

## Systemic Lipoplatin Infusion Results in Preferential Tumor Uptake in Human Studies

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**Abstract.** *Lipoplatin™, a liposomal formulation of cisplatin, was developed with almost negligible nephrotoxicity, ototoxicity and neurotoxicity, as demonstrated in preclinical and Phase I human studies. A polyethylene-glycol coating of the liposome nanoparticles is supposed to result in tumor accumulation of the drug by extravasation through the altered tumor vasculature. We explored the hypothesis that intravenous infusion of Lipoplatin results in tumor targeting in four independent patient cases (one with hepatocellular adenocarcinoma, two with gastric cancer and one with colon cancer) who underwent Lipoplatin infusion followed by a prescheduled surgery ~20 h later. Direct measurement of the platinum levels in specimens from the excised tumors and normal tissues showed that the total platinum levels were on average 10-50 times higher in malignant tissue compared to the adjacent normal tissue specimens; most effective targeting was observed in colon cancer, with an accumulation up to 200-fold higher in colon tumors compared to normal colon tissue. Of the several surgical specimens, gastric tumors displayed the highest levels of total platinum suggesting Lipoplatin as a candidate anticancer agent for gastric tumors; gastric tumor specimens had up to 260 micrograms platinum /g tissue, that was higher than any tissue level in animals treated at much higher doses. Fat tissue displayed a high accumulation of total platinum in surgical specimens in three different patients, correlating to the lipid capsule of cisplatin in its Lipoplatin formulation. It was also inferred that normal tissue had more platinum trapped in the tissue but not reacted with macromolecules, whereas tumor tissue displayed platinum that reacted with cellular macromolecules; the data were consistent*

*with a model where Lipoplatin damages more tumor compared to normal cells. In conclusion, Lipoplatin has the ability to preferentially concentrate in malignant tissue both of primary and metastatic origin following intravenous infusion to patients. In this respect, Lipoplatin emerges as a very promising drug in the arsenal of chemotherapeutics.*

Cis-dichlorodiammineplatinum (II) (cisplatin) has continued to play a central role in chemotherapy, for the treatment of epithelial malignancies, for over 20 years, since its serendipitous discovery in 1965 (1) and its identification in 1969 (2, 3, reviewed in 4, 5). The antitumor properties of cisplatin are attributed to the kinetics of its chloride ligand displacement reactions leading to DNA crosslinking activities; indeed, once inside the cell, the lower chloride ion content of the cytoplasm allows the two chloride groups of cisplatin to exchange with water yielding the diaquo (hydroxo) species. Although cisplatin reacts directly with sulfur groups (such as glutathione), the reaction of cisplatin with DNA depends on its prior hydrolysis to hydroxo complexes that are much more reactive with nitrogen and oxygen donor groups on proteins and DNA. The diaquo species of cisplatin was found, in early studies, to react with pyrimidines and substituted pyrimidines and to achieve spectacular cures of ascites Sarcoma 180 tumors in Swiss mice (6). Intensive work towards the improvement of cisplatin, with hundreds of platinum drugs tested, resulted in the introduction of carboplatin, and of oxaliplatin, used only for a very narrow spectrum of cancers.

Cisplatin, carboplatin, oxaliplatin and most other platinum compounds induce similar types of damage to DNA and their tumor killing properties largely depend on their ability to induce apoptosis; this is mediated by activation of signal transduction leading to the death receptor mechanisms as well as mitochondrial pathways. Cisplatin elicits crosslinks in DNA, confronting the DNA repair machinery. Furthermore, cisplatin alters signal transduction and apoptotic pathways cross-talking to other

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signaling pathways, such as those leading to up-regulation of transcription factor and nuclear/cellular enzymes (reviewed in 7-9).

