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(54) **MEDICAL USE OF SPLA2 HYDROLYSABLE LIPOSOMES**

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(57) **ABSTRACT**

The present invention relates to medical use of liposomes, more particular the first medical use of sPLA2 hydrolysable liposomes. Such liposomes may be used for targeted delivery of therapeutic agents to cancerous tissue and in such embodiments; the therapeutic agents are typically small molecule antitumor agents. Other aspects of the inventions relates to methods of reducing the side effects of therapeutic agents, e.g. reducing nephrotoxicity, neurotoxicity and gastrointestinal toxicity of a therapeutic agent. Yet another aspect of the present invention relate to methods of prolonging the therapeutic effect of a therapeutic agent.

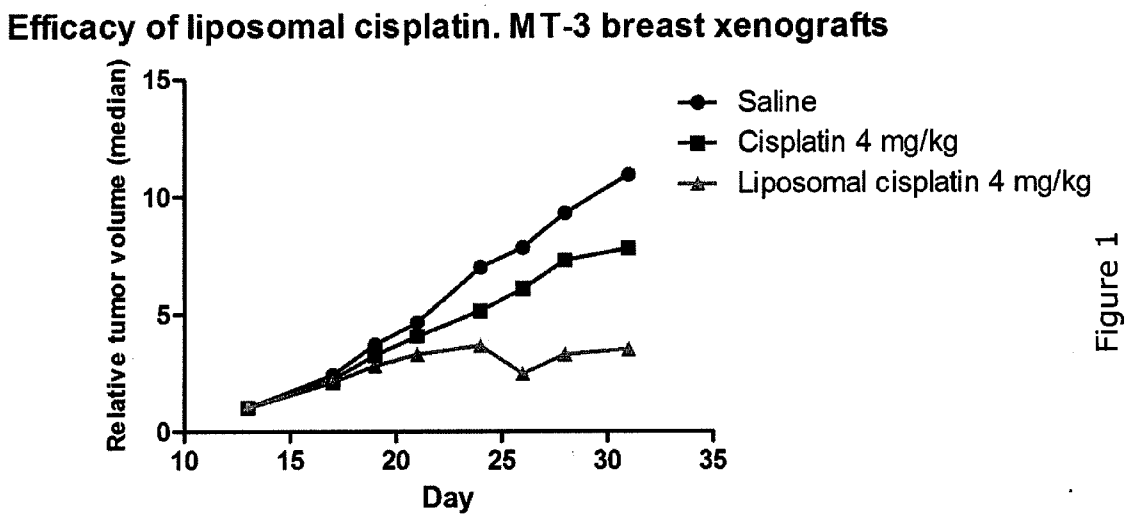
Efficacy of liposomal cisplatin. MT-3 breast xenografts
Relative tumor volume (median)
15
10
5
0
10
15
20
25
30
35
Day
Saline
Cisplatin 4 mg/kg
Liposomal cisplatin 4 mg/kg

Figure 1

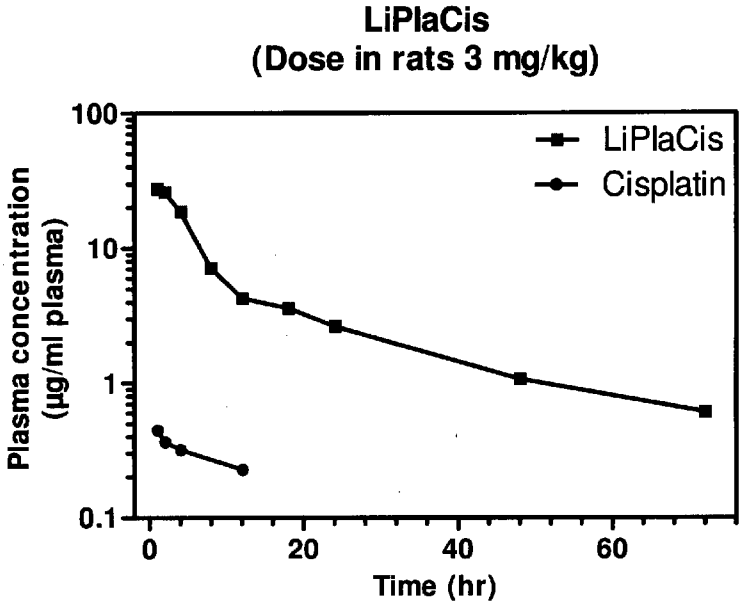
LiPlaCis
(Dose in rats 3 mg/kg)
Plasma concentration
(µg/ml plasma)
100
10
1
0.1
0
20
40
60
Time (hr)
LiPlaCis
Cisplatin

Figure 2

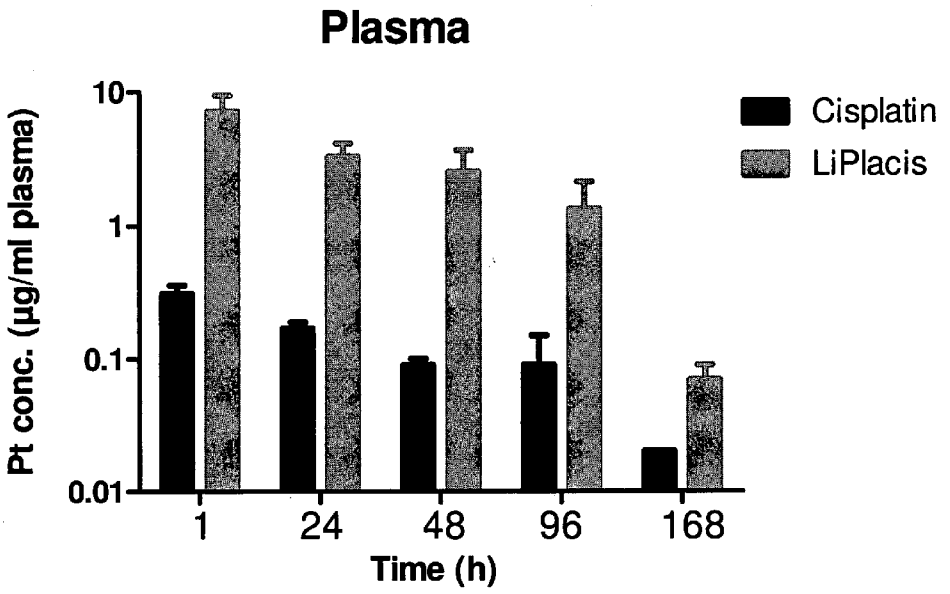
Plasma
Pt conc. (µg/ml plasma)
10
1
0.1
0.01
1
24
48
96
168
Time (h)
Cisplatin
LiPlacis

Figure 3

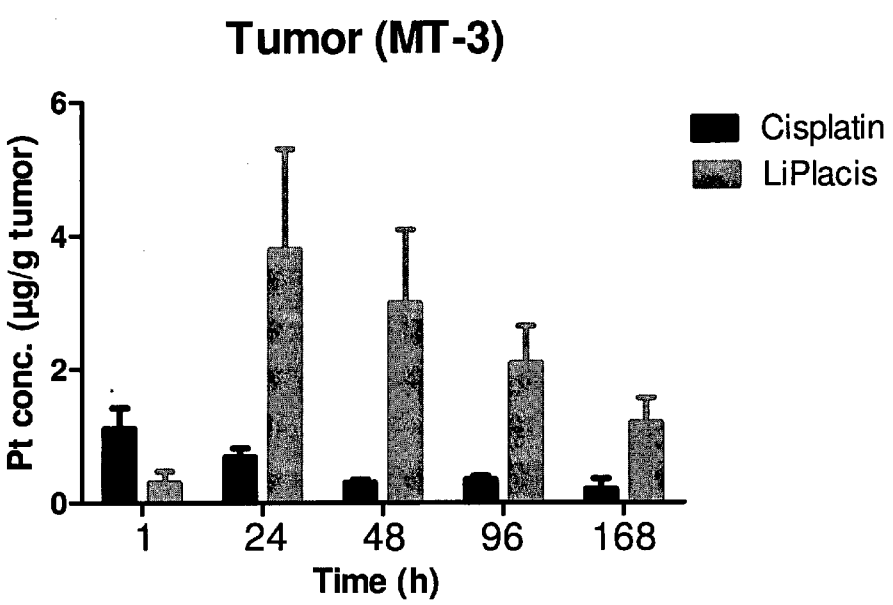
Tumor (MT-3)
Pt conc. (µg/g tumor)
6
4
2
0
1
24
48
96
168
Time (h)
Cisplatin
LiPlacis

Figure 4

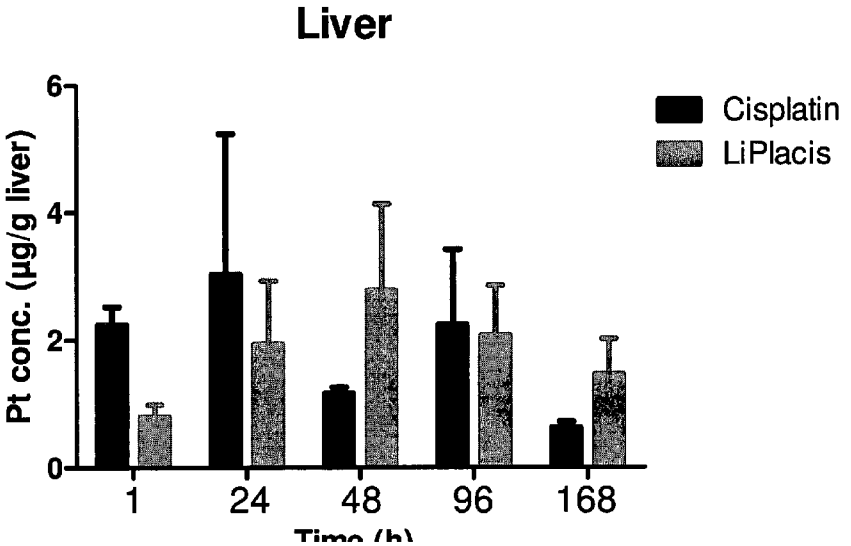
Liver
Cisplatin
LiPlacis
Pt conc. (µg/g liver)
6
4
2
0
1
24
48
96
168
Time (h)

Figure 5

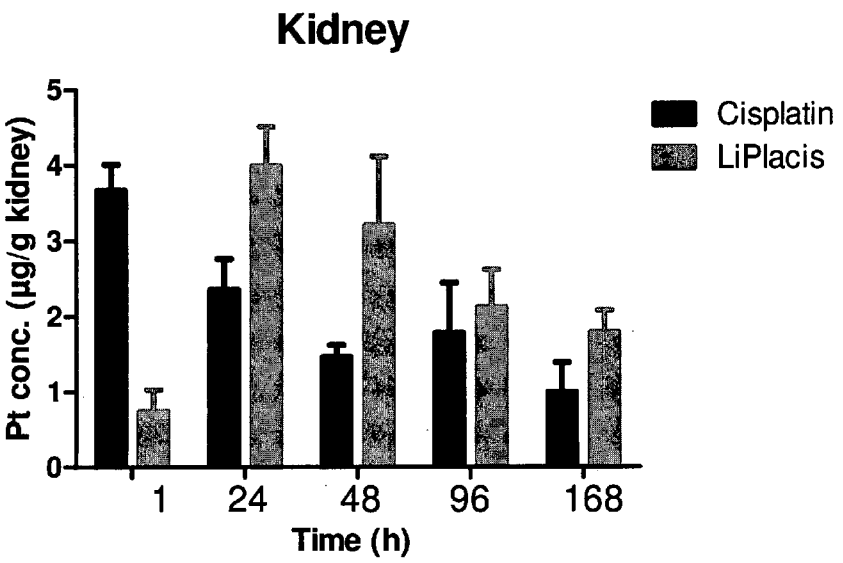
Kidney
Pt conc. (µg/g kidney)
0
1
2
3
4
5
1
24
48
96
168
Time (h)
Cisplatin
LiPlacis

