COMPARISON OF TWO SALINE LOADING PROTOCOLS FOR PREVENTING NEPHROTOXICOSIS ASSOCIATED WITH HIGH-DOSE CISPLATIN

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Veterinary Medical Sciences

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September 30, 1994
Blacksburg, Virginia

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(ABSTRACT)

Cisplatin is an antineoplastic drug used to treat malignant tumors in human beings and dogs. Nephrotoxicosis was initially considered dose limiting. The use of saline loading and hypertonic saline administration protocols allowed dose escalation, reduced nephrotoxicosis, and increased remission rates in the treatment previously poorly responsive malignant tumors in human beings.

A pilot study was performed to determine efficacy of 4-hour saline loading in providing renal protection for dogs receiving high-dose cisplatin (150 mg/m^2 IV). Two beagles were saline loaded (25 ml/kg/hr of 0.9% NaCl, IV) for 4 hours and infused with cisplatin (150 mg/m^2). We demonstrated that high-dose cisplatin (150 mg/m^2 IV) can be administered to dogs without biochemical evidence of acute nephrotoxicosis; however gastrointestinal toxicoses (fibrinonecrotic enteritis) and severe myelosuppression (leukopenia) were incompatible with patient survival and therefore, dose limiting.

In another study we compared efficacy of hypertonic saline with normal saline in preventing nephrotoxicosis associated with administration of high-dose cisplatin (90 mg/m^2 IV) to dogs. In this study we demonstrated that a single IV dose of cisplatin (90

mg/m^2) can be administered to dogs in normal saline (0.9%) or hypertonic saline (7%) in combination with 4 hour saline loading (25 ml/kg/hr) without evidence of reduced renal function as measured by exogenous creatinine clearance. Platelet numbers were significantly increased in dogs that received cisplatin in hypertonic saline.

Nephrotoxicosis was not dose limiting in either study. Future studies should attempt to determine the efficacy and toxicoses of multiple doses of cisplatin (90 mg/m^2) administered in hypertonic saline to tumor bearing dogs.

Acknowledgements

To my wife Olga; your love, encouragement and patience made the residency and writing of this thesis a more pleasurable experience. Now we can move on to *our* dreams.

To my mother; your support and sacrifices were immeasurable. They will not be forgotten.

To Dr. Dru Forrester; for reviewing the multiple drafts, coping with the world's worst deadline pusher, and making the residency a more comfortable experience. I wish you all the best.

To Dr. Geoffrey Saunders; your calmness in the heat of battle and taste in shirts is exemplary.

To Dr. Greg Troy; for setting examples by actions, always being a professional, and performing like you really enjoy veterinary medicine.

To Dr. Jeff Wilcke; for teaching me everything I know about pharmacology, reeling me in when I went too far out, and cynicism in appropriate dosages, routes and intervals.

To Megan Irby, Jeanette Baker, Carolyn Sink, Donna Burton, and Jennifer Newhall; for caring for my dogs, helping collect the data, performing accurate analyses and providing encouragement at appropriate times. Without your help this project would have never been so successful.

To Mr. James E. Kenny; for mixing the cisplatin--even on the weekends.

To Michelle Marini; you taught me more about statistics without ever realizing it. I really mean it.

To Dr. Kim May and Dr. Darlene Blischock; you helped me when no one else would. I will never forget your kindness to me and my dogs.

To Maura Wood; your assistance and patience in the writing of this manuscript are greatly appreciated.

To my research dogs; your images are forever in my memory. The knowledge obtained from your sacrifice will not be lost.

Dedication

This Master's thesis is dedicated to my father the late Pearson R. Fallin and to my grandfather the late Alton P. Underwood. Without their wisdom, inspiration, patience, and sacrifice my career in veterinary medicine would be a dream unfulfilled.

"Stress is perceived."

Walter B. Gross DVM, MS, PhD

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LITERATURE REVIEW

Cisplatin: Development and History in Human and Veterinary Medicine

History. Despite the tremendous amount of time, personnel, and resources devoted to development of new drugs for treatment of neoplasia progress has been slow and major break-throughs rare. However, as in the case of Dr. Barnett Rosenberg who discovered antineoplastic effects of cisplatin, landmark scientific events sometimes result from an experiment gone awry.

In 1965, experiments originally intended to study effects of an electric field on growth of *Escherichia coli* revealed "a new and interesting effect" when bacteria were grown in a continuous culture chamber containing platinum electrodes.[1] Turbidity of nutrient broth decreased 1 hour after initiation of the electrical field across electrodes.[1] Microscopic examination of effluent from the culture chamber demonstrated cessation of bacterial cell division, but continued growth in the form of long filaments.[1] Thus, cell division was inhibited and cell growth continued, albeit abnormally. These changes were attributed to approximately 10 ppm of a product of electrolysis that formed on electrodes only when the nutrient medium contained ammonium chloride.[1] Further experiments demonstrated the compound responsible for this effect was Peyrone's Chloride, *cis*-dichlorodiammineplatinum (II), CDDP, or cisplatinum, hereafter referred to by the compounds generic name, cisplatin.[2] Initial testing of the new drug in a mouse-tumor model revealed that cisplatin inhibited development of solid Sarcoma-180 tumor.[3] A new antineoplastic drug had been discovered -- by accident.

Development of cisplatin in human medicine. Remarkably, phase I clinical trials with terminal human cancer patients were initiated by 1971. The first reports were cautiously optimistic describing clear tumor regression at expense of renal and gastrointestinal toxicoses of unprecedented severity.[4] Extreme morbidity associated with these toxicoses prompted many researchers to abandon cisplatin as a possible chemotherapeutic agent; however, in 1974 it was found to be extremely effective in treating testicular and ovarian cancer, tumors that previously had been unresponsive.[5,6] About the same time, it was discovered that renal toxicity associated with cisplatin could be acceptably controlled by hydrating the patient. Addition of fluid loading to the administration protocol markedly reduced renal toxicosis of cisplatin, with little or no loss of anticancer activity.[7] Fluid loading also allowed administration of cisplatin doses up to three times the previous limit without compromising patient's renal function.[7] In 1984 administration of cisplatin in hypertonic saline proved to almost completely eradicate renal toxicity.[8] This allowed administration of high-dose cisplatin (100-200 mg/m^2) and provided complete response rates for 85-95% of patients with bulky testicular cancer and partial response rates of 60-80% for ovarian cancer.[9,10]

Presently, cisplatin is one of the most widely used chemotherapeutic drugs in the human oncologist's armamentarium.[11] It continues to demonstrate significant efficacy against a wide variety of tumors that were previously difficult to treat such as epithelial and mesenchymal tumors, particularly head and neck carcinomas, ovarian carcinomas, gastrointestinal adenocarcinomas, and osteogenic sarcomas.[12] Although incorporation of

hypertonic saline greatly ameliorated renal toxicosis, gastrointestinal, hematologic, and neurologic toxicoses became more prevalent with high-dose protocols.[12]

Development of cisplatin in veterinary medicine. Despite widespread use of dogs as toxicologic and pharmacologic models during development of the drug, little information was available regarding clinical efficacy of cisplatin in veterinary patients prior to 1984. The first reported use of cisplatin to treat a veterinary patient with cancer was in 1984; five dogs received cisplatin (60 mg/m^2) following lung lobectomy for treatment of primary pulmonary adenocarcinoma with 2 dogs demonstrating measurable response following chemotherapy.[13] By 1985 the veterinary literature regarding cisplatin was limited to a literature review and the above mentioned report.[13,14] Over the next 8 years numerous journal articles and abstracts addressed the use, toxicity, and efficacy of cisplatin in veterinary patients. Although patient numbers were low in most clinical reports, certain trends were demonstrated. Renal toxicity was prevalent and prevented safe administration of doses greater than 70 mg/m^2 despite use of various saline loading protocols.[15-17] Cisplatin also caused significant gastrointestinal toxicoses manifested primarily as emesis.[18] Finally, administration of cisplatin provided clinical responses when used to treat a wide variety of carcinomas and sarcomas, particularly osteogenic sarcoma.[19-24]

Pharmacology

Cisplatin is a planar, inorganic complex formed by a central platinum atom surrounded by two chlorine atoms and two ammonia groups (Figure 1).[3] Following intravenous (IV) administration of cisplatin (31.5 mg/m^2) to dogs, a biphasic excretion pattern was demonstrated. The rapid-phase half-time (α) was 22 minutes and the slow-phase half-time (ß) was 5 days.[25] This can be described by the mathematical formula for a two compartment model:[26]

$$\text{rate of loss} = Ae^{-\alpha t} + Be^{-\beta t}$$

and

$$t_{½\alpha} = 0.693/\alpha, \quad t_{½\beta} = 0.693/\beta$$

This excretion pattern also was consistently reported in mice, rats, sharks, and human beings.[27,28] Rapid-phase and slow-phase elimination half-lives remained constant in dogs with massive prehydration and mannitol-induced diuresis.[29] During the first 4 hours after administration of cisplatin (31.5 mg/m^2 IV), plasma concentrations decreased by 90% with 60-70% of the applied dose recovered in the urine.[25] Plasma concentrations of platinum were readily measured 12 days after treatment with no significant change between days 4 and 12.[25]

Pharmacokinetics of cisplatin (70-77 mg/m^2 IV) were unaltered by pretreatment with mannitol or fluid loading in dogs.[29] However, time of administration (90 mg/m^2 IV) had significant effect on pharmacokinetics of cisplatin.[30] Mean urinary rate of excretion of total platinum was increased, whereas mean plasma residence time of ultrafiltrable

platinum was decreased in dogs treated at 4 pm, compared with dogs treated at 8 am.[30] This resulted in significantly reduced cisplatin-induced renal toxicosis in dogs treated at 4 pm.[30] Reduction of cisplatin nephrotoxicity by optimal circadian dosing also was demonstrated in rats.[31]

In vitro plasma protein binding studies demonstrated that only 10% of cisplatin was protein-bound in dogs 1 hour after treatment while 90% was protein-bound 24 hours after treatment.[25] This suggested a stereotypic alteration of the cisplatin molecule or protein binding characteristics that resulted in initial rapid excretion of cisplatin and later delayed excretion.[32] Another explanation was that large doses of platinum were bound with a high rate constant but low-affinity, while lower concentrations of platinum favored binding with a low rate constant and high-affinity mechanism.[25]

After IV administration of cisplatin (31.5 mg/m^2) to dogs, initial concentrations of platinum were highest in kidneys, gonads, spleen, and adrenal glands but remained significantly increased only in kidney, liver, ovary, and uterus where a tissue to plasma ratio of 3 to 4 was maintained as long as 6 days after treatment.[25] Excretion of cisplatin occurred almost exclusively through the kidneys.[27] In dogs receiving cisplatin (31.5 mg/m^2 IV) total 48-hour urinary recovery was 76%.[25] Dogs that received fluid loading or mannitol prior to cisplatin (70-77 mg/m^2 IV) demonstrated 90%-100% urinary recovery of platinum at 48 hours.[29]

Cisplatin was rapidly distributed in the extracellular fluid after IV and intraperitoneal administration to rats and mice.[26] Cisplatin reacted primarily by exchange

of labile chlorides for water or hydroxyl ions in a classic aquation reaction.[33] The sequential steps of the aquation reaction were as follows:

$$cis\text{-}Pt(NH_3)_2Cl_2\ H_2O \rightarrow cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ + Cl^-$$

$$\downarrow H_2O$$

$$cis\text{-}Pt[(NH_3)_2(H_2O)_2]^{++} + Cl^-$$

The positively charged aquation product was thought to be the active molecule that reacted with intracellular DNA.[34] Presence of high chloride ion (Cl^-) concentrations (>5 meq of Cl^-/L) would drive the aquation reaction to the left. Intracellular concentration of Cl^- for most cells was 4 mEq/L while extracellular fluid concentration of Cl^- was 104 mEq/L.[33] Therefore, in extracellular fluid the majority of cisplatin was in the inactive or electrically neutral form. Since no evidence existed for a carrier molecule, cisplatin was hypothesized to enter the cell by passive processes across the cell membrane.[35] Upon entering the low Cl^- intracellular space, cisplatin was thought to form positively charged aquation products in high concentrations. When this aquation reaction occurred extracellularly, ie, in hypotonic solutions, positively charged compounds failed to readily enter the cytoplasm and formed platinum compound dimers and trimers.[36] Platinum dimers and trimers were suggested to be responsible for the organ toxicity that occurs with cisplatin therapy.[36]

Cytotoxic effects of cisplatin were attributed to direct binding of the compound to DNA.[37] Synthesis of DNA was prevented by cisplatin alteration of the DNA template. The *cis* position of Cl^- and ammonia groups allowed rapid and bifunctional binding of DNA.[36,38] The *cis* isomer appeared to form intrastrand and interstrand crosslinks, usually between guanine-guanine groups (Figure 2).[36,39] Such bidentate binding prevented DNA template replication in mammalian cells.[40] DNA cross-links increased with time after drug was removed, and appeared to be repaired slowly.[41] Stereochemical properties of the *trans*-platinum isomer prevented it from exhibiting any toxic or anticancer activity.[32,42] Exact mechanisms of action for cytotoxicity of cisplatin have yet to be completely resolved.

Therapeutic Indications for Cisplatin in Dogs

Use of cisplatin for treatment of cancer in dogs has increased dramatically since 1984. Cisplatin (60-70 mg/m^2 IV) has demonstrated efficacy against a wide variety of epithelial and mesenchymal origin malignant tumors in dogs.[20] Partial remissions and improved survival times have been reported in dogs with transitional cell carcinoma of the urinary bladder, nasal adnenocarcinoma, thyroid carcinoma, metastatic mesenchyoma, and squamous cell carcinoma.[19,43-47]

Perhaps the most significant effect of cisplatin administration has been reported in treatment of osteosarcoma. Administration of cisplatin (60-70 mg/m^2 IV) and amputation resulted in significantly improved survival times that were 122-197 days

greater than dogs receiving amputation alone.[22,24,48-50] Limb-sparing surgical techniques incorporating IV administration of cisplatin, intralesional infusion, or cisplatin-containing intralesional implants have demonstrated survival times greater than patients undergoing amputation alone.[51-56] Adminsistration of cisplatin (70 mg/m^2 IV) was not effective for treatment of metastatic osteosarcoma.[57]

Intracavitary administration of cisplatin was developed in human beings for delivery of higher concentrations of active drug directly to the site of abdominal and pleural space neoplasms.[58] Pharmacokinetic studies of cisplatin administered intraperitoneally (IP) to dogs allowed development of similar protocols for use in dogs.[58,59] Administration of intracavitary cisplatin (50 mg/m^2 IP q 28 days) was associated with palliation and control of malignant pleural and/or abdominal effusion in 5 of 6 dogs.[60] Three of the dogs were diagnosed with mesothelioma and demonstrated complete resolution of effusion ranging from 129 to $>$306 days.[60] Two of the three dogs diagnosed with carcinomatosis demonstrated a complete response of 255 and $>$807 days after a single treatment.[60]

Cisplatin recently was incorporated into multiple modality cancer protocols in dogs. Local hyperthermia combined with intralesional cisplatin chemotherapy provided a 40% complete response rate in dogs with carcinomas, sarcomas, and melanomas.[61] Administration of cisplatin with orthovoltage radiation provided significantly longer survival times in dogs with tonsillar squamous cell carcinoma than dogs receiving chemotherapy alone.[62] Preliminary research regarding pharmacokinetics of IV cisplatin

in combination with focal hyperthermia and radiation therapy in tumor-bearing dogs suggested that trimodal cisplatin therapy may improve tumor response.[63]

Addition of cisplatin to the veterinary oncologist's therapeutic armamentarium allowed important advances in treatment of dogs diagnosed with cancer. However, many obstacles remain to be overcome. Nephrotoxicosis has limited the maximal safe dose of cisplatin in dogs to 70 mg/m^2. Myelosuppression, although frequently reported in dogs receiving cisplatin, has yet to be evaluated at doses greater than 70 mg/m^2. Cisplatin-induced gastrointestinal toxicity often deters owners from subjecting their animal companions to rigorous chemotherapy. These toxicoses have prevented evaluation of high-dose cisplatin in dogs for treatment of tumors currently nonresponsive to conventional therapy. Development of effective high-dose protocols that cause minimal patient morbidity should be a priority of future cisplatin research in veterinary medicine.