Platinum drugs produce a distinct pattern of side-effects depending on their reaction with blood components, tissue biodistribution, half-life in plasma, ability to cross the cell membrane in tissue targets and molecular mechanisms, including reactivity to various types of macromolecules, especially DNA, type of DNA reaction products, extent of damage to normal compared to malignant tissue, rate of repair of DNA damage by particular cell types and a number of other parameters. The clinical use of cisplatin is impeded by acute toxicities, such as nausea and vomiting (GI tract toxicity), and cumulative dose-dependent chronic side-effects of nephrotoxicity, ototoxicity and neurotoxicity, as a result of damage to cells in these tissues (reviewed in 7, 10). Since the reduction of the renal side-effects with hydration and of the gastrointestinal side-effects with antiemetics, neurotoxicity is the most important adverse effect associated with cisplatin chemotherapy, occurring at a rate of 47% among 292 ovarian cancer patients treated with cisplatin combinations at conventional doses in randomized controlled studies compared to 25% of patients treated with the non-cisplatin-containing regimens (11). The symptoms include numbness, tingling, paresthesiae in the extremities, difficulty in walking, decreased vibration sense in the toes, deep tendon reflexes, loss of the ankle jerks, difficulty with manual dexterity, difficulty with ambulation from a deficit in proprioception and gait disturbances; less frequent symptoms from cisplatin neurotoxicity include retrobulbar neuritis, encephalitic symptoms, autonomic neuropathy, cerebral herniation, seizures, cortical blindness, ophthalmologic effects and vertigo (12-15). Unfortunately, neuropathy is long-term, with significant worsening of the symptoms in the first 4 months (16) that may persist for over 52 months after stopping cisplatin treatment (17). Higher platinum concentrations in tissues from the peripheral nervous system (peripheral nerves and dorsal root ganglia) compared to tissues from the central nervous system (brain, spinal cord) seem to correlate with clinical symptoms of peripheral neuropathy (18). Attempts to prevent cisplatin-induced neurotoxicity may involve treatment of patients with nucleophilic sulfur thiols (amifostine, diethyldithiolcarbamate and others), adrenocorticotrophic hormone analogs (ORG 2766) and calcium channel antagonists (19). Nevertheless, the clinical relevance of the protective effect is small (20-22). A randomized double-blind placebo-controlled study using the natural protective agent glutathione (glutamyl-cysteinylglycine), in 50 patients with gastric cancer undergoing cisplatin+5-FU+epirubicin+leucovorin dose-intensive chemotherapy (23), showed reduced neuropathies in the glutathione arm.

Ototoxicity symptoms include tinnitus (ringing in the ears), deafness and hearing difficulties, especially in noisy environments or difficulty in hearing high frequency sounds such as the ticking of a watch or the telephone ringing (24). The most important factor predicting persistent ototoxicity was a cumulative dose of over 600 mg/m<sup>2</sup> cisplatin in testicular cancer patients (25). The mechanism of ototoxicity involves loss of inner and outer hair cells of the basal turns of the cochlear, loss of spinal ganglion cells and degeneration of the stria vascularis (26, 27), caused by induction of apoptosis in these cell types (reviewed in 8).

The unique anticancer properties of cisplatin have prompted numerous attempts to either improve its therapeutic efficacy or to reduce its side-effects without compromising therapeutic efficacy. Modifications have focused either on formulations of cisplatin aimed at enhancing its tumor-targeting to minimize damage to nonmalignant tissue and to lower the side-effects, or on chemical modifications to the molecule to derive molecules of better quality.

Lipoplatin™ is a novel formulation of cisplatin into liposomes composed of dipalmitoyl phosphatidyl glycerol (DPPG), soy phosphatidyl choline (SPC-3), cholesterol and methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine (mMPEG2000-DSPE). In a previous work, Lipoplatin was shown to be less toxic than cisplatin in animals and to cause reduction of tumors after intraperitoneal or intravenous injection to human breast MCF-7 or prostate LNCaP xenografts (28); histological examination of the treated tumors from mouse xenografts was consistent with apoptosis in the tumor in a mechanism similar to that 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 free of kidney injury (29). In a Phase I study involving 27 patients, Lipoplatin dose escalation was administered as second- or third-line chemotherapy; Lipoplatin showed no nephrotoxicity and it lacked the other serious side-effects of cisplatin up to a dose of 125 mg/m<sup>2</sup> every 14 days; the MTD was above 350 mg/m<sup>2</sup> as a single infusion (30).

