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Pharmacokinetics and distribution of liposomal busulfan in the rat: a new formulation for intravenous administration

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Abstract The plasma pharmacokinetics and tissue distribution of busulfan (Bu) were investigated after intravenous injection of free Bu (D-Bu) and freshly prepared liposomal Bu (L-Bu). Liposomal Bu was prepared using L-α-phosphatidylcholine, 1,2-dioleolyl-*sn*-glycero-3-phosphate, and cholesterol. The liposomes formed were unilamellar vesicles measuring 220 ± 14 nm in diameter and containing a Bu concentration of 0.31 ± 0.03 mg/ml. The half-life of Bu in the present formulation was determined to be 8.7 ± 2.7 days at 4 °C. The liposomes in the new formulation were stable for 20 days at 4 °C. After the intravenous administration of L-Bu or D-Bu (dissolved in a mixture of DMSO, ethanol, and propylene glycol) to the rats a higher bone marrow exposure to Bu as expressed in AUC marrow/AUC blood was achieved using L-Bu as compared with D-Bu (1.59 and 0.83, respectively). A higher distribution volume was observed for L-Bu as compared with D-Bu (1.39 versus 0.67 l/kg, respectively). The elimination half-lives were significantly longer in both blood and marrow after the administration of L-Bu as compared with D-Bu (2.52 and 3.08 versus 1.53 and 1.75 h, respectively). The new liposomal Bu showed linear pharmacokinetics within the range of 0.5–3.5 mg/kg, which is comparable with that obtained for D-Bu. A slight difference was observed in systemic exposure to L-Bu as compared with D-Bu as expressed in AUC (9.93 and 11.82 μg h ml^{-1}, respectively). The distribution study using ^{14}C-labeled Bu showed that the radioactivity was significantly higher over 18 h in the bone marrow (3-fold) and spleen (2-fold; $P < 0.01$) in a comparison of L-Bu with D-Bu. However in the brain, lungs, and heart the distribution of radioactivity after the administration of L-Bu was significantly lower ($P < 0.05$) than that obtained using D-Bu. On the basis of the present study, the new formulation of liposomal Bu seems to be a promising preparation for clinical trails, since it appears to target bone marrow and spleen with no accumulation in the liver or other organs known for Bu toxicity.

Key words Busulfan · Liposomes · Pharmacokinetics · Bone marrow transplantation · Bioavailability

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Introduction

Busulfan is an alkylating agent that, in combination with cyclophosphamide (Bu/Cy), is widely used in high doses as a part of the myeloablative regimen before both allogeneic (BMT) and autologous (ABMT) bone marrow transplantation [9, 26, 34]. Bu was introduced as an alternative to total body irradiation (TBI). Moreover, the therapeutic efficacy of the combination Bu/Cy is considered to be equivalent to that of TBI/Cy and to have the advantage of avoiding TBI, especially in pediatric patients.

Bu is given only p.o., usually every 6 h over 4 consecutive days. During the last decade the disposition and pharmacokinetics of high-dose Bu have been investigated in both adults and children. Recent studies have shown alterations in Bu disposition in pediatric patients as compared with adults, including lower levels of drug exposure, minimal toxicity, and higher rates of engraftment failure.

A wide interpatient variability in Bu kinetics has been reported by many authors. Age, circadian rhythmicity,

hepatic function, disease, drug-drug interaction, and absorption were pointed out as factors affecting Bu disposition [17, 19, 45]. Many authors have expressed their concern about pediatric patients, and several attempts have been made to modify and increase the dose for younger children [44, 46]. Those attempts have resulted in an increased rate of toxicity such as veno-occlusive disease and seizures [11, 43]. This increase in toxicity is most probably due to the variability in Bu bioavailability combined with higher doses. We have reported a variation in Bu bioavailability in both adults and children after a dose of 2 mg. This variation was 2-fold in adults and 6-fold in young children [19].

