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ORIGINAL ARTICLE

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Plasma pharmacokinetics and urinary excretion of the polyamine analogue 1, 19-bis(ethylamino)-5, 10, 15-triazanonadecane in CD_2F_1 mice

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Abstract The pharmacokinetics of 1, 19-bis(ethylamino)-5, 10, 15-triazanonadecane (BE-4-4-4-4) were determined in CD_2F_1 female mice after administration of i.v. bolus doses of 20 mg/kg (approximately the dose lethal to 10% of the study animals, $\sim LD_{10}$) as well as 15, 10, and 5 mg/kg and after s.c., i.p., or p.o. doses of 20 mg/kg. BE-4-4-4-4 in plasma and urine was derivatized with dansyl chloride and measured by gradient high-performance liquid chromatography (HPLC) with fluorescence detection. Data were modeled by noncompartmental and compartmental methods. The declines observed in plasma BE-4-4-4-4 concentrations after i.v. delivery of 20, 15, 10, and 5 mg/kg were modeled simultaneously using an interval of 2000 min between doses and were best approximated by a two-compartment, open, linear model. The time courses of plasma BE-4-4-4-4 concentrations after i.p. and s.c. delivery were fit best by a two-compartment, open, linear model with first-order absorption. Peak plasma concentrations of BE-4-4-4-4 measured following an i.v. dose of 20 mg/kg ranged between 30 and 33 μg/ml, the terminal elimination half-life was 94 min, and the volume of distribution (V_{dss}) was 850 ml/kg. The plasma pharmacokinetics of BE-4-4-4-4 were linear with dose. BE-4-4-4-4 (0.5 and 2.0 μ*M*) in mouse plasma was approximately 67% protein-bound. Bioavailabilities after i.p., s.c., and p.o. delivery were 40%, 50%, and approximately 3%, respectively. Urinary excretion of parent BE-4-4-4-4 in the first 24 h after dosing accounted for less than 30% of the delivered dose. As BE-4-4-4-4 proceeds toward and undergoes clinical evaluation, the data and analytical method presented herein should prove useful in formulating a dose-escalation strategy and, possibly, evaluating toxicities encountered.

Key words BE-4-4-4-4 · Polymines · Pharmacokinetics

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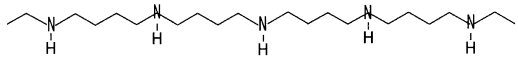
Introduction

The naturally occurring polyamines putrescine, spermidine and spermine are simple, aliphatic amines that are found in all eukaryotic cells [12]. Neoplastic cells often grow more rapidly and have much higher cellular polyamine contents than do normal cells [12]. Auxotrophic mutants deficient in key polyamine enzymes and specific inhibitors of polyamine biosynthesis have been used to demonstrate that polyamines are essential for cell growth and proliferation [12]. Because of this relationship between polyamines and cell growth, manipulation of polyamine biosynthesis and the use of polyamine analogues to interfere with polyamine biochemistry presents a very attractive anticancer chemotherapeutic strategy [5, 7, 8, 10, 11, 13, 14, 18].



1,19-Bis(ethylamino)-5,10,15-triazanonadecane

(BE-4-4-4-4)

(NSC-640506)

Fig. 1 Chemical structure of BE-4-4-4-4

Polyamines are transported into most cells via a carrier-mediated mechanism with a distinct specificity for polyamine-like molecules [4, 15]. Significantly, this transporter is more active in proliferating cells than in nonproliferating cells [4] and, therefore, utilization of this transport system might afford polyamine analogues selectivity for neoplastic cells and tissues [20].

1, 19-Bis(ethylamino)-5, 10, 15-triazanonadecane (BE-4-4-4-4; Fig. 1) is a new antineoplastic polyamine analogue synthesized following theoretical and physicochemistry studies of how spermidine and spermine analogues interact with DNA [2]. BE-4-4-4-4 is a pentamine with ethyl groups attached to each end. In that each of the five amino groups is protonated at physiological pH, BE-4-4-4-4 carries five positive charges. BE-4-4-4-4 was synthesized and tested because of data indicating that it binds tightly to DNA but does not promote DNA condensation. Although the ethyl groups were initially included to block metabolism of BE-4-4-4-4 by plasma polyamine oxidases, they may also enhance the cytotoxic effectiveness of the compound [2, 3].

