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Role of calcium in modulation of toxicities due to cisplatin and its analogs: a histochemical approach

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Cisplatin, carboplatin and paclitaxel (taxol) are potent antineoplastic agents with associated toxicities, especially gastrointestinal and nephrotoxicity that are their dose-limiting factors in clinical oncology. In an attempt to elucidate their mechanism(s) of toxicity, liver and kidney tissues from normal and drug treated rats and dogs were evaluated for changes in various dehydrogenase and non-specific lipase enzymes. Histochemically, cisplatin treatment induced an inhibition of all the enzymes studied except glucose-6-phosphate dehydrogenase and non-specific lipases, where there was a significant increase. Supplemental treatments with calcium [1 ml of 1.3% $CaCl_2$/day in rats and 2.50 mg (150 000 USP units) ergocalciferol plus 1000 mg of elemental calcium as TUMS 500 (EffeCal)/day in dogs] seem to protect against severe gastrointestinal toxicity and nephrotoxicity.

Key words: Calcitriol, calcium, cisplatin, dehydrogenases, ergocalciferol, lipases, nephrotoxicity, stomach bloating.

Introduction

Cisplatin (*cis*-dichlorodiammineplatinum II), a broad spectrum anticancer agent, has received intense attention as to its mechanism of action since its serendipitous discovery.[1] Coupled to its anti-tumor activity are many severe toxic side effects. More specifically, it is the nephrotoxicity and gastrointestinal toxicities associated with cisplatin use that have become the dose-limiting factor in some cases. Nephrotoxicity manifests pathologically as renal tubular damage which results in elevation of the blood urea nitrogen and serum creatinine levels.[2] Hydrating patients markedly diminishes kidney toxicity associated with cisplatin without a major loss of anticancer activity.[3] Antioxidants and thiol containing compounds, such as sodium thiosulfate, have become part of the treatment regimen in an attempt to alleviate nephrotoxicity.[4] Despite these advances, nephrotoxicity is still a major concern and limitation in cisplatin chemotherapy treatment due to the extremely high uptake by the kidneys, which is approximately three times the uptake of any other organ.[5]

Carboplatin (*cis*-diammine-1,1-cyclobutane dicarboxylate platinum II) is an analog of cisplatin with a similarly proposed mechanism of action and comparable effectiveness that has demonstrated reduced toxicity.[6] Consequently, carboplatin has been suggested as an alternate to cisplatin use. However, cisplatin still remains the drug of choice. Cisplatin in combination with many other anticancer agents has proven to be very effective.[7] Specifically, cisplatin plus taxol (paclitaxel) has become increasingly prevalent in clinical treatment.[8] Combination therapy is capable of producing very high response rates and is seemingly less toxic than either of the two drugs when administered alone.[9]

Rats do not have vomiting reflexes. Gastrointestinal toxicity in rats is manifest as bloating of the stomach and severe diarrhea. Dogs, however, do show vomiting reflexes and diarrhea in response to cisplatin treatments. Calcium supplements have been shown to prevent stomach bloating in rats, protect enzyme function and preserve overall organ function by minimizing the disruption of cellular homeostasis initiated by cisplatin treatment.[10–12] The present study was undertaken to characterize cisplatin-induced changes in the various dehydrogenases involved in the glycolysis process and Kreb's cycle responsible for ATP production using kidney and liver tissues of rats and dogs. Effort was made to explore the protective effects of calcium supplements on the dehydrogenases to prevent severe toxicities associated with cisplatin treatment.

Materials and methods

Animals

Male Wistar rats (Charles Rivers Laboratory,

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Wilmington, MA) weighing 160–200 g were used in the various experiments over a period of 17 months. Animals were kept on a 12 h light/12 h dark cycle with access to laboratory animal food and water *ad libitum* in accordance with the NIH Guide for Care and Use of Laboratory Animals. Animals received i.p. injections of freshly prepared cisplatin, carboplatin, taxol, cisplatin plus taxol, cisplatin plus 1 ml of 1.3% calcium chloride or 0.5 ml of 10% calcium gluconate, or 0.85% sodium chloride or 5% glucose. Various concentrations and dosages are shown in Table 1.

Male dogs weighing 70–95 lb were kept on a 12 h light/12 h dark cycle. The dogs had free access to water and food. Normal dogs were infused with 50 ml of 10% calcium gluconate (23.25 meq calcium) three times a day for 3 days. Blood samples were taken before and after calcium gluconate administration at 30 min intervals for up to 10 h. Normal dogs also received oral supplements of 0.5 μg of calcitriol (Roche Laboratories, Nutley, NJ) every day for 3 days. Blood and urine samples were taken at 3 h intervals for 3 days. A group of normal dogs were also given orally 2.50 mg (500 000 USP units) of ergocalciferol (Banner Pharmacaps, Elizabeth, NJ) and 1000 mg of elemental calcium as TUMS 500 (EffeCal) (SmithKline Beecham, Pittsburgh, PA) daily for 15 days before treating them with cisplatin (see Table 1) and every day after cisplatin infusion to maintain an elevated level of serum calcium. Blood and urine samples were collected at 12 h intervals for 3 days after cisplatin treatments and once daily there after (see Table 1).