A dose reduction or adjustment based on AUC and/or the limited-sampling model is one strategy that has been proposed to minimize toxicity and achieve higher drug efficacy [4, 20, 36, 43]. The other attempt was to develop an intravenous administration form. Recently, two studies reported the use of an intravenous form of Bu in the rat and the dog. These investigations utilized dimethylsulfoxide (DMSO) and dimethylacetamide (DMA) as solvents for the drug [2, 6]. However, despite the promising results, these organic solvents have their own toxicity [22, 25, 27, 29, 40, 47], which must especially be considered when a patient is to receive DMSO in connection with marrow infusion.

In the present study we utilized the liposome incorporation technique to develop an intravenous form of Bu. We report a liposomal form (that is known to be a nontoxic carrier for drug delivery) for intravenous administration of Bu. We also demonstrate the stability of the liposomal form and its pharmacokinetics in a rat model at doses of 0.5–3.5 mg/kg. Finally, we investigated and compared the distribution of ^{14}C-busulfan into rat organs as a liposomal and free busulfan forms.

Materials and methods

Materials

Bu [1,4-bis(methanesulfonoxy)butane] was obtained from Sigma Chemicals (St. Louis, USA). [1,4 -^{14}C]-Succinic acid was purchased from Amersham (UK). Cholesterol, L-α-phosphatidylcholine (egg, 100 mg/ml), and 1,2-dioleolyl-*sn*-glycero-3-phosphate (monosodium salt, 20 mg/ml) were obtained from Avanti Polar Lipids Inc. (Alabama, USA). [1,4-^{14}C]-Bu with a specific activity of 2 mCi/mmol was synthesized as described in our previous work [14].

Liposomal-encapsulated busulfan

The lipids L-α-phosphatidylcholine (EPC), 1,2-dioleolyl-*sn*-glycero-3-phosphate (DOPA), and cholesterol at a molar ratio of 9.45:1:9.4 were dissolved in chloroform. Bu or [^{14}C]-Bu was added. The mixture of lipids and Bu was dried by evaporation to a thin film coating the inside of a round glass vessel. Any trace of solvent was removed under a gentle stream of nitrogen. The mixture was then hydrated with 25 ml of glucose (50 mg/ml, pH 4.0) or 25 ml of sodium chloride (9 mg/ml). Multilamellar vesicles were formed by vortexing of the lipid-aqueous mixture. The suspension was transferred to an Extruder (LiposoFast 50, Avestin, Ottawa, Canada) and extruded five times under nitrogen through two stacked polycarbonate filters of 100-nm pore size. Bu concentrations were determined before and after filtration to determine the entrapment efficiency. The total phospholipid content was 16 mg/ml. Liposome-encapsulated [^{14}C]-Bu was assessed for ^{14}C to determine the injected dose of radioactivity.

The vesicle size distribution for the final liposomal preparation and the stability of the liposomal preparation were studied over 20 days at 4 °C using both dynamic light scattering (Malvern Autosizer) and laser diffraction (Malvern Mastersizer). The liposomal preparation was also examined for free crystals of Bu using electron microscopy. All preparations of Bu and/or [^{14}C]-Bu were encapsulated into liposomes under aseptic conditions.

Analysis

Bu, liposomal Bu, and the internal standard 1,5-bis(methanesulfonoxy)pentane were converted to 1,4-diiodobutane and 1,5-diiodopentane, respectively. The determination of Bu concentrations for pharmacokinetics and stability studies of liposomes was performed using gas chromatography with electron-capture detection as described previously [13]. Measurement of the radioactivity in different organs was performed using a liquid scintillation counter (model 1217, LKB, Wallac, Turku, Finland).

Animals

The study protocol was approved by the Animal Care Committee of the Karolinska Institute prior to experimentation. All experiments were designed according to the guidelines established by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources. Male Sprague-Dawley rats (200–230 g) were used (B&K Laboratories, Sollentuna, Sweden). The animals were kept in a fully climatized room at a constant temperature and humidity on a 12-h light/dark cycle and were fed standard pelleted food and water ad libitum. Rats were anesthetized with ether, injected intravenously into the tail vein with either liposomal [^{14}C]-Bu (1.5 ml) or [^{14}C]-Bu (0.25 ml) dissolved in DMSO: ethanol: propylene glycol at a ratio of 0.35:0.25:0.40. The injections of free Bu were followed by 1.25 ml of saline solution to compensate for the volume differences.

