UNIVERSITY OF CINCINNATI

May 6 , 2003

I, Patricia Elizabeth Grillo ,

hereby submit this as part of the requirements for the degree of:

Master of Science

in Biopharmaceutics

It is entitled The Clinical Pharmacokinetics of Carboplatin Coadministered with Paclitaxel and SU5416, a Novel Anti-angiogenic Agent

Approved by:

Paul Kaye Desai 5/6/03

5/6/03

5/6/03

The Clinical Pharmacokinetics of Carboplatin Coadministered with Paclitaxel and SU5416, a Novel Anti-angiogenic Agent

A thesis submitted to the

Division of Research and Advanced Studies
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

Master of Science

in the Department of Pharmaceutical Sciences
of the College of Pharmacy

2003

by

Patricia Elizabeth Grillo

BA Physics, BA Chemistry 1993;
BS Clinical Laboratory Science 1996
Miami University Oxford, Ohio

Committee Chair: Dr. Pankaj B. Desai

THE CLINICAL PHARMACOKINETICS OF CARBOPLATIN COADMINISTERED WITH PACLITAXEL AND SU5416, A NOVEL ANTI-ANGIOGENIC AGENT

SU5416 is a novel, anti-angiogenic compound that was investigated in Phase I and II combination chemotherapy trials. These investigations included determining the potential influence of SU5416 on the pharmacokinetics and pharmacodynamics of anti-cancer drugs. The goal of this study was to evaluate the clinical pharmacokinetics of carboplatin, a widely used anti-cancer agent, when coadministered with SU5416. For this purpose, 15 subjects with advanced malignancies were administered a carboplatin dose calculated using the Calvert formula to achieve an AUC of 6 mg/ml min. Carboplatin was administered as a 30 minute IV infusion on day 1 of 2 consecutive three weeks cycles. A 3 hour IV infusion of 175 or 225 mg/m^2 paclitaxel proceeded the carboplatin infusion. All subjects received infusions of SU5416 on days 4, 8, 11, 15 and 18 of each cycle. A SU5416 dose of 85 mg/m^2 was administered to 4 subjects, while 11 subjects received 145 mg/m^2. Serial blood samples were collected following the carboplatin infusion at timed intervals on day 1 of cycles 1 and 2. Plasma ultrafiltrate was prepared using the Amicon MSP-1 micropartition system. Plasma and ultrafiltrate were analyzed for total and free platinum using inductively coupled plasma mass spectrometry. Pharmacokinetic analysis was performed employing the non-compartmental approach (WinNonlin 3.1). Overall, no major intercycle variability in the carboplatin pharmacokinetics was observed. Statistical evaluation indicated that at the above dosing regimens, SU5416 does not significantly ($p>0.05$) alter the carboplatin plasma and ultrafiltrate pharmacokinetics. Thus, it appears that the addition of SU5416 does not result in drug-drug interactions with platinum anti-cancer agents.

ACKNOWLEDGEMENTS

I would like to extend my thanks to the following:

Sugen Inc. – for providing the funds necessary for this project.

The Grillo Family – my parents Mary and Vincent, my sister Kathy and my brother John for their love, support and encouragement during the completion of this project and always.

Dr. Pankaj B. Desai – for supervising my research and my education at the College of Pharmacy.

Dr. Abdul Jazieh and Dr. Jane Pruemer – for taking time out of their busy schedules to serve on my graduate committee.

The Department of Chemistry, University of Cincinnati – for allowing me to use their instrumentation. A special thanks to Dr. Joseph Caruso, Dr. Maria Montes-Bayon and Jason Day for allowing the use of their lab for some of my sample preparation, for their assistance with the method development and for training me in the use of the ICP-MS.

Murad Melhem and Rucha Sane – for their friendship, support and assistance during the completion of this project.

TABLE OF CONTENTS

Page number

LIST OF TABLES

Page number

LIST OF FIGURES

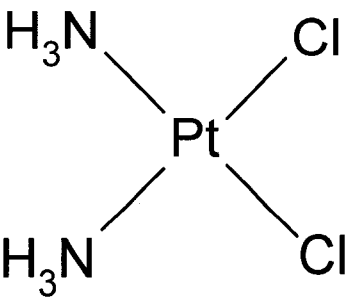
Page number

1. INTRODUCTION

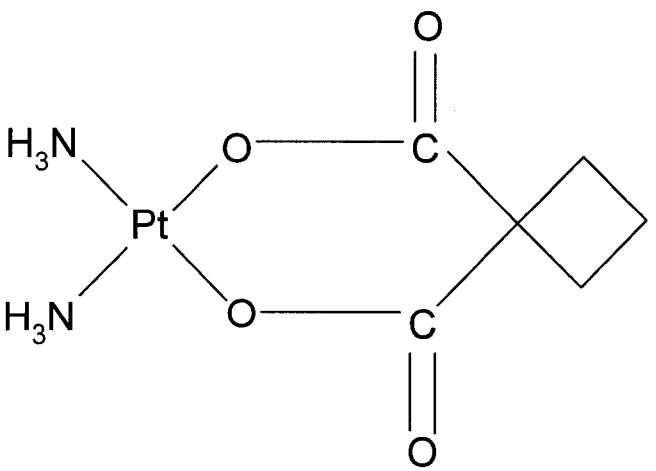
1.1 Carboplatin

1.1.1 Carboplatin background

The discovery of the platinum anti-cancer complexes occurred in the early 1960's by Dr. Barnett Rosenberg and his colleagues at Michigan State University. Dr. Rosenberg was studying the possible effects of an electrical field on the growth processes of bacteria. Platinum electrodes were used to generate the electrical field. A cessation of bacterial cell wall division was observed one to two hours after a current was applied to the nutrient medium. The hypothesis arose that some new chemical agent was being formed in the medium by electrolysis and was inhibiting cell division. This new agent was later identified as cisplatin (cis-diamminedichloroplatinum(II)). [Rosenberg *et al.*, 1965]. These experiments were later expanded upon to determine if cisplatin could inhibit tumor cell division, resulting in the realization of cisplatin's utility as a chemotherapeutic agent. Cisplatin was the first platinum complex to be introduced into clinical trials in the 1970s, and soon became a widely accepted treatment for metastatic testicular cancer, metastatic ovarian cancer and several head and neck cancers [Arkey, 1999]. The chemical structure of cisplatin is shown in figure 1a. Cisplatin has an extensive toxicity profile which includes ototoxicity, neurotoxicity and dose limiting nephrotoxicity. This profile caused researchers to set out to find a "second generation" platinum complex that would exert a similar clinical action, but have fewer toxicities. This research resulted in the discovery of carboplatin (cis-diammine-1,1-cyclobutanedicarboxylatoplatinum(II). The chemical structure of carboplatin is shown in



(a)



(b)

Figure 1: a) chemical structure of cisplatin; b) chemical structure of carboplatin.

figure 1b. Carboplatin is structurally different from cisplatin by the replacement of its chloride atoms with an oxygenated bidentate cyclobutane decarboxylate group [McKeage, 1995]. Carboplatin has been indicated in the treatment of advanced ovarian carcinoma, and is helpful in the treatment of some head, neck and lung cancers [Arkey, 1999].

1.1.2 Cytotoxic action of carboplatin

Carboplatin exerts its cytotoxic effect by targeting the DNA of tumor cells [Knox *et al.*, 1986]. The exact mechanism by which carboplatin is transported across the tumor cell membrane is not known. Once inside the cell, carboplatin undergoes an activation process known as aquation [Los *et al.*, 1991]. During this process, the cyclobutane bidentate ligand is replaced by two water molecules (figure 2). The product of this substitution reaction is a positively charged, electrophilic, reactive diaquo species. Once the substitution reaction occurs, carboplatin is in its reactive form and is able to react with nucleophilic sites such as DNA [Murry, 1997].

The reactive form of carboplatin binds covalently to DNA, resulting in the formation of intrastrand crosslinks between either adjacent guanine residues or adjacent guanine and adenine residues (figure 3). These crosslinks are referred to as Pt-DNA adducts. The formation of interstrand Pt-DNA adducts can and will occur as well but in much smaller numbers. The Pt-DNA adducts will inhibit DNA separation or result in the abnormal separation of DNA during cell division causing cell death and tumor destruction [Lipp, 1999].

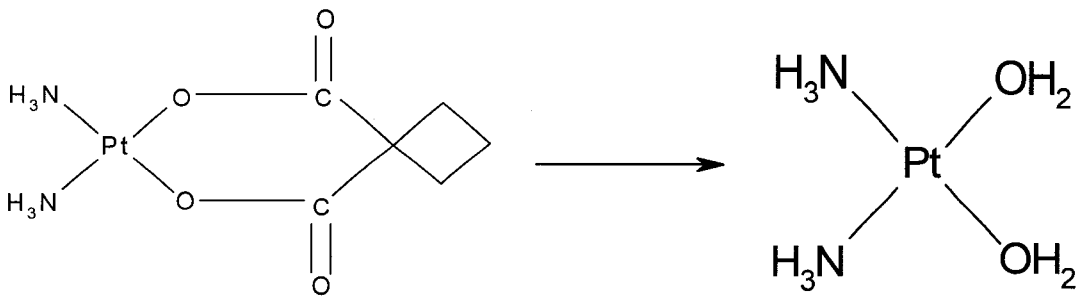


Figure 2: Cellular activation of carboplatin. The reactive form of carboplatin results from the substitution of the cyclobutane bidentate ligand with two water molecules.

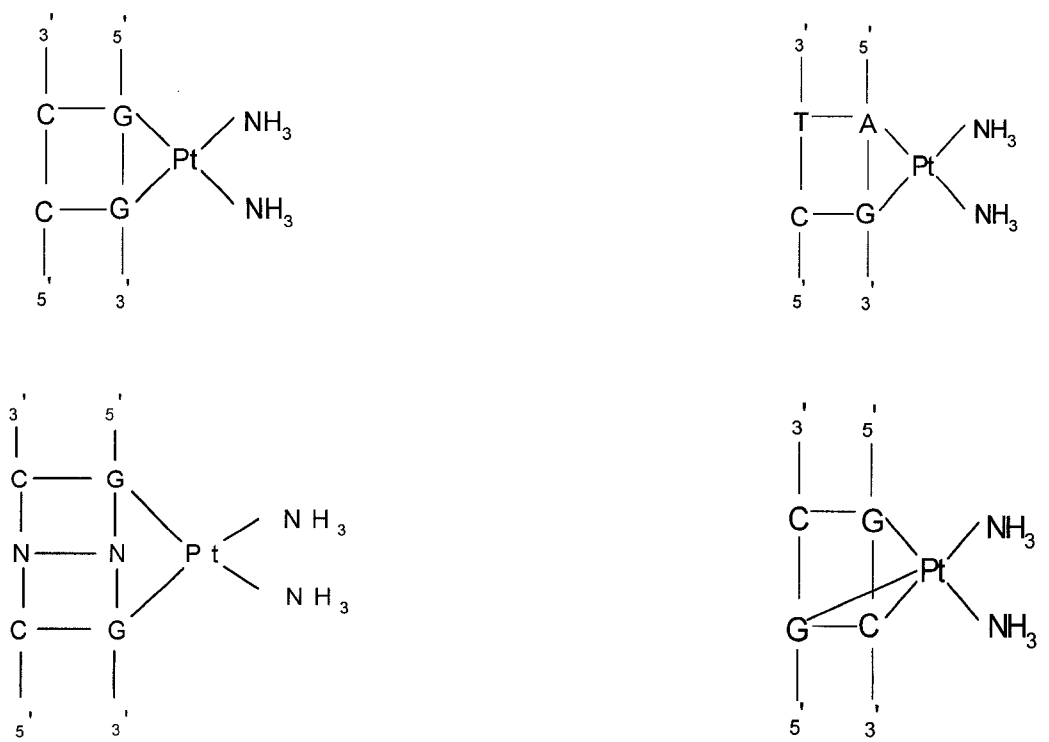


Figure 3: Formation of interstrand and intrastrand platinum-DNA adducts. The formation of these adducts will inhibit or impair DNA separation, resulting in tumor cell death

1.1.3 Toxicology of carboplatin

Carboplatin demonstrates minimal nephrotoxicity and has a low incidence of neurotoxicity and ototoxicity [Arkey, 1999]. It is moderately to highly emetogenic which is usually controllable by antiemetic therapy. Hematological toxicity is the dose limiting toxicity of carboplatin. This myelosuppression generally manifests itself as thrombocytopenia more so than leucopenia or anemia. Carboplatin dosage intervals are dependent upon platelet recovery, which usually occurs four weeks following treatment [McKeage, 1995].

1.1.4 Administration of carboplatin

A study by Van Hennik *et al.,* [1989] determined the oral bioavailabilities of total and free carboplatin to be 2.9-5.0%. Due to this low bioavailability, carboplatin is administered by IV infusion, generally over 30 minutes [Van der Vijgh, 1991]. Formulation in saline may result in the conversion of carboplatin to the more toxic cisplatin. The carboplatin formulation should contain a 5% dextrose solution to prevent this conversion. Intraperitoneal administration had been used to treat patients with residual ovarian cancer. This allows for exposure of the tumor cells to higher concentrations of carboplatin for longer periods of time [Calvert *et al.,* 1993].

