Cancer Chemother Pharmacol (1996) 37: 401–408

ORIGINAL ARTICLE

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Formulation and stability of busulfan for intravenous administration in high-dose chemotherapy

Received: 22 November 1994/Accepted: 14 May 1995

Abstract The bifunctional alkylating agent busulfan (Bu) was solubilized in a cosolvent mixture of anhydrous dimethylacetamide (DMA), polyethylene glycol 400 (PEG400), and water at a ratio of 1:2:2 (v/v/v), to achieve a Bu concentration of 3 mg/ml, a preparation that would be suitable for parenteral administration in high-dose chemotherapy preceding bone marrow transplantation. The complete formulation was stable for more than 54 h at room temperature (RT, 22° C). An accelerated stability study of Bu in anhydrous DMA or DMA/PEG400 (1:2) as stock solutions indicated shelf-lives of 191 and 180 days respectively, at RT, and 8.2 and 7.5 years, respectively, at 4° C. Although the complete formulation with Bu was very hypertonic, hemolysis studies indicated that the formulation would be safe for intravenous (i.v.) administration, since it would be rapidly diluted to harmless tonicity levels in the blood. Cytotoxicity studies of the complete formulation in vitro proved that Bu retained its activity when dissolved in the complete vehicle. A preliminary pharmacokinetic study in a rodent model after the i.v. administration of Bu at a dose of 1 mg/kg body weight yielded high plasma concentrations of Bu for at least 5 h after injection.

Key words Busulfan · Intravenous formulation · Parenteral formulation · High-dose chemotherapy · Malignant disease

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Introduction

Busulfan (1,4-butanediol dimethanesulfonate) (Bu) is a bifunctional alkylating agent whose main use for many years has been confined to low-dose administration as palliative treatment of chronic myelogenous leukemia (CML) and other myeloproliferative syndromes [1–5]. Its dose-limiting side effect, myelosuppression, has been utilized by Santos and Tutschka to create a rodent model of aplastic anemia [6, 7]. The experience gained in this model was subsequently employed when Bu was combined with cyclophosphamide (Cy) in high doses and introduced as conditioning therapy (BuCy) for leukemia patients undergoing bone marrow transplantation (BMT) [8–10]. Bu was administered orally at 1 mg/kg body weight every 6 h for 16 doses, and Cy i.v. at 50 mg/kg daily for 4 days [8], or 60 mg/kg daily for 2 days (10, 11), on the days following Bu. The antileukemic effects of the BuCy regimens are impressive [8–14]. BuCy is also very appealing in that it alleviates the need for a radiation facility, which would be mandatory if total body irradiation (TBI) were used as part of pretransplantation conditioning therapy.

Unfortunately, not only the achieved antileukemic effect but also the clinical side effects of BuCy appear related to the effective dose that reaches the systemic blood circulation [15–17]. This is a serious dilemma, considering that orally administered Bu may exhibit a highly variable and quite unpredictable intestinal absorption. Further, most patients receiving oral high-dose Bu experience substantial gastrointestinal irritation, leading to nausea and frequently also to vomiting [8, 15]. This results in loss of a considerable amount of drug, making the potential error margin in dose delivery substantial.

Up until now there has been no parenteral formulation available for human use to overcome these problems. The poor aqueous solubility of Bu has been the

major obstacle to the development of such a formulation [18, 19]. Due to the unavailability of a stable, parenterally acceptable Bu formulation, the bioavailability of clinically used high-dose oral Bu is unknown, but it appears to fluctuate; the variations in plasma concentrations between different subjects receiving oral Bu on a fixed-dose schedule are significant [15–17, 20, 21], making it virtually impossible to accurately estimate an appropriate replacement dose in case of vomiting. Further, the bioavailability of oral Bu has been recently reported to vary up to twofold between subjects when compared to a parenteral reference dose of 2 mg, using drug dissolved in DMSO and injected i.v. [22]. Finally, Bu is rapidly metabolized in the liver [23–25], and often causes severe hepatotoxicity, so-called veno-occlusive disease (v.o.d.), which is dose-limiting in patients treated with high-dose Bu. It is not known whether v.o.d. reflects a significant hepatic first-pass effect caused by the high local Bu concentrations when the drug in the portal vein passes through the liver, or whether v.o.d. would occur to the same extent also when Bu enters the liver via the systemic circulation. The finding of suspected Bu crystals in liver tissue obtained postmortem from a patient that had died from Bu-induced v.o.d. would intuitively be compatible with the former hypothesis [26].

