8-1991

The effects of localized hyperthermia on blood flow and cisplatin pharmacokinetics

Peni Lynn Ausmus

To the Graduate Council:

I am submitting herewith a thesis written by Peni Lynn Ausmus entitled "The effects of localized hyperthermia on blood flow and cisplatin pharmacokinetics." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

Donita L. Frazier, Major Professor

We have read this thesis and recommend its acceptance:

Karla J. Matteson, Bergein F. Overholt

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Signature Peni Lynn Ausmus

Date July 24, 1991

THE EFFECTS OF LOCALIZED HYPERTHERMIA ON BLOOD FLOW AND CISPLATIN PHARMACOKINETICS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Peni Lynn Ausmus

August 1991

DEDICATION

This work is dedicated to the

precious memory of my sister

Danielle Marie Ausmus

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my major professor, Dr. Donita L. Frazier, for her guidance, expertise, and enthusiasm in my research. Gratitude is also extended to Dr. Karla J. Matteson, a committee member, who took interest in my studies and career. And, for his wise counsel and advice, I am grateful to Dr. Bergein F. Overholt who also participated on my committee. The financial support and travel opportunities provided by the Thompson Cancer Survival Center and UTCVM Department of Environmental Practice are also gratefully acknowledged.

I would like to thank those students who offered vital assistance with my research surgeries: Gretchen Simpson, Lorna McLeod, and Blake Chandler. A very special thanks goes to Dr. Masoud Panjehpour, Dr. Mark Peterson, Mary Ann Barnhill, and August Wilke for freely sharing their knowledge on various subjects with me.

I would also like to recognize those people in the UTCVM Toxicology and Laser/Hypethermia Laboratories for their comradery: Kim Abney, Gina Barbaro, Sherry Cox, Jessica Orvis, Rick Sneed and Reneé Teunissen van Manen.

Acknowledgements would not be complete without recognizing my friends who took great interest this

research and helped me to maintain balance in my life while in graduate school: Angie Ayers, Rick McCullah, Greg McIntyre, and Rhonda Pack.

Most importantly, I would like to thank my Christian parents, Rita and Paul Patterson whose loving support and sacrifices made my education possible. Additionally, I wish to acknowledge my grandmothers, Idessa Powell and Mary Ausmus, and extended family: the Ausmuses, Gibsons, Leavitts, Smiddys and Taylors.

Lastly, I would like to give special thanks to Shawn Hirt for the constant love and support he provided throughout my graduate studies and to his parents, Sam and Melanie Hirt, for their encouragement and for providing relaxing weekends in Nashville during this endeavor.

If any of you lack wisdom, let him ask of God that giveth to all men liberally and upbraideth not; and it shall be given him. St. James 1:5

ABSTRACT

This study was undertaken to gain knowledge of the effects of localized hyperthermia (43°C, 1 hr) on blood perfusion rates as well as cisplatin pharmacokinetics in Sprague-Dawley rats with induced mammary adenocarcinomas.

Rats were administered either cisplatin alone, local hyperthermia alone (43°C for 1 hr), cisplatin plus hyperthermia, or neither. Blood flows in tumor and normal tissues were measured at different times relative to hyperthermia. Cisplatin was administered to another group of rats at times corresponding to those in the hyperthermia only group. Drug concentrations were determined in tumors and plasma.

Overall, treatment means of pharmacokinetic parameters (distribution and elimination half-lives, area under the plasma concentration versus time curve, clearance, A and B intercepts, α and β rate constants) were not significantly different (ANOVA). However when pairwise comparisons were made, there were significant differences in several pharmacokinetic parameters.

The distribution half-life of the drug only group was significantly shorter ($p<0.05$) than the group receiving drug one hour before, at the beginning of, and one hour after a hyperthermia treatment. The average distribution

half-life of rats receiving drug 1 hour before heat was significantly longer ($p<0.05$) than groups receiving drug only, drug at the beginning of hyperthermia , or drug a hour after hyperthermia. The elimination half-life of the group receiving drug 1 hr before heat was significantly longer ($p<0.05$) than all other groups. Clearance was significantly greater ($p<0.05$) when drug was administered at the end of heat than when it was given 1 hr after hyperthermia.

The volume of distribution of the central compartment of the group given drug at the end of hyperthermia was significantly greater ($p<0.05$) than the group which received drug 1 hour after hyperthermia. The steady state volume of distribution was significantly greater ($p<0.05$) when drug was given 1 hour before hyperthermia than when drug was given at the beginning of heat, end of heat, or 1 hour after heat.

The average tumor concentration of the group of rats receiving drug at the beginning of heat was significantly greater ($p<0.05$) than the average for the group in which rats where given drug at the end of a hyperthermia treatment. A Pearson correlation analysis revealed that tumor drug concentration was directly correlated to the area under the curve and the A intercept and indirectly

correlated to the rate of clearance and volume of distribution of the central compartment.

Similarly, blood flow did not differ significantly; however, the data reveals a trend. Blood flow was highest immediately after a hyperthermia treatment and slowed over a three hour period to reach an approximate untreated value at three hours post treatment.

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CHAPTER I

INTRODUCTION

Hyperthermia, defined as 43°C, 1 hr, is a useful adjuvant to radiation and chemotherapy of cancer (Kim et al., 1982; Luk et al., 1984; Burholt et al.; and Carde et al., 1981). In chemotherapy, the effect of cancericidal agents depends on the amount of the agent delivered to tumor cells within the tumor (Long and Repta, 1981). Most chemotherapeutic agents are also toxic to normal tissue. So, a technique that would increase blood flow preferentially to a neoplasm could be used advantageously to deliver greater concentrations of a chemotherapeutic agent to malignant tissue and less to normal tissue thereby sparing normal tissue.

It is known that heat induces an increase in blood flow accompanied by dilation of vessels and an increase in permeability of the vascular walls in tissue (Crile, 1963; Dewey et al., 1979; and Dickson and Calderwood, 1980). It can then be postulated that the proper sequencing of drug administration and hyperthermia treatment might enable delivery of chemotherapeutic agent to a tumor in greater quantity and then to "trap the drug" in the tumor for maximum therapeutic value. <u>In vitro</u> studies involving

clonogenic assays have shown that heat plus adriamycin or mitomycin C in cells from spontaneously occurring human carcinomas (Akiyoshi et al., 1986) is synergistic for cell killing. The same result was obtain using hyperthermia and thioTEPA, BCNU or cisplatin in HA1 Chinese hamster cells (Hahn, 1979) as well as adriamycin and bleomycin in EMT6 mammary sarcomas (Hahn et al., 1975). Bioassays consisting of injections of cells previously exposed to heat plus adriamycin, 5-fluorouracil, and vincristine into mice also showed synergistic killing (Adwankar and Chitnis, 1984). Although it has been reported that elevated temperatures enhance the cytotoxicity of cisplatin both in vitro (Hahn, 1979) and in vivo (Dickson and Ellis, 1974), additional knowledge of alterations in drug pharmacokinetics, i.e. plasma clearance, distribution, biotransformation, and excretion, in the presence of hyperthermia is needed in order to elucidate the proper sequencing of the two modalities of therapy for optimal effect.

The objectives of this study were 1) to determine the effect of localized hyperthermia (43^{o}C for 1 hr) on the plasma and tissue pharmacokinetics of cisplatin when drug is administered at different times relative to hyperthermia in rats with dimethylbenz[a]anthracene-induced mammary adenocarcinomas and 2) to correlate

hyperthermia-induced changes in tumor blood flow with cisplatin concentrations.

CHAPTER II

REVIEW OF LITERATURE

I. Hyperthermia

Introduction

Records of the use of hyperthermia in the treatment of cancer and various other ailments date back to ancient times. The first documented evidence that elevated temperatures might have a selective effect on tumors is ascribed to Busch (1866) who reported the disappearance of a histologically verified sarcoma of the face after two attacks of erysipelas. Many reports from the late nineteenth and early twentieth centuries describe the regression of primary and secondary tumors after infection with pyrogenic bacteria (Bruns, 1887; Coley, 1893, 1896; and Rohdenburg, 1918).

In 1912 Lambert made the first recorded observation that cultured neoplastic cells have greater thermosensitivity than normal cells. He found that mouse and rat sarcoma cells are more thermosensitive than normal mesenchymal cells. Thermosensitivity refers to the sensitivity of a cell to the damaging effects of heat. Research by Vollinar in 1941 supported the findings by showing that tumor cells (Ehrlich ascites carcinoma and

Jensen's sarcoma) were killed at temperatures between 40°C and 42°C, whereas normal spleen cells resisted exposure and were not killed at temperatures up to 43°C. Through the years a large body of evidence has accumulated that supports the theory that tumors are selectively vulnerable to heat treatment. As a general rule, it has been found that cell damage occurs in tumor cells at lower temperatures (41°C-44°C) than that required to affect normal cells. These temperatures can be attained *in vivo* without damage to the surrounding normal tissue (Cavaliere et al., 1967; Giovanella et al., 1973; Levine and Robbins, 1970; Muckle and Dickenson, 1971; and Overgaard and Overgaard, 1972).

Thermoregulatory Mechanisms

All cells within homoiotherms must maintain a relatively narrow temperature range for life to exist. Temperatures above or below the norm set off a wide variety of physiological mechanisms of heat production and loss that allow the body to return to the normal temperature range.

Each living cell has its own heat generating apparatus. Heat that is generated is directly dependent on the metabolic activity and is basically a result of oxidation of nutrients that enter each cell and a by-

product of the production of useful energy for the continued activity of all cells. The activity of muscular contraction is responsible for the generation of most body heat. Twenty percent of heat production is accounted for by glandular secretory activity, especially from the liver (Chaffee and Lytle, 1980). Food uptake is an additional factor involved in heat production which results in the stimulation of increased smooth muscle activity, glandular secretion, and "specific dynamic action" required for protein digestion (Hornback, 1984).

Endocrine glands are intimately involved in thermoregulation with both the thyroid and adrenal glands playing a significant role. Epinephrine acts to rapidly increase heat production by stimulating the activity of visceral organs such as the heart. A more prolonged increase in heat generation is achieved by the release of thyroxine by the thyroid gland which potentiates the calorigenic action of epinephrine.

Thermal radiation, convection, conduction, and evaporation are the major physical processes involved in body heat loss (Guyton, 1981). The body responds to heat stress by: suppression of endocrine gland activity responsible for metabolic cellular activity, muscle shivering, and chemical thermogenesis; skin blood vessel dilation caused by inhibition of the sympathetic centers

in the posterior hypothalamus which causes vaso-constriction; increased water evaporation through the skin and lungs; heat loss through urine and feces; and evaporation and convection heat loss of inspired air (Hornback, 1984).

Thermal radiation losses can be altered by clothing as well as by physiological mechanisms that change skin temperatures, i.e., redistribution of blood, variations in blood volumes, and changes in circulation rate. The circulatory system plays a significant role in shifting heat rapidly and efficiently to various organs of the body. Through vasomotor responses, heat can be transferred quickly and efficiently to cooler organs through the amount and pattern of blood flow. Direct venous convection of heat from the working muscle to the skin is the principal factor responsible in heat transfer (Hardy, 1961).

Effects of Anesthesia on Thermoregulation and Blood Flow

Since the body temperature of anesthetized rats may decrease to 30-32^{o}C, and tumor temperatures may be far lower depending on the type of anesthetic used, anesthesia can have a profound effect on blood flow (Mueller-Klieser and Vaupel, 1987). It is important to know the biological effects of the anesthetics that are being considered for

use in a study. Pentobarbital and ketamine were the anesthetics considered for this graduate project.

Pentobarbital may induce a central depression of respiration. As a consequence, tumor oxygenation may be impaired through a reduced O_2 concentration in the tumor arterial blood (Harvey, 1985). However, Green (1975) reported that respiratory depression is a major problem only when animals suffer from chronic respiratory disease, whole body temperature is allowed to fall, or after repeated administration of anesthetic. Pentobarbital anesthesia may influence tumor blood flow by changing the mean arterial blood pressure and/or by causing alterations in the local distribution of the blood supply (Mueller-Klieser and Vaupel, 1987). These effects on blood flow are mediated mainly through a reduction in cardiac output and also through relaxation of the vascular smooth muscles which leads to an initial arteriolar vasodilation after administration of the anesthetic (Mueller-Klieser and Vaupel, 1987). Besides the direct effects mentioned above, pentobarbital can influence circulation in two indirect ways: 1) reducing the systemic levels of adrenaline and vasopressin by restriction of both the adrenal and the pituitary gland (Kawaue and Iriuchijima, 1982), and 2) causing a dramatic drop in the animals' core temperature of up to $5^{o}C$ which may cause changes in

regional blood distribution due to thermoregulatory processes (Green, 1975). Pentobarbital would be a poor choice for this research project because of the effects on respiration and blood flow mentioned above.

Ketamine, a dissociative anesthetic, is considered superior to pentobarbital with regard to effects on the vasculature; however, disadvantages with ketamine include poor muscle relaxation, purposeless movements, occasional irrational responses to stimuli, and a large variability in analgesia (Marshall and Wollman, 1985). For the purposes of this study in which muscle relaxation was not important and repeated administration of anesthetic was sometimes necessary, ketamine was chosen as the anesthetic in combination with acepromazine.

The addition of acepromazine malate has been shown to improve skeletal muscle relaxation and recovery (Wright, 1982). Acepromazine malate has been shown to decrease arterial blood flow and cause a significant increase in central venous pressure in dogs (Popovic et al., 1972), as well as cause hypotension in the horse (Parry et al., 1982) and cat (Colby and Sanford, 1981). However, acepromazine in combination with ketamine induces a shorter depressant effect upon the mean arterial pressure, heart, and respiratory rate than ketamine and xylazine combinations (Sanford and Colby, 1982). The combination

of ketamine and acepromazine was used in this investigation.

Mechanisms of Hyperthermia-Induced Cytotoxicity

A number of morphological and biochemical modifications occur when mammalian tumor cells are exposed to supranormal temperatures *in vitro* and *in vivo*. Among these are surface blebbing and a marked increase in nuclear size (Levine and Robbins, 1970). From a morphological point of view, the main target appears to be the nucleolus. Retraction of the intranucleolar chromatin occurs along with a disappearance of the nucleolar reticulum. This is followed by a loss or reduction of the granular nucleolar component (Heine et al., 1971; and Simard et al., 1967, and 1969).

Biochemically, thermal exposure affects several aspects of cell metabolism. Inhibition of glycolysis (Westermark, 1927), oxygen consumption (Cavaliere et al., 1967), and synthesis of proteins and nucleic acids (Mondovi et al., 1969b) are all documented results of hyperthermia with DNA synthesis being most severely affected (Levine and Robbins, 1970).

It is possible that the increased thermosensitivity of neoplastic cells may be an intrinsic property due to changes in cell metabolism and physiology that occur when

a cell undergoes neoplastic transformation. However, tumor cells may be more sensitive to heat because of their aneuploidy. Evidence supporting this theory includes the low thermal tolerance of the specific germinal epithelium of testes (Collins and Lacy, 1969) and the observation that in yeast, diploid cells appear to be 2.5 times less sensitive to the lethal effect of 52^{o}C incubation than haploid cells (Wood, 1956). However, no general correlation can be evidenced between the degree of aneuploidy and heat sensitivity (Giovanella et al., 1973).

A second explanation for increased thermosensitivity is the increased rate of growth of tumor cells (Bender and Schramm, 1966). Levine and Robbins (1970) reported that normal "diploid" fibroblasts were not affected by exposure to 42^{o}C while in the resting state, whereas, in the growing state they were almost as damaged as the heteroploid "cancer" cells that were heated. Subsequent research both supports (Dickson and Shah, 1972) and contradicts (Giovanella et al., 1970) this explanation.

Many studies investigating the mechanism of hyperthermic cell killing have concentrated on tumor metabolism <u>in vitro</u>. Findings include a specific inhibition of tumor cell respiration (Mondovi et al., 1969a) and decreased incorporation of labeled precursors into DNA, RNA, and proteins of malignant cells at elevated

temperatures (Mondovi et al., 1969b). Several other investigations have confirmed these findings (Muckle and Dickson, 1971; Dickson and Shah, 1972; and Dickson and Suzanger, 1976). The above changes occurring in cellular biochemistry in different phases of the cell cycle (Mitchison, 1971) were also found to have a bearing on thermal cell killing. Another mechanism by which hyperthermia may cause cell death has been proposed by Hardesty et al. (1981) who have identified two proteins with inhibitory activity within cancer cells that were activated by heat. Both these factors caused inhibition of protein synthesis and subsequent death in cancer cells.

Generally, hyperthermia delays the progression of cells through all phases of the cell cycle although certain phases of the cycle are more sensitive than others (Bhuyan, 1979). Cultured cells are specifically heat sensitive in the phase of DNA replication (S phase) (Westra and Dewey, 1971; and Palzer and Heidelberger, 1973b) with metaphase being the phase of replication that is most sensitive to the effects of elevated temperatures (Sisken et al., 1965). Hyperthermic cell killing was shown to be enhanced by drugs that stimulated DNA or protein synthesis or inhibited RNA synthesis (Palzer and Heidelberger, 1973a).

Hyperthermia Techniques

The early translations of Ramajama (2000 B.C.), Hippocrates (400 B.C.), and Galen (200 A.D.) record the use of ferrum candens (red-hot irons) and chemical caustics in the treatment of cancer. Fluid immersion, irrigation, regional perfusion, and electromagnetic or sound waves are some of the modern means of applying local or regional hyperthermia.

