ANTICANCER RESEARCH 24: 2193-2200 (2004)

Low Renal Toxicity of Lipoplatin Compared to Cisplatin in Animals

PRASAD DEVARAJAN[1], RIDWAN TARABISHI[1], JAYA MISHRA[1], QING MA[1], ANDREAS KOURVETARIS[2], MARIA VOUGIOUKA[2] and TENI BOULIKAS[2,3]

[1]*Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229, U.S.A.;*
[2]*Regulon, A.E., 7 Grigoriou Afxentiou, Alimos 17455, Greece;*
[3]*Regulon, Inc., 715 North Shoreline Blvd, Mountain View, CA 94043, U.S.A.*

Abstract. *Cisplatin is one of the most widely used and effective chemotherapeutic agents for the treatment of several human malignancies. Although the effectiveness of cisplatin is high, its toxicities justify the demand for improved formulations of this drug. A liposomal formulation of cisplatin, Lipoplatin™, was developed in order to reduce the systemic toxicity of cisplatin. Mice and rats injected with cisplatin developed renal insufficiency with clear evidence of tubular damage, but those injected with the same dose of Lipoplatin were almost completely free of kidney injury. The maximum levels of total platinum in rat kidneys after intraperitoneal bolus injection of cisplatin or Lipoplatin at similar doses were similar, but the steady state accumulation of total platinum in the kidney was 5 times higher for cisplatin compared to Lipoplatin. This is proposed as one mechanism to explain the low renal toxicity of Lipoplatin.*

The introduction of cisplatin and later of carboplatin were milestone achievements in molecular oncology (1). Cisplatin is one of the most widely used and effective chemotherapeutic agents for the treatment of several human malignancies (2, 3). A number of additional platinum drugs are undergoing clinical trials, a great number are being evaluated in cell cultures or animal models, and an even larger number of platinum compounds have already been synthesized, tested and abandoned. The success of cisplatin lies in its ability to induce DNA damage, resulting in bulky adducts as well as intra- and inter-strand crosslinks (4); the cell, then, needs to activate sophisticated DNA repair pathways for their elimination. Cisplatin adducts and crosslinks can arrest DNA synthesis by inhibiting DNA polymerase-catalyzed chain elongation at the replication fork in proliferating cells such as tumor cells. In addition, platinum drugs can induce oxidative stress and activate stress-signaling pathways and apoptotic pathways in tumor cells (5, 6).

The efficacy of cisplatin is dose-dependent, but the significant risk of nephrotoxicity frequently hinders the use of high doses to maximize its antineoplastic effects (7, 8). Cisplatin accumulates in cells from all nephron segments but is preferentially taken up by the highly susceptible proximal tubule cells within the S3 segment, which bear the brunt of the damage (9-13). Nephrotoxicity following cisplatin treatment is common and may manifest after a single dose with acute renal failure, or may present with a chronic syndrome of renal electrolyte wasting. Despite various hydration protocols designed to minimize the nephrotoxicity, approximately one-third of patients who receive cisplatin develop evidence of acute renal failure (14-16). This can have major consequences in terms of mortality and morbidity, especially in the face of co-morbid conditions such as those related to the primary malignancy (17, 18). Several therapeutic maneuvers have proven to be efficacious in the treatment of cisplatin-induced nephrotoxicity in animals (12). However, successful human experiences have remained largely anecdotal (19). The development of less toxic alternatives to cisplatin has therefore remained a major challenge.

In this study, we report the properties of a new drug termed Lipoplatin™, a liposomal formulation of cisplatin developed in order to reduce its systemic toxicity. When compared with cisplatin, an equal dose of Lipoplatin resulted in significantly less structural and functional evidence of nephrotoxicity in mice and rats. The maximum levels of total platinum in rat kidneys after intraperitoneal bolus injection of cisplatin or Lipoplatin at similar doses were similar, but

Correspondence to: Dr. Prasad Devarajan, Nephrology & Hypertension, MLC 7022, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, U.S.A. Tel: (513) 636-4531, Fax: (513) 984-9770, e-mail: prasad.devarajan@cchmc.org

Key Words: Cisplatin, Lipoplatin, nephrotoxicity.

the steady state accumulation of total platinum in kidney was 5 times higher for cisplatin compared to Lipoplatin. These pharmacokinetic differences may account, at least in part, for the low renal toxicity of Lipoplatin.

Materials and Methods

Preparation and characteristics of Lipoplatin. Cisplatin was purchased from Heraeus (Hanau, Germany)/Flavine (Florida, USA) (mw 300). The lipid shell of Lipoplatin is composed of 1,2-dipalmitoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) also known as dipalmitoyl phosphatidyl glycerol (DPPG, mw 745), purchased from Lipoid GmbH (Ludwigshafen, Germany), soy phosphatidyl choline (SPC-3, mw 790) also purchased from Lipoid GmbH, cholesterol (CHOL, mw 386.66) from Avanti Polar Lipids (Alabama, USA) and methoxy-polyethylene glycol–distearoyl phosphatidylethanolamine lipid conjugate (mMPEG2000-DSPE, mw 2807, Genzyme (Basel, Switzerland). The ratio of cisplatin to lipids is 8.9% cisplatin: 91.1% total lipids (w/w). Repeated extrusions are performed using a Thermobarrel Extruder (Northern Lipids Inc., Vancouver BA, Canada) through membranes of 0.2 μm, 0.1 μm, 0.08 μm and 0.05 mm pore sizes (Whatman, CA, USA) under pressure in ultrapure nitrogen. About 15 passages are used and the average particle diameter and size distribution at a 90˚ angle are controlled with dynamic light scattering ($N4^+$ nanoparticle analyzer, Beckman-Coulter, CA, USA). The type of liposome particles used in Lipoplatin™ is a proprietary formulation of an average size of 110 nm. The anionic lipid DPPG gives to Lipoplatin its fusogenic properties with respect to entrance through the cell membrane. The total lipid to cisplatin ratio in Lipoplatin is 10.24 mg lipid/mg cisplatin. The content of Lipoplatin in cholesterol is 11.6% (w/w) of the total lipid. Lipoplatin is provided in 50-ml clear glass vials of 3mg/ml (concentration refers to cisplatin).