Because Lipoplatin nanoparticles bear a PEG-coating, it can be inferred that intravenous infusion of this drug will result in its preferential distribution at tumor sites *via* extravasation through the leaky tumor vasculature, in analogy to PEGylated DOXIL/Caelyx liposomal doxorubicin (31). The objective of the present study was to investigate the possibility of platinum accumulation in solid human tumors after Lipoplatin infusion in cancer patients. Single doses of 100 mg/m<sup>2</sup> Lipoplatin were used, which are known not to cause any adverse reactions (30).

## Patients and Methods

*Preparation and characteristics of Lipoplatin.* Cisplatin was purchased from Heraeus/Flavine (mw 300, Hanau, Germany). The lipid shell of Lipoplatin is composed of 1,2-dipalmitoyl-sn-glycero-

3-[phospho-rac-(1-glycerol)] (sodium salt) (DPPG, mw 745, Lipoid GmbH, Ludwigshafen, Germany), soy phosphatidyl choline (SPC-3, mw 790 Lipoid GmbH), cholesterol (CHOL, mw 386.66, Avanti Polar Lipids, Alabaster, Alabama, USA) and methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine lipid conjugate (mMPEG2000-DSPE, mw 2807, Genzyme, Liestal, Switzerland). The ratio of lipid: cisplatin is 10.24: 1.0 (w/w). The content of Lipoplatin in cholesterol is 11.6% (w/w) of the total lipid. It is calculated that 1.3 mg cholesterol are injected per mg cisplatin in its Lipoplatin formulation.

The type of liposome particles used in Lipoplatin is a proprietary formulation of an average size of 110 nm. Lipoplatin is provided in 50-ml opaque glass vials of 3 mg/ml (concentration refers to cisplatin). Although cisplatin is light-sensitive, its Lipoplatin formulation appears to be resistant to disintegration by light because liposomes shield the drug. However, as a precaution, storage in the dark is recommended. Lipoplatin is stored at 4°C with an expiry date of two years.

**Study design.** The criteria of patient selection included: histologically confirmed type of malignancy; operable stage of disease; patients signed informed consent. Lipoplatin was used at an underdose as monotherapy (100 mg/m<sup>2</sup>), known from previous studies not to cause adverse effects that would interfere with the recovery of the patients or would complicate surgery (such as the ability of wound repair). The MTD of Lipoplatin is above 350 mg/m<sup>2</sup> (30).

The protocol was approved both by the ethics committee of the Errikos Dunant Hospital and by the Hellenic Drug Organization, Greece. The procedures followed were in accordance with the Helsinki Declaration of the World Medical Association.

**Patient characteristics.** This study involved four patients at stage III of their disease with the following characteristics: Patient 1: A female patient with hepatocellular adenocarcinoma. Patient 2: A male patient with gastric cancer and liver metastasis. Patient 3: A male patient with gastric cancer. Patient 4: A male patient with colon cancer.

**Patient evaluation and treatment.** Before study entry, all patients underwent the following evaluation: complete medical history and physical examination, tumor measurement or evaluation, WHO performance status, an electrocardiogram and a neurological examination. Prior to Lipoplatin infusion, complete hematology and serum chemistry including cholesterol, triglyceride levels, blood urea, serum creatinine, liver function tests and urine analysis were performed. Staging was determined by chest and abdominal CT scans, and occasional magnetic resonance imaging endoscopy (gastroscopy) and colonoscopy.

All patients were infused with a single dose of 100 mg/m<sup>2</sup> Lipoplatin as a single agent diluted in 1 L 5% dextrose during a period of 8 h.

**Analysis of plasma for platinum.** Blood was drawn before Lipoplatin infusion as well as prior to the operation and placed in EDTA-containing centrifuge tubes. The blood was then centrifuged to remove cells and total plasma was analyzed for total platinum (*i.e.* free plus protein-bound plus liposomal), using both the Perkin Elmer Analyst AA200 (flame) and Perkin Elmer AA 700 Graphite Furnace Atomic Absorption Spectrometers equipped with a

platinum lamp. Plasma platinum levels at 20 h from the start of Lipoplatin infusion were ~2 µg/ml, in accordance with previous studies (30).

**Analysis of surgical specimens for platinum.** During surgery (performed at ~20 h from Lipoplatin infusion start) specimens were obtained (0.1-1 g) from the primary or metastatic tumor, the adjacent normal tissue and, in some cases, from colon fat or muscle tissue.