The various forms of electromagnetic energy seem to cause tissue heating by a similar mechanism. Energy is transferred into tissue by a field interaction that causes oscillation of ions in the tissue, or changes in the magnetic orientation of molecules, which is locally converted into heat.

Among the options for heating superficial tumors are capacitive-type electrodes, pancake inductive coils, radiofrequency needle electrodes, ultrasound transducers, and microwave applicators (Strohbehn and Douple, 1984). Capacitive-type electrodes are simple, but tend to deposit much greater power in the fat layers than in muscle tissue. Inductive coils put most of their energy in the muscle layer, but the power deposition pattern tends to be nonuniform. Radiofrequency is employed using two parallel planes of stainless steel needles implanted near the tumor boundary. An RF voltage (typically 0.5-1 MHz) is applied

to the two planes of needles resulting in currents between them which heat the tissue due to its resistive properties. Coaxial microwave antennas radiate power to the surrounding tissue; however, because most of this power is absorbed by the tissue close to the antenna, multiple antennea must be inserted into the tumor. Similarly, superficial microwave applicators suffer from the fact that they are not able to deposit significant power more than a few centimeters below the surface. Lower frequencies have a deeper depth of penetration; however, at the lower frequencies of interest the wavelength is large compared to the dimension of the applicator and the energy is not confined to a well-defined beam.

Ferromagnetic seeds can be deposited in the tumor volume during surgery after which the patient is placed in a large concentric coil for a hyperthermia treatment. If the frequency is low enough (<2 MHz), more energy is deposited into the tumor volume than into surrounding normal tissue. An advantage of this system is that seeds can be left in for extended amounts of time with less risk of infection. The wavelength of ultrasound transducers is small (typically 1 mm) in the frequency range where there is good penetration resulting in a well collimated beam. However, ultrasound is not a good modality for heating

tumors over bone or in the brain or thorax because of impedance mismatches between soft tissue and either air or bone which causes most of the energy to be reflected at these types of interfaces.

The major problem when using electromagnetic applicators is that only the designer has control over the energy radiated into the tissue. The actual temperature distribution is a function of the absorption properties of anatomical structures, thermal conductivity, and blood flow. Therefore, even if the system delivers power to a region containing a tumor, the hottest region within the patient may not be where the maximum power is deposited.

Electromagnetic energy produced by a laser (Light Amplification by the Stimulated Emission of Radiation) can also be used to induce hyperthermia. The Nd:YAG laser contains neodymium, a rare earth metal, in a yittrium-aluminum garnet which emits near-infrared radiation of wavelength 1,064 nm when an excitation source is introduced. The Nd:YAG laser was chosen to induce hyperthermia in this research project. The primary reason for choosing this modality was the size of the tumors at treatment (approximately 1 cm) which would not permit the introduction of multiple electrodes for radiofrequency techniques. Also there is no ultrasound applicator of the appropriate size for selectively heating the small tumor

area. In order to monitor actual temperatures within the tumor during heating, three thermocouples (surface, middle, and deep to the tumor) were used to record temperatures every 30 seconds.

Tumor Blood Flow

Heating of body tissues is influenced by blood flow to tissues. In the resting muscle only a fraction of the total number of capillaries are open at any given time due to the action of the precapillary sphincter muscles in the arterioles (Mellander and Johansson, 1968). A rich blood supply tends to prevent an excess build up of temperature. Using a mathematical model, Patterson and Strang (1979) determined the relationship between blood flow and the production of temperatures by external heat sources and found that blood flow through the tissue had a profound effect on temperatures obtained within the heated volume. They reported the time to achieve a certain temperature is directly dependent upon blood flow and that if heat input is too low or blood flow is too high, the desired temperature will not be reached. The differential blood flow between tumor and normal surrounding tissue is a critical factor in heat retention. If the tumor blood flow is higher than that present in the surrounding tissue, the critical temperature required for tumor cell

kill can not be obtained without the risk of significant injury to normal tissue unless selective heating of the tumor only occurs.

Hyperthermia causes dilation of the small capillaries and smaller blood vessels. The resulting increased blood supply to the tissues eventually exceeds the actual metabolic demand of the cells, and as a result, leads to increased oxygen tension and alkalosis occurs (Selawry et al., 1958). It has been demonstrated that the tissue oxygen tension and local blood flow are primarily dependent on the temperature of the tissue (Bicher et al., 1980). Below 41^{o}C, the blood flow to the tissue is increased with a resultant increase in tissue oxygen tension. Above 41^{o}C, a collapse of the microcirculation to the tissues occurs with time and a lower oxygen tension is seen (Hornback, 1984). Histologically, evidence of collapse of the microcirculation with thrombosis of smaller vessels has been shown following hot water perfusions (44^{o}C) of bladder tumors (Ludgate et al., 1978). Using the same therapeutic temperature, Endrich et al. (1979) also observed that collapse of microcirculation occurred, especially during the early growth phase of tumors.

Von Ardenne and Reitnauer (1980) studied the effects of heat on the microcirculation in Wister rats

transplanted with DS carcinosarcomas. Histological sections of tumor tissue were taken before and after heating. Following heat applications, there were markedly enlarged vessels with reduced red blood counts (RBC) and low pH with reduced microcirculation only in the tumor vessels. It was proposed that the RBC's became rigid due to lysosomal enzymes that had been discharged from leukocytes.

As the temperature of the tissue rises, an increase in metabolic rate of the cell is produced (approximately 10 to 15% for each $1^{o}C$ rise in temperature) until the metabolic demands of the cells can no longer be provided; then vascular heat injury begins (DuBois, 1936; and Lusk, 1928). One of the earliest pathological signs of thermal injury following capillary dilation is a rise in capillary blood volume and pressure which leads to increased capillary permeability and oozing of fluid into the interstitial tissue, clinically manifested as edema (Hornback, 1984b).

The ability to differentially heat tumors and eliminate possible complications to normal tissue is primarily a matter of blood flow (Patterson et al., 1979; and Hume et al., 1979). For this reason blood supply to tumors and that of the surrounding normal tissue becomes of critical importance. It should be pointed out that

tumor tissue develops after blood vessels and tissue are already in existence. As the tumor grows, it expands and displaces adjacent normal tissue and does not produce an integrated deliberate blood vessel pattern as seen in normal tissue. Blood vessels are ingulfed by the engulfing tumor, but a well-integrated system of blood flow seen in normal tissue is not established in growing malignant tumors (Lindgren, 1945). This diminished blood supply to malignant tumors decreases the supply of nutrients to the center core of the tumors and renders them relatively ischemic. This decreased tumor blood flow can be demonstrated by measuring the lowered oxygen tension and increased lactic acid production in the core of tumors (Urbach, 1961).

Many early researchers felt that the early necrosis commonly seen in larger tumor masses resulted from the poor and irregular supply of blood vessels of most malignant tumors (Borst, 1902; and Ribbert, 1911). However, there were several early researchers who erroneously believed that the blood supply to tumors was actually increased over that seen in normal tissues due to the following observations: (1) arteriovenous fistula are frequently present on the surface of tumors, (2) histological sections of some malignant tumors are rich in blood vessels, and (3) a rapidly growing tumor would

require a large supply of nutrients (Foulds, 1954; Hornback, 1984; and Bierman et al.,1952).

In fact, vascular proliferation is induced in the early stages of tumor growth. Later, large sinusoids and blood filled diverticuli develop which end blindly in the center of the tumor due to gradual obliteration of the vessel (Algire and Chalkley, 1945). The average diameter of tumor capillaries does not remain constant as it does in normal tissue but instead increases with tumor size (Hilmas and Gillette, 1974; and Vogel, 1965). Also, of note is the fact that the newly formed tumor vessels have a single-layered endothelial wall which lacks the more resistant external coat seen in normal tissue vasculature.

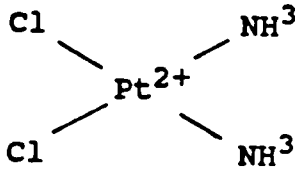
In 1907 Goldmann found that small tumor transplants were richly supplied with open vessels, and as the tumor grew larger, the rich vascular network was seen only at the periphery of the tumor with necrosis being the dominant feature in the central core of the tumor (also Cataland et al., 1962). Guillo and Grantham (1961a and 1961b) have studied blood supply and exchange of nutrient fluids between rodent host and induced tumors. They found that tumors had a consistently haphazard network of interconnected capillaries and major differences in blood supply between tumor and normal tissues.

Because of the importance of blood flow in this graduate project, tumors were induced rather than transplanted to simulate tumor vasculature in spontaneously arising tumors. A spontaneous tumor model was not chosen due to time and financial considerations.

II. Cisplatin

History

cis-Diamminedichloroplatinum (II) (cisplatin) is an inorganic, water-soluble, platinum containing complex. The II indicates the valence of platinum. The structural formula of cisplatin is as follows:



cis-dichlorodiammineplatinum(II)

(cisplatin)

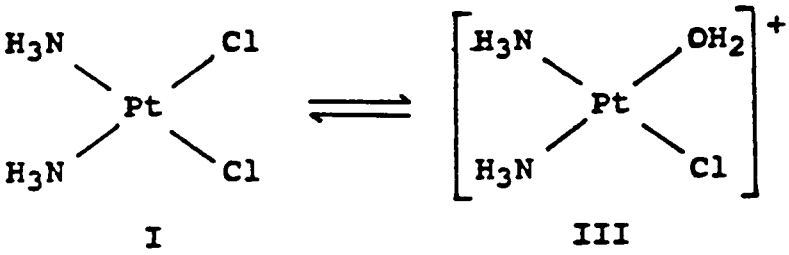
Platinum coordination complexes were first found to be cytotoxic agents in 1965 when Rosenberg and his co-workers observed growth inhibition of Escherichia coli when electrical current was delivered between platinum electrodes. Further studies indicated that the formation of inorganic platinum-containing compounds in the presence

of ammonium and chloride ions was the cause of the bacterial growth inhibition (Rosenberg et al., 1965, 1967). However, the renal toxicity caused by the chemotherapeutic agent precluded clinical use until Cvitkovic and co-workers demonstrated substantial reduction in renal toxicity in both animals (Cvitkovic et al., 1977) and man (Hayes et al., 1977) by utilizing a protocol which included prehydration and mannitol-induced diuresis.

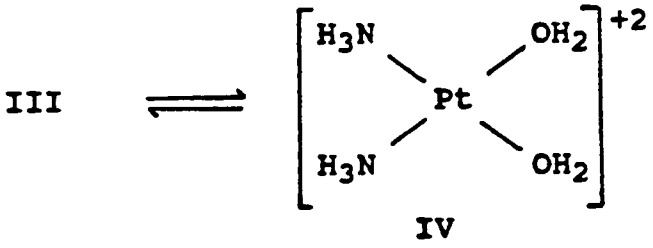
Today a variety of platinum analogues have been synthesized; however, cisplatin is the one most prevalently used in cancer therapy. The corresponding complex with ammonia residues in the trans configuration lacks antitumor activity. Cisplatin is effective in combination chemotherapy for a variety of types of cancer including metastatic testicular and ovarian carcinoma, tumors of the bladder, head, neck, and endometrium, as well as lymphomas and some neoplasms of childhood (Rozencweig et al., 1977; Sternberg et al., 1977; Randolph et al., 1978; Yagoda et al., 1978; Einhorn and Williams, 1979; Conners, 1982; Zwelling and Kohn, 1982; Roberts, 1983; Hacker et al., 1984; and Symposium, 1984). However, this agent exhibits pronounced nephrotoxicity and ototoxicity.

Mechanism of Action

Cisplatin enters the cell by diffusion. Hydrolysis of chloride is probably responsible for the formation of the activated species of the chemotherapeutic agent. Martin and co-workers (Reishus and Martin, 1961; and Lee and Martin, 1976) have demonstrated the stepwise replacement of the chloride ligands by water as shown in equations (1) and (2).



(1)



(2)

The existence of the aquated forms of cisplatin has been established by chloride ion titrimetry (Reishus and Martin, 1961; and Lee and Martin, 1976).

Since the aquated forms of cisplatin have been shown to be very reactive, much speculation on the mode of action of cisplatin has centered on the aquated forms of the drug. Numerous reports of the interaction of cisplatin and other platinum drugs with DNA constituents in aqueous systems exists (Horacek and Drobnik, 1971; and Lim and Martin, 1976). However, most of the studies have been conducted under *in vitro* conditions containing little or no chloride.

The platinum complexes can react with DNA, forming both intrastrand and interstrand cross-links. The N(7) of guanine is very reactive, and cross-links between adjacent guanines on the same DNA strand are the most readily demonstrated. It is very likely that the geometry of the *cis*, rather than the *trans*, formation is more favorable for the formation of both intrastrand and interstrand cross-links. The formation of interstrand cross-links is a relatively slow process and occurs to a much smaller extent. At present, there is no conclusive association between a single type of biochemical lesion and cytotoxicity.

Spectrophotometric studies have shown that the affinity of cisplatin for DNA is markedly lowered when the chloride levels are increased, supporting the speculation that the aquated form of cisplatin is involved in the

interaction with DNA constituents (Horacek and Drobnik, 1971). Rosenberg (1978) and Lippard (1978) have suggested that in the intracellular fluids where the chloride content is approximately 3×10^{-3} M, which is only 3 per cent of the concentration found extracellularly, cisplatin is converted to aquated platinum species which react with DNA constituents to exert the observed therapeutic action.

The specificity of cisplatin with regard to the phase of the cell cycle appears to differ with regard to the cell types. The effects on cross-linking are most pronounced during the S phase. Even though cisplatin is mutagenic, teratogenic, and carcinogenic, an increased incidence of secondary tumors has not yet been reported.

In addition to its reactivity with DNA, cisplatin can react with other nucleophiles, such as thiol groups of proteins. It is speculated that certain of the toxic effects of the drug, such as nephrotoxicity, ototoxicity, and intense emesis, may result from such reactions. This has led to experimental testing of "rescue" techniques that employ molecules with high affinity for heavy metals.

Another formulated mode of action of cisplatin invokes enhanced antigenicity of tumor cells. Rosenberg (1978) has suggested that cisplatin exerts its anticancer activity by derepression of virally coded information, which is normally latent, in some cancer cells. This

results in enhanced antigenicity of the tumor cells and eventual destruction of immune response of the subject. However, the documentation for this hypothesis is minimal. In summary, the mechanism of cisplatin as a chemotherapeutic agent is not fully known.

Absorption, Fate, and Excretion

Cisplatin is not effective when administered orally. This drug exhibits low aqueous solubility (approximately 1 mg ml^{-1} while therapeutic doses are usually in the range of 50-100 mg m^3) (Long, 1979;) and susceptibility to nucleophilic substitution reactions (Basolo and Pearson, 1967; and Belluco, 1974) which makes it difficult to develop acceptable methods of administration. Aqueous solutions of cisplatin have been shown to degrade via nucleotide displacement of the chloride ligands in water (Reishus and Martin, 1961; and Lee and Martin, 1976). The reaction of cisplatin with naturally occurring biomolecules, such as histidine (Volshtein and Luk'yanova, 1966) and methionine (Volshtein and Mogilevkina, 1965; and Volshtein and et al., 1967) has been reported. The reactivity of cisplatin with plasma proteins has also been documented (Litterst et al., 1976; Gormley et al., 1979; and Bannister et al., 1977). Only nonprotein bound cisplatin is active against tumors (Long and Repta, 1981).

The formation of stable dosage forms of this drug has been difficult due to these physiochemical properties. The instability of cisplatin in aqueous media due to reversible aquation requires that solutions of cisplatin for intravenous injection contain chloride ion to stabilize the drug (Greene et al., 1979; and Hineal et al., 1979). The stability of cisplatin has been shown to be good (less than 5% degradation) for periods up to 24 hours in the presence of as little as 0.45 per cent (w/v) sodium chloride (Hineal et al., 1979). However, even with good stability of the reconstituted dosage form, there is no assurance that cisplatin is stable in plasma or whole blood following intravenous administration. Such considerations arising from the physiochemical properties of this drug, have resulted in some questions as to the platinum species responsible for production of the anticancer activity associated with this drug.

The administration of cisplatin by rapid intravenous injection rather than infusion significantly alters the pharmacokinetic behavior. Following rapid injection, half-lives of 156 min (Bannister et al., 1977) and 220 min (Gormley et al., 1979) have been reported for the disappearance of platinum from plasma ultrafiltrate (nonprotein bound). When given by infusion instead of rapid injection, the ultrafiltrate plasma half-life is

shorter and the amount of drug excreted is greater (Patton et al., 1978). Gormley et al. (1979) also reported that the extent of platinum-protein association exceeded 90 per cent after 24 hours at initial cisplatin levels of up to 5mcg ml^{-1}. This degree of plasma protein binding leads to prolonged total cisplatin plasma half-lives (67 hr)(Gormley et al., 1979).

High concentrations of cisplatin are found in the kidney, liver, intestine, and testes, but there is poor penetration into the CNS. Only a small portion of the drug is excreted by the kidney during the first 6 hours; after 5 days up to 43% of the administered dose is recovered in the urine in humans. The extent of biliary or intestinal excretion of cisplatin is unknown (Zwelling and Kohn, 1982; and Weimann and Calabresi, 1985).