Mouse model of cisplatin nephrotoxicity. We utilized a well-established murine model in which the structural and functional consequences of cisplatin-induced nephrotoxicity have been previously documented (20-23). Briefly, male Swiss-Webster mice (Taconic Farms, Germantown, NY, USA), weighing 25-30 g, were housed with 12:12 hour light:dark cycle and were allowed free access to food and water. Mice (n=5) were given a single intraperitoneal injection of cisplatin, at the dose of 20 mg/kg body weight. It has been previously shown that this dose results in tubule cell necrosis and apoptosis, and impaired renal function within 3-4 days after the cisplatin injection (20-23). Control mice (n=8) received an equal volume of saline, and the Lipoplatin-treated animals (n=5) were given a single intraperitoneal injection of Lipoplatin, at the dose of 20 mg/kg body weight. The animals were placed in metabolic cages (Nalgene, Rochester, NY, USA), and the urine was collected on a daily basis. Five days after the injection, the animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), the abdominal cavity opened and blood obtained *via* puncture of the inferior vena cava for measurement of serum creatinine with a quantitative colorimetric assay kit (Sigma, St. Louis, MO, USA). The mice were sacrificed, the kidneys were perfusion-fixed *in situ* with 4% paraformaldehyde in PBS and both kidneys harvested. One half of each kidney was snap-frozen in liquid nitrogen and stored at -70˚C until further processing; a sample was fixed in formalin, paraffin-embedded and sectioned (4 μm). Paraffin sections were stained with hematoxylin-eosin and subjected to the TUNEL assay. The other half of each kidney was embedded in OCT compound (Tissue-Tek) purchased from Miles Laboratories Inc (Naperville, Illinois, USA) and frozen sections (4 μm) obtained for immunohistochemistry.

Rat model of cisplatin injury. We utilized a well-established rat model in which the structural and functional consequences of cisplatin-induced nephrotoxicity have been previously documented (24-26). Briefly, male rats (Taconic Farms, Germantown, USA), weighing 150 g, were housed with a 12:12 hour light:dark cycle and were allowed free access to food and water. Rats (n=5) were given a single intraperitoneal injection of cisplatin, at the dose of 5 mg/kg body weight. It has been previously shown that this dose results in tubule cell necrosis and apoptosis, and impaired renal function within 3-4 days after the cisplatin injection (24-26). Control rats (n=5) received an equal volume of saline, and the Lipoplatin-treated animals (n=5) were given a single intraperitoneal injection of Lipoplatin, at the dose of 5 mg/kg body weight. The animals were placed in metabolic cages (Nalgene), and the urine was collected on a daily basis. Five days after the injection, the animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), the abdominal cavity opened, and blood obtained *via* puncture of the inferior vena cava for measurement of serum creatinine by quantitative colorimetric assay kit (Sigma). The rats were sacrificed and the kidneys processed for microscopy as described above for mouse kidneys.

Apoptosis assay. For the detection and quantitation of kidney cell apoptosis, we utilized the TUNEL assay, as previously described (27-29). The ApoAlert DNA Fragmentation Assay Kit was obtained from Clontech (La Jolla, CA, USA). Paraffin sections were deparaffinized through zylene and descending grades of ethanol, fixed with 4% formaldehyde/PBS for 30 minutes at 4˚C, permeabilized with proteinase K at room temperature for 15 minutes and 0.2% Triton X-100/PBS for 15 minutes at 4˚C, and incubated with a mixture of nucleotides and TdT enzyme for 60 minutes at 37˚C. The reaction was terminated with 2X SSC, the sections washed with PBS, and mounted with Crystal/mount (Biomeda, Foster City, CA, USA). TUNEL-positive apoptotic nuclei were detected by visualization with a fluorescent microscope.

Histopathology scoring. Kidney sections of 4 microns were stained with hematoxylin-eosin and scored for histopathological damage to the tubules in a blinded fashion, as previously described (30, 31). Each parameter was assessed in five high-power fields (40X) in the inner cortex and outer medullary regions (where the tubular damage was most evident) and an average determined for each section. The parameters included tubule dilatation, tubule cast formation and tubule cell necrosis. Each parameter was scored on a scale of 0 to 4, ranging from none (0), mild (1), moderate (2), severe (3), to very severe/extensive (4).