We utilized the rational principle of cosolvency, or solvent blending, to enhance the aqueous solubility of Bu, and formulated a preparation that fulfils the clinical requirements of chemical stability and negligible solvent system toxicity. In this communication we report on the composition and stability of this novel Bu formulation, which promises to be acceptable for clinical i.v. use. We also demonstrated that Bu in this vehicle retains its cytotoxic properties and, finally, determined the plasma pharmacokinetics in a rat model after i.v. administration of the new Bu formulation at 1 mg/kg body weight.

Materials and methods

Chemicals and disposables

Bu, diethyldithiocarbamic acid sodium salt (DDTC), Polyethylene glycol 400 (PEG400), Iscove's modified Dulbecco medium (IMDM), fetal bovine serum (FBS), and Dulbecco's phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Chemical Co. (St. Louis, Mi.). *N*, *N*-Dimethylacetamide (DMA) was obtained from Aldrich Chemicals (Milwaukee, Wi.). The internal standard for the HPLC procedure, *N*-(2,6-difluorobenzoyl)-*N'*-[3, 5-dichloro-4-(3-chloro-5-trifluoromethyl-pyridin-2-yloxy)phenyl]-urea (CGA-112913), was a gift from Ciba-Geigy (Basel, Switzerland). Acetonitrile, tetrahydrofuran, acetone, and methanol (HPLC grade) were purchased from VWR Scientific (Houston, Tex.). Agar (Bacto Agar™) was obtained from Difco Laboratories. (Detroit, Mich.). Plastic ware for tissue culture was obtained from Corning Glass Works (Corning, N.Y.). Borosilicate glass tubes were purchased from VWR Scientific. Pentobarbital sodium (Nembutal™ Sodium) was obtained from Abbott Laboratories (Chicago, Ill.).

Quantitation of Bu

The extraction procedures and HPLC assay have been described in detail previously [27]. The methods follow in brief.

Extraction and derivatization of Bu in aqueous, non-protein-containing solutions

Bu-containing solution (500 µl) was mixed with 20 µl internal standard solution (CGA-112913; 200 µg/ml in methanol), and then with 500 µl DDTC stock solution (1.17 *M* in water). The mixture was mixed on a vortex machine for 30 s and rotated for 5 min on a Tube Rotator™ (Scientific Equipment Products, (Baltimore, Mo.). The derivatized Bu (DDCB; 1,4-bis[diethyldithiocarbamoyl]butane) and the CGA-112913 were extracted from the reaction mixture with 2 ml ethyl acetate, followed by centrifugation for 10 min (International Clinical Centrifuge, Model CL; International Equipment Co., Needham, Mass). A 1-ml aliquot of the ethyl acetate layer was withdrawn and evaporated to dryness under compressed air with a Meyer N-Evap Analytical Evaporator (Organomation Associates, Northborough, Mass). The residue was reconstituted by mixing on a vortex machine for 10 s with 1 ml of the mobile phase and subjected to HPLC.

Extraction and derivatization of Bu in plasma

After mixing 20 µl CGA-112913 (40 µg/ml in methanol) with 200 µl Bu-containing plasma, 200 µl acetonitrile was added, and the mixture was vortexed for 30 s to precipitate the proteins. After centrifugation for 3 min at 13,000 rpm (HBI microcentrifuge, Fisons, Oxbridge, UK), the supernatant (360 µl) was transferred to a borosilicate glass tube containing 400 µl water. Subsequently, 200 µl 1.17 *M* DDTC was added to yield DDCB. The DDCB and CGA-112913 were then extracted from the reaction mixture with 2 ml ethyl acetate and evaporated to dryness under compressed air at 45° C. The residue was reconstituted in 200 µl methanol and then purified through Sep-Pak cartridges (1 ml, Millipore Corp., Bedford, Ill.) that had been conditioned by seven washes with methanol, followed by two washes with water. The DDCB and CGA-112913 were eluted from the Sep-Pak cartridges with 2 × 250 µl methanol and 2 × 500 µl ethyl acetate under vacuum and the combined eluates were evaporated to dryness under compressed air at 45° C. The residue was then reconstituted with 200 µl of the mobile phase (see below) and subjected to HPLC.

