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Bioavailability and pharmacokinetic parameters for 5-ethyl-2′-deoxyuridine

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Abstract

Pharmacokinetic parameters for 5-ethyl-2′-deoxyuridine (EDU) were determined following intravenous (iv) and oral (po) dosing in male Balb-C mice and iv dosing in male Sprague-Dawley rats. The concentrations of EDU in blood after 100 mg/kg iv bolus injections into mice and rats were consistent with a two compartment kinetic model. Based on this kinetic model, EDU showed a very short distribution half-life of 1.4 ± 0.7 min in mice and 1.3 ± 0.1 min in rats. The elimination half-life of EDU in rats following iv bolus injection, was substantially (18.5 ± 1.0 min) shorter than that in mice (24.1 ± 2.9 min). The mean residence time (MRT) of EDU was also substantially longer in mice (25.8 ± 4.9 min) compared to rats (11.0 ± 2.9 min). However, clearance of EDU was similar in both rats and mice. Although the biotransformation of EDU was similar in mice and rats, cleavage of the EDU glycoside bond was less extensive in mice than in rats. EDU showed a 49% bioavailability in mice after a 100 mg/kg po dose. The concentration of EDU in blood after a po dose provided the best fit to a one compartment model. The maximum blood concentration of EDU (C_{max}) was 2.4 ± 0.2 μg/g of blood which attained 31.1 ± 1.2 min (T_{max}) after a 100 mg/kg po dose. The AUC of 5-ethyluracil (EU) after a po dose of EDU was significantly higher ($P < 0.05$) than after an iv dose of EDU. This observation indicates that EDU undergoes degradation by phosphorylases present in the gastrointestinal tract and/or by presystemic metabolism.

Keywords: 5-Ethyl-2′-deoxyuridine; Pharmacokinetics; Bioavailability; Phosphorolysis

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1. Introduction

5-Ethyl-2′-deoxyuridine (EDU) is a well documented antiviral nucleoside. EDU exhibits antiviral activity against herpes simplex virus type-1 (HSV-1), type-2 (HSV-2) and vaccinia virus (De Clercq and Shugar, 1975; Gauri and Malorny, 1967; Kulikowski and Shugar, 1974; Swierkowski and Shugar, 1969; Cheng et al., 1976). EDU is also effective in the treatment of herpes keratitis in rabbits (Elze 1979; Gauri 1968), systemic herpetic infection in mice (Davis et al., 1978 and Davis et al., 1979) and cutaneous herpes infections in guinea pigs (Spruance et al., 1985), and it increased the survival time of HSV-encephalitic mice (Davis et al., 1978 and Davis et al., 1979). Clinical studies have indicated that EDU offers promise for the treatment of ocular herpes (Elze 1979; Gauri 1968; Martent 1975). A study involving the treatment of patients with recurrent genital herpes using EDU 3% cream indicated reduced viral shedding, reduced signs of herpes and good drug-tolerance (Sacks et al., 1991).

The metabolism of EDU has been studied extensively in vivo and in vitro. EDU undergoes rapid catabolic degradation by pyrimidine nucleoside phosphorylase, which results in cleavage of the glycoside bond to produce 5-ethyluracil (EU). EU undergoes further biotransformation to 5-(1-hydroxyethyl)uracil (HEU) (Cheraghali et al., 1994a; Joly and Williams, 1991; Buchele et al., 1989; Kaul and Hempel, 1985). However, most of the reported kinetic data are based on iv doses of EDU. There are no reported data regarding kinetic and/or metabolic data for EDU after po dosing.

Some pyrimidine nucleoside antiviral drugs, such as iodoxuridine, cannot be administrated po due to their rapid degradation by either intestinal phosphorylase and/or by similar hepatic enzymes after passage from the gastrointestinal (GI) tract into the liver via the portal vein (Welch and Prusoff, 1960). However, some pyrimidine nucleosides show acceptable oral bioavailability. It has been reported that intraperitoneal administration of EDU protected mice against herpes simplex encephalitis (Davis et al., 1978), which indicates that EDU must be sufficiently bioavailable to reach the general circulation.

In this study, bioavailability and pharmacokinetic parameters for EDU were determined following iv and po dosing in male Balb-C mice and iv dosing in Spraque-Dawley rats.

