA cylinder

Drug release from PLGA cylinders through three mechanisms: (a) diffusion through the polymer matrix (Demirdere et al., 1991; Pitt et al., 2003; Pradhan and and Vasavada, 1994); (b) diffusion after solubilization in connected channels through the matrix (Heya et al., 1991; Hutchinson and Furr, 1990); and (c) release after matrix degradation (Hutchinson and Furr, 1990; Shah et al., 1992). The release rate and mechanism from biodegradable polymer matrix are highly dependent on the drug and polymer properties of the polymer and on the matrix characteristics, e.g. drug contents, porosity and surface area etc..

Hydrophilic drug and hydrophobic drug are dispersed differently in PLGA matrix. Hydrophilic drug are not miscible with PLGA in organic solvent. Small crystals and particles exist in the matrix. Some of the particles connect together to form channels leading to the open ends of the matrix. The particles present in the channels can be released by the diffusion-through-channel mechanism. The particles present as isolated particles swell absorb water under the driving force of osmotic pressure, which
results in swelling and leading to rupturing of some thinner and weaker parts of the PLGA matrix and thereby generates channels, a mechanism known as osmotic pressure induced diffusion-through-channel. Osmotic pressure can also drive water to penetrate the PLGA wall to generate a drug solution flow toward the open ends, as in an elementary osmotic pump. This mechanism is called osmotic convection flow release. Finally, the isolated drug particles in a strong PLGA matrix, which was not broken under osmotic swelling, are unreleasable until PLGA mass degradation occurs and the drug is released along with PLGA mass loss. For hydrophobic drug, such as paclitaxel, it can be dissolved in organic solvent with PLGA. Thus, when the solvent evaporates, the existence of polymer will prevent large crystal or particle formation. Analysis of result indicated that the diffusion-through-channel does not play an important role in paclitaxel release. Since the water solubility of paclitaxel is low (maximum reported water solubility of 35µM, (Ringel and Horwitz, 1991)), the osmotic pressure will be low. The high hydrophobicity also makes water up-take insignificant. Therefore, the contribution of osmotic induced release was not significant.

The degradation of PLGA occurs due to the bulk hydrolytic degradation of the ester bonds. The half life of poly(ester) bonds are about 3.3 yrs (St.Pierre et al., 1986), which is relatively slow. The polymer belongs to the bulk-eroding polymer, for which degradation and erosion are not confined to the surface of the device. Therefore, the size of the device will remain constant for a considerable portion of time during its application. In this case, the degradation process does not contribute the paclitaxel release, either since drug delivery will be diffusion controlled rather than degradation controlled.
All these facts might explain why paclitaxel has much lower release rate from polymer, compared to other hydrophilic drug, such as carmustine, 4-hydroperoxycyclophosphamide (Fung et al., 1998).

2.4.2 Increased drug release by incorporation of lactose.

In this study, it was shown lactose could increase paclitaxel release rate, with an exponential relationship between the release rate and lactose loading. Studies in our lab revealed that increase of specific area of PLGA microsphere resulted in increase of release rate (paper in preparation). Presence of water-soluble ingredients, lactose in this study, can increase the porosity, thereby increasing the surface area. The exponential equations suggested there was a minimum value to form connected channels by lactose. If one line was drawn across the first two points and the other across the latter three points in Figure 2.6, the two lines would cross at a point close to 60% of lactose loading, similar to Preparation E. Although the application of the equation might be limited for PLGA with different viscosity and composition, it could be used as a reference when designing a formulation with certain release profile.

Biodegradable porous polymer scaffolds have been used for temporal templates for tissue regeneration. There have been several methods to produce highly porous biodegradable polymer scaffolds such as compressed mesh of nonwoven polymer (Mikos et al., 1993; Mooney et al., 1996), solvent casting/salt leaching (Mikos et al., 1994; Miskos et al., 1993), emulsion freeze drying (Whange et al., 1995), expansion form pressurized carbon dioxide (Harris et al., 1998), phase separation (Nam and Park, 1999; Park et al., 1998), and 3-D printing technique (Park et al., 1998). Because the
requirement for dimension and distribution of the pores in the matrix is not so strict for drug delivery as for tissue regeneration, solvent casting/salt leaching was used in this study. This method also has the advantage of easy operation and no need for complex equipment.

2.4.3 The effect of IPM on paclitaxel-loaded cylinder

IPM, an ester, has been widely used as a vehicle in many cosmetic and pharmaceutical preparations because of its skin penetration enhancing property, its safety and its compatibility with a wide range of compounds. Co-encapsulation of IPM, can lead to a faster drug release from PLGA matrix by decreasing the transition temperature (Blanco and Alonso, 1998; Sansdrap and Moes, 1998). It was also suggested that IPM could form release channels for paclitaxel in PLGA matrix (Wang et al., 1996).

It has been reported IPM increase permeation of drugs through skin mainly by increasing diffusion coefficients (Suh and Jun, 1996). One of the proposed mechanisms is the so-called lipid fluidizing effect (Leopold and Lippold, 1995). By inserting into intercellular lipids, IPM makes the environment less rigid for drugs to penetrate. It has also been suggested that IPM can interact with drug-binding proteins, which increases free fraction of the drug and thereby, increases penetration. Although IPM has been widely studied for its skin penetration enhancing effect, little study has been done to elucidate if it can enhance drug penetration inside tumor. Whether IPM can also increase paclitaxel penetration in tumor is something worth studying.
2.5 Conclusions

Two types of paclitaxel-loaded mini cylinders were developed for introprostatic implantation, solid cylinders and porous cylinders. Through creating pores and channels by lactose, porous cylinders showed increased release rate. The release rate in both initial-burst phase and zero-order-rate phase had exponential relationship with the lactose-loading percentage. Incorporation of IPM further increased release rate. Gamma sterilization did not significantly affect the paclitaxel release rate.
<table>
<thead>
<tr>
<th>Preparation</th>
<th>IPM (%)</th>
<th>Lactose/PLGA</th>
<th>Drug Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>0.05:1</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
<td>0.5:1</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>2:1</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>4:1</td>
<td>5</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>6:1</td>
<td>5</td>
</tr>
<tr>
<td>H</td>
<td>5*</td>
<td>4:1</td>
<td>5</td>
</tr>
</tbody>
</table>

*: the ratio of IPM to PLGA is the same as in preparation B.

Table 2.1 Composition of Materials Used a Series of Preparations. Cylinders were prepared by solvent extrusion method. The composition of PLGA is 50/50, ratio of lactide to glycolide.
Figure 2.1  Cross-section morphology change of two types of cylinders before and after release, solid and porous cylinders, examined by SEM.
Figure 2.2  Sequential morphology change of porous cylinder cross-section during in vitro release. The pictures shown here are SEM of cross-section for Preparation F, which are representative of the porous cylinders (Preparations E to H).
Figure 2.3 The effect of lactose on the release profile. Preparations A, C and D are solid cylinders with increasing lactose to PLGA ratio, i.e., 0:1, 0.05:1, 0.5:1 (Table 2.1). Preparations E, F and G are porous cylinders with lactose to PLGA ratio of 2:1, 4:1 and 6:1, respectively. Details for preparations A, C and D were depicted in the upper left insert. Each point and error bar represents Mean ± Stdev (n=3). Data points are connected by straight lines.
Figure 2.4 The effect of isopropyl myristate (IPM) on the release profile. The upper panel shows the effect of IPM on the solid cylinder. Preparation B has the same composition as preparation A, except the IPM, which is 30% of the PLGA (w/w) (Table 2.1). The lower panel shows the effect of IPM on the porous cylinder. Compared to Preparation F, Preparation H was added with IPM, as much as 30% of the PLGA (w/w). Each point and error bar represents Mean ± Stdev (n=3). Data points are connected by straight lines.
Figure 2.5 The effect of gamma irradiation on the release profile. The dose for gamma sterilization was 25 kGy. Each point and error bar represents Mean ± Stdev (n=3). Data points are connected by straight lines.
Figure 2.6. Exponential relationships between lactose loading and release rate. The upper panel (a) shows the correlations of the initial release rate and the lactose loading for Preparations C, D, E, F and G. The release rate for each point was obtained from the slope of linear regression of the initial release percentage in the first twelve hours versus time ($r^2 > 0.95$); the lower panel (b) shows the correlations during the zero-order phase. The release rate was obtained from the slope of the linear regression of the release percentage after 6 days versus time ($r^2 > 0.94$). The dotted lines were fitted by nonlinear regression, using SAS, with $r^2 = 0.99$ for (a) and 0.97 for (b).
3.1 Introduction

Prostate cancer is the most common malignancy in man. The American Cancer Society estimated that 230,110 new cases and 29,900 prostate cancer-related deaths occurred in the United States in 2004 (American Cancer Society, 2004). Over the past decade, substantial improvements in diagnosis and staging of the disease have been made with the combined use of digital rectal examination, measurement of serum PSA levels, and transrectal ultrasound. Earlier diagnosis of prostate cancer has resulted in a stage migration, with an increased proportion of men diagnosed with organ-confined disease (Gleave et al., 1996; Stanford et al., 1996). It is imminent to provide the patients with more treatment options with less morbidities and high quality of life. Thus, effective nonsurgical treatments that can eradicate the localized tumors in the prostate represent an attractive alternative treatment. A biodegradable implant at the site of the action, which releases the drug locally at a controlled rate for a decided period of time would provide high local concentrations and also minimize the potential of side effects and toxicity (Domb et al., 1993; Friend and Pangburn, 1987; Poznansky and Juliano, 1984).
Paclitaxel has shown clinical efficacy against ovarian cancer, breast cancer, head and neck cancers and non-small cell lung cancer (Horwitz, 1992; Rowinsky and Donehower, 1995; Schiff and Horwitz, 1981). Paclitaxel enhances tubulin polymerization, promotes microtubule assembly and stabilizes microtubule dynamics, and thereby inhibits cell proliferation and induces apoptosis (Jordan et al., 1996; Schiff and Horwitz, 1981). Paclitaxel is a good choice for local drug delivery for prostate cancer because (a) of its demonstrated clinical activity of paclitaxel against systemic prostatic cancer (Hudes et al., 1997), (b) the highly binding of paclitaxel to macromolecules may result in high prostatic tissue concentrations, (c) it is a hydrophobic agent which may provide higher tissue penetration than a hydrophilic drug.

Last chapter described how we developed a biodegradable delivery device with a long cylindrical shape to enable prolonged regional paclitaxel administration to localized tumors. The long shape is preferred over a preparation of microspheres, which often escape in retrograde direction along the injection needle tract. In addition, the long shape might also enhance drug distribution throughout the target tissue. Among all the formulations screened from last chapter, we chose the formulation with lactose:PLGA (4:1) with or without isopropyl myristate (IPM) based on the release profile and physical property. This chapter describes paclitaxel distribution 10 days after cylinder implantation and the efficacy of intratumoral implantation by mini-cylinders. The animal model used in this study was subcutaneous PC-3 tumor bearing mice model. PC-3 is a human prostate adenocarcinoma cell line, which exhibits a low acid phosphatase and testosterone-5-α reductase activity. Although tumor-bearing
mouse does not directly model tumors localized in the prostate gland, it allows for the
determination of the efficacy of the formulation against localized human prostate
tumors and the distribution of the paclitaxel inside the tumor after implantation. The
tumor growth was measured and growth inhibition was compared between different
formulations. In this study, we also tested if paclitaxel pretreatment can enhance the
efficacy of paclitaxel loaded mini-cylinders, because our lab found that the cell density
can affect drug penetration (Jang et al., 2001).

3.2 Materials And Methods

3.2.1 Chemicals and reagents

Poly(DL-lactide-co-glycolide) (PLGA), with a 50:50 LA:GA ratio and an
inherent viscosity of 0.63 dl/g (measured in hexafluorospropanol), was purchased
from Birmingham Polymers (Birmingham, AL, USA). Paclitaxel was purchased from
Hande (Yunnan, China), IPM and β-lactose from Sigma Chemical Co. (St. Louis, MO),
and Matrigel was from BD biosciences (Bedford, MA). Other chemicals and reagents
for HPLC in highest grades were purchased from Fisher Scientific Co. (Fair Lawn,
NJ). Cephalomannine, an internal standard of paclitaxel for HPLC assay, was a gift
from NIH. All chemicals were used as received.

3.2.2 Preparation of paclitaxel-loaded PLGA cylinders.

Paclitaxel-loaded PLGA cylinders were prepared using the previously
described solvent extrusion and fluid energy micronization (FEM) methods with the
following modification. Briefly, PLGA and paclitaxel were dissolved in acetone. For
IPM-containing cylinders, IPM was also dissolved to form uniform solution. Lactose
was added last. The mixture was stirred to evaporate the extra solvent and the paste
was loaded into a 5-ml syringe and slowly extruded into the silicone tubing (Manostat, New York, NY) with 0.78 mm (1/32”) I.D. to form a cylindrical monolithic device. The extruded product was dried in a hood at room temperature for 24 hours, then in a vacuum oven at 45 °C for another 24 h before testing. The compositions of the preparations are listed in Table 3.2.

To generate release channels, Preparations F, H and I in Table 3.1 were incubated for one hour in the release medium composed of PBS, pH 7.4, and 0.02% Tween 80 (w/v). Preliminary studied showed the operation could dissolve most of the lactose and produce pores and channels, while only about 1% of paclitaxel was release into the medium. The cylinders were washed and lyophilized. All the cylinders were sterilized by γ irradiation at a dose of 25 kGy in the Ohio State University Research Reactor (OSURR) at the Nuclear Reactor Laboratory (NRL).

3.2.3 Animal protocol

Male 6-8 week old nude mice (BALB/c strain) were purchased from Charles River Laboratory (Montreal, Quebec, Canada). One million PC-3 cell suspended in 50 µl of RPMI 1640 were mixed with 50 µl of Matrigel and injected via 27-gauge needle subcutaneously into the flank region of and anesthetized mouse.

3.2.4 HPLC analysis of paclitaxel

Paclitaxel was analyzed using our previously published method with minor modification (Song and Au, 1995). Briefly, the internal standard, cephalomannine 2 µg/100 µl in methanol was mixed with 100 µl of plasma or 40 mg of tissue. The plasma samples were extracted twice with 1 ml of ethyl acetate. The mixtures
containing tumor tissue were homogenized in 4 ml ethyl acetate with a homogenizer (Tekmar Co., Cincinnati, OH, USA) for 1 min. The probe of the homogenizer was then washed with another 4 ml of ethyl acetate for 15-20 sec. The extracts were centrifuged at 3,000 rpm for 10 min. The supernatant was transferred to another tube and evaporated to dryness under nitrogen. The residue was reconstituted in 100 µl of acetonitrile and analyzed using column-switching HPLC.

The HPLC stationary phase consisted of a clean-up column (Novapak C8, 75×3.9 mm ID, 4 µm particle size from Waters Associated, Milford, MA) and an analytical column (Bakerbond C18, 250×4.6 mm ID, 5 µm particle size from J.D. Baker, Phillipsburg, NJ). Samples were injected into the clean-up column and eluted with clean-up mobile phase consisting of 37.5% acetonitrile in water at a flow rate of 1 ml/min. Concurrently, the analytical mobile phase consisting of 49% acetonitrile was directed through the analytical column at a flow rate of 1.2 ml/min. The fraction from 8-15 min containing paclitaxel and cephalomannine was transferred from the clean-up column into the analytical column. The limit of sensitivity for paclitaxel was 10 ng/ml.

3.2.5 Implantation of cylinders

For drug distribution study, when PC-3 tumor volume reached 0.5 cm³, the mice were anesthetized and 8mm of cylinder was implanted by a 17-gauge trocar parallel to the skin. Tumor weight was measured twice a week.

3.2.6 Drug distribution study.

Preparation F was used for this study. Cylinders were implanted as described in last section. After 11 days, the mice were sacrificed; blood samples were withdrawn by
cardiac puncture. The tumors were excised and cut into two halves, perpendicular to cylinder. One half was fixed in 10% neutral buffered formalin and embedded in paraffin, sectioned by microtome. The slides were stained with hematoxylin-eosin, followed by dehydration and coverslipping for microscopic examination. The other half was frozen and sectioned. The tissue samples were pooled by the distance away from the cylinder, as illustrated in Figure 3.1.