BE-4-4-4-4 is accumulated by a variety of brain-tumor cell lines, causes depletion of the natural polyamines, and does not significantly induce the polyamine metabolic enzyme, spermine/spermidine acetyltransferase. BE-4-4-4-4 is both growth-inhibitory and cytotoxic. BE-4-4-4-4 has been proven active in vitro against several human brain-tumor cell lines [2] and has also shown in vivo activity against human brain, lung, and colon xenografts implanted in athymic nude mice [6]. Because simultaneous exposure of cells to spermine can block the uptake and, hence, the growth-inhibitory and cytotoxic activities of BE-4-4-4-4, it is likely that BE-4-4-4-4 enters cells via the polyamine-specific transport protein and affects cell growth by a polyamine-interactive mechanism. As part of the continued development of this promising polyamine analogue, studies were undertaken to develop analytical methodology for measurement of the compound in biological matrices and to perform pharmacokinetics studies in mice that had received BE-4-4-4-4 at a variety of doses and by several routes of administration. These studies form the basis of this report.

Materials and methods

Reagents

BE-4-4-4-4 was obtained from the Developmental Therapeutics Program, National Cancer Institute (Bethesda, Md., USA). *N*,*N*′-dimethylhexanediamine and sodium tetraborate decahydrate were purchased from Aldrich Chemical Co. (Milwaukee, Wis.), and trichloroacetic acid was purchased from EM Science Industries (Gibbstown, N.J.). $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 7H_2O$ were Baker Analyzed reagents (J.T. Baker Chemical Co., Phillipsburg, N.J.). Dansyl chloride (specially purified grade) was purchased from Pierce (Rockford, Ill.) as a 100-mg/ml solution in acetone. High-performance liquid chromatography (HPLC)-grade hexane, ethyl acetate, and acetonitrile were obtained from EM Science, J.T. Baker Chemical Co., and EM Science, respectively.

BE-4-4-4-4 was dissolved in sterile 0.154 *M* NaCl such that i.v. bolus doses of 5, 10, 15, or 20 mg/kg could be delivered in a volume of 10 ml/kg. For i.p., s.c., or p.o. studies, BE-4-4-4-4 was dissolved in sterile 0.154 *M* NaCl such that doses of 20 mg/kg could be delivered in a volume of 10 ml/kg.

Mice

Specific-pathogen-free, adult, female CD_2F_1 mice (5–6 weeks of age) were obtained from the Animal Program administered by the Animal Genetics and Production Branch of the National Cancer Institute. Mice were allowed to acclimate to the University of Maryland Animal Facility for at least 1 week before studies were initiated. To minimize exogenous infection, mice were maintained in conventional cages in a separate room and were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH number 85–23, 1985). Ventilation and air flow in the Animal Facility were set to 12 changes/h. Room temperatures were regulated at $72 \pm 2°F$, and the rooms were on automatic 12-h light/dark cycles. Mice received Purina 5001 Chow and water ad libitum except on the evening prior to dosing, when all food was removed and withheld until 4 h after dosing. Sentinel mice were maintained in the animal room and remained free of specific pathogens as determined by MAP (murine antibody profile) testing at intervals throughout the study period. BE-4-4-4-4 was given parenterally by bolus, and the oral dose of BE-4-4-4–4 was delivered by a 22-gauge gavage needle.

Sampling

Three mice were studied at each time point. Blood was collected by cardiac puncture at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, 360, 480, 720, 960, 1,200, and 1,440 min after dosing with BE-4-4-4-4 or at 5 min after injection of a vehicle control. Blood was stored on ice until centrifuged at 1,500 *g* for 10 min to obtain plasma. Sets of animals to be sampled at 480, 960, 1,200, or 1,440 min after BE-4-4-4-4 administration were gang-housed in metabolism cages, and urine was collected on ice until animals were killed for blood sampling. Plasma, urine, and dosing solutions were stored frozen at $-70°C$ until analysis.

Analysis of BE-4-4-4-4

Concentrations of BE-4-4-4-4 in plasma and urine were determined by HPLC using a modification of the method of Kabra, et al. [9]. Briefly, 100 µl of plasma or urine was placed into a 1.5-ml microcentrifuge tube and mixed with 10 µl of 50 µ*M* *N*,*N*′-dimethylhexanediamine internal standard. Samples containing BE-4-4-4-4

concentrations greater than the highest concentration in the standard curve were diluted such that they fell within the linear range. *N*,*N*′-Dimethylhexanediamine was used in place of the 1,6-diaminohexane internal standard described by Kabra, et al. [9] so as to reduce the potential for differences in derivatization between the primary amines of 1,6-diaminohexane and the secondary amines present in BE-4-4-4-4. After 10 µl of 50% trichloroacetic acid had been added to each tube, tubes were mixed for 30 s and then centrifuged for 15 min at 12,000 *g* at 4°C. The deproteinized plasma supernatants were then derivatized with dansyl chloride as follows. An 80-µl sample of each deproteinized supernatant was added to a derivatization vial and mixed with 100 µl of 1.0 *M* borate buffer (pH 10) and 50 µl of a freshly prepared 10-mg/ml solution of dansyl chloride in acetonitrile. This mixture was vortexed for 30 s and incubated at 70°C for 10 min. Due to concern about potential photodecomposition of dansylated derivatives, all of these procedures were performed in a dark environment. Vials were allowed to cool to room temperature, and dansylated products were extracted into 120 µl of a 1:1 mixture of hexane:ethyl acetate. A 100-µl sample of the organic phase was subjected to HPLC analysis.