2. Materials and methods

5-Ethyl-2′-deoxyuridine and 5-ethyluracil were purchased from the Sigma Chemical Co. and were used without further purification. Male Balb-C mice (20–22 g) and male Spraque-Dawley rats (380–420 g) were purchased from the University of Alberta Health Sciences Animal Services Facility. Three animals were used for each experiment. All animal studies were performed according to the Canadian Council on Animal Care guidelines.

The biotransformation and pharmacokinetics of EDU were investigated in mice and rats using EDU doses of 100 mg/kg. EDU was dissolved in 0.1 ml PEG 400/water (30:70, v/v) and injected into the lateral tail vein of mice, or administered orally (po)

using a stainless steel gavage syringe. Animals were sacrificed using carbon dioxide at 2, 5, 10, 20, 30, 60, 90, and 120 min post iv injection, and at 5, 10, 20, 30, 45, 60, 90, and 120 min after a po dose. Blood samples (about 0.7 ml) were drawn from the heart. In the oral bioavailability study, animals were deprived of food, but not water, 4 h before and during the experiment. For the study in rats, EDU (0.1 ml) was injected via an implanted jugular vein catheter. Blood samples (0.2 ml) were collected at 3, 8, 18, 35, 60, 120 and 180 min post injection of EDU; the catheter was washed by injection of heparinized normal saline (0.4 ml) into the jugular vein catheter after dosing and following each sampling procedure.

All blood samples collected in these experiments were mixed with 2 ml of methanol immediately after sampling, and the mixture was shaken for 15 min in a mechanical shaker. The mixture was then centrifuged for 10 min at $1000 \times g$ and the resulting supernatant fraction was filtered through a Sep-Pak (C18, Waters Millipore) cartridge. Each Sep-Pak cartridge was preconditioned by washing with methanol (3 ml) and then water (2 ml). The filtrate from the supernatant was dried under a stream of nitrogen gas and the residue obtained was dissolved in methanol (400 μl).

A 40 μl aliquot of this solution was subjected to quantitative high performance liquid chromatography (HPLC) analysis using the HPLC system described previously (Cheraghali et al., 1994a). All separations and quantitative analyses were carried out using a Waters Radial-Pak C18 reverse phase cartridge column (10 μ, 8 mm $\times$ 10 cm). The identity of EDU, EU, and/or HEU in blood samples were confirmed by comparison of their retention times to those of authentic samples. The pharmacokinetic constants were determined using the PCNONLIN program (SCI software, ClinTrials Co., Kentucky). Statistical significance levels of data were tested using the *t*-test for independent samples. The concentration of EDU and EU in blood samples, as a function of time, was plotted using the Sigmaplot program (Jandel Scientific).

Blood level data following iv injection of EDU into mice and rats showed an excellent fit to a two compartment model, but data following po administration of EDU were best fit to a one compartment model. Blood clearance (Cl) was calculated according to the equation 1.

$$Cl = dose_{iv}/AUC_{iv} \quad (1)$$

AUC_{iv} is the value derived from the computer calculation for the area under the blood concentration-time curve.

The systemic availability of the po dose was determined from the ratio of AUC of unchanged drug after po and iv doses based on equation 2.

$$f = AUC_{po}/AUC_{iv} \quad (2)$$

3. Results

Pharmacokinetic parameters and blood concentration profiles for EDU after iv and po administration into mice and rats are shown in Tables 1–2 and Fig. 1. Since it was previously observed that (5*R*,6*R*)-5-bromo-6-ethoxy-5-ethyl-5,6-dihydro-2′-deoxyuri-

Table 1
Pharmacokinetic parameters for EDU after a 100 mg/kg iv bolus injection in mice and rats, based on a two compartment model

Species	AUC of EDU ($\mu g.g^{-1}.min$)	$T_{1/2}\ \alpha$ [a] (min)	$T_{1/2}\ \beta$ [b] (min)	Cl [c] ($ml.min^{-1}.kg^{-1}$)	MRT [d] (min)	AUC of EU [e] ($\mu g.g^{-1}.min$)
Mouse #1	698.71	0.77	18.47	143.12	15.96	224.13
Mouse #2	596.03	0.69	25.67	167.73	29.53	267.01
Mouse #3	559.38	2.86	28.30	178.77	31.96	323.96
mean ± S.E.	618.1 ± 41.7	1.4 ± 0.7	24.1 ± 2.9	163.2 ± 10.5	25.8 ± 4.9	249.2 ± 53.8
Rat #1	689.11	1.30	16.53	145.11	5.65	1300.15
Rat #2	459.71	1.37	19.04	217.53	11.54	323.87
Rat #3	866.84	1.10	19.87	115.36	15.86	169.05
mean ± S.E.	671.9 ± 117.8	1.3 ± 0.1	18.5 ± 1.0	159.3 ± 30.3	11.0 ± 2.9	597.7 ± 354.1