High-pressure liquid chromatography assay

The liquid chromatograph (Consta-Metric I, LDC Analytical, Riviera Beach, Fla.) was equipped with a 100-µl sample loop (Valco, Houston, Tex.), a fixed-wavelength UV detector monitoring at 254 nm (UV-III Monitor™, LDC Analytical), a Microsorb-MV column (5 µm particle size, C-18, 25 cm × 4.6 mm i.d.; Rainin Instruments Co., Woburn, Mass.), and a chart recorder (Linear Instrument, Irvine, Calif.). The isocratic mobile phase was a mixture of acetonitrile, tetrahydrofuran, and water at a ratio of 11:4:5 (v/v/v, pH 4.2). The flow rate used was 1.2 ml/min and the recorder's chart speed was 20 cm/h. The HPLC assay was linear within a Bu concentration range of 0.15 to 10 µg/ml for Bu extracted from aqueous non-protein-containing solutions and from 0.15 to 3.0 µg/ml for Bu in plasma samples [27].

Solubilization studies

The aqueous solubility of Bu was increased several-fold, 6–30 times, by utilizing different water miscible solvents, such as PEG400,

40–50% in water. Unfortunately, the time required to reach equilibrium solubility was in excess of 10 h, which would allow uncontrollable hydrolysis of the drug. It was therefore decided to utilize an organic solvent, DMA, which instantly solubilized Bu. A solution of Bu in DMA (15 mg/ml) was then added to a mixture of PEG400/water in various final ratios. All solutions were filtered through a 0.45-μm silver membrane filter (Selas^T, Nuclepore, Costar Cambridge, Mass.) and mounted in a 25-mm diameter filter holder. In separate experiments we verified that only negligible Bu amounts were lost during the filtration. Bu was quantitated by HPLC after appropriate dilution and derivatization as above [27].

Stability studies

The long-term stability of Bu dissolved in anhydrous DMA, either alone or mixed with PEG400 was examined in an accelerated stability study [28]:

1. Bu, 25 mg/ml in anhydrous DMA, was stored at 4° C, 22° C, 40° C, 60° C, and 80° C.
2. Bu, 5 mg/ml in anhydrous DMA/PEG400 (ratio 1:2, v/v), was stored at 4° C, 22° C, 40° C, 60° C, 80° C, and 100° C.
3. Bu, 3 mg/ml in the complete DMA/PEG400/water solvent (ratios 1:2:2, v/v/v), was stored at room temperature (RT) only.

Bu in the various solvents as above was incubated in sealed tubes. At various times triplicate samples were withdrawn, equilibrated to RT, and analyzed quantitatively for Bu by HPLC after appropriate dilution and derivatization as described above [27].

Osmotic pressure measurement

Osmotic pressures of the complete DMA PEG400/water solvent, with and without the addition of 3 mg/ml Bu, were evaluated. Human blood, double distilled water, and 154 m*M* NaCl were used as biological references. All measurements were made with an Advanced Digimatic Micro-Osmometer, model 3MO Plus (Advanced Instruments, Norwood, Mass.). Prior to each assay the osmometer was calibrated using calibration standard solutions (Clinitrol^T; Advanced Instruments).

Hemolytic potential

The hemolytic potential of the complete solvent was evaluated with and without 3 mg/ml Bu using a modification of the technique of Reed and Yalkowsky [29]. The complete DMA/PEG400/water solvent (100 μl), with or without 3 mg/ml Bu was mixed with heparinized human blood to yield solvent fractions of 5–55% by volume. The mixtures were incubated at RT for 2 min to allow hemolysis. Further hemolysis was then quenched by adding 5 ml normal saline (NS). The samples were vortexed and centrifuged at $1000 \times g$ for 5 min. The supernatant was discarded, and the pellet washed with 5 ml NS to remove adsorbed drug or solvent. Distilled water was added to a ratio of 10:1 (to initial blood volume) to lyse the remaining erythrocytes. After centrifugation and appropriate dilution with distilled water, the absorbance was measured at 540 nm (Beckman spectrophotometer model 26; Beckman Scientific Instruments division, Irvine, Calif.). The control samples with the solvent replaced by NS were similarly evaluated to correct for solvent-induced hemolysis. The relative fraction of unhemolyzed cells was determined from a standard curve (see below), and plotted against the logarithm of the solvent fraction of the mixture [29]. The ratio of solvent to blood at which 50% hemolysis occurred was then determined.