The HPLC system used a Hewlett-Packard (Palo Alto, Calif.) 1040L liquid chromatograph equipped with a 5-µm Adsorbosphere C18 column (length, 250 mm; inside diameter, 4.6 mm; Alltech Associates, Inc., Deerfield, Ill.) and a Brownlee NewGuard RP18 precolumn (Alltech Associates, Inc.). Separation of endogenous polyamines and BE-4-4-4-4 was accomplished with a gradient elution program starting at 70% acetonitrile in 10 m*M* sodium phosphate buffer (pH 5.8) and increasing linearly to 100% acetonitrile over 15 min. The flow rate of the mobile phase was 1.7 ml/min. Column eluent was monitored with a Hewlett-Packard 1046A programmable fluorescence detector set with excitation and emission wavelengths of 340 and 515 nm, respectively. The signal from the detector was processed with a Hewlett-Packard 3395 integrator so as to integrate the area under each peak eluted. The concentration of BE-4-4-4-4 in each sample was determined by calculating the ratio of the BE-4-4-4-4 peak area to that of the corresponding internal standard peak and comparing that ratio to a concomitantly performed standard curve prepared in the same matrix.

No endogenous material was found to interfere with detection of BE-4-4-4-4, internal standard, or any of the endogenous polyamines (Fig. 2A). Under the method described, internal standard eluted at approximately 7.5 min, spermidine eluted at approximately 6.8 min, spermine eluted at approximately 9 min, and BE-4-4-4-4 eluted at approximately 12.4 min (Fig. 2B). For the assay as described, the lower limit of quantitation was 22.3 ng/ml (62.5 n*M*) [17]. The assay was linear between 22.3 and 3,570 ng/ml (10 µM). Within-day coefficients of variation were between 2% and 4% at triplicate standard curve values of 44.6, 89.3, 178.5, 357, 714, 1,428, 2,856, and 3,570 ng/ml, and between-day coefficients of variation for triplicate 89.3- and 1,428-ng/ml standards were 2.9% and 1.8%, respectively. When stored on ice for up to 24 h, BE-4-4-4-4 was stable in mouse plasma and urine. These studies utilized triplicate 2.0-µ*M* samples assayed after 0, 1, 4, 8 and 24 h and were performed twice. Studies examining the stability of BE-4-4-4-4 in plasma extracts showed the compound to be stable after deproteinization with trichloroacetic acid, derivatization with dansyl chloride, and extraction into a 1:1 mixture of hexane:ethyl acetate, with no time delay occurring between sequential steps. Although dansylated derivatives are light-sensitive, there was no decomposition or loss of compound with time when the assay was performed in darkened rooms and the autosampler on the HPLC was protected from light. Specifically, no decomposition was observed in standard curves or quality-control samples analyzed at the beginning and end of each run.

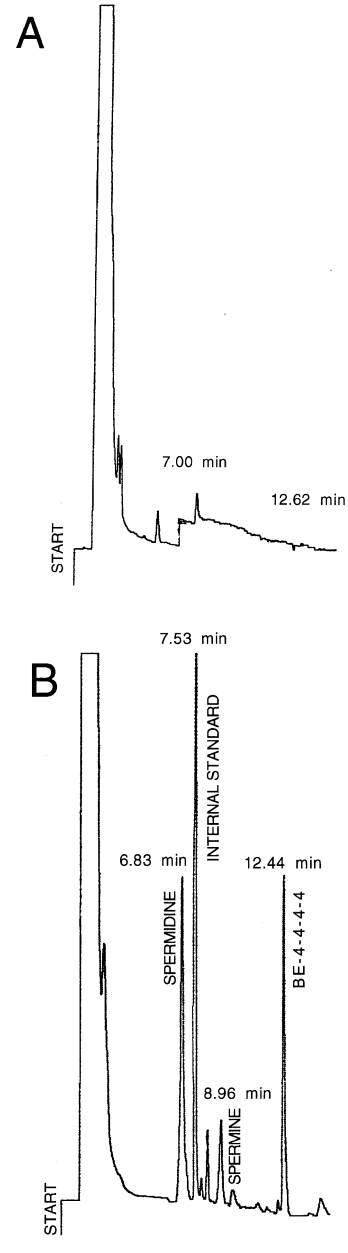


Fig. 2A, B HPLC tracings of **A** control mouse plasma and **B** mouse plasma containing 100 µ*M* spermine, 100 µ*M* BE-4-4-4-4, 500 µ*M* spermidine, and 50 µ*M* *N*,*N*′-dimethylhexanediamine (INTERNAL STANDARD)