Figure 6

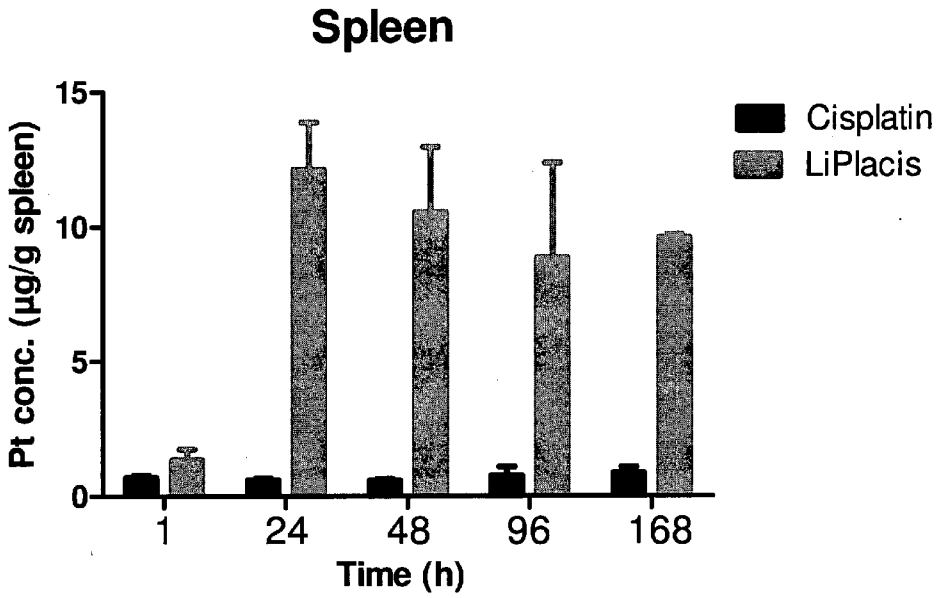
Spleen
Pt conc. (µg/g spleen)
15
10
5
0
1
24
48
96
168
Time (h)
Cisplatin
LiPlacis

Figure 7

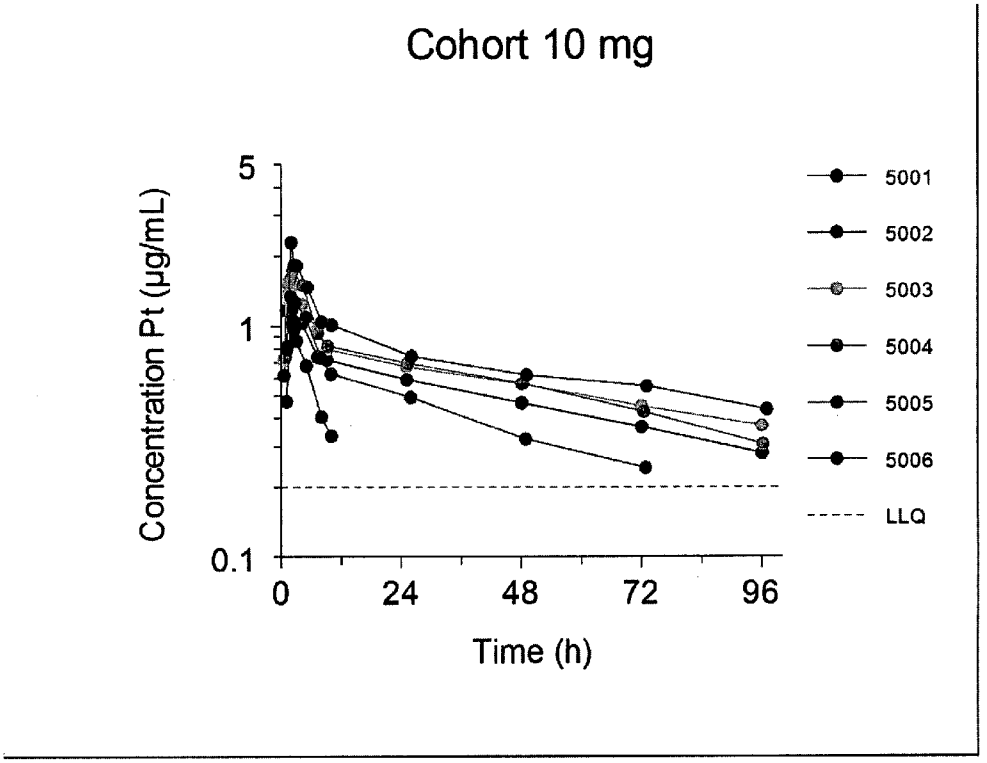
Cohort 10 mg
Concentration Pt (µg/mL)
5
1
0.1
0
24
48
72
96
Time (h)
5001
5002
5003
5004
5005
5006
LLQ

Figure 8

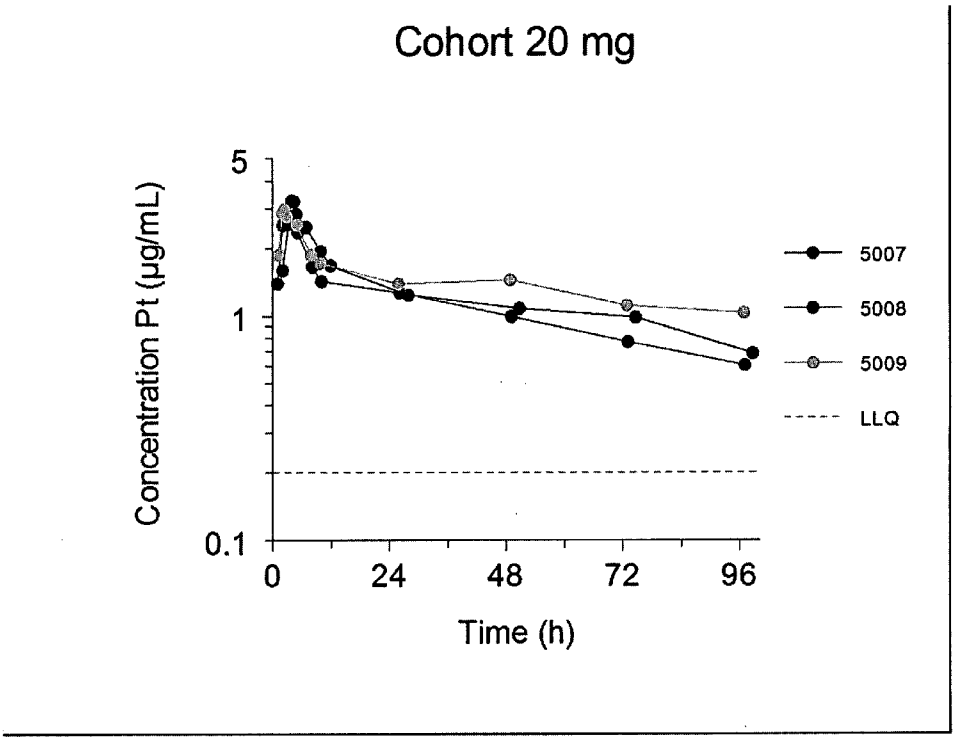
Cohort 20 mg
Concentration Pt (µg/mL)
5
1
0.1
0
24
48
72
96
Time (h)
5007
5008
5009
LLQ

Figure 9

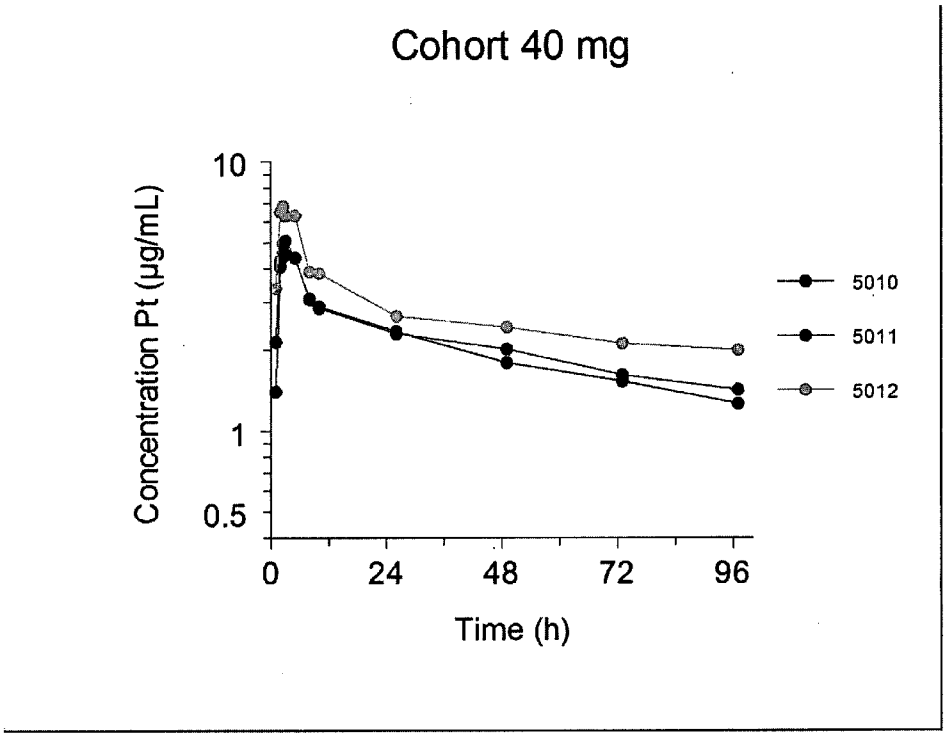
Cohort 40 mg
Concentration Pt (µg/mL)
10
1
0.5
0
24
48
72
96
Time (h)
5010
5011
5012

Figure 10

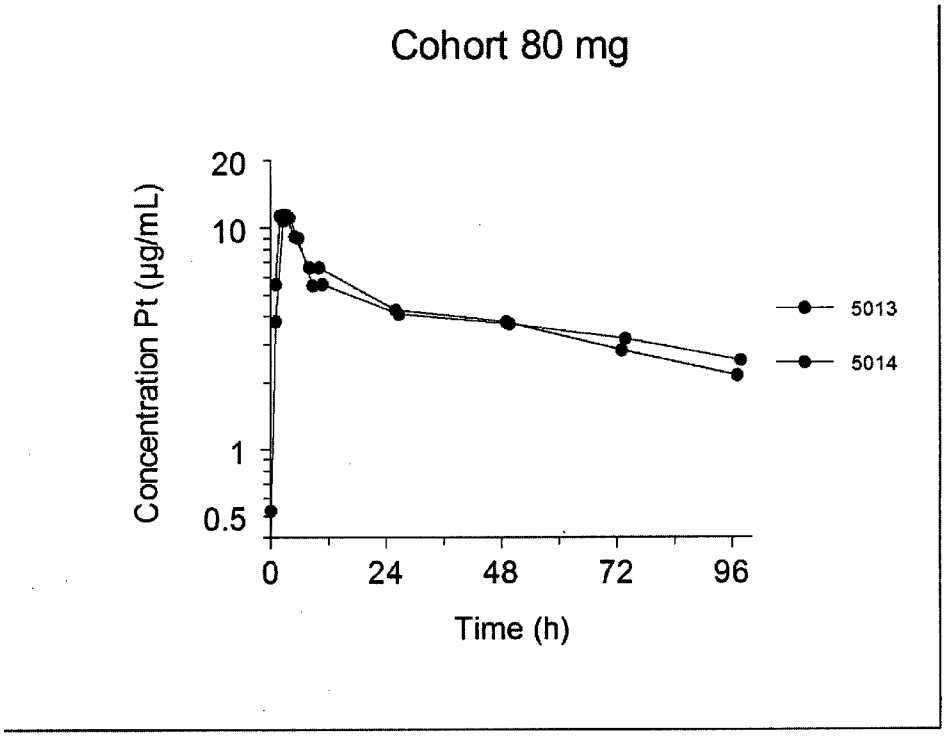
Cohort 80 mg
Concentration Pt (µg/mL)
20
10
1
0.5
0
24
48
72
96
Time (h)
5013
5014