Cisplatin Toxicoses

General overview. Nephrotoxicosis initially was considered to be the dose-limiting toxicity associated with administration of cisplatin to human beings and animals.[11] However, it was not considered a clinical problem when doses of 40-70 mg/m^2 were administered to human beings that had received adequate hydration.[64] The advent of hypertonic saline protocols allowed doses of 200 mg/m^2 to be used in human beings without significant nephrotoxicity.[8] However, high-dose cisplatin greatly exacerbated gastrointestinal toxicity and revealed a plethora of new toxicoses, such as peripheral

neuropathy, ototoxicity, myelosuppression, hypersensitivities, hypomagnesemia, and seizures.[8,65-71]

In dogs, the dose-limiting toxicosis associated with administration of cisplatin (60-70 mg/m^2) was nephrotoxicosis.[29,72] However, mild to moderate myelosuppression appeared to be commonly reported in veterinary clinical trials.[15,16,19,20,22,48] Gastrointestinal toxicity manifested as vomiting was the most common toxicity.[18,22,30] Cardiovascular toxicity and ototoxicity have been sporadically reported in the veterinary literature.[15,19,73]

Gastrointestinal toxicoses. Clinically important gastrointestinal toxicosis associated with administration of cisplatin include nausea and/or vomiting in the acute (within 24 hours of drug administration) or delayed (after 24 hours) situation. Nausea and vomiting occur in virtually all human and canine patients receiving cisplatin. In human beings, incidence of nausea ranged from 50% to 100% and incidence of acute vomiting ranged from 17% to 100%.[74] Delayed nausea and vomiting were seen in 93% of human beings receiving high-dose cisplatin (120 mg/m^2).[65] Nausea and vomiting occurred in 100% of canine patients in several veterinary studies.[15,16,75] Gastrointestinal toxicity was found to be dose-dependent in human beings and dogs.[18,76] Vomiting was more likely to occur when cisplatin was administered IV than when given intra-arterially to dogs.[18]

The mechanism of acute gastrointestinal toxicity originally was thought to involve direct stimulation of the chemoreceptor trigger zone located in the vicinity of the area

postrema, a richly vascularized circumventricular organ located on the caudal margins of the fourth ventricle.[77] However, this theory was almost abandoned after it was shown that cisplatin-induced emesis could be abolished via a vagotomy and sympathectomy in ferrets.[78] Recently it was demonstrated in dogs and human beings that selective antagonists of serotonin type-3 receptors (5-hydroxytryptamine$_3$ [5-HT$_3$] antagonists) were very effective against nausea and vomiting associated with chemotherapy.[79,80] The antiemetic effect of these drugs may be mediated centrally in the area postrema and associated structures of the emetic reflex such as the nucleus tractus solitarius, all of which have a very high density of 5-HT$_3$ receptors.[80] Additional sites of action may be found on the 5-HT$_3$ receptors located on the vagus nerve or enteric neuronal elements in the gastrointestinal tract.[80] The precise site(s) and mechanism(s) of action of different cytotoxic treatments that induce emesis remain(s) to be determined, but appear(s) to involve a common action on a 5-HT$_3$ system.

A more severe form of gastrointestinal toxicosis manifested as severe hemorrhagic colitis (3-5 days post-infusion) was described in early toxicologic studies of dogs receiving cisplatin (30-199 mg/m^2).[29,72] These dogs also had severe azotemia and it was not clear whether gastrointestinal toxicoses were due to uremia or a primary gastrointestinal toxicoses associated with cisplatin. Fatal post-cisplatin hemorrhagic gastroenteritis has been described in one dog, 4 days after administration (60 mg/m^2, IV) for treatment of prostatic adenocarcinoma.[20]

Very few clinical trials have compared efficacy of various drugs used to prevent cisplatin-induced emesis in dogs. Metoclopramide (1 or 3 mg/kg, SC) was found to be the most effective antagonist of cisplatin-induced emesis in dogs compared with haloperidol (1 mg/kg, SC), chlorpromazine (0.3, 1, 3 mg/kg, SC), and nabilone (0.1 mg/kg, IV).[81] Metoclopramide originally introduced as a dopamine antagonist antiemetic, was later discovered to partially inhibit 5-HT_3 receptors.[82] This may explain the activity associated with metoclopramide in ameliorating cisplatin-induced emesis.

In human beings corticosteroids have an apparent synergistic antiemetic effect when used in conjunction with lorazepam/prochlorperazine, droperidol,chlorpromazine,or metoclopramide.[83-86] It has been postulated that corticosteroids inhibit prostaglandin synthesis in the emetic center, unfortunately there are no experimental data to prove this.[87]

Butorphanol has been reported to prevent 100% of acute emetic episodes associated with administration of cisplatin (60 mg/m^2, IV) to dogs.[88] The mechanism of action of butorphanol is not very well understood; however, it is postulated that it is a dopamine antagonist similar to haloperidol and droperidol.[89]

In recent clinical trials with dogs, granisetron, and ondansetron, both potent 5-HT_3 antagonists, demonstrated significant reduction in cisplatin-induced emesis when compared with metoclopramide.[90,91] Unfortunately expense of these drugs precludes them from being used in most veterinary patients. Batanopride and RG12915, two new 5-HT_3

antagonists currently are under investigation in dogs for prevention of cisplatin-induced emesis.[92,93]

Myelosuppression. Myelosuppression initially was not considered a clinically important problem in human beings receiving cisplatin; however, advent of high-dose protocols revealed severe myelosuppression, particularly leukopenia and thrombocytopenia, to be prevalent and often life threatening.[12,67] Cisplatin-induced myelosuppression appeared to be dose-related in human beings.[12] The day of white blood cell count nadir varied with schedule of drug administration; leukopenia occurred between days 6 and 26, whereas onset of thrombocytopenia varied from days 10 to 26.[76] In human beings, leukopenia and thrombocytopenia were attributed to cumulative doses of cisplatin and usually considered reversible.[76,94] Administration of cisplatin dosages greater than 200 mg/m^2 IV resulted in severe life threatening myelosuppression in human beings that prevented further dose escalation.[67]

In dogs, cisplatin-induced myelosuppression appeared to be dose-dependent.[72] Review of the veterinary literature revealed thrombocytopenia and leukopenia to be reported with equal frequency. Nadirs for white blood cells in dogs varied from being bimodal (days 6 and 15) to a single nadir (day 7 or 8) depending on dose and administration protocol.[16,29] Early toxicologic studies in dogs demonstrated severe myelosuppression evidenced by bone marrow hypocellularity after administration of high-

dose cisplatin (106 mg/m^2 to 290 mg/m^2).[72] Information regarding effects of saline loading protocols on myelosuppression after high-dose cisplatin have not been reported.

Neurotoxicoses. The advent of high-dose cisplatin protocols in human beings revealed dose-limiting neurotoxicoses usually manifested as ototoxicity or peripheral neuropathy, but occasionally as seizures or blindness.[66] In rhesus monkeys and guinea pigs cisplatin-induced ototoxicity resulted in death of the hair cells in the organ of Corti.[95,96] Cisplatin-induced ototoxicity in human beings may be associated with tinnitus, audiogram abnormalities only, or clinical hearing loss.[74] Clinical deafness was demonstrated in 6% of human patients receiving high-dose cisplatin.[74]

Ototoxicity has been reported in two dogs that received cisplatin.[15,19] One dog became deaf after receiving 4 doses of cisplatin (70 mg/m^2 IV, q 21 days).[15] This dog had concurrent acute renal failure and blindness.[15] Another dog experienced transient deafness after 3 treatments with cisplatin for transitional cell carcinoma (50 mg/m^2 IV); this dog also was azotemic.[19] Audiometric changes have not routinely been evaluated in dogs receiving cisplatin.

Sensory peripheral neuropathies, manifested as paresthesia, loss of proprioception, or vibratory sensation have been reported in human beings receiving cisplatin.[6,66,94] Based on results of electromyogram and nerve conduction studies, cisplatin initially was thought to cause primary segmental demyelination. [6,94] However, sural nerve biopsies of affected individuals demonstrated primary axonopathic changes with secondary

segmental demyelination.[97] This confirmed that cisplatin-induced neuropathy was a distal axonal neuropathy.[97] Cessation of cisplatin therapy usually stabilizes the disease, however, recovery is variable.[98]

Hypomagnesemia. Hypomagnesemia was the most prominent electrolyte disorder seen in human beings secondary to administration of cisplatin.[70] Hypomagnesemia was demonstrated in 53% to 88% of human patients treated with cisplatin; hypocalcemia was demonstrated concurrently with hypomagnesemia in 5.8% of patients.[99] Severity and incidence of hypomagnesemia was shown to be dose-related.[100] Hypomagnesemia usually occurred without concurrent renal failure.[101] Although most often subclinical, severe hypomagnesemia can cause tetany, weakness, and cardiac problems.[102] Severe hypomagnesemia has been associated with clinical hypocalcemia and/or hypokalemia in human beings.[103,104]

Cisplatin-induced hypomagnesemia has been attributed to renal wasting of magnesium ions.[100,105] Some research has implicated the distal tubule (ascending limb of the loop of Henle) as the site of magnesium wasting (failure of absorption).[105] Others have implicated proximal tubular dysfunction as the source of increased fractional excretion of magnesium.[100] Concurrent hypocalcemia that occurs with hypomagnesemia was associated with low serum parathyroid hormone (PTH) concentrations and/or end-organ resistance to PTH induced by hypomagnesemia.[106,107] There are no reports of cisplatin-induced hypomagnesemia in the veterinary literature; however, increased urinary

fractional excretion of magnesium was reported in dogs receiving one dose of cisplatin (90 mg/m^2 IV) in the morning versus dogs receiving cisplatin in the evening.[30]

Hypomagnesemia was successfully treated in human beings by IV or oral supplementation of magnesium.[108] Interestingly, hypocalcemic or hypokalemic patients with clinical signs responded only after supplementation of magnesium and their respective deficient electrolyte.[103,104]

Nephrotoxicosis. Initial toxicologic studies of cisplatin in dogs demonstrated dose-dependent renal toxicity.[29,72] Although the exact mechanism of renal toxicity was unknown, it was believed that nephrotoxicoses occurred because cisplatin is eliminated primarily by the kidneys.[25,27] After administration of cisplatin (20-22 mg/m^2 IV) to dogs, urinary concentrations increased rapidly with 50 to 60% of the administered dose recovered in urine within 4 hours.[25] Rapid elimination of cisplatin in urine resulted from net tubular secretion in dogs.[109] In one study, cisplatin was excreted primarily by the kidneys in dogs and tissue concentrations of cisplatin remained highest in kidneys for 6 days.[25]

Renal blood flow and glomerular filtration rate (GFR) did not change significantly immediately after IV administration of cisplatin (129-155 mg/m^2 IV) to dogs.[110] It was demonstrated by clearance of lithium that delivery of fluid out of the proximal, straight segment of the renal tubule increased significantly.[111] Also, renal clearance and perfusate disappearance of *p*-amminohippuric acid was markedly depressed in dogs after

administration of cisplatin, suggesting impaired proximal tubular transport of organic ions.[112] These findings support the theory that cisplatin-induced nephrotoxicity was initiated by impairment of proximal tubular functions.[111]

Water molecules were theorized to react with the high urine concentration of cisplatin in the proximal tubule.[113] The aquation reaction was driven to the right producing reactive electrophiles, cytotoxic platinum hydroxyl complexes and their dimers and trimers (Figure 3).[113] It was hypothesized that these post-aquation platinum species were responsible for injury that occurred to renal tubular epithelial cells (Figure 3).[114] At a subcellular level, cisplatin appeared to be toxic to renal tubular mitochondria.[115] Electron microscopy demonstrated post-cisplatin mitochondrial swelling, degeneration, and vacuolization in renal tubules of rats.[115]

Damage to the proximal tubule caused increased delivery of fluid from the proximal tubule to the thin, descending limb of Henle's loop, which promoted increased reabsorption of sodium and water beyond the proximal tubule.[111,116] However, the increased rate of reabsorption did not compensate completely for increased delivery of sodium from proximal tubules as evidenced by increased fractional excretion of sodium and water after administration of cisplatin to dogs.[116]

Two to 3 days after administration of cisplatin to dogs (20-22 mg/m^2 IV) renal blood flow was reduced by 33% and GFR by 78%.[111,116] The mean arterial pressure and cardiac output of these dogs remained unchanged, thus this subacute change in renal hemodynamics was attributed to increased renal vascular resistance.[116] Lithium clearance

studies used to evaluate absolute and fractional reabsorption rates of sodium and water in proximal and distal segments demonstrated increased urine water loss and sodium excretion during this period.[110] This suggested that cisplatin also induced subacute distal tubular malfunction. Histologically this was confirmed by presence of patchy necrosis in proximal and distal tubules 48 to 72 hours after administration of cisplatin.[116]

Cisplatin nephrotoxicosis was clinically manifested as polyuria and azotemia.[29,30,72] Polyuria usually occurred following administration of cisplatin to rats in two distinct phases.[11] The first phase of cisplatin associated polyuria was observed 24 to 48 hours after drug administration at which time urine osmolality decreased without reduction of GFR.[11] A second phase of polyuria occurred between 72 and 96 hours after administration of cisplatin.[11,117] This phase was associated with reduced GFR and usually persisted.[11] Serum urea nitrogen and creatinine peaked 7 to 8 days after administration of cisplatin to dogs with IV fluids (80 mg/m^2 IV in 5% dextrose and 0.45% NaCl) and without fluids (90 mg/m^2 IV early morning), respectively.[29,30]

Severity of nephrotoxicoses associated with administration of cisplatin severely dampened initial enthusiasm generated by the drug's tremendous anticancer activity.[26] Through trial and error it was discovered that pretreatment with fluid loading greatly reduced cisplatin-induced nephrotoxicity and actually allowed administration of doses up to three times the previous limit in human beings.[7] It also was discovered that slow infusion of the drug over 6 to 8 hours ameliorated nephrotoxicoses in human beings without compromising anticancer activity.[118]

In veterinary medicine the earliest protocols for administration of cisplatin to dogs varied greatly as to length of fluid loading, type of fluid, dose of cisplatin, and period of drug infusion.[13,20] These early protocols were hybrid extrapolations of regimens used in human beings that utilized various combinations of fluid loading and diuretics.[119] Addition of diuretics such as mannitol or furosemide to protocols was not universal. Anecdotally, it was thought that these protocols reduced amount and severity of nephrotoxicoses in dogs treated with cisplatin, but published studies evaluating protocols were not available until 1988.[16]

The first protocol documented to allow administration of cisplatin (70 mg/m^2 IV) to normal dogs without evidence of nephrotoxicity involved a 6-hour saline loading procedure (0.9% saline, 18.3 ml/kg/hr IV).[16] Endogenous creatinine clearance, GFR measured by scintigraphy, serum urea nitrogen, serum creatinine, and electrolytes remained normal throughout the study period.[16] Renal lesions attributable to cisplatin were not demonstrated histopathologically.[16] This protocol later was utilized in 61 dogs with histologically confirmed malignant tumors receiving cisplatin (70 mg/m^2 IV) every 21 days for one to six cycles.[120] Clinically evident renal disease (ie, increased serum urea nitrogen and creatinine) developed in 4 dogs (6.6%), 3 of which had pre-existing renal disease, therefore it appeared that this protocol was effective in preventing nephrotoxicosis in dogs without renal disease.[120]

Another study of 64 dogs with malignant neoplasia receiving 1 to 4 doses of cisplatin (70 mg/m^2 IV) utilized a 4-hour saline loading protocol (0.9% NaCl, 25

ml/kg/hr, IV).[121] Results were similar to the 6-hour protocol with 5 dogs (7.8%), 2 of which had pre-existing pyelonephritis, developing clinically evident renal disease.[121] Compared with pretreatment values, there was a significant increase in median serum creatinine concentrations in all dogs that received a third and/or fourth treatment.[121]

A 1-hour saline loading protocol was evaluated in normal dogs receiving cisplatin (70 mg/m^2, IV, q 21 days) for 4 treatments.[15] Compared to pretreatment values, serum creatinine concentrations significantly increased and endogenous creatinine clearances significantly decreased after 4 treatments.[15] One dog died of renal failure after the fourth dose of cisplatin.[15] Caution was recommended before using this protocol in dogs pending further evaluation.[15]

One of the more unique methods of reducing cisplatin-induced renal toxicoses was based on circadian changes in urine production of dogs and rats. Dogs receiving cisplatin (90 mg/m^2 IV) at 4 pm demonstrated significantly less evidence of nephrotoxicosis than dogs receiving cisplatin at 8 am.[30] Careful timing of the 4- or 6-hour saline loading protocols so that cisplatin infusion occurred in the evening might further decrease renal toxicosis.