Clinical Toxicity

The major toxicity caused by cisplatin is dose-related, cumulative impairment of renal tubular function which may occur at any time during therapy. When higher doses or repeated courses of the drug are given, irreversible renal damage, which appears as tubular necrosis, may occur (Schaeppi et al.,1973). Ototoxicity caused by cisplatin is manifested by tinnitus and hearing loss in the high frequency range (4000 to 8000 Hz). It

can be unilateral or bilateral, tends to be more frequent and severe with repeated doses, and may be more pronounced in children. Marked nausea and vomiting occur in most patients. Mild-to-moderate myelosuppression may occur with transient leukopenia and thrombocytopenia. Electrolyte disturbances, including hypomagnesemia, hypocalcemia, hypokalemia, and hypophosphatemia, have been encountered. Hypocalcemia and tetany secondary to hypomagnesenia have been observed, and routine measurement of magnesium concentrations in the plasma is recommended. Hyperuricemia, peripheral neuropathies, seizures, and cardiac abnormalities have been reported. Anaphylactic like reactions, characterized by facial edema, bronchoconstriction, tachycardia, and hypotension, may occur within minutes after administration and should be treated by intravenous injection of epinephrine and with corticosteroids or antihistamines (Weimann and Calabresi, 1985). Also skin reactions called platinosis have been observed.

Cisplatin has immunosuppressive activity. Rejection of skin grafts and graft-verses-host responses are suppressed in animals, as is mitogenesis in lymphocytes stimulated by phytohemaglutinin (Conners, 1982; Zwelling and Kohn, 1982; Roberts, 1983; Hacker et al., 1984; and Symposium, 1984).

Synergism of Cisplatin with Hyperthermia

Elkon et al. (1982) demonstrated that cisplatin could be used in combination with hyperthermia without increasing the risk of nephrotoxicity. Using clonogenic assays, it has been demonstrated by Hahn (1979) that heat plus a variety of chemotherapeutic agents, including cisplatin, are synergistic for cell killing. Also, thermo-chemosensitivity screening (40.5^{o}C and 42.5^{o}C) by Calabro et al. (1989) demonstrated significant potentiation for cisplatin. Additional *in vitro* studies (Herman et al., 1988; Marmor, 1979) suggest that heat and drug must be administered close together in time in order to obtain synergistic killing. For example, thermal enhancement of cytotoxicity of cisplatin in the thermoresistant melanoma line M14 has been demonstrated only when the two modalities were given simultaneously (Greco et al., 1987). Synergism has also been achieved when hyperthermia preceded cisplatin; however, this regimen was less effective at impairing colony-forming ability. In contrast, experiments performed by Herman and Teicher (1988) showed the greatest delay in regrowth of implanted FSa11C fibrosarcoma cells to occur when cisplatin preceded hyperthermia and radiation. Rather than measuring decreased regrowth of cells as a function of increased drug uptake of drug into the cell, this

graduate study measured increased drug concentrations in tumor cells as an indicator of increased drug uptake into those cells.

In vivo studies have pointed out the importance of the dose of heat and rate of heating in attaining an optimal effect of drugs on cells. Marmor (1979) has shown that a threshold temperature dose is required for synergism of some drugs. Less cell killing has been demonstrated with a 3 hour transition from 37°C to 42°C than with heating to peak temperature over 30 minutes or with immediate exposure to 42.4°C plus cisplatin (Herman et al., 1982). This in vitro study suggested a change in drug uptake due to a decrease in cell permeability, altered subcellular targets or altered cell repair was dependent on the rate and/or duration of heating.

III. Tumor Physiology

Background

Rodent models of normal and tumor tissues have been the basis for the majority of advances in human cancer biology and therapy over the last few decades. These advances range from the discovery of new anticancer drugs to understanding the mechanism of action of other modalities to defining the biology of cancer.

Each independently arising tumor (spontaneous or induced) is a unique biologic entity with an individual biologic behavior with its own characteristic histologic appearance and an individual response spectrum to chemotherapeutic agents. A particular murine or human tumor model is usually chosen to answer a defined question; consequently, it is a common finding that the answer provided by one particular model will often not apply to many other tumors.

The vascularity and blood flow in tumors differs between the tumor center and the periphery (Endrich et al., 1979). This difference in tumor perfusion is dependent on the tumor's diameter and the specific site in the tumor. According to work done by Hermens (1973) the periphery of a tumor contains approximately 5% hypoxic cells while 80% of the cells at the tumor's center are hypoxic. Not only does the oxygenation status of tumor cells depend upon their position in the tumor, but circulation and oxygenation of tumors show fluctuations in time (Intaglietta et al., 1977; and Reinhold et al., 1977). This phenomenon known as vasomotion is thought to be a part of the normal regulatory system.

The physiology of blood circulation is very important in experimental tumor therapy research. Blood flow and blood perfusion, their fluctuations and, therefore,

probably the oxygenation of tumor cells as well depend upon tumor size, the depth and type of anaesthesia, the intensity of respiration, the temperature of the animal, as well as other variables.

Tumor blood flow is a critical parameter with regard to the O_2 and substrate supply and to the drainage of metabolic waste products in tumor tissue. Thus, tumor blood flow is decisive for the special micromilieu of cancer cells in vivo, and therefore for the susceptibility of tumors to nonsurgical therapies.

Tumor Models

There are six basic types of rodent tumor models; spontaneous, genetically predisposed, chemically induced, and transplantable tumors. Spontaneous animal tumors most closely model human cancer in terms of antigenicity, growth fraction, cell loss, and extent of differentiation. However, spontaneous tumors suffer from a number of disadvantages including the difficulty of accumulating sufficient homogenous tumors for experimentation and, most importantly, the lack of therapeutic response assays to investigate cellular or mechanistic questions. Spontaneous mammary tumors in asplenic mice which are heterogenous for athymia are often used as a model for metastasizing mammary tumors of humans because of the

similarities in the development of the disease including genetic and immunologic factors, hormonal influence and dependency, and possible viral association (Mitchell et al., 1982).

An example of a genetically predisposed tumor model is the Dupont Oncomouse which is a transgenic strain developed by microinjection of the oncogene from the mammary tumor virus MMTV (Anon., 1989). With the exception of the oncogene, the mice are physiologically and anatomically normal. The tumors that develop are adenocarcinomas with an incidence of 50% in females at 150 days of age. A major disadvantage of this model is the relative high expense of the animals.

Chemical induction is another approach for rodent tumor models. Adolescent female rats (approximately 50 days old) are fed a single bolus of carcinogen such as 9, 10-dimethyl1-1,2-benzanthracene (DMBA), a polycyclic aromatic hydrocarbon, by gastric catheter while under anesthesia. Tumors appear within four weeks. By 6 months an 80% incidence can be expected. Up to six months the tumors are primary adenocarcinomas that are vascular and difficult to remove completely. After six months, some encapsulated fibroadenomas develop.

Another alternative is transplantable tumors. Cells from an existing tumor of the same or another species are

injected into rodents which subsequently develop tumors. These tumors are well characterized and highly reproducible; however, they are usually anaplastic, lack differentiation, posses extensive cell selection and are far removed from the tumor of origin (Siemann, 1987). Even though this is a relatively inexpensive and simple procedure, there is some debate over whether the tumors which develop have a vasculature comparable to those arising naturally and therefore whether transplanted tumors effectively model human cancer (Rockwell,1977; Steel, 1977; and Steel et al., 1971). Other rare methods of tumor induction include physical induction by means of UV light or plastic films and viral induction.

It is important to know the immunogenicity of a given tumor model in the animal strain in which it will be used because of the possible induction of an antitumor immune response in the host. Immunogenicity is the ability to induce a tumor rejection response in suitably preimmunized host. Spontaneous tumors are infrequently immunogenic (Baldwin and Embleton, 1969; Embleton and Middle, 1981; Hewitt et al., 1976; Middle and Embleton, 1981; and Wrathmell and Alexander, 1977). The immunogenicity of virally-induced tumors depends on the status of the hosts. The tumors of virus-free hosts are almost always immunogenic (Sjögren, 1962) while virus-infected host

rarely have immunogenic tumors (Morton et al., 1969). Similarly, physically induced tumors are immunogenic in the case of induction by UV light (Kripke, 1974) and exhibit rare immunogenicity when plastic films are utilized for tumor induction (Klein et al., 1963).

The frequency of immunogenic tumors that are chemically induced depends on the particular agent used. For example, tumors induced by asbestos are nonimmunogenic (Brown et al., 1980) while the majority of tumors that arise following administration of a polycyclic hydrocarbon exhibit immunogenicity (Baldwin and Embleton, 1969; and Baldwin and Embleton, 1971).

Hormones are of crucial significance for the survival of some cancers of humans and animals. Two opposite changes of hormonal status can cause extinction of such cancers: (1) deprivation of sources of essential hormones and (2) hormone interference by administration of large amounts of critical compounds such as testosterone or 17b-estradiol plus progesterone. In human mammary carcinoma the measurement of the cytoplasmic estrogen receptor is a useful determinate of the endocrine responsiveness of patients with metastatic disease (McGuire et al., 1982).

Murine DMBA-induced tumors were chosen as the tumor model for the purposes of this study for several reasons. The induction of these tumors is relatively simple, and

there is a high incidence of superficially located, hormone-dependent primary adenocarcinomas within a relatively short time frame. Also, the animals used (normal, female Sprague-Dawley rats) are relatively inexpensive. The tumor is well tolerated by the host until the mass hinders locomotion or a septicemia or toxemia results from the ulceration and necrosis. Rats with large tumors lose weight and die. Mammary tissue is widely distributed in the subcutis of murine rodents, mammary tumors may be found behind the shoulders, on the ventral abdomen and flank, and around the tail base.

Studies of the rat altered the course of research on breast cancer due to the similarity in human cancer and that caused by the administration of DMBA in rats (Huggins, 1967). DMBA-induced tumors resemble human tumors in their response to oopherectomy, hypophysectomy and androgen therapy, and histology of human breast cancer (Young et al., 1963); however, metastases are unusual in rats whereas lung metastases are very common from human mammary carcinomas. Human mammary carcinomas are on the whole less highly differentiated than rodent carcinomas, but the range of differentiation is much the same in each species. In contrast, carcinomas in cats and mice have a regularity of pattern that make them unlike the great majority of human tumors. Mammary tumors are the most

common canine neoplasms and bear a strong resemblance to that of man (Prier and Brodey, 1963; Schneider, 1970); however, due to the expense of purchase and upkeep these animals were not chosen for this study.

It seems that sensitivity to polycyclic hydrocarbons is maximal early in life. Spraque-Dawley rats were found to be most sensitive to oral doses of the carcinogen DMBA between the 50th and 65th days of life. By the time the animals are 100 days old, tumors are much more difficult to induce (Huggins, Grand and Brillantes, 1961). This may be related to a particularly favorable hormonal environment at that age or to the rate of mammary gland growth and the frequency of cell division.

DMBA is an environmental contaminant generated as a by-product of the incomplete combustion of fossil fuels and other materials (Dipple, 1976). Rats exposed to this carcinogen show a suppression of both humoral (Ward et al., 1984), and cell-mediated (Dean et al., 1986), and spontaneous immunity (Yamashita, 1982) which may be mediated by the interleukin-2 pathway (Pallardy et al., 1989). It has been proposed that DMBA provides a hormone state conducive to tumor development by desensitizing lactotrophs to dopamine and releasing prolactin by direct estrogen-like actions on the pituitary (Pasqualini et al., 1988). This carcinogen exerts the following effects on

the rat: 1) profound depression of incorporation of thymidine in DNA (Huggins et al., 1965); 2) augmentation of the production of messenger RNA (Loeb and Gelboin, 1964); and 3) induction of synthesis of a soluble enzyme, menadione reductase (Williams-Ashman and Huggins, 1961) and of microsome-bound enzymes and other proteins (Arcos et al., 1961); and 4) causes cancer of the recipient (Huggins and Fukunishi, 1964).

DMBA exhibits structural similarity to 17β-estradiol (Glusker, 1979) and is considered an estrogenomimetic agent because it has been shown <u>in vitro</u> to mimic the actions of 17β-estradiol; the depletion of membrane dopamine receptors and parallel stimulation of prolactin release (Pasqualini et al., 1988). Therefore, in addition to initiating neoplastic changes at the mammary gland level, DMBA may also create a hormonal environment which increases the sensitivity of the mammary gland to tumorogenesis by direct estrogen-like actions on the anterior pituitary (Pasqualini et al., 1988). Because of the hormone dependence that it exhibits, the DMBA tumor model is also being used to model human cancer in studies of the antitumor effects of a medroxyprogesterone acetate, a synthetic progesterone derivative (Spreafico et al., 1982).

Tumors of the Mammary Gland

In comparative studies, both similarities and differences have been shown between rat and human mammary tumors (Russo et al., 1990). The spontaneous occurrence of mammary gland tumors of rats has been observed since early in this century. Tumors of the mammary gland were first induced in the 1930's with the aid of estrogen (Young and Hallowes, 1973). Some induced mammary tumors of rats respond to hormone treatment by adrenalectomy, oophorectomy, hypophysectomy, and androgen therapy. Some spontaneous human mammary tumors also respond to these procedures and agents, but in both species some mammary tumors respond to none of these hormonal influences. It should be noted that scirrhous carcinoma, the common breast tumor of women, has not been identified in any other species. Mammary cancers in women invariably metastasize if untreated whereas most spontaneous and many carcinogen-induced mammary carcinomas in rats do not metastasize. Some rat mammary tumors having the histological appearance of cancer regress and disappear upon the removal of the initiating agent. Disappearance of mammary cancer in women happens extremely infrequently.

CHAPTER III

MATERIALS AND METHODS

I. Experimental Design

Eighty rats with dimethylbenz[a]anthracene-induced adenocarcinomas were used in the study. Each treatment group consisted of 7 or 8 rats. Rats were randomly assigned to one of the following groups when their tumors reached approximately 1 cm in size.

A. Hyperthermia (HT) only
   1. Rats euthanized at the end of HT
   2. Rats euthanized 1 hour after HT
   3. Rats euthanized 2 hours after HT
   4. Rats euthanized 3 hours after HT

B. Cisplatin (CDDP) only *

C. Cisplatin and Hyperthermia **
   1. CDDP 1 hour before HT
   2. CDDP at the beginning of HT
   3. CDDP at the end of HT
   4. CDDP 1 hour after the end of HT

D. Control group with mammary adenocarcinomas

* all rats given cisplatin were euthanized two hours after drug administration.

** times correspond with A

Rats administered hyperthermia only were used to evaluate the effects of hyperthermia on tumor blood flow. Rats administered cisplatin alone or cisplatin plus hyperthermia were used to evaluate the effect of hyperthermia on plasma and tumor cisplatin concentrations. Control rats receiving neither drug nor heat were used for control blood flow and pharmacokinetic determinations. A separate study indicated that there was no difference in tumor blood flow of rats receiving hyperthermia alone when compared to hyperthermia plus cisplatin (Ausmus, 1991). All rats were anesthetized for pharmacokinetic and blood flow studies to preclude differences in these parameters between anesthetized and awake animals.

II. Source of Rats

Following approval of the use of animals in this project by the Animal Care and Concerns Committee, Sprague-Dawley rats were obtained from Taconic Laboratories of Germantown, New York. They were received at the age of approximately 43 days and housed in individual or double cages in the laboratory animal

facilities of the University of Tennessee College of Veterinary Medicine. Rats were given food and water ad libitum and exposed to a 12 hour light: darkness cycle.

III. Induction of Mammary Adenocarcinomas

The carcinogen 7, 12-Dimethylbenz[a]anthracene (95% carcinogenic) was obtained from Sigma Chemical Company, St. Louis, Missouri. This polycyclic aromatic hydrocarbon was dissolved in sesame oil to a concentration of 10mg/ml using heat and stirring when necessary. Isoflurane was used to anesthetize the animals in an approved Class II hood. An anesthesia machine was used to mix the isoflurane with oxygen. A funnel was attached to the anesthesia outlet by a piece of tubing. Rats were held in the funnel until a light plane of anesthesia was reached. Individual doses (10mg/ 100g rat wt) of the carcinogen were then administered via an 18 gauge, 2" Perfektum laboratory animal intubation needle which had a ball diameter of 2.25 mm (Baxter). Rats regained consciousness quickly and were returned to their cages. Tumor latency period ranged between 4 weeks and 2.5 months. Mammary adenocarcinomas were confirmed histopathologically in all rats. Tumor incidence was approximately 85 percent.

IV. Hyperthermia Treatment

Rats were anesthetized with 0.25 ml dose of a 1:1 mixture of ketamine (100 mg/ml) and acepromazine (0.2 mg/ml) intramuscularly for hyperthermia treatment. Subsequent doses of ketamine were given as needed to maintain the plane of anesthesia. A Surgical Laser Technology Nd:YAG laser system with an output of 1064 nm was used to induce localized hyperthermia (target temperature = 43^{o}C for 1 hr). The power was set at 10 watts and the laser was put in a pulse mode with a pulse interval of 1 second. The energy was delivered to the tumor via a fiber with the tip placed 10-12 cm above the tumor surface. The total amount of energy used during a hyperthermia treatment ranged from 5000-16000 joules with the limit for energy set at 120000 joules. The upper and lower control temperatures were set at 43.5 and 43.2^{o}C, respectively. Fifty and 25^{o}C were chosen as the respective abnormal upper and lower temperatures. This safety mechanism triggers a shut down of the laser system if the temperature is not within the set range (25^{o}C-50^{o}C). The laser was interfaced to a computer and thermometry unit which provided feedback to control the tumor temperature. Temperature was monitored by thermocouples placed superficial to, in the middle of, and deep to each tumor. Superficial cooling was achieved

using forced moist oxygen flow from a nebulizer when necessary.