To generate the standard curve, the following procedure was used. The minimum volume ratio of water to blood to yield complete hemolysis using distilled water as the lyzing agent was 10:1. A solution of hemolyzed blood was then mixed with whole blood in ratios of 0:1, 1:4, 1:1, 4:1, and 1:0, to a final volume of 100 μl. NS (5 ml) was added to quench further hemolysis. After the intact erythrocytes had been pelleted, they were washed once in 5 ml NS. The pellet was lysed in 1 ml distilled water, the resulting absorbance was measured as above, and the data plotted as fraction of unlyzed erythrocytes versus absorbance [29].

In vitro cytotoxicity

The in vitro cytotoxicity of the complete vehicle with and without Bu was assessed after exposure of human HL-60 myeloid leukemia cells [30] to Bu dissolved in the complete solvent or, as a positive control in acetone, for 60 min at 37° C. The cells were then washed twice in ice-cold PBS and suspended in IMDM supplemented with 20% FBS and 0.3% agar as viscous support. After incubation for 8 days at 37° C in a humidified atmosphere of air containing 5% CO_2, clones of ≥ 50 cells arising from clonogenic cells that had survived the Bu exposure were counted under an inverted phase microscope. Survival curves were constructed [31].

Animal experiment

The plasma concentration versus time profile of Bu after an i.v. dose of 1 mg/kg of Bu in the complete DMA/PEG400/water solvent was established in a male Sprague-Dawley rat (Sasco Corp., Omaha, Neb.), as follows. Under pentobarbital sodium (50 mg/kg) anesthesia, a silicone catheter was introduced into the animal's jugular vein, and 24 h later the Bu was injected i.v. Blood samples were drawn at various times up to 5 h from the jugular catheter into heparinized microcentrifuge tubes. The samples were immediately cooled in an ice/water bath, and the plasma was separated after centrifugation at 12,000 rpm in an Eppendorf centrifuge (Brinkmann Instruments, Westbury, N.Y.) and frozen at −20° C until extraction. All samples were extracted and derivatized, and Bu was quantitated using HPLC (see above, and references 27 and 32).

Results

Quantitation of Bu in solvent systems and in blood

Since the Bu molecule is devoid of a chromophore for UV absorbance in spectrophotometry, it had to be derivatized to allow UV quantitation in the HPLC assay. Bu in protein-free aqueous solution could be directly derivatized with DDTC to DDCB. However, when the drug was extracted from plasma, it was necessary to precipitate the plasma proteins and to eliminate other plasma components that interfered with the HPLC analysis.

The baseline resolution in the HPLC assay was achieved with retention times of 7.5 min for DDCB and 9 min for CGA-112913.

Solubility and stability

The water-miscible organic solvent DMA was used as a primary solvent to improve the poor aqueous

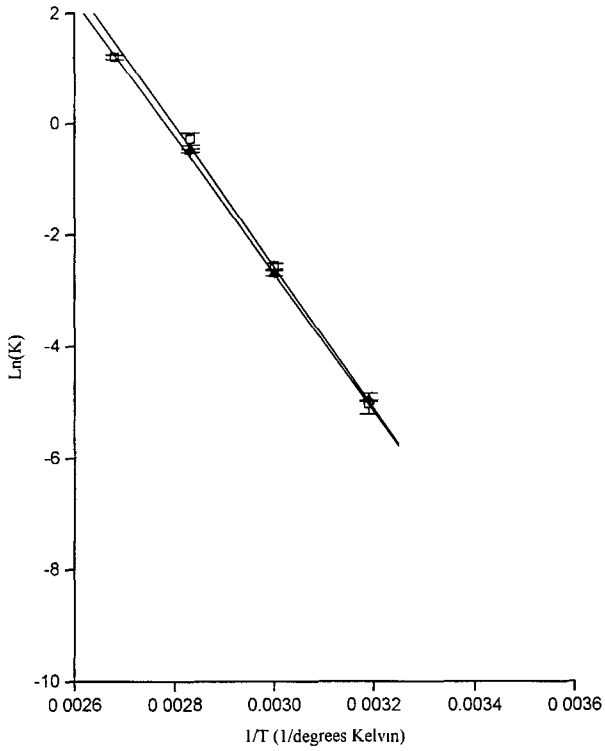

Fig. 1 Arrhenius plots for the degradation of Bu in DMA (▲), and in DMA/PEG400 (□), (ratio 1:2, v/v). Each point represents the mean of three independent determinations obtained in the accelerated stability study (*bars* ± SD). For details see Materials and methods

