Original Research

Biodistribution and antitumoral effect of long-circulating and pH-sensitive liposomal cisplatin administered in Ehrlich tumor-bearing mice

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Abstract

Cisplatin (CDDP) is one of the most active cytotoxic agents and has been widely used in the treatment of peritoneal carcinomatosis by the intraperitoneal (i.p.) route. However, CDDP, a low-molecular-weight compound, is rapidly absorbed by the capillaries in the i.p. serosa and transferred to the bloodstream, inducing the appearance of systemic side-effects, such as nephrotoxicity. Furthermore, the i.p. CDDP chemotherapy is limited to patients whose residual tumor nodules are less than 0.5 cm in diameter after surgical debulking. The failure of i.p. therapy is attributed to the poor penetration of CDDP into larger tumors. One strategy to improve drug delivery in the peritoneal region and reduce toxicity is the use of drug delivery systems. The objective of the present work was to evaluate the biodistribution and antitumoral effect of long-circulating and pH-sensitive liposomes containing CDDP (SpHL-CDDP), as compared with free CDDP, after their i.p. administration in Ehrlich ascitic tumor-bearing mice. After administering a 6 mg/kg single i.p. bolus injection of either free CDDP or SpHL-CDDP, ascitic fluid (AF), blood and organs (kidneys, liver, spleen and lungs) were collected and analyzed for CDDP content. The area under the CDDP concentration–time curve (AUC) obtained for AF and blood after SpHL-CDDP administration was 3.3-fold larger and 1.3-fold lower, respectively, when compared with free CDDP treatment, thus indicating its high retention within the peritoneal cavity. The determination of the ratio between AUC in each tissue and that in blood (Kp) showed a lower accumulation of CDDP in kidneys after SpHL-CDDP treatment. The SpHL-CDDP treatment demonstrated a significant uptake by the liver and spleen. SpHL-CDDP treatment led to a higher survival rate of mice with initial or disseminated peritoneal carcinomatosis than CDDP treatment. These results indicate that SpHL-CDDP may be useful for i.p. chemotherapy due to their greater concentration in the peritoneal cavity.

Keywords: cancer, liposomes, cisplatin, biodistribution, pharmacokinetics, antitumoral effect

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Introduction

The peritoneal cavity is a common site for metastatic disease arising from intra-abdominal primary tumors, such as hepatic, ovarian, colon and stomach tumors.[1–3] At least one-third of the cases of epithelial ovarian cancer are associated with the production of ascite.[4] Cis-diamminedichloroplatinum (II), or cisplatin (CDDP), is an effective chemotherapeutical drug that has been used for the treatment of peritoneal carcinomatosis after

intraperitoneal (i.p.) administration.[2,5] However, water-soluble and low-molecular-weight drugs, such as CDDP, enter the systemic circulation rapidly after i.p. injection. This fact leads to a low concentration of the drug in the peritoneal cavity and increases the risk of systemic toxicity, such as that observed after free CDDP i.p. administration.[6] Furthermore, i.p. CDDP chemotherapy is limited to patients whose residual tumor nodules are less than 0.5 cm in diameter after surgical debulking.[7] The failure of i.p. therapy is attributed to the poor penetration of CDDP within larger tumor masses. To achieve an optimal drug penetration within the tumor, the use of a high concentration and a longer time of contact with the tumor are required. On the other hand, a high CDDP concentration in the peritoneal cavity can lead to a high plasma concentration and, consequently, the appearance of systemic side-effects, such as nephrotoxicity and mielotoxicity.[8] One strategy to improve drug delivery in the peritoneal region and reduce toxicity is the use of drug delivery systems. In our research group, we have developed long-circurlating and pH-sensitive liposomes containing CDDP (SpHL-CDDP) that showed a three-fold higher value of median lethal dose (LD_{50}) than that obtained for free CDDP after their i.p. administration in mice. Hematological investigation did not reveal any alteration of red and white blood cell counts on SpHL-CDDP administration in mice. In addition, SpHL-CDDP treatment did not cause pronounced alterations in the blood urea and creatinine levels, nor did it induce morphological alteration in the kidneys of the mice.[9] These liposomes are composed of dioleoylphosphatidylethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS) and distearoylphosphatidylethanolamine-polyethyleneglycol$_{2000}$ (DSPE-PEG_{2000}). In an acidic medium, such as tumor sites, the CHEMS undergoes protonation, followed by the destabilization of liposomes and the release of CDDP. Therefore, it can be suggested that the release of CDDP in this specific site is responsible for the elimination of side-effects. The study of tissue distribution is important to understand the toxicology and therapeutical efficacy of this new formulation of CDDP. Therefore, the present work aimed to evaluate the tissue distribution and antitumoral effect of SpHL-CDDP and free CDDP after their i.p. administration in Ehrlich ascitic tumor-bearing mice.