Samples were blindly analyzed for platinum content as follows: 0.1-1.0 g tissue was ground and homogenized into two times its volume (0.2-2 ml) of saline. The sample was centrifuged at 21,000 xg for 2 min in a refrigerated microfuge. The supernatant was assayed for platinum levels. The tissue pellet was further treated with one time its original volume (0.1-1.0 ml) of 10% SDS, centrifuged at 21,000 xg for 2 min and the supernatant was analyzed for platinum levels as above. In some cases, the pellet was further digested with proteinase K in the presence of 10% SDS overnight at 65°C and the supernatant was analyzed for platinum levels. Platinum levels were normalized to µg platinum/g tissue. Standard cisplatin curves of known platinum concentration were used to calculate the platinum levels in all extracts.

## Results

**Targeting hepatocellular adenocarcinoma with Lipoplatin.** A 49-year-old female with hepatocellular adenocarcinoma was infused with as a single dose of 100 mg/m<sup>2</sup> Lipoplatin as a single agent during a period of 8 h the day before surgery. There were no acute side-effects as a result of this infusion. Surgery was the option chosen by her physicians. During surgery, specimens were removed (at approximately 18 h from infusion start) from the liver tumor, the adjacent normal liver tissue, a colon metastatic tumor, the adjacent normal colon tissue and colon fat. The specimens were processed as described in Patients and Methods and the platinum levels in the saline-soluble and SDS-soluble fractions were assayed. The total platinum in the saline-soluble material represents cisplatin that is trapped in the tissue and either has not reacted with macromolecules, or has formed coordination complexes with soluble molecules such as glutathione. Platinum levels in the SDS-soluble fraction represent cisplatin bound to macromolecules that are not soluble in saline extraction, but are solubilized by SDS rupture of macromolecules and cell structures, as well as by SDS rupture of nuclei, cellular membranes and liposomes. Their sum gives the total platinum and was expressed as µg platinum /g tissue.

The hepatocellular adenocarcinoma tissue accumulated about 38 µg total platinum /g tissue compared with 19 µg total platinum /g normal liver tissue. Thus, the liver tumor had taken up twice as much total platinum after Lipoplatin infusion compared to the adjacent normal liver tissue (Table I).

The difference between Lipoplatin accumulation in a colon metastasis compared to adjacent normal colon tissue was even more dramatic. The colon metastasis took up

Table I. *Platinum levels in tumor and normal tissue in a patient with hepatocellular carcinoma and colon metastases.*

Patient 1: Hepatocellular adenocarcinoma	Pt ( $\mu\text{g/g}$ tissue) in saline or SDS fractions	TOTAL Pt ( $\mu\text{g/g}$ tissue)	Ratio tumor Pt / normal Pt
liver tumor saline	5.18	38.36	1.96
liver tumor SDS	33.18		
normal liver tissue saline	16.45	19.61	49.53
normal liver tissue SDS	3.16		
colon metastasis saline	4.44	6.61	0.13
colon metastasis SDS	2.17		
normal colon tissue saline	0.06	0.13	1.79
normal colon tissue SDS	0.08		
colon fat saline	0.96	1.79	
colon fat SDS	0.83		

about 50 times more Lipoplatin than a normal colon tissue specimen (Table I). The data demonstrate a preferential uptake of Lipoplatin by liver tumor, following intravenous infusion, compared to normal liver tissue. There was a difference in the overall accumulation of the liposomally-encapsulated drug in the liver and colon tumors; overall the uptake of Lipoplatin by the liver tumor was about 6 times higher than the colon metastatic tumor (38.36 *versus* 6.61  $\mu\text{g}$  total platinum /g tissue, Table I). Such differences could arise from the biodistribution properties of the drug, but also from differences in vascularization between the liver tumor and its colon metastasis.

Our extraction method using saline gives an indication of platinum trapped in the tissue (saline-soluble), whereas the subsequent SDS extraction indicates platinum that has entered the tumor cell and/or has reacted with macromolecules. Table I indicates that the liver tumor contained about 5  $\mu\text{g}$  platinum trapped in the tissue /g tissue and 33  $\mu\text{g}$  platinum that had reacted with macromolecules /g tissue. On the contrary, normal liver tissue contained about 16 mg platinum trapped in the tissue /g tissue and 3  $\mu\text{g}$  platinum that had reacted with macromolecules /g tissue (Table I).

