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# Pharmacokinetics of fotemustine and BCNU in plasma, liver and tumor tissue of rats bearing two lines of Walker 256 carcinoma

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**Summary.** The plasma and tissue pharmacokinetics of fotemustine (diethyl-1-[3-(2-chlorethyl)-3-nitrosoureido]-ethylphosphonate) and BCNU (1,3-*bis*-[2-chlorethyl]-1-nitrosourea) were investigated in healthy control rats and in animals bearing either the nitrosourea-sensitive line A (W256/A) or the nitrosourea-resistant line B (W256/B) of Walker 256 carcinoma. The antitumor activities of these nitrosoureas were similar following i.v. doses ranging from 10 to 40 mg/kg. For both drugs, the survival of tumor-bearing rats was lower in the W256/B than in the sensitive W256/A line. Some sex differences were observed, female rats being more responsive than males to both drugs. Nitrosourea concentrations were assayed in plasma and tissues by differential pulse polarography so as to assess whether the pharmacokinetics could explain the differences in antitumor activity. The antineoplastic effects of fotemustine seemed to be influenced by its pharmacokinetics. The plasma AUC of the intact nitrosourea was higher in females than in males. Fotemustine was cleared 2–5 times more slowly than BCNU from tumor tissue, and its clearance was higher in W256/B- than in W256/A-bearing rats. This suggests that the antitumor activity in the responsive line might partly be due to longer exposure of the growing tumor to the drug. The distribution volume of both nitrosoureas in plasma was higher in tumor-bearing animals than in healthy controls, indicating that the tumor tissue probably constitutes an additional distribution space.

## Introduction

Diethyl-1-[3-(2-chloroethyl)-3-nitrosoureido]-ethylphosphonate (fotemustine) is a potent cytotoxic nitrosourea [30] that is active against many experimental tumors both in vivo and in vitro [9, 10, 14, 15, 22]. Its antitumor activity is accompanied by delayed, dose-related, mainly hematological toxicity similar to that of other nitrosoureas [29].

The toxicity and pharmacokinetics of anticancer drugs are usually studied in normal, healthy adult animals. However, the presence of a tumor can affect drug availability as a result of structural and functional changes in the host [1, 3, 11] such as impaired renal function [11, 24], depletion of adipose tissue [11, 16] and alterations in metabolic capacity [1, 28]. Pharmacokinetic changes can be correlated with the activity of antitumor compounds [11, 12], although the former are not necessarily followed by fundamental changes in therapeutic effects. In general, proliferation of neoplastic tissue may modify the distribution and metabolism of a drug [1, 11], and the altered availability of an antitumor agent in malignant tissue may be of crucial importance for the curative effect.

Two lines of Walker 256 carcinoma maintained in our laboratory differ in their sensitivity to antitumor drugs [8, 11], their histological characteristics and metastatic capacity [18] as well as their induction of anorexia and cachexia in the host animal [19]. The present study compared the pharmacokinetics and antitumor effects of fotemustine and 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (BCNU) on the nitrosourea-sensitive (W256/A) and -resistant (W256/B) Walker carcinoma lines. The pharmacokinetics of fotemustine and BCNU were determined in plasma, liver and tumor tissue, which either are important for nitrosourea distribution, activation and deactivation or are targets of their action.

## Materials and methods

*Chemicals.* Fotemustine was supplied by Servier Laboratories (Fleury les Aubrais, France) and BCNU (1,3-bis-[2-chlorethyl]-1-nitrosourea) was provided by Simes (Milan, Italy). Nitrosoureas were dissolved in 95% ethanol (50 mg/ml) and diluted with 0.9% NaCl before use. All reagents used in nitrosourea assays were of analytical grade.

*Abbreviations:* BCNU, 1,3-*bis*-[2-chlorethyl]-1-nitrosourea; W256/A, Walker carcinoma 256 line A; W256/B, Walker carcinoma 256 line B