Materials and methods

Materials

CDDP was purchased from Quiral Química do Brasil S.A. (Juiz de Fora, Brazil). DSPE-PEG_{2000} and DOPE were supplied by Lipoid GmbH (Ludwigshafen, Germany). CHEMS was purchased from the Sigma Chemical Company (St Louis, MO, USA). Sodium chloride was purchased from Merck (Rio de Janeiro, Brazil). The solvents were of analytical grade and all other chemicals were available commercially.

Irradiation of CDDP

The CDDP was irradiated according to that described by Leal *et al.*[10] A sample of CDDP (2 mg) was placed inside a cadmium capsule of 1 mm thickness. This was then irradiated within a TRIGA MARK I IPR R-I, CDTN reactor (General Atomics, San Diego, CA, USA), applying an average thermal and epithermal neutron flux of 6.4×10^{11} and 4.4×10^{10} neutrons $\times$ cm^{-2} $\times$ s^{-1}, respectively, at 100 kW. The irradiation time was eight hours. After eight hours of irradiation, the radiolabeled CDDP (CDDP*) was allowed to decay for 24 hours.

Liposome preparation

Initially, chloroform aliquots of DOPE, CHEMS and DSPE-PEG_{2000} (lipid concentration of 40 mmol/L, molar ratio of 5.7:3.8:0.5, respectively) were transferred to a round-bottom flask and submitted to evaporation. The lipid film obtained was dissolved in ethyl ether and then added to the 2 mg/mL CDDP solution prepared in the 0.9% w/v NaCl solution. The ratio of aqueous and ether phases was equal to 1:3, respectively. The resulting mixture was submitted to fast vortex agitation to produce a water/oil-type emulsion. Next, ethyl ether was evaporated, resulting in the formation of liposomes. These liposomes were calibrated by extrusion through 0.4- and 0.2-μm polycarbonate membranes (5 cycles for each). Finally, the unencapsulated CDDP was eliminated by ultracentrifugation (Ultracentrifuge, Sorvall Ultra 80, Waltham, MA, USA) at 150,000 $\times$ g at 10°C for 80 min.

Liposome characterization

The liposomes were characterized by their encapsulation percentage and size. The encapsulation percentage of CDDP into liposomes was determined by the quantification of gamma radiation in non-purified and purified liposomes. The radioactivity was measured by an automatic scintillation apparatus covering an energy window of 50–150 keV (ANSR, Abbott, Chicago, IL, USA). The counting time was four minutes and the sample volume employed was in agreement with the optimum volume of the equipment (1.5 mL). The encapsulation percentage (EP) was calculated by means of the following equation:

$$EP = \frac{\text{CDDP}^* \text{ amount in purified SpHL-CDDP}}{\text{CDDP}^* \text{ amount in non-purified SpHL-CDDP}} \times 100$$

The mean diameter of SpHL-CDDP was determined by the quasi-elastic light scattering, at 25°C, and at an angle of 90°, using the 3000HS Zetasizer equipment (Malvern Instruments, Worcestershire, UK). The measurements were performed in triplicate and the samples were diluted using a 0.9% w/v NaCl solution.

Ehrlich ascitic tumor model

The Ehrlich ascitic tumor was grown in female Swiss mice (25–30 g) by means of i.p. injection of the Ehrlich cell line. The mice were kept in an area maintained in a standardized light/dark cycle and had free access to food and water. The Ehrlich cell line was kindly supplied by Dr Jorge Luiz Pesquero (Department of Physiology and Biophysics,

Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, Brazil). Tumor cells were inoculated intraperitoneally into mice and the ascitic fluid (AF) was collected after 8 d. A viable tumor cell suspension was then prepared at a density of approximately 1.0×10^7 cells/mL and stored in liquid nitrogen. Tumor cells (1×10^6 cells) were transplanted intraperitoneally in 25–30 g female Swiss mice (School of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, Brazil). Tumors were allowed to grow for 7 d. All protocols were approved by the Ethics Committee for Animal Experiments at the Federal University of Minas Gerais and comply with the guidelines for the care and use of laboratory animals recommended by the Institute of Laboratory Animal Resources.

Tissue distribution studies

Two groups of Ehrlich ascitic tumor-bearing female Swiss mice were used in the experiments. The first group was treated with SpHL-CDDP* and the second with free CDDP*. The treatments were performed by i.p. route, and the dosage was of 6 mg/kg. At the indicated times (1, 2, 4, 8, 12, 24, 48 and 72 h), the mice ($n = 5$ for each time interval) were anesthetized with a mixture of xylazine (7.5 mg/kg) and ketamine (60 mg/kg). Blood was collected immediately by means of a cardiac puncture and the AF was recovered directly from the abdominal cavity. Spleen, lungs, liver and kidneys were removed, washed with distilled water, dried with filter paper and weighed. The radioactivity of the blood, AF and organs was measured by an automatic scintilation apparatus covering an energy window of 50–150 keV (ANSR, Abbott). The counting time was four minutes, and a standard dosage containing the same injected amount was counted simultaneously in a separate tube, which was determined to be 100% of radioactivity. The results were expressed as the percentage of injected dose/g (%ID/g) of tissue and percentage of injected dose (%ID) found in AF. The data were statistically analyzed by the unpaired *t*-test ($P < 0.05$). The tissue–blood partition coefficient (Kp) was determined by dividing the area under the tissue concentration–time curve (0–72 h) by the area under the blood concentration–time curve (0–72 hours).