Pharmacokinetic studies. Rats were injected intraperitoneally with 5 mg/Kg cisplatin or Lipoplatin. At different time-points, the animals were sacrificed, the kidneys removed, homogenized in saline plus SDS, and total platinum concentration was determined with furnace atomic absorption (Perkin Elmer AA700). Because of the lower toxicity of Lipoplatin compared to cisplatin, additional sets of rats were treated with two higher doses of Lipoplatin (30 and 45 mg/Kg), and the concentration of total

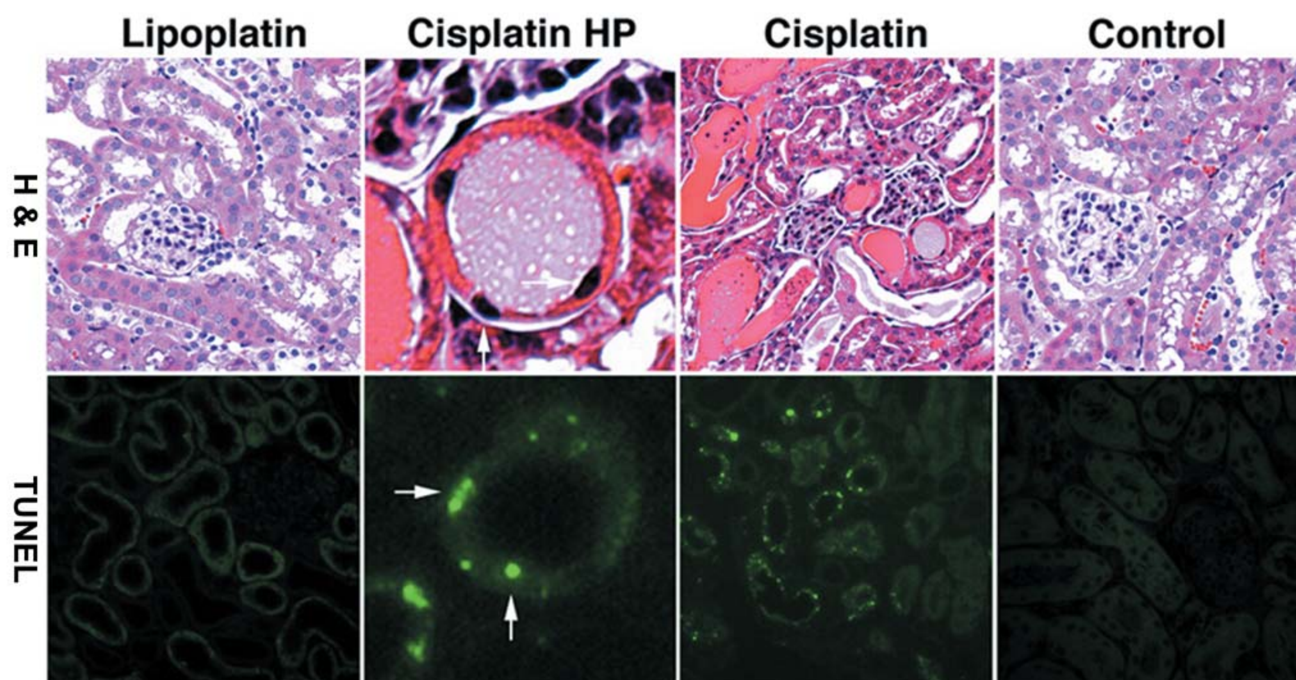

Figure 1. *Cisplatin administration results in tubule cell necrosis and apoptosis in a mouse model. Mice were injected with intraperitoneal cisplatin (20 mg/kg) and kidney sections obtained after 5 days. H & E, hematoxylin-eosin stain, showing tubular dilatation, luminal debris, and flattened epithelium in cisplatin-treated kidneys. At high-power (HP), cisplatin-treated tubules displayed condensed intensely-stained nuclei (arrow), indicative of apoptosis. TUNEL staining, showing TUNEL-positive nuclei in cisplatin-treated kidneys. At high-power, tubules displayed condensed, fragmented nuclei characteristic of apoptosis (arrow). Kidneys treated with Lipoplatin showed only minimal tubular changes. Panels labeled High Power (HP) are at 100X magnification and the others are at 20X. The figure represents five independent experiments.*

platinum in the kidney determined at different time-points as mentioned above. The same was not feasible with cisplatin, since doses of greater than 10 mg/kg resulted in animal death.

Other materials and methods. All chemicals were purchased from Sigma unless otherwise specified. A colorimetric assay kit for the determination of N-acetyl-β-D-glucosaminidase (NAG) in the urine was obtained from Roche (Basel, Siwtzerland).

Results

Lipoplatin results in decreased structural kidney damage in mice. We utilized a well-established murine model in which the structural and functional consequences of cisplatin-induced nephrotoxicity have been previously documented (20-23). Mice were given a single intraperitoneal injection of cisplatin, at the dose of 20 mg/kg body weight. This resulted in tubule cell necrosis, as evidenced in sections stained with hematoxylin-eosin by the presence of tubular dilatation, luminal debris and flattened epithelium (Figure 1). Also documented were tubule cells undergoing programmed cell death, indicated by condensed intensely-stained nuclei. This was confirmed by TUNEL assay, which showed the condensed, fragmented nuclei characteristic of apoptosis (Figure 1). No necrosis or apoptosis was detected in the control kidneys. In striking contrast with the cisplatin-treated animals, kidneys from mice treated with Lipoplatin showed only minimal changes of necrosis (Figure 1).