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**Table 1.** Antitumor effects of fotemustine and BCNU on Walker 256 lines A and B

| Tumor line | Dose (mg/kg) | Fotemustine | | | BCNU | | |
|---|---|---|---|---|---|---|---|
| | | LTS/total | MS (days) | T/C%[a] | LTS/total | MS (days) | T/C%[a] |
| W256/A: | | | | | | | |
| Males | Saline | 0/4 | 22± 3 | | 0/4 | 24± 3 | |
| | 10 | 2/4 | 46± 8 | 209 | 2/4 | 43±10 | 179 |
| | 15 | 3/4 | 52± 8 | 236 | 4/4 | >60 | >250 |
| | 20 | 4/4 | >60 | >273 | 4/4 | >60 | >250 |
| | 25 | 4/4 | >60 | >273 | 3/4 | 53± 7 | 221 |
| | 30 | 4/4 | >60 | >273 | 3/4 | 53± 7 | 221 |
| | 40 | 2/4 | 39±12 | 177 | 3/4 | 56± 4 | 233 |
| Females | Saline | 0/3 | 24± 3 | | 0/4 | 26± 3 | |
| | 10 | 4/4 | >60 | >250 | 4/4 | >60 | >231 |
| | 15 | 4/4 | >60 | >250 | 4/4 | >60 | >231 |
| | 20 | 4/4 | >60 | >250 | 3/4 | 59± 1 | 227 |
| | 25 | 3/4 | 56± 4 | 233 | 4/4 | >60 | >231 |
| | 30 | 4/4 | >60 | >250 | 2/3 | 51± 5 | 196 |
| | 40 | 2/4 | 51± 6 | 212 | 3/4 | 56± 5 | 215 |
| W256/B: | | | | | | | |
| Males | Saline | 0/2 | 16± 2 | | 0/4 | 14± 1 | |
| | 10 | 0/4 | 15± 1 | 94 | 0/4 | 16± 0 | 114 |
| | 15 | 0/4 | 23± 2 | 144 | 0/4 | 19± 2 | 136 |
| | 20 | 2/4 | 40±12 | 250 | 0/4 | 25± 7 | 179 |
| | 25 | 0/4 | 23± 1 | 144 | 1/4 | 32± 9 | 229 |
| | 30 | 0/4 | 23± 2 | 144 | 2/4 | 44±10 | 314 |
| | 40 | 0/4 | 27± 5 | 169 | 1/4 | 30±10 | 214 |
| Females | Saline | 0/4 | 16± 2 | | 0/4 | 16± 1 | |
| | 10 | 3/4 | 49±11 | 306 | 0/4 | 22± 2 | 138 |
| | 15 | 0/4 | 23± 4 | 144 | 1/4 | 37± 9 | 231 |
| | 20 | 2/4 | 39±12 | 244 | 2/4 | 41±11 | 256 |
| | 25 | 1/4 | 31±10 | 194 | 1/3 | 34±13 | 212 |
| | 30 | 1/4 | 32±10 | 200 | 0/4 | 29± 1 | 181 |
| | 40 | 1/4 | 29±10 | 181 | 1/4 | 33± 9 | 206 |

LTS, Long-term survivors (>60 days); MS, mean survival (±SE)

[a] Mean survival of the treated group (T) as a percentage of the untreated control (C) value

*Animals.* Male and female Crl:CD(SD)BR rats (Charles River Italy, Calco) aged 6–7 weeks were selected and randomly assigned to the experimental groups after quarantine. Animals were housed under standard conditions (220±1°C, 55%±10% relative humidity, 12-h light/dark schedule) and fed an "open formula" diet (Altromin MT, Rieper, Italy) with water ad libitum.

*Tumors.* Fragments (approx. 100 mg) of proliferating W256/A or W256/B tissue were transplanted subcutaneously in the right-hand scapular region under standard conditions according to NCI protocols for screening of chemical agents [17].

*Treatments.* For evaluation of antitumor activity, rats bearing W256/A or W256/B carcinoma (four males and four females per dose) received fotemustine or BCNU (10, 15, 20, 25, 30 and 40 mg/kg) intravenously at 6 days after tumor transplantation. Controls were given the same volume of saline solution (4 ml/kg). Body weight was recorded every 2 days beginning on day 0 for up to 60 days; mortality was followed daily. Starting on day 4, the sagittal and transversal tumor diameters were measured to assess tumor weight [17]. Mean survival (MS) was calculated using the logit method [6], and the percentage of increase in the mean life span of treated rats over controls (T/C%) was determined. Animals living for >60 days after tumor transplantation were considered to be long-term survivors (LTS) [17] (see Table 1).