Pharmacokinetic analysis

The clearance (CLtb), steady-state volume of distribution (Vdss), area under the curve from time zero to infinity (AUC), and terminal half-life ($t_{1/2}$) were estimated by non-compartmental analysis with the LaGrange function [19] as implemented by the LAGRAN computer program [16]. In addition, the individual concentrations of BE-4-4-4-4 in plasma versus time following i.v. administration were fit simultaneously to two- and three-compartment, open, linear models with the program ADAPT II [5] using least-squares estimation weighted with linear inverse variance of the output error. The four i.v. doses were modeled as four separate boluses in a multiple dosing regime, with each dose being followed by a 2,000-min interval. This time was sufficient for washout without carryover from the previous dose. The plasma concentration versus time profiles obtained after s.c. and i.p. administration were modeled as independent dosing events using two- and three-compartment, open, linear models with first-order absorption. In each case, the suitability of the

two-versus three-compartment model was determined on the basis of model discrimination analyses using Akaike's information criteria (AIC) [1].

Determination of BE-4-4-4-4 binding to proteins in mouse plasma

Solutions of BE-4-4-4-4 (0.5 and 2.0 μ*M*) were prepared in phosphate-buffered saline (2.7 m*M* KCl, 1.2 m*M* KH_2PO_4, 138 m*M* NaCl, pH 7.4) or mouse plasma and placed into Amicon Centrifree ultrafiltration devices (Amicon Division of W.R. Grace, Inc., Beverly, Mass.). Ultrafiltrates were prepared by centrifugation at 2,000 *g* for 20 min. Concentrations of BE-4-4-4-4 in 100 μl aliquots of both the original sample and the ultrafiltrate were determined with the dansylation procedure and HPLC system described above.

Results

Plasma pharmacokinetics

Because no information was available with regard to the toxicity of BE-4-4-4-4 after i.v. administration to mice, preliminary efforts were directed at defining a tolerable i.v. dose. BE-4-4-4-4 was dissolved in 0.154 *M* NaCl, and doses of 50, 35, or 21 mg/kg were delivered in a maximal volume of 10 ml/kg to groups of male and female mice (five mice per sex per group). The 50-mg/kg dose proved lethal within 1 min to all of the mice injected. Of the ten mice injected with 35 mg/kg, one died at 2 min after dosing and the remaining mice did not gain weight during the ensuing 7 days. The mice treated with 21 mg/kg showed no obvious adverse effect of treatment through the first 7 days of observation. Therefore, plans were made to use a dose of 35 mg/kg in initial pharmacokinetics studies of i.v. administration of BE-4-4-4-4. However, the 35-mg/kg dose proved lethal to the first three mice injected in the pharmacokinetics study, the result being that 20 mg/kg was selected as the highest dose for use in studies investigating the pharmacokinetics of BE-4-4-4-4 after i.v. administration.

After i.v. bolus delivery of BE-4-4-4-4 to mice, plasma concentrations of BE-4-4-4-4 declined in a manner suitably modeled with a two-compartment, open, linear model (Fig. 3, Table 1). In mice injected with 20 mg/kg, peak plasma BE-4-4-4-4 concentrations ranged between 31 and 33 μg/ml, and BE-4-4-4-4 was detectable in plasma for up to 480 min after injection (Fig. 3A, Table 1). At the 20-mg/kg dose, the BE-4-4-4-4 AUC was 958 $\mu g\,ml^{-1}\,min$, which corresponded to a CLtb of 20.9 $ml\,min^{-1}\,kg^{-1}$ (Table 1). The i.v. administration of BE-4-4-4-4 at 15, 10, or 5 mg/kg resulted in appropriately lower plasma concentrations and AUC values (Fig. 3, Table 1). As indicated by the proportional relationship between the BE-4-4-4-4 dose and the AUC (Table 1), the BE-4-4-4-4 CLtb did not vary systematically across the 4-fold range of doses studied, remaining between 14.8 and 20.8 $ml\,min^{-1}\,kg^{-1}$ (Table 1).

