Systemic Lipoplatin Infusion Results in Preferential Tumor Uptake in Human Studies

TENI BOULIKAS[1], GEORGE P. STATHOPOULOS[2], NIKOLAOS VOLAKAKIS[1] and MARIA VOUGIOUKA[1],

[1]Regulon, Inc. 715 North Shoreline Blvd., Mountain View, California 94043, U.S.A. and Regulon AE, Grigoriou Afxentiou7, Athehs 174 55;
[1]Errikos Dunant Hospital, Mesogeion 107, Athens 15128, Greece;

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 ~20h later. Direct measurement of platinum levels in specimens from the excised tumors and normal tissues showed that total platinum levels were on the 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.*

Correspondence to: Dr Teni Boulikas, Ph.D., Regulon, A.E., 7 Grigoriou Afxentiou, Alimos, Athens 17455, Greece; Tel: +30-210-9858454; Tel/Fax: +30-210-9858453, e-mail: teni@regulon.org

Key words: Lipoplatin, cisplatin, tumor targeting, gastric cancer, colon cancer, hepatocellular cancer.

Cis-dichlorodiammineplatinum (II) (Cisplatin) continues 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 toward improvement of cisplatin, and 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 has the talent to elicit crosslinks in DNA confronting the DNA repair machinery. Furthermore, cisplatin alters signal transduction and apoptotic pathways crosstalking to other signaling pathways such as those leading to upregulation of transcription factor and nuclear/cellular enzymes (reviewed in 7-9).

Platinum drugs elicit 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, extend 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 of 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 in 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 extremeties, 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), adrenocorticotropic hormone analogs (ORG 2766) and calcium channel antagonists (19). Never the less, 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-cysteinyl-glycine) in 50 patients with gastric cancer undergoing cisplatin+5-FU+epirubicin+leukovorin 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^2 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 in formulations of cisplatin aimed at enhancing its tumor targeting to minimize damage to nonmalignant tissue and to lower the side effects or to chemical modifications on the molecule to derive molecules of better qualities.

Lipoplatin^{TM}, is a novel liposomal formulation of cisplatin formulated 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 for 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^2 every 14 days; the MTD was above 350 mg/m^2 as a single infusion (30).

Because Lipoplatin nanoparticles bear a PEG-coating it can be inferred that intravenous infusion of this drug shall 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^2 Lipoplatin were used that 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). 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), soy phosphatidyl choline (SPC-3, mw 790 Lipoid GmbH), cholesterol (CHOL, mw 386.66, Avanti Polar Lipids) and methoxy-polyethylene glycol–distearoyl phosphatidylethanolamine lipid conjugate (mMPEG2000-DSPE, mw 2807, Genzyme). 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 being 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 being provided in 50-ml opaque glass vials of

3mg/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 it is recommended to be stored in the dark. Lipoplatin is stored at 4 ^{0}C with an expiration date of two years.

Study design. Criteria of patient selection included: histologically confirmed type of malignancy; operable stage of disease; patients gave their signed informed consent. Lipoplatin was used at underdose as monotherapy (100 mg/m^2) 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^2 (30).

The protocol was approved both by the ethics committee of the Errikos Dunant Hospital and by the Hellenic Drug Organization. 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, 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^2 Lipoplatin as single agent diluted in 1 L 5% dextrose during a period of 8h.

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. 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 20h from the start of Lipoplatin infusion were ~3 μg/ml in acordance with previous studies (30).

Analysis of surgical specimens for platinum. During surgery (performed at ~20h from Lipoplatin infusion start) specimens were obtained (0.1-1g) 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. 0.1-1.0g tissue was ground and homogenized into two times its volume (0.2-2 ml) of saline. The sample was centrifuged at 21,000g 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 21,000g 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 ^{o}C and the supenatant 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 platinum levels in all extracts.

Results

Targeting hepatocellular adenocarcinoma with Lipoplatin. A 49-year female with hepatocellular adenocarcinoma was infused with a single dose of 100 mg/m^2 Lipoplatin as single agent during a period of 8h the day before surgery. There were no acute side effects as a result of Lipoplatin infusion. Surgery was the option decided by her treating physicians. During surgery specimens were removed (at approximately 18h 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 Materials and Methods and the platinum levels in the saline-soluble and SDS-soluble fractions were assayed. The total platinum on 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 represents 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. In other words the liver tumor had uptaken twice as much total platinum after Lipoplatin infusion compared to adjacent normal liver tissue (Table 1).