Figure 11

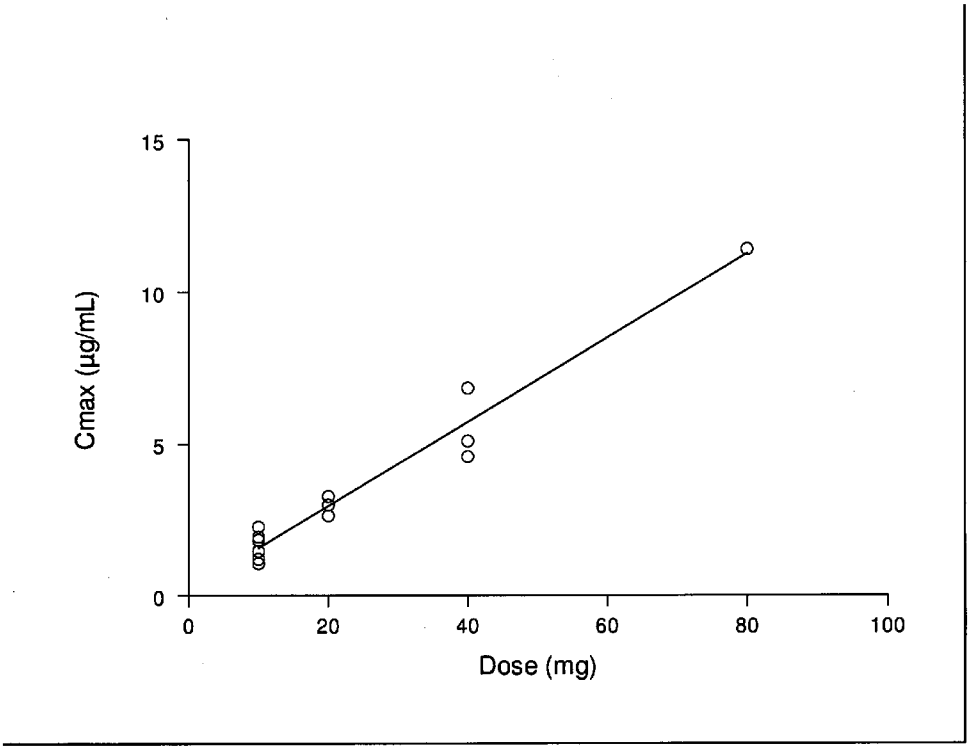

Figure 12

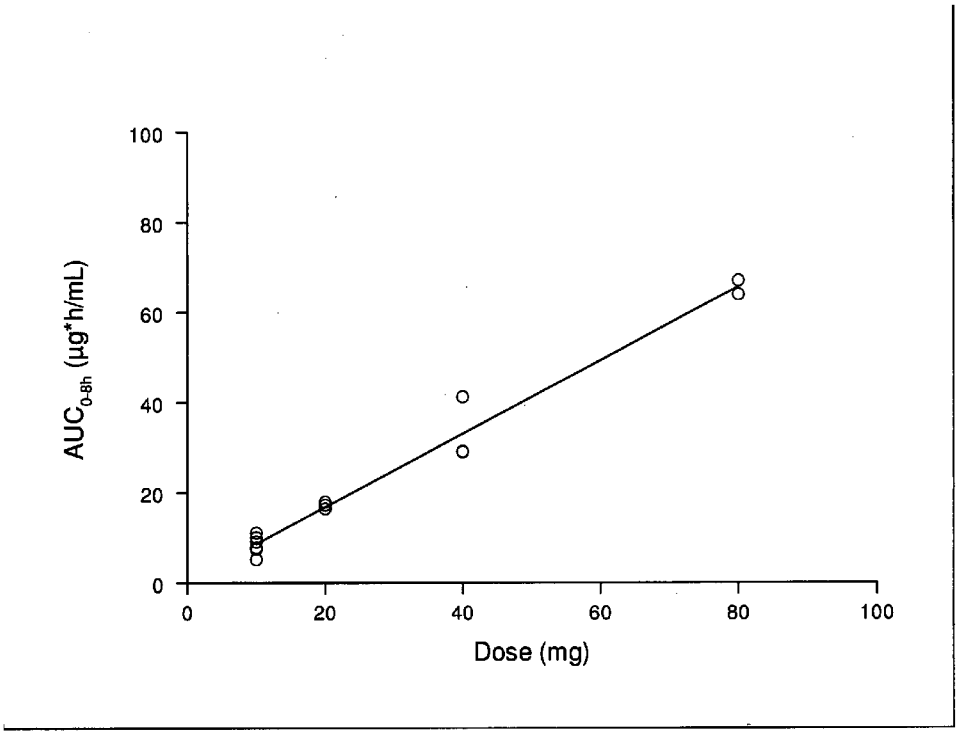

Figure 13

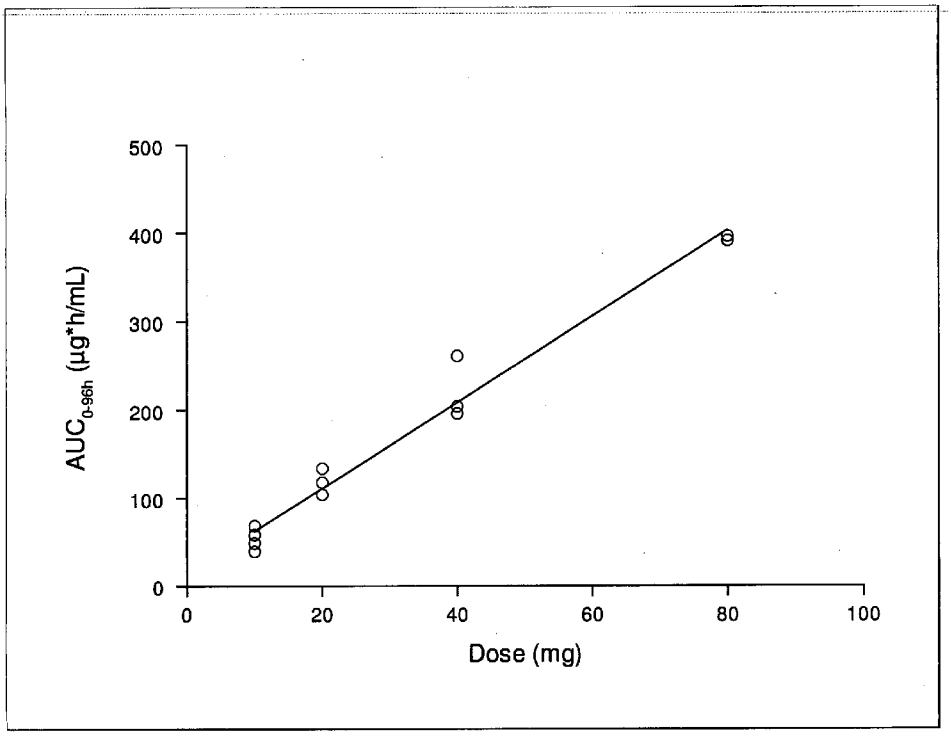
500
400
300
200
100
0
$AUC_{0\text{-}96h}$ (µg*h/mL)
0
20
40
60
80
100
Dose (mg)

Figure 14

Subject No.	Gender	Age	Diagnosis	History of Cisplatin	Dose, mg/subject	1st Cycle	2nd Cycle	3rd Cycle	Comments
1	F	63	Breast Cancer	No	10	OK	OK	OK	PD
2	F	49	Melanoma	No	10	Allergic reaction, grade 2-3	Allergic Reaction, grade 3. DLT	Not done	Off study after 2 cycles
3	F	75	Adenoid Cystic Carcinoma	No	10	OK	OK	OK	PD after 15 cycles
4	M	51	Oesopharyngeal Cancer	No	10	OK	OK	OK	PD after 3 cycles
5	M	61	Prostate Cancer	No	10	Allergic reaction grade 2	OK	OK	PD after 6 cycles
6	F	44	Oropharyngeal cancer	No	10	OK	OK	Not done	Off study after 2 cycles due to PD
7	F	39	Parotic Cancer	?	20	OK	OK	OK	PD after 3 cycles
8	M	56	Urothelial-cell Cancer	?	20	OK	OK	OK	PD after 3 cycles
9	M	64	Parotic Cancer	?	20	OK	OK	OK	PD after 6 cycles
10	M	66	Melanoma	?	40	OK	OK	Not done	PD after 2 cycles
11	M	71	Prostate Cancer	?	40	OK	OK	Not done	Off-study after 2 cycles.
12	F	45	Breast Cancer	?	40	OK	Not done	Not done	Off study after first cycle. PD
13	M	62	Urothelial-cell Cancer	?	80	OK	OK	OK	
14	F	50	CUP	?	80	Allergic reaction grade 2	Allergic reaction grade 3	Not done	Off study after 2 cycles
15	M	53	Hypopharyngeal cancer	?	80	OK	OK	Pending	

Figure 15

Pt. Nr.	Dose	Sex	Age	Tumor	Cycle	Day	WBC	ANC	PLT	Hgb	Nau	Vom	Dia	Oral	Renal	Fever	Neuro	Fatigue	Other	DLT	Respons
5001	10 mg	F	63	breast	0		1	.	.	.	.	.	.	.	.	.	.	.	ASAT 2, ALAT 2, LDH 1		
					1		1	.	1	.	.	.	.	.	.	.	.	.	skin 1 (dry desquamation), ASAT 2, ALAT 2		
					2		1	.	.	.	.	.	.	.	.	.	.	.	AST 2, ALT 2		
					3		.	.	1	.	.	.	.	.	.	.	.	.	AST 1, ALT 1, LDH 1		PD
					off																
5002	10 mg	F	49	melanoma	0		.	.	.	.	.	.	.	.	.	.	.	.	pain 1		
					1		.	.	1	.	1	.	.	.	.	.	.	2	allergic reaction 3, rash 1, anorexia 1, AST 2, ALT 2, GGT 1	X	
					2														allergic reaction 3 (despite pre-medication), medication stop		
					off																
5003	10 mg	F	75	adenoidcystic carcinoma	0		.	.	.	.	.	.	.	.	.	.	.	.			
					1		.	.	.	.	.	.	.	.	.	.	.	.	GGT 1		
					2		.	.	.	.	.	.	.	.	.	.	.	.			
					3		.	.	.	.	.	.	.	.	.	.	.	.	AST 1, cholesterol 1		SD
					4		.	.	.	.	.	.	.	.	.	.	.	.	GGT 1, hypercholesterol 1		
					5		.	.	.	.	.	.	.	.	.	.	.	.	GGT 1, hypercholesterol 1		
					6		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1		SD
					7		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1		
					8		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1		
					9		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1, glucose 1		SD
					10		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1, glucose 1		
					11		.	.	.	.	.	.	.	.	.	.	.	.	hypercholesterol 1		