At appropriate times (0.17, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, and 24 h) the animals (three animals for each time point) were anesthetized with pentobarbital (50 mg/kg) and blood samples were drawn via heart puncture (3–4 ml). Plasma was immediately separated from blood cells at 4000 rpm and stored at −20 °C until analysis.

The animals were killed under anesthesia and about 1 g of the lungs, heart, liver, brain, spleen, kidney, and testis were removed, washed free of blood in isotonic saline solution, and weighed. The marrow was removed from the femur by flushing with 1 ml of saline solution, which was weighed before and after the flushing. The femur was also weighed before and after the flushing (as a control of the marrow weight). All organs were kept at −20 °C before assay either for the Bu concentration or for the radioactivity distribution.

Preparation of biological samples

The distribution of the radioactivity representing Bu and its metabolites was studied in the organs. About 400 mg of each organ was minced and homogenized in 1 ml of saline solution. Duplicates of the homogenized samples (0.4 ml) were transferred into glass counting vials and solubilized with 1 ml of Soluene-350/isopropanol (1:1) for 2 h at room temperature. To decolorize the samples, 0.2 ml of 30% hydrogen peroxide was added after solubilization was completed. The vials were incubated with hydrogen peroxide at

50 °C for 30 min. After a cooling step, 15 ml of Hionic Fluor was added. The vials were kept at room temperature for 2 days to allow chemiluminescence to decay and were then counted for ^{14}C radioactivity.

Pharmacokinetics and evaluation

Mean values and standard deviations were calculated from the experimental data. The concentration-time curves were adjusted to the data sets via nonlinear iterative least-squares regression analysis. Curve modeling was performed according to classic one- or two-compartment open models. The pharmacokinetic parameters were calculated on a PC using WINNONLIN version 1.5.

The symbols used are: BW – body weight (kg) (in kilograms), C_0 – intercept at time zero (in micrograms per milliliter), $t_{1/2\alpha}$ – distribution half-life (in hours), $t_{1/2\beta}$ – elimination half-life (in hours), V_d – distribution volume, C_p – concentration in plasma, Cl_{tot} – total body clearance, C_{bm} – concentration in bone marrow, and $AUC_{0-\infty}$ – area under the concentration-time curve, (in microgram per milliliter times hour), estimated using the trapezoidal rule. Statistical analysis (*F*-test, Student's *t*-test, and variance analysis) was performed using STAT-MATE (Graph-Pad, version 2.0).

Results

Measurement of the encapsulation efficiency and stability of liposomes and liposomal Bu

The incorporation of Bu into liposomes as shown in Fig. 1 was more efficient when NaCl was used as a solvent. Liposomes were loaded with 0.83 ± 0.08 mg/ml ($n = 10$) as compared with 0.31 ± 0.03 mg/ml when glucose (50 mg/ml, pH 4.0) was used. The effect of the filtration can also be seen in Fig. 1, which shows a loss of Bu after the first filtration, followed by a constant concentration both in sodium chloride and in glucose. The effect of different initial concentrations on the final concentrations of Bu encapsulated in liposomes is illustrated in Fig. 2. Similar results were obtained using

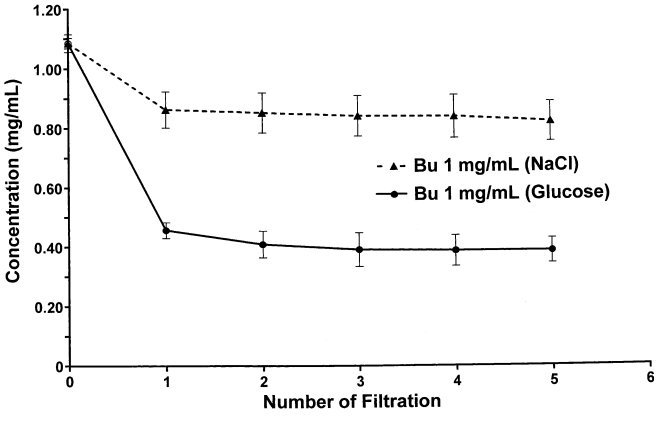

Fig. 1 Effect of solvent (glucose and sodium chloride) and of the number of filtration steps on the final concentration of Bu in the liposomal preparation