Use of hypertonic saline for administration of cisplatin to human beings greatly improved the therapeutic index of cisplatin, allowing doses up to 200 mg/m^2 to be administered without significant nephrotoxicity.[8] Recently, it was demonstrated that administration of cisplatin (70 mg/m^2 IV) in 3% saline solution (6.5 ml/kg) over a 20-minute period to normal dogs without IV fluids did not result in significant reduction in

exogenous creatinine clearance, an estimate of GFR.[75] In addition, histologic changes associated with cisplatin administration were not identified.[75] Despite lack of statistically different changes in creatinine clearance, post-treatment values were lower than pre-treatment values.[75] Although potentially a very convenient protocol, further studies were needed before hypertonic saline could be recommended as a vehicle for administration of cisplatin to veterinary patients, especially when repeated doses were administered.

Other methods also have been used to prevent cisplatin-induced nephrotoxicosis. Methimazole, a drug commonly used to treat hyperthyroidism, recently was found to significantly reduce cisplatin-induced (80 mg/m^2 IV) biochemical and histologic changes of renal disease in dogs.[122] Aminophylline, 8-cyclopentyl-1,3-dipropylxanthine, and glycine have demonstrated significant amelioration of cisplatin-induced renal failure in rats.[123-125] Effects of these drugs in tumor laden animals or dogs have not been reported.

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Cis Trans

Figure 1. *Cis* and *trans* isomers of platnum (II) molecule

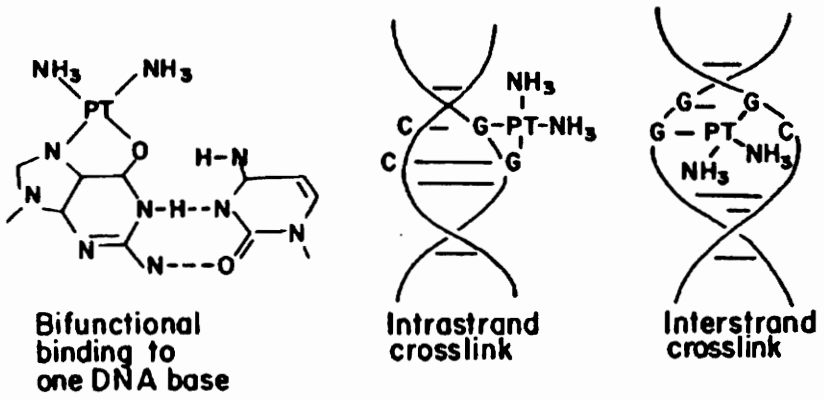

Figure 2. Cisplatin binding configurations to DNA. PT = platinum; C = cysteine; G = guanine. (Reproduced with permission from Page R, Cisplatin, a new antineoplastic drug, *Journal of the American Veterinary Medical Association* 1985;186:288-290).

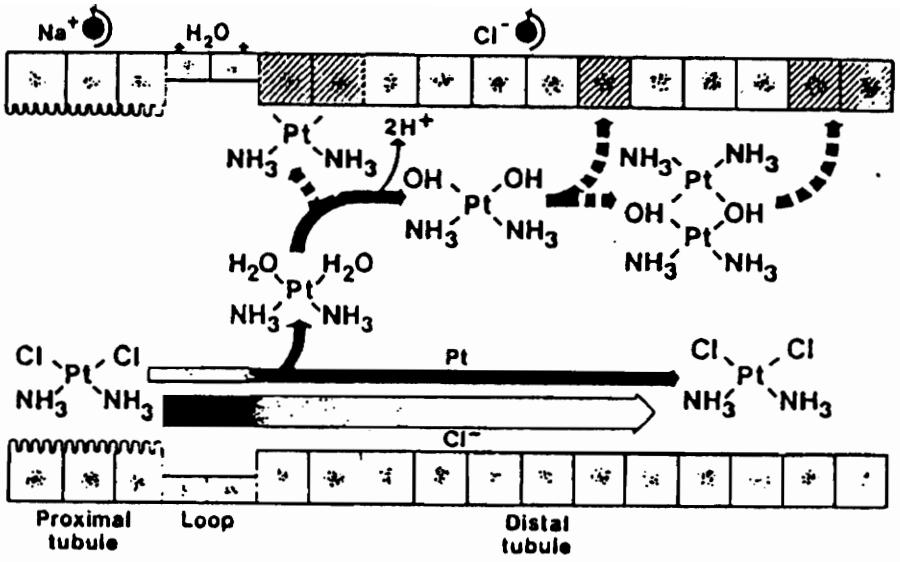

Figure 3. Hypothetical mechanism of cisplatin-induced renal tubular injury secondary to toxic aquation reaction products. In the presence of high chloride concentrations, the *cis* isomer maintains its configuration and is excreted without causing renal tubualar damage.In the prescence of low chloride concentrations the *cis* isomer reacts with water molecules to form toxic dimers, trimers, and hydroxyl complexes which cause renal tubular necrosis. (Reproduced with permission from Earhart RH, Improvement of the therapeutic index of cisplatin by pharmacologically induced chloruresis, *Cancer Research* 1983; 43:1191).

HIGH-DOSE CISPLATIN: PILOT EVALUATION OF TOXICOSES USING 4-HOUR INTRAVENOUS SALINE LOADING PROTOCOL IN TWO BEAGLE DOGS

Summary

Cis-diamminedichloroplatinum (cisplatin) is a cell-cycle phase-nonspecific chemotherapeutic agent effective against tumors of mesenchymal and epithelial origin in human beings and domestic animals. Its efficacy and toxicoses are dose-related. Toxicoses include moderate myelosuppression, neurotoxicosis, nausea, vomiting, and dose-limiting nephrotoxicosis. Incorporation of saline loading or hypertonic saline protocols recently has allowed dosages of 150-200 mg/m^2 to be successfully administered to human beings and has proven effective in treating tumors previously nonresponsive to lower doses of cisplatin. Utilization of a 4-hour saline loading protocol provided excellent renal protection for dogs receiving cisplatin (70 mg/m^2 IV).

The purpose of this pilot project was to determine efficacy of 4-hour saline loading in providing renal protection for dogs receiving high-dose cisplatin (150 mg/m^2 IV). Two mature, healthy, female beagles were saline loaded (25 ml/kg/hr of 0.9% NaCl, IV) for 3 hours, then infused with 150 mg/m^2 cisplatin mixed in 0.9% NaCl IV over 20 minutes, and loaded again with saline (25 ml/kg/hr of 0.9% NaCl, IV) for 1 hour. Both dogs vomited 6 to 8 times within 6 hours of administering cisplatin. Results of hemograms and serum chemistries were monitored every 48 hours. Mild hypomagnesemia occurred in both dogs 48 hours after treatment. Protracted episodes of vomiting began 70 hours after infusion of cisplatin and hemorrhagic diarrhea was noted approximately 78 hours after infusion. Severe leukopenia and moderate hypoalbuminemia occurred by 96 hours after treatment. Serum urea nitrogen, creatinine,

and phosphorous concentrations remained within reference ranges. The condition of both dogs continued to deteriorate and signs of sepsis were observed. Both dogs were humanely euthanized and necropsies were performed. Fibrinonecrotic enteritis was noted on gross examination. Histologic findings included necrotic enteritis with hyperplasia of mucosal glandular crypts, pulmonary edema and multifocal necrosis of the lungs, pancytopenia of bone marrow, and mild colitis. Kidneys were normal histologically.

We conclude that utilization of the 4-hour saline loading protocol prevented clinically significant acute nephrotoxicosis associated with administration of cisplatin (150 mg/m^2 IV); however, gastrointestinal and bone marrow toxicoses were unacceptable in these two dogs.

Introduction

Cis-diamminedichloroplatinum (cisplatin, Platinol® Bristol Laboratories) is a heavy metal co-ordination compound with proven antineoplastic activity against a variety of solid tumors in human and veterinary patients.[1] Cisplatin is used to treat human germ cell, ovarian, bladder, head, neck, gastrointestinal, esophageal, and lung cancers.[2] In dogs, cisplatin has demonstrated efficacy against transitional cell carcinoma of the urinary bladder, nasal adenocarcinoma, and thyroid carcinoma; however, more promising results have been noted in treatment of appendicular osteosarcoma.[3-7] Recently cisplatin demonstrated efficacy for treatment of canine mesothelioma and abdominal carcinomatosis, two diseases previously considered untreatable.[8] Extrapolation of data from studies in human beings infers that higher doses of cisplatin could prolong survival times in dogs with cancer; however, this remains to be proven. In addition, it is important to determine toxicoses associated with high-dose cisplatin (150 mg/m^2 IV) in dogs and evaluate protocols to prevent their occurrence.

Objectives of this pilot project were to determine efficacy of a previously reported saline loading protocol in providing renal protection for dogs receiving high-dose cisplatin (150 mg/m^2); to describe histologic changes that occur in kidneys of dogs after a single dose of cisplatin; and to determine occurrence, onset, and extent of myelosuppression, hypomagnesemia, and gastrointestinal toxicoses (eg, emesis).[9]

Materials and Methods

Animals. Two adult female beagle dogs were acquired from a commercial vendor and acclimated for a period of approximately 3 weeks prior to initiation of the study. Inclusion in the study was dependent on normal results of physical examination and laboratory evaluation including complete blood and platelet counts, serum chemistries, heartworm test (Knott's), and urinalysis. Dogs were dewormed for intestinal parasites with fenbendazole[a] (50 mg/kg SID for 3 consecutive days) twice at 3-week intervals before the acclimation period. Zinc sulfate fecal flotations were negative for intestinal parasites and *Giardia*. Both dogs received vaccinations for distemper, parvovirus, adenovirus 2, parainfluenza, and leptospirosis 6 months prior to beginning the study.

Administration of cisplatin. On day 0, 0.9% saline IV (25 ml/kg/hr) was administered IV to both dogs through an indwelling catheter for 3 hours. Afterwards, cisplatin[b] (150 mg/m^2) was mixed in 0.9% saline (8.3 ml/kg) and administered IV over a 20-minute period. After completion of cisplatin infusion, 0.9% saline was continued IV (25 ml/kg/hr) for 1 hour.

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[a]Panacur Granules 22%, Hoechst-Roussel Agri-Vet Co., NJ.

[b]Platinol, Bristol-Myers Squibb Co., Evansville, Ind.

Administration of antiemetics. In anticipation of nausea and vomiting, all dogs received metoclopramide[c] (0.2 mg/kg IV) and dexamethasone sodium phosphate[d] (0.22 mg/kg IV) 30 minutes prior to administration of cisplatin. Immediately prior (time 0) to infusion of cisplatin another dose of metoclopramide (0.2 mg/kg IV) was administered; this was repeated 1 hour after infusion.

Laboratory evaluation and animal monitoring. Measurements of complete blood cell and platelet counts and serum chemistries, including magnesium, were performed immediately prior to administration of cisplatin and every 24 to 48 hours thereafter until termination of the study. Dogs were continuously monitored by attendants for vomiting, diarrhea, attitude, and appetite throughout the entire study. Physical examinations were performed twice daily.

Termination of study. The study was designed to be terminated 21 days after infusion of cisplatin. Should dogs develop debilitating illness that was nonresponsive to substantial supportive care for 48 hours or if they exhibited pain or discomfort that proved nonresponsive to mild analgesics, they would be humanely euthanized.

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[c]Solopak Laboratories Inc., Elkgrove Village, Ill.

[d]Vedco, St. Joseph, Mo.

Gross and histologic examinations. On the last day of the study, both dogs were euthanized by administration of sodium pentobarbital[e] and necropsies were performed. Sections of bone marrow, kidney, small intestine, colon, mesenteric lymph node, lung, heart, and pancreas were fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined by light microscopy.

Results

Clinical findings. Both dogs vomited 6 to 8 times within 6 hours of administering cisplatin. Protracted episodes of vomiting began 70 hours after infusion of cisplatin and hemorrhagic diarrhea was noted approximately 78 hours after infusion in both dogs.

Laboratory findings. Severe leukopenia and moderate hypoalbuminemia (Tables 1, 2) were evident in both dogs by 96 hours after treatment with cisplatin. Mild hypomagnesemia occurred in both dogs 48 hours after treatment (Tables 1, 2). Serum urea nitrogen and creatinine concentrations remained within reference ranges (Tables 1, 2). After development of hemorrhagic diarrhea supportive treatment was initiated consisting of IV fluids (lactated Ringers solution 60 ml/kg/day plus extrinsic losses) and antibiotics (sodium ampicillin 20 mg/kg IV, q 6 h). Both dogs continued to deteriorate despite supportive care for 48 hours. One criteria for termination of the study had been

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[e]Beuthanasia-D Special, Schering-Plough Animal Health Corp., Kenilworth, NJ.

met, therefore, both animals were humanely euthanized by pentobarbital injection 120 hours after administration of cisplatin.

Gross necropsy findings. Small intestine of both dogs was diffusely red and covered by green fibrinonecrotic exudate that extended from the duodenum to the ileum. These lesions were most severe in the duodenum and decreased toward the ileum (Figure 2). The lungs appeared congested in both dogs. Hemorrhage was noted in mesenteric lymph nodes and bone marrow.

Histologic findings. Bone marrow demonstrated marked hypoplasia of hematopoietic cells with all cell lines present and complete in their maturity (Figure 3). Small intestinal villi were either absent or severely blunted. Surface of small bowel was covered by fibrinonecrotic exudate that contained numerous bacteria (Figure 4). Mucosal glands exhibited marked epithelial hyperplasia with failure of differentiation, focal metaplasia, and loss of goblet cells. Intestinal crypts were dilated, contained necrotic cells, and were lined by attenuated, hyperplastic epithelium. Lymphoid depletion was noted in ileal Peyer's patches. Occasional dilated crypts containing necrotic cells and debris were present in the colon. Kidneys exhibited occasional fetal glomeruli, but otherwise were normal. Mild hemorrhage and lymphoid depletion were noted in the sinusoids of mesenteric lymph nodes. Diffuse mild congestion and edema were present in the lungs. Heart, liver, and stomach were normal.

Additional findings. Immunofluorescence for parvovirus was negative. Bacterial culture of the small intestine demonstrated 4+ growth of *Campylobacter jejuni*.