Blood and urine analysis

Blood and urine samples were analyzed for ionic calcium levels using a 634 calcium pH analyzer. Blood urea nitrogen (BUN) and creatinine levels were monitored in accordance with the established methods.[13]

Table 1. Experimental design

Group	Animal	No. group	Treatment	Interval	Day of sacrifice
Ia	rats	12	cisplatin (9 mg/kg) in 0.85% NaCl	1 bolus injection	5 days after last drug treatment
b		12	cisplatin (9 mg/kg) plus 1 ml daily injections of 1.3% $CaCl_2$ or 0.5 ml of calcium gluconate	1 bolus injection	5 days after last drug treatment
		12	carboplatin (50 mg/kg) in 5% glucose	1 bolus injection	5 days after last drug treatment
d		6	control [0.85% NaCl or 5% glucose (10 ml/kg)]	1 bolus injection	5 days after last drug treatment
IIa	rats	12	cisplatin (1.8 mg/kg) in 0.85% NaCl	5 consecutive days	2 days after last injection
b		6	cisplatin (0.9 mg/kg) in 0.85% NaCl	5 consecutive days	2 days after last injection
c		12	taxol (4 mg/kg) in 0.85% NaCl	5 consecutive days	2 days after last injection
d		6	taxol (2 mg/kg) in 0.85% NaCl	5 consecutive days	2 days after last injection
e		6	cisplatin (1.8 mg/kg) and Taxol (4 mg/kg)	5 consecutive days	2 days after last injection
f		12	cisplatin (0.9 mg/kg) and Taxol (2 mg/kg)	5 consecutive days	2 days after last injection
g		6	control (10 ml/kg of 0.85% NaCl)	5 consecutive days	2 days after last injection
IIIa	dogs	2	50 ml of 10% calcium gluconate (23.25 meq calcium) in 100 ml of 0.85 NaCl[a]	3/day × 5	–
b		2	daily oral supplements of calcitriol (0.5 μg) and two Tums500 tablets plus calcium gluconate as above[b]	3 days	–
c		2	2.5 mg (50 000 USP units) ergocalciferol plus 2 Tums500[c]	5 days	–
d		2	cisplatin (1.8 mg/kg) in 500 ml 0.85% NaCl was infused over 3 h plus 2.5 mg daily oral supplements of ergocalciferol and two Tums500 (calcium carbonate) tablets (1000 elemental calcium)[c]	every 21 days × 3	40 days after last infusion
e		2	control (500 ml of NaCl infusion over 3 h)	every 21 days × 3	40 days after last infusion

[a]Blood samples were collected every 20 min before and after calcium gluconate administration for 4 h.
[b]Blood samples were collected before and every 2 h after calcium gluconate administration for 12 h.
[c]Blood samples were collected at 12 h intervals for 15 days.

Tissue collection

The rats in the various groups were anesthetized with CO_2 and killed by decapitation at each sampling interval (see Table 1). The tissues (kidneys and livers) were quickly excised and mounted on cryostubs in OCT medium (Miles Laboratories, Elkhart, IN) and frozen at −20°C until use. Sections (10 μm) were cut using a cryostat microtome (Miles Laboratories, Elkhart, IN) for enzymatic analysis.

The dogs were given a lethal dose of sodium pentobarbital (325 mg/kg). The kidney and liver tissues were quickly excised and mounted on cryostubs in OCT medium (Miles Laboratories) and frozen at −20°C. Sections (10 μm) were cut for enzymatic analysis.

Histochemical studies

Dehydrogenase localization. Frozen sections (10 μm) of normal and variously treated animal tissues were picked up on standard glass coverslips and allowed to dry at room temperature. Succinate dehydrogenase (SDH, EC 1.3.99.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), β-hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.41) and glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) were then localized, histochemically, by the standard Nitro BT method.[14] The enzyme activity of various dehydrogenases was demonstrated by using a medium containing 100 mM phosphate buffer (pH 7.4) with 32% (w/v) polyvinyl alcohol, 0.3 mM NAD or 0.8 mM NADP, 4 mM $MgCl_2$, 10 mM of the enzyme specific substrate and 5 mM of the tetrazolium salt. Fresh frozen sections were incubated at 37°C for 20 min with preheated media. The substrate or the coenzymes were omitted from the incubation media to serve as controls. Tissue sections were also boiled in distilled water to denature the endogenous enzymes and serve as control. Non-specific lipase (EC 3.1.1.3) activity was localized by incubation of tissue sections in a medium according to the standard methods.[15,16] Boiled tissue sections served as a control. All slides were viewed with a Zeiss photomicroscope II and micrographs of random sections and random areas were prepared for quantitative analysis.

Quantitative analysis. Staining intensity was based on an arbitrary scale from very intense response (+++++) to intense response (++++) to moderate response (+++) to poor response (++) to very poor response (+) to no response (−). Tissue sections were randomly evaluated, and in case of the kidney tissues the cortex and medulla were considered in a subjective determination of enzyme localization response. To avoid any variations in the staining intensities due to section thickness approximately five transmission images from five sections each of the normal, cisplatin, carboplatin, taxol, cisplatin plus taxol and cisplatin plus calcium treated tissues, as well as control slides, were examined by the Zeiss 10 laser scanning confocal microscope (LSM). Quantitative analyses were made using the 'histogram' computer program. Random areas of the tissues were analyzed for staining intensity by the computer and a representative histogram detailing 'gray scale values' was produced for each enzyme. Statistical analysis was performed and gray scale values were then converted to percentages based on normal staining being equivalent to 100% intensity.[17]

Results

Effects of various treatments on dehydrogenases

Histochemically, of all the dehydrogenases studied (MDH, SDH, GDH, β-HBDH, IDH and G-6-PDH), only G-6-PDH demonstrated an increase after cisplatin, carboplatin and taxol treatments (see Table 2). Thus,