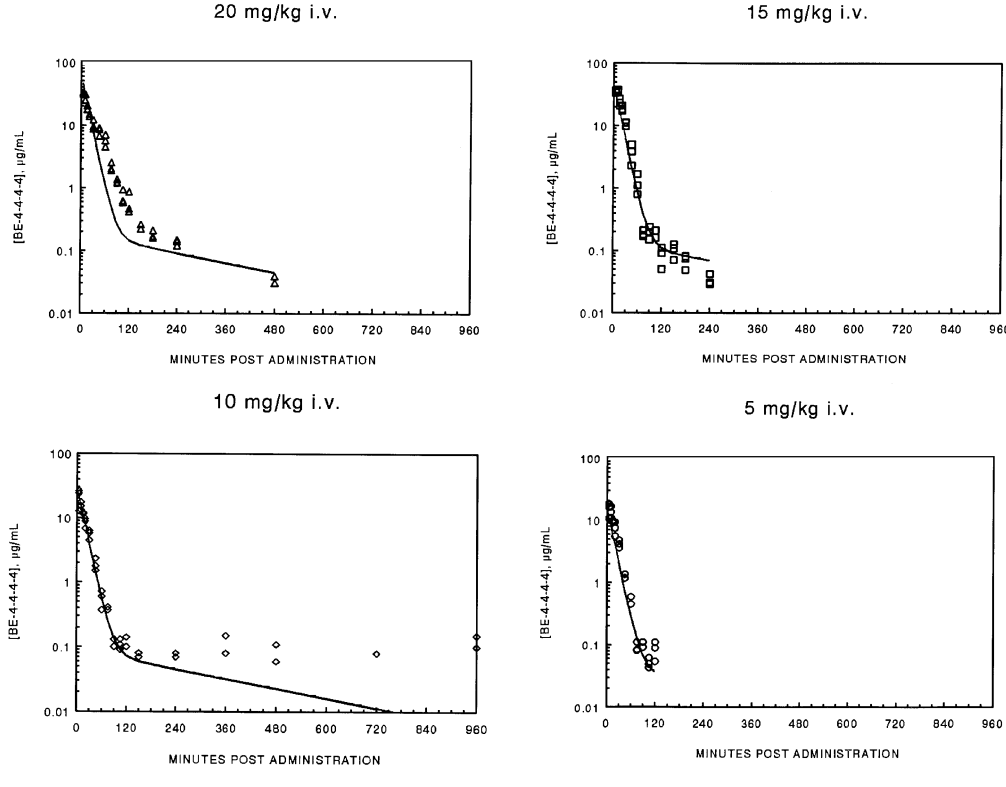


Fig. 3 Disappearance of BE-4-4-4-4 from plasma with time after various i.v. doses (*Symbols* Concentrations of BE-4-4-4-4 measured in plasma of individual animals, *solid line* predicted fit

Table 1 Pharmacokinetic analysis of BE-4-4-4-4 plasma concentration versus time profiles (*NA* Not applicable)

Noncompartmental analysis							
Dose (mg/kg)	Route	F[a] (%)	AUC ($\mu g\,ml^{-1}$ min)	k_{el} (min^{-1})	$t_{1/2}$ (min)	V_{DSS} (ml/kg)	CL_{TB} ($ml\,min^{-1}\,kg^{-1}$)
5	i.v.	–	338	0.029	24	290	14.8
10	i.v.	–	627	0.0007	924	8,426	15.9
15	i.v.	–	852	0.0103	67	376	17.6
20	i.v.	–	958	0.007	94	850	20.8
20	i.p.	40	382	0.016	42	1,873[b]	NA
20	s.c.	50	475	0.007	105	1,977[b]	NA
20	p.o.	3	29	0.013	53	47,653[b]	NA
Compartmental analysis							
Dose (mg/kg)	Route	k_a (min^{-1})	k_{10} (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	V_c (ml/kg)	
5–20[c]	i.v.	–	0.064	0.0039	0.0031	307	
20	i.p.	0.038	0.332	0.024	0.0021	181	
20	s.c.	0.059	0.055	0.0046	0.0024	850	
20	p.o.	–	–	–	–	–	

[a] F is the fraction of the dose that is bioavailable
[b] Apparent V_{DSS}
[c] Data from all animals treated i.v. with doses of 5, 10, 15, and 20 mg/kg were modeled simultaneously as described in Materials and methods