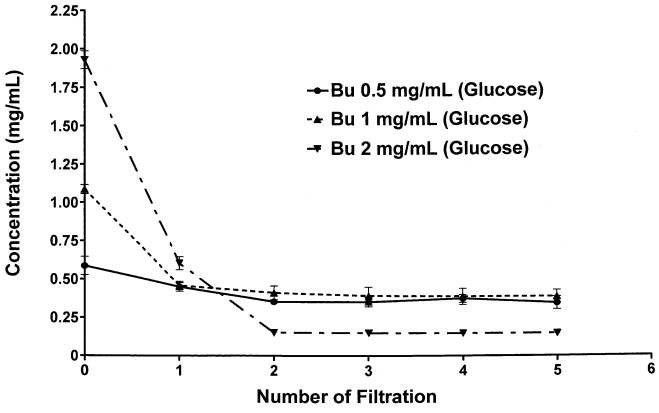

Fig. 2 Effect of initial concentrations and of the number of filtration steps on the final concentration of Bu in liposomes using glucose as a solvent

different concentrations with sodium chloride (data not shown). An initial concentration of 0.5–1 mg/ml in a glucose solution resulted in a final concentration of 0.31 mg/ml after filtration. However, when the initial concentration was increased to 2 mg/ml the final concentration dropped to less than 0.1 mg/ml. Similar results were observed when NaCl was used as a solvent (data not shown). In all animal experiments the glucose was used as a solvent.

The stability of Bu incorporated in liposomes (0.3 mg/ml) was studied in glucose solution. The degradation half-life of liposomal Bu was determined to be 8.7 ± 2.7 days at 4 °C. In ten different preparations of liposomal Bu in glucose the size of liposomes proved to be normally distributed, with the peak occurring at 220 ± 14 nm after five filtration steps. Dynamic light scattering and laser diffraction showed that the size of the liposomal preparation was stable for 20 days at 4 °C, after which the liposomes started to build aggregates. No free crystal of Bu was observed in the liposomal preparation using electron microscopy during the period of study (20 days). However, free crystals were found when sodium chloride was used as a solvent. Moreover, the stability of the liposomes in the preparation was lower in NaCl (about 5 days).

Organ distribution

Bu and liposomal Bu distribution was studied using a ^{14}C-labeled compound. The distribution of [^{14}C]-Bu entrapped in liposomes was compared with that of an equal amount of free [^{14}C]-Bu dissolved in DMSO: ethanol: propylene glycol in different organs. The distribution of the total radioactivity in these organs is listed in Table 1. The radioactivity for both administration forms were calculated as the AUC for each organ (1 g) from time 0 to 18 h after the treatment. As can be seen, a significant accumulation ($P < 0.01$) was observed in both bone marrow (3-fold) and the spleen

Table 1 Distribution of radioactivity in rat organs (dpm/g) after the administration of equal doses of [^{14}C]-Bu (3.5 mg/kg) either as liposomal Bu (L) or as Bu dissolved in DMSO (D)[a]