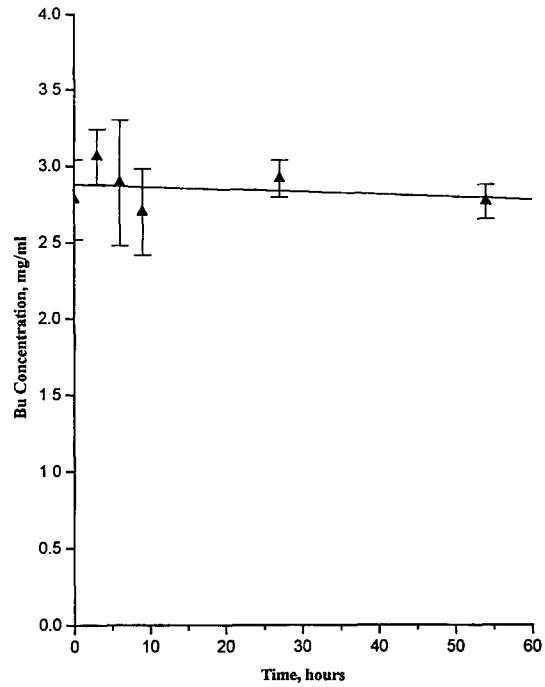

Fig. 2 Stability of Bu (3 mg/ml) in the complete DMA/PEG400/water formulation at RT. Each point represents the mean of three independent determinations (*bars* ± SD)

solubility of Bu. The drug instantly dissolved to ≥ 75 mg/ml in DMA, and in DMA-PEG400 (ratio 1:2), a stable solution of 6 mg/ml was obtained.

The shelf-lives of Bu in anhydrous DMA and DMA/PEG400 at RT and at 4° C were determined in an accelerated stability study, using extrapolations of stability at several different temperatures. There was no discernible degradation of Bu in DMA at a concentration of 25 mg/ml over more than 160 days at RT. At temperatures above RT, drug degradation followed first-order kinetics, with half-lives of 101.6, 10.2, and 1.1 days at 40, 60 and 80° C, respectively. A similar stability profile was established for Bu dissolved at 5 mg/ml in DMA/PEG400, where Bu degraded with half-lives of 120.5 days, 9.9 days, 23 h and 6 h, at 40, 60, 80, and 100° C, respectively. Figure 1 shows the Arrhenius plots of the logarithm of the first-order rate constants against the reciprocal of the absolute temperatures at which the accelerated stability studies were performed for Bu in DMA and DMA/PEG400. From these graphs the shelf-lives of Bu were calculated to be about 191 and 180 days at RT and to 8.2 years and 7.5 years at 4° C in the respective DMA and DMA/PEG400. The activation energy was computed to be 25.3 kcal/mole under the described conditions.

PEG400/water and water were added to the primary Bu solvent to yield the complete solvent systems with a final Bu concentration of 3 mg/ml. The drug remained stable in the complete formulation at this concentration for more than 54 h at RT (Fig. 2).

Osmolarity and hemolytic potential of the solvent system

The complete DMA/PEG400/water solvent was hyperosmolar (4653 mOsm/kg), having more than 15 times the osmolarity normally found in plasma. The addition of Bu at a concentration of 3 mg/ml did not significantly change the osmotic pressure of the complete formulation (Table 1).

The standard curve for relative hemolysis obtained as previously described was linear (Fig. 3), and it was used to establish the hemolytic potential of the complete DMA/PEG400/water solvent at various dilutions. The addition of Bu to the complete solvent did not appreciably change its hemolytic capacity (Fig. 4). The initially high hemolytic potential of the complete solvent rapidly decreased when the formulation was diluted in blood; it became negligible when the blood-solvent ratio exceeded 15 (Fig. 4).

In vitro cytotoxicity of Bu

The complete DMA/PEG400/water solvent was itself non-toxic to the HL-60 cell line at the various dilutions

Table 1 Osmotic pressures of the formulation with and without Bu. Values are means (SD) (*n* number of independent determinations)

Solution	*n*	Osmotic Pressure (mOsm/kg)
Water	3	0.67 (0.47)
Saline	3	285.33 (0.94)
Blood	3	318.00 (2.16)
DMA/PEG400/Water 1:2:2 v/v/v	6	4653.33 (8.50)
DMA/PEG400/Water 1:2:2 v/v/v, Bu 3 mg/ml	6	4416.67 (22)