1.1.5 Carboplatin in combination therapy

Carboplatin is commonly used in combination regimens. When administered in conjunction with 5-fluorouracil, etoposide or cyclophosphamide no pharmacokinetic interactions are observed [Duffell and Robinson, 1997]. One of the most widely used and

successful combinations pairs carboplatin with paclitaxel. This was based on a series of studies that showed synergistic interactions *in vitro*, *in vivo* and in clinical trials. There is no sequence dependent toxicity observed and the degree of thrombocytopenia observed is less than that observed when carboplatin is administered as a single agent [Giaccone *et al.*, 1995]. The carboplatin/paclitaxel combination has become the standard treatment for lung and ovarian cancer.

1.1.6 Pharmacokinetics of carboplatin

Carboplatin pharmacokinetics follow an open two compartment model with elimination from the central compartment. Pharmacokinetics are linear up to a dose of 2400 mg/m^2 [Duffull and Robinson, 1997]. Following IV infusion, carboplatin is widely distributed into the tissues. High concentrations of drug can be found in the kidneys, liver, skin and tumor tissue [Lipp, 1999]. Carboplatin, free platinum and total platinum all have relatively similar concentration vs. time profiles over the first six hours (figure 4). Distribution half-lives for all three species are approximately one hour. Beyond six hours the concentration of total platinum remains higher than the concentration of free platinum and carboplatin. This is an indication that protein binding is slow, and occurs to a small extent [Van der Vijgh, 1991]. The profiles of free platinum and carboplatin remain similar until approximately ten hours, indicating that metabolism of carboplatin is slow. During this time, there are few or no carboplatin metabolites in the circulation [Van der Vijgh, 1991]. Carboplatin is excreted predominately by the kidneys through glomerular filtration. Within 24 hours of administration 70-80% of the administered dose can be recovered unchanged in the urine [Van der Vijgh, 1991]. The total body clearance

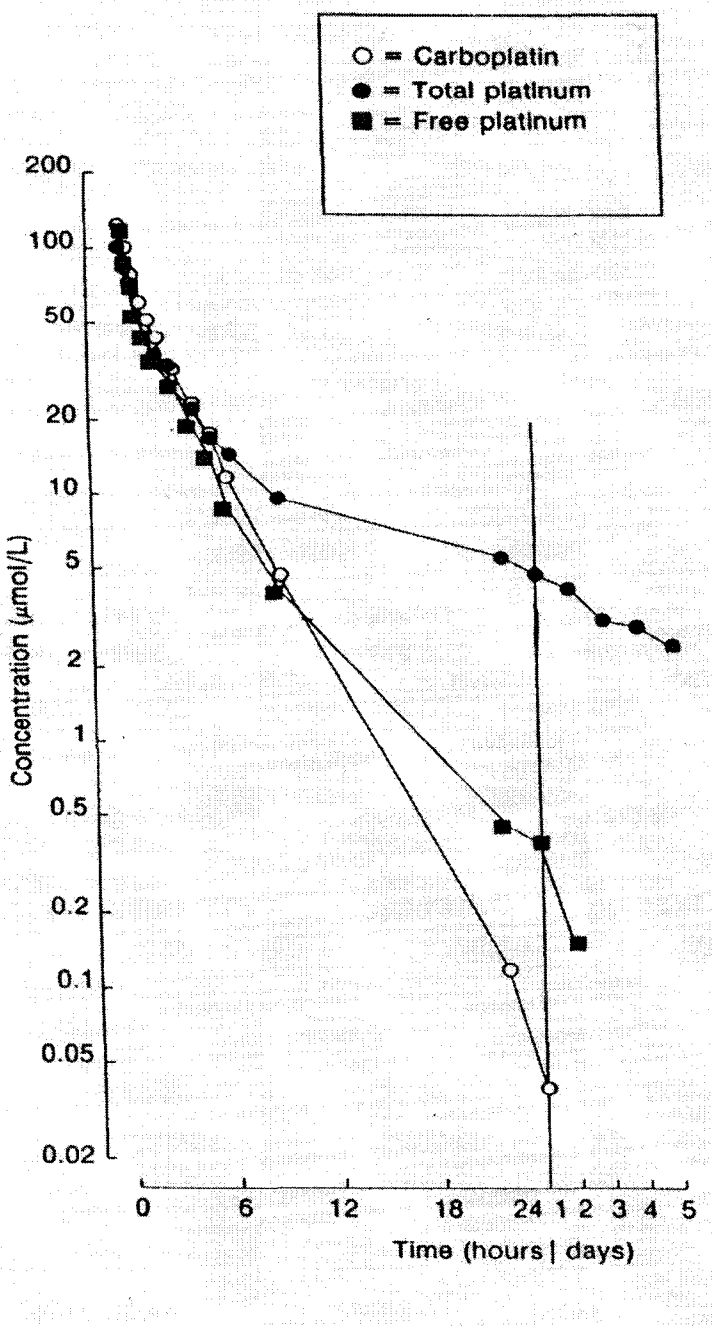


Figure 4: Concentration-time profiles of carboplatin, total platinum and free platinum. A carboplatin dose of 350 mg/m^2 was administered by IV infusion over 37 minutes (*Clin Pharmacokinet 21(4); 1991*).

of carboplatin is variable (20-200 ml/min) and correlates well with the patient's glomerular filtration rate (GFR). The total body clearances for free platinum and carboplatin are similar, while the total body clearance of total platinum is generally lower. This is attributed to irreversible protein binding. Elimination of carboplatin and free platinum follow monophasic elimination while total platinum follows biphasic elimination. This is due to the formation of both low and high molecular weight platinum-protein complexes. Macromolecules with low molecular weight will be excreted rapidly while high molecular weight complexes must first undergo enzymatic degradation. These complexes may have half-lives ranging from several days to several years [Lipp, 1991]. The second elimination phase can be seen in figure 4 when the x-axis switches from hours to days.

Carboplatin is metabolized slowly and to a small extent. This is evidenced by the high percentage of unchanged drug in the urine and the slight divergence of the concentration-time profiles of free platinum and carboplatin.

1.1.7 Dosing strategy for carboplatin

It has long been known that cancer patients demonstrate high pharmacokinetic variability due to impairment of renal, hepatic and biliary function. Due to these differences, individualization of drug therapy is often necessary, but unavailable. The sensitivity of tumor cells to chemotherapeutic agents is generally not known, so cancer drugs are given at a fixed dose without regard to an individual's needs [Masson and Zamboni, 1997]. The lack of therapeutic drug monitoring assays for anti-cancer agents makes it difficult to observe if these fixed doses are providing adequate, toxic or sub-

therapeutic drug levels to the patient. This uncertainty makes it clear that better, more specific methods are needed for determining dosing regimens.

It has been shown over the years that the correlation between effect and drug exposure is described most accurately by the extent of systemic exposure as opposed to absolute dose [Chatelut *et al.,* 1995]. In pharmacokinetics, the degree of systemic exposure is determined by measurement of the area under the concentration-time curve (AUC). AUC is defined as the ratio of the amount of drug that reaches the systemic circulation and the clearance of the drug: AUC = Dose/Cl_{total}. As such, AUC is one of the most important pharmacokinetic predictors of clinical response.

For a compound such as carboplatin which is cleared almost exclusively by filtration: $Cl_R \approx Cl_{total;}$ $Cl_{total} \approx$ GFR. Therefore, GFR may be substituted for $Cl_{total:}$

$$AUC = \frac{Dose}{GFR}$$

From this relationship, it can be seen that patients with an increased GFR will have decreased exposure and efficacy. Conversely, patients with a decreased GFR will have greater exposure and increased toxicity. This relationship was used by Calvert *et al.*, [1989] to determine a formula for AUC directed dosing of carboplatin. This formula is based on the concept of a target AUC, or an AUC that will yield maximum efficacy while minimizing toxicity. The target AUC was determined based upon a retrospective study of previously treated patients [Calvert *et al.*, 1989]. From this study the following target AUCs were determined:

Previously untreated patients: AUC = 7 mg/ml min
Previously treated patients: AUC = 5 mg/ml min
Patients on combination therapy: AUC = 4.5-5 mg/ml min

Solving for dose: dose = target AUC X GFR. The final formula proposed by Calvert:

DOSE (mg) = Target AUC (mg/ml min) X [GFR+25] ml/min

where 25 represents the percentage of drug that is not excreted in the urine within the first 24 hours [Calvert *et al.* 1989]. The Calvert formula is widely used today to determine the appropriate dose of carboplatin. When using this formula, it is important to remember that the total dose of carboplatin is calculated in mg and not mg/m^2 [Arkey, 1999].

1.1.8 Analysis of carboplatin

Carboplatin is present in the blood as two species: carboplatin and decarboxylated free platinum. This results from the *in vivo* metabolism of carboplatin to diamminoplatinum and a non-platinum carboxyl moiety. The platinum containing species is able to react with proteins irreversibly [Duffull and Robinson, 1997]. For accurate pharmacokinetic modeling three distinct species may be measured: carboplatin, total platinum and free or ultrafiltrable platinum [Van der Vijgh, 1991]. Ultrafiltrate may be prepared using a commercially available micropartition system. The most commonly used system is the Amicon MSP-1 system. For best results, the ultrafiltrate should be obtained immediately following the collection of plasma samples and should be stored at -20°C until analysis [Van Warmerdam *et al.*, 1995].

The methods used for analysis of carboplatin can be divided into two categories: selective methods for the analysis of carboplatin and non-selective methods for the analysis of elemental platinum. The most common method used for carboplatin determination is high performance liquid chromatography (HPLC). Methods used for the

determination of elemental platinum include atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) [De Waal *et al.,* 1990].

1.1.9 Inductively coupled plasma mass spectrometry

In this study we employed inductively coupled plasma mass spectrometry for the analysis of elemental platinum. ICP-MS is an analytical technique that uses an inductively coupled plasma source to dissociate a sample into its constituent atoms or ions, then uses a mass spectrometer to select, identify and quantitate these ions. This technique has several advantages over AAS that make it a more attractive option for elemental analysis. ICP-MS is able to carry
out rapid, multi-element analysis of all elements in the periodic table, as well as individual isotopes. In addition, it is capable of detecting and measuring concentrations of analytes at very low levels. Current instrumentation is able to measure concentrations down to one to ten parts per trillion (ppt) in solution [Taylor, 2001].

ICP-MS requires that the elements which are to be analyzed be in solution, preferable aqueous [Thomas, 2001a]. The analyte solution is introduced through a peristaltic pump to a nebulizer, which converts the liquid sample into an aerosol. This aerosol consists of droplets of varying size with a mean diameter range of 1-80 μm [Taylor, 2001]. The plasma, a highly energetic, inert ionized gas, is inefficient at dissociating large droplets, so the fine droplets of the aerosol are separated from the larger droplets using a spray chamber [Thomas, 2001a]. The fine aerosol then emerges from the exit tube of the spray chamber and is transported via a carrier gas to the plasma torch. Within the torch, the plasma is formed by the inductive coupling of a high

frequency energy to a gas flow of argon. The resulting ionized gas is at a temperature of 7,000-10,000 K. This high thermal energy results in the atomization and subsequent ionization of the sample. These ions are then sent to the mass spectrometer where they are detected, identified and quantitated. A schematic of the instrumental components of an ICP-MS is shown in figure 5.

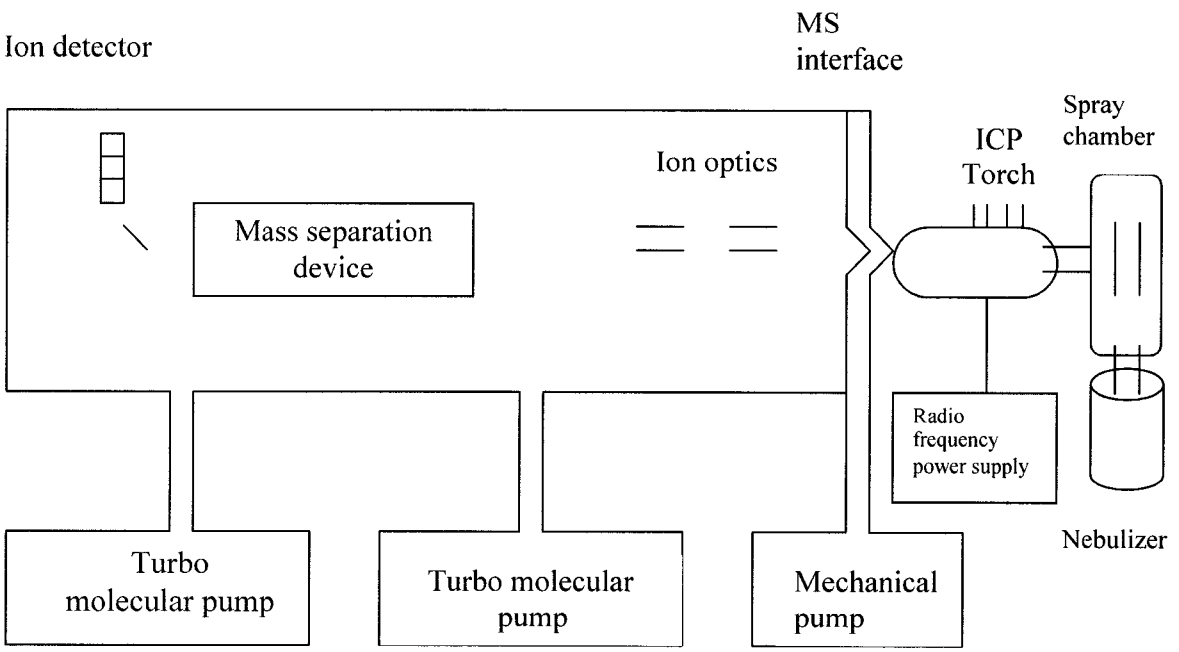


Figure 5: Schematic of ICP-MS (*Spectroscopy 16(6); 2001*)

1.2 Angiogenesis

1.2.1 Angiogenesis and tumor formation

Tumor formation commences with the mutation of a single cell. This mutation can be the result of one or more adverse events such as the absence of tumor suppressor genes, the inactivation of tumor suppressor genes or the activation of oncogenes [Rosen, 2000]. This single cell can grow to be a tumor one to two millimeters in size by using existing blood vessels to supply the nutrients and oxygen necessary for growth. Once the tumor reaches two millimeters in size, the pre-existing blood vessels are only able to provide adequate nutrients and oxygen to the outermost cells of the tumor. In order to prevent itself from becoming necrotic or apoptotic, the tumor must undergo neovascularization [Fong *et al.,* 1999]. This process of forming new blood cells is called angiogenesis and is an essential component in the growth of solid tumors. Generation of new capillaries provides the tumor with the nutrients it needs for proliferation, as well as a means for tumor cells to exit the primary tumor site and travel to other parts of the body to form metastases [Zetter, 1998]. Angiogenesis is mediated by the production of angiogenic factors including angiogenic enzymes, endothelial specific receptors, chemokines and growth factors [Liekens *et al.,* 2001].