Pharmacokinetic study

In this study, the concentrations of radioactive CDDP in the blood were expressed as a mean of %ID/g. Pharmacokinetic parameters were determined using the WinNonlin software, version 03.1A (Pharsight, Mountain View, CA, USA). A non-compartmental analysis model 200 (extravascular administration) was used with the log/linear trapeizoidal rule. Parameters, including terminal half-life ($T_{1/2\lambda z}$), maximum radioactivity (C_{max}), time to reach C_{max} (T_{max}), blood clearance (Cl/F), apparent volume of distribution during the terminal phase (V_z/F) and the area under the CDDP concentration–time curve (AUC) were determined. Pharmacokinetic parameters associated with the terminal phase were calculated using the last three measured time points to estimate the terminal half-life.

Antitumoral activity

The antitumoral activity of the formulations containing CDDP was evaluated in a Ehrlich ascitic tumor-bearing experimental animal model. Initially, 2.5×10^6 cells were transplanted intraperitoneally in 25–30 g female Swiss mice. Then, the animals were submitted to different treatments on the third (TR3) or seventh (TR7) day after the inoculation of tumor cells. These treatment schedules were chosen to verify the treatment efficacy using initial and disseminated peritoneal carcinomatosis models, respectively. Free CDDP or SpHL-CDDP were administered by i.p. route, at a dose of 10 mg/kg, into Ehrlich ascitic tumor-bearing Swiss mice. This dose was chosen because we demonstrated in previous studies that toxicity signs are more evident only from a dose of 10 mg/kg of free CDDP.[9] Animals in the control groups were treated with 0.9% (w/v) NaCl solution or blank liposomes (SpHL) using the same volume of respective formulations containing CDDP. The number of animals for each treatment was equal to 20. The survival rate was noted three times per week up to 30 d after each treatment. The death of the mice was noted daily up to 30 d after each treatment. Survival analysis was performed using the Kaplan–Meier method, while the different groups were compared by the log-rank test. A probability value of <0.05 was also considered to indicate a significant difference. Behavior modifications and weights of the mice were monitored after administration of each treatment. From the body weight data, the weight variation over time was calculated as a ratio to the initial body weight.

Results

Liposome characterization

The percentage of encapsulation of CDDP* in SpHL-CDDP was equal to $29.7 \pm 3.3\%$. The mean diameter of SpHL-CDDP was 148.9 ± 6.7 nm, showing a good homogeneity (polydispersity index equal to 0.08). These data were obtained from three experiments.

Tissue distribution

After a single i.p. injection of SpHL-CDDP, the ascitic tumor showed a higher retention of CDDP at 72 h compared with administration of free CDDP (Figure 1). The AUC value for AF was 3.3-fold higher after SpHL-CDDP treatment (973.1 and 281.2%ID × h in SpHL-CDDP and free CDDP-treated mice, respectively) (Table 1). In the blood, the maximum CDDP uptake occurred at two hours ($2.8 \pm 0.2\%$) and 1 h ($1.5 \pm 0.1\%$) after SpHL-CDDP and free CDDP administration, respectively (Figure 2). In the kidneys, the maximum uptake of CDDP occurred at 1 h for both treatments ($5.5 \pm 0.7\%$ and $1.4 \pm 0.2\%$ for CDDP and SpHL-CCDP treatment, respectively). During the entire experiment, a lower uptake of CDDP by kidneys after SpHL-CDDP treatment, as compared with that observed when the mice were treated with free CDDP (Figure 3) could be observed. The AUC value for liposomal CDDP

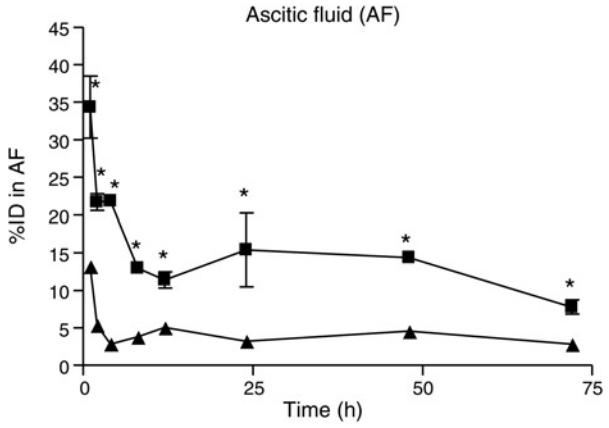

Figure 1 Concentration–time profile of CDDP in ascitic fluid after i.p. administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. The asterisks indicate a statistically significant difference between free CDDP and SpHL-CDDP treatments ($P < 0.05$). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