*Targeting a liver metastasis from a primary gastric cancer.* A male with gastric cancer and liver metastasis (Patient 2) was treated with 100  $\text{mg/m}^2$  Lipoplatin as single agent using an

Table II. *Platinum levels in liver metastasis and normal liver tissue in a patient with gastric cancer.*

Patient 2: Gastric cancer with liver metastasis	Pt ( $\mu\text{g/g}$ tissue) in saline or SDS fractions	TOTAL Pt ( $\mu\text{g/g}$ tissue)	Ratio tumor Pt / normal tissue Pt
liver metastasis saline	34.51	131.15	6.26
liver metastasis SDS	96.64		
normal liver tissue saline	16.94	20.94	
normal liver tissue SDS	4.00		
normal fat saline	8.38	51.91	
normal fat SDS	43.53		

8-h infusion one day before surgery. During surgery, specimens were removed from the liver metastatic tumor, the adjacent normal liver tissue and fat. The liver metastatic specimen displayed a total amount of 131.15  $\mu\text{g}$  platinum /g tissue compared to 20.94  $\mu\text{g}$  platinum /g normal liver tissue (Table II). This gives a 6.26 higher level of platinum in the liver metastatic tumor compared to the normal liver tissue; it is emphasized that the comparison here is between a gastric tumor in liver with liver tissue. Fat removed during surgery appears to display significant levels of total platinum (51.91  $\mu\text{g}$  platinum /g tissue), supposedly relating to the lipophilic nature of the drug.

*Targeting gastric tumors.* A male with gastric cancer (Patient 3) was treated with 100  $\text{mg/m}^2$  Lipoplatin as a single agent given during an infusion period of 8 h. During surgery, two different specimens were obtained from the stomach tumor, the adjacent normal stomach tissue and fat. The samples were blindly analyzed for platinum content. Both specimens of gastric tumors appeared to accumulate the highest amounts of platinum among all the specimens analyzed in this study (262.62 and 66.38  $\mu\text{g}$  Pt/g tissue, respectively, Table III). We surmized that the difference in platinum levels between the same tumor may arise from the different locations of the specimens in the tumor mass and probably relate to differences in vascularization. This needs to be further explored in future studies with a simultaneous examination of vascularization. However, the tumor specimens displayed 40 and 10 times, respectively, more platinum compared to normal stomach tissue (Table III). Consistent with findings in Patients 1 and 2, the platinum trapped in tumor specimen 1 was 42  $\mu\text{g/g}$  tissue *versus*



Table III. Platinum levels in a gastric tumor and its adjacent normal gastric tissue.

Patient 3: Gastric cancer	Pt (µg/g tissue) in saline or SDS fractions	TOTAL Pt (µg/g tissue)	Ratio tumor Pt / normal Pt
stomach tumor 1 saline	42.17	262.62	39.87
stomach tumor 1 SDS	220.45		
stomach tumor 2 saline	28.46	66.38	10.08
stomach tumor 2 SDS	37.92		
normal stomach tissue saline	2.62	6.59	
normal stomach tissue SDS	3.97		
normal fat tissue saline	10.36	41.12	
normal fat tissue SDS	30.76		

220 µg/g tissue of platinum that reacted with macromolecules. Specimen 2 had 28 µg/g tissue-trapped platinum *versus* 37 µg/g tissue that reacted with macromolecules. Normal stomach tissue displayed 2.6 µg/g tissue *versus* 4.0 µg/g tissue platinum that was trapped *versus* macromolecule-bound. Again, stomach tumor 1 appeared to have about 5 times more platinum in the form that presumably reacted with macromolecules and thus induced damage to cells compared to platinum simply trapped in the tissue (220:42, Table III), whereas specimen 2 and normal gastric tissue had only 1.5 times more or about the same platinum in the two fractions.

Consistent with the findings in Patient 2, the fat tissue in Patient 3 also displayed significant levels of platinum (41 µg/g fat tissue).