In order to quantify the differences in structural damage, kidneys from cisplatin- and Lipoplatin-treated mice were scored for histopathological damage to the tubules in a blinded fashion, as previously described (30, 31). Using an arbitrary scoring system ranging from 0-4 for the criteria of tubule dilatation, tubule cast formation and tubule cell necrosis, mice treated with cisplatin showed a significantly greater degree of structural injury, as shown in Figure 2. This observation also held true for apoptosis rate, which was significantly diminished in the Lipoplatin-treated mice, down to values similar to saline-treated control mice (Figure 2).

As further evidence for differences in the renal response to cisplatin *versus* Lipoplatin, we determined the urinary excretion of N-acetyl-β-D-glucosaminidase (NAG), a previously described sensitive urinary marker for tubule cell injury (28). As shown in Figure 3, there was a significant increase in urinary NAG excretion at day 4 and day 5 following cisplatin injection when compared to Lipoplatin-treated mice.

Lipoplatin results in decreased functional kidney damage in mice. It was next of interest to examine the functional

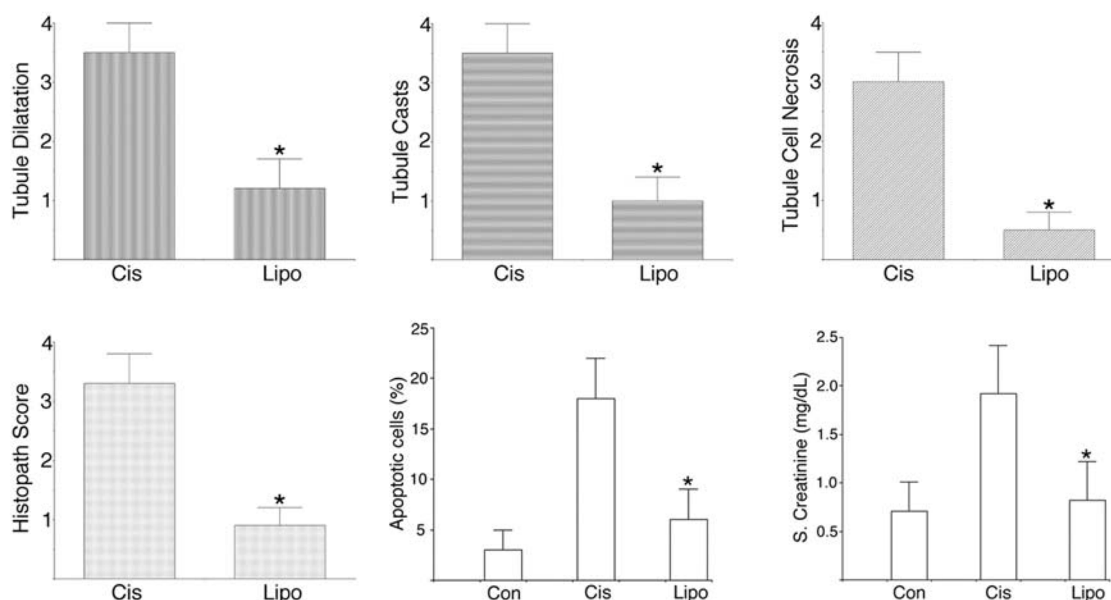

Figure 2. *Cisplatin administration results in tubule cell necrosis and apoptosis and altered renal function in a mouse model. Using an arbitrary histopathological scoring system ranging from 0-4 for the criteria of tubule dilatation, tubule cast formation and tubule cell necrosis, mice treated with cisplatin showed a significantly greater degree of structural injury (top three panels). Bottom left panel shows an overall tubule damage score, obtained by averaging the scores for the three individual criteria. Bottom middle panel shows number of apoptotic cells per 100 counted. Bottom right panel shows results of serum creatinine measurements. Values represent means±SD from five independent experiments. *$p<0.05$ for cisplatin versus Lipoplatin.*

correlate of the decrease in structural kidney damage induced by Lipoplatin. Measurement of serum creatinine levels revealed that, while cisplatin treatment results in a significant reduction in kidney function, mice treated with Lipoplatin maintained kidney function at values comparable to saline-treated controls (Figure 2).

Lipoplatin results in decreased structural and functional kidney damage in rats. We utilized a well-established rat model in which the structural and functional consequences of cisplatin-induced nephrotoxicity have been previously documented (24-26). Rats were given a single intraperitoneal injection of cisplatin, at the dose of 5 mg/kg body weight. This resulted in tubule cell necrosis and apoptosis very similar to that observed in mice (not shown). In striking contrast with cisplatin-treated animals, kidneys from rats treated with Lipoplatin showed only minimal changes of necrosis and apoptosis. The results of the histopathological scoring, apoptosis rate and serum creatinine measurements are shown in Table I. While cisplatin-treated rats displayed a significant reduction in kidney function, animals treated with Lipoplatin maintained kidney function at values comparable to saline-treated controls.

Lipoplatin results in decreased platinum accumulation in rat kidney. Pharmacokinetic analysis of total platinum accumulation in the kidney revealed that both cisplatin and Lipoplatin result in the same maximum level of total platinum in the kidney (10 μg platinum/g kidney), which is reached at about 9 min from injection start for cisplatin and at about 13 min from injection for Lipoplatin (Figure 4A). However, within 20-30 min from injection the total platinum in the kidney after cisplatin remains high (5-6 μg platinum/g kidney), whereas the levels after Lipoplatin gradually decrease, reaching 1 μg platinum/g kidney in about 1.5 h. This difference in maintained for the total time examined (~150h); thus, the steady state of total platinum concentration reached is different for the two drugs. At a dose of 5 mg/Kg cisplatin, a concentration of 4-5 μg platinum/g kidney is reached at a steady state (1-160 hours from injection). On the contrary, treatment of rats with Lipoplatin at 5 mg/Kg results in a steady state level of only 1 μg platinum/g kidney in the same time-frame (Figure 4B).