Table 2. Average enzyme histochemical staining intensity for normal, treated and control tissues[a]

Treatment	SDH	GDH	HBDH	MDH	IDH	G6PDH	Lipase
Normal	++++	++++	++++	++++	++++	+++	+++
Cisplatin	+	+	+	+	+	+++++	+++++
Cisplatin plus calcium	+++	+++	+++	+++	+++	+++	+++
Carboplatin	++	++	++	++	++	++++	++++
Cisplatin plus taxol	+++	+++	+++	+++	+++	++++	++
Taxol	+++	+++	+++	+++	+++	++++	++
Control	−	−	−	−	−	−	−

+++++, very intense reaction; ++++, intense reaction; +++, moderate reaction; ++, poor reaction; +, very poor reaction; −, no reaction.

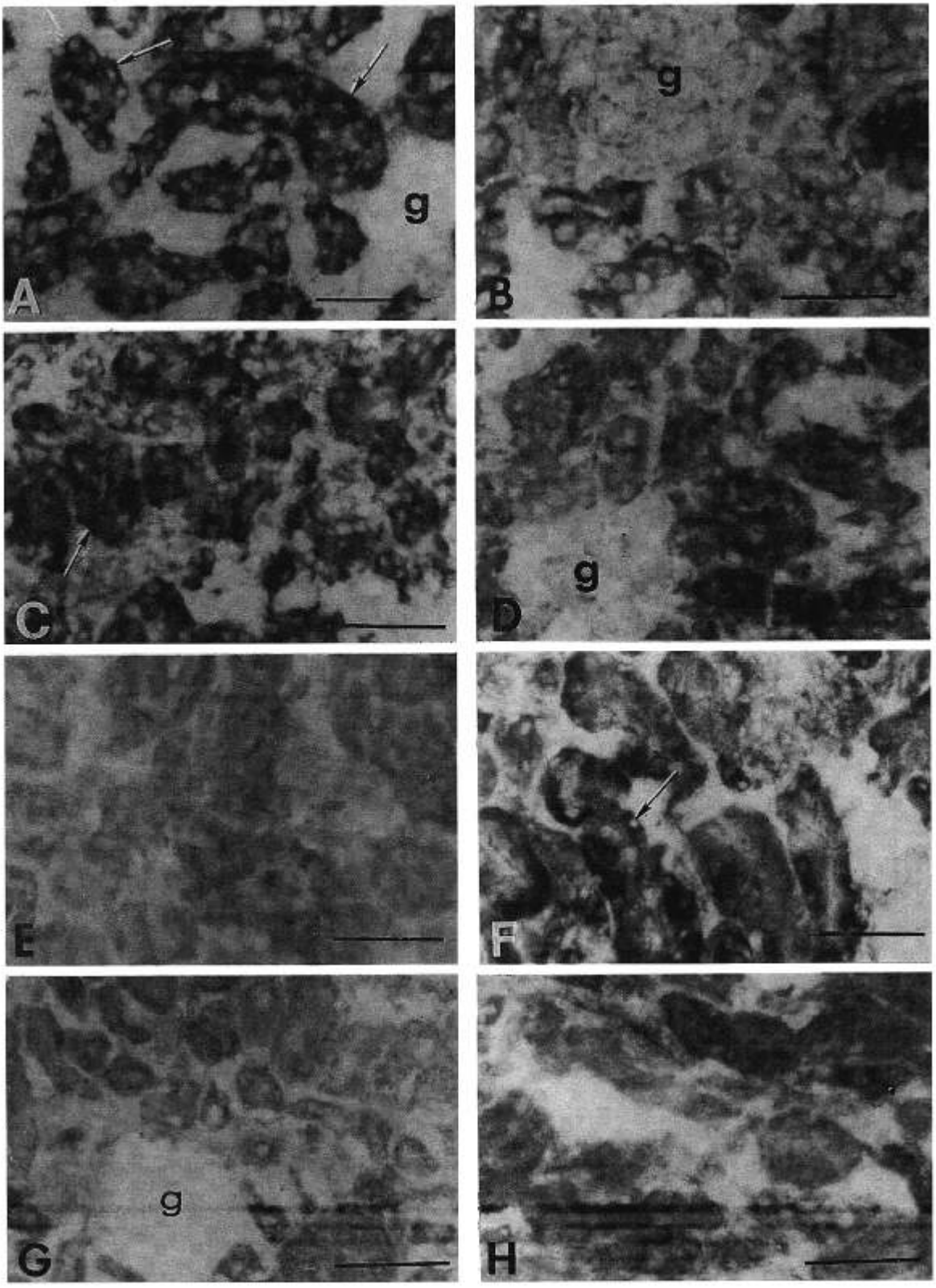

Figure 1. Light micrographs (A–D) showing the distribution of MDH and (E–H) G-6-PDH activity in the rat kidney sections. (A) MDH distribution in normal kidney (arrows) sections, (B) 5 days after cisplatin (9 mg/kg) treatment, (C) 5 days after calcium plus cisplatin (9 mg/kg) treatment and (D) 5 days after cisplatin (9 mg/kg) plus taxol (4 mg/kg). Note the significant decrease in the enzyme levels after cisplatin treatment. Enzyme levels after calcium plus cisplatin and cisplatin plus taxol treatments are

for the sake of brevity and simplicity, only MDH and G-6-PDH will be discussed in detail here.