In these studies, plasma concentrations of BE-4-4-4-4 declined rapidly with an initial half-life of 5–15 min. As the BE-4-4-4-4 dose was sequentially reduced from 15 to 10 and, finally, 5 mg/kg, the time after i.v. injection during which the compound could be detected in plasma decreased. Because of this assay limitation, estimations of the plasma elimination $t_{1/2}$ from individual data sets obtained by noncompartmental analysis could not be considered reliable (Table 1). Therefore, in an attempt to address this issue with a different methodological approach, we modeled the plasma concentration data from the individual mice at all dose levels simultaneously, first to a two-compartment, open, linear model and, subsequently, to a three-compartment, open, linear model (Fig. 3). For this compartmental modeling, we assumed that the dose events were separated by 2,000 min, a time sufficient that there be no BE-4-4-4-4 remaining from the previous dosing event. The individual data points recorded for each dose as well as the predicted values obtained using the two-compartment model are shown in Fig. 3. As based on model discrimination analysis, in which the AICs for the two- and three-compartment models were calculated to be 1,310 and 1,312, respectively, the two-compartment model was judged as providing a better fit to the data. The coefficients of variation for estimated parameters from the two-compartment model were small. For the parameters k_{12}, k_{21}, k_{10}, and V_c they were 5.15%, 9.27%, 0.98%, and 1.8%, respectively. The parameters for a two-compartment, open, linear model that describes the decline in plasma concentrations of BE-4-4-4-4 after i.v. dosing are presented in Table 1.

The i.p. administration of BE-4-4-4-4 at a dose of 20 mg/kg produced detectable plasma concentrations of BE-4-4-4-4 by 5 min after injection (Fig. 4). Peak plasma concentrations of BE-4-4-4-4 were maintained for between 5 and 15 min after i.p. administration. Thereafter, plasma concentrations of BE-4-4-4-4 declined; however, BE-4-4-4-4 was detectable in plasma for as long as 240 min after i.p. injection (Fig. 4). When modeled in a noncompartmental manner, the AUC of BE-4-4-4-4 associated with i.p. delivery of a 20-mg/kg

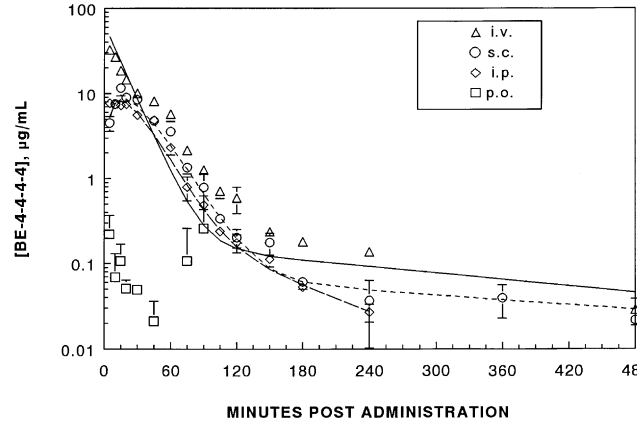


Fig. 4 Disappearance of BE-4-4-4-4 from plasma with time after administration of 20 mg/kg by various routes (*Symbols* mean plasma concentrations ± SD of BE-4-4-4-4, *lines* predicted fits, *open triangles* values recorded after i.v. administration, *open diamonds* values obtained after i.p. administration, *open circles* values obtained after s.c. administration, *open squares* values obtained after p.o. administration

dose was 382 μg ml^{-1} min, corresponding to a bioavailability of 40%. When calculated in this manner, the terminal $t_{1/2}$ of BE-4-4-4-4 was 42 min. The time course of plasma BE-4-4-4-4 concentrations associated with i.p. administration of 20 mg/kg was also well described by a two-compartment, open, linear model with first-order absorption from the peritoneal cavity (Figs. 3, 4, Table 1). When analyzed in this manner, a k_a value of 0.038 min^{-1} was calculated. Inclusion of a third compartment did not result in a better fit to the data as the AICs for two- and three-compartment models were 242 and 250, respectively. The coefficients of variation of parameter estimates ranged from 2.4% for k_a to 121.2% for k_{21}.

The s.c. administration of BE-4-4-4-4 at a dose of 20 mg/kg also produced detectable plasma concentrations of BE-4-4-4-4 by 5 min after injection (Fig. 4). In contrast to the time course of plasma BE-4-4-4-4 concentrations observed after i.p. administration, plasma concentrations of BE-4-4-4-4 increased for the first 15 min after drug delivery and then declined during the remaining period of study. BE-4-4-4-4 was detectable in plasma for as long as 480 h after s.c. injection (Fig. 4). When modeled in a noncompartmental manner, the AUC of BE-4-4-4-4 associated with s.c. delivery of a 20-mg/kg dose was 475 μg ml^{-1} min, corresponding to a bioavailability of 50%. When calculated in this manner, the terminal $t_{1/2}$ of BE-4-4-4-4 was 105 min. The time course of plasma BE-4-4-4-4 concentrations associated with s.c. administration of 20 mg/kg was also well described by a two-compartment, open, linear model with first-order absorption (Fig. 4, Table 1). When analyzed in this manner, a k_a value of 0.059 min^{-1} was calculated. As was the case with i.p. delivery of BE-4-4-4-4, addition of a third compartment to the model did not result in a better fit to the data. The AICs for two- and three-compartment models were 263 and 267, respectively.