For the pharmacokinetic study, W256/A- and W256/B-bearing rats and healthy controls (four animals each per time point) were given an i. v. bolus (10–20 s) of fotemustine or BCNU (20 mg/kg) in the tail vein at 6 days after tumor transplantation. Lightly anesthetized animals were killed at pre-set intervals (2, 5, 10, 15, 20, 30, 60, 120 and 180 min) by exsanguination from the carotid artery, and the blood was collected and mixed with 3.8% sodium citrate (10:1, v/v) adjusted to pH 5.5.

Liver and proliferating tumor tissue were quickly isolated, weighed, minced and homogenized (1:5, v/v) with 0.1 M citric acid (pH 2). The plasma samples or tissue homogenates were frozen and stored in a dark environment at –20° C. Our experience indicates that the photosensitivity of fotemustine must be borne in mind, together with the temperature and pH of the solution. These factors seemed to influence the recovery of fotemustine [4]. A differential pulse polarographic assay, has been applied for determination of nitrosoureas in biological samples; the lower limit of the linear assay was 0.02 and 0.3 µg/ml for BCNU and fotemustine, respectively [2, 4]. Animal experiments were done according to the standards of humane care [21, 31]; all experimental protocols and procedures were approved by the institutional Animal Care and Use Committee.

## Results and discussion

Fotemustine is an aminophosphonic acid derivative of chlorethylnitrosourea that was designed to achieve better passage through the cell membrane [23] and enhanced antitumor activity [10, 14]. In comparison with BCNU, fotemustine does not inhibit glutathione reductase [5], is less mutagenic [9] and less immunodepressant [32] and achieves better penetration into the brain [22, 25].

**Table 2.** Pharmacokinetic parameters of fotemustine and BCNU in the plasma of healthy and of Walker/A- and Walker/B-bearing rats after an i. v. bolus of 20 mg/kg

| Parameters | Fotemustine | | | BCNU | | |
|---|---|---|---|---|---|---|
| | Controls | Walker/A | Walker/B | Controls | Walker/A | Walker/B |
| Males: | | | | | | |
| $AUC_{0\rightarrow\infty}$ (μg $ml^{-1}$ min) | 595 | 527 | 346 | 191 | 213 | 216 |
| *C* total (ml/min) | 6.46 | 7.64 | 11.64 | 20.97 | 18.75 | 18.54 |
| $t_{1/2\alpha}$ (min) | 8 | 10 | 12 | 2 | – | 1 |
| $t_{1/2\beta}$ (min) | 60 | 74 | 52 | 15 | 8 | 15 |
| $V_d$ (ml) | 250 | 810 | 880 | 213 | 213 | 411 |
| Females: | | | | | | |
| $AUC_{0\rightarrow\infty}$ (μg $ml^{-1}$ min) | 781 | 547 | 505 | 141 | 176 | 162 |
| *C* total (ml/min) | 4.35 | 6.04 | 6.53 | 23.52 | 18.72 | 20.40 |
| $t_{1/2\alpha}$ (min) | 5 | 14 | 12 | 2 | – | 6 |
| $t_{1/2\beta}$ (min) | 56 | 63 | 103 | 13 | 11 | 46 |
| $V_d$ (ml) | 157 | 547 | 966 | 177 | 309 | 1,366 |

Plasma levels as fitted by a mono- or bi-exponential equation: $C_t = Be^{-\beta t}$, $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, where $C_t$ is the concentration (μg/ml) at time *t*, *A* and *B* are coefficients of the function, and α and β are the elimination constants ($min^{-1}$). Half-life was calculated by the formula $t_{1/2} = ln\ 2/\beta$. The AUC was calculated by trapezoidal integration ($AUC_{0\rightarrow 30\ min}$ and $AUC_{30\rightarrow 180\ min}$) and extrapolated to infinity using the formula $AUC_{0\rightarrow\infty} = c/\beta$. The equation used for total clearance was $C$ = dose/AUC, and that applied for volume of distribution was $V_d$ = dose/*C*

*Antitumor activity*

Fiebig et al. [13] found that BCNU inhibited the growth of Walker carcinoma by about 80% and increased the life span of the animals. Our results on LTS and complete inhibition of tumor growth at optimal doses indicate that both fotemustine and BCNU are active over a wide range of doses against W256/A carcinoma (Table 1). This line is also sensitive to other antitumor agents [8, 12].