3.2.7 Tumor growth inhibition study

When the tumors reached around 300 mm$^3$, the animals were divided into 6 groups. The descriptions of treatment for each group are listed in Table 3.2. The length and width of tumor were measured using a caliper and the tumor volume was calculated by: $(\text{tumor length}) \times (\text{tumor width})^2/2$. Group 1 and 2 were pretreated with Taxol 24 hours before cylinder implantation. A dose of 10 mg/kg was given through tail vein. Group 1 was given blank cylinder (Preparation I) and Group 2 received paclitaxel loaded cylinder with IPM (Preparation H). Group 3, 4 and 5 were given normal saline, i.v. bolus. Twenty four hours later, 3 different cylinders were implanted as shown in Table 3.2. The effect of pretreatment, Paclitaxel-IPM-cylinders and also IPM could be studied by comparing the tumor growth in different groups. The comparisons are listed in Table 3.3.

3.2.8 Statistical Analysis

Statistical significance for the tumor growth rate was assessed by ANOVA for repeated measures using SAS software (SAS Institute, Cary, North Carolina).
3.3 Results

3.3.1 Drug release and distribution form cylinder implants

After 11 days of implantation, large necrotic area was found around cylinder (indicated by A in Figure 3.1). The cells in this area have no nuclei, and no cell structure. The necrotic area extended to about 3mm from the cylinder. Next to that region were some condensed cells and apoptotic cells (area B). This area extended to about 5mm. Farther away was healthier cells (area C). The results show efficacy of this administration form for local chemotherapy against prostate cancer, which is limited to a highly localized distribution area.

After 11 days of implantation in nude mice, the plasma concentration was below detection limit (10 ng/ml). Drug distribution in the tumor was shown in Figure 3.2. Tissue concentrations after 10 days of release (~10% or 35 mcg paclitaxel released) declined from 23 ± 5 mcg/g at 0-2 mm from the cylinder, to 0.7 ± 0.1 mcg/g at 2-5 mm, to <0.02 mcg/g at distances >5 mm from the cylinder. 1.25 ± 0.56% of loaded drug was found in tumor tissue. The amount of residue drug was measured, 98.0 ± 5.3% of loaded drug found in residue cylinders.

3.3.2 Tumor growth inhibition by paclitaxel-loaded mini-cylinders

Figure 3.3 shows tumor growth curve for six groups of animals as listed in Table 3.2. Solid cylinder (Preparation A) did not inhibit tumor growth compared to the control group, Group 3. Group 2, 4 and 5 observed tumor growth inhibition. Follow-up study showed there were 2 mice in both 2 and 4, and 1 mouse in group 5 were cured. This suggested that paclitaxel-loaded cylinders could inhibit tumor growth. Group 2 and 4 received paclitaxel-IPM-cylinder implantation, while Group 2 was given
systemic Taxol 24 hours before the implantation and Group 4 was given saline. For the first a few days, tumors in Group 2 with pretreatment had shrunk more than Group 4. In the following period of time, tumor volumes in Group 2 were observed to be smaller than tumor volumes in Group 4, although the growth curves showed similar trends. Compared to Groups 2 and 4, animals in Groups 5 were implanted with paclitaxel-cylinder (without IMP). The tumors volume decreased for the first three weeks and then began to increase. The tumor shrank much less than Groups 2 and 4. The tumor volumes in Group 1 and 3 kept increasing for all the experimental period. Animals in Groups 1 and 3 were pretreated by Taxol and saline, respectively. And both groups were implanted with blank IPM-cylinders. There were no significant difference between the two groups. The difference between groups was compared by ANOVA for repeated measurement and the result was listed in Table 3.3.

3.3.3 Host toxicity

Animal toxicity was monitored by body weight changes. For all the groups, the body weights fluctuated within 10% of the original weight (Figure 3.4). The biggest body weight drop was the first 4 days. This might due to anesthetization and surgeries.

3.4 Discussion

Research has been done for the suppression of human prostate tumor growth in mice by the intratumoral injection of a slow-release polymeric paste formulation of paclitaxel (Jackson et al., 2000). In the treatment group, tumors decreased in volume from a mean value of 43mm$^3$ to nonpalpable, compared with the control group, in which tumors increased from 30 to 1000mm$^3$. In this study, the penetration of the drug was not a big problem since tumor volume was small when the mice received
treatment. However, in real case, drug need to penetrate longer distance than 3-5 mm to take effect. The study did not show the penetration of the drug. Our results suggested that the penetration of the drug need to be enhance to improve therapeutic effect.

Although in vitro study showed that about 15% drug was released in vitro within 11 days, the amount of drug released of the in vivo study was far less than that. This might be due to the limited amount of liquid inside tumor. The released drug was not removed fast enough and a saturated solution was formed surrounding the cylinder, which limited drug release. Thus, caution should be taken when correlating *in vitro* studies and *in vivo* studies.

It has been shown that systemic treatment with paclitaxol can decrease the tumor cell density and enhance drug penetration (Jang et al., 2001). In this study, comparison between groups with pretreatment and without pretreatment (Groups 2 and 4) showed that with pretreatment, tumors shrank faster in the first a few days. This may be due to the pretreatment caused lower cell density during the first a few days, so that drug released from cylinders can have better penetration. The other reason might be the pretreatment can delay tumor growth for a short period of time. After the systemic paclitaxel were cleared, both groups showed similar growth rate.

IPM has been widely used as a vehicle in many cosmetic and pharmaceutical preparations because of its skin penetration enhancing property, its safety and its compatibility with a wide range of compounds. Studies in Chapter 2 showed co-encapsulation of IPM can lead to a faster drug release from PLGA cylinder. This might be due to the decreased transition temperature and co-solvent effect by IPM (Blanco and Alonso, 1998; Sansdрап and Moes, 1998).
It has been reported IPM increase permeation of drugs through skin mainly by increasing diffusion (Suh and Jun, 1996). One of the proposed mechanisms is the so-called lipid fluidizing effect (Leopold and Lippold, 1995). By inserting into intercellular lipids, IPM makes the environment less rigid for drugs to penetrate. It has also been suggested that IPM can interact with drug-binding proteins, which increases free fraction of the drug and thereby increases penetration. Although IPM has been widely studied for its skin penetration enhancing effect, little study has been done to elucidate if it can enhance drug penetration inside tumor. Whether IPM can also increase paclitaxel penetration in tumor is something worth studying.

3.5 Conclusion

In this chapter, the penetration of paclitaxel from cylinders was studied at 11 days after implantation. It was found that tissue concentrations declined from $23 \pm 5$ mcg/g at 0-2 mm from the cylinder, to $0.7 \pm 0.1$ mcg/g at 2-5 mm, to $<0.02$ mcg/g at distances $>5$ mm from the cylinder. The concentration in plasma was below detection limit (5ng/injection). Histological evaluation showed extensive necrosis extending to $\sim 3$ mm from the cylinder, with increased apoptosis up to $\sim 4$ mm from the cylinder. The porous, but not solid, paclitaxel-loaded cylinders inhibit tumor growth and incorporation of IPM enhanced the efficacy greatly, although the mechanism is not clear. Systemic pretreatment with Taxol showed further enhanced treatment efficacy.
Table 3.1  **Composition of Materials Used a Series of Preparations.** Cylinders were prepared by solvent extrusion method. The composition of PLGA is 50/50, ratio of lactide to glycolide.
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
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<tr>
<td>1 (n=5)</td>
<td>i.v. Taxol + Preparation I</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>i.v. Taxol + Preparation H</td>
</tr>
<tr>
<td>3 (n=4)</td>
<td>i.v. normal saline + Preparation I</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>i.v. normal saline + Preparation H</td>
</tr>
<tr>
<td>5 (n=5)</td>
<td>i.v. normal saline + Preparation F</td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>i.v. normal saline + Preparation A</td>
</tr>
</tbody>
</table>

Table 3.2 Description of treatments for tumor growth inhibition study.
Table 3.3  **Statistic comparisons between groups.** The significance was assessed by ANOVA for repeated measures using SAS. The asterisk (*) denotes significant difference between the two groups (α < 0.05)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>F Value</th>
<th>Pr &gt; F</th>
<th>Effect</th>
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<td>31.6</td>
<td>&lt;.0001</td>
<td>Porous cylinder treatment with pretreatment</td>
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<tr>
<td>Group2 Group4</td>
<td>3.43</td>
<td>0.083</td>
<td>Taxol pretreatment</td>
</tr>
<tr>
<td>Group3 Group4*</td>
<td>15.7</td>
<td>&lt;.0014</td>
<td>Porous cylinder treatment</td>
</tr>
<tr>
<td>Group4 Group5*</td>
<td>5.82</td>
<td>0.028</td>
<td>IPM</td>
</tr>
<tr>
<td>Group1 Group3</td>
<td>0.14</td>
<td>0.72</td>
<td>Taxol treatment</td>
</tr>
<tr>
<td>Group3 Group6</td>
<td>0.56</td>
<td>0.47</td>
<td>Solid cylinder treatment</td>
</tr>
</tbody>
</table>
Figure 3.1  H.E. staining for PC-3 s.c. tumor after 10 day’s cylinder implantation (preparation G). Arrow indicates where the cylinder was implanted. b, c and d are magnified pictures for area A, B and C.
Figure 3.2  Paclitaxel distribution inside tumor 10 days after implantation.
Figure 3.3  PC-3 s.c. tumor growth inhibition by cylinder implantations. Group 1 (filled circle), pretreated with Taxol at a dose of 10 mg/kg given through tail vein. Twenty four hour later, blank IPM-cylinders were implanted. Group 2 (open circle), same pretreatment, implanted with Paclitaxel-IPM-cylinders 24 hours later. Group 3 (filled triangle), pretreated with saline, implanted with blank IPM cylinders. Group 4 (open triangle), pretreatment with saline, implanted with Paclitaxel-IPM-cylinders. Group 5 (filled square), pretreatment with saline, implanted with Paclitaxel-cylinders. Tumors were measured twice a week using calipers, and their volume were calculated by the following formula: width$^2 \times$ length / 2.
Figure 3.4  No toxicity from cylinders observed in terms of body weight. Changes in body weight of the animals were expressed as percentage of initial body weight. Animals with well established, subcutaneously implanted PC-3 tumors were randomized and pretreated with either 10 mg/kg of Taxol (Group 1 and 2) or saline (Group 3, 4 and 5). Cylinders were implanted twenty four hours later, as denoted in the legend.
CHAPTER 4
INTERSPECIES PHARMACOKINETIC SCALING OF LOW DOSE SURAMIN
AS A CHEMOSENSITIZER

4.1 Introduction

Suramin is an aromatic polysulfonated compound that has been evaluated since the 1980’s as an anticancer agent, and has shown anti-tumor activities in several malignancies, most notably in prostatic carcinoma (Myers et al., 1990; Tkaczuk et al., 1992; Tu et al., 1998). In the preclinical studies, suramin’s antiproliferative activity was dose dependent and less effective at concentrations of 50 micromolar ($\mu$M) or less of continuous exposure. Based on these findings, most studies evaluating suramin as a therapeutic agent in humans have targeted concentrations of 200 $\mu$M or higher. At these doses, suramin appears to have modest to moderate anticancer activity in hormone refractory prostate carcinoma (Calvo et al., 2001; Eisenberger and Reyno, 1994; Rosen et al., 1996; Small E et al., 2000), marginal activity in recurrent high-grade gliomas (Grossman et al., 2001), and no or insignificant activity in non-small cell lung (Mirza et al., 1997), breast, colorectal (Falcone et al., 1995) and renal carcinomas (Dreicer et al., 1999). However, severe neurological toxicity (sensory motor axonal and progressively disabling demyelinating neuropathy) is generally associated with steady state plasma
concentrations of 275 µM or higher (Bitton et al., 1995; Chaudhry et al., 1996; Stein et al., 1989). Therefore, careful clinical pharmacokinetics studies have been performed to find a strategy to circumvent the problems of seemingly unpredictable pharmacokinetic behavior and narrow therapeutic range for suramin (Kobayashi et al., 1995; Reyno et al., 1995; van Rijswijk et al., 1992).

In contrast to the high concentrations of suramin required for its antiproliferative activity, inhibition of some growth factors to their receptors occurs at substantially lower concentrations. For example, basic FGF (bFGF), a growth factor that has been implicated in neovascularization and tumor growth (Danesi et al., 1993; Pesenti et al., 1992) is inhibited at suramin concentrations below 50 µM (Song et al., 2000). Au and collaborators recently reported that elevated levels of acid FGF (aFGF) and bFGF in solid and metastatic tumors confers broad spectrum resistance to chemotherapy drugs with diverse structures and mechanisms of action, and that low concentrations/doses of suramin that are devoid of antitumor activity (or toxicity) reverse the FGF-induced chemoresistance in vitro (Song et al., 2000; Zhang et al., 2001). In addition, in mice with well established lung metastases, low doses of suramin (10 mg/kg, twice weekly x 3 weeks) yielding plasma concentrations of between 10 to 20 µM, enhanced the antitumor effect of paclitaxel; the combination resulted in tumor eradication in 42% of animals compared to zero in paclitaxel alone group, a 9-fold greater reduction of the density of nonapoptotic cells and a 30% increase in the apoptotic cell fraction (Song et al., 2001). Suramin is currently under investigation in a Phase II trial as an enhancer of the

Because the concept of using low dose suramin as a chemosensitizer was not reported by Au’s lab until recently, no pharmacokinetics studies of low dose suramin were reported in either humans or animals. In this study, the pharmacokinetics of low dose suramin was studied in mice, rats and dogs. The dose given to the animals in this study ranged from 6.75-10 mg/kg.

The objective of this study was to develop dosing regimen for suramin in humans to achieve a low concentration range of $10 – 50 \mu M$. Two pharmacokinetic scaling plots were employed to predict suramin concentration profile in human based on the animal studies, the complex Dedrick plot and the dienetichron plot. In pharmacokinetics, the physiological time scale and parameters are usually obtained by transforming clock time and absolute physical parameters into forms that become species invariant. This concept was first applied by Dedrick et al. to methotrexate in his elementary Dedrick plot, in which both time and drug plasma concentration were transformed based on allometric exponents for clearance and steady state volume of distribution, in respect to body weight (Bischoff et al., 1970). One of the limitations in the elementary Dedrick plot, which requires the exponent of allometric equation to be unity, was lifted in the complex Dedrick plot developed by Boxenbaum etc. (Boxenbaum and Ronfeld, 1983) and thereby broadened the application of interspecies scaling. However, there was still a big portion of drugs, for which a lack of good allometric relationship across species existed. It was found that lifespan of species is correlated with metabolic rate and drug disposition.
(Denckla, 1975). As shown in this study, the product of CL and maximum lifespan potential (MLP) has a better allometric relationship than CL alone in the correlation with body weight, suggesting the disposition and elimination of suramin to be dependant on MLP. By adjusting the clock time with MLP, the dienetichron plot gave a better prediction of suramin concentration profile in human than the complex Dedrick plot, as shown by our phase I study in NSCLC patients.

There are a few applications for this study. Firstly, suramin has a long half life, essentially no \textit{in vivo} metabolism (Collins et al., 1986). The major elimination route for suramin is through renal excretion. Allometric relationships for suramin established in this study provide insights into the pharmacokinetic behavior of this type of drugs. Secondly, as bFGF is widely expressed in some animal neoplasms, suramin is a potential chemosensitizer for chemotherapy in small animals. The interspecies study should provide basis for the development of dosing regimen in new species for veterinary oncology practice. The good allometric relationship of distribution and elimination parameters of suramin should also provide solid ground for later scaling of the physiology-based pharmacokinetics model in animals to humans and thereby to predict tissue concentration profile in humans.
4.2 Materials and Methods

4.2.1 Chemicals and reagents

Suramin sodium used in mice, rats and dogs were purchased from Sigma, as 500 mg powder in 10-ml vials. The vials were reconstituted with 0.9% sodium chloride and further diluted to the desired dose, then filtered with 0.22 µm sterile syringe filter from Millipore (Bedford, MA). The suramin used in human patients was supplied by the NCI, Division of Cancer Treatment and Diagnosis (DCTD) as sterile power, 600 mg in 10-ml vials. The vials were reconstituted with sterile water, resulting in a 100 mg/ml solution. The dose was further diluted to the desired concentration with 0.9% sodium chloride or 5% dextrose in water.