Because of the observed lower toxicity of Lipoplatin compared to cisplatin, rats were treated with two higher doses of Lipoplatin (30 and 45 mg/Kg) and the concentration of total platinum in the kidney determined at different time-points. The maximum total platinum reached was significantly higher that that shown in Figure 4, and proportional to the increased dose. At 30 mg/Kg Lipoplatin, the maximum total platinum is about 35 μg platinum/g kidney, while at 45 mg/Kg Lipoplatin it is about 75 μg platinum/g kidney. These maxima were reached at approximately 22 min from injection (Figure 5A). In spite

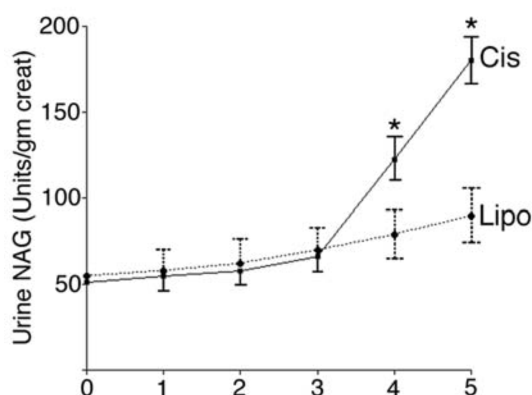

Figure 3. *Cisplatin administration results in urinary excretion of NAG. Values represent means±SD from five independent determinations at each time-point as shown (days). *p<0.05 for cisplatin versus Lipoplatin.*

Table I. *Values are means±SD obtained from rats subjected to a single intraperitoneal injection of cisplatin (5 mg/kg) or Lipoplatin (5 mg/kg) and examined 5 days later. *p<0.05 versus control; #p<0.05 for lipoplatin versus cisplatin.*

	Control (n=5)	Cisplatin (n=5)	Lipoplatin (n=5)
Tubule Dilatation	0	3.5±0.5*	1.0±0.5*#
Tubule Casts	0	3.6±0.4*	1.0±0.4*#
Tubule Cell Necrosis	0	3.0±0.5*	0.6±0.3*#
Histopath Score	0	3.4±0.5*	0.9±0.3*#
Apoptosis (%)	2±0.6	22.0±4.0*	4.0±1.5#
S. Creatinine (mg/dL)	0.52±0.1	2.5±0.5*	0.66±0.2#

of these large differences in maxima, the steady state levels reached at 120 h post injection were comparable to those of the much smaller 5 mg/Kg Lipoplatin dose, as shown in Figure 5B.

Discussion

Platinum drugs such as cisplatin remain the cornerstone of present day chemotherapy regimens not only for lung, ovarian, bladder, testicular, head and neck and gastrointestinal (epithelial malignancies), but also against a number of metastatic or advanced malignancies including cancers of the breast, melanoma, prostate, mesothelioma, nasopharynx, pancreas, leiomyosarcomas and most other advanced cancers (1-3). Newer formulations of experimental and already tested platinum compounds will continue to play an important role in cancer treatment, especially in combination with radiation therapy and emerging gene therapies (6). Their success relies on their ability to arrest DNA synthesis, induce oxidative stress and activate stress-signaling pathways and apoptotic pathways in tumor cells (5, 6). The efficacy of cisplatin is dose-dependent, but the significant risk of nephrotoxicity frequently hinders the use of high doses to maximize its antineoplastic effects (7, 8). Cisplatin leads to both necrosis and apoptotic death of kidney tubule cells and can result in acute renal failure even after a single dose (10-14). This can have major clinical consequences, especially in the face of co-morbid conditions such as those related to the primary malignancy (17, 18). Successful treatment of cisplatin-induced acute renal failure in humans has remained largely anectodal (19). Development of less toxic alternatives to cisplatin has, therefore, remained a major challenge.

In this study, we report the properties of a new drug termed Lipoplatin™, a liposomal formulation of cisplatin developed in order to reduce the systemic toxicity of cisplatin. The liposome particles used in Lipoplatin are a proprietary formulation of an average size of 110 nm. The PEG polymer coating and the small size supposedly bestow upon the Lipoplatin particles the ability to concentrate preferentially in tumors compared to normal tissue (in a mechanism also involving the altered vasculature of the tumor during angiogenesis), as shown in patients (Stathopoulos *et al.*, in preparation). Presumably the Lipoplatin particles can pass undetected by macrophages and immune cells, can remain in circulation for long periods in body fluids, can redistribute in tissues and can extravasate preferentially to infiltrate solid tumors and metastases through the altered and, often compromised, tumor vasculature. Lipoplatin has been previously shown to possess significant antineoplastic activity in mouse xenografts, resulting in apoptotic cell death of breast and prostate tumors in SCID (severe combined immunodeficient) mice (32). Human clinical trials with Lipoplatin are currently under way. It is encouraging to note in the present animal study that, when compared with cisplatin, an equal dose of Lipoplatin resulted in significantly less structural and functional evidence for nephrotoxicity in mice and rats. The incidence of renal tubule cell necrosis and apoptosis following Lipoplatin was comparable to that in control animals, and overall kidney function was preserved after Lipoplatin.