Enteral administration of BE-4-4-4-4 at a dose of 20 mg/kg produced very low plasma concentrations of drug (Fig. 4). Peak concentrations of approximately 0.325 μg/ml were observed at 5 min after delivery of BE-4-4-4-4 by gavage, and during the next 40 min they decreased to below the level of quantification of the HPLC system used in these studies. When modeled in a noncompartmental manner, the AUC of BE-4-4-4-4 associated with gavage delivery of a 20-mg/kg dose was 29.1 μg ml^{-1} min, corresponding to a bioavailability of approximately 3%. Compartmental modeling of these data was not pursued.

BE-4-4-4-4 binding to plasma proteins

When 178-ng/ml (0.5 μ*M*) and 714-ng/ml (2.0 μ*M*) solutions of BE-4-4-4-4 in mouse plasma were passed through Centrifree devices, the concentrations of BE-4-4-4-4 measured in the ultrafiltrates were approximately 33% of those detected in the ultrafiltrates of solutions of the same concentrations made in phosphate-buffered saline (data not shown). Thus, approximately 66% of the BE-4-4-4-4 appears to be bound to murine plasma protein. It is noteworthy that concentrations of BE-4-4-4-4 measured in ultrafiltrates produced from solutions of BE-4-4-4-4 in phosphate-buffered saline were approximately 80% of those detected in the solutions initially placed into the devices, implying a 20% loss of drug due either to decomposition or to binding to the ultrafiltration membrane.

Table 2 Urinary excretion of BE-4-4-4-4

Dose (mg/kg)	Route	0–4 h Excretion[a] (%)	4–24 h Excretion[a] (%)
5	i.v.	0.10	0.26
10	i.v.	4.60	0.71
15	i.v.	2.80	0.41
20	i.v.	9.40	0.50
20	p.o.	0.13	0.08
20	s.c.	9.20	3.50
20	i.p.	21.40	5.20

[a] Values are based on the cumulative urine sample obtained from 3 mice gang-housed in metabolism cages and were calculated as a percentage of the total dose delivered to those 3 mice

Urinary excretion of BE-4-4-4-4

When BE-4-4-4-4 was delivered i.v. at a dose of 20 mg/kg, urinary excretion of the parent compound accounted for only 9.4% of the dose in the first 4 h after injection, with approximately 9.90% of the dose being accounted for by urinary excretion in the 24-h period after injection (Table 2). When BE-4-4-4-4 was injected i.p. at 20 mg/kg, urinary excretion of the parent compound accounted for 21.4% of the dose in the first 4 h after injection, with 26.6% of the dose being accounted for by urinary excretion of the parent compound in the 24-h period after i.p. injection. The s.c. delivery of BE-4-4-4-4 at a dose of 20 mg/kg was associated with urinary excretion of 9.2% of the delivered dose as the parent compound in the first 4 h after injection and 12.7% of the delivered dose as the parent compound in the 24-h period after s.c. injection.

Discussion

BE-4-4-4-4 is one of a number of analogues that have been developed to exploit differences in polyamine biosynthesis and uptake between cancer cells and normal cells. BE-4-4-4-4, maintained at concentrations of 5 μ*M* in media for 6–7 days has shown activity in vitro against several human brain-tumor cell lines [2, 3]. Furthermore, BE-4-4-4-4 reduced the concentrations of

polyamines by 24 h after treatment [3]. BE-4-4-4-4 has also shown in vivo activity against human brain (U251-MG and U87-MG), lung (A549), and colon (HCT116) xenografts implanted in athymic nude mice. In these studies, BE-4-4-4-4 doses of 5 mg/kg were given i.p. twice daily on an 11-day cycle (4 days of treatment, 3 days off treatment, 4 days on treatment) [6]. The only toxicity observed in the tumor-bearing mice treated with BE-4-4-4-4 on this schedule was loss of between 4% and 12% of body weight during treatment.