In a series of papers published in the 1970's, Dr. Judah Folkman postulated that if the growth of a tumor is dependent upon the formation of new blood vessels, then the inhibition of angiogenesis should inhibit tumor expansion. The targets for this inhibition should be the angiogenic factors [Zetter, 1998]. In addition to reducing tumor expansion, inhibiting or decreasing the extent of angiogenesis that occurs in a given tumor should reduce the number of tumor cells that are able to enter the general circulation. As a

consequence, there should be a decrease in the number of metastases that occur [Folkman, 1972].

1.2.2 The process of angiogenesis

As mentioned, angiogenesis is the process of generating new capillaries from pre-existing blood vessels. This process plays a major role in the formation of the body's blood vessels during embryonic development. In adults, the proliferation rate of endothelial cells is very low compared with that of other cell types and angiogenesis virtually ceases. Exceptions include corpus luteum formation, wound healing, pregnancy and tumor growth and metastasis [Liekens *et al.,* 2001].

The process of new capillary formation begins with endothelial cells of existing blood vessels degrading the underlying basement membrane of neighboring tissue [Liekins *et al.*, 2001]. Matrix metalloproteinase enzymes produced by these endothelial cells break down the extracellular matrix (ECM). Once the ECM has been completely degraded, "leader" endothelial cells migrate into the stroma of the tissue. The leader cells are followed by "proliferating" endothelial cells which eventually organize into hollow tubes, thus creating a new network of blood vessels [Rosen, 2000]. Production of proliferating endothelial cells is stimulated by a variety of growth factors, many of whom are released by the degraded ECM. Three classes of growth factors have been identified: vascular endothelial growth factor (VEGF) and angiopoietins, which target endothelial cells; direct acting molecules such as angiogenic enzymes, cytokines and chemokines that activate a variety of target cells in addition to endothelial cells; and indirect acting molecules that stimulate angiogenesis by the release of direct acting factors from

macrophages, tumor cells or endothelial cells [Liekens *et al.,* 2001]. Of these factors, VEGF is most commonly linked with tumor neovascularization [Ellis *et al.,* 2000].

1.2.3 Vascular endothelial growth factor

The VEGF family consists of six known members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, orf virus VEGF (VEGF-E) and PlGF (placental growth factor). VEGF is expressed in many different tissues and in many different cell types. Two high affinity cognate receptors for VEGF have been identified on the vascular endothelium: VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR) [Liekens *et al.,* 2001]. VEGFR-2 is exclusively expressed in endothelial cells and plays a key role in endothelial cell differentiation [McMahon, 2000]. These receptors are cell surface receptor tyrosine kinases (RTKs). The VEGF RTKs belong to a larger family of RTKs which are all implicated in signal transduction pathways that impact cell proliferation, differentiation and migration. RTKs are single pass transmembrane receptors that transduce extracellular signals to intracellular responses [Heldin, 1996]. VEGF RTKs are characterized by an intracellular tyrosine kinase domain and seven immunoglobulin like domains extracellularly (figure 6). Binding of VEGF to two adjacent receptors forms an active dimer. This active dimer catalyzes the transfer of the gamma phosphate of ATP to tyrosine residues in the protein substrates. This protein tyrosine autophosphorylation is essential in the regulation of cell growth, shape, differentiation and migration [Heldin, 1996].

VEGF and its receptors have been implicated in angiogenesis that occurs in a variety of solid tumors such as breast, colon, bladder and endometrial cancer [Fong *et al.,*

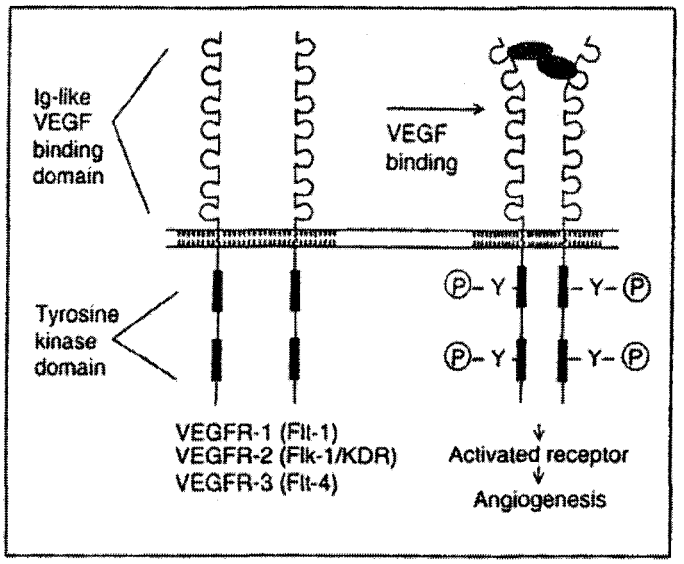


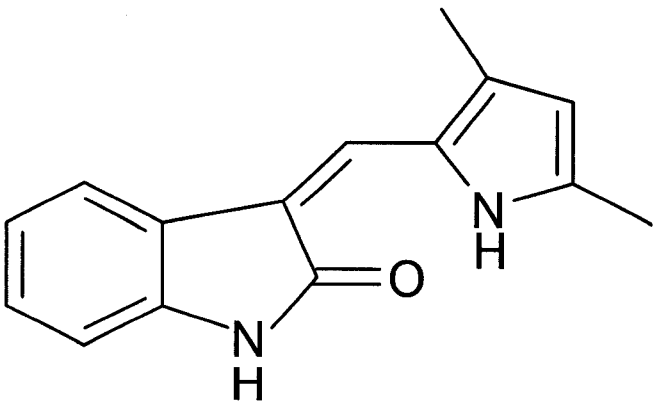
Figure 6: Structure of vascular endothelial growth factor tyrosine kinase receptors *(The Oncologist 5(suppl 1); 2000).*

1999]. Several strategies have been developed that target the VEGF pathway in an effort to inhibit tumor angiogenesis. SU5416, developed by Sugen Inc. is a selective and potent small molecule RTK inhibitor that blocks VEGF mediated receptor signaling [Mendel *et al.*, 2000].

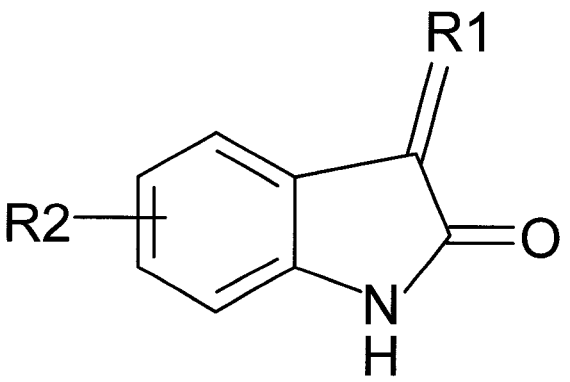
1.3 SU5416

1.3.1 Development of SU5416

The chemical name of SU5416 is 3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-indolin-2-one (figure 7a). This structure is a modification of the indolin-2-one core structure (figure 7b). This core structure was first recognized as an inhibitor for RTKs while investigating small molecule inhibitors of fibroblast growth factor (FGF) RTKs [Mohammadi *et al.*, 1997]. Elucidation of the crystallographic structure of the catalytic domain of FGF RTK revealed that the core of the indolin-2-one structure occupied the site normally bound to the adenine of ATP [Sun *et al.*, 1998]. This binding to the ATP site was shown to have an inhibitory effect on the FGF RTK. This knowledge of the competitive binding of the ATP site was then applied to finding an inhibitor of the VEGF RTK. A structural activity relationship (SAR) study was done around the indolin-2-one core with a variety of substitutions at the R_1 and R_2 positions. The results of this SAR showed many different degrees of specificity and potency with respect to inhibition of VEGF RTK. Biochemical analysis of these inhibitory properties identified several compounds with inhibition profiles that were worthy of further development. SU5416 was one of these compounds. It was identified as a highly potent and selective inhibitor of the VEGF RTK. While analysis was unable to determine why this was so, it is possible that the hydrophobicity of



(a)



(b)

Figure 7: a) Chemical structure of SU5416; b) indolin-2-one core structure

SU5416 may induce conformational changes in the binding site [Mendel *et al.,* 2000]. SU5416 was the first of these substituted indolin compounds to enter clinical development.

1.3.2 Pharmacokinetics and toxicity of SU5416

The pharmacokinetics and toxicity of SU5416 have been assessed in a phase I dose escalating trial. Sixty three subjects with advanced malignancies were given an infusion of SU5416 twice a week at thirteen dose levels ranging from 4.4-190 mg/m^2 /day [Rosen *et al.,* 1999]. SU5416 was shown to be well tolerated with phlebitis, headaches with nausea, change in urine color and increase in liver transaminases observed. No hematologic or organ toxicity was observed. The dose limiting toxicity was severe headache accompanied by projectile vomiting at 190 mg/m^2. This condition resolved within 24-48 hours and the patients continued at the maximum tolerated dose of 145 mg/m^2 [Cropp and Hannah, 2000]. Induction of metabolism was observed in all subjects and was attributed to pretreatment with dexamethasone, the parent drug or a metabolite. Two metabolites, the carboxylic and carboxymethyl biotransformations of SU5416 are formed. The pharmacokinetic parameters were estimated to be: volume of distribution in the central compartment 11.8 L; volume of distribution at the steady state 22 L and elimination half-life 55 minutes [Cropp *et al.,* 1999]. Clearance was estimated to be 52 L after eight infusions [Rosen *et al.* 1999].

SU5416 is primarily eliminated by hepatic metabolism. The cytochrome P450 enzymes CYP1A2 and CYP3A4, both inducible enzymes, have specifically been identified as participants in the metabolism of SU5416. CYP3A4 is the most abundant

isozyme in humans and is responsible for the metabolism of more than 60% of all marketed drugs. Several studies have indicated that SU5416 may induce the metabolism of concomitant medications that are also CYP substrates, among them paclitaxel [Rosen *et al.* 2000]. SU5416 is also thought to autoinduce its metabolism. The influence of SU5416 on renal function and the elimination of drugs primarily eliminated via the renal route has not been investigated.

1.4 Angiogenic inhibitors vs. standard therapies

Anti-angiogenic therapy offers two major advantages over standard chemotherapeutic regimens. The targets for standard chemotherapy agents are tumor cells which are genetically unstable and prone to mutations. This instability can often lead to drug resistance. Endothelial cells, on the other hand are genetically stable with spontaneous mutations rarely occurring. Therefore, resistance to anti-angiogenic compounds is less likely to occur [Liekens *et al.,* 2001]. In addition, the targeting of dividing endothelial cells as opposed to tumor and other proliferating cells will not produce toxicities commonly associated with cancer treatments such as myelosuppression and gastrointestinal discomfort [Rosen, 2000].

Despite these advantages, antiangiogenic compounds are not to be viewed as competition or replacement for standard therapies. These compounds are cytostatic in nature and are not powerful enough to challenge a large tumor cell burden. Therefore, this type of therapy is viewed as a potential partner for current cytotoxic compounds [Carter, 2000]. Together it is hoped these compounds will be more effective in

controlling tumor growth and metastases, thus optimizing survival chances for cancer patients.

2) SPECIFIC AIMS

From the previous discussion, it is clear that the development and use of angiogenic inhibitors offers a promising new approach for the treatment of cancer. Optimal treatment regimens may one day see the use of these compounds in conjunction with current therapies. One such potential combination could add SU5416 and/or a structurally similar analog to the already successful paclitaxel/carboplatin regimen. In phase I/II clinical trials, SU5416 was administered to study participants along with paclitaxel and carboplatin. As with any combination therapy regimen, it is important to determine whether any drug-drug interaction exists between SU5416 and co-administered compounds. Carboplatin is excreted predominately by the kidneys through glomerular filtration. As previously mentioned, the influence of SU5416 on renal function and on compounds that are eliminated renally has not been investigated. Therefore, the specific aim of this study was to assess the influence of SU5416 on the pharmacokinetics of carboplatin in subjects with advanced malignancies. For this purpose, serial blood samples were drawn from study participants on day 1 of two consecutive cycles. Carboplatin was administered on day 1 of each cycle; SU5416 on days 4, 8, 11, 15 and 18 of each cycle. Samples drawn during cycle 1 were drawn prior to any administration of SU5416 and thus contain carboplatin in the absence of SU5416. Samples drawn during cycle 2 contain carboplatin in the presence of SU5416. Samples from both cycles were processed and analyzed for platinum, a major component of carboplatin. A non-compartmental approach was employed to determine the pharmacokinetic parameters from the platinum data. Statistical analysis of the pharmacokinetic parameters and

comparison of pharmacokinetic parameters to historic values was then carried out to determine if the pharmacokinetics of carboplatin are significantly altered by SU5416.