Figure 16

					12		.	.	.	.	.	.	.	.	.	.	.	hypercholesteroi 1		SD
					13		.	.	.	.	.	.	.	.	.	.	.			
					14		.	.	.	.	.	.	.	.	.	.	.	AST 1		
					15		.	.	.	.	.	.	.	.	.	.	.	GGT 1		PD
					off															
5004	10 mg	M	51	esophagus	0		.	.	.	.	.	.	.	.	.	.	.			
					1		.	.	.	.	.	.	.	.	.	1	.	1	liverfunctioncisorder 1, sweating 2		
					2		.	.	.	1	.	.	.	.	.	.	1	1	GGT 1		
					3		.	.	.	.	.	.	.	.	.	.	1	1	sweating 1, anorexia 1, GGT 1, dysphagia 1		PD
					off															
5005	10 mg	M	61	prostate	0		.	.	.	.	.	.	.	.	.	.	.			
					1		.	.	.	1	.	.	.	.	1	.	.	1	allergic reaction 2, pyrosis 2, nervosity 1, proteinuria/hematuria 1		
					2		.	.	.	1	.	.	.	.	1	.	.	1	pyrosis 2, proteinuria/hematuria 1		
					3		.	.	.	1	.	.	.	.	1	.	.	1	proteinuria/hematuria 1		
					4		.	.	.	1	1	1	.	.	.	.	.	1	proteinuria 1		
					5		.	.	.	1	.	.	.	.	.	.	.	1	proteinuria 1		
					6		.	.	.	1	.	.	.	.	.	.	.	1			PD
					off															
5006	10 mg	F	44	oropharynx	0		.	.	.	.						1	1	pain 1		
					1		.	.	.	1	.	.	.	.	.	.	1	1	pain 1, AF 1, GGT 3, ALT 2, AST 1, thrombosis 3 (tumor related)		
					2		.	.	.	.	2	2	.	.	.	.	.	1	infusion reaction 2, thrombosis 3 (probably central line related), ALT 3 / ALT 2 (prob. Comedication related)		PD

Figure 16

					off															
5007	20 mg	F	39	parotic ca	0		.	.	.	.	2	.	.	.	.	.	1	1	anorexia 1		
					1		.	.	.	.	1	.	.	.	.	.	1	1	infusion reaction 2, anorexia 1, pain 2, GGT 1		
					2		.	.	.	.	.	.	.	.	.	.	1	1	GGT 1, anorexia 1, pain 1		
					3		1	.	.	1	.	.	.	.	1	1	2	2	GGT 1, pain 1, bili 2, obstipation 1, dyspnea 2, infection 3		PD
					off															
5008	20 mg	M	56	urothelial-cell ca	0		.	.	.	1	.	.	.	.	1	.	1	.	dyspnea 1, cough 1		
					1		.	.	.	1	.	.	.	.	1	.	1	.	cough 1, AF 1		
					2		.	.	.	1	.	.	.	.	1	.	1	1	cough 1, AF 1, LDH 1, dyspnea 1, hypophosphatemia 2, GGT 1, glucose 1		
					3	15	.	.	.	1	.	.	.	.	1	.	1	1	AF 1, GGT 1		PD
					off															
5009	20 mg	M	64	parotic ca	0		.	.	.	.	.	.	.	.	.	.	.	.	GGT 1, pain 1		
					1		.	.	.	1	1	.	.	.	1	.	.	1	GGT 1, pain 1, bili 1		
	hydratio n				2		.	.	.	1	1	.	1	.	.	.	.	1	pain 1, GGT 1		
					3		.	.	.	1	.	.	.	.	.	.	.	1	pain 1		SD
					4		.	.	.	1	.	.	.	.	.	.	.	1	GGT 1, pain 1, ureum 1, AST 1, ALT 1, headache 1		
					5		.	.	.	1	.	.	.	.	1	.	.	1	GGT 1, pain 1, ureum 1, AST 1, ALT 1, headache 1, hypophosphatemia 1, tinnitus 1		
					6		.	.	.	1	.	.	.	.	1	.	.	1	tinnitus 1, GGT 1, pain 1		PD
					off															
5010	40mg	M	66	melanoma	0		.	.	.	.	.	.	.	.	.	.	.	.			
					1		.	.	.	.	.	.	.	.	.	.	.	.			

Figure 16

					2		.	.	.	.	.	.	.	.	.	.	.			
					3		.	.	.	.	.	.	.	.	.	.	.			PD
					off															
5011	40 mg	M	71	prostate	0		1	.	.	1	.	.	.	.	.	.	.	hematuria 1		
					1		1	.	.	1	.	.	.	.	.	1	.	hematuria 1, proteinuria 1, pain 2		
					2		1	.	.	1	.	.	.	.	.	.	2	hematuria 1		
					off													on patients request		
5012	40 mg	F	45	breast	0		.	.	.	.	.	.	.	.	.	1	1	alopecia 1, dyspnea 1, pain 1, GGT 2, AST 2, ALT 1, AF 1		
					1		.	.	.	.	1	.	.	.	.	1	1	anorexia 1, pain 1, alopecia 1, AF 1, GGT 3, AST 2, ALT 2, LDH 1		PD
					off															
5013	**80 mg**		**62**	**urothelial-cell ca**	**0**		.	.	.	.	.	.	.	.	.	.	.	**GFR 1, hematuria 1, proteinuria 1**		
					1		.	.	.	.	1	.	.	.	.	1	.	**dyspnea 1, anemia 1, GFR 1, hematuria 1, proteinuria 1, abdominal pain 2, leucocyturia 1**		
					2		1	.	.	.	.	.	.	.	.	.	.	**urinary infection, anemia 1, GFR 1, hematuria 1, proteinuria 1, leucocyturia 1, fever 2 2 days after infusion with recurrent UTI**		
					3	1														
S1-01 5014	80 mg	F	50	CUP	0		.	.	.	.	.	.	.	1	.	.	1	obstipation 1, dyspnea 1, pain 1, edema 1, AST 1, ALT 1, GGT 1		

Figure 16

					1		.	.	.	1	1	.	.	.	.	.	.	2	infusion reaction 2, AST 1, LDH 1, hypercalcemia 2, GGT 1, ALT 1, anorexia 1, dyspnea 1,		
					2														treatment discontinued due to infusion reaction 3		
					off																
S2-06 5015	**80 mg**	**M**	**53**	**hypopharyngeal ca**	**0**														**Hyponatremia**		
					1		.	.	.	.	.	.	.	.	.	.	.	.	**hyponatremia 3, leucocyturia, haematuria, proteinuria**		
					2																
S1-03 5016	**120 mg**	**M**	**78**		**0**																
					1																

Figure 16

MEDICAL USE OF SPLA2 HYDROLYSABLE LIPOSOMES

FIELD OF THE INVENTION

[0001] The present invention relates to liposomal drug delivery systems and their use in therapy.

BACKGROUND

Liposomes for Drug Delivery

[0002] Liposomes are microscopic spheres which were developed as drug delivery vehicles/systems in the 1980s. The first liposome-based pharmaceuticals were approved for commercial use in the 1990s.

[0003] Liposomes have three distinct compartments that can be used to carry various compounds such as drugs: The interior aqueous compartment; the hydrophobic bilayer; and the polar inter-phase of the inner and outer leaflet. Depending on the chemical nature of the compound to be encapsulated it will be localised to either of the compartments.

[0004] Liposomes are considered a promising drug delivery system since they passively target tumor tissue by using the pathophysiological characteristics of solid tumors such as hyperplasia and increased vascular permeability but also a defect in lymphatic drainage. These features facilitate extravasation of nanoparticles and the liposomes can be retained in the tissue for longer time due to the enhanced permeability and retention effect (EPR).

[0005] The property of liposomes as drug delivery vehicles is crucially dependent on their surface charge, permeability, solubility, stability etc. which is significantly influenced by the lipids comprised in the liposome composition. In addition, the drug to be encapsulated in the liposome may need further requirements to be considered in preparing a stable liposome formulation.

[0006] Considerations regarding safety and drug efficacy require that liposome formulations maintain their properties, i.e. remain stable, from the time of preparation until administration. Furthermore, it is desirable that such formulations are intact during the transport in the treated subject until they reach the target site where the drug is specifically released.

[0007] Therapeutic use of negatively charged liposomes may induce non-IgE-mediated hypersensitivity reactions seen in patients treated with liposomal products. These adverse reactions are thought to be a result of anaphylatoxin production through complement activation.

[0008] Repeated dosing of PEGylated liposomal formulations has in some cases resulted in an Accelerated Blood Clearance (ABC-phenomenon) leading to a fast clearance from the bloodstream and corresponding increased accumulation in liver and spleen when compared to the first dose. The ABC-phenomenon may cause unintended release of encapsulated compound in organs having accumulated liposomes. Moreover, the ABC-phenomenon is typically non-desired as it may prevent the liposomes from accumulating at intended sites.

[0009] Various targeting strategies for liposomes have been described, e.g. conjugation to cell specific ligands such as antibodies.

sPLA2 Hydrolysable Liposomes

[0010] Another approach has been suggested based upon elevated levels of secretory phospholipase A2 (sPLA2) in cancerous tissue and also at sites of inflammation. The basic idea is that liposomes can be prepared which are hydrolysable by sPLA2 and that hydrolysis by sPLA2 leads to release of the drug encapsulated within the liposome. Moreover, the products of sPLA2 hydrolysis, a lysolipid and a fatty acid act as permeabilizers of cell membranes leading to increased cell uptake of the drug. Since sPLA2 levels are elevated in the cancerous tissues and at sites of inflammation, sPLA2 activated liposomes may be used to preferentially deliver encapsulated drugs to such sites.

[0011] A number of documents have described sPLA2 activated liposomes, but therapeutic applications have so far not been described.

[0012] WO0158910 described sPLA2 activated liposomes comprising prodrugs of mono-ether lyso-phospholipids. This document also described encapsulation of additional bioactive compounds. However, no therapeutic use of the described liposomes was disclosed.

[0013] WO0176555 suggested the use of a lipid-based drug delivery system for treatment of diseases or conditions associated with a localized increase in extracellular sPLA2 in cutaneous or subcutaneous tissue of a mammal, for administration of a prodrug of an ether-lysolipid that is activated by sPLA2. The system further comprised a so-called edge active compound. This document did not disclose topical application to a mammal such as a human. Hence no therapeutic use of the described liposomes was disclosed.

[0014] WO0176556 suggested the use of a lipid-based drug delivery system for treatment or prevention of a parasitic infection selected from Leishmaniasis, Tryponosomiasis, malaria, Entaboeba, Histolyticasis and "Oriental liver fluke chlomorchis sinensis", wherein the system comprised prodrugs in the form of lipid derivatives that are activated by sPLA2. The liposomes may contain an additional bioactive compound. No actual treatment of the mentioned infections was demonstrated nor was the liposomes administered to a mammal such as a human.

[0015] WO06048017 and WO07107161 did also describe sPLA2 activated liposomes, but without any disclosure of medical treatment.

[0016] Andresen et al, 2005a (Andresen TL, 2005) discussed triggered activation and release of liposomal prodrugs and drugs in cancer tissue by sPLA2. Among others, the authors disclosed data from an experiment showing inhibition of tumour growth in the MT-3 breast xenograft mouse model. Cisplatin encapsulated in sPLA2 degradable liposomes (DSPC/DSPG/DSPE-PEG2000, no amounts of the individual lipids were given) showed increased inhibition of tumour growth as compared to an equivalent amount of free cisplatin. The authors also noted that in in vitro experiments, the sPLA2 degradable liposomes loaded with cisplatin were more cytotoxic than free cisplatin possibly due to an additive membrane perturbing effect of the hydrolysis products, lysolipid and fatty acids. This effect might be useful for facilitating transmembrane diffusion of cisplatin into intracellular target sites. Whether this effect can lead to adverse side effects of sPLA2 activated liposomes was not discussed.