The difference between Lipoplatin accumulation in a colon metastasis compared to an adjacent normal colon tissue was even more dramatic. The colon metastasis uptook about 50 times more Lipoplatin than a normal colon tissue specimen (Table 1). 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 overal 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 that the colon metastatic tumor (38.36 versus 6.61 μg total platinum / g tissue, Table 1). 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 1

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

Patient 1: Hepatocellular adenocarcinoma	Pt (μg/g tissue) in saline or SDS fractions	TOTAL Pt (μg/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	
normal liver tissue SDS	3.16		
colon metastasis saline	4.44	6.61	49.53
colon metastasis SDS	2.17		
normal colon tissue saline	0.06	0.13	
normal colon tissue SDS	0.08		
colon fat saline	0.96	1.79	
colon fat SDS	0.83		

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

Patient 2: Gastric cancer w liver metastasis	Pt (μg/gr tissue) in saline or SDS fractions	TOTAL Pt (μg / gr 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		

indicates that the liver tumor contains about 5 μg platinum trapped in the tissue / g tissue and 33 μg platinum that has reacted with macromolecules /g tissue. On the contrary, normal liver tissue contains about 16 μg platinum trapped in the tissue / g tissue and 3 μg platinum that has reacted with macromolecules /g tissue (Table 1).

Targeting a liver metastasis from a primary gastric cancer. A male with gastric cancer and liver metastasis (Patient 2) was treated with 100 mg/m^2 Lipoplatin as single agent using an 8h 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 μg platinum/ gr tissue compared to 20.94 μg platinum/ gr normal liver tissue (Table 2). This gives a 6.26 higher level of platinum in the liver metastatic tumor compared to 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 signifacant levels of total platinum (51.91 μg platinum/ gr tissue) supposedly relating to the lipophilic nature of the drug.

Targeting gastric tumors. A male with gastric cancer (Patient 3) was treated with 100 mg/m^2 Lipoplatin as single agent given during an infusion period of 8h. During surgery two different specimens were obtained from the stomach tumor, the adjacent normal stomach tissue, and fat. Samples were blindly analyzed for platinum content. Both specimens of gastric tumors appear to accumulate the highest amounts of platinum among all specimens analyzed in this study (262.62 and 66.38 μg Pt/g tissue respectively, Table 3). We surmized that the difference in platinum levels between the same tumor may arise from the differnt locations of the specimens in the tumor mass and likely to relate to differences in the vascularization. This needs to be further explored in future studies with a simulataneous examination of vascularization. However, either tumor specimen displayed 40 and 10 times respectively more platinum compared to normal stomach tissue (Table 3). Consistent with findings in patients 1 and 2, the platinum trapped in tumor specimen 1 was 42μg/g tissue versus 220μg/g tissue of platinum that reacted with macromolecules. Specimen 2 had 28 μg/g tissue traapped platinum versus 37 μg/g tissue that reacted with macromolecules. Normal stomac tissue displayed 2.6 μg/g tissue versus 4.0 μg/g tissue platinum that was trapped versus macromolecule-bound. Again stomach tumor 1 appears to have about 5 times more platinum in the form that presumambly reacted with macromolecules and thus induces damage to cells compared to platinum simply trapped in the tissue (220:42, Table 3) whereas specimen 2 and normal gastric tissue has only 1.5 times more or about the same platinum in the two fractions.

Consistent with 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^2 Lipoplatin as single agent during a period of 8h. During surgery specimens were obtained from the colon tumor (in duplicate), the adjacent normal colon tissue, muscle and fat. 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 4). This gives 204- and 140-fold higher levels of platinum between the colon tumor and normal colon tissue. Consistent with findings in Patients 2 and 3 the fat tissue in Patient 4 also displayed significant levels of platinum (20 μg/g

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

Patient 3: Colon cancer	Pt (μg/gr tissue) in saline or SDS fractions	TOTAL Pt (μg / gr 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		

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/gr tissue) in saline or SDS fractions	TOTAL Pt (μg / gr 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		

fat tissue). The muscle tissue had very small amounts of platinum (0.36 μg platinum/g tissue).

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 cases of 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 Lipolatin was found to be 60-114 h in patients compared to less than 6h for cisplatin (30). Futhermore, 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 levels of total platinum (up to 263 μg cisplatin /g tissue, Table 3) followed by liver tumors (up to 38 μg cisplatin /g tissue, Table 1). The lowest levels of total platinum accumulation among tumor specimens was observed in a metastatic colon tumor from a primary hepatocellular cancer (6.6 μg cisplatin /g tissue, Table 1) and two primary colon tumor specimens from a colon cancer patient (11.2 and 7.7 μg cisplatin /g tissue, respectively, Table 4). Following the suggestion that Lipoplatin nanoparticles have the intrinsic property to extravasate 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 high degree of vascularization it displayed a high accumulation of total platinum in surgical specimens excised from three out of four patients (52 μg cisplatin /g fat tissue, Table 2; 41 μg /g fat tissue Table 3; 20 μg cisplatin /g fat tissue Table 4). This might correlate to the liposomal nature of Lipoplatin and the lipophilicity of the drug.