4.2.2 Intravenous kinetics studies in mice

Female BALB/c nu/nu mice were purchased from Charles River Laboratory. All mice were given food and water ad libitum, and were cared for according to institution guidelines. One million HT-29 cells suspended in 100 µl of normal saline were injected via 27-gauge needle subcutaneously into the flank region of anesthetized mice, when they were 4-5 weeks old. Suramin solution was given when the mice were 7-8 weeks old and the tumor volume was about 350 mm³. The pretreatment body weights were 18.2 ± 2.0 g. The mice were randomized based on body weight and tumor sizes. A suramin dose of 10 mg/kg was administered intravenously via a tail vein. The concentration of the dosing solution was 1 mg/ml and the dosing volume ranged from 160 to 200 µl. Blood samples were collected via cardiac puncture with the animals under anesthetization at the following times after dosing: 0.1, 0.5, 1, 2, 4, 6, 24, 72 and 168 h (n=4 or 5 for each time
point). The samples were centrifuged and the plasma was used for high performance liquid chromatography (HPLC) analysis.

4.2.3 Intravenous kinetics studies in rats

Four male Copenhagen rats (224 ± 14 g) from Charles River Laboratory were anesthetized with ether through inhalation. The carotid artery of rats was catheterized as described in the literature (Jang et al., 2001). Animals were allowed to recover for 4 to 5 h and then given a suramin bolus dose of 10 mg/kg intravenously through a tail vein. Blood samples were collected at the following times after dosing: 0.083, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 168 h. The samples were centrifuged and the plasma was used for HPLC analysis.

4.2.4 Intravenous kinetics studies in dogs

Four beagles donated by Battelle (Columbus, OH) were used, weighing 11.4 ± 0.4 kg. The dogs were housed for one week before the experiment. All animals were fasted overnight, but allowed access to water. The cephalic veins on both legs were catheterized, one for drug infusion and the other for blood sampling, so as to prevent drug contamination. A suramin dose of 6.75 mg/kg was infused over half an hour. The drug solution was 2.7 mg/ml and the dosing volume ranged from 27.5 to 29.5 ml. At each sampling time point, 3 ml of blood was taken and the same volume of heparinized saline was injected back. Blood samples were collected at the following times after the infusion: 0.083, 0.5, 1, 2, 4, 6, 9, 12, 24 hrs and 2, 3, 7, 14, and 21 days. Samples were centrifuged and the plasma was used for HPLC analysis.
4.2.5 **Intravenous kinetics studies in human patients**

We finished a Phase I study of low dose suramin as a chemosensitizer in patients with advanced non-small cell lung cancer (NSCLC). The details of the treatment protocol can be found in the literature (Villalona-Calero et al., 2003). Briefly, the treatment was administered on an inpatient or outpatient basis, in 3-week cycles. The drug administration schedule was as follows, a 30 min infusion of suramin, followed immediately by a 3-hr infusion of paclitaxel (starting at 175 mg/m² and escalated to 200 mg/m² after the suramin dose was established), and then a 1-hr infusion of carboplatin. The carboplatin dose was calculated using the Calvert equation (Calvert et al., 1989) to yield an AUC (area under the plasma concentration-time curve) of 6 mg-min/ml. In the first 6 patients, the initial suramin dose during the first treatment was 240 mg/m², given as a single dose. The initial target suramin concentrations were between 10 to 50 µM over 72 hr following the initiation of suramin infusion. Results in these patients indicated that while 5 of 6 patients achieved the target suramin concentration of 10-20 µM at 72 hr, all 6 patients showed peak levels exceeding 50 µM. We also found that most of the AUC of paclitaxel and carboplatin was attained in the first 48 hr (i.e., >92% and >99% of their total respective AUC) (manuscript in preparation). The target duration of suramin concentrations were then adjusted to 10-50 µM over 48 hr following the initiation of suramin infusion, which was the duration when paclitaxel and carboplatin are present at therapeutically significant levels. This goal was achieved by giving the total suramin dose in two split doses, with two-thirds of the dose given on the first day and the remaining one-third given 24 hr later. Blood samples were collected at 1.5, 3.5, 12, 24, 48, 72 hr...
after infusion, 3 days before next treatment, and right before next treatment. Samples were centrifuged and plasma was used for HPLC analysis.

**4.2.6 Extraction of suramin from plasma**

Suramin in plasma was extracted with previously published method with minor modifications (Kassack and Nickel, 1996). Briefly, 100 µl plasma was mixed with 100 µl of 0.5 M tetrabutylammonium bromide (pH 8.0), 20 µl of internal standard (trypan blue, 100 µg/ml), and vortexed for 30 sec. After the addition of 300 µl of acetonitrile and vortexing for 30 sec, the samples were stored at 4°C for at least 2 hr. The samples were then centrifuged at 1,000 g for 5 min. The supernatant was analyzed by HPLC. The extraction yield was >90%.

**4.2.7 HPLC analysis of suramin**

Suramin concentrations were determined using a previously published HPLC method with minor modifications (Kassack and Nickel, 1996). Briefly, HPLC was performed on an Agilent 1100 series chromatography system: Agilent quaternary pump G1311A equipped with degasser G1379A, autosampler G1313A and UV detector G1314A. Recording and processing of detector signals were performed on a HP computer KAYAK XM600 model with the HP HPLC ChemStation Rev. A.09.03. The analytical column used Agilent 100 × 2.1 mm I.D., and the guard column used Agilent 20 × 2.1 mm I.D., both filled with MOS Hypersil 5 µm (RP-8). The capillaries were 0.25 mm I.D. The effluent was a mixture of 80% A and 20% B. A was a mixture of 100 ml of methanol and of 400 ml of 0.02 M phosphate buffer (pH 6.5) containing 6.25 mmol/l of tetrabutylammonium hydrogensulfate. B was 100% methanol. Linear gradient conditions
were as follows: 0 min: A=80%, B=20%; 0-8 min: A=46.4%, B=53.6%; 8-12 min: A=80%, B=20.0%. A flow-rate of 0.6 ml/min was maintained. UV detection at 330 nm was applied. The detection limit of suramin was 0.2 µg/ml of plasma.

### 4.2.8 Pharmacokinetic analysis

Pharmacokinetic parameter estimation was accomplished using WinNonLin version 4.0 (Pharsight Co. software, Mountain View, CA). Total body clearance (CL), steady state volume of distribution (Vss) and terminal half lite (\(T_{1/2, \text{term}}\)) were derived from the model-dependent parameters.

### 4.2.9 Interspecies scaling

Interspecies scaling of Vss and AUC employed simple allometric method only. The simple allometric equation was written as follows:

\[
Y = aW^b \quad \text{Eq. 4.1}
\]

where \(Y\) was the parameter of interest, \(W\) was body weight, \(a\) and \(b\) were the coefficient and exponent of the allometric equation, respectively. The log transformation of equation (1) was represented as follows:

\[
\log Y = \log a + b \log W \quad \text{Eq. 4.2}
\]

where \(\log a\) was the y-intercept, and \(b\) was the slope.

Scaling of CL employed two methods, the first one being same as above. The other approach incorporated maximum life-span potential (MLP) in the equation, which was described as follows:

\[
CL \times MLP = aW^b \quad \text{Eq. 4.3}
\]

The log transformation of equation (3) was written as follows:
Log (CL*MLP) = log a + b log W  

Pharmacokinetic parameters, Vss, AUC, CL and (CL*MLP) were plotted on a log-log scale against body weight (W). The linear regression of the logarithmic values was calculated by the least-squares method to obtain the coefficient (a) and exponent (b) (Boxenbaum, 1984).

### 4.2.10 The dienetichron plot and complex dedrick plot

The dienetichron plot was based on allometric exponents of CL*MLP and Vss as described by Boxenbaum (Boxenbaum, 1984). The concentration and time points from animal species were transformed as $C^*$ and $T^*$:

$$C^* = \frac{C_{\text{animal}}}{(Dose_{\text{animal}} / W_{\text{animal}})^{b'}}$$  \hspace{1cm} \text{Eq. 4.5}

$$T^* = \frac{T_{\text{animal}}}{(MLP_{\text{animal}} \times W_{\text{animal}})^{b'-b}}$$  \hspace{1cm} \text{Eq. 4.6}

where $b$ and $b'$ were allometric exponents relating CL*MLP and Vss with body weight, respectively, across three species, mice, rats and dogs. Concentrations of suramin in humans were obtained by follows:

$$C_{\text{human}} = C^* \times \frac{Dose_{\text{human}}}{W_{\text{human}}^{b'}}$$  \hspace{1cm} \text{Eq. 4.7}

$$T_{\text{human}} = T^* \times MLP_{\text{human}} \times W_{\text{human}}^{b'-b}$$  \hspace{1cm} \text{Eq. 4.8}

All the points of $C_{\text{human}}$ with corresponding $T_{\text{human}}$ were fitted to the biexponential disposition with WinNonLin program.

For the purpose of comparison, the complex Dedrick plot, a plot without MLP, was performed. The complex Dedrick plot was chosen instead of the elementary Dedrick because the exponent in equation (1) for $V_{\text{ss}}$ was not equal to 1 (Boxenbaum, 1984). The concentration and time points from animal species were transformed as $C^*$ and $T^*$:
\[ C^* = \frac{C_{\text{animal}}}{(Dose_{\text{animal}} / W_{\text{animal}}^{b'})} \]  \hspace{1cm} \text{Eq. 4.9}\\
\[ T^* = \frac{T_{\text{animal}}}{( W_{\text{animal}}^{b' - b})} \]  \hspace{1cm} \text{Eq. 4.10}\\

where \( b \) and \( b' \) were allometric exponents relating \( Cl \) and \( V_{ss} \) with body weight, respectively, across three species, mice, rats and dogs. The concentrations of suramin in humans were obtained as follows:

\[ C_{\text{human}} = C^* \times \frac{\text{Dose}_{\text{human}}}{W_{\text{human}}^{b'}} \]  \hspace{1cm} \text{Eq. 4.11}\\
\[ T_{\text{human}} = T^* \times W_{\text{human}}^{b' - b} \]  \hspace{1cm} \text{Eq. 4.12}\\

All the points of \( C_{\text{human}} \) with corresponding \( T_{\text{human}} \) were fitted to the biexponential disposition with WinNonLin program.
4.3 Results

The goodness of fit by two- and three-compartment models was compared. Although a three-compartment model gave a better fit based on Akaike Information Criterion and the Schwartz Criterion, both models yielded similar total body clearance of suramin (<15% difference). In addition, the detection limit and the treatment protocol have limited sampling time for decent estimation of terminal half-life in a three-compartment model. Thus, a two-compartment model was used in this study.

4.3.1 Pharmacokinetics Study in Mice, Rats and Dogs

Suramin plasma concentration profiles in mice, rats and dogs are depicted in Fig. 1., and pharmacokinetic parameters are listed in Table 1. In all three species, the peak plasma concentrations (C_max) were above 50 µM. In mice, C_max at 5 min was 94 µM and it dropped to 30 µM after half an hour. The half-lives of distribution phase and elimination phase were 0.29 hour and 1.4 days, respectively. In rats, similarly, C_max at 5 min after i.v. injection was 160 µM and it dropped to 51 µM in 4 hr. The half-lives of distribution phase and elimination phase were 3.1 hours and 6.1 days, respectively. Suramin was given by i.v. infusion in dogs, resulting in a lower peak concentration of 62 µM at 5 min after infusion. The half-lives of distribution phase and elimination phase were 3.5 hours and 8.8 days, respectively.

4.3.2 Pharmacokinetics Study in Human Beings

A total of 85 treatment cycles were given to 15 patients. Three patients were taken off protocol within two days after the first treatment cycle. The first 12 patients received 240 mg/m² for the first cycle. The average dose for subsequent cycles was 158 ± 41
mg/m². The loading doses for the other 3 patients were calculated using an empirical equation, which was based on the first 12 patients’ plasma concentration-time profiles and developed by NONMEM population pharmacokinetics analysis. Details can be found elsewhere (manuscript in preparation). Fig. 2 shows the plasma concentration-time profiles of suramin during the first cycle of treatments in the first 12 patients. Pharmacokinetic parameters were estimated from all cycles of the 13 patients who received more than 1 treatment cycle and are listed in Table 1. When suramin was given in single doses, the average peak concentration was 77 µM, measured 1 hr after infusion and dropped to 62 µM in 2 hr, and simulation showed tha concentration would drop below 50 µM within 3 hr. When suramin was given in split doses, the average peak concentration was 49 µM after the first dose and 43 µM after the second dose. Compared to other high dose suramin pharmacokinetics, low dose suramin has a bigger total body clearance (0.025 L/hr/m² vs. 0.013² L/hr/m (Jodrell et al., 1994), 0.0046 L/hr vs. 0.0062 L/hr (Chijioke et al., 1998)), smaller steady state volume of distribution (8.5 L/m² vs. 13.6 L/ m² (Jodrell et al., 1994), and 16 L vs.4 20.6 L (Chijioke et al., 1998)), and a shorter terminal half life (11 days vs. 41 days (Jodrell et al., 1994) and 92 days (Chijioke et al., 1998)).

4.3.3 Interspecies Scaling of Low Dose Suramin Pharmacokinetics

The exponents for allometric scale of CL, Vss and CL*MLP across three animal species were used for the dienetichron plot and the complex Dedrick plot to predict the human plasma concentration-time profile. The predicted concentration-time profile in humans was comparable with that observed in the first 12 patients with the loading dose
of 240 mg/m² in our Phase I trial (fig. 3). The model-predicted $t_{1/2,\alpha}$ and $t_{1/2,\beta}$ to be 7.4 hours and 11 days, respectively, deviating from the observed average values by 7.5% and 39%. CL and Vss were estimated to be 0.037 L/hr/m² and 13 L/ m², respectively, deviating from the observed mean values by 42% and 9.2%. Agreement between predicted and observed values indicated the validity of the approach.

The complex Dedrick plot predicted plasma concentration-time profile less well than the dienetichron plot (Fig. 4). The $t_{1/2,\alpha}$ and $t_{1/2,\beta}$ predicted by this method were 5.1 hours and 9.0 days, respectively, deviating from the observed average values by 36% and 50%. The clearance was 0.056 L/hr/m², and steady state volume of distribution was 15 L/ m². The deviations from observed values were 114% and 31%, respectively. The AIC value was 343 for the dienetichron plot, and 962 for the complex Dedrick plot, showing significant difference (p<0.0001) (Yamaoka et al., 1978).

After the pharmacokinetic parameters of suramin in humans were obtained, interspecies scaling across four species, i.e. mice, rats, dogs and humans, was attempted. CL, Vss and AUC showed good allometric relationships to body weight (Fig. 5), with r-square values of 0.971, 0.995 and 0.971 respectively. The correlation was further improved when MLP was incorporated in the scaling of CL ($r^2=0.984$), suggesting MLP an important parameter in interspecies scaling for suramin.
4.4 Discussion

The prediction of human responses to drugs from data obtained in animals can be of considerable significance in the process of drug development. Despite criticism (Heusner, 1987), interspecies scaling has been successfully used to predict the pharmacokinetic parameters from animals to human. In this study, interspecies scaling were applied successfully for dosing regimen design.