3) MATERIALS AND METHODS

Carboplatin was purchased from the Sigma Chemical Company (St. Louis, MO USA). Rhodium 10 ppm standard was purchased from SPEX CertiPrep (Metuchen, NJ USA). Optima grade nitric acid was purchased from Fisher Scientific (Pittsburgh, PA USA). Argon gas used during analysis was supplied by the Department of Chemistry, University of Cincinnati (Cincinnati, OH USA).

3.1 Study design

This clinical trial was a single center, dose escalating Phase I/II study whose aim was to examine the pharmacokinetics and toxicity of SU5416 when administered intravenously in combination with paclitaxel and carboplatin. Patients were eligible to participate if 175 mg/m^2 paclitaxel every three weeks was acceptable therapy for their advanced malignancies. Malignancies included, but were not limited to AIDS related Kaposi's sarcoma, bladder, breast, head/neck and lung cancer. Patients had to be at least 18 years of age, had no significant liver or renal abnormalities, an absolute neutrophil count >1500/mm^3, hemoglobin >8.5 g/dl and a platelet count >80,000/mm^3 . Full recovery from any reversible effects of previous therapies was necessary before enrollment in this study. Subjects were ineligible to participate if they had experienced: any other investigational therapy within four weeks of the start of the study, surgery within six weeks of the start of the study or a myocardial infarction or a diagnosis of angina within six months of the start of the study. Subjects with a history of insulin dependent diabetes mellitus, non-insulin dependent diabetes with vascular disease or

diabetic ulcers or acute or chronic pancreatitis were excluded from participating. Patients chosen to participate in this study were not to receive any other chemotherapeutic agents, growth factors or immunotherapeutic agents while enrolled in the study. Patients with a known allergy to Cremophor® or a Karnofsky Performance Status <60% were not eligible to participate. The trial was conducted at UCLA's Jonsson Cancer Center, Los Angeles, California.

3.2 Dosing regimen and sample collection

15 subjects were administered a paclitaxel dose of 175 mg/m^2 or 225 mg/m^2 over three hours of day one of consecutive three week cycles. This was followed by a carboplatin dose calculated by the Calvert formula for a target AUC of 6 mg/ml min as a 30 minute infusion. All subjects received an infusion of SU5416 on days 4, 8, 11, 15 and 18 of each cycle. Four subjects received 85 mg/m^2 and eleven subjects received 145 mg/m^2 SU5416. For determination of carboplatin pharmacokinetics, serial blood samples were collected following the carboplatin infusion at the following times (hours): 0, 0.5, 1.0, 1.5, 3.5, 5.5, 8.5, and 20.5. Blood samples were centrifuged at 3500 rpm for 15 minutes to separate plasma from the cells. Ultrafiltrate samples were prepared using the Amicon MSP-1 micropartition system. Both plasma and ultrafiltrate samples were transferred to cyrovials labeled with patient's initials, collection date and time, cycle number and day number. Samples were sent frozen from the Jonsson Cancer Center to the University of Cincinnati College of Pharmacy and stored at -70°C until analysis.

3.3 Sample preparation

When preparing standards, quality control samples and patient samples for analysis, the assumption was made that the density of the solutions was 1gm/ml. The samples were therefore prepared by weight using Ohaus analytical plus balance, assuming 1gm = 1ml. This eliminated the need for volumetric glassware. Samples were prepared in 30 ml HDPE bottles (Nalgene).

3.3.1 Preparation of platinum standards:

A platinum working standard of 5.25 ppm was used to prepare all calibration standards and quality control samples. The working standard was prepared from a stock platinum solution of 525 ppm. The stock platinum solution was prepared using commercially purchased carboplatin (CBDCA) as a platinum source. For the preparation of the stock platinum solution of 525 ppm:

$$0.01\text{gm CBDCA} \times \frac{1\text{mole CBDCA}}{371.3\text{gm CBDCA}} \times \frac{1\text{mole Pt}}{1\text{mole CBDCA}} \times \frac{195\text{ gm Pt}}{1\text{ mole Pt}} = 0.00525\text{gm Pt}\ (5.25\text{mg Pt})$$

$$\text{For a 10 ml solution: } \frac{5.25\text{ mg Pt}}{10\text{ ml}} = \frac{0.525\text{ mg}}{\text{ml}} \times \frac{1000\ \mu\text{g}}{1\text{ mg}} = 525\text{ ppm}$$

For a 525 ppm stock platinum solution, 0.01gm of carboplatin was dissolved in 10 ml 2% nitric acid. For a working solution of 5.25 ppm platinum, 200 µl of stock platinum solution was diluted to a final volume of 20 ml with 2% nitric acid.

3.3.2 Preparation of the internal standard:

Although ICP-MS instrumentation is relatively stable, some instrument drift can occur. This drift can be corrected for by the use of an internal standard. The internal

standard will normalize all data to a non-analyte isotope that is present in all samples and standards in the same concentration. An ideal internal standard for ICP-MS analysis should be a mono-isotope species with a similar mass and ionization efficiency as the element it is being applied to, and should not have interferences in the sample matrix. Fairly uncommon elements are often used. Rhodium (Rh) is the most commonly used element because of its position in the mass range [Pyke, 2000], and was used in this analysis. A working Rh solution was prepared from a commercially purchased 10 ppm Rh standard. For a working solution of 1 ppm, 2 ml of the stock solution was diluted to a final volume of 20 ml using 2% nitric acid. The target concentration of internal standard in each standard, QC and patient sample was 10 ppb. 40 µl of Rh working solution was added to each 4 ml sample to achieve this desired concentration.

3.3.3 Preparation of the calibration curve

Day to day variations in instrument performance require that all appropriate standards be run each time the instrument was used. A calibration curve from 0-100 ppb platinum was constructed and run with each patient run. Preparation of the calibration standards is summarized in table I. Each calibrator was brought to a final volume of 4 ml with 2% nitric acid.

Prior to analyzing any patient samples, the absence/presence of matrix effects were evaluated by comparing plasma and ultrafiltrate curves to an aqueous calibration curve. Plasma, ultrafiltrate and ultrapure water were spiked at concentrations ranging from 0-160 ppb platinum. After ICP-MS analysis, calibration curves were constructed and

Calibrator(ppb)	Rh(µl)	Pt(µl)
0	40	0
10	40	7.6
25	40	19
50	40	38
100	40	76

Table I : Preparation of calibration standards

Time Point(hrs)	Rh (µl)	Sample(µl)	Dilution Factor
0	40	10	400
0.5	40	10	400
1	40	10	400
1.5	40	10	400
3.5	40	40	100
5.5	40	40	100
8.5	40	80	50
20.5	40	200	20

Table II: Preparation of patient samples

equations were generated. From comparison of the slopes of these equations it was determined that there were no matrix effects that would interfere with analysis and an aqueous calibration curve could be constructed for analysis along with patient samples.

3.3.4 Preparation of quality control samples

Three quality control specimens are run with each patient run. The concentrations of these specimens were 10 ppb, 50 ppb and 100 ppb. The QC samples are prepared following the procedure used for preparing the calibrators of the same concentrations (table I).

3.3.5 Preparation of the patient samples

All plasma samples were centrifuged for ten minutes prior to sampling for dilution. Table II summarizes the dilutions made for all patient samples. Adjustments were made on a case by case basis. Ultrafiltrate specimens were brought to a final volume of 4 ml with 2% nitric acid. Plasma specimens were brought to a final volume of 4 ml with ultrapure water to prevent precipitation of plasma proteins in the acid.

3.4 Analysis of specimens

Analysis was carried out using a modification of an unpublished method for elemental analysis. The settings for the Perkin-Elmer Sciex Elan 6000 were as follows: sample uptake flow rate, 1 ml/min; nebulizer gas flow, 0.82 L/min; ICP RF power, 1000 watts; number replicates per sample, 50; acquisition mode, peak hopping; detector mode,

pulse; wash solution, 2% nitric acid. Inter-day and within day variation associated with the platinum assay was less than 5%.

3.5 Data analysis

Raw data was obtained in the form of mean counts per second (n=50) signal intensity for Pt 194, 195, 196 and Rh. An example of the data output is shown in figure 8. The mean intensity of the signal was directly proportional to the concentration of the element in the sample. A calibration curve was constructed by plotting [(signal Pt 195/signal Rh) X Rh concentration (ppb)] vs. Pt 195 concentration (ppb) of the standards (figure 9). Least squares regression produced an equation which was used to calculate the concentration of elemental platinum in the QC and patient samples.

3.6 Pharmacokinetic analysis

Platinum pharmacokinetics were determined using WinNonlin computer software, version 3.1 (Pharsight Corp., MountainView, CA USA). A non-compartmental approach was employed. Using this approach, the elimination rate constant, λ_z, was determined graphically from the slope of the terminal phase of the concentration-time curve for each subject. Ideally, a minimum of three points from the terminal phase was used for this determination. The software performed regression analysis and computed an adjusted correlation coefficient (R^2) for each regression. Goodness of fit of the regression curve was based on a number of diagnostic criteria which included visual inspection of the fit, correlation coefficient and residual analysis. λ_z was determined from the slope of this regression curve. The terminal half-life in hours was then calculated from the relationship $t_{1/2} = 0.693/\lambda_z$. The total area under the curve (AUC) was determined by the addition of

$AUC_{0\to t}$ and $AUC_{t\to\infty}$. $AUC_{0\to t}$ was calculated by the trapezoidal rule using the observed data points:

$$AUC_{0\to t} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2} (C_i + C_{i+1})$$

$AUC_{t\to\infty}$ was determined by the factor C_{last} / λ_z, where C_{last} is the last measurable non-zero concentration and λ_z is the previously determined elimination rate constant. Units for AUC are mg/ml min. Total clearance was calculated from the relationship Cl = (dose/AUC) X F. Since F=1 for an IV infusion, this relationship simplified to Cl = dose/AUC. Units for clearance are ml/min. Once clearance was calculated, that value was used to determine the volume of distribution using the equation $Vd=Cl / \lambda_z$. Volume of distribution is given in liters.

3.7 Statistical analysis

Statistical analysis was conducted using SAS statistical software, version 7.0 (SAS Institute, Cary, NC USA). Pharmacokinetic parameters for carboplatin in the absence and presence of SU5416 were compared by one way analysis of variance (ANOVA). Statistical significance between cycles was assumed if $p<0.05$.

Quantitative Analysis – Summary Report

Sample Id: 100PPB QC 04-06-01
Sample Date/Time: Friday, April 06, 2001 16:36:25
Sample Description:
Solution type: Sample
Blank File: c\elandata\Dataset\default\Blank.063
Number of Replicates: 50
Peak Processing Mode: Average
Dual Detector Mode: Pulse
Current Dead Time (ns): 55
Acq. Dead Time (ns): 55
Cumulative Autodilution Factor: 1

Sample File:
Method File: c:\elandata\Method\Pt-Rh.mth
Dataset File: c:\elandata\Dataset\default\100PPB QC 04/06/01.640
Tuning file: default.tun
Optimization file: default.dac
Calibration File: c:\elandata\System\pt1.cal
Calibration Type: External Calibration

Summary

Intensities

Analyte	Mass	Meas. Intens. Mean	Meas. Intens. SD	Blank Intensity	Blank Intens. SD
Pt	194	540261.87	45134.6142	250.8061	53.9045
Pt	195	561464.47	51054.0489	242.0057	43.0488
Pt	196	410023.28	36842.8791	176.6749	50.1069
Rh	103	156748.51	15348.3768	184051.3656	15216.6690

Figure 8: Data output from ICP-MS

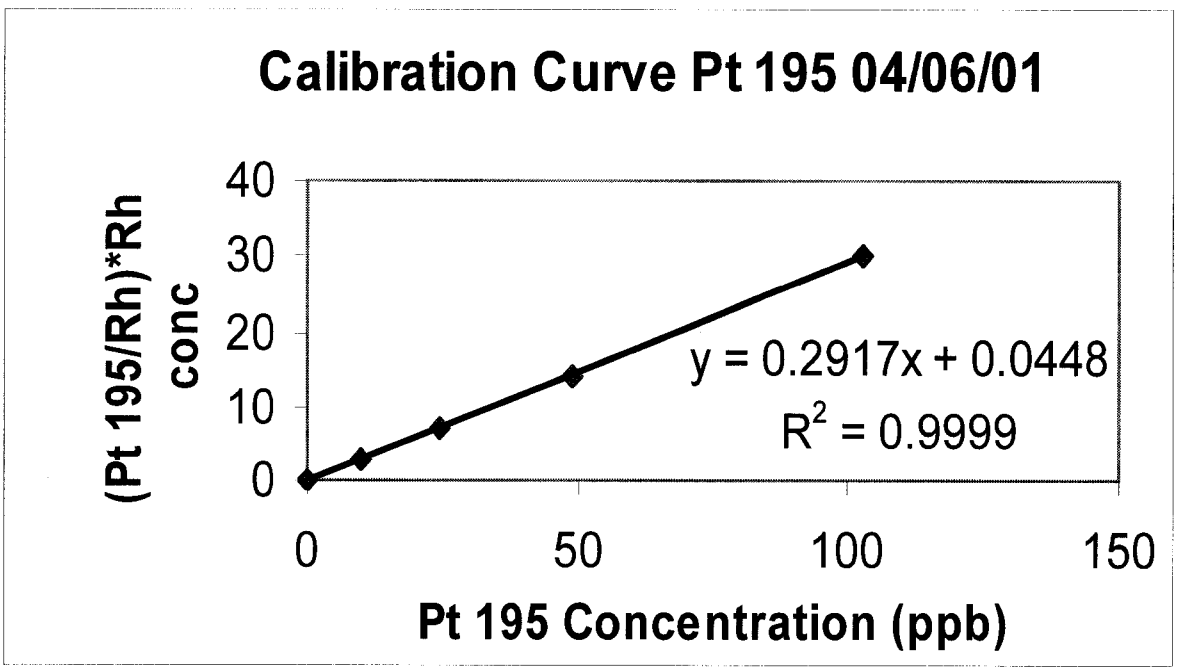


Figure 9: Platinum 195 calibration curve

4) RESULTS

The purpose of this study was to determine if any statistical and/or observable difference exists in the pharmacokinetics of carboplatin in the absence and presence of SU5416, a novel anti-angiogenic inhibitor. To make this determination, serial blood samples were collected from fifteen subjects on day 1 during two consecutive chemotherapy cycles. Plasma and ultrafiltrate samples were analyzed for elemental platinum using ICP-MS and free and total platinum concentrations were calculated. These concentrations were then used to determine AUC, Cl, $t_{1/2}$ and Vd.