In the current study, the maximally tolerated dose of BE-4-4-4-4 given as an i.v. bolus was 20 mg/kg. Doses of both 50 and 35 mg/kg delivered as i.v. boluses were lethal to CD_2F_1 mice. Plasma pharmacokinetics after i.v. administration of BE-4-4-4-4 appeared to be linear over a 4-fold dose range (5–20 mg/kg). After i.v. administration of 20 mg/kg, plasma concentrations of BE-4-4-4-4 were maintained at or above the in vitro effective concentration of 5 μM (1.78 μg/ml) for 75 min. When lower doses of BE-4-4-4-4 were given by the i.v. route, concentrations of BE-4-4-4-4 in plasma were maintained above 5 μM for shorter periods: for 45 min after administration following 15- and 10-mg/kg doses of BE-4-4-4-4 and for 30 min after a 5 mg/kg dose. This reflected the limited volume of plasma that could be obtained from individual mice, which restricted the sample available for HPLC analysis. If the HPLC system described in this paper were to be applied to clinical samples, such restrictions would not be operative, and the increased sample size available for assay could result in a 10 to 20-fold reduction in the lower limit of quantitation. The bioavailability of BE-4-4-4-4 was reasonable after both i.p. and s.c. administration, being 41% and 50%, respectively, and effective plasma concentrations of BE-4-4-4-4 were attained after administration of BE-4-4-4-4 by either of these routes. After administration of a 20-mg/kg dose by the i.p. or s.c. route, plasma concentrations of BE-4-4-4-4 were maintained at or above 5 μM for at least 60 min. These data suggest that both of these routes would also be acceptable for the administration of BE-4-4-4-4 in efficacy trials. In contrast, after p.o. administration of 20 mg/kg, plasma BE-4-4-4-4 concentrations never reached effective in vitro concentrations, as peak concentrations never exceeded 0.11 μM.

Data from Dolan et al. [6] clearly indicate that BE-4-4-4-4 given i.p. at doses of 5 mg/kg twice daily is sufficient to delay the growth of several human tumor xenografts in nude mice. These data suggest that the concentrations of BE-4-4-4-4 required for in vivo activity might be lower than those based on in vitro studies or that concentrations of BE-4-4-4-4 measured in the plasma of nude mice might be higher than those detected in the plasma of CD_2F_1 mice after the administration of similar doses. Alternatively, plasma concentrations of BE-4-4-4-4 might not adequately reflect tumor concentrations of the compound. After 7 days of in vitro exposure to 5 μM BE-4-4-4-4, uptake of BE-4-4-4-4 by U-87 tumor cells was approximately 0.010 μmol/10^6 cells [2]. If the tumor volume of 10^6 cells is assumed to be 1 μl, then the concentration of BE-4-4-4-4 achieved in 1 ml of packed tumor cells would be 10 μmol/ml, or 10 mM. This would represent a roughly 2,000-fold concentration of the compound in the tumor cells over the concentration in the medium. Furthermore, although BE-4-4-4-4 was bound to plasma protein, it was not bound to as great an extent as many other therapeutic agents, with approximately 33% of the drug being free. Thus, it may be that tumor cells are capable of accumulating and retaining BE-4-4-4-4 even though plasma concentrations of the drug do not remain above 5 μM for very long periods.

Simultaneous modeling of the plasma concentration versus time data from all four i.v. studies was undertaken because there were large differences in the terminal elimination constant when each individual data set was modeled separately by noncompartmental analysis. However, log-linear plots of the data indicated that a true terminal phase was missed at the lower doses of BE-4-4-4-4 because plasma concentrations of BE-4-4-4-4 fell below the detection limit of the assay before the terminal phase was reached. Therefore, to use information about the terminal elimination rate associated with a 20-mg/kg i.v. dose of BE-4-4-4-4, the individual data from mice at all four dose levels were modeled simultaneously as four separate dose events separated by an interval sufficient to eliminate carryover from the previous dose event. This novel approach, although more complex, selected parameters that represented all the data points.

The current study of the plasma pharmacokinetics of BE-4-4-4-4 in CD_2F_1 mice provides data that can form a basis for assessing concentrations of BE-4-4-4-4 associated with toxicity and in vivo activity in murine testing. That BE-4-4-4-4 has proved active in a number of tumor models after i.p. administration of 5 mg/kg suggests that the drug is effective in vivo at plasma concentrations lower than those projected from in vitro testing. That BE-4-4-4-4 was not available by the p.o. route implies that this is not an acceptable route for its administration. Furthermore, data from the current study could prove useful in planning initial doses and dose-escalation schemes when BE-4-4-4-4 is introduced into phase I clinical trials. Although a complete acute-toxicity study was not performed to define accurately the dose lethal to 10% of mice (LD_{10}), there was no evidence of nonlinearity in the pharmacokinetics of BE-4-4-4-4 at the i.v. doses studied, and, as based on our preliminary toxicity observations, it is unlikely that the validated LD_{10} would be substantially higher than the 20-mg/kg i.v. dose used as our maximally tolerated dose. Therefore, the pharmacokinetic data presented could be extrapolated if pharmacokinetically guided dose escalation were truly desired.

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