Sections of normal kidney and liver tissues, incubated for MDH localization, demonstrated a dark blue granulation with intense diffuse staining throughout the cytoplasm of the cells. In the normal kidney, staining intensity and localization was approximately the same in both the cortical and medullar regions with the proximal and distal tubules having equally pronounced enzyme localization. No staining was demonstrated within the nuclei or glomeruli (Figure 1). Similarly, sections of normal liver stained for MDH showed dark and diffuse staining throughout the cytoplasm of the hepatocytes. For the sake of uniformity most of the observations described here are restricted to the pericentral regions of the liver.

In cisplatin treated tissues, MDH staining was significantly decreased compared to normal; however, after cisplatin and calcium treatment, MDH staining and localization were very similar to that of the normal tissues (Figure 1). Kidney tissue from cisplatin plus taxol treated kidneys had MDH enzyme intensities greater than cisplatin treatment alone but still was less than that seen in normal tissues. MDH localization in liver from rats treated with cisplatin and taxol followed a similar pattern where hepatocyte staining was less than normal but greater than that of cisplatin treatment. MDH after taxol or carboplatin treatment demonstrated only a slight decline in activity from normal.

Sections of normal tissues stained for G-6-PDH localization demonstrated a diffuse staining throughout the cytoplasm. Further, staining intensity and localization was approximately the same in both the cortical and medullar regions of the kidney with the proximal and distal tubules showing a pronounced enzymatic activity (Figure 1E). However, in all animals after various drug treatments, G-6-PDH staining was much more enhanced (Figure 1F) compared to the normal tissues with the exception of calcium plus cisplatin treatment where the G-6-PDH levels were similar to the normal tissues (Figure 1G). G-6-PDH activity after carboplatin was still more than the normal tissues but was less than those after cisplatin treatments. G-6-PDH activity after treatments of cisplatin plus taxol and taxol alone was slightly increased compared to the normal (Figure 2 and Table 2).

Effects of various treatments on non-specific lipase activity

Under the light microscope non-specific lipase activity was observed as brown diffuse granulation throughout the tissues (Figure 3). In the kidneys, staining was similar in the proximal and distal tubules of the cortex and medulla. No significant glomerular staining was observed. Cisplatin treatment caused an elevation in lipase throughout the kidney tubules compared to the normal (Figure 3). Since the tissues were not counterstained for morphological details no distinction could be made between the proximal and distal tubules. Similarly, hepatocyte staining for non-specific lipase after cisplatin treatment was enhanced. After cisplatin plus calcium treatment in both the kidney and liver (Figures 3 and 4 and Table 2) the level of lipase was close to that of the normal tissues. Cisplatin plus taxol treated tissues and taxol treated tissues both demonstrated a decreased lipase activity as compared to the normal tissues. Carboplatin treatment demonstrated an increased lipase activity compared to normal tissues; however, these increases were less than those after cisplatin treatments (Table 2).

Dog serum concentrations of $[Ca^{2+}]_i$ after calcium gluconate, calcitriol and ergocalciferol

In the initial experiment infusion of calcium gluconate raised the level of inorganic calcium in the blood from 1.4 to 2.5 mmol/l within 15–20 min. However, this peak declined to the baseline just as quickly as it was achieved. Repeated injections of calcium gluconate at 1 and 2 h intervals also failed to maintain a constantly higher level of serum calcium and the peak which reached as high as 3.0 mmol/l declined to a normal level in about 2 h (Figure 5). Similarly, calcitriol administration also raised the level of serum calcium quickly but did not stay high for a long time. In order to maintain a constantly higher than normal levels of serum calcium, ergocalciferol and calcium carbonate in the diet proved to be much more effective (Figure 6). Higher than normal levels (1.5 mmol/l) of serum calcium were achieved by day 7 through ergocalci-

close to normal. G-6-PDH in (E) normal kidney section, (F) 5 days after cisplatin (9 mg/kg) treatment, (G) 5 days after calcium plus cisplatin treatment and (H) 5 days after cisplatin (9 mg/kg) plus taxol (4 mg/kg). Note a significant increase in the enzyme levels after cisplatin treatment which is suppressed after calcium supplements. g, glomerulus. Original magnification ×420. Bars=40 μm.