[0017] Andresen et al, 2005b (Andresen T L J. S., 2005) also disclosed data from an experiment showing inhibition of tumour growth in the MT-3 breast xenograft mouse model.

[0018] Even in view of the references discussed above, it is unclear whether sPLA2 activated liposomes can be used therapeutically. They may e.g. be rapidly cleared by the cells of the RES because of their typically negative charge. They may also simply be too leaky for therapeutic use. Another very unpredictable parameter is toxicity of the sPLA2 lipo-

somes. As mentioned, the products of sPLA2 mediated hydrolysis, lysolipids and fatty acids, may lead to unintended side effects e.g. through permeabilization of cell membranes. Moreover, drug release at unintended sites may occur if sPLA2 is present at increased levels at sites other than in tumours. Such unintended drug release may have detrimental consequences and prevent therapeutic use of sPLA2 activated liposomes. Drug release at unintended sites may be caused by unanticipated elevated sPLA2 levels at such sites.

[0019] The therapeutically use of negatively charge liposomes could involve non-IgE-mediated hypersensitivity reactions seen in patients treated with liposomal products. These reactions are thought to be a result of anaphylatoxin production through complement activation.

[0020] Repeated dosing of PEGylated liposomal formulations has in some cases resulted in an Accelerated Blood Clearance (ABC-phenomenon) leading to a fast clearance from the bloodstream and corresponding increased accumulation in liver and spleen when compared to the first dose. The ABC-phenomenon may course unintended release of encapsulated compound in organs having accumulated liposomes.

Treatment Using Cisplatin

[0021] Free cisplatin formulations have some serious side effects. The most important are listed below:

[0022] Nephrotoxicity—The major dose-limiting toxicity of cisplatin is dose-related and cumulative renal insufficiency. The administration of cis-platin using a 6- to 8-hour infusion with intravenous hydrationhas been used to reduce nephrotoxicity. However, renal toxicity still can occur after utilization of these procedures.

[0023] Ototoxicity—Ototoxicity has been observed in up to 31% of patients treated with a single dose of cisplatin 50 mg/m2, and is manifested by tinnitus and/or hearing loss in the high frequency range (4,000 to 8,000 Hz). Ototoxic effects may be related to the peak plasma concentration of cisplatin.

[0024] Hematologic—Myelosuppression occurs in 25% to 30% of patients treated with cisplatin. Leukopenia and thrombocytopenia are more pronounced at higher doses (>50 mg/m2). Anemia occurs at approximately the same frequency as leukopenia and thrombocytopenia

[0025] Gastrointestinal—Marked nausea and vomiting occur in almost all patients treated with cisplatin, and are occasionally so severe that the drug must be discontinued. Nausea and vomiting usually begin within 1 to 4 hours after treatment and last up to 24 hours.

[0026] Serum Electrolyte Disturbances—Hypomagnesemia, hypocalcemia, hyponatremia, hypokalemia, and hypophosphatemia have been reported to occur in patients treated with cisplatin and are probably related to renal tubular damage.

[0027] Neurotoxicity—Neurotoxicity is usually characterized by peripheral neuropathies. The neuropathies usually occur after prolonged therapy (4 to 7 months); however, neurologic symptoms have been reported to occur after a single dose.

[0028] Hepatotoxicity—Transient elevations of liver enzymes, especially SGOT, as well as bilirubin, have been reported to be associated with cisplatin administration at the recommended doses.

[0029] Thus, treatment using free cisplatin has a number of potential side effects and there is a need for cisplatin formulations with a reduced risk of side effects.

SUMMARY OF THE INVENTION

[0030] In a first aspect, the present invention provides sPLAs hydrolysable liposomes for medical use. The sPLA2 hydrolysable liposomes preferably comprise a therapeutic agent such as a small molecule antitumor agent.

[0031] Other aspects of the present invention relates to methods of reducing the side effects of therapeutic agents, e.g. reducing nephrotoxicity, neurotoxicity and gastrointestinal toxicity of a therapeutic agent.

[0032] Yet another aspect of the present invention relate to methods of prolonging the therapeutic effect of a therapeutic agent.

BRIEF DESCRIPTION OF THE FIGURES

[0033] FIG. **1**. Efficacy of LiPlaCis towards MT-3 (human breast carcinoma) xenografts. Nude mice with exponentially growing tumors were treated once weekly with 4 mg/kg cisplatin or LiPlaCis. The control group was treated with (saline). The cisplatin and saline-treated groups received three injections whereas the LiPlaCis-treated mice only received two injections due to toxicity. See example 2 for details.

[0034] FIG. **2**. Rats (BrlHan:WIST@Mol(GALAS)) (3 rats/treatment) were injected with 3 mg/kg cisplatin or LiPlacis and blood was withdrawn at the indicated time points. After acid digestion, the plasma fraction was analyzed for platinum content using ICP-MS. See example 2 for details.

[0035] FIGS. **3-7** Pharmacokinetics and biodistribution in nude mice bearing MT-3 xenografts. Nude mice (3 mice/timepoint/treatment) with exponentially growing MT-3 tumors were injected with a single dose of cisplatin or LiPlaCis (3 mg/kg). After blood withdrawal, the mice were sacrificed at the indicated time points and tumors and organs were dissected, washed and snap frozen. After acid digestion, the platinum content was measured by ICP-MS. See example 4 for details.

[0036] FIGS. **8-11** Cisplatin concentration in blood as a function of time after administration of LiPlaCis. See example 6 for details.

[0037] FIGS. **12-14** Cisplatin concentration in blood as a function of administered amount of LiPlaCis. See example 6 for details.

[0038] FIG. **15** Summary of phase 1 data. See example 6 for details.

[0039] FIG. **16**. Detailed phase 1 data. WBC WBC~white blood cells, ANC~absolute neutrophil count, PLT~platelets, Hgb~hemoglobin, Nau~Nausea, Vom~vomiting, Dia~diarrhea. See example 6 for details.

DISCLOSURE OF THE INVENTION

[0040] The present inventors have carried out in vivo studies with sPLA2 hydrolysable liposomes (also herein termed sPLA2 activated liposomes). When sPLA2 hydrolysable liposomes loaded with cisplatin (herein also termed LiPlaCis) was administered to tumor mice models, an increased efficacy as compared to administration of free cisplatin was often observed. However, increased efficacy was also often entailed by increased toxicity leading to death of mice.

[0041] Nonetheless, the present inventors initiated a phase 1 dose escalation trial of cisplatin encapsulated in sPLA2

hydrolysable liposomes in patients with advanced or refractory tumors. The primary endpoint of the study was safety and tolerability of cisplatin encapsulated in sPLA2 hydrolysable liposomes (also termed LiPlaCis).

[0042] The main conclusions of the study were:

[0043] LiPlaCis has a tolerable tox profile at clinically relevant doses.

[0044] LiPlaCis enables administration of at least the same dose of cisplatin as administration of free cisplatin, which is surprising in view of non-clinical data.

[0045] LiPlaCis reduced nephrotoxicity as compared to administration of free cisplatin, which is typically dose limiting for cisplatin.

[0046] LiPlaCis reduced nausea and vomiting as compared to administration of free cisplatin

[0047] The MTD (maximum tolerated dose) of LiPlaCis given every 3 weeks was determined to be above 80 mg per treatment cycle, which is surprising in view of the MTD predicted from animal experiments.

[0048] The RD (recommended dose) of LiPlaCis given every 3 weeks was determined to 80 mg per treatment cycle or higher.

[0049] LiPlaCis can be administrated without hydration, which is required for administration of free cisplatin. LiPlaCis can be administered on an outpatient basis.

[0050] In particular, LiPlaCis had good safety and tolerability profile compared to free cisplatin formulations in terms of nausea, diarrhea, vomiting, anemia, neuropathy, nephrotoxicity and ototoxicity.

[0051] Thus, the present invention has made medical use of sPLA2 hydrolysable liposomes available and in its broadest aspect provides a sPLA2 hydrolysable liposome for use in therapy, preferably treatment of humans.

sPLA2 Hydrolysable Liposomes

[0052] sPLA2 hydrolysable liposomes for use in therapy according to the present invention are defined in more detail in the following embodiments. In its broadest embodiment, the term sPLA2 hydrolysable liposomes refer to liposomes that are hydrolysable under physiological conditions, particular in cancerous tissue.

[0053] Preferably, the sPLA2 hydrolysable liposomes comprises between 20% and 45% (mol/mol) of an anionic lipid. The content of anionic lipid affects important characteristics of the liposome, such as the rate of sPLA2 mediated lipid hydrolysis of the liposome and also the immune response toward the liposome.

[0054] As the content of anionic lipid increases, so does the rate of lipid hydrolysis by $sPLA_2$ (and the release of drug). It has been demonstrated that a reasonable rate of hydrolysis can be achieved by an anionic lipid content between 20% and 45%. Thus, in one embodiment, the content of anionic lipid is at least 20%. In another embodiment, the content of anionic lipid is no more than 45%. In yet another embodiment, the anionic lipid content of the liposome is selected from the group consisting of between 20% and 45%, between 25% and 45%, between 28% and 42%, between 30% and 40%, between 32% and 38% and between 34% and 36%.

[0055] As mentioned, also the immune response toward the liposomes is affected by the content of anionic lipid. Thus, the clearance rate of the liposome in the body may be reduced by keeping the content of the anionic lipid in the liposome below a certain level and the present inventors have recognized that the content of anionic lipid in the liposome can be used to strike a balance between hydrolysis rate of $sPLA_2$ and clearance by the reticuloendothelial system.

[0056] Preferably the anionic lipid is a phospholipid and preferably, the phospholipid is selected from the group consisting of PI (phosphatidyl inositol), PS (phosphatidyl serine), DPG (bisphosphatidyl glycerol), PA (phosphatidic acid), PEOH (phosphatidyl alcohol), and PG (phosphatidyl glycerol). More preferably, the anionic phospholipid is PG. Preferably, the lipids comprise stearoyl chains. Thus preferably PG is DSPG etc.

Hydrophilic Polymers

[0057] In a preferred embodiment, the sPLA2 hydrolysable liposome for use in the present invention further comprises a hydrophilic polymer selected from the group consisting of PEG [poly(ethylene glycol)], PAcM [poly(N-acryloylmorpholine)], PVP [poly(vinylpyrrolidone)], PLA [poly(lactide)], PG [poly(glycolide)], POZO [poly(2-methyl-2-oxazoline)], PVA [poly(vinyl alcohol)], HPMC (hydroxypropylmethylcellulose), PEO [poly(ethylene oxide)], chitosan [poly(D-glucosamine)], PAA [poly(aminoacid)], polyHEMA [Poly(2-hydroxyethylmethacrylate)] and co-polymers thereof.

[0058] Most preferably the polymer is PEG with a molecular weight between 100 Da and 10 kDa. Particular preferred are PEG sizes of 2-5 kDa (PEG2000 to PEG5000), and most preferred is PEG2000.

[0059] The inclusion of polymers on liposomes is well known to the skilled artisan and can be used to increase the half-life of the liposomes in the bloodstream, presumably by reducing clearance by the reticuloendothelial system. Moreover, the inclusion of polymers affects sPLA2 hydrolysis.