The lower toxicity of Lipoplatin compared to cisplatin may be due to alterations in its pharmacokinetics, preferential localization to tumors containing compromised vasculature and differences in cellular uptake. In the present study, the maximum level of total platinum after intraperitoneal bolus injection of cisplatin or Lipoplatin at similar doses was the same, but the steady state accumulation of total platinum in the kidney was five times greater for cisplatin compared to Lipoplatin. This is important, since is well known that cisplatin-induced nephrotoxicity is a delayed and duration-dependent phenomenon. In this report the first evidence for renal tubule cell damage was not evident until day 4 after cisplatin injection, even when using sensitive urinary biomarkers such as NAG (Figure 3). Changes in serum

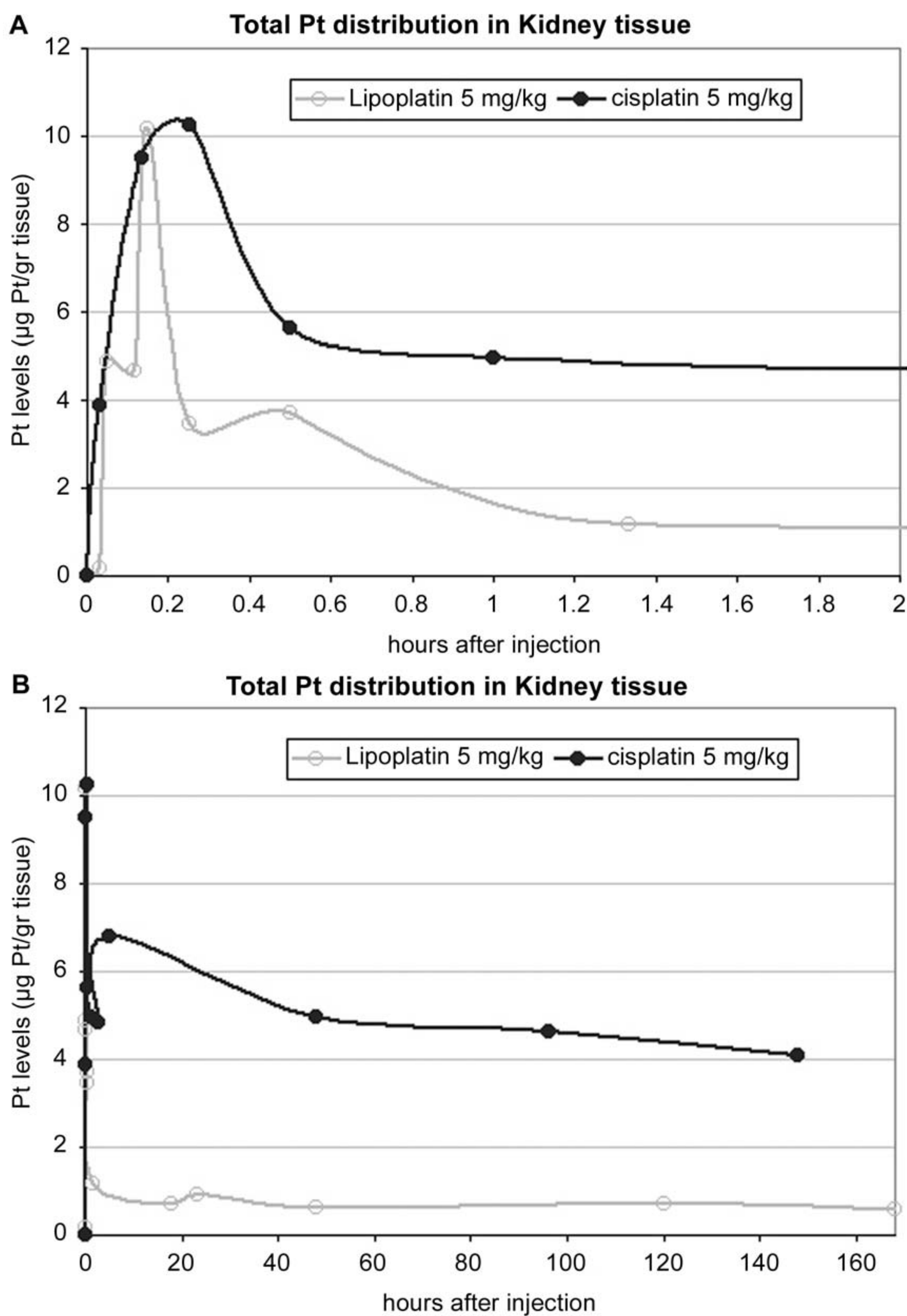

Figure 4. *Treatment of rats with 5 mg/Kg cisplatin or Lipoplatin results in differences in total platinum concentration in the kidney. Points are averages of duplicates. Both cisplatin and Lipoplatin result in the same maximum level of total platinum in the kidney (Figure 4A). However, a steady state of total platinum concentration is different for the two drugs (Figure 4B).*