Cisplatin, in combination with other drugs, is being 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-HT3-receptors (reviewed in 7).

This study as well as a previous one (30) show no side effects after a single 8h-infusion of Lipoplatin at 100 mg/m^2 (from full blood analysis seven 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 1-3) an observation that suggests one additional mechanism for 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^2 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 increase remarkably the plasma longevity of liposomes, in a way similar to the erythrocyte coated with a dense layer of carbohydrate groups to evade 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 talent to induce monofunctional DNA adducts as well as intrastrand and interstrand crosslinks. 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 will be 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 μg platinum per g tissue in type of tumors other than those examined here (liver, gastric or colon tumors). This approach would give important clues on 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 3) 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 dose in 96h (48) compared to 40% of the total dose in 72 h for Lipoplatin (30).

Animal studies have shown the distribution of Lipoplatin in the animal tissues after i.v. or 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 μg platinum/ml in plasma (28) and 45 μg 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 μg platinum/g tissue in kidney at 24h and and to about 2 μg platinum/ml in plasma. The dose used in rats was 18 times higher than the 100 mg/m^2 dose used here in humans. Nevertheless, the absolute platinum levels reached in human tissue at 24h from injection (38.36 μg/g tissue in liver tumor, 19.61 μg/g tissue in normal liver see Table 1; 262.62 μg/g in gastric tumor, see Table 3) demonstrate an astonishing high level 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 5μg/ml and is attained at 6h from infusion start; plasma platinum levels drop to lower amounts (~ 1-2 μg/ml) at 24h (30). On the contrary, platinum levels in all tumors examined are much higher than the -2 μg/ml plasma at 20 h (Tables 1-4). The data presented here show that a preferential uptake of Lipoplatin by tumors is established and is quite high at 20h under conditioins where the levels of platinum in plasma drop to below 2 μg/ml. In addition, the maximum plasma levels of total platinum reached in human patients (5μg/ml) were much lower than the total platinum levels attained in certain tumors especially gastric cancer (262 μg/g tissue, Table 3). It will be interesting to determine how early 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 1-3 show that tumor specimens contain more SDS-extracted platinum compared to saline-extracted platinum whereas normal tissue contains more saline-extracted platinum compared to SDS-extracted platinum. 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 1), the liver metastasis from gastric cancer compared to normal liver tissue (Table 2) and to gastric tumor compared to normal gastric tissue (Table 3). 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 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 1-3). 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 Lipolatin 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 able to target tumors and eliciting 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 a higher damage to the cancer tissue compared to most other tissues in the body of the patient is promising by minimizing the side effects.

The preliminary clinical studies reported here provide a 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 merits a careful assessment. Lipoplatin™ is currently under Phase II and PhaseIII clinical evaluations.

Acknowledgements

We are grateful to John Papadimitriou (surgeon, Errikos Dynan Hospital, Athens) for the tumor specimens and to Ioannis Stathopoulos for monitoring the patients. We thank Georgios Deliconstantinos and Vasiliki Viliotou for help with atomic absorption. We are also indebted to Stavros Kottaridis for helpful comments on the manuscript.