When we designed our Phase I trial of suramin with NSCLC patients, we set the target concentration as 15 µM at 72 hours. The initial dose was derived from the literature (Jodrell et al., 1994), and confirmed by interspecies scaling. The confirmation was necessary because nonlinear pharmacokinetics was observed in rats for suramin (McNally et al., 2000). In a previous report, C\textsuperscript{14}-suramin was given to rats at a dose of 300 mg/kg and the disposition of suramin was studied by auto-radiography. Comparing suramin pharmacokinetic parameters from their study with our study, the CL was 33% lower, the Vss and terminal half life were 4 and 6 fold higher, respectively. Using the dienetichron plot, it was predicted that suramin peak concentration should be over 50 µM if a single dose was given, and should drop below 50 µM in 2 hr, while the peak concentration should be below 50 µM if a split dose was given. Same as predicted by the dienetichron plot, peak concentration higher than 50 µM was observed in our first 6 patients in Phase I trial. This problem was avoided by giving split doses in all the other patients. The dienetichron plot successfully extrapolated pharmacokinetics of low dose suramin from animal species to human beings.
It has been reported that suramin was essentially unmetabolized \textit{in vivo} (Collins et al., 1986), although very low levels of highly polar metabolites were recovered in bile in an isolated perfused rat liver study (Coleman and Adjepon-Yamoah, 1986). Urinary excretion accounted for elimination of most of the drug (Collins et al., 1986). Interspecies scaling of CL of drugs appears to be relatively successful for the drugs which are mainly excreted via kidney (Mordenti, 1985; Paxton et al., 1990). On the contrary, scaling of the CL among species has been less successful if drug metabolism is the primary route for elimination. This may be because species differ in the nature of metabolites and also in the rate and extent of metabolism (Dedrick and Bischoff, 1980). Good correlations between the log Cl of suramin and the log W further confirms this observation.

Clearance is the most important pharmacokinetic parameter for characterizing drug removal from the body. However, clearance cannot be accurately predicted for all drugs using the simple allometric equation (Heusner, 1987). Over the years many theories and different approaches have been proposed to address this issue. One approach is to predict clearance on the basis of species weight and MLP. Another approach uses both body weight and brain weight. Mahmood and Balian proposed the rule of exponents, trying to standardize different approaches (Mahmood and Balian, 1996). Based on this rule, if the exponent of CL in the simple allometry equation is less than or equal to 0.70, simple allometry is to be used for the prediction of clearance. The MLP approach is taken when the exponents of the simple allometry are between 0.71 and 0.99, and brain weight is taken when the exponents of simple allometry are $\geq 1.0$. In this study, the exponent of simple allometric equation for clearance of suramin across mice, rats and dogs was 0.77,
consistent with this rule. Retrospective scaling across four species showed higher R-
square value for the CL x MLP approach than CL alone (0.984 vs. 0.971). Besides total
clearance, many investigators have attempted to predict the unbound clearance of drugs.
The rationale for predicting unbound rather than total clearance is that plasma protein
binding of many drugs varies considerably from one species to another. As a result, the
distribution and elimination of drugs may be variable in different species. However, after
Mahmood reviewed 20 drugs and compared the two approaches, he concluded unbound
clearance could not be predicted any better than total clearance (Mahmood and Balian,
1996). Besides this reason, this study did not try to predict the unbound clearance because
of the concern of very low fraction of free suramin (0.44% in human plasma). When the
free drug fraction is so low, as in this case, a little fluctuation in free fraction would result
in big difference in pharmacokinetic parameters.

Among all the various pharmacokinetic parameters studied in the literature, the
volume of distribution often yields the most statistically significant allometric
relationship (Paxton et al., 1990). For many drugs, the exponent (b) of the allometric
equation for the volume of distribution approaches unity as observed in the present study,
0.991. In other words, the volume of distribution is often approximately proportional to
body weight (Boxenbaum, 1984).

The interspecies scaling has wide application in veterinary clinical practice.
Cancer is one of the major causes of death in pet animals. Chemotherapy plays an
important adjuvant role among various cancer treatments and has been proved to prolong
patients’ median survival time (Berg et al., 1992; Berg et al., 1995; Hammer et al., 1991;
Ogilvie et al., 1996). It has been reported bFGF is expressed in small animals malignancies and presents a prognostic factor for treatment outcome (Allen et al., 1996). Veterinarians have shown strong interests in testing the chemosensitization effect of suramin in dogs and cats (personal communications) because of the promising results of our Phase I trial in NSCLC patients (Villalona-Calero et al., 2003). Findings from this study should help to design dosing regimen of suramin in new species.

In summary, interspecies scaling of the pharmacokinetics of suramin was attempted by using data from three animal species and extrapolating to humans. The dienetichron plot provided a useful tool to determine the dose in our Phase I trial. Our results indicate that Cl, Vss and AUC of suramin follow a well-defined size-related relationship. Pharmacokinetic results obtained from this study may be used to predict pharmacokinetics in an untested species in veterinary oncology practice.
<table>
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Table 4.1 Pharmacokinetic parameters of suramin in mice, rats, dogs and humans determined by i.v bolus or infusion. ‡: calculated from values of 26 ± 5 ml/hr/m²; †: calculated from values of 11.8 ± 6.7 L/m².
Figure 4.1 Suramin plasma concentration-time profile in mice, rats and beagles. Mice and rats received suramin by i.v. bolus through tail veins. Dogs received suramin by i.v. infusion over 30 minutes through cephalic veins. Closed circles represent observed concentration. Each point represents a mean ± SD (n = 4 or 5). The lines show predicted profiles based on a 2-compartment model as estimated using WinNonLin.
Figure 4.2 Suramin plasma concentration-time profile in human patient with NSCLC in first cycle. Profile of first two cohorts of 12 patients are depicted. These patients received a loading suramin dose of 240 mg/m², either given by single doses (Panel A, total of 6 treatments) or by split doses (Panel B, total of 6 treatments) as described in Methods and Results. Closed circles represent observed concentration. Data are mean ± SD. Data points are connected by straight lines.
Figure 4.3 Suramin plasma concentration–time profile extrapolated by the dienetichron plot vs. observed profile in NSCLC patients. Suramin was given by single dose (Panel A) or by split doses (Panel). Solid line represents extrapolated profile. Open circles represents observed individual concentration in first cycle in first two patient cohorts.
Figure 4.4 Surmin plasma concentration–time profile extrapolated by the complex Dedrick plot vs. observed profile in NSCLC patients. Suramin was given by single dose (Panel A) or by split doses (Panel B). Solid line represents extrapolated profile. Open circles represents observed individual concentration in first cycle in first two patient cohorts.
Figure 4.5 Interspecies scaling of CL, CL*MLP, Vss and AUC of suramin across 4 species. The regression line was determined by the least square analysis. Allometric equations and correlation coefficients (R^2) of the regression lines are indicated. AUC is normalized over dose given to individual subject.
CHAPTER 5

POPULATION-BASED PHARMACOKINETIC ANALYSIS TO IDENTIFY THE DOSE OF SURAMIN AS A CHEMOSENSITIZER IN TUMOR-BEARING DOGS

5.1 Introduction

We recently reported that elevated levels of aFGF and bFGF, in solid and metastatic tumors confers broad spectrum resistance to chemotherapy drugs with diverse structures and mechanisms of action, and that low concentrations/doses of suramin (10-50 µM) that are devoid of antitumor activity (or toxicity) reverse the FGF-induced chemoresistance both in vitro and in vivo (Song et al., 2001; Song et al., 2000; Zhang et al., 2001). Suramin concentrations above 50 µM were found to antagonize chemotherapy action in in vitro and in vivo studies (Zhao, 2003). Based on these findings, suramin is currently evaluated in several human Phase I/II clinical trials as a chemosensitizer in combination with different drugs, including paclitaxel, carboplatin, and 5-FU. In the past, many treatment modalities developed for human cancers have been successfully applied to dog patients. The promising results in human clinical trials for suramin as a chemosensitizer has led to this Phase I trial in dog patients to test the chemosensitization effect of suramin in combination with doxorubicin for various tumor types.

In view of the narrow therapeutic index, one major task is to develop approaches to maintain suramin in the target concentration range. The wide variation in demographic
characteristics of dog patients, for example in body weight and body composition, may make this more challenging in dog patients than it is in human patients. The nonlinear mixed-effect approach (as implemented in NONMEM) provides a useful tool to identify the major covariates (fixed effects), that correlate with, or explain the variability on the investigated population (random effects). By using population-based pharmacokinetic (PPK) analysis and expressing the population pharmacokinetic parameter as a function of covariates, this modeling approach can predict the most likely outcome for each individual subject.

The objective of the present study was to identify the sources of the variability in the suramin pharmacokinetics and to use this information to determine the suramin dose for individual patients. The patients were divided into 2 groups. Data from the first 14 dogs were used to develop an empirical equation to calculate the appropriate dose and data from the following 7 patients were used to validate the equations.
5.2 Material and methods

5.2.1 Study Population Description

Data from a Phase I clinical trial testing the combination of low dose suramin and doxorubicin were used in this population pharmacokinetic analysis. Dogs with solid tumors or lymphoma for which no effective therapy existed or standard therapy was declined, or which had become chemotherapy-resistant were eligible for this study. Breeds and tumor types were not screened. The total population of 21 patients was divided into two subsets following recruiting order. The first 14 patients were designated as index set and the later 7 patients formed the validation set. Demographic and clinical characteristics are summarized in Table 5.1.

5.2.2 Treatment Protocol

Physical examination and complete blood cell count (CBC) were performed prior to each treatment cycle. Suramin was given to patients by i.v. infusion over 30 min. The dose was fixed at 6.75 mg/kg in the first 14 patients and adjusted in the next 7 patients based on the pharmacokinetic information obtained from the previous patients. Doxorubicin at a dose of 30 mg/m², or 1 mg/kg if the body weight was less than 15 kg, was given 3 hours after the suramin infusion in the first 14 patients and 4 hours after the suramin infusion in the next 7 patients. Thirty minutes prior to receiving doxorubicin, patients were premedicated with 2 mg/kg diphenhydramin intramuscularly. Subsequent treatment doses of doxorubicin were reduced by 25% if grade 3 or 4 hematological toxicity occurred. Treatment was scheduled at 2 week-intervals. Treatment was continued until disease progression or owners’ noncompliance. Evidence of drug toxicity was
monitored by CBC, the evaluation of the history obtained from the owners, physical examination and echocardiograms.

5.2.3 Blood Sampling and Data Collection

Blood was sampled from the jugular or cephalic veins at the following time points: immediately before the initiation of suramin infusion, 5 min, 30 min, 90 min, 150 min after the end of suramin infusion, immediately before the initiation of doxorubicin infusion, 30 min after the end of doxorubicin infusion, 1 day and 7 days after suramin infusion. If the day after infusion was a weekend or a holiday, the samples were drawn on the next workday. Samples were centrifuged and the plasma was taken and analyzed by high performance liquid chromatography (HPLC), as described elsewhere (Kassack and Nickel, 1996).

5.2.4 Pharmacokinetic Model Structures.

The suramin data was analyzed using a nonlinear mixed-effects model (NONMEM Version V, UCSF, San Francisco, CA).

**Kinetic Model.** Analysis of the suramin plasma concentration-time data by standard methods indicated that while suramin disposition was adequately described with a 2-compartment model (initial and terminal half-lives were 4 hours, and 11 days, respectively), the area under the terminal phase accounted for most of the total area-under-time-concentration-curve (AUC), i.e., ~90%. To simplify the population pharmacokinetics model and hence the equation for dosing calculations, the intended end product of this model, we elected to use a one-compartment pharmacokinetic model, and only included time points during the terminal phase (i.e., greater than 4 times the half-life of the initial phase). Specifically, these time points were at 24 hr, 1 week after the
treatment and immediately prior to the next treatment (usually at 2 weeks after the treatment).

The one-compartment kinetic model depicting plasma concentrations as a function of clearance (CL) and volume of distribution (V) is described by equation 5.1:

$$C_{ij} = \frac{Dose}{V_j} \cdot e^{\left(\frac{CL_j}{V_j}\right) \cdot \text{time}_i}$$  

Eq. 5.1

where $C_{ij}$ is the predicted plasma concentration at a particular time $i$ for a patient $j$; $CL_j$ and $V_j$ are the values of clearance and volume of distribution in patient $j$. The NONMEM subroutines describing this model are supplied as pre-written programming codes ADVAN1, TRAN2 in the PREDPP library of the NONMEM software.

**Structural Model.** The influence of dog clinical characteristics (fixed effects) on the average population values of pharmacokinetic parameters was assessed, using the following general linear regression models:

$$CL_{typ} = \theta_1 + \theta_2 \cdot Cov_2 + \theta_3 \cdot Cov_3 + \ldots + \theta_n \cdot Cov_n$$  

Eq.5.2

$$V_{typ} = \theta_{n+1} + \theta_{n+2} \cdot Cov_{n+2} + \theta_3 \cdot Cov_{n+2} + \ldots + \theta_z \cdot Cov_z$$  

Eq.5.3

where $CL_{typ}$ and $V_{typ}$ represent typical population values of total body clearance and volume of distribution for individuals defined by $Cov_1, Cov_2, \ldots, Cov_n$, respectively; $Cov$ is a fixed effect (e.g. body surface area (BSA), serum creatinine concentration) and $\theta$ is the estimated proportionality constant that quantifies the influence of the fixed effect on the pharmacokinetic parameters. A literature review of clinical variables related to pharmacokinetic parameters describing suramin disposition was conducted. On the basis of the diagnostic plots for the residues against weight, body surface area, age, gender, breed, plasma albumin and protein concentration, serum creatinine, bicarbonate, and
BUN concentration, 6 final regression models were selected for testing. The covariates in the models include body surface area (BSA), gender, total plasma protein concentration, serum creatinine concentration and bicarbonate concentration.

**Statistical Model.** Error in the concentration and pharmacokinetic parameters was described by a constant coefficient of variation term (proportional error), according to the following statistical models:

\[ C_{ij} = f(p_j, \text{Dose}, t) \times (1 + \varepsilon_{ij}) \quad \text{Eq.5.4} \]

\[ CL_j = CL_{typ} \times (1 + \eta_{CLj}) \quad \text{Eq.5.5} \]

\[ V_j = CL_{typ} \times (1 + \eta_{vj}) \quad \text{Eq.5.6} \]

where \( f \) was the pharmacokinetic model (Eq. 5.1); \( p_j \) was the set of kinetic parameters, i.e. \( CL_j \) and \( V_j \); the intra-individual error term \( \varepsilon_{ij} \) accounted for the residual variability in the data caused by assay error, model misspecification, intra-individual variability, and any other source of variability not accounted for by the inter-individual (\( \eta_j \)) error terms; \( \eta_{CLj} \) and \( \eta_{vj} \) are normally distributed random variables with a mean value of zero, and respective variances, \( \omega^2_{CL} \), and \( \omega^2_V \). Preliminary analysis indicated that a proportional error model was more suitable than an additive error model. In this analysis, model was considered better if NONMEM fitting resulted in a lower value for the NONMEM objective function. The objective function in mixed effect modeling is analogous to the sum of squared errors in nonlinear regression.

**5.2.5 Model Build-up**

Model building was accomplished through a stepwise procedure, starting with the simplest model (without covariates) and consequently adding covariates one by one and testing its significance. The difference in objective function values obtained for the full
and reduced models is approximately $\chi^2$ distributed with degrees of freedom equal to the difference in number of fixed effect parameters in the compared models. To determine whether a covariate should be incorporated into the model, the difference in the objective function values of the models, obtained with or without adding the candidate covariate was calculated. A reduction in the objective function values of more than 3.9 (i.e., $\chi^2$ value associated with $P < 0.05$ for 1 degree of freedom) was required for inclusion into the full model for $CL^\text{typ}$ or $V^\text{typ}$.

5.2.6 Model Refinement

As additional covariates were included in the model, the previously added parameters might become less important. A backward deletion strategy was used to determine which candidate covariates should be retained in the final population model (Mandema JW et al., 1995). A covariate was retained if the objective function increased at least 7.9 upon removal of the covariate ($\chi^2$ value associated with $P < 0.005$ and 1 degree of freedom). This conservative approach ensured that the final model only contained the most significant covariates.