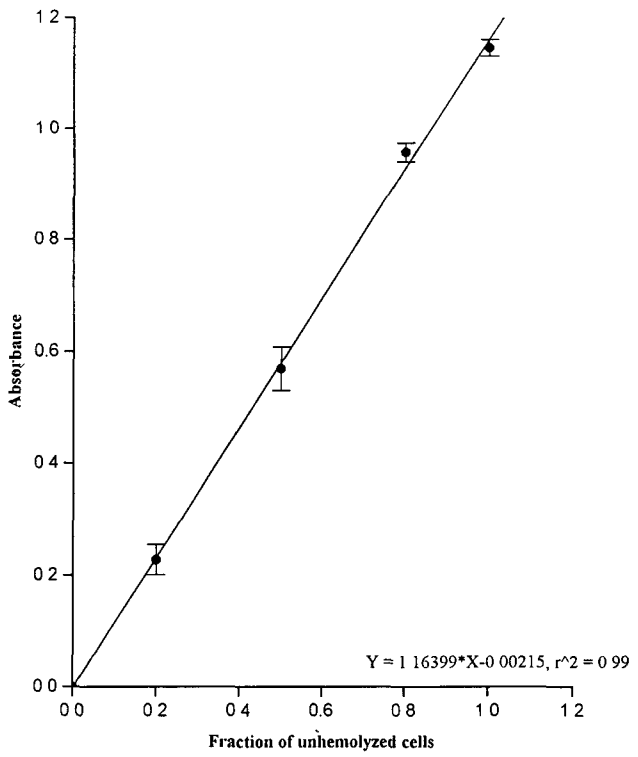

Fig. 3 Standard curve for the hemolysis study. Each point represents the mean of three separate determinations (*bars* ± SD). See Materials and methods for details

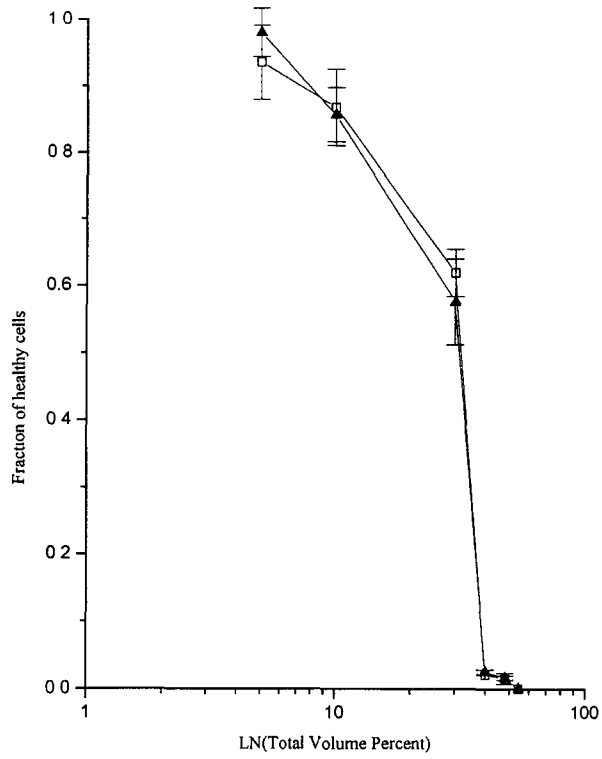

Fig. 4 Hemolytic potential of the DMA/PEG400/water formulation (ratios 1:2:2) with (▲) and without (□) Bu. Each point represents the mean of three separate determinations (*bars* ± SD)

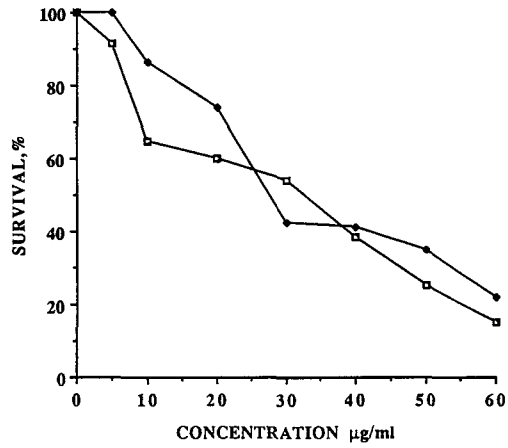

Fig. 5 Survival of clonogenic cells from the HL-60 cell line, after in vitro exposure for 60 min at 37° C to increasing concentrations of Bu dissolved in the complete DMA/PEG400/water formulation (□), or in acetone (◆) as primary solvents. For details see Materials and methods

where Bu cytotoxicity was recorded. When the Bu was solubilized in this solvent system, it retained its cytotoxic activity, comparable to that of Bu dissolved in a non-toxic amount of acetone (Fig. 5). Thus, there was a gradual concentration-dependent cytotoxicity to the clonogenic HL-60 cell population with a calculated IC_{50} of about 30 µg/ml for Bu in the DMA/PEG400/water solvent.