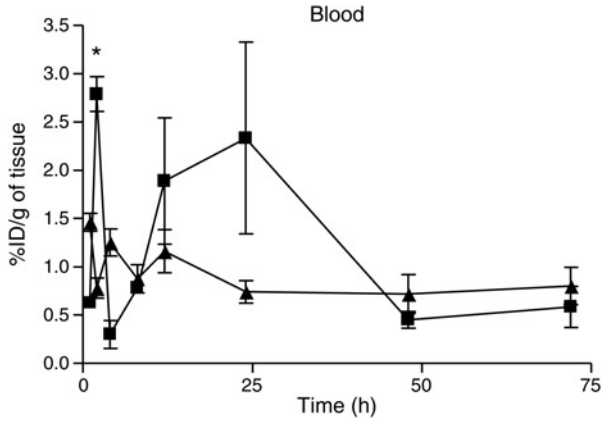

Figure 2 Concentration–time profile of CDDP in blood after i.p. administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. The asterisk indicates a statistically significant difference between free CDDP and SpHL-CDDP treatments ($P < 0.05$). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

was 1.5-fold lower than that obtained after free CDDP administration (Table 1). In addition, the CDDP showed a lower renal perfusion after SpHL-CDDP injection when compared with free CDDP treatment. The Kp values were equal to 1.8 and 3.8 for SpHL-CDDP and free CDDP treatments, respectively (Table 2). The uptake of CDDP by organs of the mononuclear phagocytic system (liver and spleen) after free CDDP administration was found to be low (Figures 4 and 5). In contrast, mice treated with SpHL-CDDP showed a high uptake of CDDP by these organs. In the spleen, the CDDP radioactivity peak was attained 12 h (46.2 ± 7.0%) after the injection of SpHL-CDDP (Figure 4). The AUC value was 6.7-fold higher after SpHL-CDDP treatment as compared with that obtained after free CDDP treatment (Table 1). The liposomal CDDP, in comparison with the free CDDP treatment, revealed a greater affinity to the spleen tissue, which was demonstrated by a high Kp value (Table 2). In the liver, the CDDP reached its maximum level at 24 h (24.4 ± 3.1%) after SpHL-CDDP injection (Figure 5). The AUC value was five-fold higher when the mice were treated with SpHL-CDDP, as compared with free CDDP treatment (Table 1). The CDDP uptake following both treatments proved to be extensive (Kp values were higher than the unit values). However, the CDDP accumulation in the liver proved to be higher for the SpHL-CDDP treatment than for the free CDDP treatment. In the lungs, the distribution profile was similar after SpHL-CDDP and free CDDP injections (Figure 6). The maximum uptake of CDDP occurred at 1 h (2.6 ± 0.5%) and 2 h (8.5 ± 1.5%) for free CDDP and SpHL-CDDP treatment, respectively. The lung AUC for both treatments were similar (86.1 and 80.0% ID × h, respectively). However, the Kp value proved to be equal to the unit value after the SpHL-CDDP treatment, which indicated that CDDP did not diffuse from the vascular space to the pulmonar tissue. In contrast, after the administration of free CDDP, an amount of CDDP underwent extravasation to the lungs (Table 2).

Table 1 CDDP concentration–time curve (AUC) values for AF and different organs at 72 h after SpHL-CDDP or free CDDP administration*

	$AUC_{(0\rightarrow72\ h)}$ SpHL-CDDP	$AUC_{(0\rightarrow72\ h)}$ CDDP
AF	973.1	281.2
Kidneys	147.2	224.7
Spleen	1501	224.5
Liver	1010	199
Lungs	86.1	80

CDDP, cisplatin; AUC, concentration–time curve; AF, ascitic fluid; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP
*The units of AUC were expressed as %ID/g × h for blood and organs and %ID × h for AF

Pharmacokinetic study

Figure 2 illustrates the concentration of CDDP from blood versus time after i.p. injection of free CDDP and

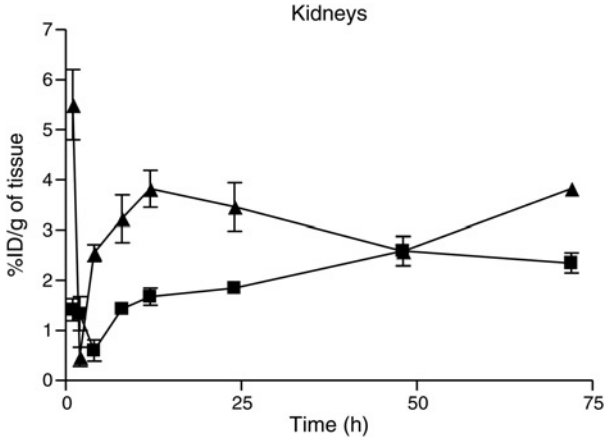

Figure 3 Concentration–time profile of CDDP in kidneys after i.p. administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