V. Cisplatin Administration

Cisplatin (Platinol) was obtained from Bristol Laboratories as a lyophilized powder in vials that contain 10 or 50 mg of drug. The agent was prepared by adding sterile H_2O to achieve a final concentration of 1mg/ml. Aluminum reacts with and inactivates cisplatin; therefore needles or other equipment containing aluminum were not used to prepare or administer the drug. Because this antineoplastic agent is mutagenic, carcinogenic and teratogenic, it was prepared in a Class II hood by a researcher wearing a protective gown, latex gloves, and a respirator mask (BioSafety Systems, Inc.). A plastic backed absorbent pad was used where the chemotherapeutic agent was being prepared and under the administration work area. Cisplatin was covered after preparation due to the photosensitivity of the compound.

Rats were anesthetized using the same procedure as for hyperthermia treatment. At various times relative to hyperthermia, the tail vein was catheterized using a 24 gauge 3/4in Insyte catheter (Deseret). After flushing the catheter with 0.9% NaCl to insure placement, cisplatin was administered at a dose of 10mg/kg (20mg/m^2) of body

weight. Any residual cisplatin was flushed through the catheter with saline. Following the bolus injection, blood collections were taken at these times: immediately, 30 min., 1 hr, and 2 hr without volume replacement therapy.

At each time point the vein was catheterized and approximately 0.7 ml of blood was collected in a heparinized Microtainer Tube (Becton Dickinson) and placed on ice for up to 30 min. Samples were centrifuged at 2500 RPM for 10 min. at 4^{o}C (Sorvall RT6000B). After spinning, the plasma was transferred to an ultrafiltration membrane (Amicon 2100, CF-50) and centrifuged at 1000 rpm in a refrigerated superspeed centrifuge (Sorvall RC-5B) for 30 min at 4^{o}C. The resulting ultrafiltrate, containing only the platinum fraction not bound to plasma proteins, was stored at -70^{o}C until analysis.

VI. Method of Platinum Analysis

Ultrafiltrate Analysis

Flameless atomic absorption spectroscopy was used to determine the amount of platinum in the ultrafiltrate of the rat plasma according to the method described by El-Yazigi and Al-Saleh (1986). An Instrumentation Laboratory spectrophotometer equipped with a Thermo Jarrell Ash model 188 furnace atomizer was employed in this analysis. A

standard curve was obtained by adding platinum standard (Sigma Laboratories) to control rat-plasma ultrafiltrate which had been diluted 1:10 with 0.2% nitric acid. The absorption of the atomized platinum was measured at 265.9 nm and the furnace conditions were:

	Dry	Pyr1	Pyr2	Atom	Clean
Temp.	120	750	900	2000	2000
Ramp	45	10	10	0	
Hold	15	0	0	4	0
Purge	1	2	2	0	3
AIR ASH			Int		
Pk Area	0.3 sec		Delay = 0.2 sec		

The standard curve was constructed by plotting the absorbance versus the platinum concentration.

A 1:10 dilution of the experimental samples was also achieved using 0.2% nitric acid. After vortex-mixing, 25μl of the diluted sample was directly pipetted into a cuvette designed for direct deposit. Further dilution of the sample was performed whenever necessary. Triplicate absorbance readings were taken for each sample.

Tissue Analysis

Tissues were prepared for analysis according to the method described by McGahan and Tyczkowska (1987). A sample containing approximately 200 mg of each tumor was weighed out and incubated overnight at room temperature in 0.5 ml of concentrated nitric acid. It was then boiled for approximately 5 minutes after which 30% H_2O_2 (0.5 ml) was added and the solution boiled again for approximately 5 minutes. The resulting clear yellow solution was analyzed directly without dilution. Furnace parameters were the same as those used for plasma ultrafiltrate analysis above with the exception of an integration delay of 0.6 seconds.

VII. Blood Flow Determination

Rats were anesthetized using the protocol described under Hyperthermia Materials and Methods. A Harvard surgery board was used to properly position the rats during surgery.

Blood flow determinations were made using a modification of the reference sample method described by Malik et al. (1976). The left ventricle was catheterized through the right carotid artery. The tip of this catheter was a 6cm section of PE 10 polyethylene tubing which had been inserted and glued into a 10 cm PE 50

polyethylene catheter. Another PE 50 polyethylene catheter was inserted into an internal iliac artery. All catheters were filled with heparinized saline (50 U/ml). Approximately 12,000 radioactively-labeled microspheres (^{113}Sn), suspended in 0.46 ml saline, were injected through the ventricular catheter over a period of 10 sec and then flushed with 0.1-0.3 ml saline. Reference blood samples were withdrawn from the iliac arterial catheter using a withdrawal pump (Model 351: Sage Instruments). The sample was withdrawn at approximately 0.30 ml/min beginning at the start of injection and continuing for 70 sec. At the end of 70 sec, a 0.45 ml dose of T-61 euthanasia solution was administered through the ventricular catheter.

Various organs and tissues were removed, briefly rinsed in H_2O, and placed in pre-weighed vials. Radioactivity was determined using a Cobra 5005 gamma counter (Packard Instrument Company). Blood flow was calculated from the measured activity (counts/min) according to the relation:

$$\text{Blood flow} = \frac{(\text{tissue cpm})(\text{ref blood wt})}{(\text{ref blood cpm})(\text{tissue wt})}$$

VIII. Pharmacokinetic Evaluation

Pharmacokinetic profiles were determined for each of the experimental groups. Venous blood (0.7 ml) was collected immediately, at 30 min, 1 and 2 hr after cisplatin administration. Tumors were collected 2 hr after drug administration for measurement of cisplatin tissue concentration. All samples were placed on ice immediately, blood was centrifuged within one half hour after collection and stored at -70°C until analysis. Cisplatin concentrations were measured using atomic absorption spectrophotometry as described in Section VI.

Pharmacokinetic parameters were determined using an automated curve-stripping program (R-Strip). Calculation of parameters was as follows:

A = Cp_o = extrapolated drug concentration in the central compartment at time = 0

α = slope of the distribution phase

B = y intercept of the elimination phase

β = slope of the elimination phase

Vd_C = dose/Cp_o

$$Vd_{SS} = \frac{\text{dose } [(A/\alpha^2)+(B/\beta^2)]}{[(A/\alpha)+(B/\beta)]^2}$$

$t_{\frac{1}{2}\alpha} = \ln 2/\alpha$

$t_{\frac{1}{2}\beta} = \ln 2/\beta$

$CL = dose/AUC$

AUC was calculated by the trapezoidal method (Gibaldi and Perrier, 1982). Vd_C and VD_{SS} represent volumes of distribution of the central compartment and steady state, respectively. CL is the whole body clearance; $t_{½\alpha}$ and $t_{½\beta}$ are the half-lives in the distribution and elimination phases, respectively. Statistical comparison of these parameters was performed using the General Linear Model (SAS, Cary, NC).

CHAPTER IV

RESULTS AND DISCUSSION

I. Heating Profiles

Tumor temperature profiles of rats administered hyperthermia (43^{o}C for 1 hr) are shown in Table 1. Only rats in which temperatures achieved at least 42^{o}C in the center of the tumor were used in the study. Approximately ten rats were eliminated from the study after receiving hyperthermia treatments because they did not meet this requirement. This suggests that this heating technique will achieve temperature profiles believed to be in the therapeutic range (Song et al., 1980) in approximately 89% of small tumors. Experience in thermocouple placement and use of the laser resulted in better temperature profiles; therefore an experienced user could expect to achieve a greater percentage of adequate treatments. It should also be noted that only two thermocouples were placed within the tumors; one which provided feedback to the Nd:YAG laser and one which provided the temperature profiles recorded in Table 1. The other thermocouples were (1) just beneath the skin and superficial to the tumor and (2) deep to the tumor. It is expected that temperature profiles varied somewhat throughout the tumor; however,

Table 1. Temperatures (^{o}C) of rat mammary adenocarcinomas treated with localized hyperthermia for 1 hr using a Nd:YAG laser (Mean ± SEM)
* Refers to the location of the thermocouple

RAT #	SURFACE*	CENTER	BASE
555	43.5 ± 0.1	44.0 ± 0.1	43.9 ± 0.1
632	43.2 ±0 .1	43.5 ± 0.0	41.0 ± 0.0
442	36.6 ± 0.4	43.1 ± 0.2	38.4 ± 0.1
641	42.9 ± 0.2	43.4 ± 0.2	41.6 ± 0.1
329	42.6 ± 0.1	43.4 ± 0.1	42.3 ± 0.2
676	44.4 ± 0.2	43.7 ± 0.1	42.3 ± 0.1
446	42.3 ± 0.2	42.4 ± 0.2	40.8 ± 0.2
529	43.1 ± 0.2	43.6 ± 0.1	42.5 ± 0.2
504	41.5 ± 0.0	43.7 ± 0.1	43.3 ± 0.2
583	42.7 ± 0.1	43.5 ± 0.1	41.9 ± 0.2
541	43.5 ± 0.1	44.2 ± 0.1	43.6 ± 0.1
281	34.3 ± 0.2	43.1 ± 0.2	40.2 ± 0.2
521	38.6 ± 0.1	43.3 ± 0.1	41.6 ± 0.1
513	43.8 ± 0.1	43.6 ± 0.2	43.3 ± 0.2
544	45.4 ± 0.1	43.3 ± 0.1	43.5 ± 0.2
524	43.0 ± 0.1	43.7 ± 0.1	43.3 ± 0.1
533	43.7 ± 0.1	43.5 ± 0.2	42.8 ± 0.1
444	45.4 ± 0.2	44.7 ± 0.2	43.5 ± 0.1
478	43.1 ± 0.2	43.4 ± 0.1	41.8 ± 0.1
488	43.0 ± 0.2	43.1 ± 0.1	40.6 ± 0.1
463	43.2 ± 0.2	42.6 ± 0.1	42.9 ± 0.1
530	43.1 ± 0.1	43.5 ± 0.2	42.8 ± 0.1
451	44.2 ± 0.1	43.7 ± 0.1	41.9 ± 0.1
540	44.7 ± 0.2	43.6 ± 0.1	41.9 ± 0.2
582	43.5 ± 0.2	44.1 ± 0.2	43.8 ± 0.2
534	43.7 ± 0.2	44.1 ± 0.2	42.8 ± 0.2
453	43.5 ± 0.1	44.0 ± 0.1	43.5 ± 0.1
438	40.7 ± 0.1	42.8 ± 0.1	43.2 ± 0.1
430	43.9 ± 0.1	42.9 ± 0.1	41.8 ± 0.1
507	43.7 ± 0.1	42.2 ± 0.1	41.4 ± 0.1
510	42.8 ± 0.1	43.5 ± 0.1	42.8 ± 0.1
469	43.3 ± 0.2	42.8 ± 0.1	43.2 ± 0.1
579	42.7 ± 0.2	43.7 ± 0.2	42.8 ± 0.1
564	44.4 ± 0.1	43.5 ± 0.0	44.3 ± 0.1
408	37.4 ± 0.2	43.5 ± 0.2	41.9 ± 0.2
287	37.9 ± 0.2	43.5 ± 0.2	41.6 ± 0.1
535	44.8 ± 0.1	44.0 ± 0.2	43.4 ± 0.1
470	31.0 ± 0.5	42.4 ± 0.3	42.4 ± 0.3

Table 1. cont.

RAT#	Surface	Center	Base
584	43.1 ± 0.1	43.5 ± 0.1	43.9 ± 0.1
531	43.8 ± 0.1	43.9 ± 0.1	43.1 ± 0.1
649	42.8 ± 0.1	43.5 ± 0.1	44.0 ± 0.1
650	43.5 ± 0.1	43.5 ± 0.2	43.2 ± 0.2
390	34.9 ± 0.2	42.2 ± 0.1	39.9 ± 0.1
456	38.4 ± 0.1	42.3 ± 0.1	41.1 ± 0.1
457	40.3 ± 0.3	42.1 ± 0.1	42.3 ± 0.1
179	37.6 ± 0.1	43.6 ± 0.2	39.5 ± 0.2
427	42.2 ± 0.1	43.3 ± 0.3	42.2 ± 0.1
441	41.5 ± 0.2	43.3 ± 0.1	40.2 ± 0.1
588	43.6 ± 0.1	43.8 ± 0.1	43.7 ± 0.1
536	42.9 ± 0.2	42.6 ± 0.1	43.2 ± 0.1
443	42.4 ±.0.1	44.1 ± 0.2	43.1 ± 0.2
428	44.1 ± 0.1	43.8 ± 0.1	42.8 ±.0 1
307	43.3 ± 0.1	43.5 ± 0.1	43.3 ± 0.1
477	43.0 ± 0.2	43.1 ± 0.3	42.9 ± 0.1
517	42.7 ± 0.2	43.8 ± 0.1	43.6 ± 0.1
476	42.0 ± 0.2	43.7 ± 0.1	41.4 ± 0.2
471	43.2 ± 0.1	43.4 ± 0.1	43.1 ± 0.1
606	43.2 ± 0.1	43.5 ± 0.1	43.6 ± 0.1
481	43.5 ± 0.0	43.8 ± 0.0	42.9 ± 0.0
509	41.1 ± 0.1	43.6 ± 0.1	42.3 ± 0.1
489	37.7 ± 0.1	44.0 ± 0.0	38.7 ± 0.1
511	38.6 ± 0.1	43.3 ± 0.1	41.4 ± 0.0
468	40.7 ± 0.1	43.5 ± 0.1	43.3 ± 0.1
556	43.7 ± 0.1	43.3 ± 0.1	43.3 ± 0.1
646	42.0 ± 0.3	43.1 ± 0.1	41.6 ± 0.4

this variability should not be extreme due to the small size of the tumors (Rogers et al., 1988). A separate study revealed that the amount of heating of the metal thermocouple probe is neglible (Ausmus and Wilke, 1991). A typical temperature profile is shown in Figure 2.

II. Blood Flow

All rats were anesthetized using the same anesthetic protocol to preclude differences in blood flow resulting from cardiovascular effects of ketamine and acepromazine. Average blood flow values of tumor and skin at various times prior to or following hyperthermia are shown in Figure 3. Overall, mean blood flow in tumor was not statistically different (ANOVA) between treatment groups. This may be due to the small sample size and large normal variance within treatment groups. Although there was a high degree of variability in the blood flow values obtained in this study, a trend can be seen in the data. Control blood flow values were the lowest of those reported. A marked increase in blood flow occurred during hyperthermia treatment resulting in increased flow values immediately after a hyperthermia treatment. Flow returned to the approximate control value by three hours post-treatment.

It is difficult to compare blood flow values obtained

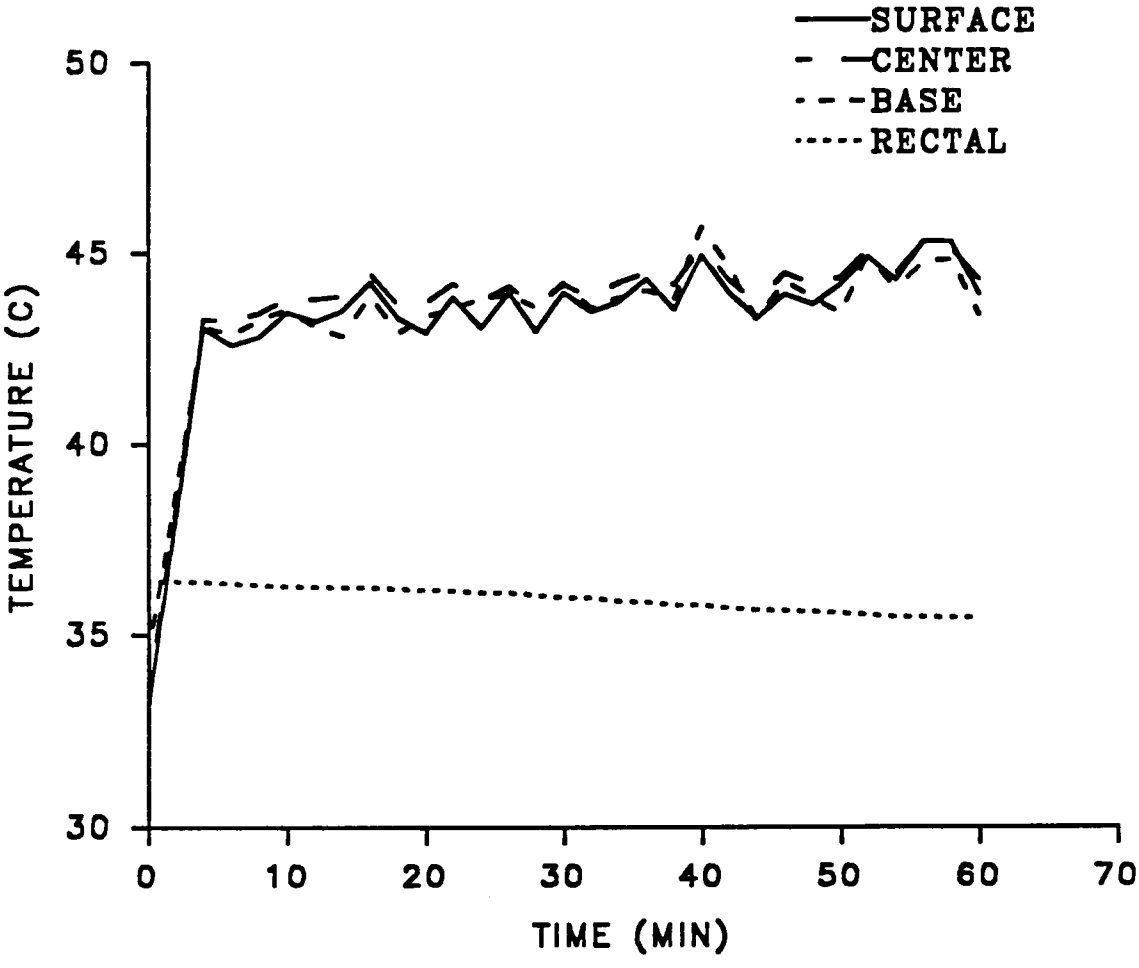


Figure 1. Tumor temperature profile for a rat administered localized hyperthermia (43°C for 1hr) using a Nd:YAG laser