Discussion

Chemotherapeutic protocols in human medicine incorporating cisplatin have demonstrated increased efficacy with increased doses.[10-12] Unfortunately, toxic effects of cisplatin, (ie, extreme nausea and vomiting, renal failure, and myelosuppression) also are exacerbated in a dose-dependent fashion.[11-13] Nephrotoxicosis is dose-limiting and characterized by reduced glomerular filtration and proximal tubular necrosis.[14] Administration of a single dose of cisplatin (80-95 mg/m^2 IV) consistently causes nephrotoxicosis in dogs, manifested by increased concentrations of serum urea nitrogen (SUN) and creatinine.[13] These higher doses also have revealed new toxicoses (ie, ototoxicity and peripheral neuropathy) that can be dose limiting in human beings.[15-17] Incidence and severity of toxicoses associated with high-dose cisplatin (greater than 90 mg/m^2) in dogs have been vaguely described.[13]

Acute renal toxicosis was not observed clinically nor histologically in either dog treated with high-dose cisplatin. Glomerular filtration was not measured; however serum creatinine, phosphorous, and urea nitrogen concentrations remained normal throughout the study. In addition there was no histologic evidence of renal tubular necrosis. It appears that 4-hour saline loading was effective in preventing acute renal toxicosis associated with administration of high-dose cisplatin administration to these two dogs.

Myelosuppression that occurs with administration of cisplatin ($\leq$ 90 mg/m^2) is mild compared with other chemotherapeutic agents.[1] Peak reduction in neutrophil count occurs around day 6 and again on day 15 after administration of cisplatin (70 mg/m^2 IV) to dogs.[18] Exact cause of myelosuppression is unknown; however, it is thought to be associated with direct damage to committed stem cells or interference with production of cytokines, [eg, granulocyte-colony stimulating factor (G-CSF)].[19] Recent availability and proven efficacy of recombinant G-CSF (Nupogen®, Amgen Corporation) in human beings and dogs has greatly improved chances of recovery from chemotherapy-associated myelosuppression.[20,21] Degree of myelosuppression associated with high-dose cisplatin has not been critically evaluated in dogs. Data from initial preclinical toxicologic evaluations vaguely elude to more severe myelosuppression with increasing doses.[13] Unfortunately, the manner in which these studies were done (ie, lack of protection from nephrotoxicosis and ensuant renal failure) does not permit accurate extrapolation of information to current protocols. Both dogs in our study demonstrated laboratory evidence of severe myelosuppression 4 days after administration of high-dose cisplatin. Clinically evident myelosupression was accompanied by histologic documentation of severe bone marrow hypoplasia. These findings support the theory that cisplatin has direct cytotoxic effects on bone marrow. It also appears that nadirs of myelosupression are dependent on dose of drug administered in that our dogs developed clinically evident myelosuppression sooner than that previously reported.[18,22]

Both dogs in this study demonstrated mild hypomagnesemia 48 hours after administration of cisplatin. It is possible this actually occurred earlier, since the first serum concentrations were measured 48 hours after infusion of cisplatin. Hypomagnesemia and concurrent hypocalcemia, although usually subclinical, have also been described as dose-dependent phenomena of cisplatin therapy in human beings.[23] Hypomagnesemia is attributed to renal wasting (decreased resorption) due to a defect in the thick ascending loop of Henle.[24]

Gastrointestinal toxicosis was manifested in two phases: one an acute episode of emesis occurring within the first 6 hours after drug administration, and the second a delayed syndrome consisting of emesis and hemorrhagic diarrhea associated with severe fibrinonecrotic enteritis. In dogs it has been demonstrated that number of emetic episodes increases as dose of cisplatin increases.[25] Thus vomiting that occurred immediately after cisplatin was anticipated despite use of antiemetics. Severe hemorrhagic enterocolitis in dogs subsequent to administration of cisplatin has been previously reported; however, it is difficult to completely discern whether the hemorrhagic diarrhea was a result of severe uremia or a cytotoxic effect of cisplatin on the rapidly differentiating intestinal cells.[13,22]

In preclinical toxicologic evaluation of cisplatin, five beagles developed renal failure and hemorrhagic enterocolitis 4 to 8 days after a single IV injection of 80 to 160 mg/m^2 or after 5 daily consecutive treatments of either 58 mg/m^2 or 32 mg/m^2.[13] Histologic changes consistent with those reported here included thickening and fusion of

small intestinal villi in addition to marked metaplasia, necrosis, and focal sloughing of the epithelium covering villi and crypts.[13] Severe hemorrhagic diarrhea that resulted in death was attributed to administration of cisplatin (60 mg/m^2 IV) to a dog; however, histologic findings and onset of clinical signs after administration of cisplatin were not described.[26] Significance of positive culture for *Campylobacter* in these dogs is unknown. Culture of *Campylobacter* from the intestine of asymptomatic commercially bred beagles is not uncommon (prevalence 35%).[27] The dogs in our study did not demonstrate any clinical evidence of campylobacteriosis such as large bowel diarrhea prior to administration of cisplatin. It could be argued that severe myelosupression associated with infusion of cisplatin resulted in severe immunosuppression and clinical campylobacteriosis. It is true that some of the histologic findings seen in dogs infected with *Campylobacter* also were seen in our two dogs (ie, exfoliation of surface epithelium, loss of goblet cells, and hypertrophy of crypt epithelium).[28] In contrast, our dogs demonstrated the most severe lesions in the proximal small bowel; those seen in dogs with campylobacteriosis usually occur in the colon.[28]

Salmonellosis and parvoviral enteritis are two diseases that could present with similar clinical signs and histologic lesions. *Salmonella* was not cultured from either dog. Both dogs had been vaccinated and direct immunofluorescence for parvovirus on sections of small bowel were negative.

In human beings high-dose cisplatin causes two distinct patterns of emesis: one an acute emetic response that occurs within the first 24 hours following administration,

and the second a delayed syndrome of emesis that develops 24 to 120 hours later.[29] Emesis that occurs within the first 24 hours is thought to be due to serotonin release secondary to cytotoxicity of enterocromaffin cells and is very responsive to 5-HT_3 receptor antagonists.[30,31] However, delayed emetic episodes are not responsive to 5-HT_3 antagonists for unknown reasons.[32] It may be that delayed emesis and severe hemorrhagic enteritis seen in our two dogs was an exaggerated model of the mechanism of delayed emesis in human beings.

Hypoalbuminemia that occurred in both dogs was attributed to severe hemorrhagic enteritis. Massive disruption of gastrointestinal epithelium evident in both dogs could easily explain sudden and significant loss of protein.

This pilot study demonstrated that a previously described saline loading protocol provided renal protection for 2 dogs receiving high-dose cisplatin; however, severe myelosuppression and gastrointestinal toxicosis preclude therapeutic use of 150 mg/m^2 at this time. Hypomagnesemia was noted but was not clinically important. Delayed onset of gastrointestinal toxicosis after administration at this dose may be similar to that associated with delayed onset emetic episodes in human beings receiving high-dose cisplatin.

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Table 1—Values of serum urea nitrogen (SUN), creatinine (SCr), magnesium (Mg), albumin, total white blood cells (WBC), segmented neutrophils (Segs), band neutrophils (Bands), lymphocytes (Lymphs), and platelets (Plts) for dog 1 before (day 0) and after (days 2, 4, and 5) administration of cisplatin (150 mg/m^2 IV) with saline loading.

Value	Day 0	Day 2	Day 4	Day 5	Reference Interval
SUN (mg/dl)	8.00	12.00	13.00	8.00	6.00 - 28.00
SCr (mg/dl)	0.60	0.70	0.50	0.60	0.80 - 1.90
Mg (mg/dl)	1.70	1.50	1.10	1.30	1.70 - 2.40
Albumin (g/dl)	3.30	3.10	2.30	1.70	2.80 - 3.60
WBC (x $10^3/\mu l$)	9.60	11.10	0.80	0.80	5.40 - 16.60
Segs (x $10^3/\mu l$)	5.57	8.99	0.14	0.32	3.20 - 10.70
Bands (x $10^3/\mu l$)	0.19	0.78	0.18	0.00	0.00 - 0.20
Lymphs (x $10^3/\mu l$)	3.07	1.11	0.49	0.74	0.80 - 5.60
Plts (x $10^4/\mu l$)	36.30	42.70	43.40	28.10	17.90 - 47.30

Table 2—Values of serum urea nitrogen (SUN), creatinine (SCr), magnesium (Mg), albumin, total white blood cells (WBC), segmented neutrophils (Segs), band neutrophils (Bands), lymphocytes (Lymphs), and platelets (Plts) for dog 2 before (day 0) and after (days 2, 4, and 5) administration of cisplatin (150 mg/m^2 IV) with saline loading.

Value	Day 0	Day 2	Day 4	Day 5	Reference Interval
SUN (mg/dl)	14.00	14.00	7.00	11.00	6.00 - 28.00
SCr (mg/dl)	0.60	0.60	0.50	0.90	0.80 - 1.90
Mg (mg/dl)	1.70	1.40	1.00	1.80	1.70 - 2.40
Albumin (g/dl)	3.50	3.40	2.50	2.20	2.80 - 3.60
WBC (x $10^3/\mu l$)	10.80	8.20	1.10	0.70	5.40 - 16.60
Segs (x $10^3/\mu l$)	5.61	6.81	0.31	0.08	3.20 - 10.70
Bands (x $10^3/\mu l$)	0.00	0.08	0.24	0.00	0.00 - 0.20
Lymphs (x $10^3/\mu l$)	4.21	0.82	0.53	0.59	0.80 - 5.60
Plts (x $10^4/\mu l$)	34.85	39.83	35.57	38.12	17.90 - 47.30

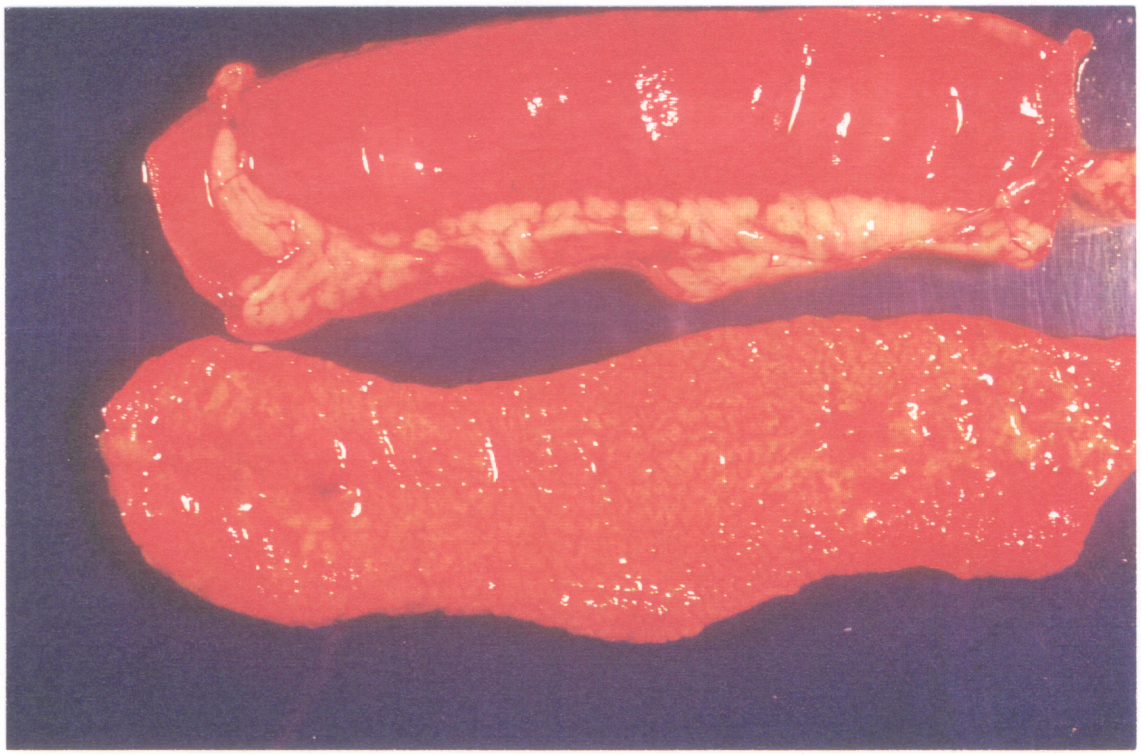

Figure 1. Gross section of small intestine from dogs 1 and 2, 4 days after receiving cisplatin (150 mg/m^2 IV). Small intestine of both dogs was diffusely red and covered by a green fibrinonecrotic exudate that extended from the duodenum to the ileum.

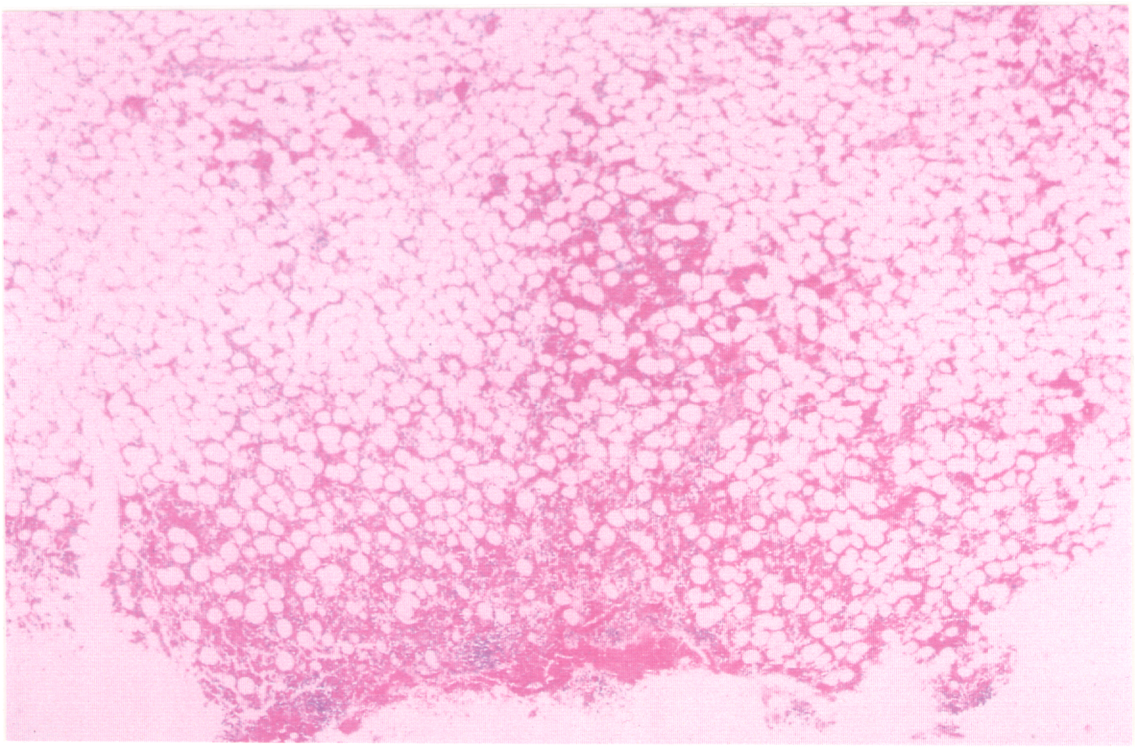

Figure 2. Histologic section of bone marrow from dogs 1 and 2, 4 days after receiving cisplatin (150 mg/m^2 IV). Bone marrow demonstrated marked hypoplasia of hematopoietic cells with all cell lines present and complete in their maturity.