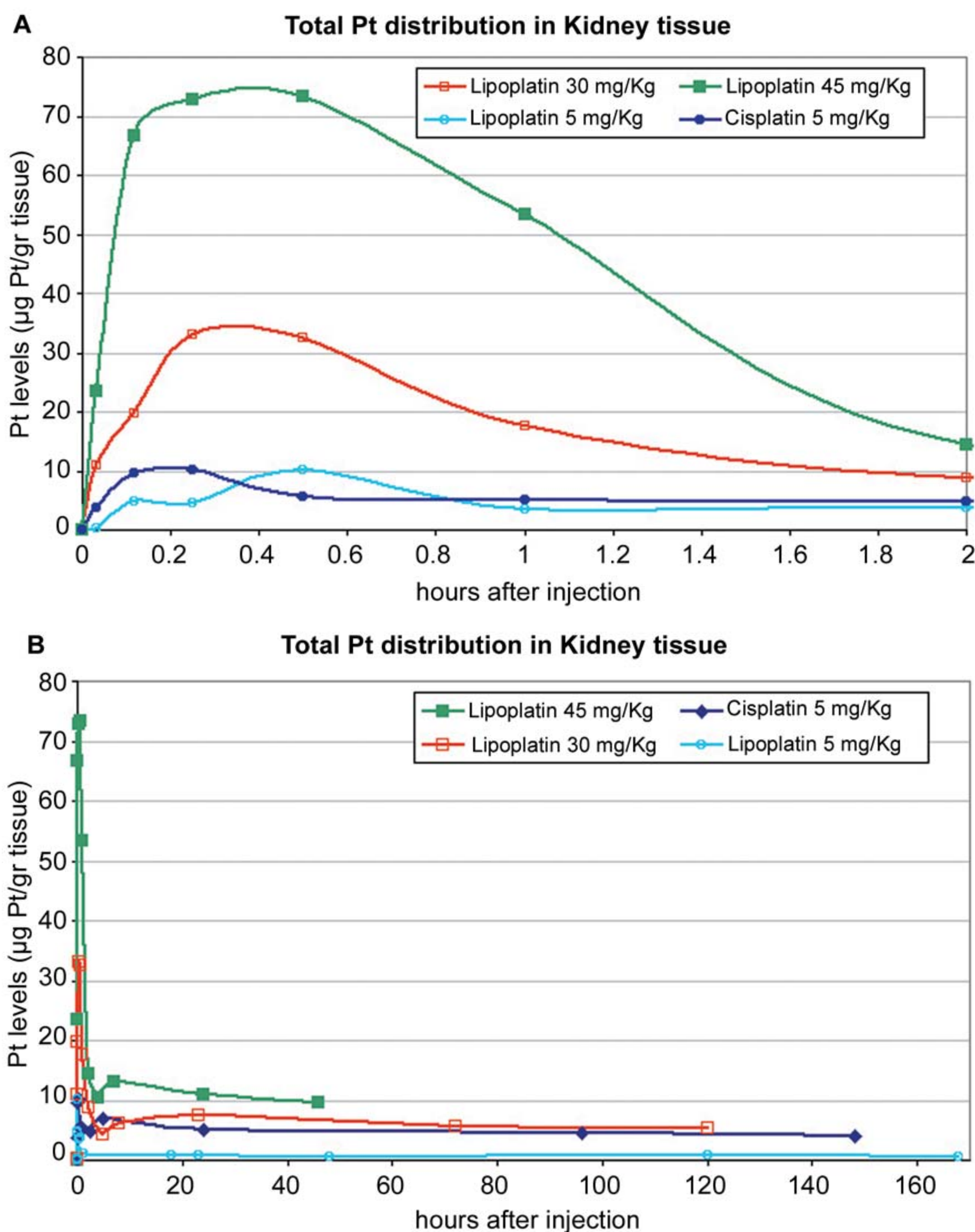

Figure 5. *Because of the lower toxicity of Lipoplatin compared to cisplatin, rats were treated with two higher doses of Lipoplatin (30 and 45 mg/Kg) and the concentration of total platinum in the kidney determined at different time-points. Points are averages of duplicates. The maximum total platinum reached is proportional to the dose (Figure 5A). However, the steady state levels are similar to those of the 5 mg/Kg dose at 120 hours from injection (Figure 5B).*

creatinine and tubule morphology were delayed until 5 days after the initial injection. Thus, it is likely that the diminished steady state accumulation of Lipoplatin may be responsible, at least in part, for its low renal toxicity in comparison to cisplatin. The initial rapid accumulation of Lipoplatin in kidney cells is presumably ineffective in causing cell death. This is because, in the normally quiescent renal proximal tubular cells, cisplatin-induced nephrotoxicity requires the

metabolism of cisplatin to a nephrotoxin, followed by induction of stress-activated kinase pathways as well as mitochondrial apoptotic pathways (5-12). In contrast, Lipoplatin remains effective in proliferating cells (such as tumor cells), where the mechanism of cell death primarily involves a rapid inhibition of DNA synthesis.

Acknowledgements

This work was supported by grants from the NIH/NIDDK to P.D. (DK53289, DK52612).