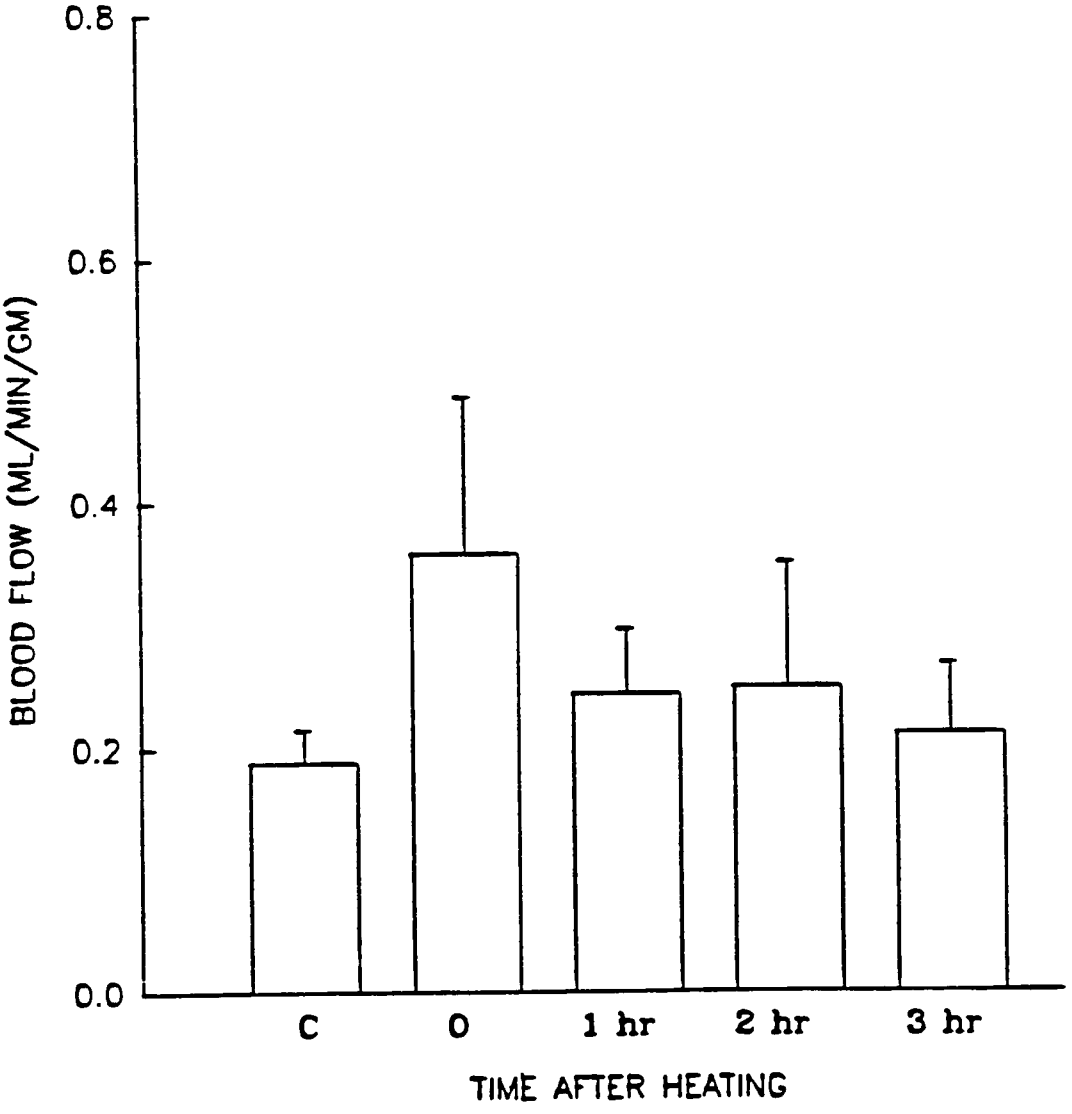


Figure 2. Average blood flow values of tumors in each treatment group C = control group.

in separate studies due to the wide variety of animal tumor models, heating protocols, and assay methods used in various studies. Table 2 describes changes in blood flow to tumors resulting from hyperthermia obtained in studies by other investigators. Considerable variability of the tumor blood flow with regard to tumor type, location, size and treatment protocol exists. Of those listed, the present research results agree most with the work of Vaupel (1983) in which blood flow was increased in 60% DS-carcinoma tumors in Sprague-Dawley rats given localized hyperthermia treatment (43-44^{o}C for 20 min).

Emami and Song (1984) reviewed hyperthermia literature and concluded that heating tissues to 40^{o}-42^{o}C caused a mild increase in blood flow in normal tissue and no change or a mild increase in tumor tissue with a return to normal perfusion rates for both tissue types after heating. The investigators found that heating in the range of 42^{o}C to 44^{o}C resulted in a marked increase in normal tissue microcirculation during heating and a return to normal flow after heating. Similarly, the blood flow to normal tissue in this graduate study exhibited a slight to marked increase during heating at these temperatures. However, the investigators reported a marked decrease in tumor blood flow after hyperthermia treatment which was not seen in this study. Since no specific time is

Table 2. Response of blood flow to localized hyperthermia in tumors of rats

TUMOR MODEL	Temp (°C)	TIME (min)	FLOW RESPONSE	REFERENCE
Walker 256 carcinoma	42-45	30-60	no significant changes flow inhibition after heating	Song et al., 1980a-c
Walker 256 carcinoma	41.3-41.6	60	no consistent flow changes	Guillo and Grantham, 1978 Guillo, 1980
BA 1112 rhabdomyo-sarcoma	41.3	60	significant flow reduction	Endrich et al., 1979
BA 1112 rhabdomyo-sarcoma	41-44	30-60	flow reduction dependent on temperature and exposure time	Emami et al., 1980
BA 1112 rhabdomyo-sarcoma	43.0/42.5	160/226	flow stoppage in 50% of the tumors	Reinhold and van den Berg-Blok, 1981
Yoshida sarcoma	42	60-180	flow reduction dependent on temp and exposure time	Dickson and Calderwood, 1980
DS-carcinosarcoma	39.5-44.0	30	flow increase at 39.5°C, flow decrease above 42°C	Vaupel et al., 1982
DS-carcinoma	43	100	no significant flow change with heat alone	Von Ardenne and Reitnauer, 1982
Walker 256 carcinoma	45	60	slight increase in blood flow in small tumors	Song et al., 1980a-c
DS-carcinoma	43-44	20	blood flow increased in 60% of tumors	Vaupel, 1983

recorded for the decrease in blood flow after hyperthermia in their studies, a direct comparison of perfusion rates cannot be made. The differences in technique of measurement and tumor size are additional factors can cause conflicting results of the present study and that of Emami and Song (1984).

When the blood flow values of heated and unheated skin from this study were compared, there was a statistically significant difference ($p<0.05$) between unheated skin and heated skin immediately after hyperthermia (Figure 3). Blood flow rates of heated skin showed a similar trend as those in tumor. There was a marked increase immediately after hyperthermia and a steady decrease in the following three hours; however, the rate did not decrease to the control value within three hours as it did in the malignant tissue. Heating invoked an increase in normal tissue that remained elevated for the three hour period of investigation. These results are in partial agreement with Dewhirst et al. (1984) who demonstrated a rapid increase then decrease in arterial flows in rat access chambers exposed to a rapid temperature elevation to 44^{o}C. Slower exposure to 42^{o}C resulted in a slower decline in perfusion rates. Table 3 lists actual blood flow values obtained in various studies. Again, variation in the tumor model, treatment

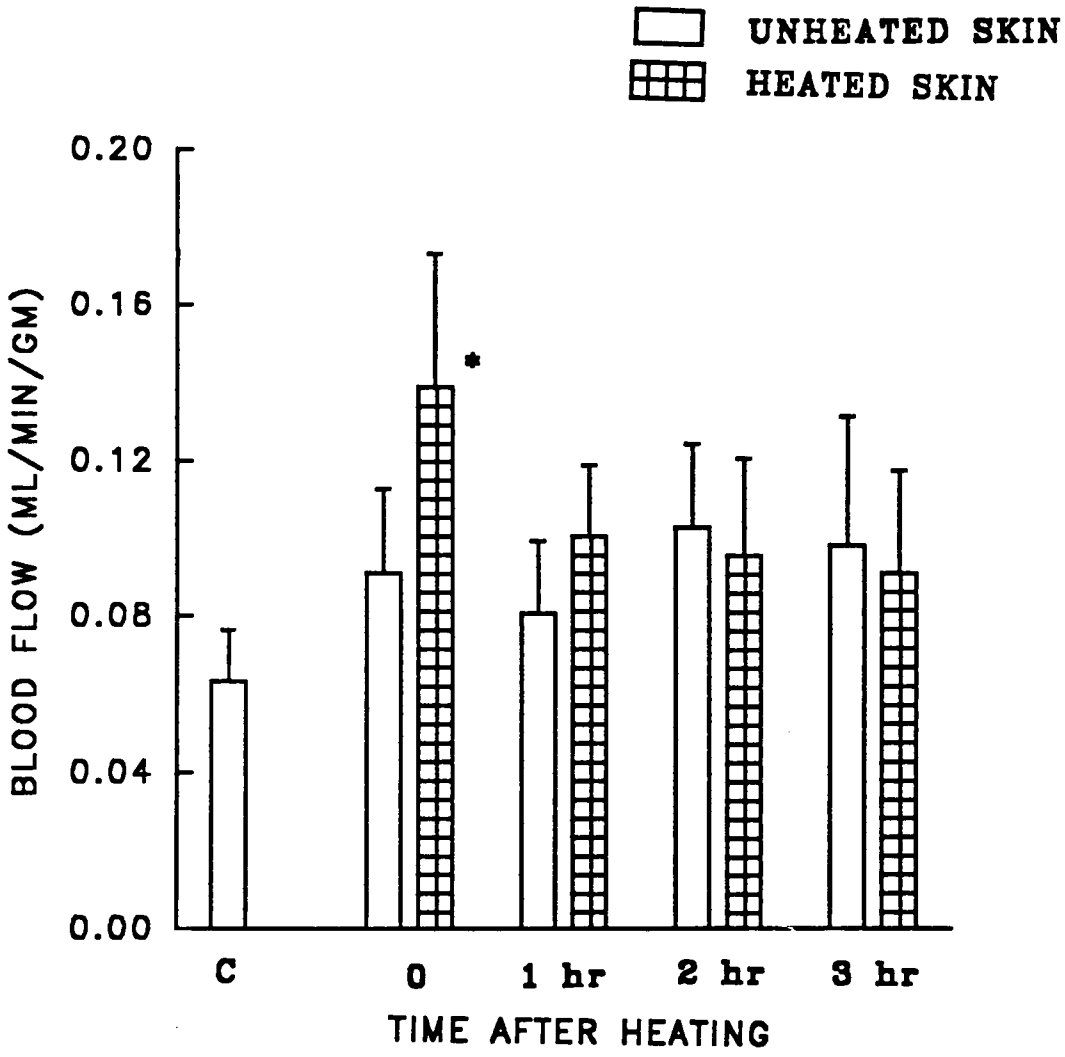


Figure 3. Average blood flow values of heated and unheated skin in each treatment group
C = control group

* significantly greater than C ($p<0.05$)

Table 3. Blood flow of various tumors under various conditions

Rat Tumor Model	Protocol	Tumor Size (g)	Blood Flow (ml/g/min) Tumor Control	Tumor Heated	Reference
Walker carcinoma 256	43°C, 1 hr	0.3- 0.7	0.48 ± 0.04	0.55 ± 0..09	Song et al., 1980
		2.0-5.0	0.15 ± 0.02	0.12 ± 0.02	
13762A mammary carcinomas	43.5°C, 1 hr	0.2-0.5	0.18	0.13	Rappaport et al., 1983
		< 1.0	0.05	0.09	
SMT-2A mammary carcinomas	44°C, 1 hr		0.47 ± 0.08	0.64 ± 0.11	Shrivastav et al., 1983
Walker tumor	43°C, 1 hr	< 0.07	0.10	0.28	Song, 1984
		> 2.0	0.16	0.14	
Walker 256 mammary carcinoma	40.2°C, 1 hr	1.7	0.02	0.39	Guillo et al., 1978
	41.8°C, 1 hr	2.3	0.26	0.21	
Novikoff's hepatoma			0.10-0.17		Guillo and Grantham 1961a
Hepatoma 5123			0.04-0.21		Guillo and Grantham 1961b
DMBA-induced adenocarcinoma			0.03		Moller and Bojsen, 1975
Nerve and brain tumors			0.44-0.79		Allen et al., 1975
					Rogers et al., 1969
Transplanted adenocarcinoma		0.1-0.9	0.40 ± 0.38		
		1.2-3.0	0.27 ± 0.21		
		3.4-4.9	0.10 ± 0.13		
		5.4-6.7	0.05 ± 0.04		
Sarcoma			0.02-0.05		Kjartansson, 1976

protocol, and method of analysis may explain differences in results.

II. Cisplatin Pharmacokinetics

Cisplatin plasma concentration versus time profiles are shown for all treatment groups (Figure 4). The predicted concentration versus time profile based on the intercepts (A and B) and rate constants (α and β) for each treatment group are shown in Figure 5. Due to the limited amounts of blood which could be collected from each rat, only the ultrafilterable fraction of plasma was used for the determination of platinum levels, corresponding to cisplatin not bound to plasma proteins.

Bajorin et al. (1986) have reported elimination half lives of nonprotein bound cisplatin (25-46 min) in humans which are consistent with those obtained in this study. Their human patients were receiving cisplatin for the first time and had adequate renal function. The peak platinum concentrations (A) achieved in the present bimodality study (dose = 20mg/m^2) differ markedly from those obtained by Campbell et al. (1983) and Jacobs et al. (1991) where peak platinum levels did not exceed 1.8 μg/ml in a twenty-four hour period following a 80mg/m^2 to administration of cisplatin to rats by bolus injection compared to slow intravenous infusion in humans.

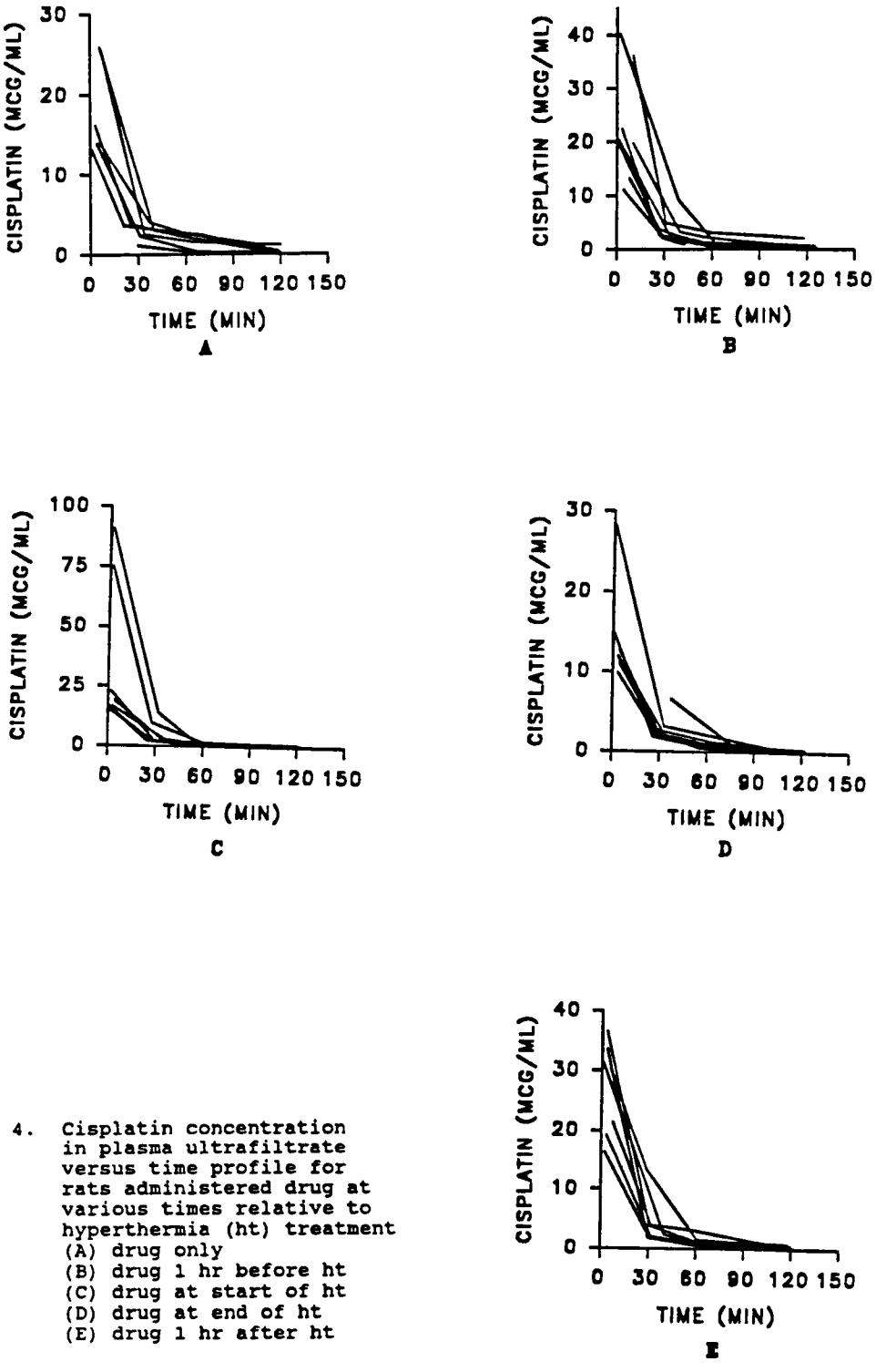


Figure 4. Cisplatin concentration in plasma ultrafiltrate versus time profile for rats administered drug at various times relative to hyperthermia (ht) treatment
(A) drug only
(B) drug 1 hr before ht
(C) drug at start of ht
(D) drug at end of ht
(E) drug 1 hr after ht