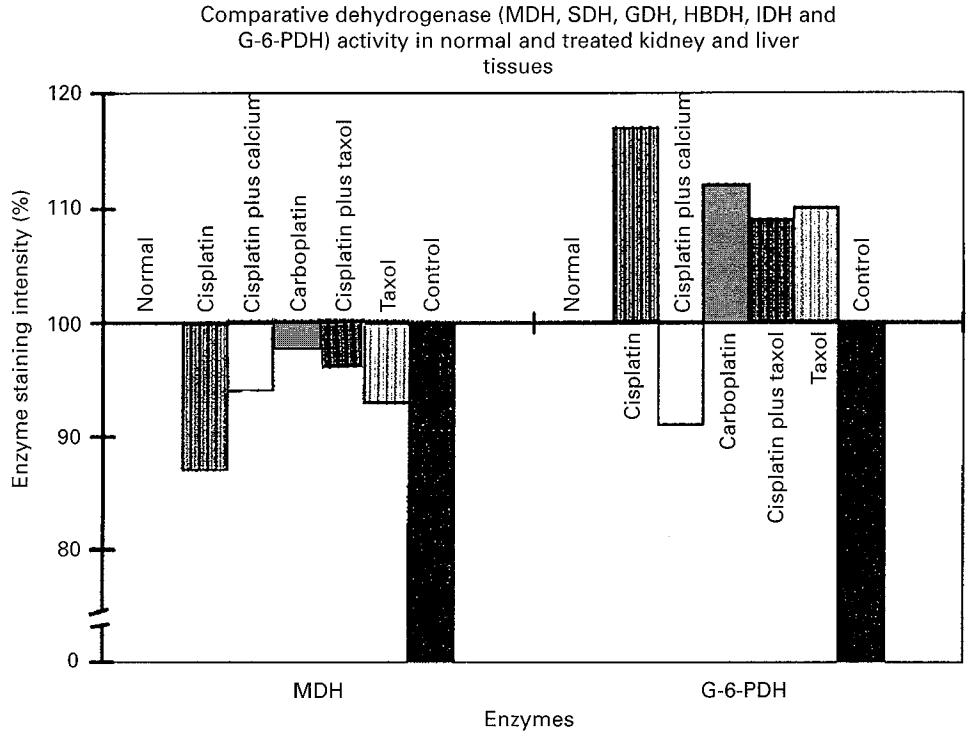

Figure 2. Bar graph showing comparative enzymatic activity of MDH and G-6-PDH in normal and variously treated rat tissues. Note that 100% staining is the baseline and represents normal tissue enzyme activity. Cisplatin inhibits MDH activity the most while calcium supplements before cisplatin treatment seem to protect the activity, whereas such pretreatments with calcium suppress the G-6-PDH activity.

ferol and Tums500 administration. Subsequent cisplatin infusions did not decrease the ionized serum calcium levels below normal (1.25–1.45 mmol/l). After three such cisplatin treatments extending over 9 weeks the blood urea nitrogen (BUN) and creatinine levels were found to be normal. In order to avoid any deleterious effects of hypercalcemia the levels of calcium were strictly monitored.

Comparison of dehydrogenase activity in normal and cisplatin plus calcium treated dog kidneys

In the cisplatin plus calcium treated tissues, the MDH intensity and localization was similar to normal tissues. The cytoplasmic staining of the tubules was even, precise and comparable to the normal tissue dehydrogenase staining. No differences were noticeable in the proximal and distal tubules of the cortex and medulla. Further, enzyme intensity and localization was approximately the same for G-6-PDH as for MDH and the other dehydrogenases studied after cisplatin and cisplatin plus calcium treatments.

Discussion

Under low intracellular chloride ion concentrations, cisplatin has been shown to hydrolyze into variously charged reactive species including monoaqua *cis*-$(NH_3)_2Pt(H_2O)^+$ and diaqua-equated *cis*-$(NH_3)_2Pt(H_2O)_2^{2+}$ forms.[18–21] It is these hydrolyzed forms of cisplatin (diol) that have been shown to be 1000 times more reactive than cisplatin. These have been shown to act through the inhibition of mitochondrial respiration by inducing uncoupling of oxidative phosphorylation.[21] This results in an efflux of calcium from the mitochondria, which is thought to play a significant role in the disruption of normal calcium homeostasis and hence cell function. Calcium is known to control many physiological functions ranging from stimulation of muscle contractions,[22] synthesis and secretion of transmitters and hormone release,[23,24] enzyme activity and membrane permeability.[25] Increased intracellular levels of calcium have been shown to be directly responsible for the polymerization or depolymerization of the microtubules and microfilaments in different cell forms. This has also been proposed as one of the mechanisms of