References

1 Rosenberg B: Noble metal complexes in cancer chemotherapy. Adv Exp Med Biol *91*: 129-50, 1977.
2 Lebwohl D and Canetta R: Clinical development of platinum complexes in cancer therapy: An historical perspective and an update. Eur J Cancer *34*: 1522-1534, 1998.
3 Trimmer EE and Essigmann JM: Cisplatin. Essays Biochem *34*: 191-211, 1999.
4 Takahara PM, Rosenzweig AC, Frederick CA and Lippard SJ: Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. Nature *377*: 649-52, 1995.
5 Levresse V, Marek L, Blumberg D and Heasley L: Regulation of platinum-compound cytotoxicity by the c-Jun N-terminal kinase and c-Jun signaling pathway in small-cell lung cancer cells. Mol Pharmacol *62*: 689-697, 2002.
6 Boulikas T and Vougiouka M: Cisplatin and platinum drugs at the molecular level. Oncol Rep *10*: 1663-1682, 2003.
7 Humes HD: Insights into ototoxicity. Analogies to nephrotoxicity. Ann NY Acad Sci *884*: 15-18, 1999.
8 Arany I and Safirstein RL: Cisplatin nephrotoxicity. Semin Nephrol *23*: 460-4, 2003.
9 Leibbrandt MEI, Wolfgang GHI, Metz AL, Ozobia AA and Haskins JR: Critical subcellular targets of cisplatin and related platinum analogs in rat renal proximal tubule cells. Kidney Int *48*: 761-770, 1995.
10 Park MS, De Leon M and Devarajan P: Cisplatin induces apoptosis in LLC-PK1 cells *via* activation of mitochondrial pathways. J Am Soc Nephrol *13*: 858-865, 2002.
11 Townsend DM, Deng M, Zhang L, Lapus MG and Hanigan MH: Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. J Am Soc Nephrol *14*: 1-10, 2003.
12 Hanigan MH and Devarajan P: Cisplatin nephrotoxicity: molecular mechanisms. Cancer Therapy *1*: 47-61, 2003.
13 Liu H and Baliga R: Cytochrome P450 2E1 null mice provide novel protection against cisplatin-induced nephrotoxicity and apoptosis. Kidney Int *63*: 1687-1696, 2003.
14 Meyer KB and Madias NE: Cisplatin nephrotoxicity. Mineral Electrolyte Metab *20*: 201-213, 1994.
15 McKeage MJ: Comparative adverse effect profiles of platinum drugs. Drug Saf *13*: 228-244, 1995.
16 Santoso JT, Lucci JA 3rd, Coleman RL, Schafer I and Hannigan EV: Saline, mannitol, and furosemide hydration in acute cisplatin nephrotoxicity: a randomized trial. Cancer Chemother Pharmacol *52*: 13-8, 2003.
17 Star RA: Treatment of acute renal failure. Kidney Int *54*: 1817-1831, 1998.
18 Lamiere N and Vanholder R: Pathophysiologic features and prevention of human and experimental acute tubular necrosis. J Am Soc Nephrol *12*: S20-S32, 2001.
19 Sheikh-Hamad D, Timmins K and Jalali Z: Cisplatin-induced renal toxicity: Possible reversal by N-acetylcysteine treatment. J Am Soc Nephrol *8*: 1640-1645, 1997.
20 Megyesi J, Safirstein RL and Price PM: Induction of $p21^{WAF/CIP1/SDI1}$ in kidney tubule cells affects the course of cisplatin-induced acute renal failure. J Clin Invest *101*: 777-782, 1998.
21 Shiraishi F, Curtis LM, Truong L, Poss K, Visner GA, Madsen K, Nick HS and Agarwal A: Heme oxygenase-1 gene ablation or expression modulates cisplatin-induced renal tubular apoptosis. Am J Physiol Renal Physiol *278*: F726-F736, 2000.
22 Ramesh G and Reeves WB: TNF-1 mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest *110*: 835-842, 2002.
23 Tsuruya K, Ninomiya T, Tokumoto M, Hirakawa M, Masutani K, Taniguchi M, Fukuda K, Kanai H, Kishihara K, Hirakata H and Iida M: Direct involvement of the receptor-mediated apoptotic pathways in cisplatin-induced renal tubular cell death. Kidney Int *63*: 72-82, 2003.
24 Sueshi K, Mishima K, Makino K, Itoh Y, Tsuruya K, Hirakata H and Oishi R: Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rat. Eur J Pharmacol *451*: 203-208, 2002.
25 Mora LO, Antunes LM, Francescato HD and Bianchi ML: The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. Pharmacol Res *47*: 517-522, 2003.
26 Ichimura T, Hung CC, Yang SA, Stevens JL and Bonventre JV: Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury. Am J Physiol Renal Physiol *286*: F552-F563, 2004.
27 Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC and Devarajan P: Differential gene expression following early renal ischemia-reperfusion. Kidney Int *63*: 1714-1724, 2003.
28 Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J and Devarajan P: Identification of NGAL as a novel urinary biomarker for ischemic injury. J Am Soc Nephrol *14*: 2534-2543, 2003.
29 Del Rio M, Imam A, DeLeon M, Gomez G, Mishra J, Ma Q, Parikh S and Devarajan P: The death domain of ankyrin interacts with Fas and promotes Fas-mediated cell death. J Am Soc Nephrol *15*: 41-51, 2004.
30 Deng J, Kohda Y, Chiao H, Wang Y, Hu X, Hewitt SM, Miyaji T, McLeroy P, Nibhanupudy B, Li S and Star RA: Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. Kidney Int *60*: 2118-2128, 2001.
31 Yokota N, Burne-Taney M, Racusen L and Rabb H: Contrasting roles for STAT4 and STAT6 signal transduction pathways in murine renal ischemia-reperfusion injury. Am J Physiol Renal Physiol *285*: F319-F325, 2003.
32 Boulikas T: Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts. Int J Oncol Rep *12*: 3-12, 2004.

Received February 18, 2004
Revised May 21, 2004
Accepted June 2, 2004