Organ	AUC (dpm × 10^4 g^{-1} h) ± SD	Ratio g organ/g blood	*P*
Blood D	9.33 ± 0.08	1.00	0.02
Blood L	6.44 ± 0.37	1.00	
Marrow D	18.72 ± 1.45	1.96	0.006
Marrow L	60.73 ± 15.48	9.42	
Liver D	11.85 ± 1.95	1.27	0.78
Liver L	12.52 ± 3.22	1.94	
Spleen D	9.92 ± 2.81	1.06	0.008
Spleen L	21.95 ± 4.17	3.41	
Kidney D	50.87 ± 1.26	5.45	0.53
Kidney L	42.97 ± 17.03	6.67	
Heart D	7.93 ± 1.18	0.85	0.01
Heart L	4.46 ± 1.46	0.69	
Lung D	9.81 ± 0.63	1.05	0.03
Lung L	7.90 ± 0.96	1.23	
Brain D	7.23 ± 1.15	0.77	0.009
Brain L	4.21 ±0.77	0.65	
Testis D	7.77 ± 1.87	0.83	0.09
Testis L	5.46 ± 1.18	0.85	

[a] Data are expressed as mean values ± SD ($n = 3$) and represent the AUC determined from time zero to 18 h. *AUC (dpm × 10^4 g^{-1} h)* represents the AUC obtained in different organs from time zero to 18 h

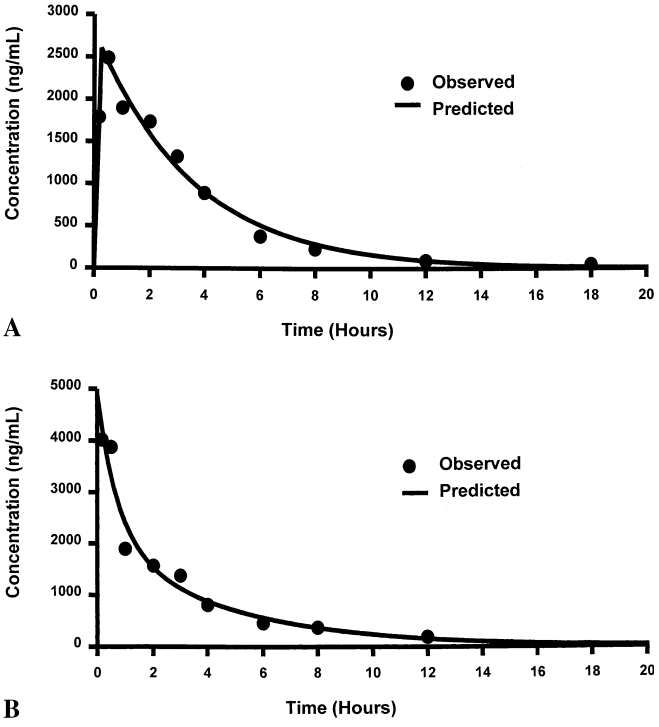

Fig. 3A Plasma time-concentration curve generated after the administration of liposomal Bu to the rat (one of three independent experiments, 2 mg/kg). **B** Plasma time-concentration curve generated after the administration of Bu dissolved in DMSO to the rat (one of three independent experiments, 2 mg/kg)

(2-fold) after the administration of L-Bu as compared with D-Bu. However, a significant ($P < 0.05$) decrease in the distribution of the radioactivity to the heart, lungs, brain, and blood was observed. The distribution of the radioactivity into the brain after the administration of L-Bu was about 58% of that obtained using D-Bu. No significant difference was seen in the distribution of radioactivity into the liver, kidney, or testis.

Pharmacokinetics

Bu in the liposomal form was well tolerated at doses ranging from 0.5 to 3.5 mg/kg. Figure 3 shows the plasma concentration-time profiles of D-Bu and L-Bu after a bolus injection of 3.5 mg/kg. The figures also show that L-Bu is eliminated from plasma according to biexponential decay, whereas D-Bu is eliminated according to a monoexponential model. All relevant pharmacokinetic parameters for L-Bu and D-Bu are listed in Table 2.

Peak plasma levels were within the range of 833–3538 ng/ml after the administration of D-Bu, which is higher than that obtained after the administration of L-Bu (range 1459–2603 ng/ml). Bu pharmacokinetics (Fig. 4) were linear within the range of 0.5–3.5 mg/kg for both D-Bu and L-Bu. The bioavailability of L-Bu as compared with D-Bu (Table 2) was 0.85. However, the distribution of liposomal Bu to the bone marrow was significantly ($P < 0.05$) higher, with the AUC marrow/AUC blood ratio being 0.08 as compared with 0.90 for D-Bu.