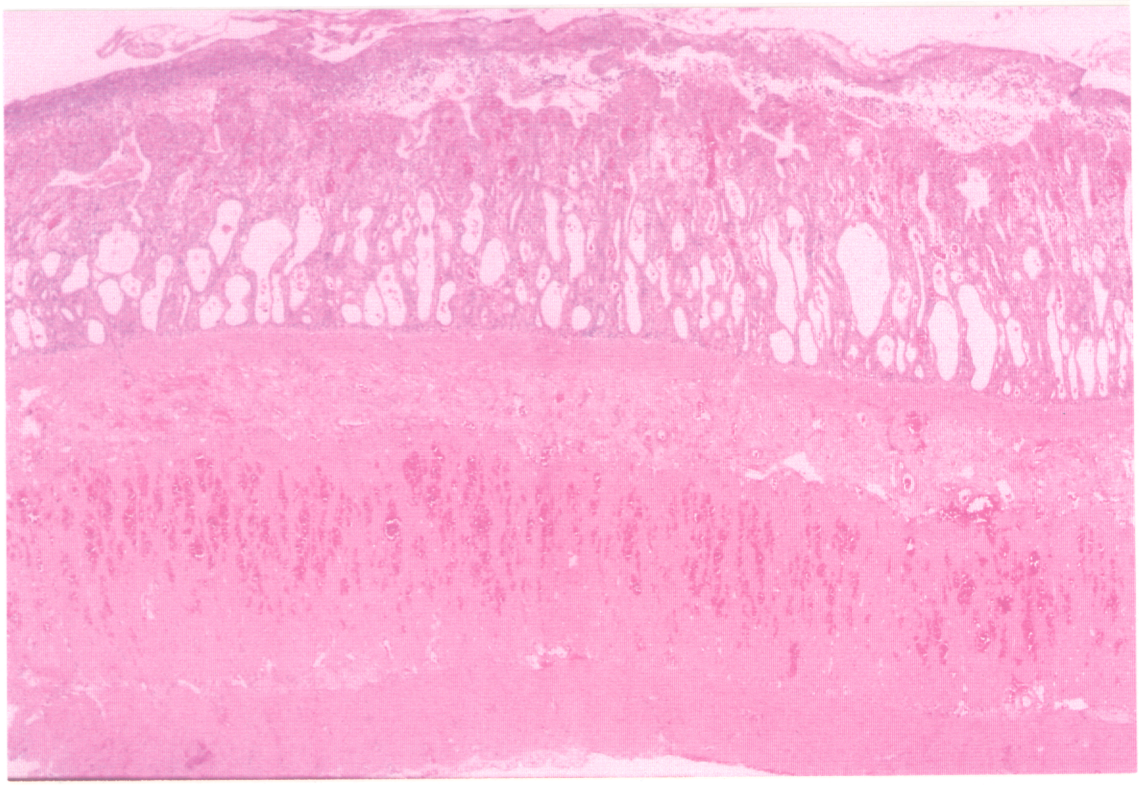

Figure 3. Histologic section of small intestine from dogs 1 and 2, 4 days after receiving cisplatin (150 mg/m^2 IV). Small intestinal villi were either absent or severely blunted. The surface of the small bowel was covered by fibrinonecrotic exudate that contained numerous bacteria. Mucosal glands exhibited marked epithelial hyperplasia with failure of differentiation, focal metaplasia, and loss of goblet cells. Intestinal crypts were dilated, contained necrotic cells, and were lined by attenuated, hyperplastic epithelium.

COMPARISON OF TWO SALINE LOADING PROTOCOLS FOR PREVENTING NEPHROTOXICOSIS ASSOCIATED WITH HIGH-DOSE CISPLATIN

Summary

This study was performed to compare efficacy of hypertonic saline with normal saline at preventing nephrotoxicosis associated with administration of high-dose cisplatin (90 mg/m^2 IV) to dogs. Twelve adult dogs were acclimated for 3 weeks. Dogs were included in the study on the basis of normal findings on physical examination and routine laboratory evaluation. Dogs were randomly assigned to 1 of 2 groups with 6 dogs each. On day 0, both groups received 0.9% saline IV (25 ml/kg/hr) for 3 hr. Afterwards, cisplatin was mixed in 7% saline (8.3 ml/kg) for group 1 dogs and 0.9% saline (8.3 ml/kg) for group 2 dogs and administered IV over 20 min. After completion of cisplatin infusion, 0.9% saline was continued (25 ml/kg/hr) for 1 hr in both groups. Exogenous creatinine clearances (ml/min/kg) were measured in all dogs prior to beginning the study (day 0) and on days 5, 12, and 21 after administration of cisplatin. Complete blood cell and platelet counts, and serum concentrations of magnesium, calcium, albumin, creatinine, and urea nitrogen were measured on day 0 and every 3 days after infusion of cisplatin for 21 days. On day 21, all dogs were euthanized and necropsies including gross and histiologic evaluation of tissues were done. Hemogram and serum chemistry data for groups 1 and 2 were compared by use of multivariate analysis of variance. Body weight data for days 0 and 21 were analyzed by use of a paired Student *t* test. The level of significance was a P value < 0.05.

All dogs demonstrated acute gastrointestinal toxicoses manifested as 1 to 4 episodes of vomiting within the first 24 hours after treatement with cisplatin. Three dogs

manifested delayed gastrointestinal toxicoses characterized by inappetence on day three or four. One of these dogs developed mild, small bowel diarrhea on day 3 that resolved within 24 hours. Mean values for exogenous creatinine clearances on days 0, 5, 12, and 21 were not significantly different when groups 1 and 2 were compared over time; however, there was a trend for increased values in group 1 dogs. Values for serum chemistries were not significantly different between groups; however, there was a trend for lower serum urea nitrogen, calcium, and albumin in group 2 dogs. Trends of lower serum creatinine and magnesium were noted in group 1 dogs. None of the dogs in either group were azotemic at any sampling period. Platelet counts were significantly higher in group 1 dogs when compared over time ($p=0.0108$). There was no significant difference between groups for total white blood cell counts, segmented neutrophils, or lymphocytes. However, there were trends for higher white blood cells, segmented neutrophils, and lymphocytes in group 1 dogs. Total white blood cell and neutrophil counts were lowest on days 6 and 15 for group 1 dogs and days 3 and 15 for group 2 dogs. Platelet counts were lowest on days 9 and 12 for groups 1 and 2, respectively. Lymphocyte counts were lowest on days 3 and 12 in group 1 dogs and days 6 and 12 in group 2 dogs. There was neither gross nor histologic evidence of toxicity attributable to cisplatin.

We conclude that high-dose cisplatin (90 mg/m^2 IV) can be administered to normal dogs using either protocol without biochemical evidence of reduced renal function. Gastrointestinal toxicoses were minimal and manageable compared with previous reports of dogs receiving high-dose cisplatin. Administration of cisplatin (90

mg/m^2 IV) in hypertonic saline was associated with higher platelet counts in dogs; however cause is unknown. This suggests that sodium chloride concentration may be related to severity of myelosuppression. Further studies are necessary before multiple cycles of high-dose cisplatin (90 mg/m^2 IV) can be safely recommended for treatment of cancer in dogs. Increasing dose of cisplatin to 90 mg/m^2 without increasing types and severity of toxicoses may provide improved response rates for treatment of cancer in dogs.

Introduction

Since its first reported use in veterinary patients in 1984, cisplatin has become one of the more frequently administered antineoplastic drugs in dogs.[1] Cisplatin is a cell-cycle phase-nonspecific chemotherapeutic agent that has exhibited efficacy against a wide variety of malignant epithelial and mesenchymal origin tumors. The most notable effect of cisplatin administration to veterinary patients has been reported in treatment of dogs with osteosarcoma. Administration of cisplatin (60 to 70 mg/m^2 IV) and amputation resulted in improved survival times that were 122 to 197 days greater than dogs treated by amputation alone.[2-6] Partial remissions and improved survival times have been reported in dogs with transitional cell carcinoma of the urinary bladder, nasal adenocarcinoma, thyroid carcinoma, metastatic mesenchyoma, and squamous cell carcinoma.[7-12]

Until recently, nephrotoxicity has been considered the dose-limiting toxicosis in human and veterinary patients. Adminstration protocols incorporating saline loading and/or hypertonic saline have allowed escalation of the dose of cisplatin and eliminated nephrotoxicosis as a dose-limiting factor in human beings.[13] Allowance of dose escalation has greatly improved therapeutic value of cisplatin in treating malignant neoplasia that previously failed to respond to other treatment. Adminstration of high-dose cisplatin (100-200 mg/m^2) provided complete response rates for 85 to 95% of men with bulky testicular cancer and partial response rates of 60 to 80% for women with ovarian cancer .[14,15]

Presently, cisplatin usually is adminstered at a dose of 60 to 70 mg/m^2 IV to dogs. Administration of the drug utilizing either a 4- or 6-hour saline loading protocol has been reported to be effective in preventing nephrotoxicosis in dogs without pre-existing renal disease.[16,17] Use of saline loading or hypertonic saline protocols for administering high-dose cisplatin has not been evaluated in dogs.

The study reported here was designed to compare efficacy of hypertonic saline (7%) with that of normal saline (0.9%) in preventing nephrotoxicity of cisplatin (90 mg/m^2 IV), to compare biochemical and hematologic parameters between groups of dogs, and to describe histologic changes that occur in kidney, bone marrow, and gastrointestinal tract. If the IV dose of cisplatin can be safely escalated to 90 mg/m^2 in dogs, more effective treatment of malignant neoplasia may follow as it has in human beings.

Materials and Methods

Acclimation of animals. This study was reviewed and approved by the University Animal Care and Use Committee of Virginia Polytechnic Institute. Twelve adult mixed-breed dogs (6 male, 6 female) were acquired and acclimated for a period of approximately 3 weeks prior to initiation of the study. Inclusion in the study was dependent on normal results of physical examination and laboratory evaluation including complete blood count, platelet count, serum chemistries, heartworm test (Knotts), and urinalysis. All dogs were free of disease during the acclimation period. They were dewormed for intestinal parasites with fenbendazole[f] (50 mg/kg q 24 h for 3 consecutive days) twice at 3-week intervals before the acclimation period. Zinc sulfate fecal floatations were negative for intestinal parasites and *Giardia*. All dogs were vaccinated for distemper, parvovirus, adenovirus 2, parainfluenza, and leptospirosis[g] 2 months prior to beginning the study.

Group assignment and animal monitoring. Dogs were randomly assigned to 1 of 2 groups with 6 dogs in each group (3 male, 3 female). Group 1 dogs received cisplatin[h] in 7% saline,[i] and group 2 dogs received cisplatin in 0.9% saline.[j] All researchers

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[f]Panacur Granules 22%, Hoechst-Roussel Agri-Vet Company, Somerville, NJ.

[g]Galaxy 6 MHP-L, Solvay Animal Health, Inc., Mendota Heights, Minn.

[h]Platinol, Bristol-Myers Squibb Co., Evansville, Ind.

[i]Fisher Scientific, Norcross, Ga.

directly responsible for adminstration of cisplatin, monitoring, and care of dogs were blinded to group assignment. Dogs were continuously monitored during the initial 24-hour period after administration of cisplatin. Physical examinations were performed twice daily throughout the experiment. Attitude, appetite, urination, defecation, and incidence of vomiting were monitored daily for 21 days. A dog was considered to have acute gastrointestinal toxicoses if it demonstrated emesis, diarrhea, or inappetance during the first 24 hours. Delayed gastrointestinal toxicoses were defined as emesis, diarrhea, or inappetence without azotemia at any time after the first 24 hours. Body weight was measured twice daily. Leash restricted exercise was provided 3 to 4 times daily. Dogs were fed a commercially available, moderately low-fat, highly digestible diet.[k]

Administration of cisplatin. On day 0, both groups of dogs were administered 0.9% saline IV (25 ml/kg/hr) for 3 hours through an indwelling cephalic vein catheter. Afterwards, cisplatin (90 mg/m^2) was mixed in 7% saline (8.3 ml/kg) for group 1 dogs and 0.9% saline (8.3 ml/kg) for group 2 dogs and administered IV over a 20-minute period. After completion of cisplatin infusion, 0.9% saline was continued (25 ml/kg/hr) for an additional hour in both groups. All dogs received cisplatin between 3 and 4 pm.

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[j]Baxter Healthcare, Bridgeport, NJ.

[k]ID dry, Hills Pet Products, Topeka, Kan.

Administration of antiemetics. In anticipation of nausea and vomiting, all dogs received metoclopramide[l] (0.2 mg/kg IV) and dexamethasone sodium phosphate[m] (0.22 mg/kg IV) 30 minutes prior to beginning infusion of cisplatin. Immediately prior (time 0) to infusion of cisplatin another dose of metoclopramide (0.2 mg/kg IV) was administered; this was repeated 1 hour post-infusion.

Exogenous creatinine clearance. Glomerular filtration rate (GFR) was estimated in all dogs prior to beginning the study (day 0) and on days 5, 12 and 21 by measuring exogenous creatinine clearance.[18] All dogs were fasted for 8 hours and water was provided *ad libitum*. An indwelling urinary catheter was placed and creatinine solution[n] (50 mg/ml) was injected subcutaneously (2 ml/kg) at time 0. Immediately after the creatinine was injected, a volume of water equal to 3% of the dog's body weight was given via stomach tube. The urinary bladder was rinsed with 100 ml of sterile saline 60 minutes after injection of creatinine solution. All urine was collected for a 20-minute period and an aliquot was submitted for measurement of creatinine.[o] Blood was collected for measurement of serum creatinine[o] at 60 and 80 minutes after injection of

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[l]Solopak Laboratories Inc., Elkgrove Village, Ill.

[m]Vedco, St. Joseph, Mo.

[n]Creatinine anhydrous, Sigma Chemical Co., St. Louis, Mo.

[o]Kodak Ektachem 700, Eastman Kodak Co., Rochester, NY.

creatinine solution. Glomerular filtration rate was calculated using a standard clearance formula.[18]

Reference intervals for exogenous creatinine clearance were derived following standard laboratory protocol. Briefly, exogenous creatinine clearances were performed on 38 adult dogs (19 of each sex) of multiple breeds. All dogs were normal on physical examination and laboratory evaluation including complete blood count, serum chemistries, and urinalysis. Mean exogenous creatinine clearances for these 38 dogs was 3.13 ± 0.76 ml/kg/min. Considering that 95% of normal dogs should have values that are within two standard deviations of the mean, a reference interval of 2.1 to 4.75 was established for our laboratory. These values are similar to those previously reported for exogenous creatinine clearance in normal dogs (4.14 ± 0.53) ml/min/kg).[18]

Laboratory evaluation. Complete blood cell and platelet counts,[p] serum magnesium,[o] and serum chemistries[o] were performed immediately prior to administration of cisplatin and every 3 days after treatment for 21 consecutive days.

Gross and histologic examinations. On day 21 of the study, all dogs were euthanized by barbiturate overdose[q] and necropsies were done. Sections of kidney, bone marrow, stomach, jejunum, ileum, colon, liver, pancreas, heart, and lung were fixed in neutral

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[p]System 9000 Automated Cell Counter, Serono Baker Diagnostics, Allentown, Penn.

[q]Beuthansia-D Special, Schering-Plough Animal Health Corporation, Kenilworth, NJ.

buffered 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined by light microscopy. A single pathologist[r] who was blinded to treatment groups examined all sections.

Statistical evaluation. Statistical analyses were performed using a microcomputer-based statistical package.[s] Results of complete blood and platelet counts and serum chemistry data for groups 1 and 2 were compared by use of multivariate analysis of variance for repeated measures. The Shapiro-Wilk test for normality of residuals was performed to insure appropriate test selection. Body weights on days 0 and 21 were analyzed by use of a paired Student *t* test. The level of significance was a P value < 0.05.