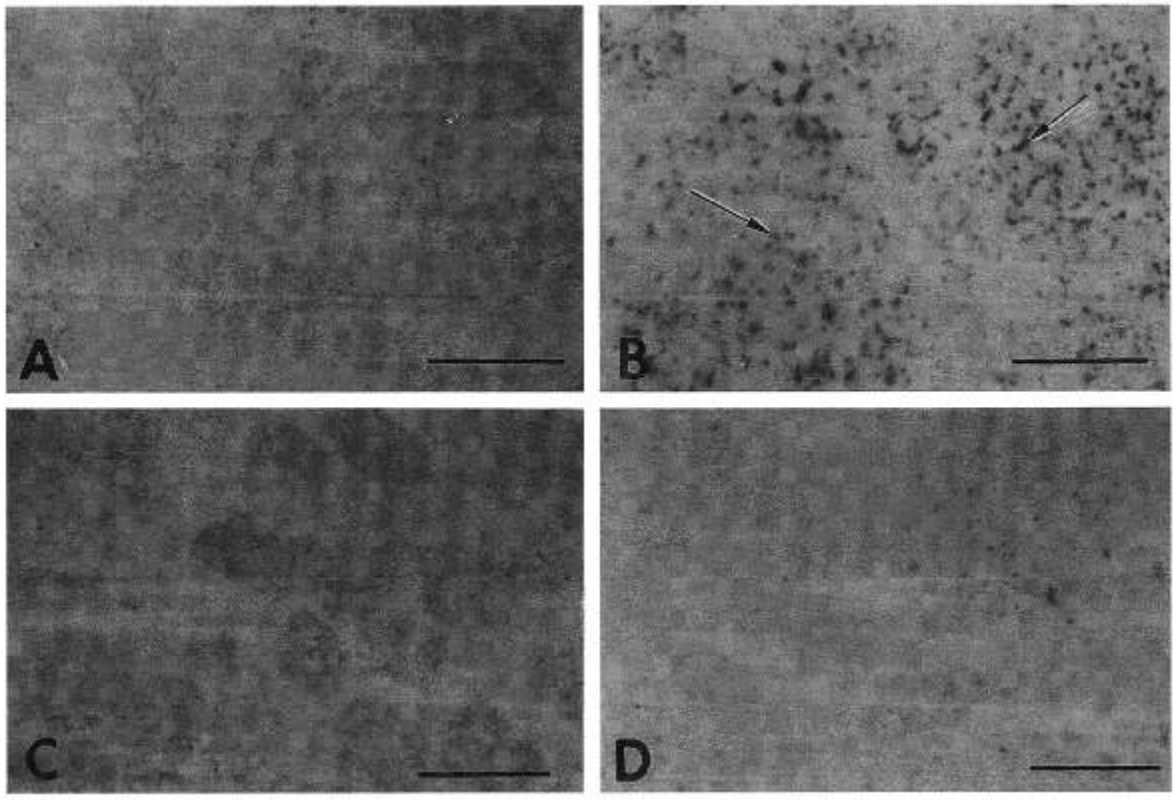

Figure 3. Light micrographs of normal and variously treated rat kidney sections showing localization of non-specific lipase activity. (A) Lipase distribution in normal kidney section and (B) after cisplatin treatment demonstrating a significant increase in lipase activity compared to normal. (C) Cisplatin plus calcium treatment showing a slight decrease in lipase localization from that of normal and (D) lipase reaction product after cisplatin plus taxol treatment demonstrating a decrease in enzyme activity compared to normal. Original magnifications $\times 420$. Bar=40 μm.

action of cisplatin by inhibiting cytokinesis even though the genomic DNA may have already replicated.[26] Calcium's role in decreasing cisplatin-induced nephrotoxicity was demonstrated by using calcium channel blockers like verapmil.[27]

Cisplatin has been shown to induce a loss in the membrane associated calcium and thiol (SH) groups,[10] which in turn changes the fluidity of the cell membrane.[28] It has been suggested that calcium ions and SH groups are involved in holding various glycoproteins in place.[29] Cisplatin has been shown to bind SH groups on proteins resulting in the modification of their structure and function.[30] Raising the levels of extracellular calcium probably keeps the diol from occupying the active sites of membrane bound calcium to cause any cascade of activities that might lead to cellular toxicities.

Biochemical studies have conclusively shown that the energy requiring calcium transport in the mitochondria can be measured by the increased oxygen consumption after diol. This oxygen consumption is directly proportional to the concentration of diol used and can be reversed by SH-rich *N*-acetyl-L-cysteine (NAC) with no time lag.[31] Cisplatin in an unhydrolyzed state does not seem to have any effect. Mitochondrial glutathione (GSH) seems to be essential in the regulation of inner mitochondrial permeability and enzyme function by keeping SH in the reduced state.[32] When the SH groups of enzymes are not maintained in a reduced form, they become inactivated.[33] Cisplatin-induced toxicities, especially nephrotoxicity, seem to be very similar to other heavy metals in regard to a decrease in the intracellular concentrations of GSH and protein bound SH groups.[34,35]

Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase are inhibited[36] with loss of mitochondrial function and metabolism, leading to a state of anoxia and ionic imbalance, cell swelling, and ultimate cell lysis.[35] Further, this binding of heavy metals, such as platinum, to SH groups also induces a rapid release of calcium from intracellular storage organelle[37] and the inactivated ATPase is unable to transport calcium and restore the equilibrium.[38] Various antioxidants and radical scavengers, and compounds rich in thiol

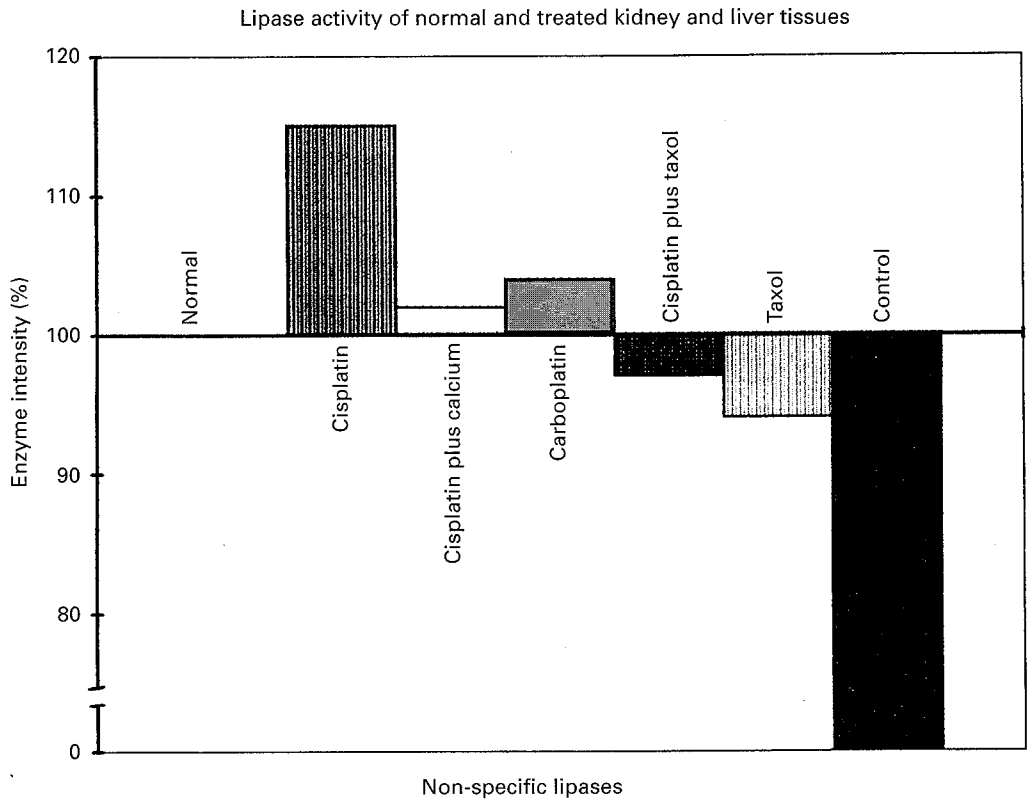

Figure 4. Graph showing comparative non-specific lipase activity for the various treatments in rats. Cisplatin shows the maximum activation of non-specific lipases while calcium supplements seem to prevent the adverse effects of cisplatin.