The distribution volumes of L-Bu were significantly higher (Table 2) than those recorded for D-Bu (mean 1.39 and 0.65 l/kg, respectively). It can also be seen that the apparent distribution volume ($V_d \times C_p/C_{bm}$) in bone marrow (which reflects the concentration of the drug in bone marrow as compared with the concentration in plasma) was significantly lower after the administration of L-Bu as compared with D-Bu. As shown in Table 2, the elimination half-lives were significantly longer in blood and marrow after the administration of L-Bu as compared with D-Bu. No significant difference was observed in clearance for L-Bu or D-Bu.

Table 2 Pharmacokinetic parameters obtained after the administration of Bu (3.5 mg/kg) either as liposomal Bu or as Bu dissolved in DMSO[a]

Parameter	Blood (DMSO)[b]	BM (DMSO)[c]	Blood (Lipo)[d]	BM (Lipo)[e]
AUC (μg h ml^{-1})	11.82 ± 2.22	9.85 ± 0.67	9.93 ± 0.15	15.82 ± 2.25
$t_{1/2}$ (h)	1.75 ± 0.37	1.53 ± 0.01	2.52 ± 0.09	3.08 ± 1.06
V (l/kg)	0.68 ± 0.06	0.80 ± 0.10[f]	1.39 ± 0.22	0.44 ± 0.05[f]
CL (ml min^{-1})	0.072 ± 0.004	0.066 ± 0.01	0.099 ± 0.02	0.055 ± 0.03

[a] Data represent mean values ± SD (n = 3)
[b] Pharmacokinetic parameters determined in plasma after dosing with Bu in DMSO
[c] Pharmacokinetic parameters determined in bone marrow after dosing with Bu in DMSO
[d] Pharmacokinetic parameters determined in plasma after dosing with liposomal Bu
[e] Pharmacokinetic parameters determined in bone marrow after dosing with liposomal Bu
[f] Apparent distribution volume in bone marrow that reflects the Bu concentration in bone marrow as compared with blood (calculated as V_d × concentration in plasma/concentration in bone marrow)

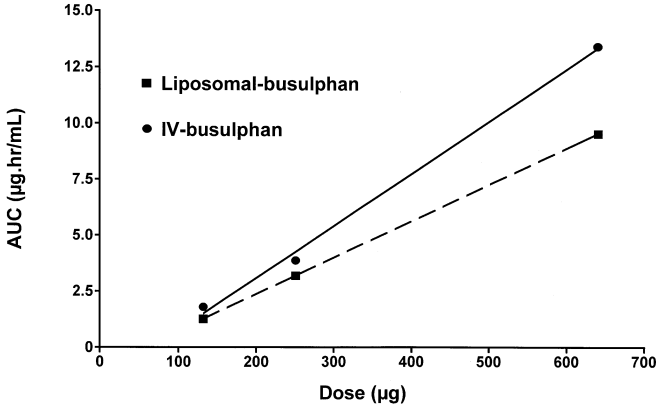

Fig. 4 Correlation between the area under the plasma concentration-time curve (AUC) and the delivered dose (0.5–3.5 mg/kg)

Discussion

During the last decade the use of Bu-based conditioning regimens has increased enormously as an alternative to TBI-based treatment for patients undergoing BMT [31, 35], especially young children [32, 38] and patients who have received TBI as a part of initial therapy. Bu-based regimens are well tolerated and have a good antileukemic effect. However, recent studies have shown that Bu-based regimens also have serious side effects such as veno-occlusive disease [12], neurotoxicity, and interstitial pneumonia (IP).