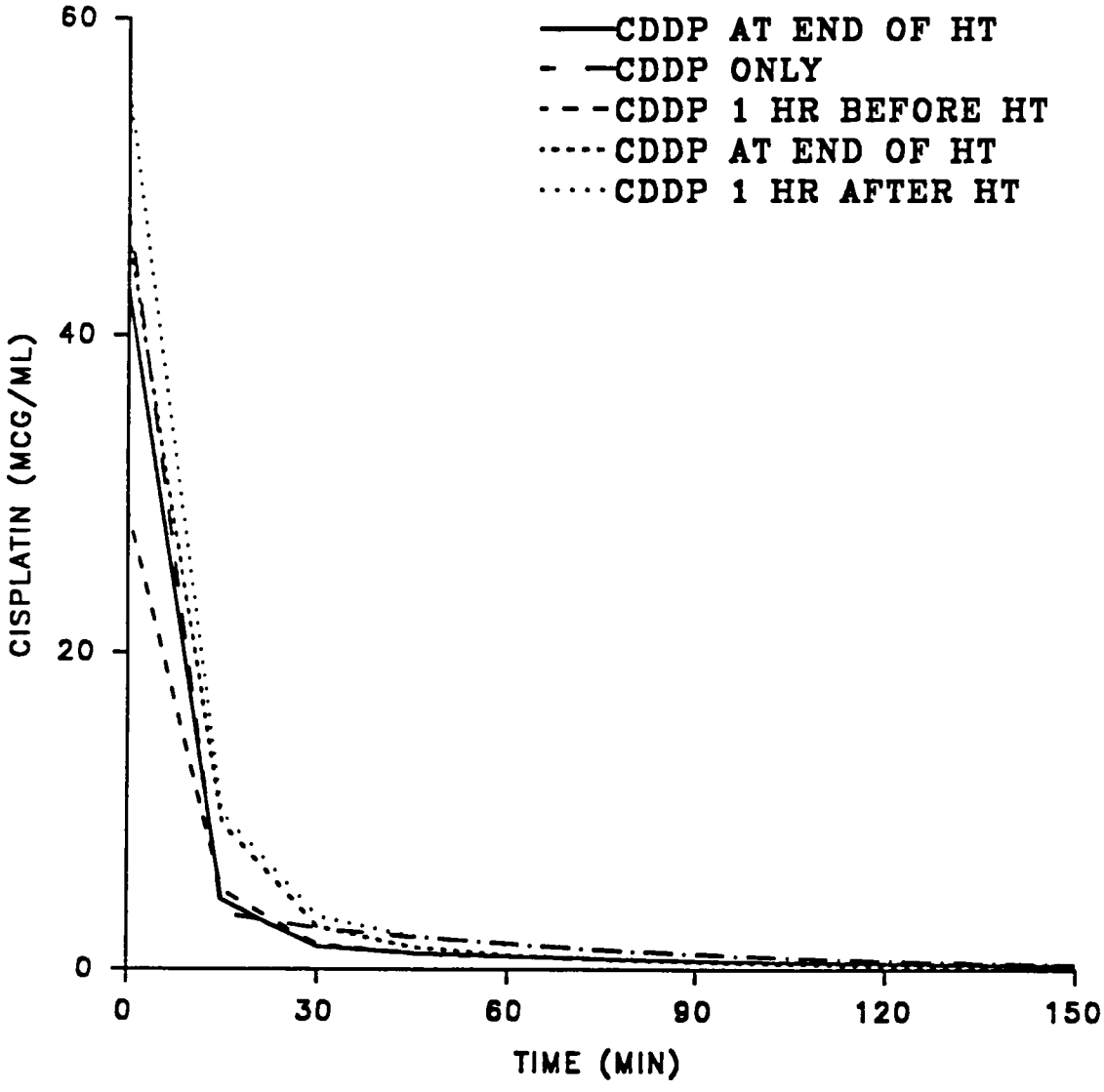


Figure 5. Average cisplatin concentration versus time profile for rats in each treatment group

Similar to blood flow values, overall treatment means of the pharmacokinetic parameters were not significantly different (ANOVA); however, when pairwise comparisons were made several of the parameters different (Table 4 and Figures 6-13).

There were no statistically significant differences in the A and B intercept between treatment groups (Figure 6). Similarly, the average α (distribution) and β (elimination) rate constants did not differ statistically between treatment group; however, it is interesting to note that each group of rats receiving hyperthermia had a much shorter α rate constant (Figure 7).

There were statistical differences in the average distribution half-life ($t_{\frac{1}{2}\alpha}$) and elimination half-life ($t_{\frac{1}{2}\beta}$) as shown in Figure 8. Because the distribution rate constant was greater for the group receiving drug only, this group had the shortest distribution half-life. In fact, the distribution half-life ($t_{\frac{1}{2}\alpha}$) of the drug only group was significantly shorter than the group receiving drug 1 hour before, at the beginning of, and at the end of hyperthermia.

Surprisingly, the average distribution half-life of rats receiving drug 1 hour before heat was significantly greater ($p<0.05$) than the groups that received drug only,

Table 4. Means and standard deviations of the pharmacokinetic parameters for each treatment group

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
		TIME OF DRUG ADMINISTRATION RELATIVE TO HYPERTHERMIA			
	DRUG ONLY	1H BEFORE HT	BEGINNING OF HT	END OF HEAT	1H AFTER HT
T1 (h)	0.06 ± 0.02[a]	0.17 ± 0.01[b]	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
T2 (h)	0.95 ± 0.28	3.2 ± 1.1[c]	0.93 ± 0.35	1.2 ± 0.33	1.1 ± 0.37
AUC (μg/ml h)	436 ± 64	460 ± 70	568 ± 169	332 ± 48	546 ± 88
A (μg/ml)	43.± 9.8	27 ± 3.3	43 ± 15	41 ± 23	51 ± 18
α (h^{-1})	0.32 ± 0.09	0.13 ± 0.01	0.12 ± 0.02	0.18 ± 0.09	0.14 ± 0.03
B (μg/ml)	4.4 ± 1.0	1.5 ± 0.87	3.2 ± 0.96	1.8 ± .63	4.3 ± 1.7
β (h^{-1})	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
CL (ml/kg/min)	5.7 ± 0.07	6.1 ± 0.72	6.7 ± 1.2	8.3 ± 1.2[d]	4.9 ± 0.78
TUMOR SIZE (mm^3)	0.71 ± 0.07	0.70 ± 0.07	0.55 ± 0.05	0.63 ± 0.05	0.77 ± 0.07[e]
TUMOR CONC (μg/g)	5.4 ± 1.1	6.7 ± 1.0	8.6 ± 2.1[f]	4.3 ± 1.1	8.5 ± 1.9
Vd_{ss} (L/kg)	0.34 ± 0.11	0.81 ± 0.35[g]	0.16 ± 0.04	0.28 ± 0.06	0.18 ± 0.06
Vd_C (L/kg)	0.08 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.13 ± 0.02[h]	0.07 ± 0.01

a = significantly shorter than groups 2, 3, and 4 ($p<0.05$)
b = significantly longer than groups 1, 3, and 5 ($p<0.05$)
c = significantly longer than all other groups ($p<0.05$)
d = significantly greater than group 5 ($p<0.05$)
e = significantly larger than group 3 ($p<0.05$)
f = significantly greater than group 4 ($p<0.05$)
g = significantly greater than groups 3, 4, and 5 ($p<0.05$)
h = significantly greater than group 5 ($p<0.05$)

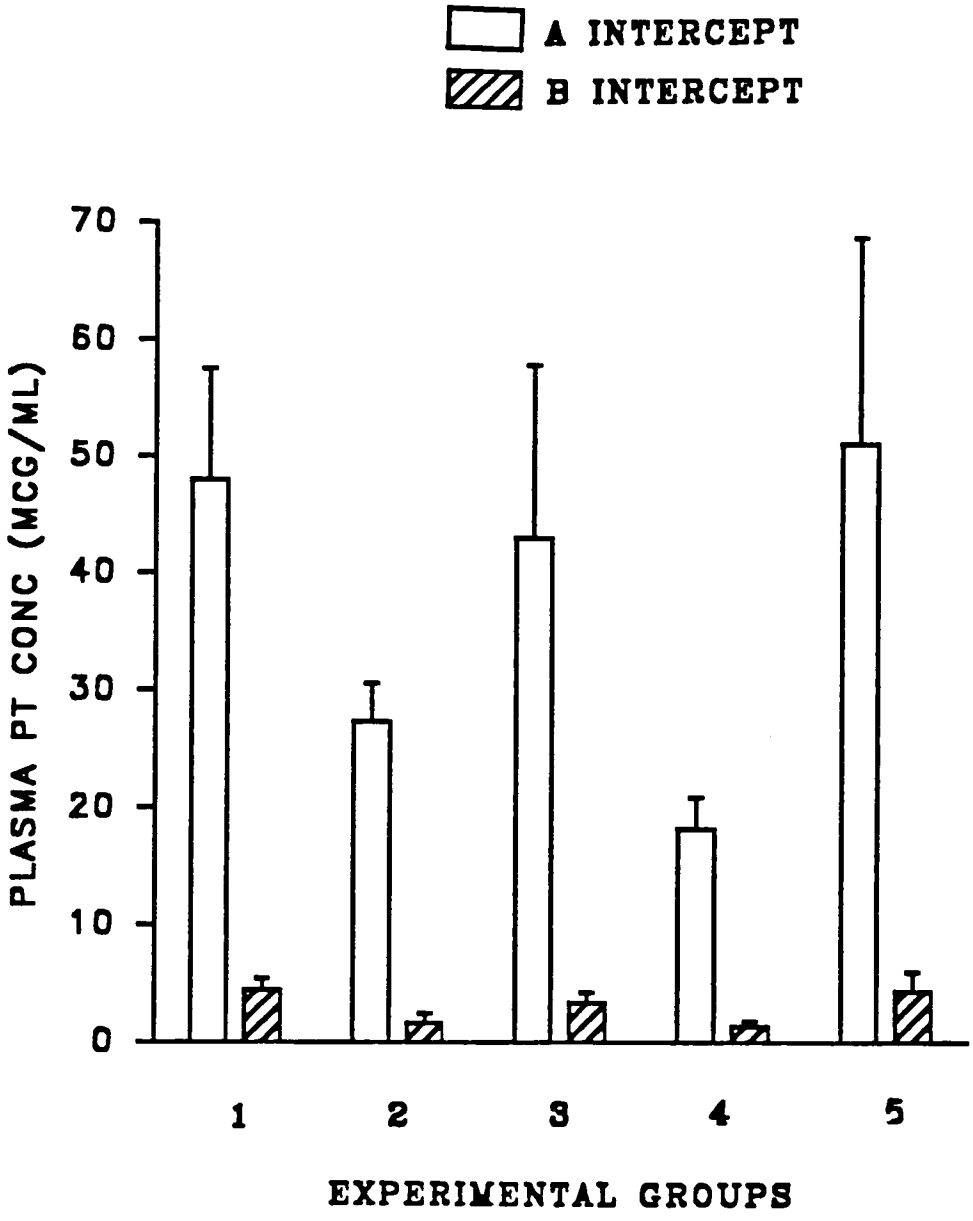


Figure 6. Mean values for the A and B intercepts of each experimental group
1 = drug only
2 = drug 1 hour before hyperthermia
3 = drug at the beginning of hyperthermia
4 = drug at the end of hyperthermia
5 = drug 1 hour after hyperthermia

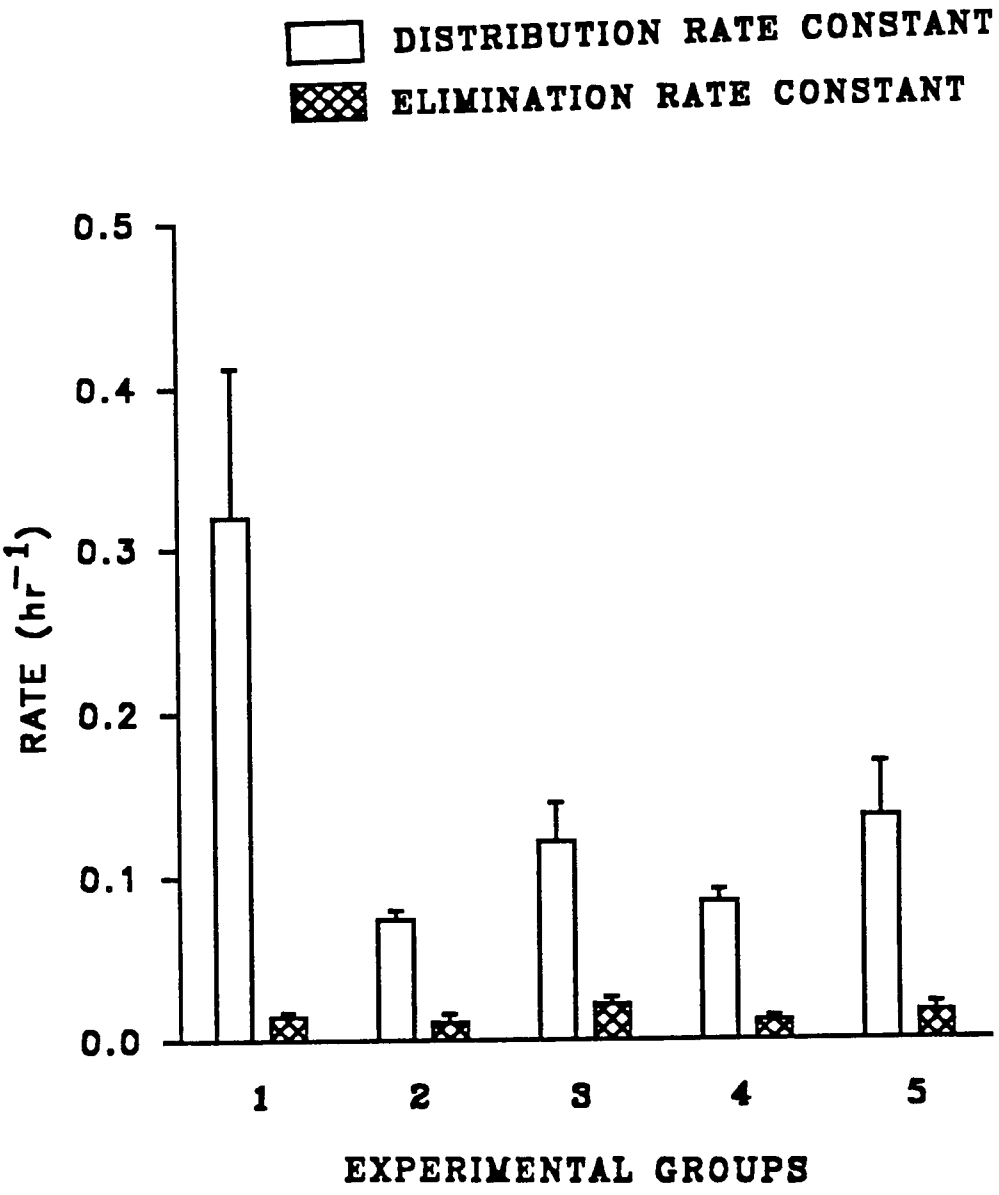


Figure 7. Mean values for the distribution (α) and elimination (β) rate constant of each experimental group
1 = drug only
2 = drug 1 hour before hyperthermia
3 = drug at the beginning of hyperthermia
4 = drug at the end of hyperthermia
5 = drug 1 hour after hyperthermia