Pharmacokinetics experiment

Figure 6 shows the plasma concentration versus time profile of Bu after the i.v. injection of 1 mg/kg body weight of Bu in the complete solvent. The plasma Bu level rapidly declined in a log/linear fashion after injection with a half-life of about 2 h. The data fit a one-compartment open model with first-order elimination kinetics.

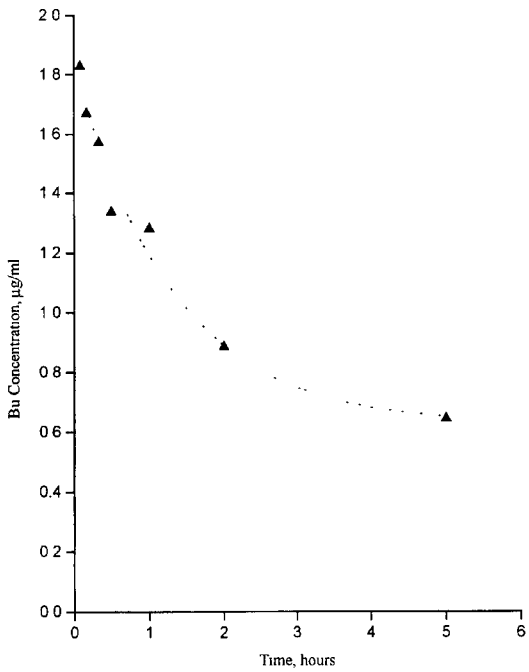

Fig. 6 Pharmacokinetic profile of Bu in plasma from a rat after i.v. injection of 1 mg/kg Bu dissolved in the complete DMA/PEG400/aqueous vehicle as described in the Materials and methods

Discussion

In the last few years Bu-based high-dose chemotherapy regimens have been established as attractive complements to TBI-based treatment for the conditioning of patients undergoing BMT. Not only can such therapy be used in the absence of access to a radiation facility, but these regimens also appear to be well tolerated and have good antileukemic efficacy [33–36]. Furthermore, it is clinically advantageous to have access to myeloablative treatments that can be used in patients with lymphoma, Hodgkin's disease and leukemia who have received radiation therapy as part of their initial management, which excludes the later use of TBI. In addition, among patients who experience recurrent disease after an allogeneic BMT, a second allogeneic BMT may still provide long-term disease-free survival [37]. A TBI-based conditioning regimen can, however, only be utilized once, and Bu-based therapy here provides an important alternative.

Unfortunately, not only the antileukemic efficacy, but also serious side effects of high-dose Bu, such as v.o.d. and grave neurotoxicity, appear related to the amount of drug that can be traced over time in the systemic circulation [16, 17]. This is a cause of concern, since any orally administered solid agents, whose absorption is dissolution-rate limiting due to low solubility, like Bu, is prone to have variable and quite unpredictable intestinal absorption. In addition, most patients receiving oral high-dose Bu experience pronounced nausea and often vomit, resulting in loss of a considerable amount of the administered doses [15]. Since the bioavailability of orally administered Bu is highly variable [22], an accurate estimate of a replacement dose is virtually impossible. This predicament makes the potential error margin in dose delivery substantial. Bu also undergoes significant hepatic first-pass metabolism, which could help to explain why high-dose Bu often causes severe hepatotoxicity, [8–13, 16, 34, 36].

Based on these considerations, a stable Bu preparation for i.v. administration would be advantageous. A prerequisite for developing such a formulation for high-dose conditioning therapy prior to BMT would be to increase the aqueous solubility of Bu by an order of magnitude. It would be desirable to improve solubility to at least 1 mg/ml to avoid the clinical risks of fluid overload with secondary congestive heart failure. Such increased solubility would also be clinically convenient, allowing easy handling and administration of Bu, assuming that repeated doses of 0.5–1 mg/kg body weight would be utilized, in analogy with current oral high-dose Bu regimens [8, 9, 10–13, 33–36].