During the last decade, many studies have shown an alteration in Bu pharmacokinetics due to age, circadian rhythm, disease stage, and drug-drug interaction [16, 17, 45]. We have also shown a great variation in Bu bioavailability [19], which was more pronounced in children (0.22–1.2 in children and 0.47–1.03 in adults). In young pediatric patients, Bu has been shown to cause little early toxicity as well as a higher rate of graft failure as compared with TBI [3, 37]. Moreover, late effects of Bu as conditioning for BMT in children have not yet been sufficiently evaluated [33]. All these factors have indeed created a great need for dose optimization and a search for a parenteral administration form.

Recently, two studies introduced two new forms for intravenous administration of Bu [2, 6]. In both studies an organic solvent was used (DMSO and DMA) to solve the solubility problem of Bu and to overcome the problems with bioavailability of the drug. However, it is well known that DMA is highly toxic to the liver at low concentration as observed in both mice and rats [27]. Kinney et al. [25] showed that rats exposed to DMA had higher serum cholesterol levels as compared with a control group. Kennedy and Sherman [22] reported that DMA caused hepatic necrosis in rabbits, whereas anemia, leukocytosis, and testicular changes were observed in rats. The other solvent used was DMSO, which was reported to induce major cardiac complications in BMT recipients [29]. DMSO is also rather toxic to corneal endothelium [40]. Yellowlees et al. [47] have reported elevated serum enzymes and anemia after i.v. administration of DMSO. Despite the acute need for an intravenous administration form of Bu and the good pharmacokinetic results obtained in both the dog and the rat in the above-mentioned studies, the late effects of using such organic solvents in combination with Bu, especially in pediatric patients, have to be evaluated.

It is known that liposomes are good carriers for many other drugs such as amphotericin B, methotrexate, cytosine arabinoside, platinum compounds, and doxorubicin [5, 8, 21, 23, 24, 30, 41]. The present study was designed to obtain preclinical information on the stability, pharmacokinetics, and biodistribution of Bu in a new form suitable for intravenous administration. Liposomes are stable microscopic vesicles formed by phospholipids and similar amphipathic lipids. According to many investigations, there are four major factors affecting the in vivo behavior and the biodistribution of liposomes. These factors are the presence of cholesterol, the size of the liposomes, the amounts of lipids, and the charge of the liposomes [7, 10]. In our present investigation we used small uncharged liposomes, which contain cholesterol. These liposomes showed higher stability in glucose than in NaCl (20 versus 5 days). The high rate of liposomal aggregation in NaCl solution is most probably due to the charge of both sodium and chloride. However, it is reasonable to consider the use of sodium chloride solution in cases in which the preparation will

be used within a few hours. All pharmacokinetics studies were run using glucose solution. The liposomes used showed a significantly longer half-life in both blood and bone marrow as compared with that obtained after intravenously injected Bu (about 2.5 versus 1.5 h, respectively; Table 2). The increase in half-life combined with the small size of liposomes resulted in a higher distribution volume and, subsequently, a higher concentration of Bu in the bone marrow (1.6-fold) as compared with that obtained after the administration of D-Bu. The longer elimination half-life combined with a higher distribution to other tissues probably explains the differences observed in the elimination of L-Bu (Fig. 3A), which follows a biexponential decay, as compared with D-Bu, which follows monoexponential decay (Fig. 3B). Moreover, peripheral blood levels of Bu were slightly lower after the administration L-Bu as compared with D-Bu (AUC 11.8 and 9.9 μg h ml^{-1}, respectively). These can be seen in the slight difference observed in clearance values obtained after the use of the two forms (Table 2).

The distribution study using the radiolabeled compound showed a high concentration of Bu and its metabolites in bone marrow and the spleen (Fig. 5). These organs are of clinical importance for BMT because they are known to accumulate leukemic cells. However, the distribution of Bu and its metabolites was significantly lower in organs known to be involved in Bu toxicity, such as the lungs and brain (Fig. 5). The low distribution to the brain is most probably due to the observation that the brain is more protected by the selective blood-brain barrier when Bu is given as L-Bu as compared with the free form (D-Bu). It has been well documented that Bu toxicity is expressed as interstitial pneumonia [1] and seizures [28, 42]. We have also reported the high distribution of Bu to both cerebrospinal fluid and the human brain [15, 18].