5.2.7 Model Validation

The performance of the final population pharmacokinetic model was tested in 7 additional patients. The correlations between the predicted concentrations and observed concentrations were investigated. The accuracy of the PPK-predicted plasma concentrations was calculated using Equation 5.7.

$$\text{Mean } \% \text{ deviation from observed concentrations} = \text{Mean} \left( \frac{C_{\text{predicted}} - C_{\text{observed}}}{C_{\text{observed}}} \times 100\% \right)$$

Eq. 5.7
To compare the dose predicted by the PPK method (referred to as PPK-Calculated Dose) to the target dose, i.e. a dose that would yield in an individual patient a plasma concentration of 15 µM suramin at 24 hr (referred to as Ideal Dose), the PPK-calculated Dose was calculated using Equation 5.8.

\[
\text{PPK - calculated Dose} = \frac{(C_{24hr, \text{target}} - C_{\text{res}} \times e^{(-\frac{\text{CL}_{\text{typ}}}{V_{\text{typ}}}) \cdot 24}) \cdot V_{\text{typ}}}{e}
\]

Eq.5.8

Where \(C_{24hr, \text{target}}\) was set as 15 µM. \(V_{\text{typ}}\) and \(\text{CL}_{\text{typ}}\) were population typical values calculated by the PPK-derived model. \(C_{\text{res}}\) was the predose residual suramin concentration, calculated from previous dose. For the first cycle, \(C_{\text{res}}\) equaled to zero. For the later cycles, \(C_{\text{res}}\) was calculated using Equation 5.9.

\[
C_{\text{res}} = \frac{\text{Dose}_{\text{previous}}}{V_{\text{typ}}} \times e^{(-\frac{\text{CL}_{\text{typ}}}{V_{\text{typ}}} \cdot t_{\text{interval}})}
\]

Eq. 5.9

Where \(\text{Dose}_{\text{previous}}\) was the dose for the previous cycle, and \(t_{\text{interval}}\) was the time interval between the two treatments. The Ideal Dose to achieve the target concentration, i.e. 15 µM at 24 hr, was estimated by Equation 5.10.

\[
\text{Ideal Dose} = \frac{\text{administered dose} \times C_{24hr, \text{target}}}{C_{24hr, \text{observed}}}
\]

Eq. 5.10

Where \(C_{24hr, \text{observed}}\) was the concentration observed at 24 hr. Similarly, the accuracy of the PPK-predicted dose was calculated using Equation 5.11.
Mean % deviation from Ideal Dose = \( \text{Mean} \left( \frac{\text{Ideal Dose} - \text{Simulated Dose}}{\text{Ideal Dose}} \times 100\% \right) \)

Eq. 5.11
5.3 Results

5.3.1 Patients Characteristics

There were 14 patients in the learning set and the 7 patients in the validation set, which received between 2 to 10 treatment cycles (median, 3 cycles), for a total of 80 treatment cycles (Table 5.1). The patient population included different breeds and tumor types. Numbers were fairly balanced between the two groups, except for greyhound in the breed category. It is noteworthy that only two greyhounds, a breed with an exceptionally high lean muscle ratio, were included in the study. The population was characterized by large variation in size. For example, the body weight ranged from 3 kg (a fox terrier) to 46 kg (a golden retriever), showing a 15-fold difference. An aged population was indicated by the median age, as being 8.8 years in the learning population set and 8 years in the validation population set. Table 5.1 also summarizes the ranges of the parameters, which potentially have effects on the disposition of suramin and were screened during model build-up as covariates, i.e. plasma protein, serum albumin, creatinine, and bicarbonate concentrations. Compared to the normal ranges, all the relevant parameters either fell in the boundaries, or within 30% of the up and low limits, except the platelet, which tends to have an acute change because of inflammation and chemotherapy.

5.3.2 Model Build-up

During the model build-up, the covariates that were possibly correlated with the pharmacokinetic parameters were screened, and included in the model, if shown significant, one by one (Table 5.2). Statistical analysis was performed to compare two models before and after the addition of one more parameter. The basic model did not
include any covariate. After adding BSA, the minimum values were significantly decreased. When gender was considered as a fixed effect, the model was improved further. Based on the minimum value of objective functions, serum creatinine concentration significantly improved the model performance, while the rest of the parameters, i.e. plasma protein concentration, albumin concentration and bicarbonate concentration did not further improve the model significantly. Model 6 in Table 5.2 was the full model for the subsequent refinement.

5.3.3 Model Refinement

During the step of model refinement, the statistical significance of each parameter in the model was tested with a more restrictive criterion (P<0.005) for the full model and the subsequent reduced models, to find a final model. In this step, 4 parameters, when set to null value, did not affect the performance of the model significantly (Table 5.3). The reduced model with the lowest value of objective function was tested for further refinement (Model 3 in Table 5.3). Each parameter in Model 3 was then set to null value, and three reduced model were obtained with the difference of MVOF less than 7.9, compared to Model 3 (Models 11, 12 and 13). Notably, Model 12 eliminated serum creatinine concentration from the model. Therefore, both Models 11 and 12 were tested for further refinement (Models 14, 15, 16). Between Model 12 and Model 14, although Model 14 contained fewer parameters, Model 12 provided the simplest form by eliminating the covariate of creatinine concentration in the equation. Therefore, Model 12 was chosen as the final model. Comparisons of the basic model, full model and final model are summarized in Table 5.4. Figure 5.1 depicts the correlation between the observed concentrations and the predicted concentrations for the three models,
respectively. The correlation between predicted values and observed values were drastically improved with the $r^2=0.35$ in the basic model, $r^2=0.77$ in the full model and $r^2=0.79$ in the final model. The difference between the full model and the final model was minor.

5.3.4 Model Validation

Two endpoints were used to validate the final model, by predicted concentrations and by predicted doses. The model was applied to the 7 patients in the validation set and the correlation between the predicted concentrations and measured concentrations was examined (Figure 5.2.a). Good correlation was shown between the predicted and observed concentrations ($r^2=0.64$). The accuracy of plasma concentration prediction, evaluated according to Equations 5.7, showed a mean deviation of 17%. Notably, there was a cluster of data points, which were more distant from the unit line than others, and belonged to one greyhound. Because the objective for this study was to determine an effective dosing regimen, the correlation between simulated dose and the ideal dose was also explored ($r^2=0.62$, Figure 5.2.b). The accuracy of dose prediction by the PPK model, evaluated according to Equation 5.11 showed a mean deviation -32%. Data points in the two circles belonged to the two greyhounds in the validation set, respectively (Patient I and II), suggesting that greyhound may have different disposition for suramin. In Figure 5.2.b, the solid line across the graph is a unit line and two dashed lines delineate the ideal dose range to achieve 10 to 20 µM at 24 hr. Other than the two greyhounds, all the patients either fell in the range, or on the line. Taking the two greyhounds into account, the concentrations at 24 hr after suramin infusion ranges from 11 to 33 µM, which were
within the chemosensitization range of suramin. Therefore, the model provides a fairly good prediction.

5.4 Discussion

The present study demonstrates the use of PPK analysis to identify an empirical equation for calculating the suramin dose that would yield the target plasma concentration range in tumor-bearing pet dogs. The empirical equation was derived from 14 patients and validated in additional 7 patients.

Although suramin has been evaluated as an anticancer agent in humans since the 1980’s (Myers et al., 1990; Tkaczuk et al., 1992; Tu et al., 1998), information regarding the use of suramin in domestic animals is limited. Two studies investigated antiviral effect of suramin in cats at a dose of 40 mg/kg once or twice a week (Abkowitz, 1991; Cogan et al., 1986). The pharmacokinetics and therapeutic use of suramin in dogs have not been reported previously. Suramin’s antiproliferative activity was dose dependent and less effective at concentrations of 50 micromolar (µM) or less of continuous exposure. Therefore, pharmacokinetics studies of suramin found in the literature have administered high dose suramin and targeted at therapeutic plasma concentrations of between 100 to 200 µM (Kobayashi K et al., 1996; Reyno et al., 1995). Comparing reported pharmacokinetic studies using high dose suramin with our lab’s studies using low dose, nonlinear disposition of suramin was observed in both humans and rats. Low dose suramin had a bigger total body clearance in human, e.g. 0.025 L/hr/m² vs. 0.013² L/hr/m², smaller steady state volume of distribution, e.g. 8.5 L/m² vs. 13.6 L/ m², and a shorter terminal half-life, e.g. 11 days vs. 41 days (Jodrell et al., 1994). In rats, the CL was 33% higher, the Vss and terminal half life were 4 and 6 fold lower, respectively,
when the doses were 30-fold lower, i.e. 10 mg/kg vs. 300 mg/kg. Therefore, cautions need to be taken to extrapolate the results from this study, when using suramin for other purpose instead of as a chemosensitizer in dog patients.

Au and collaborators have found an unconventional dose-response relationship for the chemosensitization effect of suramin. Chemosensitization occurred only at low doses (i.e., doses that result in 10-50 µM plasma concentrations) and not at high doses yielding between 100-200 µM plasma concentrations (Zhao, 2003). Our studies suggested that the loss of chemosensitization was, at least partly if not all, due to cell cycle blocking effect of suramin that occurred only at high concentrations above 50 µM. Blockade of cells in early phases in the cell cycle might reduce the effect of a drug that acted on the later phases of the cell cycle and thereby negated the chemosensitization. Further studies showed that cell cycles were blocked at G1 phase after 48 hours of exposure or more of high concentration suramin (> 50 µM) (paper in preparation).

Figure 5.3 depicts first cycle suramin plasma concentration profile in first fourteen patients from the learning set (panel a) and the last seven patients from the validation set (panel b). The first group received a fixed dose of suramin, and the second group received a suramin dose based on the empirical equation developed by PPK. In both groups, the peak concentration exceeded 50 µM. In the first group, suramin concentration dropped below 50 µM at 3 hours after infusion in twelve patients. At 24 hours, nine patients achieved suramin concentration above 10 µM. In the second group, suramin concentration dropped below 50 µM 4 hours after infusion in 6 out of 7 patients. At 24 hours, all patients achieved suramin concentration above 10 µM. The model
developed by population pharmacokinetic analysis helped to improve the dosing regimen for suramin.

To identify covariates for population pharmacokinetics, the processes of distribution and elimination of suramin needed to be considered. Suramin is highly protein-bound drug, i.e. 99.6% bound to plasma protein in humans (paper submitted). Therefore albumin and total protein level in plasma were screened during model build-up. The other parameters are related with elimination of suramin. Several studies have discussed the processes involved in the clearance of suramin (Collins et al., 1986; Jodrell et al., 1994; Piscitelli et al., 1997; Reyno et al., 1995). A study in patients with acquired immunodeficiency syndrome showed that suramin is essentially unmetabolized, mainly eliminated by renal clearance, and suggested glomerular filtration as the mechanism of suramin clearance by the kidney (Collins et al., 1986; Jodrell et al., 1994; Reyno et al., 1995). A subsequent study in prostate cancer patients found that the average total body clearance of suramin was about 2-fold higher than the average creatinine clearance and thereby suggested tubular secretion as a second mechanism of renal clearance (Jodrell et al., 1994; Reyno et al., 1995). The role of tubular secretion in renal elimination of suramin was further indicated by a more recent report showing that competitive inhibition of nonselective organic acid transporters at the proximal tubule by furosemide led to a 36% decrease in the total body clearance of suramin (Piscitelli et al., 1997). Creatinine clearance and serum creatinine clearance are related with glomerular filtration rate. Creatinine clearance is not readily available in dog patients. Therefore only serum creatinine concentration was examined. Suramin is a sulfate salt and exists as anion in
water. The concentration of bicarbonate was tested as a covariate during model build-up in the concern of its possible competition with suramin during renal excretion.

There were 2 greyhounds in the validation set. In both patient, the concentration were underestimated and the dose were overestimated. This may be due the special physiological characteristics of the breed. Greyhound has lean musculature, thin skin and as much as half of the body fat compared to other breeds of similar weight. Further pharmacokinetic study is warranted for suramin as a chemosensitizer in this breed.

In summary, we have identified BSA, gender, and serum creatinine as major factors accounting for >90% of the inter-individual variation of suramin PK in dogs. An empirical equation, including only BSA and gender, were derived and validated to calculate the suramin dose for target concentration, thus eliminating the need of real-time pharmacokinetic monitoring.

**ACKNOWLEDGEMENTS**

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<table>
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<th>BREAD</th>
<th>Learning set n = 14</th>
<th>Validation set n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden Retriever</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Greyhound</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Beagle</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mixed breed</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>1</td>
</tr>
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<table>
<thead>
<tr>
<th>TUMOR TYPE</th>
<th>Learning set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteosarcoma</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemangiopericytoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Bladder TCC</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Prostatic CA</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other CA</td>
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<td>1</td>
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<tr>
<td>Lymphoma</td>
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<td>0</td>
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<tr>
<td>Soft tissue Sarcoma</td>
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<tr>
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<th>Normal (range)</th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>P value♣</th>
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<tbody>
<tr>
<td>Males</td>
<td>NA</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>NA</td>
<td>4</td>
<td>1</td>
<td>0.443</td>
</tr>
<tr>
<td>Age (years)</td>
<td>NA</td>
<td>8.8 (4.0 – 15.6)</td>
<td>8.0 (2.9 – 11.0)</td>
<td>0.336</td>
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<tr>
<td>Weight (kg)</td>
<td>NA</td>
<td>30 (3 – 46)</td>
<td>35 (10 - 37.5)</td>
<td>0.617</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>NA</td>
<td>0.96 (0.2 – 1.28)</td>
<td>1.07 (0.46-1.12)</td>
<td>0.612</td>
</tr>
<tr>
<td>Number of Treatments</td>
<td>NA</td>
<td>3 (2-10)</td>
<td>5 (3-9)</td>
<td>0.190</td>
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<td>Hemoglobin (g dL⁻¹)</td>
<td>11.9 – 18.4</td>
<td>13.5 (9.2-15.1)</td>
<td>14.3 (12.2-19.6)</td>
<td>0.182</td>
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<td>WBC (x 10⁹ L⁻¹)</td>
<td>4.1 – 15.2</td>
<td>9.0 (3.5 - 16.1)</td>
<td>8.6 (3.3 – 11.8)</td>
<td>0.355</td>
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<tr>
<td>Platelets (x 10⁹ L⁻¹)</td>
<td>106 – 424</td>
<td>404 (13⁷ – 921)</td>
<td>299 (200 - 646)</td>
<td>0.443</td>
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<tr>
<td>Plasma Protein (g dL⁻¹)</td>
<td>5.7 –7.2</td>
<td>7.1 (5.9-8.2)</td>
<td>6.6 (6.7 – 7.5)</td>
<td>0.590</td>
</tr>
<tr>
<td>Albumin (g dL⁻¹)</td>
<td>2.9 – 4.2</td>
<td>3.6 (2.1 – 4.3)</td>
<td>3.7 (3 – 3.8)</td>
<td>0.533</td>
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<td>Serum Creatinine (mg/dL)</td>
<td>0.6 – 1.6</td>
<td>1.2 (0.7 – 1.8)</td>
<td>1.3 (0.7 – 2.2)</td>
<td>0.599</td>
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<tr>
<td>Serum Bicarbonate (mmol/L)</td>
<td>16 – 25</td>
<td>18 (12 – 24)</td>
<td>19 (17 – 22)</td>
<td>0.149</td>
</tr>
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</table>

*: Low platelet count in one lymphoma stage 4A patient, which might be secondary to bone marrow involvement. The patient received the standard doxorubicin dose.
♣: Comparison of genders between sets was evaluated using the Fisher exact test. Other comparisons used Student’s t-test.

Table 5.1 Summary of demographic and clinical characteristics in tumor-bearing dog patients. The patient population was divided sequentially. The first 14 patients formed the learning data set, and received fixed suramin doses of 6.75 mg/kg. The later 7 patients comprised the validation data set and received adjusted doses based on the empirical equation derived from the first population. NA, not applicable.
Table 5.2 Stepwise model build-up for the population pharmacokinetics of suramin in tumor-bearing dog patients.

Model building was started with the simplest model (without covariates). Subsequently, covariates were added one by one and tested for significant decrease of the objective function. A reduction in the objective function value by more than 3.9 ($P < 0.05$) was required for inclusion into the Full Models for CL and V.