Table 2 Tissue–blood partition coefficient (Kp) for different tissues after i.p. injection of SpHL-CDDP (A) or free CDDP (B)

Tissue	$Kp_{(A)}$	$Kp_{(B)}$
Kidneys	1.8	3.8
Spleen	18	3.8
Liver	12.1	3.4
Lungs	1	1.4

SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal; CDDP, cisplatin

SpHL-CDDP in Ehrlich ascitic tumor-bearing mice. The pharmacokinetic parameters are summarized in Table 3. The maximum radioactivity (C_{max}) and the time to reach C_{max} (T_{max}) in the blood were determined to be 3.20 μg/mL and one hour, respectively, for free CDDP treatment, and 4.3 μg/mL and two hours, respectively, for SpHL-CDDP treatment. The $T_{1/2\lambda Z}$ of free CDDP and SpHL-CDDP were 919.8 and 668.5 h after i.p. injection, respectively. The $AUC_{(0\rightarrow\infty)}$ of free CDDP and SpHL-CDDP treatments were 4185.70 and 3207.1 h.μg/mL, respectively. The $AUC_{(0\rightarrow\infty)}$ of free CDDP in the blood was 1.3-fold larger than that of SpHL-CDDP. The apparent volume of distribution during the terminal phase (V_z/F) of CDDP and SpHL-CDDP were equal to 29.2 and 30.1 mL, respectively. The blood clearance after i.p. administration (CL/F) of free CDDP (0.022 mL/h) was lower than that of SpHL-CDDP (0.031 mL/h).

Antitumoral activity

The *in vivo* antitumoral activity of SpHL-CDDP and free CDDP treatments was determined by assessing survival in Ehrlich ascitic tumor-bearing Swiss mice (Figure 7). For the TR3 treatment schedule, the median survival of the free CDDP and SpHL-CDDP treatment groups was 29 d and more than 30 d, respectively (Figure 7a). The median survival of mice treated with SpHL-CDDP was statistically longer than that of mice treated with free CDDP ($P = 0.0005$). The 0.9 % (w/v) NaCl solution and SpHL control groups showed a median survival of 21.5 and 17 d, respectively. For the TR7 treatment schedule, the group treated with SpHL-CDDP also presented a significantly longer survival period than that of mice treated with free CDDP (21 and 15 d, respectively. $P = 0.0174$) (Figure 7b). In this case, the median survival of two control groups was similar (17 d). It is noteworthy that diarrhea, ataxia, piloerection and weakness occurred in all free CDDP-treated mice, while in the group treated with SpHL-CDDP, these symptoms were not observed. These findings, as well as the body weight loss observed post-injection of free CDDP in the TR3 and TR7 treatment schedules (Figure 8), may be due to the toxicity of CDDP administered as the free form. The toxic effect provoked by free CDDP seems to be more pronounced in disseminated Ehrlich ascitic tumor-bearing mice. The maximum body weight loss was equal to 18.0% (10th day) and 24.0% (13th day) for mice presenting Ehrlich ascitic tumors into initial or advanced stages, respectively

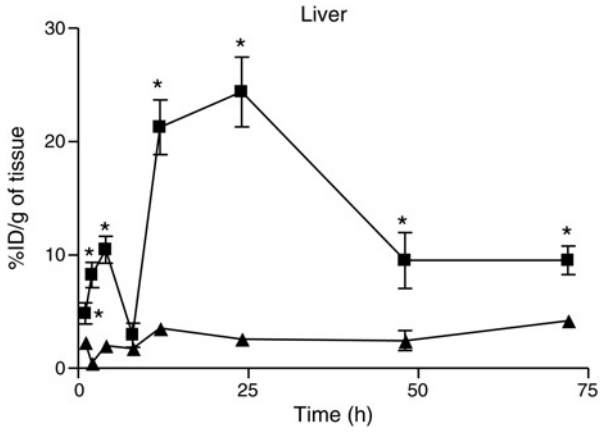

Figure 5 Concentration–time profile of CDDP in liver after i.p administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. The asterisks indicate a statistically significant difference between free CDDP and SpHL-CDDP treatments ($P < 0.05$). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

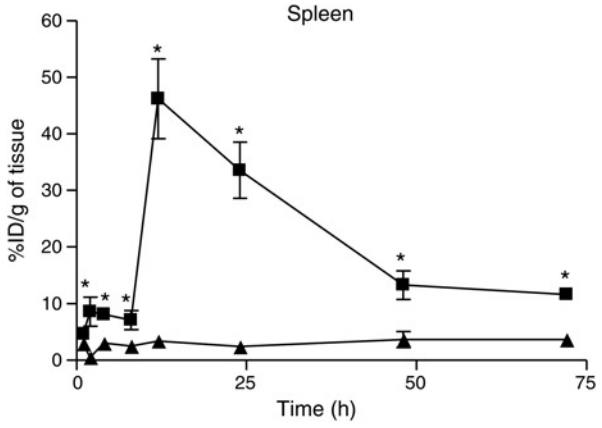

Figure 4 Concentration–time profile of CDDP in spleen after i.p. administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. The asterisks indicate a statistically significant difference between free CDDP and SpHL-CDDP treatments ($P < 0.05$). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