The stability of the novel formulation of Bu showed that the drug can be prepared and used without any degradation problem within 48 h. Another possibility to increase the stability of the novel formulation is to use the lyophilized form, which has proved to be very practical when used in Ambisome [21]. However, these forms need to be investigated. The kinetics and distribution studies showed that the pharmacokinetic parameters obtained in the rat using the liposomal form of Bu are consistent with the known pharmacokinetic parameters published about the drug in both humans and rats. However, the advantage of the new formulation can be summarized (Table 1) as higher distribution of the drug and its metabolites to bone marrow and the spleen. Moreover, a higher concentration of the drug in the marrow makes it possible to achieve the same efficacy with a lower dose, which is very interesting, especially for pediatric patients. In the new liposomal form a Bu dose of 1 mg/kg corresponds to 48 mg of lipids/kg. In an adult patient weighing 70 kg, one dose is equal to 210 ml (containing 3.36 g of lipids), which is given over 30–60 min ever 6 h. The total amount of lipids used in liposomes for 1 day of treatment (high-dose Bu) will reach 13.4 g of lipids. This amount of lipid is about 6% of the maximum tolerated dose used in parenteral nutrition (3 g/kg and day), i.e., 210 g/day.

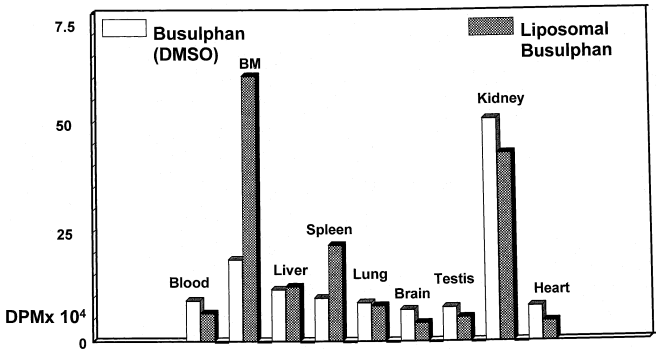

Fig. 5 Distribution of the total radioactivity obtained as the AUC (time zero to 18 h) after the administration of 3.5 mg/kg of liposomal Bu and of Bu dissolved in DMSO in rat organs. Every histogram represents the mean value for three AUCs

Over the last decade, many studies have been published about Bu pharmacokinetics in both children and adults. Many authors expressed their concern for the dose for children and the correlation between the Bu concentration during the treatment and drug toxicity, on the one hand, and the relapse risk, on the other. These investigations resulted in new dosing schedules, mostly based on the body surface area for children [38, 44, 46]. The new recommended doses for children were higher than those for adults and resulted in a higher level of systemic exposure and, probably, in enhanced drug efficacy. However, higher rates of neurotoxicity and veno-occlusive disease (VOD) were reported [39, 43]. These increases in drug toxicity were most probably due to the observation that Bu bioavailability proved to have a great degree of variation in pediatric patients [19]. Unfortunately, oral Bu has to be used nevertheless. The new liposomal form is a promising alternative to the oral form of Bu. The new form can provide a constant concentration during the treatment. It is also a better alternative to the intravenous forms reported [2, 6], where-by Bu is dissolved in organic solvents, since a combined toxicity or solvent-induced toxicity, especially for children, will be avoided. Moreover, it has been shown that the new form has lower distribution over the blood-brain barrier, which is of interest for decreasing the neurotoxicity reported in connection with high-dose Bu therapy [28, 42]. It is also of clinical relevance to realize that the distribution of this new form to the lungs is lower, which can minimize Bu toxicity in the lung (interstitial pneumonia).

It is of great importance that an intravenous administration form be developed that produces minimal systemic toxicity yet has the same pharmacological effect. The novel liposomal form is a very promising administration form since it provides higher concentrations in the target organs without increasing the distribution to the organs involved in the drug's side effects. The

efficacy and the pharmacodynamics of liposomal Bu on bone marrow and hematopoietic stem cells are currently under investigation.

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