The mean survival of saline-treated controls bearing W256/B was 16 ± 2 days, which was less than that obtained for the sensitive A line (24 ± 3 days). The total ratio of cured/treated W256/A-bearing rats was the same (19/24) for males, regardless of the nitrosourea used, and nearly the same (21/23 and 20/23 for fotemustine and BCNU, respectively) for females. This ratio was markedly lower in animals bearing the B line (2/24 males and 8/24 females; Table 1). In females bearing the A line, fotemustine and BCNU prolonged survival beyond 60 days at doses as low as 10 mg/kg, whereas the males seemed to be less sensitive, as survival beyond 60 days was reached only using higher doses (15–20 mg/kg).

None of the doses of either nitrosourea induced complete cures in W256/B transplanted rats. However, fotemustine achieved the longest survival for W256/B-bearing males (MS, 40 ± 12 days; LTS, 2/4; T/C, 250%) and females (MS, 49 ± 11 days; LTS, 3/4; T/C, 306%) at doses lower than those at which BCNU was given (Table 1). These results suggest that fotemustine may exhibit more antitumor activity than BCNU as indicated by the longer MS.

*Pharmacokinetics in plasma*

Nitrosoureas are considered to be prodrugs that are activated to alkylating intermediates in tissues [7, 26, 28]. The plasma pharmacokinetics of intact fotemustine was compared that of BCNU in W256/A- and W256/B-bearing rats and in healthy controls of both sexes (Table 2).

Fotemustine disappeared from the plasma of all groups 2–5 times more slowly than BCNU, indicating that unchanged fotemustine persists in the bloodstream for longer periods, which could improve its distribution into the tumor. Sex-related differences were evident in animals treated with fotemustine, whose total clearance was about 1.3–1.8 times lower in females than in males. No such sex difference was found for BCNU. In general, clearance of the latter was about 2.5–3 times that of fotemustine.

Hepatic metabolism of nitrosoureas accounts for only 5%–10% of their total transformation and leads to inactive derivatives [20]. The rapid distribution of fotemustine (indicated by its $t_{1/2\alpha}$) was followed by slower elimination ($t_{1/2\beta}$); concentrations of fotemustine were low but not negligible at 3 h after injection, whereas BCNU could no longer be detected after 60 min.

The distribution volume ($V_d$) of fotemustine in tumor-bearing rats was >3-fold that in healthy controls, whereas the difference was smaller for BCNU. Similar differences in the $V_d$ in solid-tumor-bearing and healthy animals have been observed for other antitumor agents [24].

*Pharmacokinetics in liver and tumor tissue*

Tissue levels of both nitrosoureas in male animals bearing the A or B line are shown in Fig. 1, and the pharmacokinet-

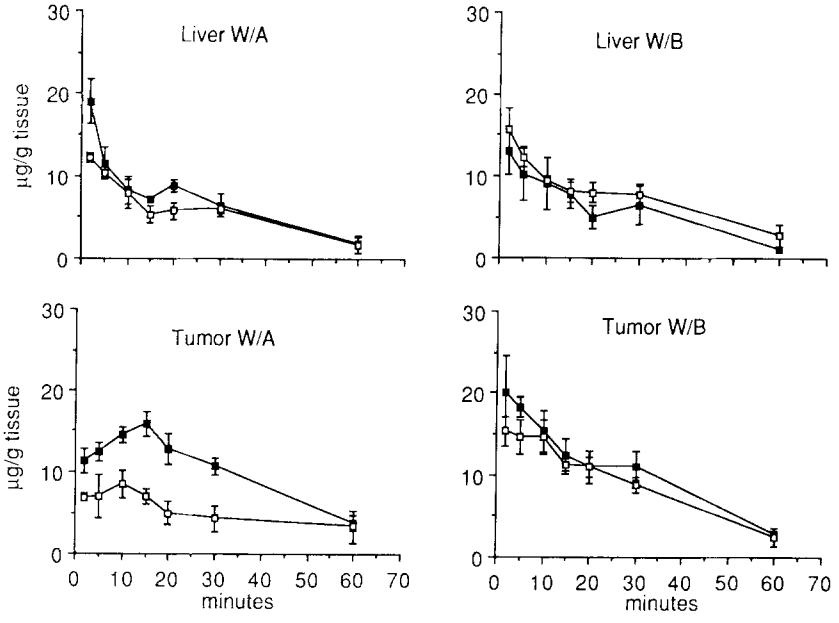


**Fig. 1.** *Upper panels:* Pharmacokinetics of fotemustine (■) and BCNU (□) disappearance from the liver of rats bearing Walker lines A and B carcinoma. Each point represents the mean value ± SE for 4 rats. *Lower panels:* Pharmacokinetics of fotemustine (■) and BCNU (□) disappearance from proliferating tissue of Walker/A and Walker/B carcinoma in rats. Each point represents the mean value ± SE for 4 rats