[a] Half-life of the distribution phase.
[b] Half-life of the elimination phase.
[c] Blood clearance of EDU.
[d] Mean residence time.
[e] EU is a metabolite of EDU.

dine, a brain-targeted prodrug of 5-ethyl-2′-deoxyuridine, exhibited different concentrations in plasma and whole blood (Cheraghali et al., 1994b), whole blood was used for calculation of pharmacokinetic parameters in this study. The log blood concentration of EDU after an iv dose decreased biexponentially with time. An excellent fit was obtained using a two compartment model (Fig. 2). Based on this model, the half-lives of the distribution phase, $t_{1/2}\alpha$, in mice and rats were very short (1.4 ± 0.7 min in mice and 1.3 ± 0.1 min in rats). However, the half-lives of the elimination phase, $t_{1/2}\beta$, were 24.1 ± 2.9 min in mice and 18.5 ± 1.0 min in rats. Total body clearance (Cl) of EDU was 163.2 ± 10.5 $ml.min^{-1}.kg^{-1}$ in mice and 159.3 ± 30.3 $ml.min^{-1}.kg^{-1}$ in rats. The mean residence time (MRT) for EDU was shorter in rats (11.0 ± 2.9 min) than in mice (25.8 ± 4.9 min).

Blood concentration data following a po dose were best fit to a one compartment model (Fig. 2). According to this model, the maximum concentration of EDU in blood, C_{max}, was 2.4 ± 0.2 $\mu g/g$ of blood at a T_{max} of 31.1 ± 1.2 min after dosing. Based on this model, EDU showed a half-life of 50.3 ± 6.2 min in mice. The AUC for EDU after

Table 2
Pharmacokinetic parameters for EDU following a 100 mg/kg oral dose in mice based on a one compartment model

Species	AUC of EDU ($\mu g.g^{-1}.min$)	$T_{1/2}$(min)	T_{max} (min)	C_{max} ($\mu g.g^{-1}$)	AUC of EU [a] ($\mu g.g^{-1}.min$)
Mouse #1	260.27	38.90	35.59	2.72	1020.13
Mouse #2	267.01	60.30	29.88	2.41	1047.04
Mouse #3	390.31	51.75	29.89	2.06	486.52
mean ± S.E.	305.9 ± 42.4	50.3 ± 6.2	31.1 ± 1.2	2.4 ± 0.2	851.2 ± 182.5

[a] EU is a metabolite of EDU.

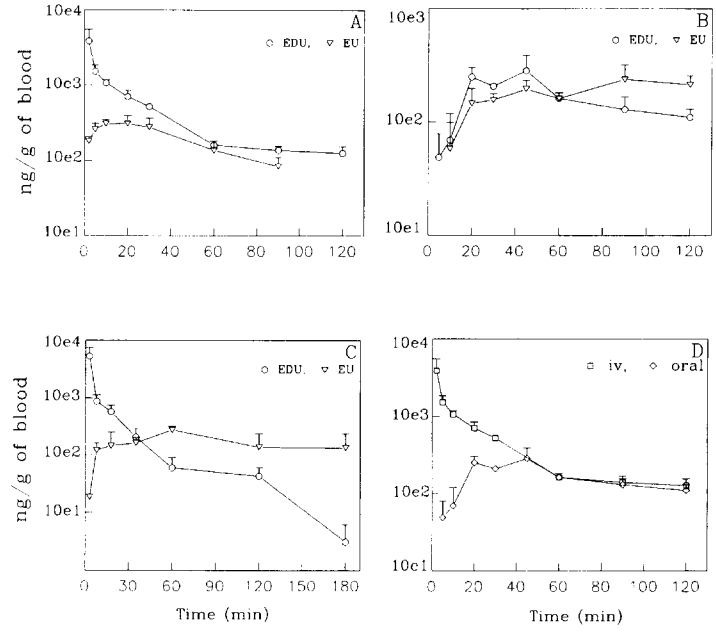

Fig. 1. Blood profile of EDU after a 100 mg/kg iv bolus injection into mice (A), and rats (C), and after a 100 mg/kg oral dose in mice (B). Extent of bioavailability of EDU in mice is shown in plot D. Data are presented as mean ± S.D. ($n = 3$).

a 100 mg/kg po dose was 305.9 $\mu g.g^{-1}$.min, which indicates a 49% bioavailability for EDU in mice.