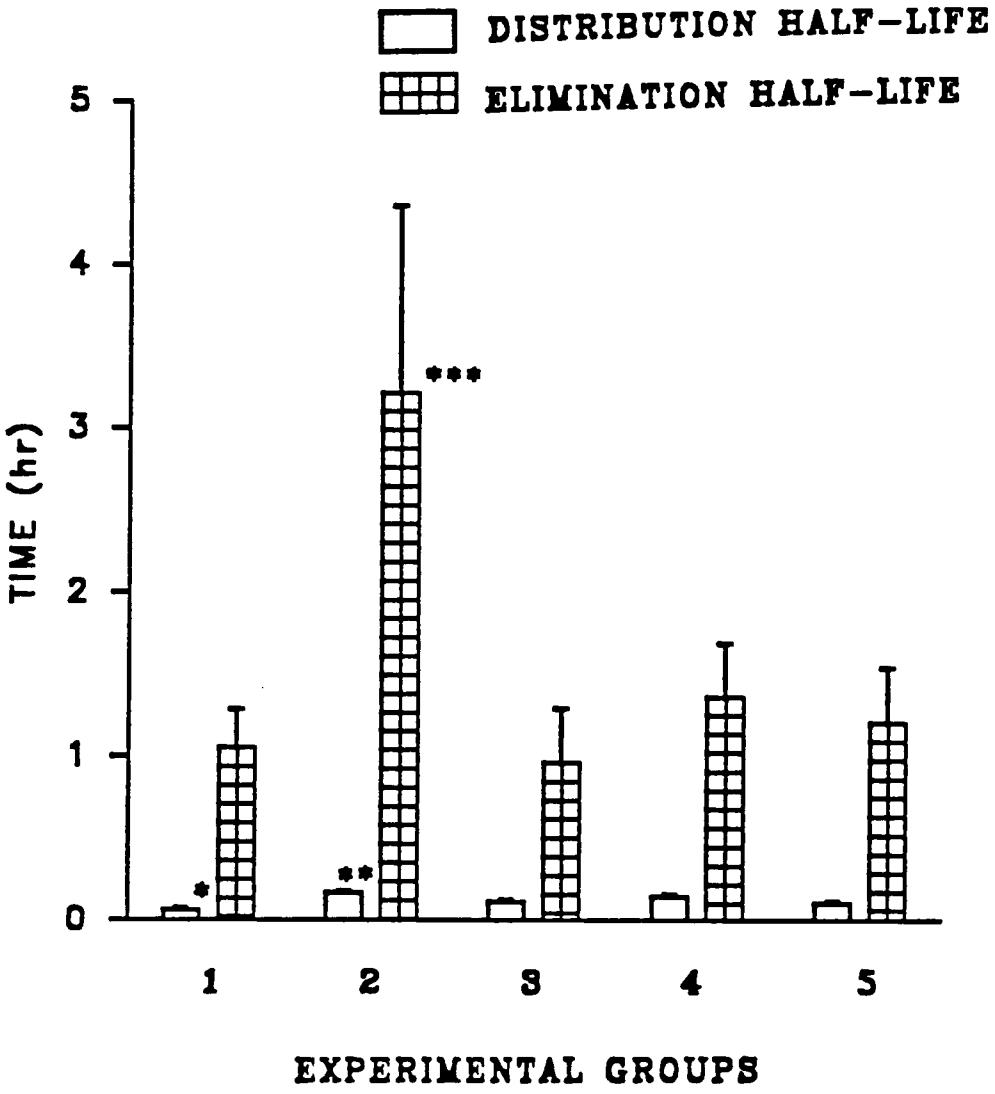


Figure 8. Mean values for the distribution ($t_{½\alpha}$) and elimination half-life ($t_{½\beta}$) of each experimental group
1 = drug only
2 = drug 1 hour before hyperthermia
3 = drug at the beginning of hyperthermia
4 = drug at the end of hyperthermia
5 = drug 1 hour after hyperthermia

* significantly shorter than groups 2, 3 and 4
** significantly longer than groups 1, 3, and 5
*** significantly longer than group 5
significance is at the $p<0.05$ level

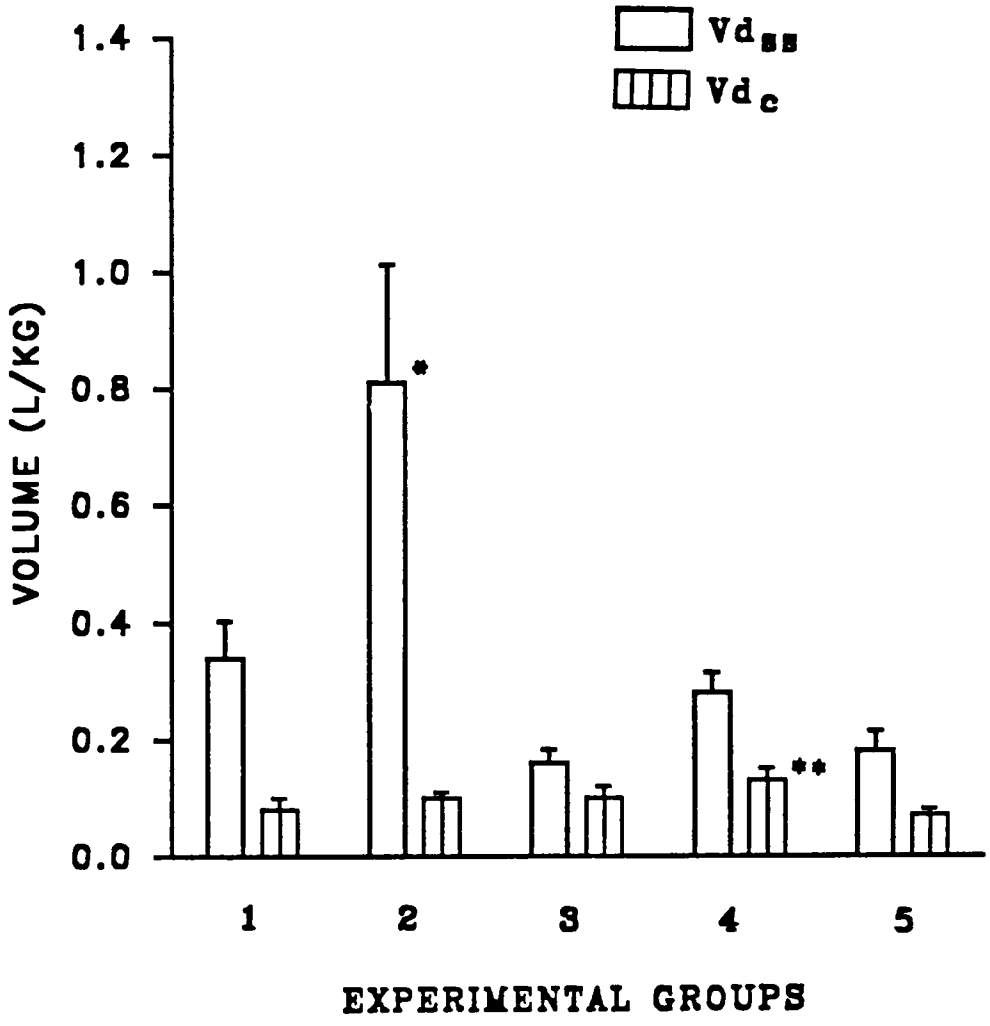


Figure 9. Mean values for the steady state volume of distribution (Vd_{SS}) and the volume of distribution of the central compartment (Vd_C) of each experimental group
1 = drug only
2 = drug 1 hour before hyperthermia
3 = drug at the beginning of hyperthermia
4 = drug at the end of hyperthermia
5 = drug 1 hour after hyperthermia

* significantly greater than groups 3, 4, and 5
** significantly greater than group 5
significance is at the $p<0.05$ level

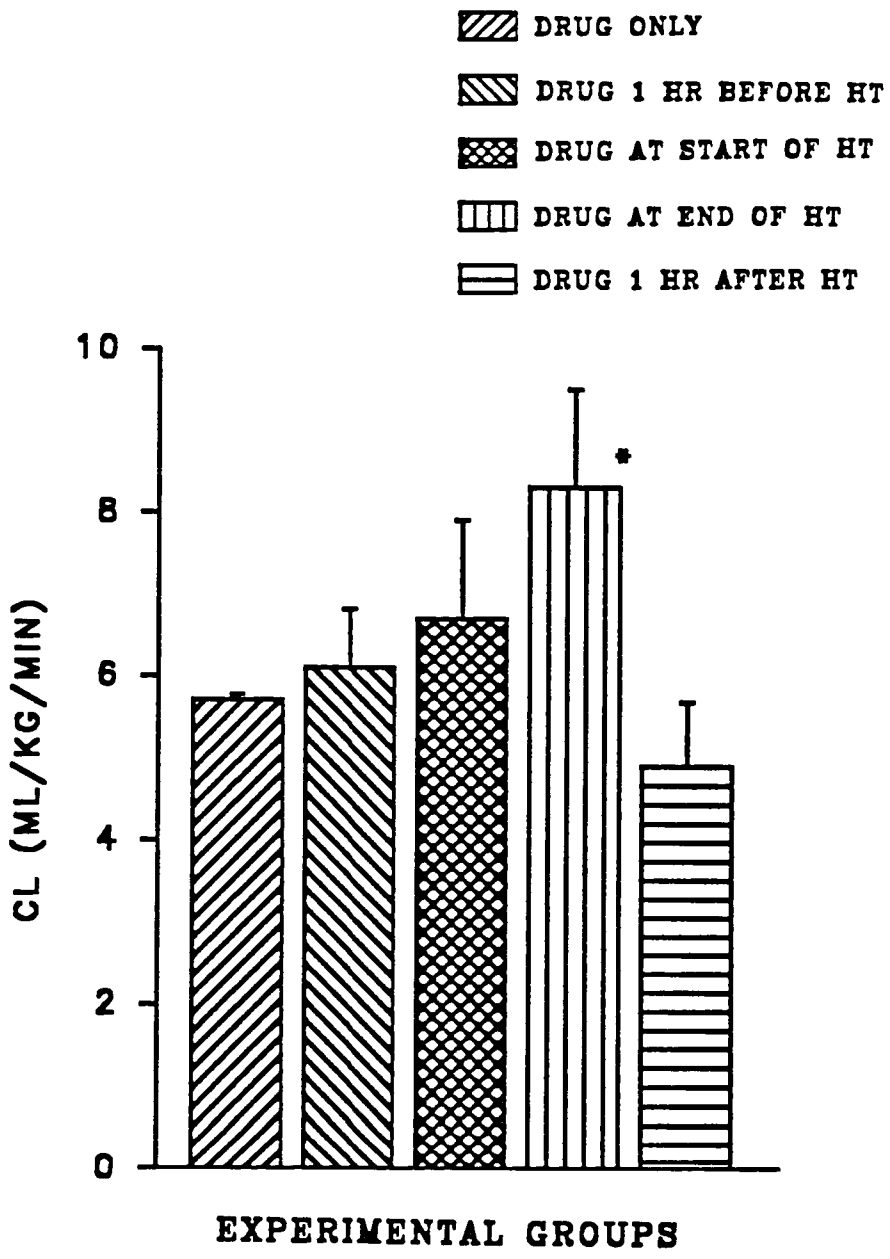


Figure 10. Mean values for whole body clearance of each experimental group

* significantly greater than group given drug 1 hour after hyperthermia ($p<0.05$)

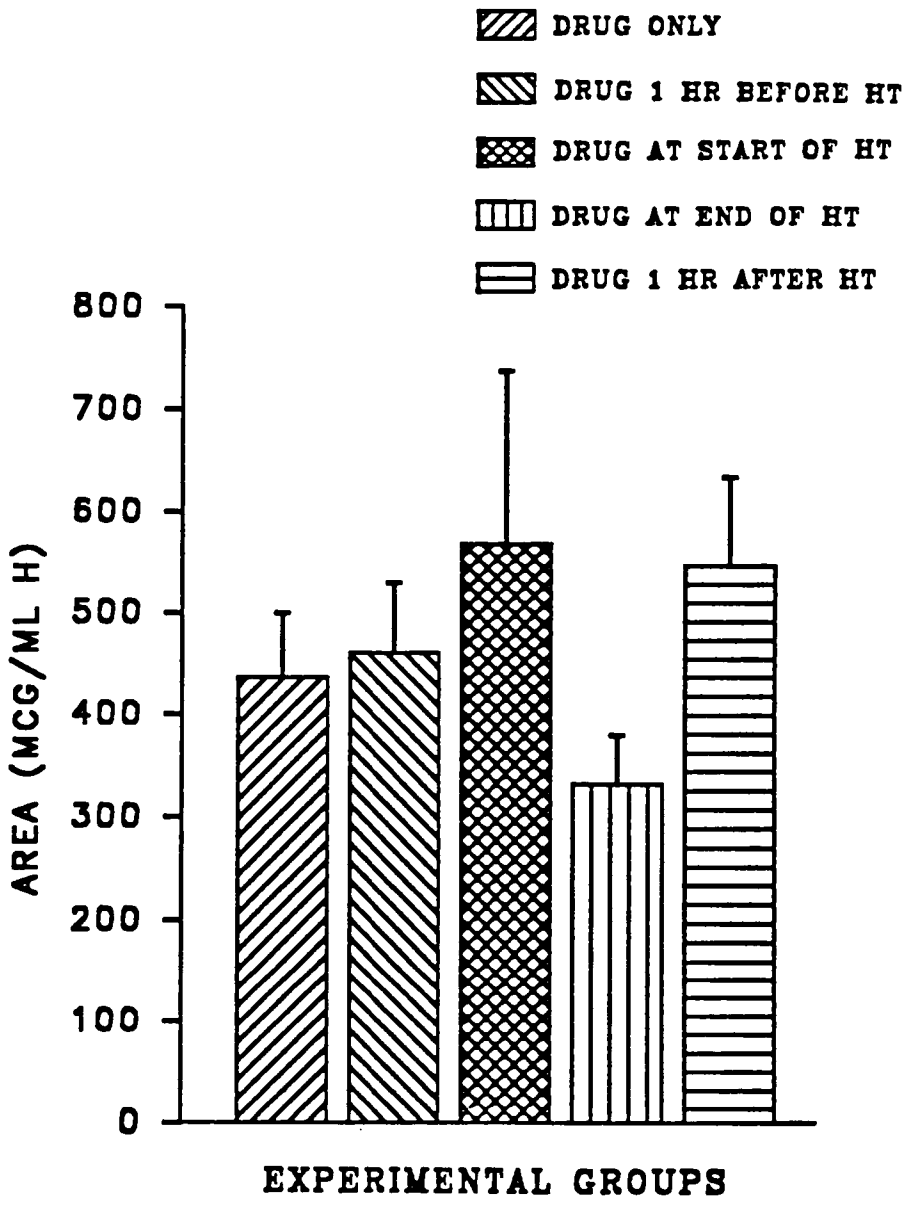


Figure 11. Mean values for area under the curve (AUC) of each experimental group

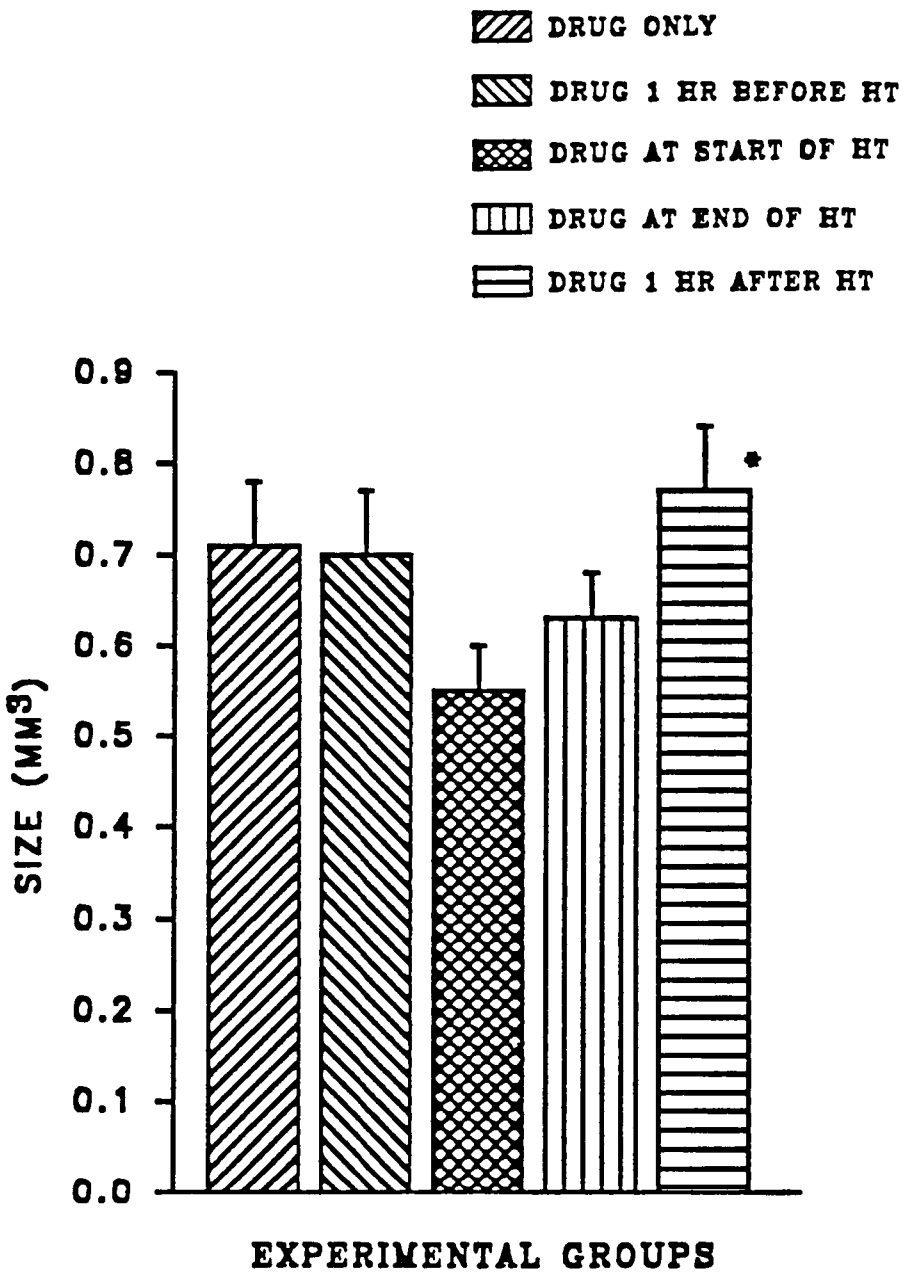


Figure 12. Mean values for tumor size of each experimental group

* significantly larger than the group given drug at the beginning of hyperthermia ($p<0.05$)