groups have been used to prevent the toxic side effects of cisplatin.[30,39,40]

NADH, which helps to maintain SH groups, declines with cisplatin treatment. Consequently, this depletion of GSH and NADH appears to result in the inhibition of some dehydrogenases.[41] This results in the uncoupling of oxidative phosphorylation[31] leading to hydroxyl radical formation and oxidative stress.[42] These free radicals attack polyunsaturated lipids and proteins and initiate lipid peroxidation.[43–45] This process becomes autolytic and causes severe damage to membrane integrity.[30,46]

Hypermetabolism is a cellular means of compensation for increased energy needs as a result of mitochondrial damage but this also leads to the additional activation of unregulated Ca^{2+}-dependent degradative enzymes such as phospholipases.[46] Phospholipases A and C are known to be activated after cisplatin treatment.[47,48] Our studies have demonstrated such increases but these can be reversed by calcium supplements only prior to cisplatin treatment. Similar to lipase activity G-6-PDH is also enhanced after cisplatin treatment. It seems that elevation of G-6-PDH activity occurs because of its unique and critical function in NADH generation and maintenance of reduced sulfydryl groups as part of the pentose phosphate pathway. Furthermore, if the pentose phosphate pathway is carried through to completion, there is the degradation of glucose to glyceraldehyde-3-phosphate, which can further be oxidized to produce ATP. G-6-PDH catalyzes the conversion of β-D-glucose-6-phosphate to 6-phosphoglucono-1,5-lactone and reducing NAD in the process.[49] Thus, G-6-PDH has a dual function in being the rate-determining step in the pentose phosphate pathway and catalyzing the above reaction. Possible lack of essential sulfydryl groups may be limiting its own inhibition allowing for increased activity. This may be a means for cells to adapt to the effects of cisplatin.

Calcium supplementation has been demonstrated to modulate cisplatin-induced toxicities.[31] The importance of maintaining higher levels of calcium than normal is clear from our studies probably for a competitive binding between $[Ca^{2+}]_i$ and *cis*$[(NH_3)_2Pt(H_2O)_2]^{2+}$. In *in vitro* experiments using MeroCalmodulin-1, a calcium-sensitive fluorescent analog of calmodulin, it has been demonstrated that only the hydrolyzed form of cisplatin is able to inhibit the conformational change which occurs after calcium binding to the molecule.[50] This conforma-

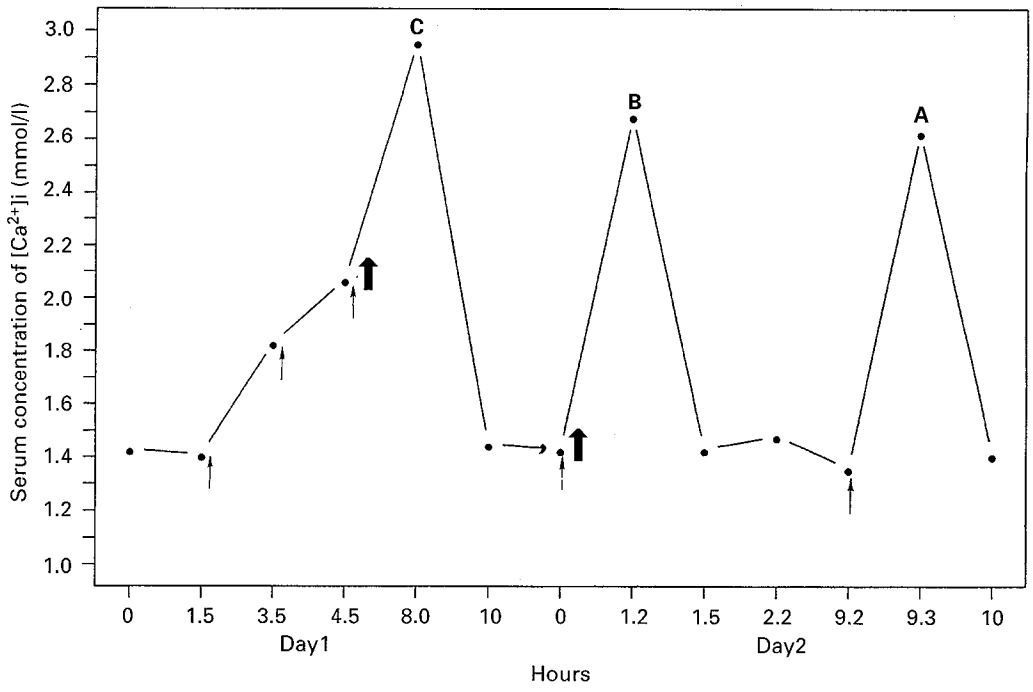

Figure 5. Graph showing the serum calcium $[Ca^{2+}]_i$ levels after calcium gluconate (23.25 meq calcium) infusion and calcium gluconate plus 0.5 μg calcitriol administration in the dogs. Note the calcium gluconate infusions (↑) did cause an elevation in the serum calcium levels but the levels come back to normal within 25–35 min (A). With calcitriol (⬆) and calcium gluconate (↑) an elevated level of calcium has been achieved but again it comes back to normal within 45–60 min (B). With continued infusion of calcium gluconate (↑) and calcitriol (⬆) an elevated level of calcium may be achieved but this also drops to normal in 2–3 h.