Results

Clinical findings. All dogs in both groups demonstrated acute gastrointestinal toxicosis manifested as 1 to 4 episodes of vomiting within the first 24 hours after treatment with cisplatin. Three dogs (1 dog from group 1, 2 dogs from group 2) manifested delayed gastrointestinal toxicosis characterized by inappetence on day three or four. One of these dogs (group 2) developed mild, small bowel diarrhea on day 3 that resolved within 24 hours. Dogs in group 1 gained 4 $\pm$ 3% of their body weight over baseline, and dogs

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[s]PCSAS, SAS Institute, Cary, NC.

in group 2 gained 3 ± 4% of their body weight over baseline (day 0); this difference was not significant. None of the dogs in either group demonstrated weight gain or loss of more than 3% between morning and evening measurements.

Exogenous creatinine clearance. Mean values for exogenous creatinine clearances on days 0, 5, 12, and 21 were not significantly different when groups 1 and 2 were compared over time (Table 1). None of the dogs in either group had a creatinine clearance lower than normal reference interval for our laboratory (Figure 1).

Biochemical findings. Serum chemistries were not statistically different between groups (Tables 2-6); however, there was a trend for lower serum urea nitrogen (Figure 2), calcium (Figure 3) and albumin (Figure 4) in group 2 dogs. Trends of lower serum creatinine (Figure 5) and magnesium (Figure 6) were noted in group 1 dogs. Dogs in both groups were hypomagnesemic during the entire study (Figure 6) with mean values for both groups below normal reference intervals on days 0, 3, 6, 9, 12, 15, 18, and 21. Trends were noted for lower serum magnesium concentrations after administration of cisplatin; however, there were no clinical signs associated with hypomagnesemia in any dogs. None of the dogs in either group demonstrated azotemia at any sampling period (Figures 2 and 5).

Hemogram findings. There was no significant difference between groups for total white blood cells, segmented neutrophils, or lymphocytes (Tables 7-9). However, there were trends for higher white blood cells (Figure 7), segmented neutrophils (Figure 8), and lymphocytes (Figure 9) in group 1 dogs. Bimodal nadirs were evident for each cell line observed. Total white blood cell counts were lowest on days 6 and 15 for group 1 and days 3 and 15 for group 2 (Figure 7). Segmented neutrophils were lowest on days 6 and 15 for group 1 dogs and days 3 and 15 for group 2 dogs (Figure 9). Lymphocyte counts were lowest on days 3 and 12 for group 1 dogs and days 6 and 12 for group 2 dogs (Figure 9). Platelet counts were significantly higher in group 1 dogs (Table 10). Platelet counts were lowest on day 9 and 12 for groups 1 and 2 respectively (Figure 10).

Gross necropsy and histologic findings. Results of necropsies revealed no gross abnormal findings in any dogs. No histologic lesions were identified in any sections of organs examined.

Discussion

Despite use of antiemetics, all dogs vomited at least one time within 24 hours after administration of cisplatin. This was not unexpected since acute vomiting associated with IV administration of cisplatin was demonstrated to be a dose-dependent phenomena in human beings and dogs.[19,20] Metoclopramide and dexamethasone were used as antiemetics in this study due to availability, lack of expense, and reported efficacy.[21]

Antiemetic effects of metoclopramide initally were attributed to blockade of dopamine receptors at the chemoreceptor trigger zone within the area postrema of the medulla.[22] Metoclopramide was later proven to be a weak 5-HT_3 antagonist as well, which explained the drug's moderate efficacy in controlling cisplatin-associated emesis.[23] The exact mechanism of corticosteroids in preventing cisplatin associated emesis has not been determined; however, inhibition of prostaglandin synthesis in the emetic center has been postulated.[24] Corticosteroids and metoclopramide have demonstrated synergistic antiemetic activity in human beings receiving cisplatin.[25] These findings led to adaption of similar antiemetic protocols in dogs including the one in our study.

Acute gastrointestinal toxicity has been attributed to release of seritonin (5-HT_3) from enterocromaffin cells of the gastrointestinal tract.[26] Centrally mediated events in the area postrema and associated structures of the emetic reflex such as the nucleus tractus solitarius also have very high concentrations of 5-HT_3 receptors.[26] Developement of pure 5-HT_3 antagonists (granisetron and ondansetron) and their subsequent success in preventing cisplatin associated emesis in human beings and dogs further support the role of 5-HT_3 receptors.[25,27-29]

Delayed gastrointestinal toxicoses have been defined as nausea, vomiting and anorexia ocurring 24 to 100 or more hours after cisplatin therapy.[30] Delayed gastrointestinal toxicoses manifested as vomiting, anorexia, and weight loss recently were described in dogs receiving cisplatin (90 mg/m^2 IV) without saline loading.[31] Dogs in that study lost 5% to 13% of their body weight during the 13 days after infusion of

cisplatin.[31] Three of the dogs in our study demonstrated transient anorexia with one dog concurrently exhibiting transient, mild, small bowel diarrhea. Intravenous hydration before and after administration of cisplatin was thought to mitigate gastrointestinal toxicoses in human beings and may be the reason for low prevalence of signs in our dogs compared with dogs previously reported to receive the same dose of cisplatin without concurrent administration of fluid.[31-33]

None of the dogs in our study demonstrated more than 3% weight loss during the study. In fact, all dogs experienced a net gain in body weight by the end of the 21-day study. Weight gain in part can be attributed to low incidence of delayed gastrointestinal toxicoses among dogs in this study. Weight gain also could be attributed in part to inadvertent dietary oversupplementation. Dogs were fed a maintenance kibble[t] according to basal energy caloric requirements during the mandatory isolation period. Upon being transferred to the research environment for acclimation, dogs were fed a moderate fat, highly digestible diet that was of slightly higher caloric content[f]. The same volume of food was fed, mildly increasing available daily caloric consumption of all dogs throughout acclimation and experimental periods.

Severe nephrotoxicity associated with intravenously administered cisplatin was initially recognized in early toxocologic trials with dogs, mice, and monkeys.[34] Phase I clinical trials of cisplatin (30 mg/m^2 IV) in human beings revealed similar findings and almost led to abandonment of the drug by the medical community.[35] Addition of fluid

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[t]Canine maintenance, Hills Pet Products, Topeka, Kan.

loading with normal saline prior to administration of cisplatin greatly reduced nephrotoxicosis associated with the drug in human beings and actually allowed escalation of the dose to 90 mg/m^2.[36] Saline loading also was successful in preventing nephrotoxicosis in dogs treated with cisplatin (70 mg/m^2)[16,17,37] Administration of cisplatin in hypertonic saline not only prevented nephrotoxicoses, but allowed dose escalation to 100-200 mg/m^2 in human beings, which was associated with increased efficacy in human beings.[13] Recently it was demonstrated that a single dose (70 mg/m^2) of cisplatin mixed in hypertonic (3%) saline could be administered to dogs without significant decrease in renal function.[38] It seemed a reasonable hypothesis that protocols utilizing saline loading, hypertonic saline, or both might allow dose of cisplatin to be safely escalated in dogs. We previously determined that cisplatin could be administered to dogs at a dose of 150 mg/m^2 using a 4-hour saline loading protocol without laboratory or histologic evidence of renal disease.[39] However, severe myelosuppression and gastrointestinal toxicity were unacceptable dose-limiting toxicoses.[39] Previous studies have demonstrated that cisplatin administered at a dose of approximately 90 mg/m^2 IV consistently produced azotemia in dogs.[31,40,41] It also has been demonstrated that dogs receiving cisplatin (90 mg/m^2) IV demonstrated varying amounts of gastrointestinal toxicoses manifested as vomiting, anorexia, or 5 to 13% weight loss.[31] We therefore selected a cisplatin dose of 90 mg/m^2 to compare effect of hypertonic saline (7%) and normal saline (0.9%) in conjunction with saline loading in preventing nephrotoxicosis. Cisplatin nephrotoxicosis was found to be dose-dependent in dogs.[34,40] However, the

exact mechanism of nephrotoxicity cisplatin has not been determined. After IV administration of cisplatin to dogs urinary concentrations increased rapidly with 50 to 60% of the administered dose recovered in urine within 4 hours.[42] Based on studies in human beings cisplatin entered the urine primarily as a result of glomerular filtration[43] and to some degree tubular secretion.[44] Recently, net tubular secretion of cisplatin was definitively demonstrated in dogs.[45] Cisplatin was excreted primarily by the kidneys in dogs and renal concentrations remained the highest for 6 days.[42] Renal blood flow and glomerular filtration rate (GFR) did not change significantly immediately after IV administration of cisplatin (129 to 155 mg/m^2) to dogs.[46] Lithium clearance and perfusate disappearance of *p*-amminohippuric acid studies supported the theory that cisplatin-induced nephrotoxicity was initiated by impairment of the proximal tubular function.[47,48]

It has been hypothesized that post-aquation platinum species were responsible for injury that occurs to tubular epithelial cells.[49] Water molecules in the dilute urine of the proximal tubule combined with high urine concentration of cisplatin. An aquation reaction ensued and the cisplatin molecule exchanged labile chloride ions for water or hydroxyl ions.[50] Products of this aquation reaction were reactive electrophiles, cytotoxic platinum hydroxyl complexes, their dimers, and trimers.[51] Maintaining the cisplatin molecule in a high chloride environment would prevent the formation of toxic aquation products in the proximal tubule and thus prevent tubular injury.[49] Hypertonic saline (4.5%) greatly reduced lethality in mice receiving cisplatin compared with mice receiving

cisplatin in distilled water.[52] Also, rats treated with hypertonic saline had significantly reduced concentrations of toxic platinum aquation species in their urine.[49] These findings support the theory that maintenance of the *cis* configuration of the platinum molecule in urine prevents nephrotoxicity and supports the hypothesis of the present study.

In our study saline loading with and without administration of hypertonic saline was effective in preserving renal function in dogs receiving cisplatin (90 mg/m^2 IV) cisplatin. Lack of statistical difference between groups can be partially explained by the standard deviations for each of the sampling periods. This may have been improved upon by increasing number of animals in each group; however, the ethical and economic considerations associated with increasing sample size made it impractical. Exogenous creatinine clearance was chosen as our method to evaluate renal function because it is technically simple, relatively inexpensive, and it closely correlates with inulin clearance, the gold standard for measuring GFR.[18,53] Collection of urine during 2 to 3 consecutive 20-minute sampling periods for calculation of exogenous creatinine clearance might also have reduced the amount of standard deviation within treatment groups and should be considered for future studies.[53]

It could also be argued that addition of hypertonic saline did not demonstrate any greater efficacy because the animals had previously been saline loaded. That is, increasing chloride concentration in the proximal tubule with hypertonic saline provided no improved protection because chloride ion concentration was saturated. Chloride concentration was found to be inversely proportional to percent mortality after

administration of cisplatin to mice; however, sodium chloride concentrations exceeding 4.5% were not evaluated.[52]

Plasma decay and urinary excretion rates for filterable platinum were not evaluated in this study, therefore, it could be argued that lack of measurable nephrotoxicity was due to altered pharmacokinetics of cisplatin. Hypertonic saline and/or saline loading could have accelerated urinary excretion of platinum, thus decreasing plasma residence time of platinum and reducing cytotoxicity. This could be significant in that the tumoricidal properties of cisplatin also could be reduced; however, experiments in rats have demonstrated that hypertonic saline does not alter antitumor activity of cisplatin.[49,52] Also, evidence of cytotoxicity was manifested as myelosuppression in our dogs. Nadirs and severity of myelosuppression, excluding platelet data, were similar to previous studies in dogs receiving cisplatin both with and without any type of concurrent fluid administration.[17,31,41]

Since none of the dogs in either group became azotemic or exhibited a creatinine clearance below normal reference intervals for our laboratory, it appears reasonable to suggest that both protocols provided adequate renal protection from toxicity associated with high dose cisplatin. Further studies are needed to evaluate efficacy of these protocols in providing renal protection following multiple cycles of cisplatin chemotherapy. Tumor reponse also should be closely monitored to evaluate alterations in cisplatin's antineoplastic properties when using these protocols.

Dogs in both groups became hypomagnesemic with mean values falling below reference intervals during all sampling periods. Increased fractional excretion of magnesium was reported in dogs receiving cisplatin (90 mg/m^2 IV) without benefit of fluid loading.[31] Although fractional excretion of magnesium was not evaluated in this study, magnesium wasting in the urine provides a reasonable explanation for the mild hypomagnesemia demonstrated in this study.

Onset and degree of myelosuppression in this study, with the exception of platelets, were similar to previous studies on dogs receiving cisplatin both with and without concurrent administration of fluid.[17,31,41] In contrast, our study demonstrated trends of higher total white cells, neutrophils, and lymphocytes in dogs that received cisplatin in hypertonic saline compared with dogs that received cisplatin in normal saline. Statistical significance may have been obtained if there had been more dogs in each treatment group. This unexpected trend may be specific to dogs; however, fluid loading alone failed to reduce amount of myelosuppression associated with administration of cisplatin to dogs in one study.[40]

Cisplatin-associated myelosuppression was demonstrated to be dose-dependent in human beings and dogs; however the mechanism of cisplatin-associated myelosuppression has not been completely determined.[34,54] Recent success of peripheral blood progenitor cell support therapy suggested that myelosuppression results from damaged progenitor cells, possibly as early as the committed colony forming units, and aberrant cytokine production, specifically granulocyte-colony stimulating factor.[55]

Increased concentration of sodium chloride in the vehicle for administration of cisplatin also may have accounted for the trends of reduced myelosuppression seen in group 1 dogs. Incorporation of saline loading and hypertonic saline (3.5%) into protocols for administering cisplatin greatly reduced incidence of nephrotoxicosis in human beings with no reduction of myelosuppression.[13,56] Despite evidence of an inversely proportional relationship between chloride concentration and severity of cisplatin associated toxicoses, administration protocols using higher sodium chloride concentrations have not been reported.[52,u] Chloride concentrations greater than 3.5% may not further reduce nephrotoxicosis, but instead reduce myelosuppression by yet unknown mechanisms. Definitive answers would require further studies.

Significantly higher platelet counts in dogs receiving hypertonic saline may have been attributed to several factors. Platelets, although derived from the same pluripotent and myeloid stem cell as erythrocytes and other white cells, were proven to be very unique in two ways. First, megakaryocytopoiesis (ie, production of platelets) involved polyploidization of precursor cells as a result of nuclear division without cytoplasmic division (endomitosis or endoreduplication).[57] Secondly, a cytokine specifically responsible for platelet production called thrombopoietin was necessary. Thrombopoietin was derived from the kidneys of mice and human embryos.[58-60] Exact location of thrombopoietin production in the kidney has not been determined. Mice injected with thrombopoietin after sublethal irradiation produced increased megakaryocyte size and

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[u]Medline search, June 28, 1994 by author.

significantly higher platelet counts than mice injected with albumin placebo.[61,62] This suggested that maintenance of thrombopoietin production reduced effects of cytotoxic injury and/or accelerated recovery from cytotoxic events. Thrombopoietin concentrations were not measured in this study; however, it was possible that the site of thrombopoietin production in the canine kidney was deleteriously affected in group 2 dogs and protected by hypertonic saline in group 1 dogs. If thrombopoietin production was not affected in dogs receiving hypertonic saline because of reduced renal tubular injury, they would be expected to have experienced less platelet-associated cytotoxicity and/or recovered more effectively. Further studies should be directed at evaluating thrombopoietin concentrations in dogs receiving cisplatin and effect of thrombopoietin supplementation. This may allow more accurate localization of thrombopoietin production centers in the kidney or completely disprove their existence in renal tissue.

In conclusion, there was no significant difference in values for exogenous creatinine clearance or serum concentrations of urea nitrogen or creatinine over time between group 1 (7% saline) and group 2 (0.9% saline) protocols. There also was no evidence of decreased renal function or histologic evidence of tubular necrosis in either group that could be attributed to administration of cisplatin. This was important in that administration of cisplatin (90 mg/m^2 IV) alone reliably produced azotemia in previous studies of dogs.[31,40,41] It appeared that degree of thrombocytopenia was favorably augmented by infusion of cisplatin in hypertonic saline with concurrent saline loading, although the cause remains unknown. Gastrointestinal toxicity was mild and manageable

using either protocol. Further studies in tumor bearing dogs, receiving multiple 21-day cycles of treatment, will be required for definitive evaluation of efficacy and toxicity of high-dose cisplatin in dogs, as well as usefulness of hypertonic saline for prevention of cisplatin-associated toxicoses.