References

1. Rosenberg B, VanCamp l, Krigas T. Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature 205:698-699, 1965.
2. Rosenberg B, VanCamp L, Trosko JE, Mansour VH. Platinum compounds: a new class of potent antitumour agents. Nature. 222:385-386, 1969.
3. Rosenberg B, VanCamp L. The successful regression of large solid sarcoma 180 tumors by platinum compounds. Cancer Res. 30:1799-802, 1970.
4. Rosenberg B: Noble metal complexes in cancer chemotherapy. Adv Exp Med Biol 91: 129-50, 1977.
5. Hill JM, Speer RJ: Organo-platinum complexes as antitumor agents (review). Anticancer Res 2: 173-86, 1982
6. Davidson JP, Faber PJ, Fischer RG Jr, Mansy S, Peresie HJ, Rosenberg B, VanCamp L. "Platinum-pyrimidine blues" and related complexes: a new class of potent antitumor agents. Cancer Chemother Rep. 59:287-300, 1975.
7. McKeage MJ: Comparative adverse effect profiles of platinum drugs. Drug Saf 13: 228-44, 1995
8. Boulikas T and Vougiouka M: Cisplatin and platinum drugs at the molecular level. Oncol Rep 10, 1663-1682, 2003
9. Boulikas T and Vougiouka M: Recent clinical trials using cisplatin, carboplatin and their combination chemotherapy drugs. Oncol Rep 11: 559-595, 2004
10. Hanigan MH and Devarajan P: Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther 1, 47-61, 2003.
11. van der Hoop RG, van der Burg ME, ten Bokkel Huinink WW, van Houwelingen C, Neijt JP. Incidence of neuropathy in 395 patients with ovarian cancer treated with or without cisplatin. Cancer 66:1697-1702, 1990.
12. Thompson SW, Davis LE, Kornfeld M, Hilgers RD, Standefer JC. Cisplatin neuropathy. Clinical, electrophysiologic, morphologic, and toxicologic studies. Cancer 54, 1269-1275, 1984.
13. Ozols RF, Young RC. High-dose cisplatin therapy in ovarian cancer. Semin Oncol 12, 21-30, 1985.
14. Cersosimo RJ. Cisplatin neurotoxicity. Cancer Treat Rev 16, 195-211, 1989.
15. Harmers FP, Gispen WH, Neijt JP. Neurotoxic side-effects of cisplatin. Eur J Cancer 27:372-376, 1991.
16. Hovestadt A, van der Burg ME, Verbiest HB, van Putten WL, Vecht CJ. The course of neuropathy after cessation of cisplatin treatment, combined with Org 2766 or placebo. J Neurol. 239:143-146, 1992.
17. Bissett D, Kunkeler L, Zwanenburg L, Paul J, Gray C, Swan IR, Kerr DJ, Kaye SB. Long-term sequelae of treatment for testicular germ cell tumours. Br J Cancer 62:655-659, 1990.
18. Gregg RW, Molepo JM, Monpetit VJ, Mikael NZ, Redmond D, Gadia M, Stewart DJ. Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. J Clin Oncol 10:795-803, 1992.
19. Alberts DS, Noel JK. Cisplatin-associated neurotoxicity: can it be prevented? Anticancer Drugs 6:369-383, 1995.
20. Gandara DR, Nahhas WA, Adelson MD, Lichtman SM, Podczaski ES, Yanovich S, Homesley HD, Braly P, Ritch PS, Weisberg SR, et al. Randomized placebo-controlled multicenter evaluation of diethyldithiocarbamate for chemoprotection against cisplatin-induced toxicities. J Clin Oncol 13:490-496, 1995.
21. Kemp G, Rose P, Lurain J, Berman M, Manetta A, Roullet B, Homesley H, Belpomme D, Glick J. Amifostine pretreatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized control trial in patients with advanced ovarian cancer. J Clin Oncol 14:2101-2112, 1996
22. Roberts JA, Jenison EL, Kim K, Clarke-Pearson D, Langleben A. A randomized, multicenter, double-blind, placebo-controlled, dose-finding study of ORG 2766 in the prevention or delay of cisplatin-induced neuropathies in women with ovarian cancer. Gynecol Oncol 67:172-177, 1997.
23. Cascinu S, Cordella L, Del Ferro E, Fronzoni M, Catalano G. Neuroprotective effect of reduced glutathione on cisplatin-based chemotherapy in advanced gastric cancer: a randomized double-blind placebo-controlled trial. J Clin Oncol 13:26-32, 1995.
24. Melamed LB, Selim MA, Schuchman D. Cisplatin ototoxicity in gynecologic cancer patients. A preliminary report. Cancer. Jan 55:41-43, 1985.
25. Bokemeyer C, Berger CC, Hartmann JT, Kollmannsberger C, Schmoll HJ, Kuczyk MA, Kanz L. Analysis of risk factors for cisplatin-induced ototoxicity in patients with testicular cancer. Br J Cancer 77:1355-1362, 1998.
26. Hinojosa R, Riggs LC, Strauss M, Matz GJ. Temporal bone histopathology of cisplatin ototoxicity. Am J Otol 16:731-40, 1995.
27. Saito T, Yamada T, Manabe Y, Yamamoto T, Saito H. Cisplatin metabolites and their toxicity on isolated cochlear outer hair cells in vitro. Acta Otolaryngol 116:561-565, 1996.
28. Boulikas T (2004) Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts. Oncol Rep 12: 3-12, 2004.
29. Devarajan P, Tarabishi R, Mishra J, Ma Q, Kourvetaris A, Vougiouka M, and Boulikas T: Low renal toxicity of Lipoplatin compared to cisplatin in animals. Anticancer Res, 24, 2193-2200, 2004.
30. Stathopoulos GP, Boulikas T, Vougiouka M, Deliconstantinos G, Rigatos S, Darli E, Viliotou V, and Stathopoulos JG: Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): Phase I study Oncology Rep. 13, 589-595, 2005.
31. Martin F and Boulikas T: The challenge of liposomes in gene therapy. Gene Ther Mol Biol 1, 173-214, 1998.