5-Ethyluracil (EU) was the major metabolite of EDU after both iv and po dosing, and HEU was detected as a subsequent metabolite of EU (Fig. 3). However, the concentration of EU in blood samples taken after a po dose of EDU was significantly higher ($P < 0.05$) than that after an iv dose.

4. Discussion

EDU followed a two compartment kinetic model after a 100 mg/kg iv bolus injection in both male Balb-C mice and male Spraque-Dawley rats. EDU also showed a very short distribution half-life in both species. However, the elimination phase of EDU was faster in rats than in mice, with half-lives of 18.5 and 24.1 min, respectively. The observation that EDU has a longer distribution half-life in mice, as well as a longer MRT (25.8 min) in mice compared to that in rats (11.0 min) indicates that in vivo elimination of EDU was slower in mice than rats. However, the observed difference between blood clearance values of EDU in the two species was not significant ($P > 0.05$).

The extremely short distribution half-life for EDU shows that early blood concentrations are a crucial determinant of pharmacokinetic constants for EDU. However, the

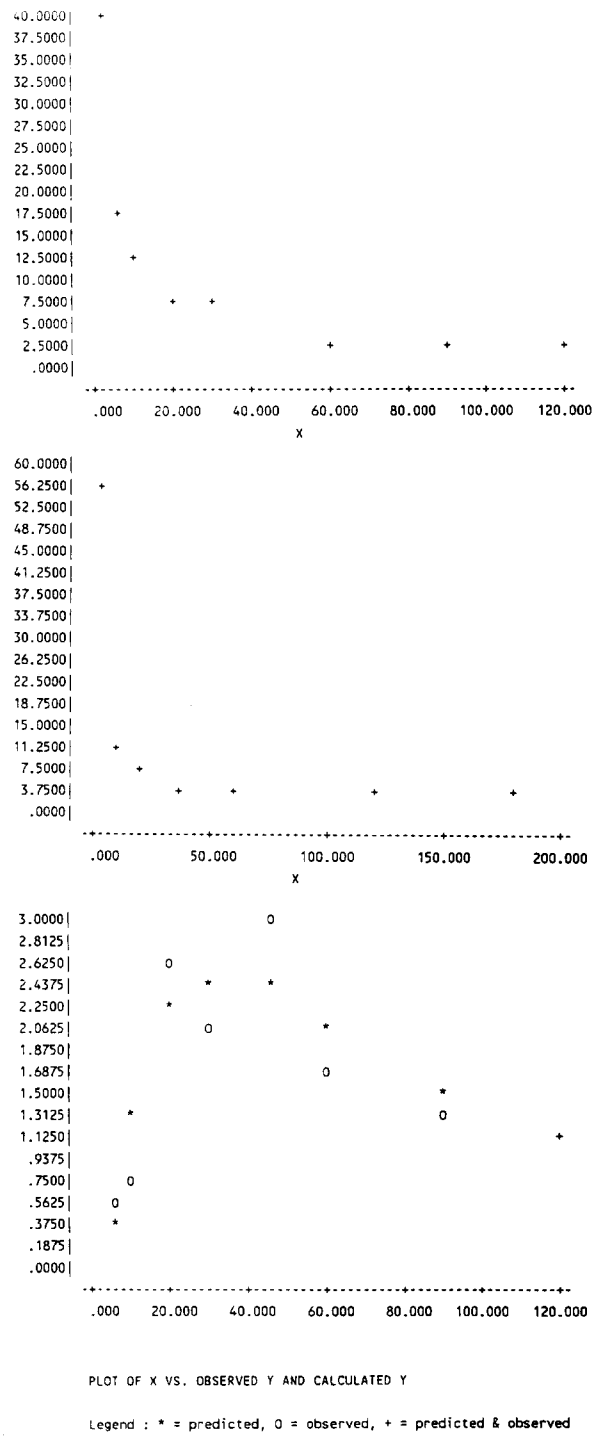

Fig. 2. Plot of observed and calculated values for mean of concentrations of EDU in blood (Y axis) after a 100 mg/kg iv bolus injection into mice (A), rats (B), and after an oral dose (C) vs. time (X axis).