[0060] Preferably, the polymer is conjugated to the head group of phospatidyl ethanolamine. Another option is conjugation to ceramide (even though this lipid is not hydrolyzable by $sPLA_2$). When the polymer is conjugated to phospatidyl ethanolamine, a negative charge is introduced and hence DSPE-PEG is regarded as an anionic lipid (contrary to DSPE which is regarded as a neutral lipid). The polymer-conjugated lipid is preferably present at an amount of at least 2%. More preferably, the amount is at least 5% and no more than 15% (mol/mol). Even more preferably, the amount of polymer-conjugated lipid is at least 3% and no more than 6%. Liposomes containing anionic phospholipids and ≦2.5% DSPE-PEG2000 have increased tendency to aggregate in the presence of calcium. This can usually be observed by formation of high viscous gel. Liposomes containing anionic phospholipids and >7.5% DSPE-PEG2000 causes the liposomes to sediment or phase separate.

[0061] Neutrally Charged Lipid Components in the Liposome

[0062] Preferably, the liposome to be used in the present invention also comprises an uncharged phospholipid selected from the group consisting of zwitterionic phospholipids comprising PC (phosphatidyl choline) and PE (phosphatidylethanolamine). Most preferably, the zwitterionic phospholipid is PC.

[0063] In contrast to anionic phospholipid, zwitterionic phospholipid serves as a charge neutral $sPLA_2$-hydrolyzable lipid component in the liposome. By combining zwitterionic- and anionic phospholipid in the same liposome, it is possible to adjust to a desired surface charge density which complies with both sufficiently high $sPLA_2$ hydrolysis and a low clearance rate in the blood.

[0064] The amount of zwitterionic phospholipid in the liposome is preferably between 40% and 75% and more preferably between 50 and 70%.

[0065] Preferably, the lipids (anionic lipids, neutral lipids and polymer conjugated lipids) comprise stearoyl chains). Thus preferably PG is DSPG, PE is preferably DSPE etc.

Ether-Phospholipids

[0066] Some or all of the phospholipids may be ether-phospholipids.

[0067] Thus, they may harbour an ether-bond instead of an ester-bond at the sn-1 position of the glycerol backbone of the phospholipid. When $sPLA_2$ hydrolyze this particular type of phospholipids, mono-ether lyso-phospholipids are produced and these are toxic to e.g. cancer cells. I.e. ether phospholipids may be seen as pro-drugs of mono-ether lyso-phospholipids and liposomes of the invention can be used to deliver such pro-drugs to the $sPLA_2$-enhanced environment of cancer cells, where the pro-drugs are activated by $sPLA_2$ hydrolysis. Ether-phospholipids have been described in EP 1254143 and WO 2006/048017, the contents of which are hereby incorporated by reference.

[0068] In one embodiment, the sPLA2 activated liposomes as used in the present invention does not comprise ether-phospholipids.

Other Pro-Drugs

[0069] The moiety released from the lipid by $sPLA_2$ to create a lysolipid may also be a drug. Thus, a liposome may comprise pro-drugs of mono-ether lysolipids, pro-drugs released from the lipid by $sPLA_2$ and other therapeutic agents, as further outlined below.

[0070] In one embodiment, the sPLA2 activated liposomes as used in the present invention does not comprise prodrugs released from the lipid by sPLA2.

Stabilizing Agent

[0071] The liposome may be stabilized by the inclusion of cholesterol as membrane component in the liposome. However, high amounts of cholesterol in the liposome have a negative effect on hydrolysis by PLA_2 and therefore it is preferred that the liposome comprises no more than 10% cholesterol. Even more preferably, the liposome comprises less than 1% cholesterol, less than 0.1% or does not comprise any cholesterol at all.

[0072] The alkyl chain length of the lipids comprising the liposome may be adjusted for optimal PLA_2 hydrolysis rate and minimum leakage of entrapped compound out of the liposome. Preferably, the alkyl chains are C18 or C16 saturated chains.

[0073] The liposomes to be used may be stabilized by exposure to divalent cations.

[0074] As described above, the liposomes may comprise pro-drugs of mono-ether lyso-lipids and/or of the moiety released from the lipid by $sPLA_2$ to create the lysolipid.

[0075] In a preferred embodiment, the liposomes comprise a bioactive compound such as a therapeutic agent (drug), which is not a pro-drug of mono-ether lysophospholipid or a mono-ether lysophospholipid. The liposome may also comprise pro-drugs of mono-ether lysophospholipid and a therapeutic agent. Preferred bioactive compounds are small molecules, peptides, proteins and nucleic acids such as plasmids and oligonucleotides. A preferred class of proteins is antibodies, more preferably monoclonal antibodies. Preferred oligonucleotides are aptamers, antisense oligonucleotides, microRNAs and siRNAs. A class of compounds of particular interest is small molecule antitumour agents such as anthracyclin derivatives, cisplatin, oxaliplatin, carboplatin, doxorubicin, paclitaxel, 5-fluoruracil, exisulind, cis-retinoic acid, suldinac sulphide, methotrexate, bleomycin and vincristine. A preferred subclass of antitumor agents is platinum based antitumor agents; cisplatin, oxaliplatin, picoplatin and carboplatin. Another class of compounds of particular interest is antibiotics and antifungals and yet another class is anti-inflammatory agents such as steroids and non-stereoids.

[0076] The therapeutic agent may be located in the interior aqueous compartment; the hydrophobic bilayer; or the polar inter-phase of the inner and outer leaflet.

[0077] Preferably, the therapeutic agent is encapsulated in the liposome, i.e. present in the interior aqueous compartment.

[0078] In another embodiment, the liposome comprises a diagnostic agent. By "diagnostic agent" is meant an agent that supports the localisation of the target tissue and/or the diagnosis of the disease and/or condition. Non-limiting examples could be contrast agents, microparticles, radioactive agents, target specific agents such as e.g. agents that bind specifically to markers associated with the disease and/or condition, etc. It is clear to a skilled person that in some embodiments the invention relates to a liposome formulation wherein the liposome comprises at least one drug as well as a diagnostic agent.

[0079] Physical-Chemical Characteristics of the Liposomes of the Invention

[0080] The liposome can be unilamellar or multilamellar. Most preferably, the liposome is unilamellar. The diameter of the liposome should be between 50 and 400 nm, preferably between 80 and 160 nm and most preferable between 90 and 120 nm.

[0081] Preferably, the Poly Dispersity Index (PDI) of the liposomal formulation of the second aspect of the invention should not exceed 0.2 and more preferable is 0.10 or less. A PDI value in this range expresses a relatively narrow particle size-distribution in the formulation.

[0082] As will be clear from the above, it is preferred that at least one of the lipids comprising the liposome is a substrate for $sPLA_2$ when present in the liposome.

[0083] In one embodiment, the liposome comprises lipids which are hydrolysed by $sPLA_2$ at the sn-3 position instead of at the sn-2 position. Such unnatural lipids and liposomes comprising unnatural lipids have been disclosed in WO 2006/048017, the content of which is hereby incorporated by reference.

[0084] In a most preferred embodiment, the liposomes to be used in the present invention comprise 70% DSPC, 25% DSPG and 5% DSPE-PEG.

[0085] When the therapeutic agent is cisplatin, the interior of the liposomes preferably comprises 0.9% NaCl and the exterior buffer solution comprises 10 mM phosphate buffer at pH 6.5, 1 mM NaCl and 10% sucrose.

Medical Use

[0086] In a preferred embodiment, the sPLA2 hydrolysable liposome is administered by injection (parenteral administration) e.g. the subcutaneous, intramuscular, intra-peritoneal, intravenous, and intrathecal routes. A preferred route is intravenous administration in form of bolus injection or infusion.

[0087] As described above, the liposome may comprise various therapeutic agents. However, preferred agents are small molecule anti tumour agents (herein also termed antineoplastic agents, cytotoxic drugs or cytostatic drugs). Cisplatin is one of these compounds and the demonstration that cisplatin encapsulated in a sPLA2 hydrolysable liposome can be used therapeutically, argues that other antineoplastic agents encapsulated in sPLA2 hydrolysable liposomes can also be used therapeutically, i.e. they will not be released from sPLA2 hydrolysable liposomes at unintended sites at a concentration which would be detrimental to the therapeutic use of sPLA2 hydrolysable liposomes encapsulating antineoplastic agents.

[0088] In a preferred embodiment, the administration of sPLA2 hydrolysable liposomes comprising a therapeutic agent enables administration of a reduced dose of the therapeutic agent as compared to administration of the free therapeutic agent. This is possible for several reasons. First, liposomal encapsulation of the therapeutic agent prolongs the half-life of the agent. Second, the targeting effect of sPLA2 hydrolysis leads to an increased concentration of the free therapeutic agent at sites of increased sPLA2 levels, e.g. at tumours.

[0089] In another preferred embodiment, the administration of sPLA2 hydrolysable liposomes comprising a therapeutic agent enables administration of an increased dose of therapeutic agent as compared to administration of the free therapeutic agent. This is possible because of the targeting effect of sPLA2 hydrolysable liposomes and can e.g. be seen by reduced nephrotoxicity of cisplatin encapsulated in sPLA2 hydrolysable liposomes as compared to free cisplatin.

[0090] LiPlaCis have been studied in a number of non-clinical toxicology studies in rats and mice. The overall purpose of these studies was to determine both the single dose and multiple dose Maximum Tolerated Dose (MTD) in the two species. These studies were conducted according to Good Laboratory Practice (GLP). In these studies the two species was found to be equally sensitive to LiPlaCis and by applying FDA rules (Reference: Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. FDA, July 2005), the human equivalent maximum tolerated dose is predicted to be 30 mg per treatment cycle. Thus, a human MTD of 80 mg or more per treatment cycle is surprising.

[0091] Preferred doses of encapsulated cisplatin are between 80 mg and 120 mg per treatment cycle with a 3 week interval, between 120 and 160 mg per treatment cycle with a 3 week interval, between 160 mg and 200 mg per treatment cycle with a 3 week interval, between 200 mg and 240 mg per treatment cycle with a 3 week interval and between 240 mg and 300 mg per treatment cycle with a 3 week interval.

[0092] The time between administrations of therapeutic agent may also be adjusted in line with the discussion of reduced/increased doses of therapeutic agent. Thus, in one embodiment, the time between administrations of therapeutic agent is prolonged as compared to the time between administrations of the free therapeutic agent. In another embodiment, the time between administrations of therapeutic agent is reduced as compared to the time between administrations of the free therapeutic agent. When the therapeutic agent is cisplatin, the time between administrations may e.g. be more than 3 weeks or less than 3 weeks.

[0093] Preferably, the disease to be treated according the invention is cancer or inflammation, preferably cancer.

Method of Treatment

[0094] A second aspect of the present invention is a method of treatment comprising administering an effective amount of an sPLA2 hydrolysable liposome as described in the first aspect of the invention to a patient in need thereof. Specific embodiments of this aspect will be apparent from the first aspect of the invention.

Method of Reducing Nephrotoxicity

[0095] A third aspect of the invention is a method of reducing the nephrotoxicity of a therapeutic agent, said method comprising encapsulating the therapeutic agent in a sPLA2 hydrolysable liposome. Preferably, the therapeutic agent is an antineoplastic agent such as cisplatin and preferably, the therapeutic agent is administered to a patient in need thereof. Other embodiments will be apparent from the first aspect of the invention.

Method of Reducing Neurotoxicity

[0096] A forth aspect of the invention is a method of reducing the neurotoxicity of a therapeutic agent, said method comprising encapsulating the therapeutic agent in a sPLA2 hydrolysable liposome. Preferably, the therapeutic agent is an antineoplastic agent such as cisplatin and preferably, the therapeutic agent is administered to a patient in need thereof. Other embodiments will be apparent from the first aspect of the invention.