<table>
<thead>
<tr>
<th>Model</th>
<th>Pharmacostatistical model</th>
<th>Intersubject variability</th>
<th>Objective Function</th>
<th>Compared to Model</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{pij} = (C_0 \times e^{-\left(\frac{CL \cdot V}{V_i}\right)} \cdot (1 + \epsilon_{ij}))$</td>
<td>$\omega^2_{CL}$</td>
<td>$\omega^2_{V}$</td>
<td>$\sigma^2$</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>$CL = (\theta_1 + \theta_2 \cdot BSA) \cdot (1 + \eta_1)$</td>
<td>$2.9E-12$</td>
<td>$2.3$</td>
<td>$0.58$</td>
<td>$618.8$</td>
</tr>
<tr>
<td></td>
<td>$V = (\theta_3 \cdot (1 + \eta_2))$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>$CL = (\theta_1 + \theta_2 \cdot BSA) \cdot (1 + \eta_1)$</td>
<td>$0.048$</td>
<td>$8.6E-5$</td>
<td>$0.14$</td>
<td>$447.0$</td>
</tr>
<tr>
<td></td>
<td>$V = (\theta_3 + \theta_4 \cdot BSA) \cdot (1 + \eta_2)$</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>$CL = (\theta_1 + \theta_2 \cdot BSA) \cdot (1 - Gender \cdot \theta_3) \cdot (1 + \eta_1)$</td>
<td>$0.032$</td>
<td>$0.0092$</td>
<td>$0.14$</td>
<td>$439.2$</td>
</tr>
<tr>
<td></td>
<td>$V = (\theta_4 + \theta_5 \cdot BSA) \cdot (1 + \eta_2)$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$CL = (\theta_1 + \theta_2 \cdot BSA) \cdot (1 - Gender \cdot \theta_3) \cdot (1 + \eta_1)$</td>
<td>$0.029$</td>
<td>$5.5E-9$</td>
<td>$0.13$</td>
<td>$431.4$</td>
</tr>
<tr>
<td></td>
<td>$V = (\theta_4 + \theta_5 \cdot BSA) \cdot (1 - Gender \cdot \theta_6) \cdot (1 + \eta_2)$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$CL = (\theta_1 + \theta_2 \cdot BSA + \theta_3 \cdot CCr) \cdot (1 - Gender \cdot \theta_4) \cdot (1 + \eta_1)$</td>
<td>$0.029$</td>
<td>$3.7E-11$</td>
<td>$0.13$</td>
<td>$427.3$</td>
</tr>
<tr>
<td></td>
<td>(full) $V = (\theta_5 + \theta_6 \cdot BSA) \cdot (1 - Gender \cdot \theta_7) \cdot (1 + \eta_2)$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Model Number</td>
<td>Null Hypothesis</td>
<td>Minimum Values of Objective Function</td>
<td>Compare to Model</td>
<td>P Value</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
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<td>-----------------</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Full model</td>
<td>427.3</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>$\eta_1 = 0$</td>
<td>439.4</td>
<td>1</td>
<td>&lt;0.005</td>
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<tr>
<td>3</td>
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<td>427.3</td>
<td>1</td>
<td>NS</td>
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<tr>
<td>4</td>
<td>$\theta_1 = 0$</td>
<td>430.2</td>
<td>1</td>
<td>NS</td>
<td></td>
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<tr>
<td>5</td>
<td>$\theta_2 = 0$</td>
<td>566.5</td>
<td>1</td>
<td>&lt;0.005</td>
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<tr>
<td>6</td>
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<td>1</td>
<td>NS</td>
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<tr>
<td>7</td>
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<td>1</td>
<td>&lt;0.005</td>
<td></td>
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<tr>
<td>8</td>
<td>$\theta_5 = 0$</td>
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<td>&lt;0.005</td>
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<tr>
<td>9</td>
<td>$\theta_6 = 0$</td>
<td>604.6</td>
<td>1</td>
<td>&lt;0.005</td>
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<td>10</td>
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<td>433.5</td>
<td>1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>$\eta_2 = 0, \theta_1 = 0$</td>
<td>430.2</td>
<td>3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>$\eta_2 = 0, \theta_3 = 0$</td>
<td>431.4</td>
<td>3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$\eta_2 = 0, \theta_7 = 0$</td>
<td>433.5</td>
<td>3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>$\eta_2 = 0, \theta_1 = 0, \theta_7 = 0$</td>
<td>435.9</td>
<td>11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>$\eta_2 = 0, \theta_1 = 0, \theta_3 = 0$</td>
<td>460.6</td>
<td>11</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>$\eta_2 = 0, \theta_3 = 0, \theta_7 = 0$</td>
<td>439.3</td>
<td>12</td>
<td>&lt;0.005*</td>
<td></td>
</tr>
</tbody>
</table>

*: the difference of MVOF of Model 12 and Model 16 was 7.904, considered statistically significant.

Table 5.3 Model refinements by elimination of redundant parameters from the full model. A backward deletion strategy was used to determine which candidate covariates should be retained in the final population model. A significant P value was taken to be 0.005, which was related with a value of 7.9.
### Table 5.4 Comparisons among basic model (Model 1), full model (Model 6) and final model (Model 12).

<table>
<thead>
<tr>
<th>Model</th>
<th>Final Estimation</th>
<th>(\omega^2_{CL})</th>
<th>(\omega^2_V)</th>
<th>(\sigma^2)</th>
<th>MVOF</th>
</tr>
</thead>
</table>
| Basic  | \(Cl=26.7\cdot(1+\eta_1)\)  \\
|        | \(V=4890\cdot(1+\eta_2)\)  \\
|        | \(C_{pij}=(C_{0i}\times e^{-\left(Cl/V\right)\cdot t_{ij}})\times (1+\varepsilon_{ij})\) | 0.92               | 2.3             | 6.5             | 575.6  |
| Full   | \(Cl=(68 \cdot BSA-3.5 \cdot CCr-5.5)\cdot(1-0.30 \cdot Gender)\cdot(1+\eta_1)\) | 0.029              | 3.7E-11         | 0.13            | 427.3  |
|        | \(V=(16100 \cdot BSA-2840)\cdot(1-\text{Gender} \cdot 0.25)\cdot(1+\eta_2)\) |                    |                  |                 |        |
|        | \(C_{pij}=(C_{0i}\times e^{-\left(Cl/V\right)\cdot t_{ij}})\times (1+\varepsilon_{ij})\) |                    |                  |                 |        |
| Final  | \(Cl=(71 \cdot BSA-11)\cdot(1-0.30 \cdot Gender)\cdot(1+\eta_1)\) | 0.029              | -               | 0.13            | 431.4  |
|        | \(V=(16800 \cdot BSA-3040)\cdot(1-0.27 \cdot Gender)\) |                    |                  |                 |        |
|        | \(C_{pij}=(C_{0i}\times e^{-\left(Cl/V\right)\cdot t_{ij}})\times (1+\varepsilon_{ij})\) |                    |                  |                 |        |

MVOF, minimum value of objective function.
Figure 5.1 Comparisons of fitness from three models. The measured plasma concentrations 24 hours after suramin infusion from the 14 dog patients, two to ten treatment cycles are plotted against concentrations predicted by the 3 models, respectively. The solid line in the middle of each graph is the unit line.
Figure 5.2 Validation of Final Model in 7 new dog patients. (a) Concentration plot. For these 7 new patients, the model-predicted concentration were calculated as discussed in Materials and Methods. The solid line represents the unit line. Only concentrations 24 hrs after suramin infusion are plotted; (b) Dose plot. For these new patients, the Ideal Dose needed to obtain a plasma concentration of 15 µM at 24 hr was calculated as discussed in Materials and Methods. The solid line represents the unit line. Two broken lines delineate the dose range corresponding to a concentration range of 10-20 µM at 24 hr. All the circled outlier values belonged to two greyhounds, as labeled in the figure.
Figure 5.3 **Suramin plasma concentration-time profiles.** Suramin was given as a fixed dose of 6.75 mg/kg in patients No. 1 to 14. For patients No. 15 to 21, the dose was calculated from the empirical equation described in Materials and Methods. Data are median values. Error bars represent the concentration range. Data points are connected by straight lines.
CHAPTER 6
TISSUE DISTRIBUTION OF INTRAVENOUS LOW DOSE SURAMIN IN TUMOR-BEARING MICE AND DEVELOPMENT OF LOCAL PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

6.1 INTRODUCTION

Suramin, an organic polyanion is a polysulfonated naphthylurea. Suramin has been commonly used for the treatment of parasitic diseases such as African trypanosomiasis and onchocerciasis since the 1920’s (Hawking, 1978). Since the 1980’s, suramin has received attention as a possible antiretroviral agent based on its ability to inhibit the reverse transcriptase of several retroviruses including human immunodeficiency virus (DeClercq, 1979; Levine et al., 1986; Mitsuya et al., 1984) and as an antineoplastic agent based on its ability to inhibit binding of several tumor growth factors to their respective receptors (Basile and Holzwarth, 1994; Bernardini et al., 1993; Coffey et al., 1987; Fujiuchi et al., 1997; Hosang, 1985; Minniti et al., 1992; Pesenti et al., 1992; Pienta et al., 1991; Pollak and Richard, 1990; Wade et al., 1992; Waltenberger et al., 1996). Multiple studies have evaluated suramin as a cytotoxic agent in the preclinical and clinical settings, where suramin was given alone or in combination with other chemotherapeutic agents. In vitro, the antiproliferative activity of suramin was concentration dependent. Suramin has little or no antitumor activity at concentrations of
<50 µM and stimulates cell growth at concentrations of <35 µM (Frommel, 1997; Kuratsu et al., 1995; Rubio et al., 1995; Taylor et al., 1992). Clinical evaluation of suramin was conducted at its maximum tolerated steady state concentrations of 100-200 µM (equivalent to approximately 150-300 µg/ml) over a relatively long duration (up to 12 weeks). At these doses, suramin appears to have moderate anticancer activity in hormone refractory prostate carcinoma (Eisenberger MA et al., 1995; Rosen et al., 1996; Small E et al., 2000), and insignificant activity in non-small cell lung, breast, colorectal, and renal carcinomas (Dreicer et al., 1999; Falcone et al., 1998; Mirza et al., 1997). Clinical results showed that suramin has no antitumor activity at plasma concentration less than 100 µM and is associated with severe toxicity at plasma concentration above 200 µM.

Our laboratory recently established acidic and basic fibroblast growth factors (aFGF and bFGF) as a cause of chemoresistance in solid tumors. These two proteins at clinically relevant concentrations induce an up to 10-fold resistance to drugs with diverse structures and action mechanisms, without altering drug accumulation. *In vitro*, suramin at concentrations ranging from 10 to 15 µM, which have no antitumor effect when used alone, completely reverses the FGF induced resistance. The ability of suramin to enhance drug activity under in vivo conditions was further evaluated in immunodeficient mice bearing well-established human xenograft tumors. In these studies, suramin was given at a low dose of 10 mg/kg in combination with different chemotherapeutic agents for a total of 6 treatments (10 mg/kg, twice weekly x 3 weeks). Results indicated that low dose suramin significantly enhanced the activities of doxorubicin, paclitaxel, and mitomycin C
in PC3 subcutaneous tumors, PC3-LN lung metastases, and RT4 subcutaneous tumors (Song et al., 2001; Song et al., 2000; Zhang et al., 2001).

The chemosensitization effect of suramin has been found in the range of 10-50 µM in the plasma or the medium. However, no data are available on the concentration in tumor and other tissue under these conditions. To understand pharmacodynamics of low dose suramin, there is a need to study pharmacokinetics of suramin in different tissues, including tumor. Tissue distribution of suramin in rats had been previously studied using high dose, i.e. 300 mg/kg (McNally et al., 2000). However, suramin exhibits nonlinear kinetics with a >2-fold higher clearance and a >5-fold shorter terminal half-life when given a dose of 10 mg/kg compared to a dose of 300 mg/kg in rats (paper in preparation). Trends in the same direction were also found in human, in that low suramin dose resulted in higher clearance and shorter terminal half-life. Therefore, the tissue distribution cannot be simply derived from the literature. The present study is to characterize the pharmacokinetics and tissue distribution of low dose suramin in tumor-bearing immunodeficient nude mice, to provide greater insight to drug distribution in the body. The suramin concentration in tumor was of particular interest because tumor was the ‘effect’ compartment. A local physiologically based pharmacokinetic model was developed, introducing the possibility of animal scale-up, which would provide a rational basis for the correlation of drug data among animal species.
6.2 MATERIALS AND METHODS

6.2.1 Chemicals and reagents

Suramin was purchased from Sigma (St. Louis, MO). Methanol and other high-performance liquid chromatographic (HPLC) grade chemicals were purchased from Fisher Scientific Company (Fair Lawn, NJ).

6.2.2 Apparatus

HPLC was performed on a Perkin-Elmer LC Analyst system (Perkin Elmer Instruments, Norwalk, CT), consisting of a solvent delivery pump (Quaternary LC-620), an automated sampler (ISS-200), and a variable wavelength UV/visible detector (LC-95). The analytes were quantified by their UV absorbance at 313 nm.

6.2.3 Animal protocol

Female BALB/c nu/nu mice (6-8 weeks of age) were used. All mice were given food and water ad libitum, and were cared for according to Institutional guidelines. The pretreatment body weights were $18 \pm 1.5$ g. On the day of experimentation, the mice were lightly anesthetized with an intraperitoneal injection of Avertine (tribromoethanol, 240 mg/kg) and a suramin dose of 10 mg/kg was given intravenously via a tail vein. The concentration of the dosing solution was 1 mg/ml and the dosing volume ranged from 160 to 200 µl.

6.2.4 Sampling

Tissue distribution after one treatment and six treatments were studied. At various time points after treatment, mice ($n = 4$ per time point) were anaesthetized with Avertine (240 mg/kg), arterial blood samples were obtained by cardiac puncture from the left
ventricles and placed into heparinized tubes on ice. The time points for one treatment were 5, 30 min, 1, 2, 4, 6, 24, 72, 168, 336 and 504 hr. The time points for six treatments were 24, 72 and 168 hr after the last treatment. The plasma fraction was obtained after centrifugation at 1,000 g for 5 min at 4°C and stored at –20°C until analysis. Tissues were quickly excised (within 10 minutes after exanguination), wrapped in aluminum foil and stored at –70°C until analysis.

6.2.5 Plasma and tissue extraction

To extract suramin from the plasma, 100 µl of plasma was mixed with 100 µl of 1 M tetrabutylammonium bromide (TBAB, pH 8.0), 20 µl of internal standard (trypan blue 100 µg/ml), and vortexed for 30 sec. After addition of 300 µl of acetonitrile and vortex for 30 sec, the samples were stored at 4°C for at least 2 hr. Samples were then centrifuged at 1,000 g for 5 min. The supernatant (50 µl) was injected directly into the HPLC. This method produced an extraction yield of >90%.

To extract suramin from tissues, the thawed tissues were weighed, minced with scissor, placed into a 15-ml glass tube and 0.4 ml of 0.5 mM of tetrabutylammonium bromide (TBAB), 4 ml of methanol, and 20 µl of trypan blue (200 µg/ml) were added. The mixture was homogenized for 1 min and washed with 4 ml of methanol. The suspension was centrifuged at 1500 g for 5 min. The supernatant was transferred and the pellet was again homogenized with another mixture of 0.4 ml of TBAB and 4 ml of methanol. Supernatant fractions of the two extracts were pooled, evaporated under air over night, reconstituted with the mobile phase, and filtered. The filtrates were then analyzed for suramin by HPLC. This method produced an extraction yield of >80%.
6.2.6 HPLC analysis of suramin

Suramin was analyzed using ion-pairing HPLC. The stationary phase consisted of an analytical column (C\textsubscript{18}, 3 µm, 4.6 x 8.3 mm, Perkin Elmer Instruments, Norwalk, CT) and a guard column (Nova-pak C\textsubscript{18} guard column insert, Waters Associates, Milford, MA). The mobile phase consisted of two solvents. Solvent 1 was a mixture of 200 ml of methanol, and 800 ml of 0.02 M phosphate buffer (pH 6.5) containing 6.25 mM of tetrabutylammonium hydrogensulfate. Solvent 2 was 100% methanol. The two solvents were delivered using a linear gradient condition, as follows: 0 min: A = 80%, B = 20%; 0.1-17 min: A = 46.4%, B = 53.6%; 17-22 min: A = 80%, B = 20%. A flow-rate of 1 ml/min was maintained. The detection limit of suramin was 0.5 µg/ml of plasma and 1.5 to 2 µg/g of tissue.