*Accumulation of platinum in a colon tumor after Lipoplatin infusion.* A male with colon cancer was treated with 100 mg/m<sup>2</sup> Lipoplatin as a single agent during a period of 8 h. During surgery, specimens were obtained from the colon tumor (in duplicate), the adjacent normal colon tissue, muscle and fat. The total platinum levels in the two colon tumor specimens were 11.26 and 7.69 µg platinum/ g tissue compared to 0.06 µg/g of normal colon tissue (Table IV). This gives 204- and 140-fold higher levels of platinum between the colon tumor and normal colon tissue. Consistent with the findings in Patients 2 and 3, the fat tissue in Patient 4 also displayed significant levels of platinum (20 µg/g fat tissue). The muscle tissue had very small amounts of platinum (0.36 µg platinum/g tissue).

Table IV. Platinum levels in a colon tumor, its adjacent normal colon tissue, as well as normal fat and muscle tissues.

Patient 4: Colon cancer	Pt (µg/g tissue) in saline or SDS fractions	TOTAL Pt (µg/g tissue)	Ratio tumor Pt / normal Pt
colon tumor 1 saline	4.42	11.26	204.72
colon tumor 1 SDS	6.85		
colon tumor 2 saline	1.86	7.69	139.70
colon tumor 2 SDS	5.83		
normal colon tissue saline	0.02	0.06	
normal colon tissue SDS	0.04		
muscle tissue saline	0.36	0.36	
muscle tissue SDS	0.00		
fat tissue saline	4.34	19.98	
fat tissue SDS	15.64		

## Discussion

*High accumulation of Lipoplatin in tumors and its low toxicity.* The present study directly shows platinum accumulation in tumors compared to normal tissue after intravenous infusion of Lipoplatin in four cancer patients. The tumor accumulation properties of Lipoplatin™ are astonishing and, in colon tumor specimens, levels that were up to 204 times higher than those attained in the normal tissue were documented. The PEG polymer coating used to camouflage Lipoplatin is suggested to be responsible for the enhanced circulation of the drug, its ability to pass undetected by the macrophages and immune cells, and its property to extravasate preferentially and infiltrate solid tumors and metastases through the altered, and often compromised, tumor vasculature (31). Indeed, the half-life of Lipoplatin was found to be 60-114 h in patients compared to less than 6 h for cisplatin (30). Furthermore, unlike other liposomal drugs endowed with tumor-targeting abilities such as Doxil/Caelyx or SPI-77, Lipoplatin is suggested to be endowed with cell membrane fusion properties because of the DPPG lipid molecule used in its formulation (28).

Among the various surgical specimens examined, gastric tumors revealed the highest level of total platinum (up to 263 µg cisplatin /g tissue, Table III), followed by liver

tumors (up to 38  $\mu\text{g}$  cisplatin /g tissue, Table I). The lowest level of total platinum accumulation among tumor specimens was observed in a metastatic colon tumor from a primary hepatocellular cancer (6.6  $\mu\text{g}$  cisplatin /g tissue, Table I) and two primary colon tumor specimens from a colon cancer patient (11.2 and 7.7  $\mu\text{g}$  cisplatin /g tissue, respectively, Table IV). Following the suggestion that Lipoplatin nanoparticles have the intrinsic property of extravasating through the altered tumor vasculature, the findings of high platinum accumulation in certain tumor specimens, especially gastric, reported here might correlate with the vascularization of the tumor. Although fat tissue lacks a high degree of vascularization, it displayed a high accumulation of total platinum in surgical specimens excised from three out of four patients (52  $\mu\text{g}$  cisplatin /g fat tissue, Table II; 41  $\mu\text{g}$  /g fat tissue, Table III; 20  $\mu\text{g}$  cisplatin /g fat tissue, Table IV). This might correlate with the liposomal nature of Lipoplatin and the lipophilicity of the drug.