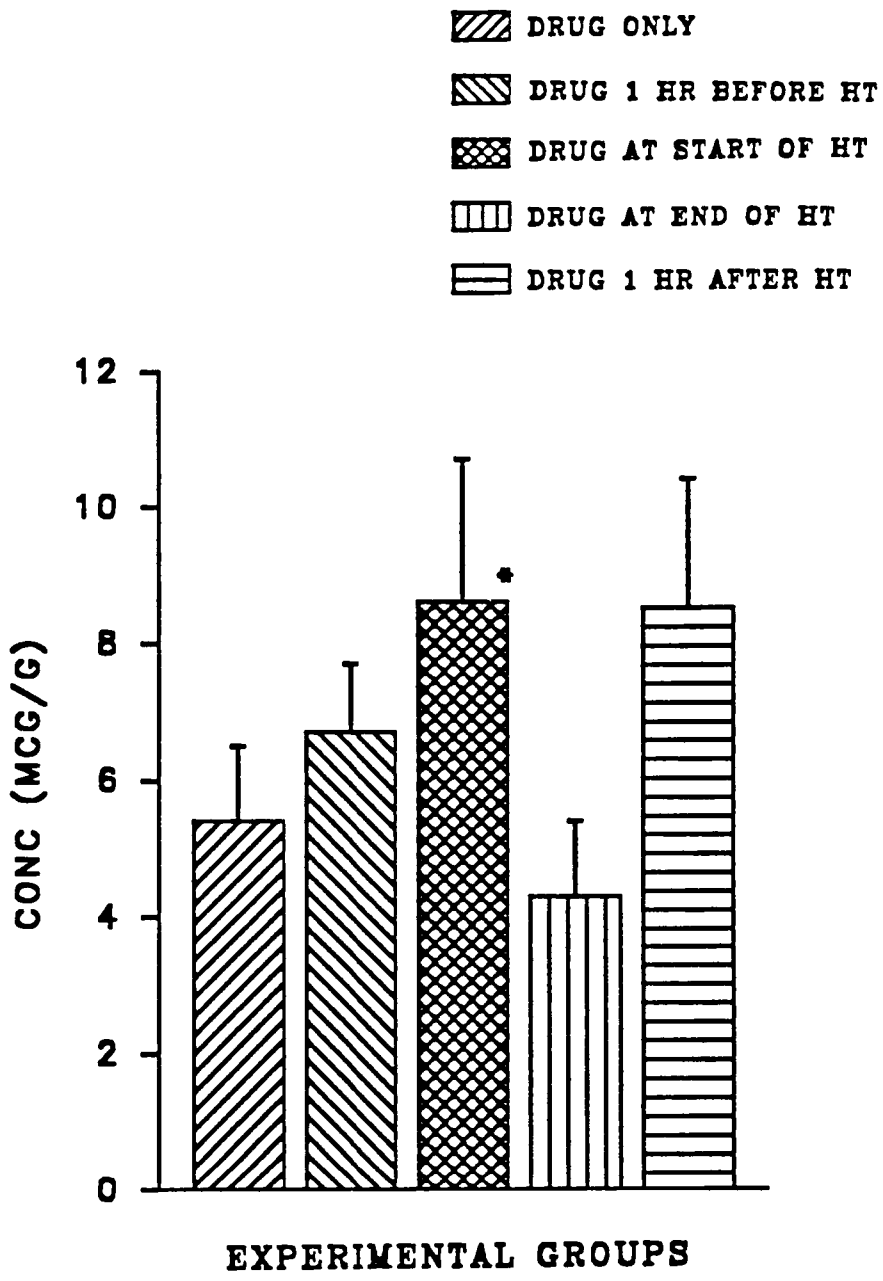


Figure 13. Mean concentration of drug in tumor tissue of each experimental group

* significantly greater than the group given drug at the end of hyperthermia ($p<0.05$)

drug at the beginning of heat, and 1 hour after heat. The elimination half-life ($t_{\frac{1}{2}\beta}$) of the group receiving cisplatin 1 hour before hyperthermia was significantly greater ($p<0.05$) than all other groups.

There was also significant differences in the volumes of distribution between the treatment groups (Figure 9). The steady state volume of distribution (Vd_{SS}) refers to the amount of drug distributed throughout the body. The Vd_{SS} was significantly greater ($p<0.05$) in the group receiving drug 1 hour before hyperthermia than in the groups that were dosed at the beginning, end, or 1 hour after of hyperthermia. This large Vd_{SS} would be in agreement with the large half-lives reported for the drug 1 hr before heat group. An increased Vd_{SS} would indicate an increase in tumor drug concentration; however, there was no increase in cisplatin concentration shown in the tumors of this group.

Rats receiving drug at the end of hyperthermia had an significantly ($p<0.05$) greater average Vd_C than the average value for rats dosed a hour after hyperthermia. The volume of distribution of the central compartment (Vd_C) indicates the amount of drug in the blood and highly perfused organs and less in the lowly perfused organs such as tumor tissue. In fact, the rats with the greatest Vd_C had the lowest concentration of drug in tumor.

Clearance was significantly greater ($p<0.05$ in this same group (drug at the end of heat) than in the groups which in which drug was administered 1 hour after hyperthermia (Figure 10). Although there was no statistically significant difference in the area under the curve (AUC) between treatment groups, it is important to note that the group receiving drug at the end of hyperthermia had the lowest AUC value (Figure 11). AUC value represents the total amount of drug in the blood. Therefore, a decrease in the AUC value represents a decreased concentration of cisplatin in the blood.

All rats were treated when their tumors were approximately 1 cm in diameter; however, rats administered drug one hour after hyperthermia had statistically larger ($p<0.05$) tumors than rats receiving drug at the beginning of hyperthermia (Figure 12).

Tumor drug concentration was significantly greater ($p<0.05$) in rats receiving drug at the beginning of heat than those given drug at the end of heat (Figure 13). In a study by Douple et al. (1988) mice with 1 cm mammary adenocarcinomas were administered cisplatin (20 mg/kg body weight). At one half hour post injection tumors were removed and analyzed for platinum concentration. The mean concentration of platinum in the tumors was 5.95 $\mu g/g$ which is very similar to the value obtain 2 hours post

injection for tumors of rats that received drug only in the present study as shown in Table 4.

Even though the group in this graduate study with the smallest average tumor size (drug at the beginning of hyperthermia) had the largest tumor drug concentration there does not seem to be a correlation between tumor size and drug concentration. For example, the group of rats with the largest average tumor size (drug 1 hour after heat) had the second highest average concentration of drug in their tumors.

According to the values obtained in this study cisplatin should be administered at the beginning of or 1 hour after a one hour hyperthermia treatment. This is in contrast to the present clinical protocol which suggest that drug be given at the beginning or end of hyperthermia.

The lowest tumor drug concentrations were seen in rats that were given drug at the end of a one-hour hyperthermia treatment. This group also had the lowest AUC value and a value for the Vd_C and CL that were significantly greater ($p<0.05$) than other values. Previously, blood flow in the tumor was shown to be increased at the end of hyperthermia (Figure 2). This suggests but does not prove that decreased tumor drug concentrations result when blood flow is increased,

presumably due to a wash-out effect where increased flow allows inadequate time for drug uptake into cells.

Because of the results obtained when a pairwise comparison was made, a Pearson correlation was performed to see which pharmacokinetic parameters were important in determining tumor drug concentration. Tumor drug concentrations were found to be directly correlated to the area under the curve (AUC) ($p<0.001$) and A intercept ($p<0.05$). Since the A intercept represents that peak plasma concentration, one possible clinical application of this information is that by obtaining a blood sample from a patient and ascertaining the peak plasma concentration, one may be able to predict the tumor drug concentration.

In the Pearson correlation analysis tumor drug concentration was indirectly correlated to clearance (CL) ($p<0.006$) and the volume of distribution of the central compartment (Vd_C) ($p<0.008$). This implies that an increase in the amount of drug which is cleared from the body or is kept in the blood and highly perfused organs results in a decrease in the drug concentration of the lowly perfused tumor tissue.

Riviere et al.(1986) has reported that at $43^{o}C$ there is a significant increase in the rate constant reflecting the decay of free cisplatin as opposed to the value obtained at $37^{o}C$. They were also able to demonstrate that

canines subjected to whole-body heating (42^{o}C for 1 hr) after receiving cisplatin (1mg/kg) had increased rate of transformation of reactive metabolites from parent cisplatin and increased volume of distribution of free platinum. An increased clearance rate (CL) was also noted in the study by Riviere et al. which was attributed to increased renal clearance, increased biotransformation, or increased rate of tissue binding. There was an increase in total clearance in the present study in all groups administered hyperthermia when compared to control rats except the one in which rats received cisplatin 1 hr after hyperthermia. The only group in which this increase was statistically significant was the group in which rats received drug at the end of hyperthermia.

If one assumes that an increase in the concentration of drug in the tumor will result in increased cell kill in the tumor, the results of the present study are in agreement with <u>in vitro</u> studies that suggest that synergistic killing requires that heat and drug be administered close together in time, if not simultaneously (Herman et al., 1988; and Marmor, 1979).

CHAPTER V

CONCLUSION

The objectives of this study were 1) to determine the effect of localized hyperthermia (43^{o}C for 1 hr) on the plasma and cisplatin pharmacokinetics of cisplatin when the drug is administered at different times relative to hyperthermia in rats with dimethyl-benz[a]anthracene-induced mammary adenocarcinomas and 2) to correlate hyperthermia-induced changes in tumor blood flow with cisplatin concentrations. According to the data it appears that certain pharmacokinetic parameters, specifically drug concentration in tumor tissue, can be altered as a result of localized hyperthermia.

Contrary to the present dosage regimen in which drug is given either at the beginning of hyperthermia or at the end of hyperthermia, the results of this work indicate that drug should be administered to patients at the beginning of hyperthermia and 1 hour after hyperthermia. According to the results of a Pearson correlation analysis, the factors which indicate a high drug concentration in the tumor tissue are a large value for the A intercept (peak plasma concentration)

and area under the curve and a low value for whole body clearance and volume of distribution of the central compartment.

The data obtained can assist in the formation of a study which can lend greater insight into the effects of hyperthermia on cisplatin pharmacokinetics. In future studies the number of subjects should be increased in order to compensate for individual variability.

Although only one hyperthermia treatment was given to an individual in this study, further study should investigate the effect of multiple heatings, if possible, and/or multiple drug treatments in order to more closely mimic the clinical protocol.

Because both hyperthermia (Kim et al., 1982; and Luk et al., 1984) and cisplatin (Burholt et al., 1979; and Carde et al., 1981) are radiation sensitizers, this third modality should be added to a research project in the future. It is well documented that the maximum cytotoxic effect is observed when radiation is delivered simultaneously with heat or during the heating interval (Dewey et al., 1980).

Hyperthermia is one of the few modalities used to treat cancer that does not of itself induce oncogenic transformation (Jarisiadis et al., 1980; Clark et al.,

1981; Watanabe et al., 1984; and Raaphort et al., 1983). This modality should be investigated fully in combination with existing modalities with the goal of potentiating, as much as possible, the antitumor activity of other types of treatment while diminishing adverse effects. A phase I-II trial of this trimodality has been conducted with very gratifying results (Bornstein et al., 1989); however, additional laboratory study is needed to define the most efficacious treatment regimen.

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APPENDIX

APPENDIX

SAFETY PROCEDURES

Cisplatin Administration

Because this antineoplastic agent is mutagenic, carcinogenic and teratogenic, it is prepared under a Class II hood by a researcher wearing a protective gown, latex gloves, and a respirator mask (BioSafety Systems, Inc.). The protective barrier gowns have closed fronts, long sleeves, and closed cuffs. A plastic backed absorbent pad is used where the chemotherapeutic agent is being prepared and under the administration work area. Cisplatin is sensitive to light and must be covered after preparation.

Disposal of materials follows the guidelines of the National Study Commission on Cytotoxic Exposure and the OSHA work-practice guidelines for personnel dealing with cytotoxic (antineoplastic) agents. Materials that come in contact with cisplatin (i.e. gowns and mats) are disposed of in a receptacle appropriately marked as "hazardous material". Syringes and needles are disposed of in a chemotainer box which is a leakproof, puncture-proof container. In order to prevent aerosolization, needles and syringes are not clipped. When full, containers are sealed, collected, and disposed of as hazardous chemical

waste. Also, a CYTA Spill Kit (Winfield) was kept in an obvious location in the laboratory to be used in the event that cisplatin was spilled.

Blood Flow Determination

A corner of the Laser/Hyperthermia laboratory was designated for use of radioactive substances and separated from the rest of the room by a laboratory work bench and a metal storage cabinet. Radiation labelling tape was used to denote the boundaries on the floor and wall of this area.

The remaining tissue from the rat is placed in two disposal bags, labeled with radioactive labeling tape specifying the type and quantity of isotope used, half life, rat #, date, and name of the investigator. This is placed in the UTCVM necropsy freezer. And, after notification, the Radiological Safety Department will appropriately dispose of the carcass. All materials used in the surgery, including needles and syringes, are disposed of in a specified 55-gallon drum supplied by the Radiological Safety Dept. Surgical instruments that are contaminated with radioactivity are washed in a sink labeled and reserved for radioactive substances. The surgery area is cleaned with all purpose cleaner and various regions of the lab (i.e. surgery table, lab bench,

etc.) are swabbed in order to check for contamination in the work space. If analysis of these with the gamma counter detects any such contamination, the region is washed with Radiacwash (Atomic Products Corporation) and surveyed again. Each investigator and assistant is supplied with a monthly film badge and ring in order to monitor his exposure to the agents used.

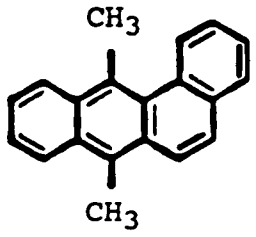
The radioactive microspheres are stored in a locked cabinet with radiation caution signs adhered to it. In the advent that a vial of spheres is dropped or otherwise broken we would follow the guidelines set forth by the Radiological Safety Department. A small spill (<3 ml) can be cleaned with a detergent such as 409 (The Clorox Company) or Radiacwash and reported to the safety dept. Larger spills would require isolation of the area and notification of the safety dept. for an appropriate clean-up.

Laser Use

Because the Nd:YAG laser emits a concentrated beam that would damage the eye, Laser-gard anti-laser protective goggles (LGS-NDGA Neodymium-Gallium Ardenide) were worn when the laser was in operation. This protective eyewear was obtained from Glendale Protective Technologies.

DMBA Administration

The structure of the highly potent carcinogen 7,12-dimethylbenz[a]anthracene is as follows:



Molecular formula

$C_{20}H_{16}$

The National Institute of Health Division of Safety has a safety data sheet that should be referred to whenever it becomes necessary to use this chemical. This agent has no known commercial or industrial use and is destroyed through photooxidation in the atmosphere and probably degrades slowly by bacteria in the soil. DMBA is rapidly absorbed through the skin and respiratory tract and may irritate tissue and induce sensitivity. All laboratory operations with this agent should be conducted in a hood and researchers should wear protective clothing when handling this agent. In the event that skin is contact, the skin should be washed with soap and water and washing with solvents and exposure to UV light should be

avoided. In the event of eye exposure, the eye should be irrigated immediately. Vomiting should be induced if ingestion occurs. After induction of tumors in laboratory animals, all waste materials from the animals for at a twenty-four hour period until the carcinogen has been metabolized and products excreted should be incinerated. All disposable materials used in the induction process should be collected in a biohazard bag and incinerated along with the waste material.

There are also specific guidelines for decontamination of equipment and glassware. If more than 1 g has been spilled or if there is an uncertainty regarding the procedures to be followed for decontamination, the NIH Fire Department should be contacted for assistance. Surfaces should be washed with large quantities of soap and water. Glassware should be rinsed in a hood with an organic solvent other than alcohol followed by soap and water. Animal cages should also be washed with soap and water.

Surplus DMBA should be disposed of according to hazard chemical waste guidelines of the NIH. Nonchemical waste, such as animal carcasses and contaminated absorbant materials, should be packaged for incineration according to the NIH medical-pathological waste disposal system.

VITA

Peni Lynn Ausmus was born in Wenatchee, Washington on January 7, 1966. She graduated from Campbell County High School, LaFollette, Tennessee in 1984 and was awarded a Presidential Academic Fitness Award.

As a flutist, she was the talent winner in the 1984 Young Miss America pageant and accepted a music scholarship to Tennessee Technological University where she majored in both music therapy and biology. In 1986, she transferred to the University of Tennessee, Knoxville to pursue a Bachelor of Science degree in Microbiology which she completed in 1988.

After completing a semester of graduate study at East Tennessee State University, she accepted a graduate research assistantship from the Thompson Cancer Survival Center and completed the degree of Masters of Science in Comparative and Experimental Medicine in August 1991.

She is a member of Alpha Epsilon Delta Pre-professional Honor Society and the LaFollette Church of God where she is actively involved in Christian education and music ministry.