6.2.7 Tissue distribution parameters

The tissue partition coefficient, K\textsubscript{p}, was estimated from the tissue-to-plasma ratios of AUC using finite AUC values up to 168 hr (Gallo et al., 1987). The finite AUC values (AUC\textsubscript{0-168 hr}) were calculated by the linear trapezoidal method from time zero to 168 hr, as follows. For plasma, the AUC before the first measured time point was calculated by the product of the time interval, i.e., 0.1 hr, and the first measured concentration. For tissues, the concentration was assumed to increase in proportion to time from zero to the first measured concentration at 0.1 hr. The AUC\textsubscript{0-0.1 hr} was calculated as half of the product of the time interval (0.1 hr) the concentration. For all the other time points, the AUC\textsubscript{time A –time B} was calculated by as follows,

\[ \text{AUC}_{\text{time A –time B}} = (C_A + C_B) \frac{(t_B-t_A)}{2} \]  

Eqn. 5.1;
Where, $C_A$ and $C_B$ are the concentrations at time point $t_A$ and time $t_B$, respectively, for either plasma or the tissue.

All the AUC from time zero to 168 hr were added up to get $AUC_{0-168hr}$ for plasma and tissues, respectively.

The apparent partition coefficient, $K_{p,\text{app}}$ was also calculated as the ratio of tissue-to-plasma concentration.

6.2.8 PBPK modeling setup

Because the decline of tissue concentration did not parallel with the decline of plasma concentration, the well-stirred perfusion limited model could not adequately predict suramin concentration in the tumor. Based on the PK profile in the tumor, a three-compartment model and a two-compartment model were proposed here. In the three-compartment model, an organ was divided into three tissue compartments with exchange of drug between them (Figure 6.1), i.e., (a) vascular space; (b) a shallow compartment, including interstitial space and cell membrane; and (c) a deep compartment, representing intracellular space. The drug concentration inside the vascular space was considered the same as in the arterial blood, Because uptake of suramin into blood cells were negligible (data not published), the concentration in the vascular space is approximately half of the measured plasma concentration. The exchange of suramin occurred between compartments a and b, and compartment b and c, following first order kinetics. The following assumptions were included in the model.

1. The association and dissociation of free suramin with albumin occur fast. And the movement of free suramin between plasma and shallow compartment is fast enough that the free suramin distribution in plasma and shallow compartment maintains
equilibrium, which is $C_{\text{free, plasma}} = C_{\text{free, shallow compartment}}$. Because the free fraction of suramin is small, therefore the free suramin concentration in the extra-cellular matrix is negligible, compared to the total suramin concentration.

(2) The association and dissociation of suramin and protein in the shallow compartment rate has rate constants ($k_{\text{ass}}$ and $k_{\text{diss}}$, respectively). The endocytosis and pinocytosis of suramin into the cell is the second rate-limiting step, with rate constants of $k_{\text{in}}$ and $k_{\text{out}}$.

(3) To simplify the equation, plasma free fraction of suramin is assumed constant in the whole plasma concentration range during the period of experiment. The preliminary study showed suramin free fractions were the same in the concentration range of $1 – 100 \mu g/ml$, measured by ultra centrifugation.

(4) During the elimination phase, the concentration in the shallow compartment is proportional to the concentration in the outflow venous plasma.

The concentration change in compartment (a) could be expressed:

$$\frac{d(V_t \cdot R_a \cdot C_{\text{blood}})}{dt} = Q \cdot (C_{\text{in}} - C_{\text{out}}) - K_a \cdot f_p \cdot C_p \cdot V_t \cdot R_b + K_{d} \cdot C_{\text{st}} \cdot R_b \cdot V_t \quad \text{Equ.6.1}$$

where $V_t$ was tumor volume at time $t$; $R_a$, blood volume fraction inside the tumor; $C_{\text{in}}$ and $C_{\text{out}}$ represented the plasma concentration entering and leaving the tumor, respectively; $Q$ was plasma flow rate perfusing the tumor; $f_p$ is the free suramin fraction in plasma; $R_b$ is volume fraction of compartment b; $C_{\text{st}}$ was suramin concentration in the shallow tissue compartment, compartment (b).

The concentration change in compartment (b) was expressed as:
\[
\frac{d(V_t \cdot R_b \cdot C_{sl})}{dt} = k_a \cdot f_p \cdot C_p \cdot V_t \cdot R_b - k_d \cdot C_{sl} \cdot R_b \cdot V_t - k_{in} \cdot C_{sl} \cdot V_t \cdot R_b + k_{out} \cdot C_{dt} \cdot V_t \cdot R_c
\]

Equ. 6.2

where \( R_c \) was volume fraction of compartment \( c \); \( C_{dt} \) was suramin concentration in the deep compartment.

The concentration in the deep compartment was expressed as:

\[
\frac{d(V_t \cdot R_c \cdot C_{dt})}{dt} = k_{in} \cdot C_{st} \cdot V_t \cdot R_b - k_{out} \cdot C_{dt} \cdot V_t \cdot R_c
\]

Equ. 6.3

The average tumor concentration was calculated as:

\[
C_{tumor} = R_a \cdot C_{blood} + R_b \cdot C_{st} + R_c \cdot C_{dt}
\]

Equ. 6.4

From equations 1-3, the following equations could be derived.

\[
C_{out} = C_{in} - \frac{V_{slopes}}{Q} \cdot R_a \cdot \frac{1}{2} \cdot C_p - \frac{V_t}{Q} \cdot R_a \cdot \frac{1}{2} \cdot \frac{dC_p}{dt} - \frac{V_t}{Q} \cdot k_a \cdot f_p \cdot R_b \cdot C_p + \frac{V_t}{Q} \cdot k_d \cdot C_{sl} \cdot R_b
\]

Equ. 6.5

\[
\frac{d(R_b \cdot C_{st})}{dt} = k_a \cdot f_p \cdot C_p \cdot R_b - k_d \cdot C_{st} \cdot R_b - k_{in} \cdot C_{st} \cdot R_b + k_{out} \cdot C_{dt} \cdot R_c - R_b \cdot C_{st} \cdot \frac{dV_t}{V_t \cdot dt}
\]

Equ. 6.6

\[
\frac{d(R_c \cdot C_{dt})}{dt} = k_{in} \cdot C_{st} \cdot R_b - k_{out} \cdot C_{dt} \cdot R_c - R_c \cdot C_{dt} \cdot \frac{dV_t}{V_t \cdot dt}
\]

Equ. 6.7

The equations were simplified as follows.

\[
\frac{dZ1}{dt} = k_a \cdot C_p - k_d \cdot Z1 - k_{in} \cdot Z1 + k_{out} \cdot Z2 - Z1 \cdot \frac{dV_t}{V_t \cdot dt};
\]

Equ. 6.8

\[
\frac{dZ2}{dt} = k_{in} \cdot Z1 - k_{out} \cdot Z2 - Z2 \cdot \frac{dV_t}{V_t \cdot dt}
\]

Equ. 6.9
\[
C_{\text{out}} = C_{\text{in}} - \frac{V_{\text{slope}}}{Q} \cdot R_a \cdot \frac{1}{2} \cdot C_p - \frac{V_t}{Q} \cdot R_a \cdot \frac{1}{2} \cdot \frac{dC_p}{dt} - \frac{V_t}{Q} \cdot k_a \cdot C_p + \frac{V_t}{Q} \cdot k_d \cdot Z1
\]

Eq. 6.10

\[Z1 = k \cdot C_{\text{out}}, \text{ for } t > 6\text{hr};\]

in which, \(Z1 = R_b \cdot C_{st}; Z2 = R_c \cdot C_{dt}; k_a' = k_a \cdot f_p \cdot R_b.\)

In the two-compartment model, the organ was divided into two compartments, including vascular space and tissue compartment, without differentiating between shallow and deep compartments. All the assumptions made for the three-compartment model were applicable here.

The concentration change in compartment (a) could be expressed:

\[\frac{d(V_t \cdot R_a \cdot C_{\text{blood}})}{dt} = Q \cdot (C_{\text{in}} - C_{\text{out}}) - k_a \cdot f_p \cdot C_p \cdot V_t \cdot R_b + k_d \cdot C_t \cdot R_b \cdot V_t\]

Eq. 6.11

The concentration change in compartment (b) was expressed as:

\[\frac{d(V_t \cdot R_b \cdot C_{\text{st}})}{dt} = k_a \cdot f_p \cdot C_p \cdot V_t \cdot R_b - k_d \cdot C_t \cdot R_b \cdot V_t\]

Eq. 6.12

The average tumor concentration was calculated as:

\[C_{\text{tumor}} = R_a \cdot C_{\text{blood}} + R_b \cdot C_t\]

Eq. 6.13

Transformations and simplification were made similarly as in the three-compartment model. The final simplified equations were simplified as follows.

\[\frac{dZ1}{dt} = k_a \cdot C_p - k_d \cdot Z1 - Z1 \cdot \frac{dV_t}{V_t} \cdot dt\]

Eq. 6.14
6.2.9 Source of physiological parameters

Five parameters were fitted in the model, including $k_a'$, $k_d$, $k_{in}$, $k_{out}$ and $k$. Other parameters were either obtained from the literature or measured in the experiment.

The values of $R_a$ and blood flow rate were calculated from the literature value as listed in Table 6.2 (Blumenthal et al., 1989; Blumenthal et al., 1992; Blumenthal et al., 2000; Herman et al., 1999; Yuan et al., 1993). The average of the listed values, 0.035 for $R_a$ and 0.052 ml/g/min for blood flow was used. The length and width of tumors were measured using a caliper and the tumor volume was calculated by: (tumor length) x (tumor width)$^2$/2. The tumor volume growth was analyzed by linear regression and the growth curves were described as: tumor volume (cm$^3$) = 0.462 + 0.00291 × time (hr). The plasma concentration was modeled by 2-compartment model, expressed as:

$$C_p = A \times \exp(-\alpha \times t) + B \times \exp(-\beta \times t)$$

Equ. 6.15

The parameters were fitted from the data of single dosing group. The concentrations in plasma and tumors were simulated for multiple-dosing group.
6.3 RESULTS

6.3.1 Mass balance

The amount of suramin dose recovered in mice was calculated as the product of individual tissue weight multiplied by the concentration of individual tissues. Suramin concentrations in plasma and the analyzed tissues (i.e., skin, lung, heart, liver, kidney, muscle, stomach, large and small intestine) at 5min were used in the calculation and the total amount of suramin recovered accounted for 90 ± 8.7 % of the administer dose (10 mg/kg).

6.3.2 Plasma and tissue concentration-time profile

Figure 6.1 shows the concentration-time profiles of low-dose suramin in arterial plasma, bladder, brain, fat, heart, kidney, liver, lung, muscle, pancreas, stomach, small intestine, large intestine spleen and tumor after an i.v. injection of 10 mg/kg suramin. Table 6.1 summarizes the pharmacokinetic parameters.

The plasma concentration–time profile showed a biphasic decline. For all the analyzed organs, tissue concentration profile of suramin showed different concentration profile as in plasma. Between organs, the concentration profiles also varied. The highest suramin concentration was found in kidney and the lowest in brain. Based on the pattern of the profiles, the organs could be put in three groups. The first group included organs that had two peaks, with the first peak occurring at an early time point, ranging from 5min to 4 hr, and the second peak occurring at a later time point, ranging from 24 hr to 1 week. Most organs belong to the first group, such as kidney, heart, lung, liver, muscle, pancreas, skin, spleen, tumor. The second group consisted of organs, which just showed one peak between 5 min to 6 hr. These organs include stomach, small intestine and large intestine.
The third group included only brain, of which the highest concentration was observed at the first time point. Among all the organs, brain was the only one, which had pharmacokinetic profile parallel to plasma profile. All the other organs showed longer suramin terminal half-life than plasma.

6.3.3 Tissue partitioning coefficient

The magnitude of distribution in individual organs was characterized by tissue partition coefficient, estimated by the finite tissue to plasma AUC ratio (Table 6.2). For all the organs, $K_p$ increased with time. The tissue to plasma AUC$_{0-168h}$ ratios higher than 1.0 were observed in the following organs, from high to low, kidney, skin, bladder, spleen, lung, fat, large intestine, small intestine and stomach. These include both highly perfused organs and poorly perfused organs. The ratios lower than 1.0 were observed in, from high to low, heart, liver, pancreas, tumor and muscle. The result suggested that perfusion was not an important deterministic factor for drug partition. The kinetics of suramin distribution in tissues was also indicated by apparent partition coefficient $K_{p, app}$, which was defined as the tissue to plasma concentration ratio (Figure 6.2). The $K_{p, app}$ increased with time, except in brain, which almost remained constant.

6.3.4 Comparisons of two local PBPK model

Figure 6.3 shows the predicted curves for tissue and plasma by 2-compartment and 3 compartment models, respectively. The concentration profile for 2-compartment showed only one peak, while 3-compartment model predicted two peaks. Both models underestimated the tumor concentration for multiple dosing. The 3-compartment model predicted the plasma concentrations better than the 2-compartment model. Table 6.3 lists the PK parameters and diagnostic parameters obtained by WinNonLin. Both AIC and
Schwarz's criteria indicated that the 3-compartment model was better than 2-compartment model.
6.4 DISCUSSION

Results of this study indicate that low dose suramin was widely distributed to bladder, lung, liver, heart, kidney, stomach, large and small intestines, muscle, pancreas, spleen, skin, and tumor tissues after intravenous administration. Only minimal amounts of suramin were detected in the brain. A mass balance analysis indicates that about 90% of the dose was found in the tissues measured, indicating these tissues as the major sites for drug distribution and accumulation.

Different concentration-time profiles were observed in different tissues. Time to peak concentration in various tissues was not consistent, and in some tissues two peaks were measured, even though plasma levels declined continuously over the course of the study. These results indicate that suramin distribution in tissues is not homogenous and involves more than one process. Chemically, suramin is a symmetric, polysulfonated naphthylurea. The presence of the six, negatively charged sulfonated groups allow this compound to bind to many proteins of biological interest, presumably because of a weak ionic interaction between these residues and basic amino acids. For example, suramin has been shown to bind to heparin-binding growth factors in the extracellular matrix and can inhibit the binding of these factors, including basic fibroblast growth factor (Acevedo et al., 1993; Mignatti et al., 1991), acidic fibroblast growth factor (Mascarelli et al., 1993; Volkin et al., 1993), Kaposi’s fibroblast growth factor (Moscatelli and Quarto, 1989), platelet-derived growth factor (Huang and Huang, 1988; Keating and Williams, 1988) and vascular endothelial growth factor (Plouet and Moukadir, 1990), to their cell surface receptors on a variety of cell types. Suramin can also bind to the cell membrane with different binding affinities to various cell lines (Stein et al., 1995). Due to the low
value of pKa (<-1) of the arylsulfonate groups, suramin remains negatively charged under all physiological conditions. Therefore, it is unlikely that the molecule can passively diffuse across cell membranes. In the absence of passive diffusion, it is most likely that suramin is internalized by a combination of the active processes of adsorptive endocytosis and pinocytosis. The common end point of both of these mechanisms is the endosomal/lysosomal compartment. For the same reason, suramin leaves the cell most likely by exocytosis. It has been shown that high dose suramin elicits a marked lysosomal storage disorder, which is similar to that observed in patients with inherited mucopolysaccharidosis (Christensen and Lullmann-Rauch, 1988; Constantopoulos et al., 1983; Gritli et al., 1993). Suramin distribution to the extracellular matrix and cell membrane might be associated with the initial tissue concentration peak. The intracellular uptake may be associated with the second peak. The high exocytosis rate in the GI organs might be the reason for the absence of a second peak in these organs. It has been reported that suramin cannot cross blood brain barrier (McNally et al., 2000). Therefore suramin detected in brain most likely derives from the blood residue contained in the brain tissue upon harvest. The blood volume fraction in brain was reported to be 0.03 in mice (Brown et al., 1997), and the plasma volume fraction would be half of the value, since RBCs do not take up suramin (Findley G.M., 1950). The brain $K_{p,\text{app}}$ remained approximately constant, ranging from 0.013 to 0.02 with an average of 0.017. The consistency confirmed our speculation. Due to detection limit, suramin was detected in time points until 6 hr.