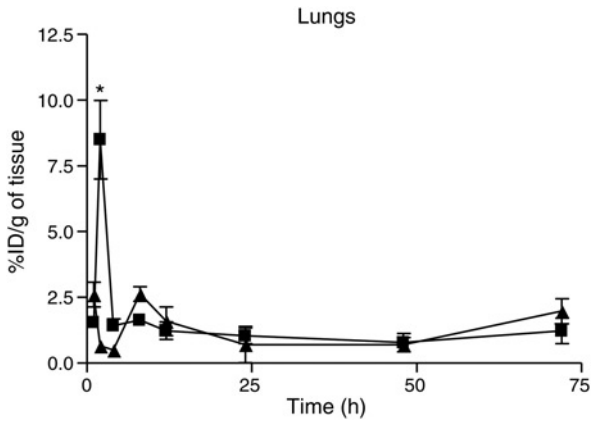

Figure 6 Concentration–time profile of CDDP in lungs after i.p. administration of SpHL-CDDP (■) or free CDDP (▲) in Ehrlich ascitic tumor-bearing mice. Results were expressed as means ± standard error. The asterisk indicates a statistically significant difference between free CDDP and SpHL-CDDP treatments ($P < 0.05$). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

Table 3 Pharmacokinetic parameters of free CDDP and SpHL-CDDP after i.p. administration in Ehrlich ascitic tumor-bearing Swiss mice*

Parameter	Unit	Free CDDP	SpHL-CDDP
C_{max}	μg/mL	3.2	4.3
$AUC_{(0\rightarrow\infty)}$	h.μg/mL	4185.7	3207.1
$T_{1/2\lambda Z}$	H	919.8	668.6
Vz/F	mL	29.2	30.1
Cl/F	mL/h	0.022	0.031

SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal; CDDP, cisplatin; C_{max}, maximum radioactivity; AUC, area under the CDDP concentration–time curve; $T_{1/2\lambda Z}$, terminal half life; Vz/F, apparent volume of distribution during the terminal phase; Cl/F, blood clearance

*Calculated with WinNonlin program for a non-compartmental model.

(Figures 8a and b). After SpHL-CDDP administration at both treatment schedules, loss of body weight was also observed. However, this fact seems not to contribute to the mortality, since it was observed only 10% of deaths at a time interval of 30 d for the TR3 treatment schedule.

Discussion

It has been demonstrated that liposomes are drug delivery systems that are able to modify tissue distribution, metabolism, and *in vivo* elimination as well as reduce the toxicity of free drugs.[11–13] In peritoneal carcinomatosis therapy, the i.p. administration of liposomes containing anticancer drugs can take advantage of the injection directly in the tumor site to deliver a high dose of cytotoxic agents while potentially reducing the toxicity and increasing the therapeutic efficacy of systemic drug administration.[2,9] These benefits result from the retention of liposomes in the peritoneal cavity over a long period of time and an improved influx in tumor cells. In this light, in order to obtain a proof of concept, it is quite important to perform a tissue distribution study of new chemotherapeutic systems. This work aimed to investigate the tissue distribution and pharmacokinetics of a novel liposomal formulation of CDDP (SpHL-CDDP, developed by our research group) in an Ehrlich ascitic tumor-bearing mice model. The localization of SpHL-CDDP near the acidic tumor site can favor the collapse of liposomes, thereby leading to its disruption and subsequent release of CDDP in this region. A higher concentration of CDDP at tumor sites can reduce the systemic toxic effects and improve the antitumor efficacy. In this study, SpHL-CDDP treatment led to a higher concentration of the drug in the AF than in the free CDDP treatment. The retention of CDDP within the abdominal cavity is desirable as regards the chemotherapy of several types of tumors that are able to produce abdominal metastasis AF.[4] Some authors have reported that CDDP is quite effective in cancer chemotherapy, including ovarian tumors and produces clinical responses when injected by i.p. route. Still, even when administered by an i.p. route, CDDP can accumulate in the renal tissue and produce toxic effects.[5,14] Our results, however, showed a decreased renal extravasation and uptake after SpHL-CDDP administration. Moreover, as expected, the lower blood $AUC_{(0\rightarrow\infty)}$ value for SpHL-CDDP treatment as compared with free CDDP treatment demonstrates the reduction of the passage of CDDP into the bloodstream after peritoneal injection. Thus, these findings can explain the absence of signs of renal toxicity after the i.p. administration of SpHL-CDDP, as observed in previous studies.[9] SpHL-CDDP also demonstrated an extensive accumulation in organs of the mononuclear phagocytic system, including the liver and spleen, as has also occurred in other biodistribution studies on liposomal preparations administered by i.p. route.[15,16] The spleen and liver were responsible for a high clearance of SpHL-CDDP and showed a high tissue–blood partition ratio. These results indicate that SpHL-CDDP has an affinity toward these organs, but it did not induce any toxicity in the liver and spleen tissues, as demonstrated by Leite *et al.*[9] In the beginning hours, the amount of SpHL-CDDP uptaken by these organs was of approximately 10%ID/g. At 12 h, an increased uptake of SpHL-CDDP, which attained 46%ID/g and 20%ID/g for splenic and hepatic tissue, respectively, could be observed. This increased accumulation in these organs may well be the result of SpHL-CDDP clearance from the peritoneum to systemic circulation through drainage into the lymphatic system. After reaching the organs of the mononuclear phagocytic system, SpHL-CDDP can be degraded, releasing CDDP from cells into the blood,