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Table 1—Mean values (± standard deviation) for creatinine clearance (ml/min/kg) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	2.76 ± 0.45	3.04 ± 0.60
5	3.30 ± 0.68	3.29 ± 0.78
12	2.84 ± 0.61	2.76 ± 0.55
21	3.17 ± 0.53	3.03 ± 0.70

$P = 0.9673$ when values from days 0, 5, 12, and 21 were compared using multivariate ANOVA for repeated measures.

Table 2—Mean values (± standard deviation) for serum urea nitrogen (mg/dl) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	14.33 ± 6.31	10.83 ± 3.25
3	14.33 ± 3.33	11.17 ± 1.60
6	16.00 ± 4.20	9.50 ± 1.05
9	14.50 ± 2.81	15.00 ± 4.24
12	13.67 ± 4.46	12.17 ± 4.96
15	19.17 ± 3.71	14.50 ± 4.04
18	15.00 ± 4.24	15.00 ± 3.41
21	14.00 ± 3.46	14.00 ± 4.60

$P = 0.7560$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 3—Mean values (± standard deviation) for serum calcium (mg/dl) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	10.61 ± 0.48	10.40 ± 0.49
3	10.54 ± 0.25	10.43 ± 0.36
6	10.42 ± 0.26	10.24 ± 0.41
9	10.63 ± 0.30	10.44 ± 0.39
12	10.59 ± 0.33	10.43 ± 0.43
15	10.74 ± 0.26	10.48 ± 0.53
18	10.52 ± 0.33	10.46 ± 0.39
21	10.61 ± 0.31	10.50 ± 0.45

P = 0.1391 when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 4—Mean values (± standard deviation) for serum albumin (gm/dl) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	3.23 ± 0.18	3.18 ± 0.15
3	3.42 ± 0.19	3.37 ± 0.16
6	3.17 ± 0.20	3.10 ± 0.17
9	3.25 ± 0.30	3.07 ± 0.18
12	3.23 ± 0.16	3.10 ± 0.17
15	3.20 ± 0.09	3.10 ± 0.16
18	3.22 ± 0.21	3.05 ± 0.18
21	3.23 ± 0.12	3.07 ± 0.15

$P = 0.1919$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 5—Mean values (± standard deviation) for serum creatinine (mg/dl) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	1.03 ± 0.15	1.03 ± 0.18
3	0.97 ± 0.12	1.08 ± 0.20
6	1.10 ± 0.24	1.23 ± 0.34
9	1.08 ± 0.19	1.12 ± 0.19
12	1.03 ± 0.19	1.12 ± 0.20
15	0.98 ± 0.15	1.06 ± 0.18
18	1.00 ± 0.17	1.07 ± 0.14
21	0.97 ± 0.15	1.05 ± 0.17

$P = 0.4863$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 6—Mean values (± standard deviation) for serum magnesium (mg/dl) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	1.67 ± 0.12	1.65 ± 0.12
3	1.55 ± 0.19	1.63 ± 0.24
6	1.48 ± 0.13	1.65 ± 0.29
9	1.48 ± 0.13	1.55 ± 0.19
12	1.53 ± 0.18	1.58 ± 0.22
15	1.50 ± 0.17	1.63 ± 0.27
18	1.55 ± 0.12	1.55 ± 0.21
21	1.48 ± 0.12	1.55 ± 0.19

$P = 0.4886$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 7—Mean values (± standard deviation) for total white blood cells (x $10^3/\mu l$) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	12.82 ± 3.96	14.63 ± 7.89
3	8.83 ± 3.30	7.07 ± 2.88
6	8.67 ± 3.50	7.33 ± 2.88
9	12.82 ± 4.71	8.90 ± 4.85
12	8.62 ± 3.26	7.25 ± 4.47
15	8.15 ± 2.25	6.10 ± 3.06
18	9.35 ± 2.09	7.17 ± 3.26
21	9.80 ± 1.56	9.15 ± 3.66

$P = 0.4316$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 8—Mean values (± standard deviation) for segmented neutrophils (x $10^3/\mu l$) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	8.27 ± 1.89	8.70 ± 5.32
3	5.40 ± 2.50	3.79 ± 1.40
6	5.15 ± 2.36	4.67 ± 1.43
9	9.34 ± 4.10	5.19 ± 3.30
12	5.14 ± 2.24	3.81 ± 3.43
15	4.22 ± 2.27	2.59 ± 1.86
18	5.63 ± 1.86	3.38 ± 1.94
21	5.82 ± 1.71	5.07 ± 2.27

$P = 0.1829$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 9—Mean values (± standard deviation) for lymphocytes (x $10^3/\mu l$) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) to normal dogs mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	2.88± 1.57	2.91 ± 1.35
3	2.06 ± 0.66	1.94 ± 0.81
6	2.75 ± 1.31	1.91 ± 0.86
9	2.72 ± 0.96	2.64 ± 1.12
12	2.18 ± 1.07	2.19 ± 0.19
15	3.01 ± 0.98	2.91 ± 0.90
18	2.87 ± 0.96	2.84 ± 0.91
21	3.02 ± 1.16	2.89 ± 1.34

$P = 0.7415$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

Table 10—Mean values (± standard deviation) for platelets (x $10^3/\mu l$) measured before (day 0) and after IV administration of cisplatin (90 mg/m^2 of body surface area) mixed in either 7% saline (group 1) or 0.9% saline (group 2).

DAY	Group 1	Group 2
0	398.67 ± 72.53	311.17 ± 73.89
3	408.83 ± 71.25	377.17 ± 85.76
6	369.83 ± 71.63	324.67 ± 99.19
9	217.83 ± 51.18	140.33 ± 50.50
12	261.67 ± 68.36	110.83 ± 43.01
15	454.67 ± 116.63	245.00 ± 77.42
18	490.00 ± 130.87	282.50 ± 69.89
21	444.17 ± 119.32	294.33 ± 76.75

$P = 0.0108$ when values from days 0, 3, 6, 9, 12, 15, 18, 21 were compared using multivariate ANOVA for repeated measures.

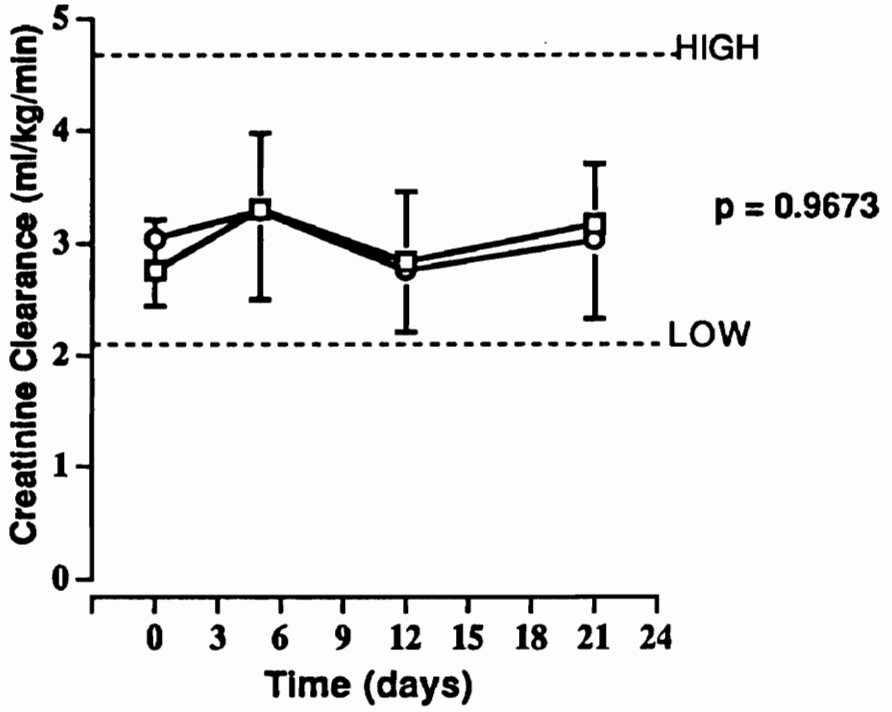


Figure 1. Exogenous creatinine clearances (mean ± SD) before (day 0) and after (days 5, 12, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

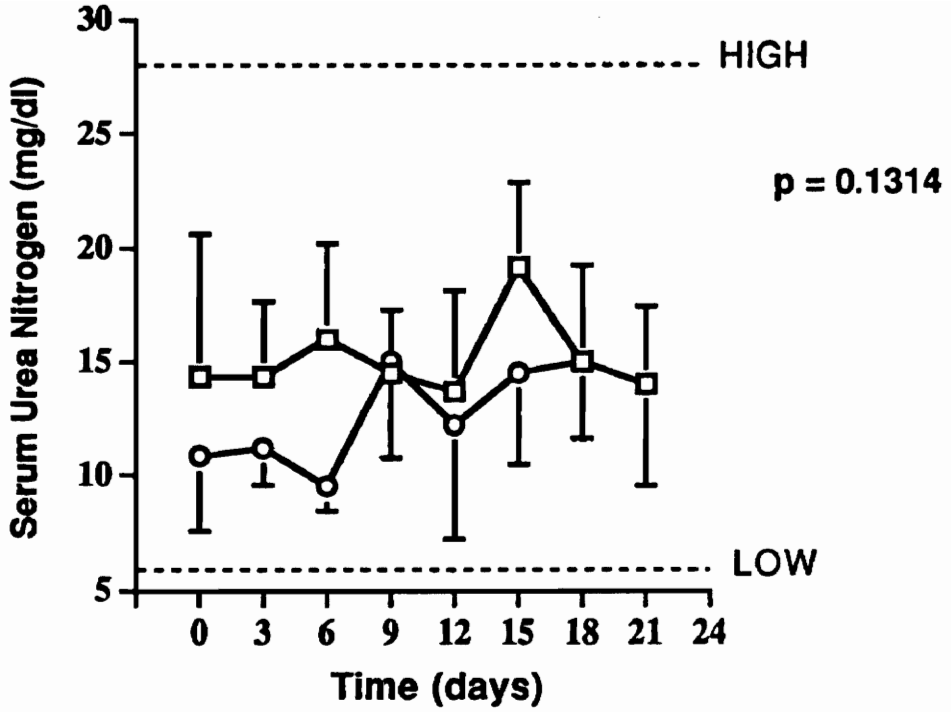


Figure 2. Serum urea nitrogen (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

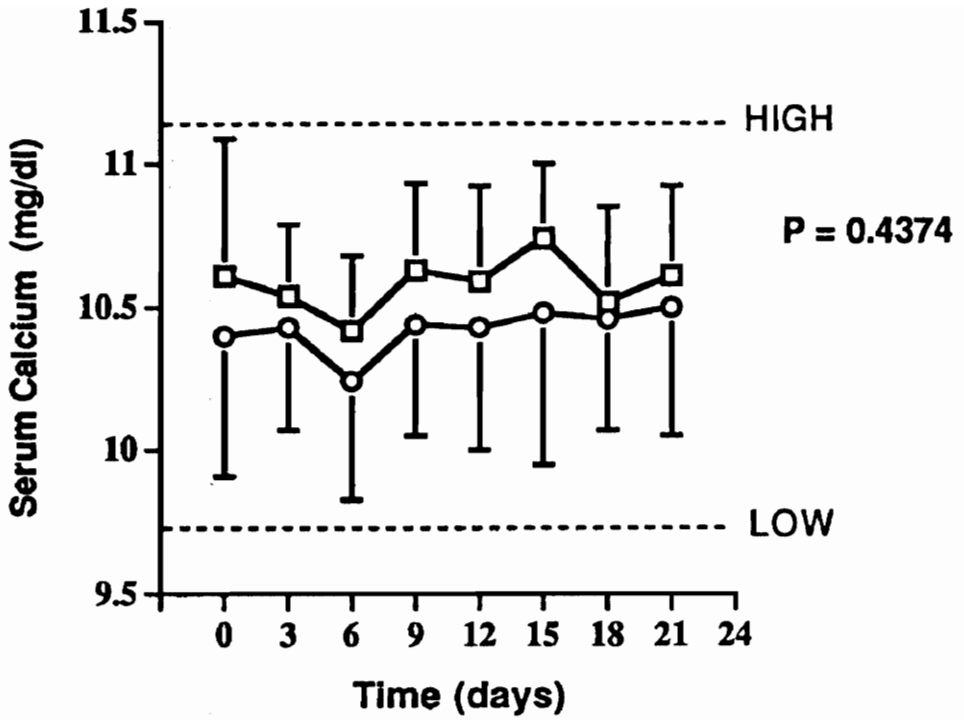


Figure 3. Serum calcium (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

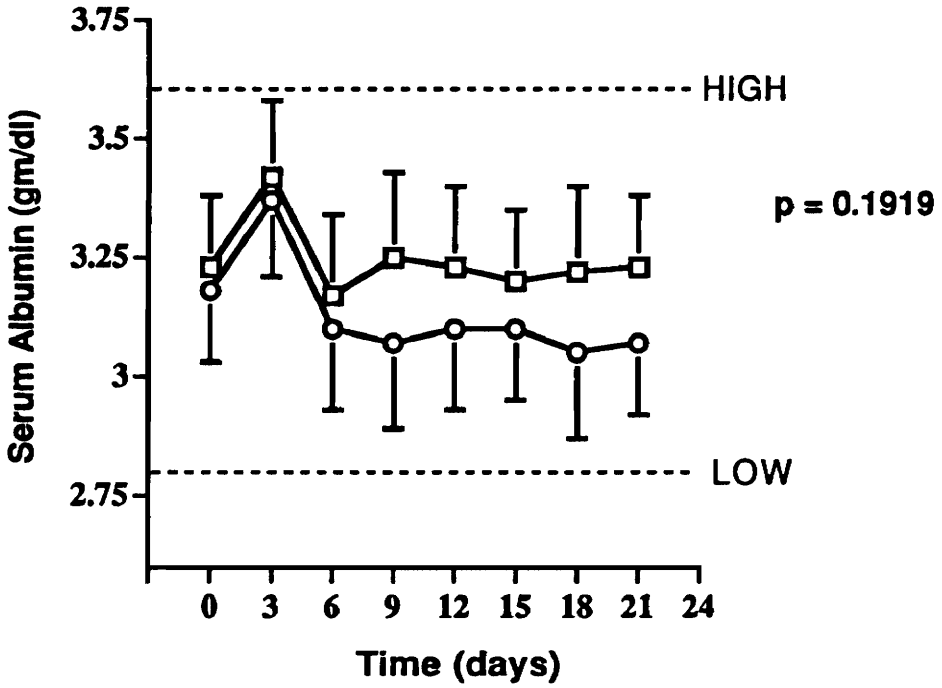


Figure 4. Serum albumin (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

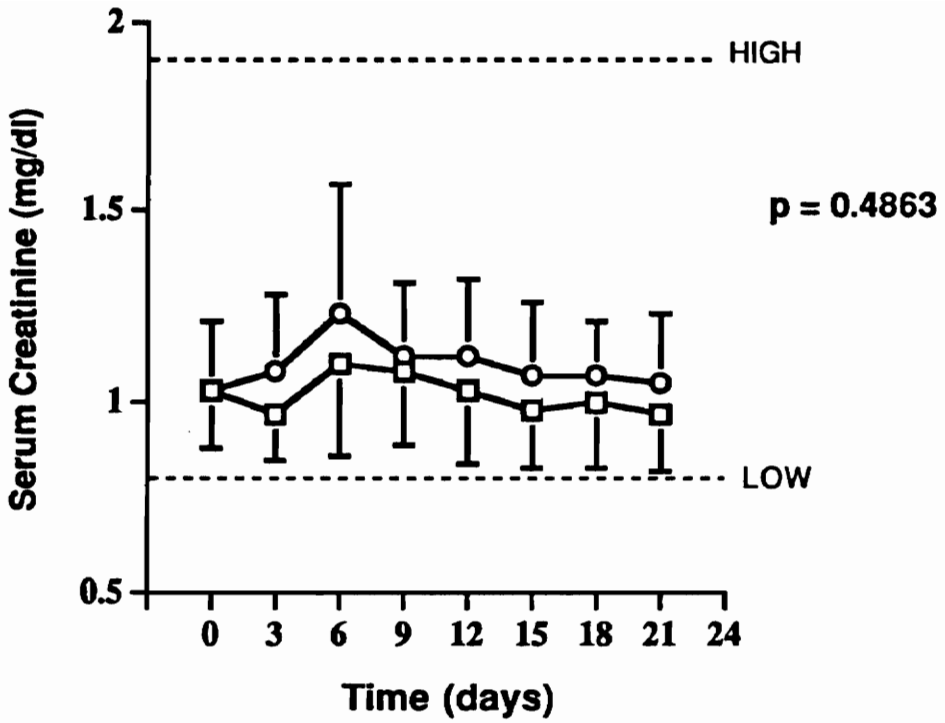


Figure 5. Serum creatinine (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