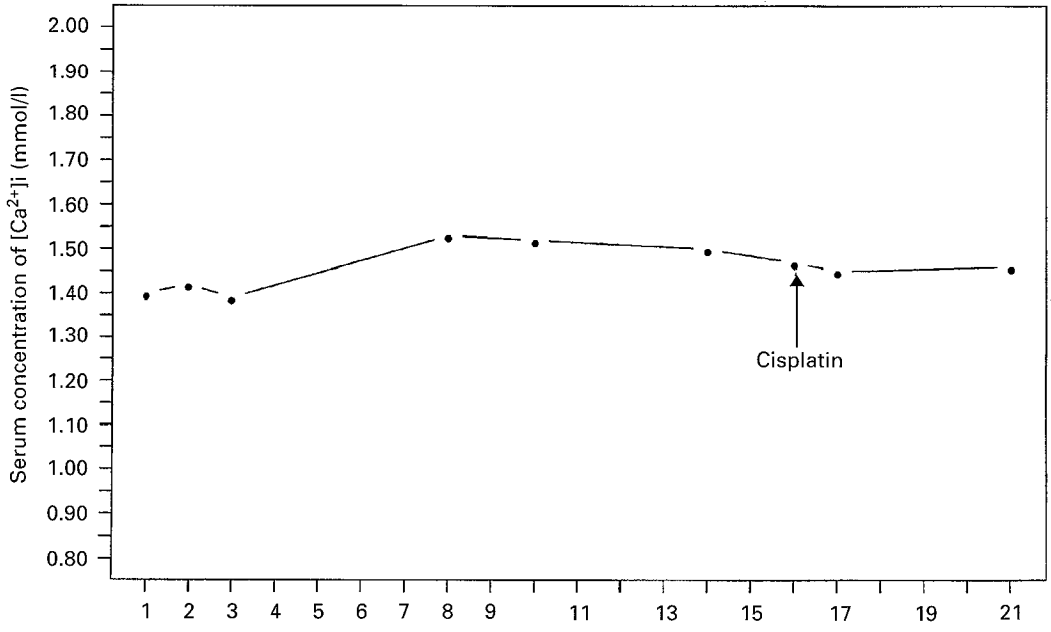

Figure 6. Graph showing an elevated level of serum calcium after daily administration of ergocalciferol (2.5 mg) and 1000 mg calcium carbonate (EffeCal). Note cisplatin administration on day 16 did not cause any significant drop in the serum calcium levels below the normal.

tional change which is calcium activated makes the molecule fluoresce.[50] Further, competitive binding studies for the calcium binding sites of the calmodulin molecule have demonstrated that it is only the diol form of cisplatin that is able to inhibit the neuronal NOS activation.[12] Similarly, acetylcholine release inhibition has been tied to the hydrolyzed cisplatin in the rat stomach smooth muscle resulting in its bloating and ulceration.[11] However, these adverse effects of cisplatin can be inhibited by calcium supplements only when calcium is administered in advance of cisplatin. If cisplatin is administered first and calcium is administered later then the various toxicities, especially nephrotoxicity, due to cisplatin are not prevented. Probably once the $[Ca^{2+}]_i$ binding sites are blocked by diol, then the normal functions are disrupted, especially various transport functions across the membranes through the inhibition of ATP.[10]

An alternative to cisplatin treatment being promoted these days is the combination therapy using cisplatin and taxol. Taxol (paclitaxel) is a diterpene plant product with no heavy metal constituents[51] and is highly active in numerous preclinical tumor models.[52] Taxol treatment is also associated with a number of toxicities such as hypersensitivity,[53] alopecia[54] and emesis.[55] Taxol's combination with cisplatin has proven extremely effective against breast cancer with high success rates.[9] With such treatment nephrotoxicity is limited, with minimal dehydrogenase inhibition and close to normal lipase activity. Taxol is eliminated by hepatic conversion, by the cytochrome P450 system,[8] which minimizes its nephrotoxicity due to limited renal exposure. It has recently been shown that taxol and cisplatin combination therapy is schedule dependent. Addition or even synergism occurs when taxol is given approximately 24 h prior to cisplatin treatment; however, when cisplatin is given prior to taxol or if they are administered at the same time, strong antagonistic interactions are observed.[56] Consequently, all of these factors are probably responsible for the appreciable decline in nephrotoxicity; the most significant being the schedule dependency of combination therapy. Nonetheless, clinical success of cisplatin plus taxol combination therapy is a viable alternative to cisplatin treatment alone.

In conclusion, cisplatin's disruption of calcium homeostasis initiates primary events such as lipid peroxidation and enzyme inhibition. These events damage the cells through mitochondrial damage, inhibition of mitochondrial function, depletion of ATP and other cofactors. This probably leads to apoptosis and tissue necrosis.[57] Thus, it seems that elevated calcium levels, via calcium supplementation, may act as another means of cyto-protection, by competing for binding sites with diol and prevent various toxicities associated with it.

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