The determination of the pharmacokinetic parameters was carried out using a non-compartmental approach. With this approach, the elimination rate constant, λ_z was determined graphically from the slope of the terminal phase of the concentration-time curve for each subject. Ideally, at least three time points should be used for this determination. For example, figure 10 shows the concentration-time profile for *total* platinum for one subject that was used for the determination of λ_z. Figure 11 shows the determination of λ_z from the concentration-time profile for *free* platinum for the same subject. The pharmacokinetic software WinNonlin performed regression analysis using the data points from the terminal phase to compute a correlation coefficient (R^2). Estimation of λ_z was then made based on the regression with the largest R^2 value. The correlation coefficient for *total* platinum was calculated to be 0.9928. For *free* platinum, the correlation coefficient was calculated to be 0.9984. Visual inspection of both figures shows that all points from the terminal phase are included in the regression. Good correlation is seen between the observed and predicted values. Had there not been a good

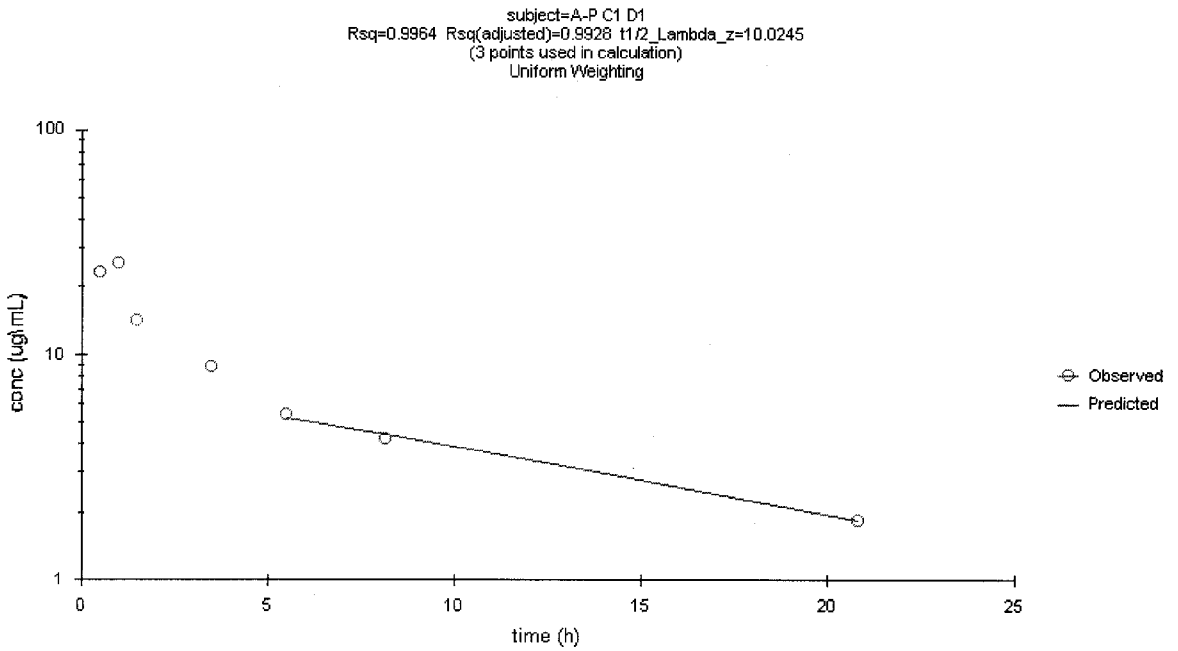


Figure 10: Determination of λ_z for total platinum, cycle 1 for one subject. Pharmacokinetic parameters for this subject were determined to be: AUC 3.66 mg/ml min, Cl 64.06 ml/min, $t_{1/2}$ 9.95 hours and Vd 55.20 liters.

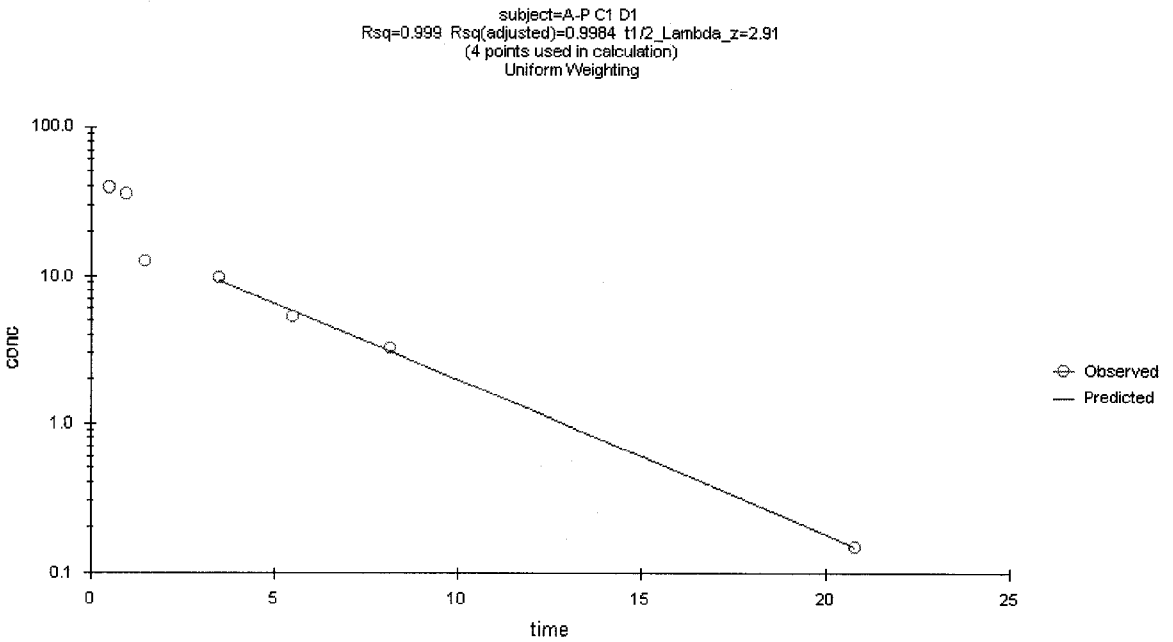


Figure 11: Determination of λz for free platinum, cycle 1 for one subject. Pharmacokinetic parameters for this subject were determined to be: AUC 3.47 mg/ml min, Cl 82.36 ml/min, $t_{1/2}$ 2.92 hours and Vd 20.82 liters.

correlation, a weighing scheme of 1/y or $1/y^2$ (where y is the observed plasma or ultrafiltrate concentration) could have been used to improve the "goodness of fit".

All subjects received carboplatin on day 1 and SU5416 on days 4, 8, 11, 15 and 18 of each three week cycle. A dose of 85 mg/m^2 SU5416 was given to 4 subjects, while 11 subjects received a dose of 145 mg/m^2. Blood samples taken during cycle 1 were drawn prior to the administration of any SU5416 and contain carboplatin in the absence of SU5416. Samples drawn during cycle 2 contain carboplatin in the presence of SU5416. Figure 12 shows a representative concentration-time profile for one subject. Curves for both free and total platinum for cycle 1 and cycle 2 are shown. Based on visual observation, is it clear that no difference exists between the two cycles. This correlation between cycles suggests that perhaps SU5416 does not alter the pharmacokinetics of carboplatin. This correlation between cycles should be observed in all subjects if this is the case. In addition to analyzing for inter-cycle variability, the data can be analyzed to assess whether the dose of SU5416 has any influence on the pharmacokinetics of carboplatin. LOW DOSE SU5416: The concentration-time profiles for *total* platinum for all subjects that received 85 mg/m^2 are shown in figure 13. The concentration-time profiles for *free* platinum are shown in figure 14. Both cycles are represented in both figures. No observational differences are apparent in either curve. The calculated mean pharmacokinetic parameters for *total* platinum of subjects who received 85 mg/m^2 SU5416 are summarized in table III. For cycle 1, these values were determined to be: AUC, 3.40 mg/ml min; Cl, 85.11 ml/min; half-life, 11.72 hours and Vd 89.45 liters. These values for cycle 2 were determined to be: AUC, 3.66 mg/ml min; Cl, 95.78 ml/min; half-life, 11.88 hours and Vd, 97.10 liters. The *free* platinum calculated mean

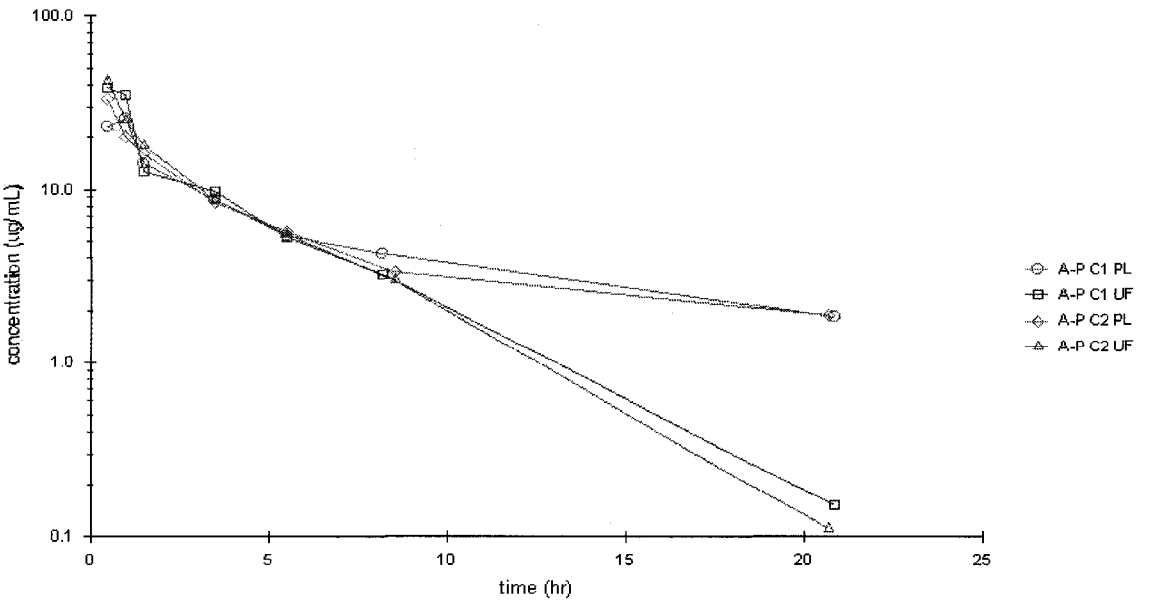


Figure 12: Representative concentration-time profile for one subject. Carboplatin dose for cycle 1 was 542 mg; carboplatin dose for cycle 2 was 636 mg; SU5416 dose was 85 mg/m^2.

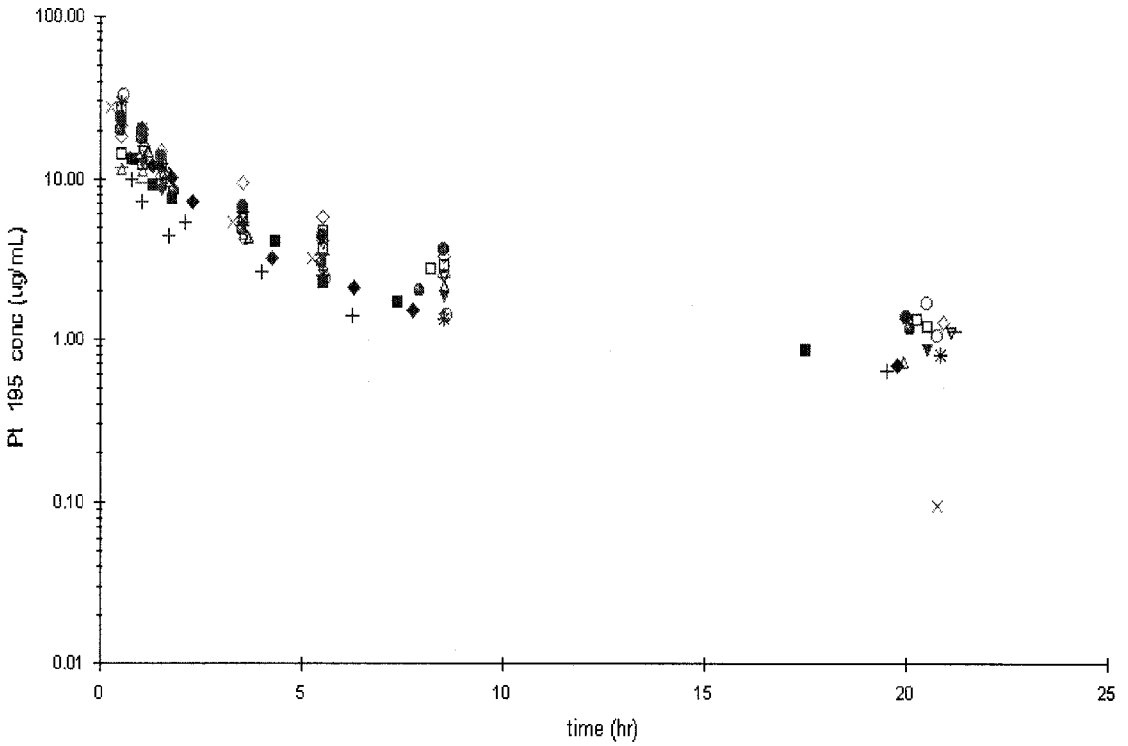


Figure 13: Concentration-time profile of total platinum in the absence and presence of 85 mg/m^2 SU5416. Cycle 1 is shown in blue; cycle 2 is shown in red. Carboplatin dose range for cycle 1 was 526-1064 mg; carboplatin dose range for cycle 2 was 636-11195 mg.