**Table 3.** Fotemustine and BCNU disappearance from the liver and tumor of Walker 256 carcinoma-bearing male rats after an i. v. bolus

| Parameters | Liver | | Tumor | |
|---|---|---|---|---|
| | Fotemustine | BCNU | Fotemustine | BCNU |
| Walker/A: | | | | |
| $AUC_{0\rightarrow\infty}$ (µg $g^{-1}$ min) | 532 | 646 | 6,316 | 1,331 |
| $t_1/_{2\alpha}$ (min) | 2 | 3 | 14 | – |
| $t^1/_2\beta$ (min) | 33 | 61 | 2,615 | 99 |
| Walker/B: | | | | |
| $AUC_{0\rightarrow\infty}$ (µg $g^{-1}$ min) | 511 | 645 | 1,045 | 649 |
| $t_1/_{2\alpha}$ (min) | 13 | 2 | 19 | – |
| $t_1/_{2\beta}$ (min) | 77 | 38 | 406 | 22 |

Tissue levels as fitted by a mono- or bi-exponential equation: $C_t = Be^{-\beta\tau}$, $C_t = Ae^{-\alpha\tau} + Be^{-\beta\tau}$, where $C_t$ is the nitrosourea concentration (µg/g) at time $t$, $A$ and $B$ are coefficients of the function, and $\alpha$ and $\beta$ are the elimination constants ($min^{-1}$). Half-life was calculated by the formula $t_{1/2} = In\ 2/\beta$. The AUC was calculated by trapezoidal integration ($AUC_{0\rightarrow30\ min}$ and $AUC_{30\rightarrow180\ min}$) and extrapolated to infinity using the formula $AUC_{0\rightarrow\infty} = c/\beta$

ic parameters are summarized in Table 3. Fotemustine and BCNU were rapidly distributed and eliminated from tissues (Fig. 1). Its rapid distribution is due to the lipophilicity or carrier-mediated transport of nitrosoureas followed by the rapid formation of alkylating entities [7, 26–28].

The liver is considered to be a well-perfused organ; the liver-to-plasma AUC ratio was close to 1 for the animals treated with fotemustine, whereas the AUC for BCNU in liver was approximately 3 times the plasma values. The high uptake of BCNU by the liver could indicate higher metabolism and inactivation of this drug as compared with fotemustine.

Metabolism of 2-chloroethylnitrosoureas is minor since 5%–10% of these drugs are enzymatically metabolized in the liver [20, 27]. However, this metabolism is fast enough to enable the metabolism of large portions of delivered doses before chemical decomposition of the drugs occurs [27]; thus, the altered metabolism in the liver due to malignancy can change the chemical activity of the drug.

The amino-1-ethylphosphonic moiety of fotemustine may be considered to be an isoester of alanine, sharing the same transport mechanisms, and this might result in this drug's availability being different from that of other nitrosoureas [23]. The slower elimination of fotemustine from tumor tissue might in some cases be an advantage, prolonging its activity.

In the present study, fotemustine seemed to disappear sooner from the plasma of tumor-bearing rats than from that of controls (Table 2), suggesting either quicker elimination or increased metabolism of the drug. Therefore the presence of a tumor may increase the rate of metabolism of fotemustine; this phenomenon has previously been described for hydrocortisone and 6-mercaptopurine. The same tumors decrease the metabolism of phenobarbital and cyclophosphamide [1].

Major differences were observed between the two nitrosoureas in proliferating tumor tissue of both lines. Fotemustine was eliminated more slowly than BCNU. AUC values were higher in rats treated with fotemustine than in those given BCNU (Fig. 1). In proliferating tumor tissue, the level of intact fotemustine as expressed by the AUC was higher than that in plasma (about 12 times higher in W256/A-bearing rats and 3 times higher in W256/B-transplanted animals), whereas the tumor-to-plasma AUC ratio ranged from 3 to 6 in rats treated with BCNU. The clearance of BCNU from W256/B or W256/A tumors was 2–5 times that of fotemustine, the AUC in tumor tissues being higher in rats bearing the A line.

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