Method of Reducing Gastrointestinal Toxicity

[0097] A fifth aspect of the invention is a method of reducing the gastrointestinal toxicity of a therapeutic agent, said method comprising encapsulating the therapeutic agent in a sPLA2 hydrolysable liposome. Preferably, the therapeutic agent is an antineoplastic agent such as cisplatin and preferably, the therapeutic agent is administered to a patient in need thereof. Other embodiments will be apparent from the first aspect of the invention

Method of Prolonging the Therapeutic Effect

[0098] A sixth aspect of the invention is a method of prolonging the therapeutic effect of a therapeutic agent, said method comprising encapsulating the therapeutic agent in a sPLA2 hydrolysable liposome. Preferably, the therapeutic agent is an antineoplastic agent such as cisplatin and preferably, the therapeutic agent is administered to a patient in need thereof. Other embodiments will be apparent from the first aspect of the invention. References

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EXAMPLES

Example 1

Preparation of sPLA2 Liposomes (LiPlaCis)

[0101] A lipid intermediate is prepared by spray-drying the following a mixture of phospholipids (70/25/5 mol % DSPC/

DSPG/DSPE-PEG2000). The lipids are dissolved in methanol and chloroform. The lipid intermediate is hydrated in an aqueous solution of the anti-cancer drug with agitation. At this step the liposomes are formed but they have a broad size distribution and is a mixture of single-layer and multiple-layer liposomes. In order to get a product with a narrow size distribution and mono-layer liposomes the hydration mixture is extruded by passing it through poly-carbonate filters of appropriate pore sizes. To remove un-encapsulated anti-cancer drug the mixture is purified. A number of techniques are available e.g. dialysis, gel-filfration and ultra-filtration. For preparations ranging from a few liters and above ultra-filtration is the preferred method. Preparations intended for parenteral administration must be sterilized e.g. by sterile-filtration.

Example 2

Efficacy in Mice

Methods

[0102] NMRI nude female mice (6-8 weeks) were inoculated subcutaneously into the left flank with $1*10^7$ cells of the human breast carcinoma cell line MT-3. Only mice carrying exponentially growing tumors were selected for the study. Treatment started when tumors had reached a size of 70-80 mm3. Animals received one dose (4 mg/kg cisplatin (Platinol), LiPlaCis or saline) weekly with intra-venous injections into the tail vein starting on day 13 after tumor transplant. Tumor growth was assessed three times a week by measuring two perpendicular diameters and tumor growth was normalized for differing starting sizes by calculating relative tumor volume. Body weight was measured three times a week. Blood samples were taken four days after the first injection to estimate white blood cells and thrombocytes by Coulter counter.

Results:

[0103] LiPlaCis was compared with cisplatin and saline in an efficacy study using MT-3 breast xenografts on nude mice. Cisplatin and LiPlaCis were given at a dose of 4 mg/kg weekly. Because of toxicity, only two injections of LiPlaCis were administrated compared to three for cisplatin and saline. LiPlaCis inhibited tumor growth significantly better than free cisplatin (FIG. **1**). The effect was apparent a week after of the first dosing and lasted till the experiment was terminated because of large tumors in the control group. One mouse died in the LiPlaCis-treated group.

Example 3

Pharmacokinetics in Rats

Methods:

[0104] Rats (BrlHan:WIST@Mol(GALAS)) were injected with 3 mg/kg cisplatin or LiPlaCis and blood was collected into heparinised tubes (Microvette CB 300 Sarsted). Samples were taken from 10 minutes up to 72 h. A blood volume of 250 μl was taken from each sampling point and immediately placed in an ice-bath and centrifuged (3000×g; 5 min) to obtain the plasma fraction. The plasma-containing tubes were frozen until shipment and subsequent digestion in HCl/HNO3/H2O2 (60/5/35 vol %) before platinum analysis using ICP-MS.

Results and Conclusion:

[0105] The experiment revealed that LiPlaCis a long-circulating liposomal form of cisplatin with a T½ of about 20-23 h compared to the 15 minutes for free cisplatin. The area under the curve (AUC) for LiPlaCis was at least 50 times that of cisplatin. See FIG. **2**.

Example 4

PK/BD in Nude Mice

Methods:

[0106] Nude BALB/c female mice (6-8 weeks) were inoculated subcutaneously into the left and right flank with 1×10^7 cells of the human breast carcinoma cell line MT-3. Only mice carrying exponentially growing tumors were selected for the study. The single dose was given when the tumors had reached a size of at least 300 mm3. Animals received 3 mg/kg cisplatin (Platinol) or LiPlaCis) by tail vein injection. The time points were 1, 24, 48, 72 and 168 h. Blood samples (500 μl) was taken immediately before sacrifice and transferred to heparinised tubes (Microvette CB 300 Sarsted), centrifuged and frozen. Post mortem, the tumors, organs and tissues (kidneys, liver, quadriceps muscle on the hind limb and spleen) were dissected, washed in saline and snap frozen. To determine platinum concentrations in plasma and tumors/tissues, the samples were digested in HCl/HNO3/H2O2 (60/5/35 vol %) and subjected to ICP-MS.

Results:

[0107] Platinum analysis in plasma showed that LiPlaCis was present at high concentrations in serum and the effect was

TABLE 1

Experimental parameters and toxicity:

Group	mice	Subst.	Treatment (days)	Dose (mg/kg/inj.)	tox deaths(d)	BWC (%) d 13-24	Optimum T/C (%) [at day]	WBC d 17 (10^6/ml)	Thromb. d 17 (10^6/ml)
A	10	Saline	13, 20, 27		0	−2		9.7 +/− 1.2	1185 +/− 89
B	10	Cisplatin	13, 20, 27	4	0	−6	71 [31]	10.6 +/− 1.5	1201 +/− 117
C	10	LiPlaCis	13, 20	4	1 (26)	−14	31 [26]*+	11.2 +/− 2.7	1058 +/− 183

BWC: Body Weight Count, difference in percentage compared to the weight before treatment.

Optimum T/C: Quote of treated tumors divided with control tumors.

WBC: White Blood Cells

Thromb: Thrombocytes

LiPlaCis appears to lead to higher bioavailability of cisplatin and induce more potent anti-tumor efficacy but has more intense side effects than free cisplatin including body weight loss and thrombopenia.

lasting for at least a week. The levels of LiPlaCis in serum were at any time-point more than an order of magnitude higher than free cisplatin. LiPlaCis also accumulated in tumors with a maximum of about 4 μg/mg tumor mass compared to about 1 μg/mg tumor for free cisplatin. There were no significant differences in platinum accumulation in the liver. There was a moderate accumulation of LiPlaCis in the kidneys whereas the highest levels of platinum could be measured in the spleen from LiPlaCis-treated animals. See FIGS. **3-7**.

Conclusion PK/BD:

[0108] LiPlaCis long-circulating liposomal form of cisplatin. LiPlaCis accumulates in tumors and also in kidneys and spleen. Cisplatin can be released from LiPlaCis in the tumor microenvironment

Example 5

Nephrotoxicity

[0109] In humans receiving cisplatin therapeutically, a major side effect and the dose limiting toxicity of cisplatin is nephrotoxicity. In this study the nephrotoxicity of cisplatin was compared with that of LiPlaCis in the rat.

Methods

[0110] Groups of five male and five female Wistar rats, 6-7 weeks old and with a body weight of 145-175 g, were given an intravenous injection of either 3 mg/kg of Cisplatin or 3 mg/kg of LiPlaCis.

[0111] Two days after the injection, two males and two females from each group were sacrificed, 7 days after the injection, one male and one female from each group were sacrificed, and 14 days after the injection the remaining two males and two females from each group were sacrificed. The animals were subjected to macroscopic pathology and absolute and relative kidney weights were recorded. Histopathology was performed on kidneys, urinary bladder and spleen from all animals.

Results and Conclusion

[0112] At necropsy, the kidney weights were generally higher after treatment with LiPlaCis, and the histopathological examination showed that treatment with LiPlaCis clearly reduced the severity of renal degenerative changes in the form of multifocal tubular basophilia/debris and diffuse tubular vacuolation and dilation. Treatment with LiPlaCis presumably also caused a lower incidence of decreased cellular density of the white pulp/periarterial sheath compared with Cisplatin. In conclusion, LiPlaCis clearly reduced the nephrotoxicity of Cisplatin in rats.

Example 6

Phase I Dose-Escalating Study to Evaluate the Safety and Tolerability of LiPlaCis (Liposomal Cisplatin Formulation) in Patients with Advanced or Refractory Tumors

Study Synopsis

Primary Objective:

[0113] 1. To evaluate the safety and tolerability of LiPlaCis given every 3 weeks

[0114] 2. To determine the maximum tolerated dose (MTD) and the recommended dose (RD) of LiPlaCis given every 3 weeks

Secondary Objectives:

[0115] 3. To evaluate the pharmacokinetics (PK) of LiPlaCis given every 3 weeks

[0116] 4. To evaluate the therapeutic efficacy of LiPlaCis given every 3 weeks

Study Design:

[0117] Open label, non-randomised dose escalation study

Study Population:

[0118] Subjects with a solid tumor not amenable to standard treatment

Number of Patients:

[0119] The precise number of patients cannot be defined, as this is dependent on the observed toxicity. Cohorts of 3 to 6 patients will be treated at each dose level until MTD is reached. It is anticipated that 30 patients could be needed to assess MTD.

Eligibility

Inclusion Criteria:

[0120] 1. Histological or cytological documented locally advanced or metastatic solid tumor refractory to standard therapy or for which no curative therapy exists.

[0121] 2. Be ≧18 years of age.

[0122] 3. Have a life expectancy 3 months.

[0123] 4. Have an ECOG performance status of 0-2.

[0124] 5. Have recovered to grade 1 or less from acute toxicities of prior treatment:

[0125] 6. ≧6 months must have elapsed since patient received cisplatin.

[0126] 7. ≧4 weeks must have elapsed since patient received any investigational medicinal product.

[0127] 8. ≧4 weeks must have elapsed since patient received any radiotherapy, or treatment with cytotoxic or biologic agents (≧6 weeks for mitomycin or nitrosoureas). No hormonal treatment is allowed except treatment with corticosteroids at physiological dose and hormonal treatment with LHRH agonists for prostate cancer.

[0128] 9. ≧2 weeks must have elapsed since any prior surgery, blood transfusions or therapy with GM-CSF. However, current use of erythropoietin will be permitted.

[0129] 10. Be in adequate condition as evidenced by the following clinical laboratory values:

[0130] a. Absolute neutrophil count (ANC)≧1.5×109/L

[0131] b. Haemoglobin is at least 9 g/dl (5.6 mmol/L)

[0132] c. Platelets≧100×109/L

[0133] d. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)≦2.5×ULN; in case of known liver metastases ALT and AST ≦5×ULN

[0134] e. Serum bilirubin ≦1.5 ULN

[0135] f. Alkaline phosphatase ≦2.5×ULN

[0136] g. Creatinine and blood urea within normal limits, unless creatinine clearance is within normal

limits (≧60 mL/min calculated according to Cockcroft-Gault formula) (see appendix 1)

[0137] 11. Patients (male and female) must be willing to practice an effective method of birth control during the study.

[0138] 12. Patient or legal representative must understand the investigational nature of this study and sign an independent ethical committee (IEC) approved written informed consent form prior to treatment.

Exclusion Criteria are the Following:

[0139] 1. Active uncontrolled bleeding or bleeding diathesis (e.g., active peptic ulcer disease).

[0140] 2. Any active infection requiring parenteral or oral antibiotic treatment.

[0141] 3. Known infection with human immunodeficiency virus (HIV) or hepatitis virus.

[0142] 4. Active heart disease including myocardial infarction or congestive heart failure within the previous 6 months, symptomatic coronary artery disease, or symptomatic arrhythmias currently requiring medication.

[0143] 5. Known or suspected active central nervous system (CNS) metastasis. (Patients stable 8 weeks after completion of treatment for CNS metastasis are eligible.)