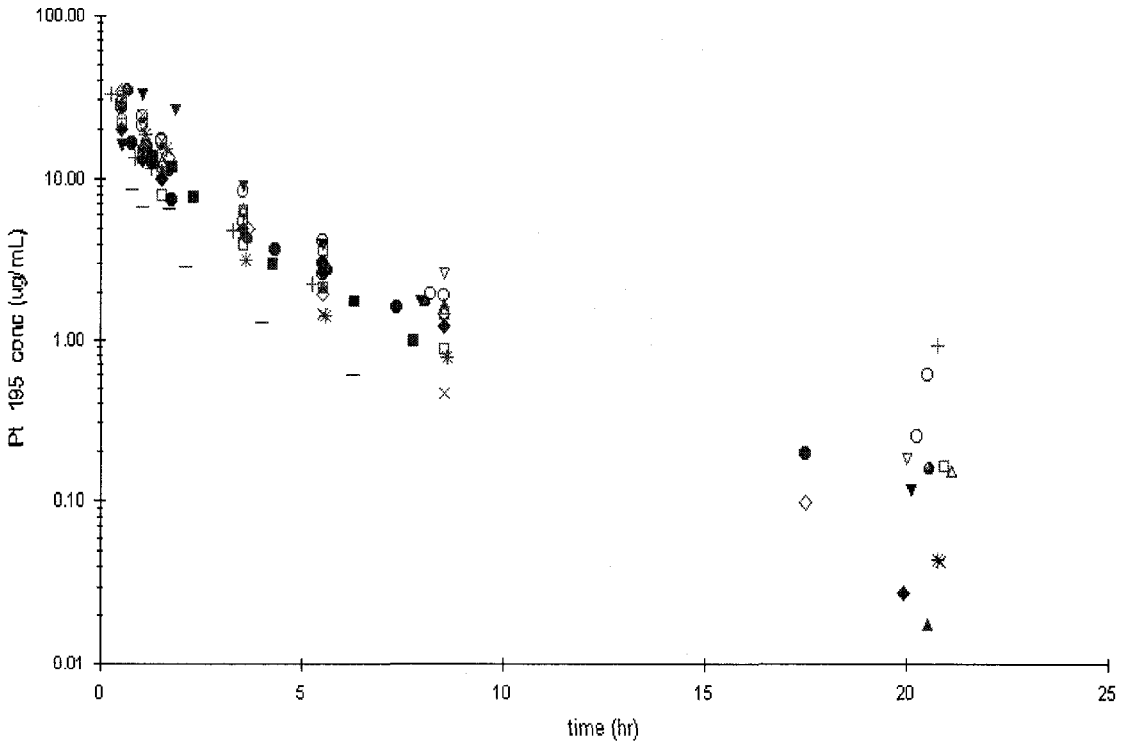


Figure 14: Concentration-time profile of free platinum in the absence and presence of 85 mg/m^2 SU5416. Carboplatin dose range for cycle 1 was 526-1064 mg; carboplatin dose range for cycle 2 was 636-1195 mg. Cycle 1 is shown is blue; cycle 2 is shown in red.

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	3.40 ± 0.67	3.66 ± 0.92	N/A	0.6582
Clearance (ml/min)	85.11 ± 34.17	95.78 ± 29.85	64.6 ± 27.90	0.632
Half-life (hours)	11.72 ± 2.31	11.88 ± 2.49	8.10 ± 7.20	0.9267
Vd (liters)	89.45 ± 45.95	97.10 ± 35.34	69.00 ± 38.50	0.785

Table III: Mean pharmacokinetic parameters of total platinum in the absence and presence of 85 mg/m^2 SU5416 (n = 4)

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	2.89 ± 0.64	3.72 ± 1.02	N/A	0.2076
Clearance (ml/min)	119.43 ± 59.95	116.24 ± 32.49	112.80 ± 36.30	0.9211
Half-life (hours)	5.50 ± 4.88	3.68 ± 1.20	3.11 ± 7.25	0.443
Vd (liters)	53.66 ± 45.78	36.38 ± 14.89	22.10 ± 6.70	0.4475

Table IV: Mean pharmacokinetic parameters of free platinum in the absence and presence of 85 mg/m^2 SU5416 (n = 4)

pharmacokinetic parameters are summarized in table IV. These values for cycle 1 were determined to be: AUC, 2.89 mg/ml min; Cl, 119.43 ml/min; half-life, 5.50 hours and Vd 53.66 liters. For cycle 2, these values were determined to be: AUC, 3.72 mg/ml min; Cl, 116.24 ml/min; half-life, 3.68 hours and Vd, 36.38 liters. Also given in tables III and IV are the p values obtained by statistical analysis of the pharmacokinetic parameters. Significant differences between cycles were assumed if the p value was <0.05. The p values for *total* platinum were calculated to be: 0.6582 for AUC; 0.6320 for Cl; 0.9267 for half-life and 0.7850 for Vd. For *free* platinum, the p values were calculated to be: 0.2076 for AUC; 0.9211 for Cl; 0.4430 for half-life and 0.4475 for Vd. Based on the p values for both total and free platinum, it can be concluded that a dose of 85 mg/m^2 SU5416 does not significantly alter the pharmacokinetics of carboplatin.

HIGH DOSE SU5416: The concentration-time profiles for *total* platinum for all subjects that received 145 mg/m^2 SU5416 in cycles 1 and 2 are shown in figure 15. The corresponding concentration-time profiles for *free* platinum are shown in figure 16. Once again, visual inspection of the figures yields no observable differences between the two cycles. The calculated mean pharmacokinetic parameters for *total* platinum are summarized in table V. For cycle 1, these values were determined to be: AUC, 3.78 mg/ml min; Cl, 84.79 ml/min; half-life, 8.48 hours and Vd 61.54 liters. For cycle 2, these values were determined to be: AUC, 4.77 mg/ml min; Cl, 78.93 ml/min; half-life, 8.89 hours and Vd 54.40 liters. Table VI summarizes the mean calculated pharmacokinetic parameters for *free* platinum. For cycle 1, these values were determined to be: AUC, 3.90 mg/ml min; Cl, 114.02 ml/min; half-life, 3.25 hours and Vd, 27.74 liters. For cycle 2, these values were determined to be: AUC, 3.72 mg/ml min; Cl, 112.91 ml/min; half-life,

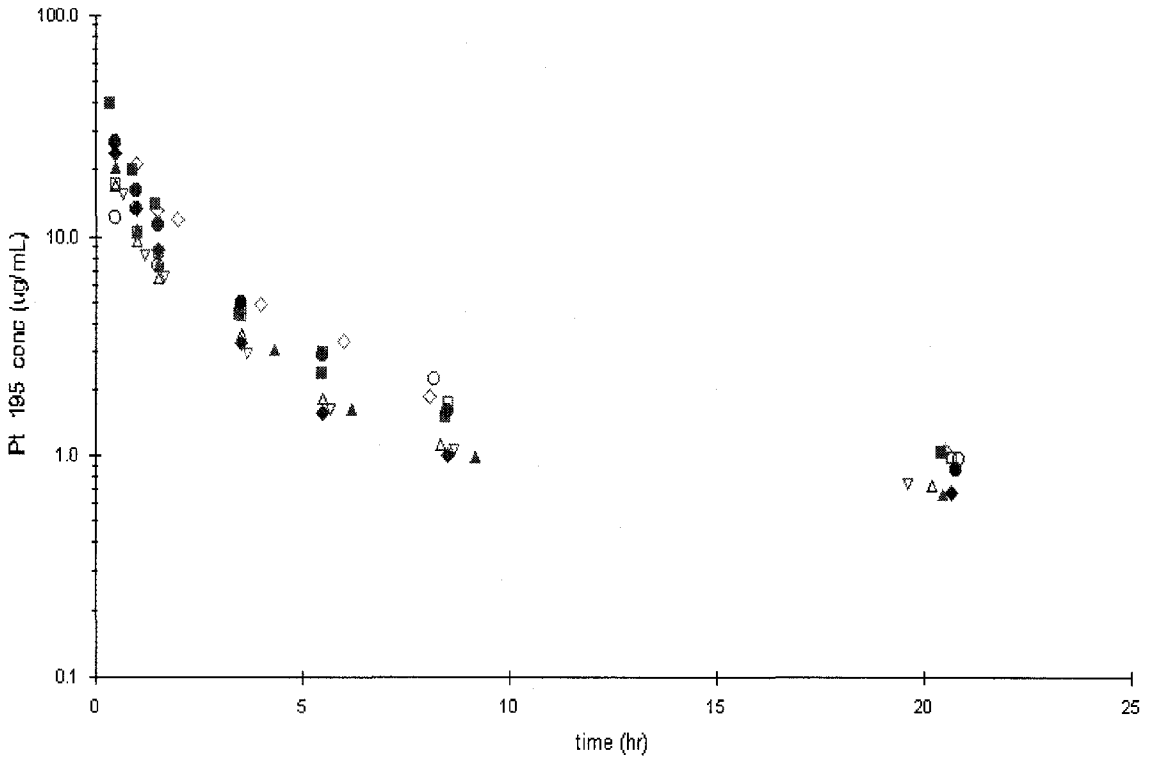


Figure 15: Concentration-time profile of total platinum in the absence and presence of 145 mg/m^2 SU5416. Cycle 1 is shown in blue; cycle 2 is shown in red. Carboplatin dose range for cycle 1 was 294-1064 mg; carboplatin dose range for cycle 2 was 445-1000 mg.

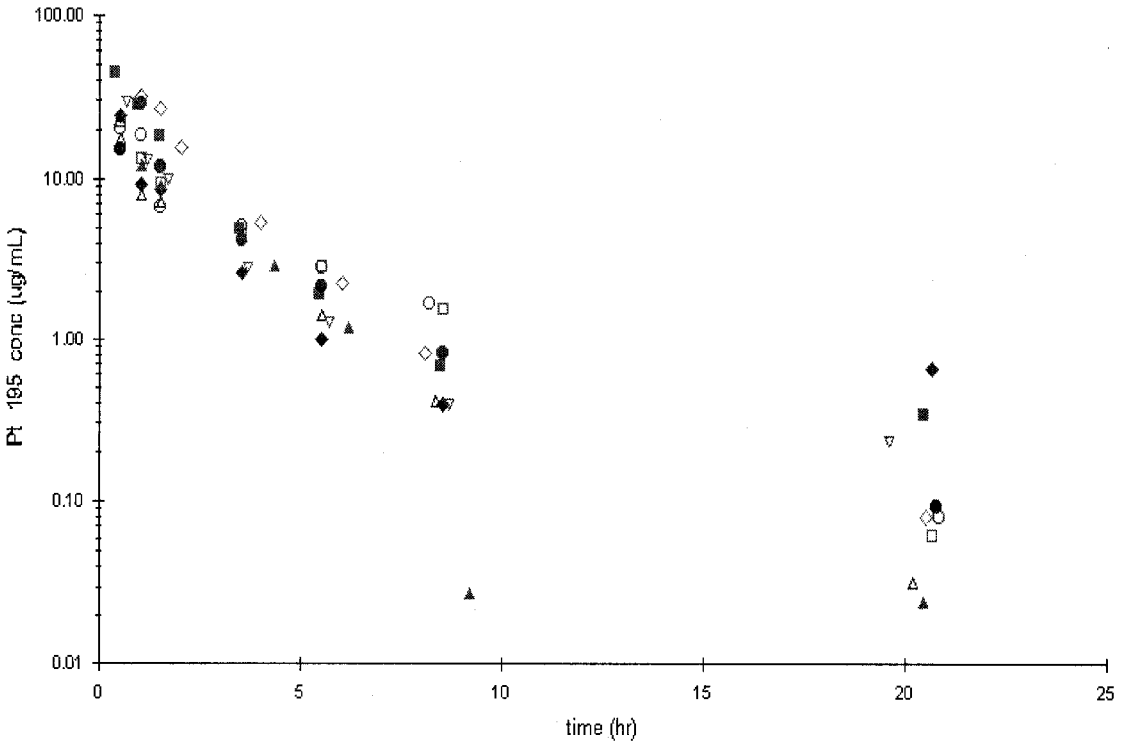


Figure 16: Concentration-time profile for free platinum in the absence and presence of 145 mg/m^2 SU5416. Carboplatin dose range for cycle 1 was 294-1064 mg; carboplatin dose range for cycle 2 was 445-1000 mg. Cycle 1 is shown in blue; cycle 2 is shown in red.