To improve the poor aqueous solubility of Bu, we employed the principle of cosolvency [38]. The cosolvency technique, or solvent blending approach, postulates that by using a chemically complex solvent system, one can achieve a strikingly higher aqueous solubility of a desired nonpolar substance [39]. This methodology has been utilized to modify the solubility of several commonly used drugs, e.g. diazepam, pentobarbital sodium, and phenytoin.

We examined several solvent systems to improve the aqueous solubility of Bu. A 6–30-fold increase in Bu aqueous solubility was achieved using this approach (data not shown). However, more than 10 h were required to achieve equilibrium solubility of Bu in PEG400/aqueous formulations (40% and 50% v/v). Such an extended solubilization time requirement is not only awkward in clinical practice, but carries with it the risk of drug hydrolysis/inactivation. The use of an organic solvent, which itself is chemically inert, should allow virtually instantaneous drug solubilization, and leave the drug undegraded. Further, the organic solvent must be both water miscible and have negligible toxicity to permit its administration in humans. DMA, which fits these criteria, has found use in pharmaceutical practice [40]. Thus, DMA in combination with lactic acid (1:10 v/v) has been safely used to solubilize m-AMSA, an intercalating agent extensively investigated for the treatment of myeloid leukemia [41–43]. A clinical phase I investigation of DMA as an antitumor agent in itself has also been performed [44]. That study concluded that DMA was safe for i.v. administration in total doses up to 80 mg/kg body weight.

Since DMA instantly dissolved Bu, we utilized anhydrous DMA to prepare a Bu stock solution. PEG400 is also acceptable for parenteral administration and is routinely used in the formulation of various drugs [45]. It was added in a mixture with DMA to facilitate the subsequent mixing with water to serve as the complete formulation. The complex DMA/PEG400/water solvent consistently provided the highest Bu concentration of the various solvent systems examined. The vehicle provided both immediate solubilization and an acceptable Bu stability of more than 54 h at RT. A stable Bu stock solution could be prepared in either anhydrous DMA or in DMA/PEG400 (1:2, v/v), with respective shelf-lives of 191 and 180 days at RT and (projected) 8.2 and 7.5 years at 4° C.

Any parenterally administered drug preparation must have negligible solvent system toxicity. The types of toxicity encountered include:
(a) the effects from an altered tonicity of the preparation compared to whole blood;
(b) solvent-induced hemolysis;
(c) solvent-related interference with lipoprotein components of cell membranes and cellular enzyme systems.
It was therefore desirable to formulate a parenteral dosage form that was both isosmotic to blood and had a low overall hemolytic/toxic potential. The current preparation was hyperosmotic, as assessed with the technique used (Table 1), and its hemolytic capability was high. However, when the vehicle was diluted more than 15 times in blood, the hemolytic effects became negligible. The cytotoxic effects of the solvent system itself were also negligible in the concentrations used *in vitro*. Since the dilution of both the drug and solvent is a function of the infusion rate as well as the blood flow in the vein used for drug administration, it appears reasonable to assume that drug infusion through a central venous catheter of divided Bu doses in volumes of about 30–60 ml over 1–2 h every 6–12 h should minimize the risks of drug precipitation and any potential solvent toxicity. Therefore, the formulation should be safe. The definitive answer to this question must, however, await the outcome of clinical trials utilizing this Bu formulation. The tonicity data in combination with the preparation's hemolytic potential may indicate that the formulation should be investigated for i.v. or intraarterial administration only. It may carry a significant potential for local tissue damage if administered intramuscularly, or if it extravasates.

The in vitro stability and cytotoxicity studies of this novel Bu formulation, in conjunction with the preliminary pharmacokinetic investigation, demonstrate that the drug remains undegraded in solution for many hours permitting convenient i.v. administration while retaining its cytotoxic efficacy; its pharmacokinetic profile in plasma after i.v. administration is consistent with published pharmacokinetic parameters. This Bu formulation holds the promise of being a much improved alternative to the currently available oral preparation for high-dose chemotherapy in combination with BMT. It warrants a thorough evaluation in a clinical setting of BMT.

Acknowledgements The skilful technical assistance of Mrs. Tara Sadeghi, and the assistance of Mrs. Eva Barcelo in the preparation of this manuscript is gratefully acknowledged. This study was supported by a generous grant from the Adler Foundation in New York, and by AML PO1-CA55164 from the National Cancer Institute.

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