[0144] 6. Autoimmune disease.

[0145] 7. Impending or symptomatic spinal cord compression or carcinomatous meningitis.

[0146] 8. Having pre-existing neuropathy, i.e., Grade >1 neuromotor or neurosensory toxicity (as defined by National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) v3.0), except for abnormalities due to cancer.

[0147] 9. Having known hypersensitivity to cisplatin or liposomes.

[0148] 10. Requiring immediate palliative treatment of any kind including surgery and/or radiotherapy.

[0149] 11. Female patients who are pregnant or breast-feeding (pregnancy test with a positive result before study entry).

[0150] 12. Unwilling or unable to follow protocol requirements.

Study Procedures:

[0151] Adverse events (AEs): From signing informed consent of study drug until 30 days after receiving the last dose of study drug. Related AEs, incl. serious AEs, are followed until returned to baseline or grade to grade 1. Physical examination, vital signs, Performance status, Blood chemistry, Urinalysis: baseline and weekly in each cycle.

[0152] Haematology: baseline, bi-weekly in cycle 1 and weekly in other cycles.

[0153] Pharmacokinetic (PK) sampling: blood and urine samples should be obtained for PK evaluation. Please see section 6.4 Clinical Pharmacology Procedures.

[0154] Tumor assessments: baseline and every 3 cycles.

[0155] Study Assessments:

[0156] Safety, as determined by physical examinations, laboratory toxicity, and the incidence and severity of adverse events.

[0157] Safety assessments: NCI Common Technology Criteria for Adverse Events (CTCAE) version 3.0, laboratory evaluations (biochemistry, haematology), vital signs, physical examination including neurological examination, ECOG performance status and body weight.

[0158] Maximum tolerated dose of LiPlaCis as determined by dose-limiting toxicities (DLTs) and the recommended dose.

[0159] Clinical response rate will be determined by radiographic criteria using RECIST.

[0160] Efficacy assessments (if applicable): overall tumor response according to RECIST (CR, PR, SD or PD).

Rationale for the Study

Rationale for Selecting Dose and Schedule:

[0161] The human starting dose is determined by using the approach suggested by FDA (Reference: Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. FDA, July 2005). Rat MTD is 3 mg/kg and the conversion factor between rat and human is 6.3 according to FDA's guideline. This gives a human equivalent dose (HED) of 0.5 mg/kg.

[0162] Mouse MTD is 6 mg/kg and the conversion factor between mouse and human is 12.3 according to FDA's guideline. This gives a human equivalent dose (HED) of 0.5 mg/kg.

[0163] Thus, when the rat and mouse MTD's are converted into human equivalent doses is evident that these two species have the same sensitivity when exposed to LiPlaCis.

[0164] The reference human body weight is according to the guideline 60 kg which correspond to a dose of 30 mg per patient (0.5 mg/kg*60 kg=30 mg). A safety factor of 3 is applied resulting in a starting dose of 10 mg per patient. This dose represents 1/10 of the recommended lowest dose of plain cisplatin products (50 to 100 mg/m2 when administered as a single dose every 3-4 weeks) assumed that a normal person's body surface area is 2 m2.

[0165] A higher safety factor is frequently used (often 10) but the findings in the non-clinical studies suggest that the toxicity of LiPlaCis determined by the intrinsic toxicity of cisplatin and that LiPlaCis less toxic compared to plain cisplatin. Starting at 10 mg per subject should then be well on the safe side. Further it should be noted that LiPlaCis not a completely new drug, but a new formulation of a very well known and widely used drug.

Rationale for Schedule and Route of Administration:

[0166] In previous clinical phase I and II studies of liposomal cisplatin formulations—e.g. SPI-077 and Lipoplatin developed by ALZA Corp. and Regulon Inc., respectively—the drug product has mainly been administered every three weeks and the median number of cycles given has been between 2 and 4. In some of these studies, patients were to receive a total of 6 cycles.

[0167] To ensure that patients can be treated optimally in terms of safety and potential efficacy, LiPlaCis will be administered every 3 weeks for up to 3 cycles or more if the patient benefits from further cycles in the opinion of the investigator.

[0168] LiPlaCis will be administered intravenously by infusion as conventional cisplatin.

Study Description

[0169] The study is an open label, dose-escalating, non-randomised phase I study of LiPlaCis in patients with advanced cancer.

[0170] LiPlaCis will be administered every 3 weeks for up to 3 cycles or more if the patient benefits from the treatment upon the investigator's judgement and if there is no evidence of progressive disease or unacceptable toxicity. Post-trial access to other care must be evaluated when patients enter the trial.

[0171] LiPlaCis will be administered with increases of 20 to 100% from the previous dose level.

[0172] The number of levels needed to reach MTD is unknown. The dose escalation of to 100% will be made based on toxicity and pharmacokinetics after discussion between the investigators and the sponsor.

[0173] A clinical (telephone) conference will be organized once the last patient in the respective cohort has completed the first cycle to discuss dose-escalation. The same panel of investigators in discussion with the sponsor (LiPlasome Pharma) will decide on the MTD and the recommended dose (RD) to be used in future phase II studies of LiPlaCis.

[0174] The MTD will be the regimen with two or more patients with DLT in a cohort of 3 or 6 patients. Following completion of all cohorts and after the MTD has been defined; a clinical conference will be organized to review the outcomes of the patients to decide on the next dose, and to determine the RD for LiPlaCis. The RD will normally be the dose level below MTD (MTD-1). RD will be the dose at which no more than 1 out of the 6 patients experience DLT in first cycle.

[0175] Three patients will be enrolled per dose level and each cohort of patients will receive LiPlaCis every 3 weeks to a total of three cycles or more or until disease progression or unacceptable toxicity occurs (please see definition of dose-liming toxicity in section 6.5). Per cohort/dose level the second and third patient can be entered simultaneously after evaluation of the first week of the 1st cycle of the first patient in that cohort.

[0176] The duration of infusion will be 1 hour and could be changed to 3 hours in case adverse events—e.g. infusion reactions—necessitate a longer duration or a temporary discontinuation of infusion.

[0177] If a dose-limiting toxicity (DLT) occurs in one of the three patients within one cohort, then three additional patients will be treated at that level. If a DLT occurs in ⅔ or ⅔ patients, the next lower dose level will be expanded to at least 6 patients. The last patients of a cohort will be observed for 3 weeks before accrual to the next higher dose level might start.

[0178] Patients will be replaced within a cohort when they go off study within 3 weeks for other reason than toxicity.

[0179] The last patient at a dose level should be observed for at least 3 weeks before the first patient at the subsequent dose level can be treated.

Antiemetics:

[0180] Initially, the study treatment will start without the use of prophylactic anti-emetics. Once two patients experience nausea and/or vomiting grade 2 or more, prophylactic use of the following anti-emetics will be introduced for the patient in question and the remaining patients.

[0181] Step 1: 5-HT3 antagonist (e.g. granisetron, ondansetron)

[0182] Step 2: Day 1: granisetron 1 mg iv and dexamethason 10 mg iv, Day2-4: dexamethason 6 mg per os

[0183] Step 3: Day 1: aprepitant 125 mg per os, granisetron 1 mg iv, dexamethason 10 mg iv; Day2-3: prepitant 80 mg per os, dexamethason 6 mg per os; Day 4: dexamethason 6 mg per os.

[0184] If a patient experiences nausea and/or vomiting of grade 2 or more, therapeutic anti-emetics may be administered including Step 0: metoclopramide. At re-treatment this patient may receive prophylactic anti-emetics at investigators decision. The anti-emetics will be administered in accordance with procedures at Erasmus MC and LUMC.

Hydration:

[0185] Hydration will not be used routinely.

[0186] However, if nephrotoxicity is observed in a patient, both pre- and post-hydration will be introduced for the remaining cycles of this patient Hydration will consist of 1000 mL glucose 2.5%/NaCl 0.45% over 4 hours prior to treatment and 3000 mL glucose 2.5%/NaCl 0.45% over 8 hours post treatment.

[0187] In accordance with the definition of MTD in case nephrotoxicity should be observed in two or more patients in a cohort of 3 or 6 patients pre- and post-hydration will be introduced for the remaining cycles of the remaining patients.

[0188] However in case nephrotoxicity is observed in different patient over different cohorts this might also be a reason to start with the introduction of additional hydration. This will be decided during dose escalation teleconferences with the investigators and the sponsor.

Study Population

[0189] The targeted population for this study are patients with histologically or cytologically documented locally advanced or metastatic solid tumor refractory to standard therapy or for which no curative therapy exists.

Number of Patients

[0190] The precise number of patients cannot be defined, as this is dependent on the observed toxicity. Cohorts of 3 to 6 patients will be treated in each cohort until the MTD and the recommended dose for phase II studies of LiPlaCis determined. It is expected that up to 30 evaluable patients could enter the study to meet the key objectives of the study. However, more patients will be enrolled if required to do so.

[0191] Prior to inclusion, the patients must give written informed consent for this study and must meet all the selection criteria listed in section 3.3. Patients who sign an informed consent but fail to meet the inclusion and/or exclusion criteria are defined as screen failures. For all patients who have consented, the investigator is to maintain a screening log that documents the screening number, patient initials, and (if applicable) reason(s) for screen failure. A copy of the log should be retained in the investigator's study file.

Results from Phase 1 Study

PK:

[0192] Pharmacokinetic data confirm that LiPlaCis a long circulatory formulation of cisplatin. The following is observed:

[0193] The observed T½ is 78 hours, which is to be compared with cisplatins T½ of less than one hour. See FIGS. **8-11**.

[0194] The pharmacokinetic profile is linear both in terms of Cmax and AUC. See FIGS. **12-14**.

[0195] Urinary excretion is significantly altered compared to cisplatin. Urine is collected from 0 to 96 hours and excretion is between 0 and 20% of the administered dose. Cisplatin urinary excretion is above 90% within 3 hours.

Tox:

[0196] LiPlaCis administered in doses up to 120 mg per treatment cycle shows no sign of nephrotoxicity, ototoxicity and neurotixicity. Further, gastrointestinal toxicity in form of nausea and vomiting have not been reported in patients receiving LiPlaCis. See FIGS. **15** and **16**.

1. A sPLA2 hydrolysable liposome for use as a medicament of humans.

2. The liposome of claim **1**, wherein the content of anionic lipid is between 20% and 45%, the content of polymer conjugated lipid is between 3% and 6% and the content of neutral lipids is between 40% and 75%.

3. The liposome of claim **2**, wherein the anionic lipid is DSPG, the neutral lipid is DSPC and the polymer conjugated lipid is DSPE-PEG.

4. The liposome of claim **1**, wherein the liposome is administered intravenous administration in form of bolus injection or infusion.

5. The liposome of claim **1**, wherein the liposome comprises a therapeutic agent.

6. The liposome of claim **1**, wherein the therapeutic agent is a small molecule antitumour agent is selected from the group consisting of: anthracyclin derivatives, cisplatin, oxaliplatin, carboplatin, picoplatin, doxorubicin, paclitaxel, 5-fluoruracil, exisulind, cis-retinoic acid, suldinac sulphide, methotrexate, bleomycin and vincristine.

7. The liposome of claim **1**, wherein the small molecule antitumor agent is a platinum based antitumor agent selected from the group consisting of cisplatin, oxaliplatin, picoplatin and carboplatin.

8. The liposome of claim **1**, wherein the dose of the therapeutic agent is reduced as compared to the standard dose of the free therapeutic agent.

9. The liposome of claim **1**, wherein the dose of the therapeutic agent is increased as compared to the standard dose of the free therapeutic agent.

10. The liposome of claim **1**, wherein liposome is administered at prolonged intervals as compared to the standard dose regiment of the free compound.

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