Cisplatin, in combination with other drugs, is used as first-line chemotherapy against epithelial malignancies and as second-line treatment against most other advanced cancers. However, cisplatin causes severe renal tubular damage, reducing glomerular filtration by inducing oxidative stress in renal tubular cells, leading to apoptotic death (10). A major dose-limiting side-effect of cisplatin is ototoxicity, leading to hearing loss by inducing apoptosis in auditory sensory cells and destruction of hair cells linked with tinnitus; this is proportional to the cumulative dose of cisplatin and independent of treatment schedules (32). Peripheral neurotoxicity is also a dose-limiting toxicity, causing loss of vibration sense, paresthesia and sensory ataxia. Acute emesis after cisplatin administration is mediated by serotonin release from enterochromaffin gut mucosal cells and stimulation of serotonin 5-HT<sub>3</sub>-receptors (reviewed in 7).

This study, as well as a previous one (30), showed no side-effects after a single 8-h infusion of Lipoplatin at 100 mg/m<sup>2</sup> (from full blood analysis 7 days after drug treatment), concomitant with higher platinum accumulation in surgical specimens. The present study provides one reasonable explanation for the lower toxicity of the drug by showing a higher uptake by the tumor tissue compared to normal tissue. In addition, Lipoplatin appears to be bound more in macromolecules in cancerous tissue, but simply trapped and not reacted with macromolecules in normal tissue (Tables I-III), an observation that suggests one additional mechanism for the lower toxicity of Lipoplatin compared to cisplatin. Of considerable importance is the fact that the neuro-, oto- and nephro-toxicity of Lipoplatin at doses up to 125 mg/m<sup>2</sup> are negligible (30).

Coating the surface of liposomes with inert materials designed to camouflage the liposome from the body's host defense systems was shown to remarkably increase their

plasma longevity, in a similar way to the erythrocyte coated with a dense layer of carbohydrate groups to evade the immune system (31, 33). Lipoplatin has a coat of polyethylene glycol (PEG), a modification used for many years to prolong the half-lives of biological proteins (such as enzymes and growth factors) reducing their immunogenicity (e.g. 34), or to coat liposomes that were then able to circulate for remarkably long times after intravenous administration, leading to a reduction in nonspecific uptake by the reticuloendothelial system (35-38).

The major mechanism of cisplatin cytotoxicity arises from its ability to induce monofunctional DNA adducts as well as intrastrand and interstrand cross-links. Cisplatin lesions are recognized by High Mobility Group (HMG) nonhistones and binding of HMG-domain proteins to cisplatin-modified DNA has been postulated to mediate the antitumor properties of the drug (39, 40). Cisplatin is an activator of stress-signaling pathways, especially of the c-Jun N-terminal kinase (JNK) family of mitogen-activated protein (MAP) kinases, in a variety of cell types leading to apoptosis (41-44). The present study suggests that alterations in signaling and apoptotic pathways is more extensive in tumor cells because of the higher accumulation of the drug in tumors compared to normal tissue, leading to their apoptotic death.

It will be important to determine the preferential Lipoplatin accumulation and the absolute amounts in  $\mu\text{g}$  platinum per g tissue in tumor types other than those examined here (liver, gastric or colon tumors). This approach would give important clues regarding the therapeutic efficacy of Lipoplatin, simply from its targeting ability and reaction with macromolecules to various tumor types following intravenous administration. The data showing a high accumulation in gastric cancer (Table III) suggest a therapeutic potential of the drug in gastric cancer to be tested in Phase II clinical trials.

Lipoplatin differs from the SPI-77 formulation of cisplatin (45-48). SPI-77 administration did not result in tumor accumulation (48). In addition, urinary excretion of SPI-77 is only 2%-6% of the total dose in 96 h (48) compared to 40% of the total dose in 72 h for Lipoplatin (30).

Animal studies have shown the distribution of Lipoplatin in animal tissues after intravenous (*i.v.*) or intraperitoneal (*i.p.*) bolus injection (28, 29). Therefore, the time from infusion start is important in determining platinum levels in various tissues. After Lipoplatin treatment of rats at 45 mg/Kg *i.p.* the maximum level reached was 23  $\mu\text{g}$  platinum/ml in plasma (28) and 45  $\mu\text{g}$  platinum/g tissue in kidney at about 20-40 min following *i.p.* injection (29). In both studies, the levels of platinum dropped to about 12  $\mu\text{g}$  platinum/g tissue in kidney at 24 h and to about 2  $\mu\text{g}$  platinum/ml in plasma. The dose used in rats was 18 times higher than the 100 mg/m<sup>2</sup> dose used here in humans. Nevertheless, the

absolute platinum levels reached in human tissue at 24 h from injection (38.36  $\mu\text{g/g}$  tissue in liver tumor, 19.61  $\mu\text{g/g}$  tissue in normal liver, see Table I; 262.62  $\mu\text{g/g}$  in gastric tumor, see Table III) demonstrate an astonishingly high level of accumulation in human tissue after *i.v.* infusion of Lipoplatin compared to the maximum level reached in rat plasma or rat kidney.