Based on the model simulation, accumulation would occur in the tumor after multiple dosing, which was confirmed by the experimental data. This raised the question if the elevated concentration would cause antagonism for other chemotherapeutic drugs. We
have defined the chemosensitization range of suramin as 10 – 50 µM, which refer to the concentrations in the medium and plasma. The tumor concentration increased beyond the range after 6 times of dosing, although the plasma concentrations were still in the range. To derive the corresponding tissue concentrations is a complicated issue, because the concentration ratio of tissue to plasma was increasing within one cycle and in the subsequent cycles at the same points after each dosing. The pharmacodynamics study of suramin, in directly relation to the concentrations in tissues instead of plasma, is warranted to clarify an optimal tissue concentration range.

The PBPK model provide basis to scale up the tissue concentrations in animals to humans. However, cautions need to be taken to predict suramin concentration in tumors other than colorectal carcinoma, because different organs have varied concentration profiles. Therefore tumors originateing from different sites might different $k_a$, $k_d$, $k_{in}$ and $k_{out}$ from the values derived from the s.c. HT-29 tumor model. Further experimental investigations of different tumor types tumor model are recommended to further refine the PBPK model.

In summary, results of the present study indicate that low dose suramin was widely distributed to different tissues and elimination from the tissue is the rate-limiting step for the clearance of suramin. Of particular interest is the differential drug distribution in different organs. Further studies are needed to determine suramin concentration-time profile in different tumor-bearing animal models.
Table 6.1 Tissue distribution parameters of suramin following i.v. bolus administration (10 mg/kg) in tumor bearing mice. AUC is estimated using the trapezoidal method. Terminal half-life was estimated using the slope of the last three time points.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;0-168h&lt;/sub&gt; (µg/ml·hr or µg/g·hr)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;tissue&lt;/sub&gt;:AUC&lt;sub&gt;plasma&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma</td>
<td>131</td>
<td>0.083</td>
<td>935</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>brain</td>
<td>1.81</td>
<td>0.1</td>
<td>0.698</td>
<td>6.3</td>
<td>0.016</td>
</tr>
<tr>
<td>heart</td>
<td>13.1</td>
<td>0.1</td>
<td>773</td>
<td>614</td>
<td>0.827</td>
</tr>
<tr>
<td>kidney</td>
<td>159</td>
<td>168</td>
<td>19,000</td>
<td>554</td>
<td>20.3</td>
</tr>
<tr>
<td>liver</td>
<td>5.23</td>
<td>0.1</td>
<td>640</td>
<td>338</td>
<td>0.684</td>
</tr>
<tr>
<td>lung</td>
<td>15.4</td>
<td>0.1</td>
<td>2,200</td>
<td>182</td>
<td>2.35</td>
</tr>
<tr>
<td>muscle</td>
<td>8.07</td>
<td>2</td>
<td>583</td>
<td>86</td>
<td>0.623</td>
</tr>
<tr>
<td>pancreas</td>
<td>11.5</td>
<td>0.5</td>
<td>606</td>
<td>181</td>
<td>0.648</td>
</tr>
<tr>
<td>skin</td>
<td>18.5</td>
<td>6</td>
<td>2,930</td>
<td>269</td>
<td>3.13</td>
</tr>
<tr>
<td>stomach</td>
<td>12.5</td>
<td>2</td>
<td>1,070</td>
<td>102</td>
<td>1.15</td>
</tr>
<tr>
<td>small intestine</td>
<td>23.1</td>
<td>0.5</td>
<td>1,100</td>
<td>159</td>
<td>1.17</td>
</tr>
<tr>
<td>large intestine</td>
<td>18.3</td>
<td>2</td>
<td>1,180</td>
<td>96</td>
<td>1.26</td>
</tr>
<tr>
<td>spleen</td>
<td>21.2</td>
<td>0.1</td>
<td>2,420</td>
<td>131</td>
<td>2.59</td>
</tr>
<tr>
<td>tumor</td>
<td>11.3</td>
<td>24</td>
<td>585</td>
<td>239</td>
<td>0.626</td>
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</table>
Table 6.2  Suramin partition coefficient and concentrations in tissues. $K_p$ was estimated by finite tissue to plasma AUC ratio at 168 hr following 10 mg/kg i.v. dose. AUC is estimated using the trapezoidal method. Concentrations was listed up to 168 hr. ND, not detectable.
### Table 6.3 Estimates of parameters for PBPK model.

Tissue and plasma concentrations from single dosing group were fitted by WinNonLin simultaneously. The diagnostic parameters were provided by the software.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k_a )</td>
<td>SSR</td>
</tr>
<tr>
<td>2-compartment</td>
<td>0.0720</td>
<td>WSSR</td>
</tr>
<tr>
<td></td>
<td>( k_d )</td>
<td>AIC</td>
</tr>
<tr>
<td></td>
<td>0.000868</td>
<td>SBC</td>
</tr>
<tr>
<td>( A )</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>( B )</td>
<td>8.91</td>
<td></td>
</tr>
<tr>
<td>( \alpha )</td>
<td>0.719</td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>0.00368</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( k_a )</td>
<td>SSR</td>
</tr>
<tr>
<td>3-compartment</td>
<td>0.515</td>
<td>WSSR</td>
</tr>
<tr>
<td></td>
<td>( k_d )</td>
<td>AIC</td>
</tr>
<tr>
<td></td>
<td>5.84</td>
<td>SBC</td>
</tr>
<tr>
<td>( k_{in} )</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>( k_{out} )</td>
<td>6.88E-5</td>
<td></td>
</tr>
<tr>
<td>( K )</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>( A )</td>
<td>77.4</td>
<td></td>
</tr>
<tr>
<td>( B )</td>
<td>7.03</td>
<td></td>
</tr>
<tr>
<td>( \alpha )</td>
<td>0.416</td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>0.00390</td>
<td></td>
</tr>
</tbody>
</table>

*: SSR, sum of squared residuals; WSSR, weighted sum of squared residuals; AIC, akaike’s information criteria; SBC, Schwarz’s criteria.
Figure 6.1 Two physiologically based pharmacokinetic (PBPK) model proposed for suramin in tumor. Both models have in common the first order kinetics between compartments, with the coefficient of $k_a$, $k_d$, $k_{in}$ and $k_{out}$. There is no elimination of suramin for the tissue compartment. The 3-compartment model (a) assumes the existence of a shallow compartment and a deep compartment in the tissue, whereas the 2-compartment model (b) assumes homogeneous tissue compartment. The single directional arrow denotes a non-instantaneous equilibration process.
**Figure 6.2  Tissue distribution of suramin.** Mice were given an intravenous bolus injection of suramin (10 mg/kg). Symbols are, plasma: open circle; tissue: closed circle. 4 mice per time point, mean ± SD. Inserts illustrate the concentration-time profile between time zero to 24 hours. Group (a), organs with two peaks. Group (b) (next page), organs with one peak, but not parallel to the plasma concentration. Group (c) (next page), organs with one peak, parallel to the plasma concentration.
Figure 6.1 continued,

(b)

(c)
Figure 6.3  Increase of apparent partition coefficient $K_{p,\text{app}}$ in tissues. $K_{p,\text{app}}$ was defined as tissue to plasma concentration ratio. Each point represents an average value from 4 mice. For legibility, only standard deviations for kidney and brain are plotted.
Figure 6.4 Measured and the best predictions of suramin concentrations in plasma and tumors in mice. The tumor-bearing mice receive either one dose (upper panels) or six doses (lower panels). Suramin concentrations in plasma and tumors after six doses were simulated using the parameters estimated by WinNonLin based fitting constants for a 3-compartment model (a) and 2-compartment model (b), respectively. Each symbol and error bar represents the mean and standard deviation, respectively. Solid (——) and dashed lines (- - -) are the PBPK best-fit prediction or simulation for plasma and tumors, respectively.
CHAPTER 7

PERSPECTIVES AND CONCLUSIONS

This dissertation has made several important contributions in the field of cancer chemotherapy, including formulation optimization and dosing optimization. Although our knowledge in tumor biology has exploded during the past decades, the existing difficulties of translating preclinical findings to relevant clinical designs have impeded the development of new effective therapeutics. The dissertation specifically addresses the problems for two drugs, paclitaxel and suramin, in respect to the problems of each drug. Paclitaxel, or any other anti-cancer drugs is not recommended for localized prostate cancer because of toxicity and low bioavailability of the drug in the prostate. A biodegradable PLGA mini-cylinder was developed for paclitaxel, which can be implanted into localized tumor in the prostate to achieve a high local drug concentration while sparing other organs. Chapter 2 and 3 are focused on the development of paclitaxel PLGA cylinders for local chemotherapy. A chemosensitizer, suramin, can enhance the activity of chemotherapeutic drugs in a narrow concentration range. This dissertation describes how the dosing regimen of suramin was optimized to achieve the synergistic concentration range. Further tissue distribution studies were conducted for the purpose to understand the dose response relationships in the effect compartment. Chapter 4, 5 and 6 are focused on dosing regimen optimization for suramin.
Treatment modalities for localized prostate cancer are very limited and associated with severe morbidities. Systemic chemotherapy is not recommended for the patients with localized disease because of the toxicity and low drug bioavailability in the prostate. Development of local chemotherapies can increase the drug concentration in the prostate while sparing other organs and systemic exposure. For this purpose, the biodegradable PLGA implants containing the anticancer drug paclitaxel were developed (Chapter 2). Being a highly hydrophobic drug, paclitaxel was releasing very slowly from the solid matrix, which problem was solved by the preparation of porous cylinders. It was a significant contribution in that it established a viable method for administering regional chemotherapy and a method for increasing release rate for hydrophobic drug, like paclitaxel. Porous PLGA matrix has been used for tissue regeneration. It is a novel idea to apply the technique to deliver a hydrophobic drug. The implants were designed such that they could be placed using commercially available implant needles, and currently used ultrasonic imaging techniques.

The cylinders were further tested in tumor bearing mice (Chapter 3). The porous paclitaxel cylinder showed tumor growth inhibition effect. Tumor volume was decreasing for three weeks and started to increase after that. It was shown the drug penetration was limited within 5 mm around the cylinder. This finding helps us to design the ideal mapping of implantation to cover the whole tumor area in future clinics when the formulation enters further development stage. Implantation of Paclitaxel-IPM-cylinders further inhibited tumor growth, which effect was enhanced by Taxol pretreatment. IPM is a commonly used ingredient in cosmetics and trans-dermal delivery to enhance drug penetration. It increased paclitaxel release rate from PLGA matrix in \textit{in vitro} condition.
Based on its general effect, it was hypothesized that IPM might also increase paclitaxel penetration in tumors, which, if confirmed, will have a great impact on the local drug delivery area.

Systemic chemotherapy is the most important treatment modality for metastasized cancer patients. However, drug resistance has been a hurdle to effective therapies for years. Our lab has found that suramin reversed FGF-induced chemoresistance in a narrow therapeutic range from 10-50 µM, with antagonism occurring above this concentration. To achieve the narrow therapeutic concentration range in human patients, pharmacokinetic studies of low-dose suramin were conducted across three species, mice, rats, and dogs (Chapter 4). The dienetichron plot, an allometric scaling plot, which transforms clock time and absolute physical parameters into forms that become species invariant, was proved a useful tool to design the dosing regimen in our Phase I clinical trial in NSCLC patients. Very good allometric relationships were established between pharmacokinetic parameters and body weight across four species, mice, rats, dogs and humans. The allometric scaling can be used to predict suramin pharmacokinetics in other species in veterinary practice.

We further performed studies to evaluate the effectiveness of low dose suramin in pet dogs with naturally occurring tumors and determined the pharmacokinetics of suramin in the dog patients, in order to identify the target suramin dose (Chapter 5). Using a nonlinear mixed-effect model (NONMEM), we developed and validated an empirical equation to calculate the dosage to achieve target suramin concentration.

For a better understanding of suramin pharmacodynamics, tissue distribution of low-dose suramin was studied in tumor-bearing mice. Suramin was found to accumulate
in tissues at various levels, with accumulation continuing for up to 1 week. The highest concentration level was observed in kidneys and the lowest in brains. A local physiologically based pharmacokinetic (PBPK) model was developed for the tumor compartment, using a 3-compartment model approach.

The pharmacokinetic study in this dissertation plays important roles in optimizing suramin dosing regimen in the future clinical trials. The interspecies scaling study facilitated us to design the first clinical trial to use suramin as a chemosensitizer in human patients. The allometric relationships can further provide reference for veterinarians when using suramin as a chemosensitizer to treat other species. The empirical equation developed in Chapter 5 were tested in patients with a variety of breeds and can be applied readily in new trials to test the chemosensitization effect of suramin for dog patients. It was indicated that, as a limit of the equation, the dose calculated from the equation might give greyhound an overdose, compared to the preset target concentration range, although the resulting concentration is still within the chemosensitization range of suramin. This warrants a phase I study in greyhound to further refine the dosing regimen. The PK/PD relationship of suramin is currently based on the concentration in the surrounding medium, i.e. culture medium or plasma. The PBPK model established in this dissertation will establish a PK profile in the organs and thereby provide basis for a more direct PK/PD relationship in the organs. In the future study, from the results of the animal model, we can scale up to humans and predict suramin concentration in different organs. By combining the \textit{in vitro} pharmacodynamics of suramin for different tumor types, suramin dose will be given to achieve the optimal concentration for a specific organ,
realizing the goal to treat tumors in an intelligent way, thereby introducing a new dosing paradigm for cancer patients.

In conclusion, the research in this dissertation spanned multiple disciplines and covered many areas of the pharmaceutical sciences, from drug delivery to pharmacokinetics, from *in vitro* to *in vivo*. In the past, the low yield of new therapeutics with clinical benefits for cancer patients have imposed us with problems both financially and ethnically. This dissertation tackles the problem by giving two old drugs in more ‘optimal’ ways, based on the understanding of the pharmacodynamics and limitations of the old drugs, instead of inventing new drugs. Hopefully, this study can not only provide certain findings facts, including a good formulation to treat localized prostate cancer, some allometric equations for further reference, an empirical dosing equation to dose dog cancer patients, suramin distribution profiles in tissues and local PBPK model, but also encourage readers to dig out the hidden values of drugs that are being developed or are already being negated.
BIBLIOGRAPHY


Ref Type: Thesis/Dissertation


Denckla, WD, 1975, A time to die: Life Sci., v. 16, p. 31-44.


Foley, JP, I M Thompson, 2000, Natural history of localized adenocarcinoma of the prostate. in Advanced Therapy of Prostate Disease, edited by Martin I. Resnick, B.C. Decker Inc.


Fujiuchi, S, Y Ohsaki, K Kikuchi, 1997, Suramin inhibits the growth of non-small-cell lung cancer cells that express the epidermal growth factor receptor: Oncology, v. 54, p. 134-140.


Heusner, AA, 1987, What does the power function reveal about structure and function in animals of different size?: Annu.Rev.Physiol, v. 49, p. 121-133.


Lewis, DH, 1990, Controlled release of bioactive agents from lactide / glycolide polymers., in R Langer (ed), Biodegradable polymers as drug delivery systems.: p. 45.


Mascarelli, F, G Fuhrmann, Y Courtois, 1993, aFGF binding to low and high affinity receptors induces both aFGF and aFGF receptors dimerization: Growth Factors, v. 8, p. 211-233.


Moscatelli, D, N Quarto, 1989, Transformation of NIH 3T3 cells with basic fibroblast growth factor or the hst/K-fgf oncogene causes downregulation of the fibroblast growth factor receptor: reversal of morphological transformation and restoration of receptor number by suramin: J. Cell Biol., v. 109, p. 2519-2527.


Ortiz, R. Implantable biodegradable drug delivery device for regional cancer chemotherapy. 2002. College of Pharmacy, The Ohio State University. Ref Type: Thesis/Dissertation


Schiff, PB, S B Horwitz, 1981, Taxol assembles tubulin in the absence of exogenous guanosine 5'-triphosphate or microtubule-associated proteins: Biochemistry, v. 20, p. 3247-3252.


Villalona-Calero,MA, M G Wientjes, G A Otterson, S Kanter, D Young, A J Murgo, B Fischer, C DeHoff, D Chen, T K Yeh, S Song, M Grever, J L Au, 2003, Phase I study of