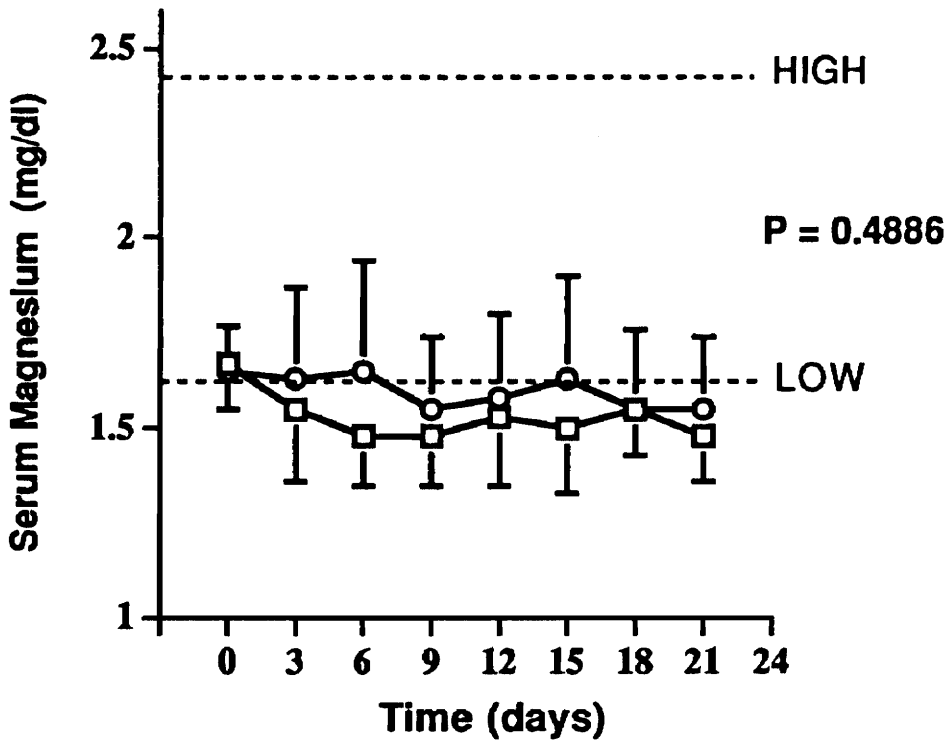


Figure 6. Serum magnesium (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

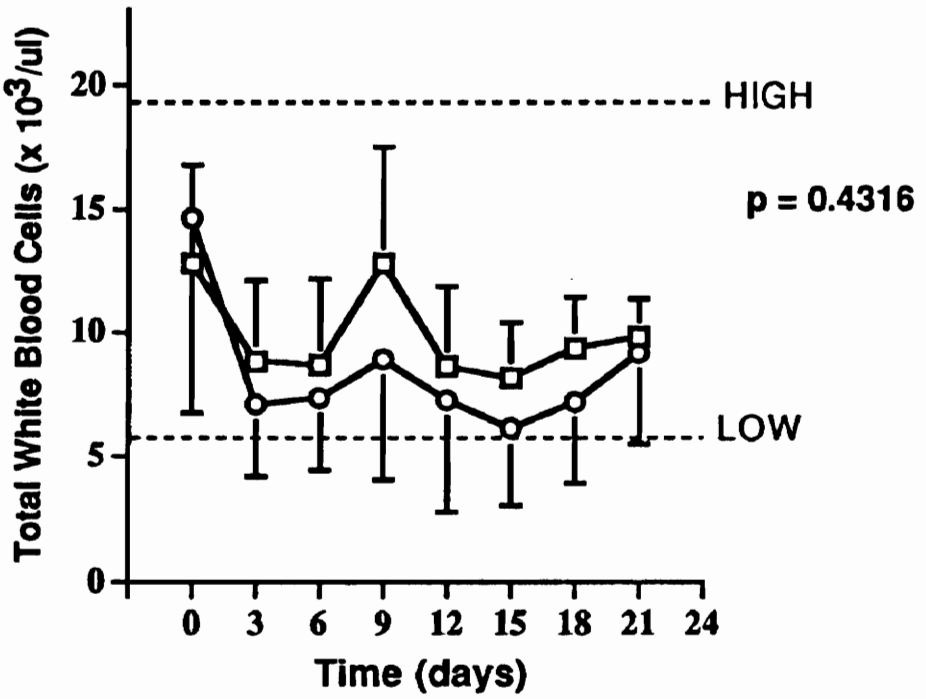


Figure 7. Total white blood cells (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

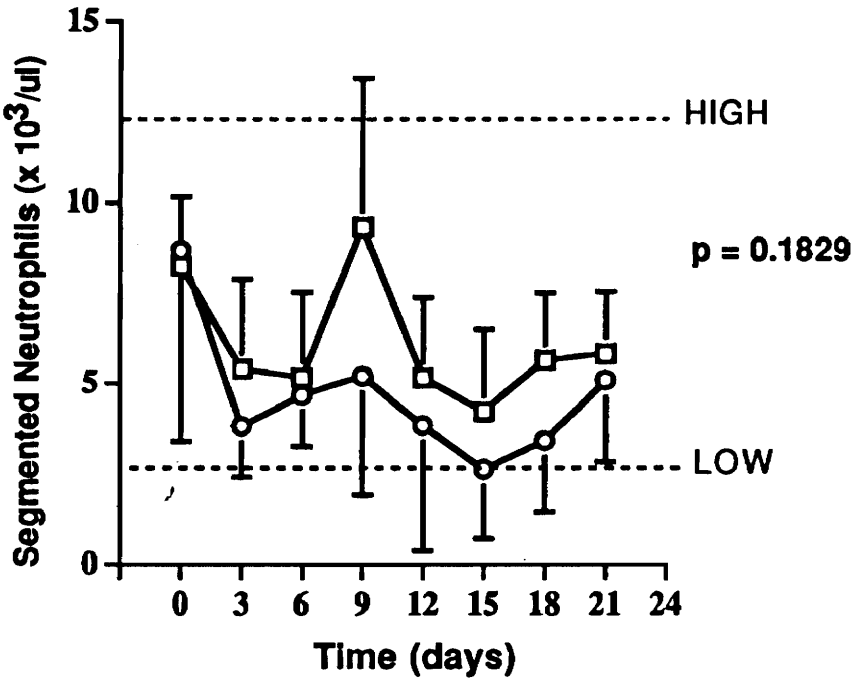


Figure 8. Segmented neutrophils (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

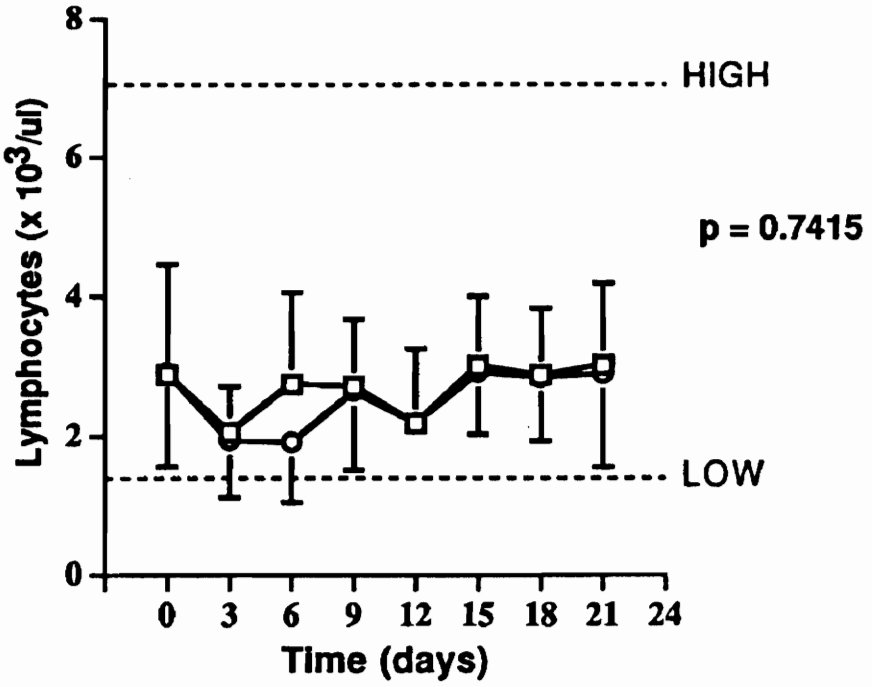


Figure 9. Lymphocytes (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

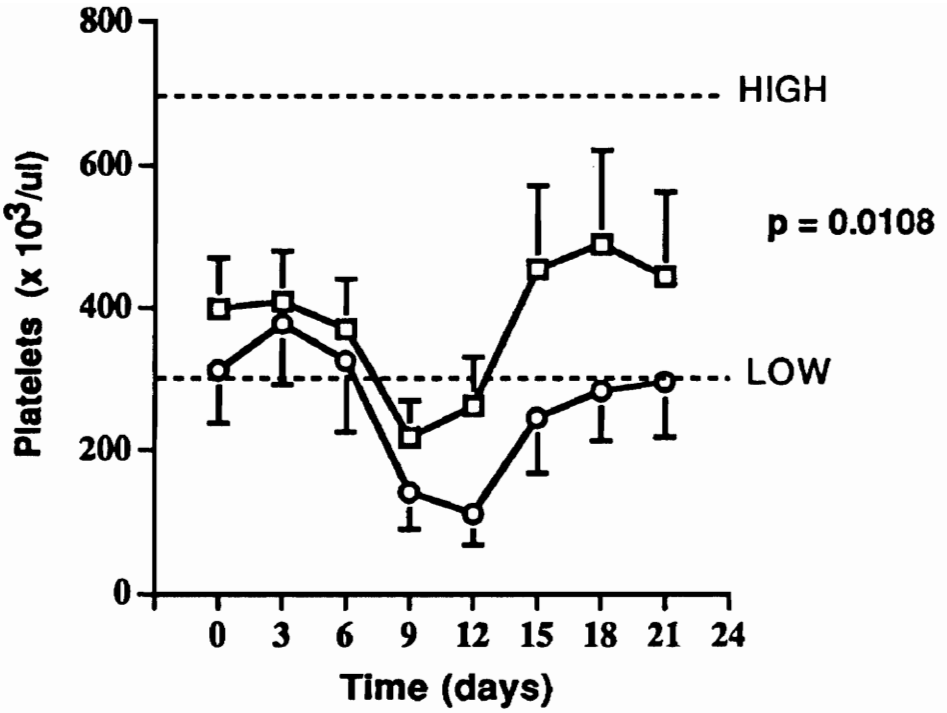


Figure 10. Platelets (mean ± SD) before (day 0) and after (days 3, 6, 9, 12, 15, 18, and 21) IV administration of cisplatin (90 mg/m^2) to group 1 dogs (—□—) in hypertonic saline (7%) and to group 2 dogs (—○—) in normal saline (0.9%). Reference interval represented by area between dashed lines (denoted high and low).

Appendix I

Individual exogenous creatinine clearances (ml/min/kg) of group 1 dogs with means and standard deviations before (day 0) and days 5, 12, and 21 after IV administration of cisplatin (90 mg/m^2) in hypertonic saline (7%).

Animal #	Day 0	Day 5	Day 12	Day 21
132	2.57	3.29	2.34	3.18
100	3.12	3.41	3.65	3.99
134	3.05	2.82	2.38	2.69
123	2.15	2.60	3.13	3.36
112	2.40	3.13	2.19	2.50
115	3.26	4.55	3.37	3.31
Mean	2.76	3.30	2.84	3.17
S. D.	0.45	0.68	0.61	0.53

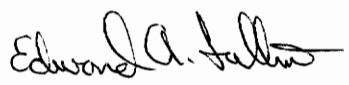
Appendix II

Individual exogenous creatinine clearances (ml/min/kg) of group 2 dogs with means and standard deviations before (day 0) and days 5, 12, and 21 after IV administration of cisplatin (90 mg/m^2) in normal saline (0.9%).

Animal #	Day 0	Day 5	Day 12	Day 21
129	2.45	3.46	2.84	3.02
133	3.50	3.64	3.74	3.97
135	3.10	3.38	2.90	3.67
106	3.95	4.45	2.54	2.04
110	2.68	2.57	2.38	2.81
124	2.54	2.25	2.18	2.65
Mean	3.04	3.29	2.76	3.02
S. D.	0.59	0.78	0.55	0.70

VITA

Edward Alton Fallin was born to Emma and Pearson Fallin on July 4, 1963, in Martinsville, Virginia. Although he attended Martinsville City Schools, Ed was fortunate to spend summers, weekends, and holidays growing up on the family farm in Floyd County. Working with his grandfather, the late Alton P. Underwood, Ed developed an interest in animal husbandry. Immediately following his fourteenth birthday, Ed applied for a work permit so he could be legally employed as a kennel assistant and head cow catcher for a new veterinarian in town - Dr. Joseph A. May. Following graduation from Martinsville High School, Ed attended Virginia Tech for 2 years of undergraduate training before being accepted in fourth class of the Virginia-Maryland Regional College of Veterinary Medicine where he received his Doctor of Veterinary Medicine degree in 1987. Although he had directed the majority of his senior electives toward equine practice, Ed settled for "a small animal job" at Albemarle Veterinary Hospital in Charlottesville, Virginia. Two years later, after a life-threatening case of Wahoo toxicity, Ed returned to his hometown of Martinsville where he was employed by Henry County Animal Clinic for almost two years. He was accepted for a small animal residency in internal medicine at the Virginia-Maryland Regional College of Veterinary Medicine after returning his alumni update information. He completed his medicine residency under the direction of Dr. Dru Forrester in December 1993. He remained as a clinician at the college until June of 1994. Ed was awarded the Vaughn merit scholarship in 1992, second place for clinical graduate research in 1993, and first place for clinical graduate research in 1994. He was inducted into the Phi Zeta Honor Society in 1994. Ed married his long-time love and best friend Dr. Olga van Beek on August 21, 1993. Ed, Olga, and Dr. Douglas Kern are currently preparing to open Veterinary Referral and Critical Care in Richmond, Virginia. He is very appreciative of the opportunities provided by the Virginia-Maryland Regional College of Veterinary Medicine and looks forward to cooperating with its faculty and staff in the future.



Edward A. Fallin, DVM