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	3.78 ± 0.90	4.77 ± 0.92	N/A	0.182
Clearance (ml/min)	84.79 ± 35.77	78.93 ± 29.34	64.60 ± 27.90	0.6199
Half-life (hours)	8.48 ± 1.61	8.89 ± 3.54	8.10 ± 7.20	0.747
Vd (liters)	61.54 ± 26.02	54.40 ± 31.12	69.00 ± 38.50	0.5521

Table V: Mean pharmacokinetic parameters of total platinum in the absence and presence of 145 mg/m^2 SU5416 (n = 11)

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	3.90 ± 1.34	3.72 ± 0.79	N/A	0.7524
Clearance (ml/min)	114.02 ± 91.53	112.91 ± 47.18	112.80 ± 36.30	0.9767
Half-life (hours)	3.25 ± 1.43	2.90 ± 0.58	3.11 ± 1.25	0.5535
Vd (liters)	27.74 ± 15.20	27.12 ± 9.43	22.1 ± 6.7	0.9247

Table VI: Mean pharmacokinetic parameters of free platinum in the absence and presence of 145 mg/m^2 SU5416 (n = 11)

2.90 hours and Vd, 27.12 liters. Statistical analysis calculated p values for *total* platinum as: 0.1820 for AUC; 0.6199 for Cl; 0.7470 for half-life and 0.5521 for Vd. The p values for *free* platinum were calculated as: 0.7524 for AUC; 0.9767 for Cl, 0.5535 for half-life and 0.9247 for Vd. Based on these p values, it can be concluded that the pharmacokinetics of carboplatin are not significantly altered at a higher dose of SU5416.

POOLED DATA: The data for all 15 subjects was analyzed to determine whether any differences exist between cycles. The concentration-time profiles for *total* platinum in the absence and presence of SU5416 for all 15 subjects are shown in figure 17. The concentration-time profiles for *free* platinum are shown in figure 18. In both figures, minor differences are observed from subject to subject but overall, no major observable differences can be noted. The calculated mean pharmacokinetic parameters for *total* platinum are summarized in Table VII. For cycle 1, these values were determined to be: AUC, 3.68 mg/ml min; Cl, 84.87 ml/min; half-life, 9.34 hours and Vd, 68.98 liters. For cycle 2, these values were determined to be: AUC, 4.31 mg/ml min; Cl, 82.43 ml/min; half-life, 10.18 hours and Vd, 72.19 liters. Table VIII summarizes the mean calculated pharmacokinetic parameters for *free* platinum. For cycle 1, these values were determined to be: AUC, 3.62 mg/ml min; Cl, 115.46 ml/min; half-life, 3.85 hours and Vd 34.65 liters. The calculated pharmacokinetic parameters for cycle 2 were determined to be: AUC, 3.72 mg/ml min; Cl, 114.29 ml/min; half-life, 3.23 hours and Vd, 30.98 liters. For *total* platinum, the p values were: 0.0975 for AUC; 0.8447 for Cl; 0.4610 for half-life and 0.8173 for Vd. The p values for *free* platinum were: 0.8254 for AUC; 0.9645 for Cl; 0.4650 for half-life and 0.6718 for Vd. Based on these p values, it can be concluded that no significant inter-cycle differences exist in any of the measured parameters. The

carboplatin pharmacokinetic parameters, with and without SU5416 present determined in our studies were compared to historical data. These values have been included in tables III-VIII. For some of these studies the number of subjects included in the pharmacokinetic analysis and the standard deviation associated with each parameter were not given, which makes it difficult for us to compare data with those previously published. However, it is apparent that our values are not different than those reported earlier. This observation not only validates the analytical and pharmacokinetic approached used in this study, but more importantly, underscores the conclusion the SU5416 does not influence carboplatin disposition.

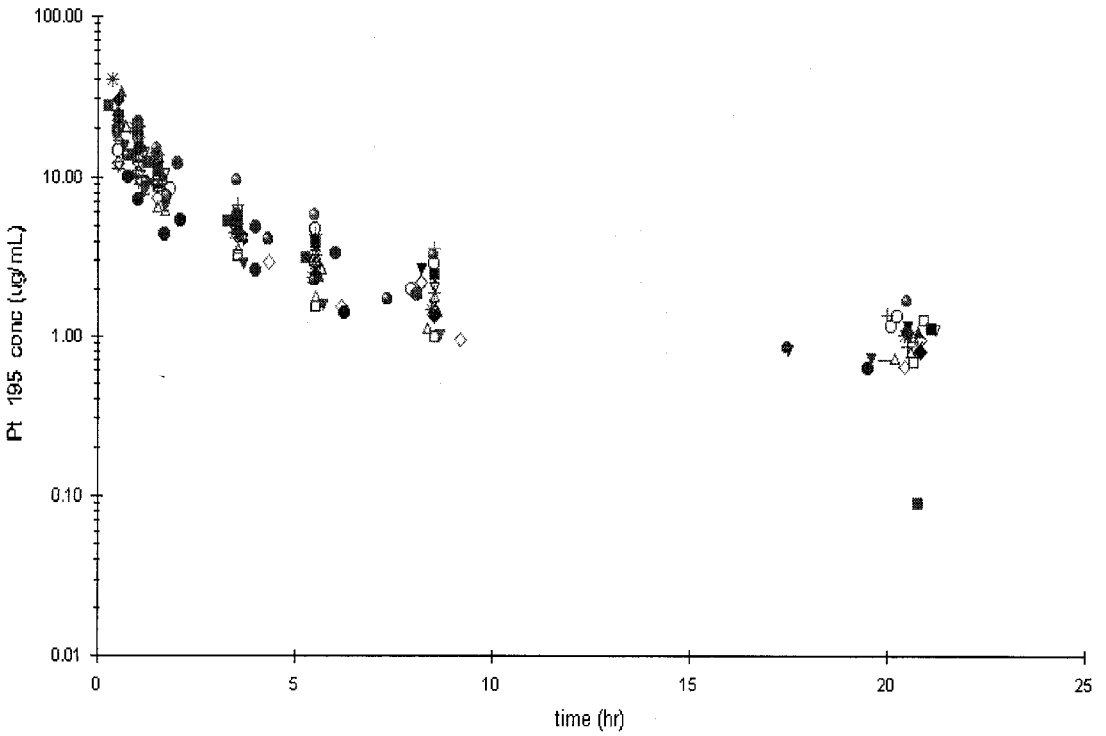


Figure 17: Concentration-time profile of total platinum in the absence and presence of SU5416 for all subjects. Cycle 1 is shown in blue; cycle 2 is shown in red. Carboplatin dose range for cycle 1 was 294-1064 mg; carboplatin dose range for cycle 2 was 445-1000 mg; SU5416 dose was 85 or 145 mg/m^2.

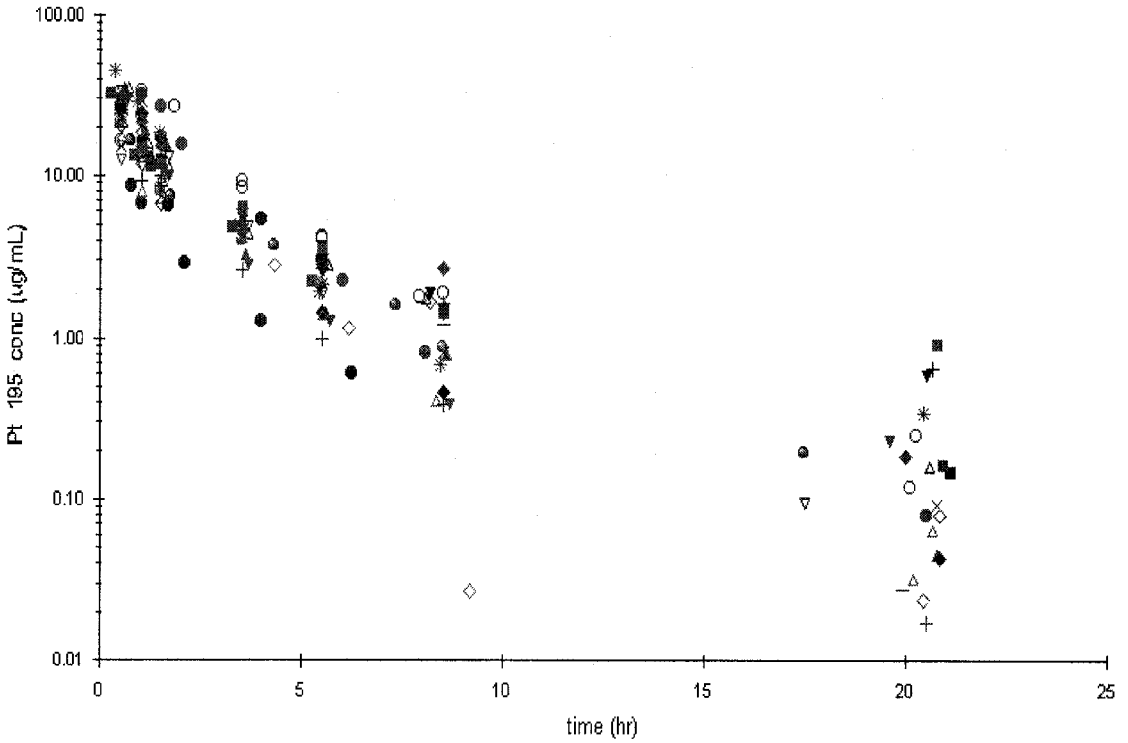


Figure 18: Concentration time-profile of free platinum in the absence and presence of SU5416 for all subjects. Cycle 1 is shown in blue; cycle 2 is shown in red. Carboplatin dose range for cycle 1 was 294-1064 mg; carboplatin dose range for cycle 2 was 447-1195mg; SU5416 dose was 85 or 145 mg/m^2.

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	3.68 ± 0.84	4.31 ± 1.05	N/A	0.0975
Clearance (ml/min)	84.87 ± 34.12	82.43 ± 28.84	64.6 ± 27.90	0.8447
Half-life (hours)	9.34 ± 2.28	10.18 ± 3.52	8.10 ± 7.2	0.4610
Vd (liters)	68.98 ± 33.15	72.19 ± 38.29	69.00 ± 38.50	0.8173

Table VII: Mean pharmacokinetic parameters of total platinum in the absence and presence SU5416 for all subjects (n = 15).

	Cycle #1	Cycle #2	Carboplatin and paclitaxel, published values	p value
AUC (mg/ml min)	3.62 ± 1.29	3.72 ± 0.85	3.22 ± 0.76	0.8254
Clearance (ml/min)	115.46 ± 82.22	114.29 ± 40.01	112.80 ± 36.30	0.9645
Half-life (hours)	3.85 ± 2.76	3.23 ± 0.93	3.11 ± 1.25	0.4650
Vd (liters)	34.65 ± 27.47	30.98 ± 12.32	22.10 ± 6.70	0.6718

Table VIII: Mean pharmacokinetic parameters of free platinum in the absence and presence of SU5416 for all subjects (n =15)

5) DISCUSSION

Angiogenesis, the formation of new blood capillaries from preexisting vessels, is thought to be essential for tumor growth and the formation of metastases. Inhibition of this process may stall tumor growth and ultimately contribute to the successful treatment of a variety of malignancies. Several proangiogenic growth factors and their receptors have been identified and have become the focus of anti-angiogenic research. Inhibitors of these growth factors are being identified and evaluated by way of molecular modeling and structure based drug design. One growth factor of particular interest is vascular endothelial growth factor, or VEGF. VEGF and its receptors have been implicated in the angiogenesis associated with a variety of solid tumors. Several strategies have been developed that target the VEGF pathway in an effort to inhibit tumor angiogenesis. SU5416, developed by SUGEN Inc. was one of the first of these compounds to enter clinical trials. Since SU5416 and related compounds are potential partners for current chemotherapeutic agents, it is necessary to evaluate the safety and efficacy of these compounds in combination therapy. To date, only a few studies have assessed the influence of SU5416 on drugs cleared by the hepatic route, since SU5416 was shown to induce the drug metabolizing enzymes CYP1A2 and CYP3A4. The influence of SU5416 on drugs cleared via the renal route in humans is not known. During pre-clinical studies in rats, high doses of SU5416 resulted in changes in the kidneys that were attributed to either SU5416 or a component of its vehicle. SU5416 is dosed almost on a daily basis - sometimes at the maximum tolerated dose - over the course of a chemotherapy cycle. Since several drugs such as the platinum anti-cancer complexes are cleared primarily by

the kidneys, it is important to assess the potential influence of SU5416 on these compounds. To this end, we undertook this study.

In phase I/II clinical trials, SU5416 was administered to study participants in conjunction with two well known and well tolerated compounds, paclitaxel and carboplatin in an effort to assess the influence of SU5416 on the pharmacokinetics of carboplatin. A three hour of infusion of paclitaxel was followed by a 30 minute infusion of carboplatin on day 1 of consecutive three week cycles. All subjects received SU5416 on days 4, 8, 11, 15 and 18 of each cycle. Serial blood samples were drawn from study participants on day 1 of two consecutive cycles. Samples drawn during cycle 1 were drawn prior to administration of SU5416 and contain carboplatin in the absence of SU5416. Samples drawn during cycle 2 contain carboplatin in the presence of SU5416. Samples from both cycles were processed and analyzed for platinum using ICP-MS. Concentrations of total and free platinum were calculated and were used to determine mean AUC, Cl, half-life and Vd values for each cycle. The pharmacokinetics of carboplatin in the absence and presence of paclitaxel were reported by Obasaju *et al.*, [1996]. It was concluded that paclitaxel does not alter the pharmacokinetics of carboplatin. Therefore, when paclitaxel, carboplatin and SU5416 are administered together, any inter cycle variation in the carboplatin pharmacokinetics can therefore be attributed to the presence of SU5416.