32. Vermorken JB, Kapteijn TS, Hart AA, Pinedo HM: Ototoxicity of cis-diamminedichloro-platinum (II): influence of dose, schedule and mode of administration. Eur J Cancer Clin Oncol 19: 53-8, 1983
33. Gabizon, A., Shiota, R., Papahadjopoulos, D.: Pharmacokinetics and tissue localization of doxorubicin encapsulated in stable liposomes with long circulation times. J. Natl. Cancer Inst. 81, 1484-1488, 1989.
34. Beauchamp CO, Gonias SL, Menapace DP, Pizzo SV: A new procedure for the synthesis of polyethylene glycol-protein adducts; effects on function, receptor recognition, and clearance of superoxide dismutase, lactoferrin, and alpha 2-macroglobulin. Anal Biochem 131, 25-33, 1983.
35. Gabizon A, Papahadjopoulos D: Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors. Proc Natl Acad Sci USA 85, 6949-6953, 1988.
36. Klibanov AL, Maruyama K, Torchilin VP and Huang L: Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Lett 268, 235-237, 1990.
37. Lasic, D.D., Martin, F.J., Gabizon, A, et al.: Sterically stabilized liposomes: a hypothesis on the molecular origin ot the extended circulation times. Biochim. Biophys. Acta 1070:187-192, 1991.
38. Torchilin VP, Klibanov AL, Huang L, O'Donnell S, Nossiff ND and Khaw BA (1992) Targeted accumulation of polyethylene glycol-coated immunoliposomes in infarcted rabbit myocardium. FASEB J. 6, 2716-2719.
39. Zamble DB, Mikata Y, Eng CH, Sandman KE, Lippard SJ: Testis-specific HMG-domain protein alters the responses of cells to cisplatin. J Inorg Biochem 91: 451-62, 2002
40. Jung Y, Lippard SJ: Nature of Full-Length HMGB1 Binding to Cisplatin-Modified DNA. Biochemistry 42: 2664-71, 2003
41. Nehme A, Baskaran R, Nebel S, Fink D, Howell SB, Wang JY and Christen RD: Induction of JNK and c-Abl signalling by cisplatin and oxaliplatin in mismatch repair-proficient and -deficient cells. *Br J Cancer* 79: 1104-1110, 1999
42. Devarajan P, Savoca M, Castaneda MP, Park MS, Esteban-Cruciani N, Kalinec G, Kalinec F: Cisplatin-induced apoptosis in auditory cells: role of death receptor and mitochondrial pathways. Hear Res 174: 45-54, 2002
43. Levresse V, Marek L, Blumberg D, 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
44. Woessmann W, Chen X, Borkhardt A: Ras-mediated activation of ERK by cisplatin induces cell death independently of p53 in osteosarcoma and neuroblastoma cell lines. Cancer Chemother Pharmacol 50: 397-404, 2002.
45. Harrington KJ, Lewanski CR, Northcote AD, Whittaker J, Wellbank H, Vile RG, Peters AM, Stewart JS: Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. Ann Oncol 12: 493-6, 2001
46. Kim ES, Lu C, Khuri FR et al: A phase II study of STEALTH cisplatin (SPI-77) in patients with advanced non-small cell lung cancer. Lung Cancer 34: 427-32, 2001
47. Veal GJ, Griffin MJ, Price E, Parry A, Dick GS, Little MA, Yule SM, Morland B, Estlin EJ, Hale JP, Pearson AD, Welbank H, Boddy AV: A phase I study in paediatric patients to evaluate the safety and pharmacokinetics of SPI-77, a liposome encapsulated formulation of cisplatin. Br J Cancer 84: 1029-35, 2001
48. Meerum Terwogt JM, Groenewegen G, Pluim D, Maliepaard M, Tibben MM, Huisman A, ten Bokkel Huinink WW, Schot M, Welbank H, Voest EE, Beijnen JH, Schellens JM: Phase I and pharmacokinetic study of SPI-77, a liposomal encapsulated dosage form of cisplatin. Cancer Chemother Pharmacol 49: 201-10, 2002