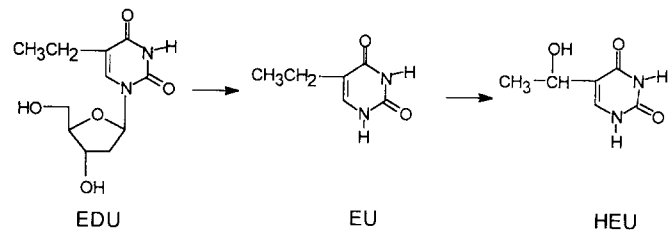

Fig. 3. Metabolism of EDU in mice and rats.

elimination half-life for EDU (24.1 min) is considerably longer than the reported value of 4.3 min for (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) (Samuel et al., 1986). The rapid in vivo clearance of EDU from blood corresponds to its in vivo distribution into the body, extraction by other tissues such as liver, renal clearance and metabolism to EU and HEU (Cheraghali et al., 1994a, Cheraghali et al., 1994c).

The biotransformation of EDU in rats and mice were similar. EDU was rapidly metabolized to 5-ethyluracil (EU) by pyrimidine nucleoside phosphorylases and EU was subsequently hydroxylated to 5-(1-hydroxyethyl)uracil (HEU) (Fig. 3). However, biotransformation of EDU was less extensive in mice than in rats. The AUC for EU, a metabolite of EDU, after iv injection of EDU into mice was lower (249.2 ± 53.8 $\mu g.g^{-1}.min$) compared to that in rats (597.7 ± 354.1 $\mu g.g^{-1}.min$). This observation indicates that renal clearance of EDU is a more important pathway for elimination of EDU in mice. In fact, it was previously observed that an iv dose of ^{14}C-labelled EDU in mice was excreted predominately in urine (Cheraghali et al., 1994b). A similar observation was reported for [2-^{14}C]-5-(2-chloroethyl)-2′-deoxyuridine (CEDU) which bears a close structural similarity to EDU, where a higher percentage of an iv dose was excreted via the urine of mice relative to that of rats (Szinai et al., 1991).

In contrast to iv dosing, EDU followed a one compartment kinetic model in mice after a 100 mg/kg po dose. The concentrations of EDU in blood samples collected after po dosing were best fitted to a one compartment model with first order input (Fig. 2). Based on the fact that EDU showed a very short distribution half-life following an iv bolus injection, it is believed that the distribution phase of EDU is superimposed on the absorption phase after po dosing. The maximum concentration (C_{max}) of EDU was 2.4 ± 0.2 $\mu g/g$ at a T_{max} of 31.1 ± 1.2 min after dosing. However, the blood profile of EU, a metabolite of EDU, after a po dose is significantly different ($P < 0.05$) compared to that after an iv dose. This observation indicates that EDU undergoes catabolic degradation by GI tract phosphorylases and/or similar hepatic enzymes before entering the circulation. These results suggests that some of the EU present in blood samples collected after a po dose arises from the absorption of EU *per se* after cleavage of the glycoside bond present in EDU. The degradation of other pyrimidine nucleosides such as 5′-deoxy-5-fluorouridine and its prodrug trimethoxybenzoyl-5′-deoxy-5-fluorouridine by phosphorylases present in the GI tract, which resulted in the production of 5-fluorouracil, was reported previously (Ninomiya et al., 1990).

EDU showed a 49% bioavailability in male Balb-C mice, which falls in the mid-range of reported values for other antiviral nucleosides. Ganciclovir, for example,

showed an oral bioavailability as low as 6%, whereas AZT showed an oral bioavailability as high as 63% (Morse et al., 1993, Klecker et al., 1987).

In conclusion, this study shows that the pharmacokinetic parameters for EDU in mice and rats are substantially different, and that phosphorolysis of EDU is less extensive in mice than in rats. EDU undergoes glycosidic bond cleavage in the GI tract and/or presystemic degradation before entering the general circulation. However, EDU showed an f value in the upper range of values reported for antiviral nucleosides. Since EDU is already marketed as a topical dosage form for treatment of herpes manifestations in some countries (De Clercq and Walker, 1986), it would be worthwhile to determine the pharmacokinetic parameters for EDU in humans and to compare these results with the reported values for other antiherpetic nucleosides such as acyclovir.

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