Mean AUC values obtained for both free and total platinum are similar and demonstrate no significant inter-cycle variability. Instrumentation and methodology available for this study allowed for the determination of elemental platinum instead of the parent compound. For consistency, the pharmacokinetic parameters were calculated

based on the concentrations obtained for elemental platinum. Platinum doses were calculated from the carboplatin doses and were used in the pharmacokinetic calculations. Therefore, the calculated mean AUC values shown in tables III through VIII are those of elemental platinum. However, the Calvert formula was used to calculate a carboplatin dose that would achieve a target AUC of 6 mg/ml min. In order to assess if this target was being achieved, platinum AUCs need to be converted to carboplatin AUCs. For the low dose SU5416 group, this conversion yielded a mean carboplatin AUC for total platinum of 6.47 mg/ml min for cycle 1 and 6.96 mg/ml min for cycle 2. For free platinum, the mean carboplatin AUC for cycle 1 was 5.50 mg/ml min and 7.00 mg/ml min for cycle 2. Carboplatin AUCs for the low dose group are all fairly close to the target AUC. For the high dose SU5416 group, the mean carboplatin AUCs for total platinum were 7.19 mg/ml min for cycle 1 and 9.00 mg/ml min for cycle 2. Mean carboplatin AUCs for free platinum were 7.42 mg/ml min for cycle 1 and 7.08 mg/ml min for cycle 2. All of the mean carboplatin AUCs for the high dose group exceed the target AUC. There are several possible explanations as to why this is so. As mentioned previously, the instrumentation available for this study allowed for the determination of elemental platinum. Measurement of elemental platinum does not allow for differentiation between carboplatin and its biotransformation products. Thus, it is possible that the platinum from these biotransformation products contributed to the measurements of platinum that were used to determine platinum concentrations and AUCs. Another explanation for the higher than expected AUCs could lie in the method used to determine subjects GFRs. At present, it is not known what method was used by Sugen to make these determinations. However, most often the creatinine clearance is measured and used as an indicator of the

GFR. This generally results in an overestimation of the GFR because it takes into account secretion as well as filtration of creatinine. The use of the overestimated GFR in the Calvert formula will lead to an overestimation of the dose, which in turn will lead to a higher than expected AUC. It is possible that the larger number of subjects in the higher dose group made the error associated with the overestimated GFR more apparent. The last possible explanation could have to do with the ages of the subjects in the high dose group. The GFR is known to decrease with age. One consequence of a decreased GFR is a decrease in the clearance of drugs eliminated by the kidneys, resulting in an increased systemic exposure or AUC. The average age of the subjects in the high dose group was 61.7 years, so this might have contributed to the higher than expected carboplatin AUCs.

Mean clearance values were comparable between cycles for both free and total platinum. The clearance values for free platinum are higher than those for total platinum. Total body clearance of free platinum is higher than that of total platinum because of irreversible protein binding. Thus, the results are consistent with what was expected. Carboplatin, and consequently free platinum, is cleared almost exclusively by filtration. The total clearance of free platinum can therefore be represented by the renal clearance, which in turn should approximate the GFR. The average normal GFR in humans is estimated to be 120 mg/ml min. The mean clearance values obtained for free platinum for both low and high dose groups correlate very well with the average GFR as well as with the historic values. The large standard deviations were to be expected due to the fact that clearance is highly variable.

Mean half-lives and volumes of distribution obtained for both free and total platinum show no significant inter-cycle variability, and correlate well with published

values. An exception is seen the volumes of distribution for free platinum (IV) for subjects that received 85 mg/m^2. Although the differences between cycles were not statistically significant, the calculated volumes of distribution are higher than the published value. This is most probably due to the small sampling set (n=4).

Overall, no major inter-cycle variability in the pharmacokinetics of free or total platinum was observed. Furthermore, there were no SU5416 dose effects observed. Statistical evaluation indicated that at the dosing regimens used in this study, SU5416 does not significantly ($p>0.05$) alter the carboplatin pharmacokinetics. Therefore it appears the pharmacokinetics of carboplatin are not affected by the addition of low or high dose SU5416 to the paclitaxel/carboplatin regimen.

In February 2002, Pharmacia, who acquired Sugen in 1999, announced it was closing the SU5416 phase III clinical trial involving patients with advanced stage colorectal cancer. The subjects were being treated with standard chemotherapy with or without SU5416. An interim safety and efficacy study showed a lack of clinical benefit in those who had received SU5416. All other trials involving SU5416 were set to be brought to the appropriate conclusions. The closing of these trials was not at all related to alteration of renal function by SU5416 and therefore does not diminish the findings and conclusions of this study. Although trials of SU5416 have been halted, trials for two structurally similar compounds continue. SU6668 is a small molecule inhibitor of VEGF receptors as well as two other angiogenic activators, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) [Ning *et al.*, 2002]. This broad spectrum RTK inhibitor is currently in phase I clinical trials for the treatment of advanced malignancies.

SU11248 is a VEGF and PDGF RTK inhibitor. Recruitment is underway for phase I clinical trials in patients with refractory hematologic malignancies [Giles, 2002].

6. REFERENCES

1. Arkey R. Physicans' Desk Reference, 53rd edition. Medical Economics Inc., New Jersey (1999).
2. Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, Siddik ZH, Judson IR, Gore ME, Wiltshaw E. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J. Clin Oncol 7(11): 1748-1756 (1989).
3. Calvert AH, Judson I, Van der Vijgh WJF. Platinum complexes in cancer medicine: pharmacokinetics and pharmacodynamics in relation to toxicity and therapeutic activity. Cancer Surv 17: 189-217 (1993).
4. Carter S. Clinical strategy for the development of angiogenesis inhibitors. The Oncologist 5(suppl 1): 51-54 (2000).
5. Chatelut E, Canal P, Brummer V, Chevreau C, Pujol A, Boneu A, Roche H, Houin G, Bugat R. Prediction of carboplatin clearance from standard morphological and biological patient characteristics. J. Natl Cancer Inst 87(8): 573-580 (1995).
6. Cropp G, Rosen L, Mulay M, Langecker P, Hannah A. Pharmacokinetics and pharmacodynamics of SU5416 in a phase I, dose escalating trial in patients with advanced malignancies. Proceedings of the 35th Annual Meeting of the American Society of Clinical Oncology: abstract 619 (1999).
7. Cropp G and Hannah A. SU5416, a molecularly targeted novel anti-angiogenesis drug: clinical pharmacokinetics and safety review. Proceedings of the 11th NCI-

EORTC-AACR Symposium on New Drugs in Cancer Therapy: abstract 262 (2000).

8. DeWaal WAJ, Maessen FJMJ, Kraak JC. Analytical methods for the quantitation of platinum anti-cancer drugs and related compounds in biological media. J. Pharm Biomed Anal 8(1): 1-30 (1990).
9. Duffull SB and Robinson BA. Clinical pharmacokinetics and dose optimization of carboplatin. Clin Pharmacokinet 33(3): 161-183 (1997).
10. Ellis LM, Takahashi Y, Liu W, Shaheen RM. Vascular endothelial growth factor in human colon cancer: biology and therapeutic implications. The Oncologist 5(suppl 1): 11-15 (2000).
11. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. Ann Surg 175(3): 409-415 (1972).
12. Fong TAT, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, Ullrich A, Hirth KP, McMahon G. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization and growth of multiple tumor types. Cancer Res 59: 99-105 (1999).
13. Giaccone G, Huizing H, Postmus PE. Dose finding and sequencing of paclitaxel and carboplatin in non-small cell lung cancer. Semin Oncol 22: 78-82 (1995).
14. Giles FJ. The emerging role of angiogenesis inhibitors in hematologic malignancies. Oncology 16(5): suppl 4 (2002).
15. Heldin CH. Protein tyrosine kinase receptors. Cancer Surv 27: 7-22 (1996).

16. Knox RJ, Friedlos F, Lydall DA. Mechanisms of cytotoxicity of anti-tumor platinum drugs: evidence that cis-diamminedichloroplatinum (II) and cis-diammine-(1,1-cyclobutanedicarboxylato)platinum(II) differ only in the kinetics of their interaction with DNA. Cancer Res 46: 1972-1979 (1986).

17. Liekens S, DeClercq E, Neyts J. Angiogenesis: regulators and clinical applications. Biochem Pharmacol 61: 253-270 (2001).

18. Lipp HP (Ed). Anticancer Drug Toxicity. Marcel Decker, Inc., New York (1999).

19. Los G, Verdegaal E, Noteborn HPJM, Ruevekamp M, DeGraeff A, Meesters EW, Huinink DTB, McVie JG. Cellular pharmacokinetics of carboplatin and cisplatin in relation to their cytotoxic action. Biochem Pharmacol 42(2): 357-363 (1991).

20. Masson E and Zamboni WC. Pharmacokinetic optimisation of cancer therapy: effect on outcomes. Clin Pharmacokinet 32(4): 324-343 (1997).

21. McKeage MJ. Comparative adverse effect profiles of platinum drugs. Drug Saf 13(4): 228-244 (1995).

22. McMahon G. VEGF receptor signaling in tumor angiogenesis. The Oncologist 5(suppl 1): 3-10 (2000).

23. Mendel DB, Laird AD, Smolich BD, Blake RA, Liang C, Hannah AL, Shaheen RM, Ellis LM, Weitman S, Shawver LK, Cherrington JM. Development of SU5416, a selective small molecule inhibitor of VEGF receptor kinase activity as an anti-angiogenesis agent. Anti Cancer Drug Design 15: 29-41 (2000).

24. Mohammadi M, McMahon G, Sun L, Tang C, Hirth P, Yen B, Hubbard S, Schlessinger J. Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science 276: 955-960.

25. Murry, DJ. Comparative clinical pharmacology of cisplatin and carboplatin. Pharmacotherapy 17(5): 140S-144S (1997).

26. Ning S, Laird D, Cherrington M, Knox S. The angiogenic agents SU5416 and SU6668 increase the antitumor effects of fractioned irradiation. Radiat Res 157: 45-51 (2002).

27. Obasaju CK, Johnson SW, Rogatko A, Kilpatrick D, Brennan J, Hamilton TC, Ozol RF, O'Dwyer PJ, Gallo JM. Evaluation of carboplatin pharmacokinetics in the absence and presence of paclitaxel. Clin Can Res 2: 549-552 (1996).

28. Pyke J. Minerals laboratory staff develops new ICP-MS preparation method. AGSO Research Newsletter 12-14 (Dec 2000).

29. Rosen L, Mulay M, Mayers A, Kabbinavar F, Rosen P, Cropp G, Hannah A. Phase I dose escalating trial of SU5416, a novel angiogenesis inhibitor in patients with advanced malignancies. Proceedings of the 35th Annual Meeting of the American Society of Clinical Oncologists: abstract 618 (1999).

30. Rosen L. Antiangiogenic strategies and agents in clinical trials. The Oncologist 5(suppl 1): 20-27 (2000).

31. Rosen L, Kabbinavar F, Rosen P, Parson M, Laxa B, Mayers A, Mulay M, Cropp G, Hannah A. A phase I/II trial and pharmacokinetic (PK) study of the vascular endothelial growth factor (VEGF) receptor pathway inhibitor SU5416 in combination with paclitaxel. Proc Am Assoc Cancer Res: abstract 2087 (2000).

32. Rosenberg B, Van Camp L, Krigas T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature 205: 698-699 (1965).

33. Sun L, Tran N, Tang F, App H, Hirth P, McMahon G, Tang C. Synthesis and biological evaluations of 3-substituted idolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases. J. Med Chem 41: 2588-2603 (1998).

34. Taylor HE. Inductively coupled plasma-mass spectrometry: practices and techniques. Academic Press, San Diego California (2001).

35. Thomas HD, Newell SD, Calvert AH, Lind MJ, Bailey N, Bodday AV. Pharmacokinetics of a combination of paclitaxel and carboplatin in a phase I dose escalation study in patients with ovarian cancer. EORTC Early Drug Development Meeting: 69 (1995).

36. Thomas R. A beginner's guide to ICP-MS: Part II: The sample introduction system. Spectroscopy 16(5): 56-60 (2001a).

37. Thomas R. A beginner's guide to ICP-MS: Part III: The plasma source. Spectroscopy 16(6): 26-30 (2001b).

38. Van der Vijgh WJF. Clinical pharmacokinetics of carboplatin. Clin Pharmacokinet 21(4): 242-261 (1991).

39. Van Hennik MB, Van der Vijgh WJF, Klein I, Vermorken JB, Pinedo HM. Human pharmacokinetics of carboplatin after oral administration. Cancer Chemother Pharmacol 23: 126-127 (1989).

40. Van Warmerdam LJC, Van Tellingen O, Maes RAA, Beijnen JH. Validated method for the determination of carboplatin in biological fluids by zeeman atomic absorption spectrometry. Fresenius J. Anal Chem 351: 777-781 (1995).

41. Zetter B. Angiogenesis and tumor metastasis. Ann Rev Med 49: 407-424 (1998).