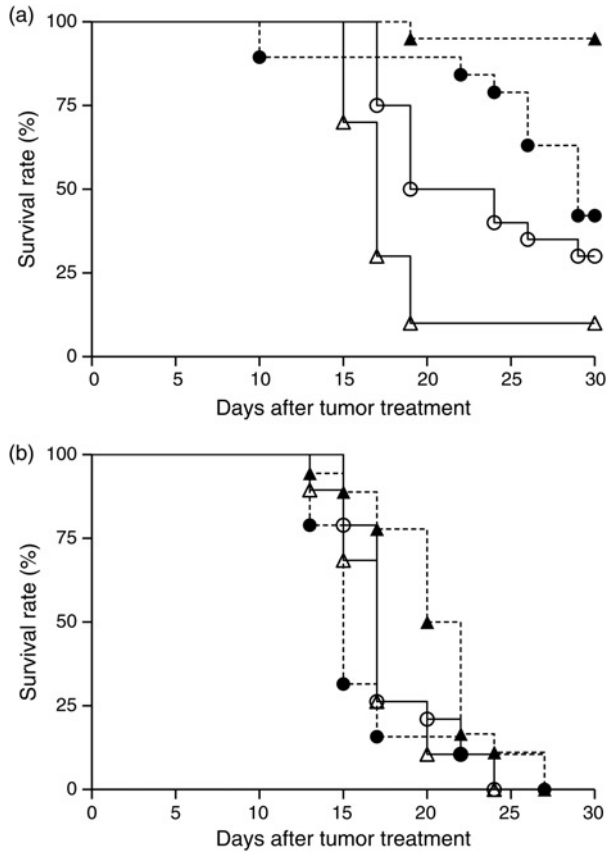

Figure 7 Survival of Ehrlich ascitic tumor-bearing Swiss mice treated with free CDDP (•) or SpHL-CDDP (▲) on the third (TR3; a) or seventh (TR7; b) day after tumor cells inoculation. The dose of CDDP in each formulation was of 10 mg/kg. The survival of the following control groups was also evaluated: SpHL (Δ) or 0.9% (w/v) NaCl solution (○). CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