A Phase I pharmacokinetic study has shown that the maximum level of platinum in plasma is 5mg/ml and is attained at 6 h from infusion start; plasma platinum levels drop to lower amounts ( $\sim 1\text{--}2\ \mu\text{g/ml}$ ) at 24 h (30). On the contrary, the platinum levels in all the tumors examined here were much higher than the 2  $\mu\text{g/ml}$  plasma at 20 h (Tables I-IV). The data presented here show that a preferential uptake of Lipoplatin by tumors is established and is quite high at 20 h under conditions where the levels of platinum in plasma drop to below 2  $\mu\text{g/ml}$ . In addition, the maximum plasma levels of total platinum reached in human patients (5  $\mu\text{g/ml}$ ) were much lower than the total platinum levels attained in certain tumors, especially gastric cancer (262  $\mu\text{g/g}$  tissue, Table III). It will be interesting to determine how early on during infusion the preferential uptake of Lipoplatin by the tumor is established and for how long it persists.

*Preferential intake of Lipoplatin by tumor cells?* Tables I-III show that the tumor specimens contained more SDS-extracted platinum compared to saline-extracted platinum, whereas the opposite was true for normal tissue. Presumably, SDS-extracted platinum represents cisplatin molecules bound to macromolecules, whereas saline-extracted platinum represents unreacted cisplatin molecules trapped in the tissue or cisplatin molecules bound to small soluble molecules. This finding suggests that a significant fraction of Lipoplatin enters the liver tumor cell as opposed to normal liver tissue (Table I), the liver metastasis from gastric cancer compared to normal liver tissue (Table II) and to gastric tumor compared to normal gastric tissue (Table III). A statistically significant number of tumor specimens need to be analyzed before final conclusions can be drawn. However, these preliminary studies suggest that the lower toxicity of Lipoplatin compared to cisplatin found in animals and in human trials (30) also arises from the fact that in normal tissue most of the platinum appears to be trapped in the tissue, whereas most of the platinum in tumors appears to have reacted with macromolecules (Tables I-III). This difference could arise: (i) from alterations in the membrane of the tumor cells rendering them more vulnerable to Lipoplatin penetration or fusion compared to the membrane of normal cells; (ii) from a more avid phagocytosis of Lipoplatin by tumor cells compared to normal cells; tumor cells might have higher phagocytotic activities than normal cells.

## Conclusion

The present study shows platinum accumulation in tumors compared to normal tissue after intravenous infusion of Lipoplatin to cancer patients. The tumor accumulation properties of Lipoplatin™ are astonishing, reaching in certain cases levels that are 200 times higher than those attained in the normal tissue. This property of Lipoplatin adds to its value as a promising formulation of cisplatin against advanced cancers (30).

It is proposed that Lipoplatin is able to target tumors and, eliciting only mild side-effects, can be administered in a dose-dense manner and, thus, have the potential to kill tumor cells more effectively in a window of opportunity linked to first-line chemotherapy before chemoresistant cells appear. Acquired chemoresistance of tumors is largely responsible for the very discouraging response rates in second- or third-line chemotherapy treatments compared to first-line treatments. The impact of dose-dense Lipoplatin treatment, allowing targeting of the tumor in a manner minimizing the likelihood of appearance of chemoresistant cells, needs to be tested in clinical trials. Also, the preferential tumor targeting of Lipoplatin, causing more damage to the cancer tissue compared to most other tissues in the body of the patient, is promising in terms of minimizing side-effects.

The preliminary clinical studies reported here provide the first evidence that the lower toxicity of the drug arises in part from its tumor-targeting properties; because of this, Lipoplatin combinations with other drugs merit careful assessment. Lipoplatin™ is currently under Phase II and Phase III clinical evaluations.

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