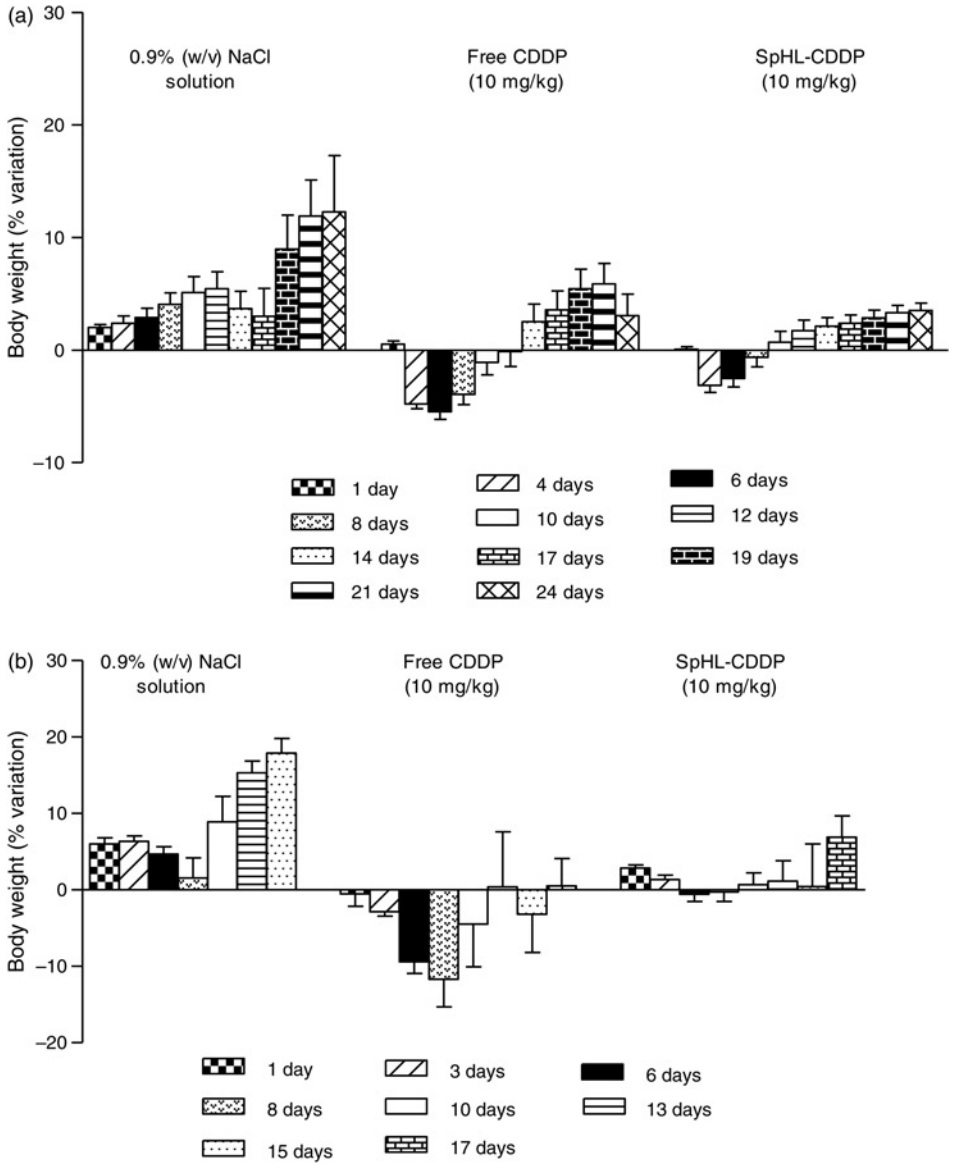

Figure 8 Body weight variations observed after i.p. administration of free CDDP or SpHL-CDDP on the third (TR3; a) or seventh (TR7; b) day after tumor cells inoculation. The dose of CDDP in each formulation was of 10 mg/kg. The body weight of the following control group was also evaluated: 0.9% (w/v) NaCl solution. Data were expressed as mean ± standard error of mean. CDDP, cisplatin; SpHL-CDDP, long-circulating and pH-sensitive liposomes containing CDDP; i.p., intraperitoneal

followed by renal elimination. This biodistribution profile may explain the lower and higher values of $T_{1/2\lambda z}$ and V_z/F, respectively, found for SpHL-CDDP treatment, as compared with free CDDP treatment (668.6 and 919.8 h and 0.031 and 0.022 mL/h, respectively). In the lungs, the AUC values were similar after both free CDDP and SpHL-CDDP injections during the investigated time intervals. However, the tissue distribution was extensive for the free CDDP treatment, and the extravasation to the pulmonary tissue did not occur after SpHL-CDDP administration (Kp values were equal to 1.4 and 1.0, respectively). These findings allow us to understand and corroborate the reduction of systemic toxicity observed in previous studies related to the acute toxicity of SpHL-CDDP after their i.p. administration.[9] In addition, according to the data obtained (Figure 8), the encapsulation of CDDP into long-circulating and pH-sensitive liposomes allowed a high retention of the drug in the abdominal cavity that probably contributed to the better survival of the mice. This effect was more remarkable in initial-stage Ehrlich tumor-bearing mice. Considering the limitation of increasing doses of free CDDP, mainly due to its toxicity, improvements in cancer therapy, through the use of SpHL-CDDP, will almost certainly be observed at high doses of this drug delivery system. Thus, experiments aimed at evaluating the *in vivo* antitumoral effect of SpHL-CDDP at high doses are currently being performed in mice bearing initial and advanced-stage tumors by our research group.

Conclusion

In conclusion, the i.p. administration of SpHL-CDDP in Ehrlich ascitic tumor-bearing female Swiss mice proved to be a potential approach to improving the bioavailability

and antitumoral efficacy of CDDP in tumors located in the abdominal cavity, such as is the case with malignant gastrointestinal and gynecological diseases. Moreover, the lower uptake of SpHL-CDDP through the renal tissue, as compared with that of free CDDP, contributes to a consequent reduction in the nephrotoxicity induced by CDDP and may well allow for the use of elevated doses during cancer chemotherapy.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. JGCA, LGM, EAL and LCM conducted the experiments. AJAW, LGVC, PRS-R, VNC and MCO were responsible for the elaboration of the approach of application of long-circulating and pH-sensitive liposomes containing cisplatin in the treatment of peritoneal carcinomatosis. MTP contributed with the preparation of radiolabeled cisplatin, ATC contributed with the experiments in relation to antitumor activity and JGCA and MCO wrote the manuscript.

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