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Pharmacokinetics and toxicity of the human immunodeficiency virus inhibitor 1-ethoxymethyl-6-phenylselenenyl-5-ethyluracil in rodents

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Abstract

1-(Ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil (E-EPSeU) has been shown to exhibit potent and selective activity against human immunodeficiency virus type 1 in vitro. The pharmacokinetics of E-EPSeU were characterized after intravenous administration of 5, 10 and 15 mg/kg to rats. Plasma and urine concentrations of E-EPSeU were determined by HPLC. The plasma protein binding of E-EPSeU averaged 86 ± 4% and the blood : plasma concentration ratio was unity. E-EPSeU concentrations after the 5 mg/kg dose were too low to reliably characterize the pharmacokinetics. The pharmacokinetics of E-EPSeU were independent of dose over the range of 10–15 mg/kg. Plasma concentrations of E-EPSeU declined in a bi-exponential manner with terminal half-life of 0.45 ± 0.12 h (mean ± S.D.). The steady-state volume of distribution was 0.091 ± 0.031 l/kg, suggesting the compound distributed primarily into blood. The systemic clearance (0.63 ± 0.13 l/h/kg) was moderate and limited, in part, by protein binding. No parent compound was detected in urine. E-EPSeU-related toxicities were observed at high doses. One rat, out of 5, died 4 h after 15 mg/kg of E-EPSeU was administered and two rats administered 20 and 25 mg/kg died within 1 h. Two mice, out of 5, administered 30 mg/kg/day of E-EPSeU intraperitoneally for 6 days died during the experiment, while significant loss of body weight was observed in the surviving mice. However, body weight of the surviving mice returned to control values within 2 weeks after E-EPSeU treatment was stopped. While toxicities were observed after

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high doses of E-EPSeU, effective anti-HIV E-EPSeU plasma concentrations were achieved without toxicities by administering lower doses.

Keywords: 1-(Ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil (E-EPSeU); Antiviral action; Pharmacokinetics; Toxicity

1. Introduction

Since 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (HEPT), was first reported as an inhibitor of human immunodeficiency virus type 1 (HIV-1) by Miyasaka et al. (1989), a number of acyclouridine derivatives have been developed and shown to have potential for the treatment of AIDS (Baba et al., 1991, 1989; Tanaka et al., 1992, 1991a,b). These compounds selectively inhibit HIV-1 through a mechanism somewhat different from other nucleoside analogues, such as AZT, in that they do not need to be phosphorylated in order to inhibit the HIV reverse transcriptase (Tanaka et al., 1991c). Furthermore, these compounds are not cross-resistant with AZT (Baba et al., 1990), suggesting that they may be effective in treating the patients affected with AZT-resistant HIV strains. Later, acyclic 6-(phenylselenenyl)pyrimidine nucleosides were synthesized utilizing selenium instead of sulfur (Goudgaon and Schinazi, 1991). These compounds not only inhibited HIV-1, but also HIV-2, in primary human lymphocytes. Recently, a novel class of non-nucleoside HIV inhibitors, 1-(ethoxymethyl)-6-(phenylselenenyl)pyrimidines (Goudgaon et al., 1992), in which a hydrogen is substituted for the primary hydroxyl group in the acyclic side chain, have been reported to inhibit HIV-1 replication in primary human lymphocytes at nanomolar concentrations. Of these compounds, 1-(ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil (E-EPSeU), exhibited potent activity selectively against HIV-1-infected peripheral blood mononuclear cells and HT4-6C cells with no observed cytotoxicities. E-EPSeU can inhibit HIV-1 reverse transcriptase, however, this may not be its only mechanism of action since the inhibition of virus replication of E-EPSeU was much greater than the effect on reverse transcriptase. Similar observations have been reported for other non-nucleoside reverse transcriptase inhibitors (Merluzzi et al., 1990; Baba et al., 1991, 1992). Virtually nothing is known about the in vivo disposition of this compound as a potential anti-HIV compound. The purpose of this study was to characterize the preclinical pharmacokinetics of E-EPSeU in rats and to investigate the effects of dose on drug disposition. Furthermore, the toxicity of the compound in mice and rats is reported.

2. Materials and methods

2.1. Materials

E-EPSeU and 5-carboranyl-2′-deoxyuridine (CDU) were synthesized following literature procedures (Goudgaon et al., 1991; Yamamoto et al., 1992; Schinazi et al., 1994). The chemical purity of the compounds determined by spectral and HPLC analysis, was greater than 98%.

2.2. Animals

Fifteen adult male Sprague–Dawley rats, weighing 280–360 g (Charles River, Wilmington, MA) and 15 6-week-old female ND4 Swiss–Webster mice, weighing 18–22 g (Harland Sprague Dawley, Inc., Dublin, VA) were used in this study. Animals were housed in a 12-h light/12-h dark cycle with controlled temperature (22°C) for an acclimation period of 7 days before the experiment. On the day before the study, the rats underwent cannulation of the right external jugular vein under ketamine : acepromazine : xylazine (50 : 3.3 : 3.4 mg/kg) anesthesia. Food was withdrawn the night before the experiment; however, water was allowed ad libitum. Free access to food was resumed 4 h after dosing. Rats were placed in metabolism cages during the experiment to facilitate urine collection.

Rats (5 per group) were randomly administered 5, 10 or 15 mg/kg E-EPSeU dissolved in 100 μl DMSO over 1 min via the cannula. Blood samples were collected prior to the administration, 0.08, 0.167, 0.25, 0.5, 0.75, 1.5, 2, 2.5, 3, 4, and 6 h after dosing from the cannula into the heparinized tubes. An equal volume of 0.9% saline was injected into the cannula to replace the blood volume. Blood samples were immediately centrifuged and plasma was frozen at −70°C until analysis. Preliminary studies indicated no adsorption of E-EPSeU to the cannulas. Urine was collected for 24 h, urine volume was measured and samples were frozen at −70°C until analysis.

Five weight-matched mice per group were administered 30 mg/kg/day of E-EPSeU dissolved in 100 μl DMSO, 100 μl DMSO or 100 μl phosphate-buffered saline (PBS) intraperitoneally for 6 days. The body weight of each mouse was measured before the experiment and on days 7, 14, 21 and 28 of the experiment. Analysis of variance was used to assess the influence of E-EPSeU on the mouse body weight. A probability level of less than 0.05 was considered statistically significant.

2.3. Protein binding and blood/plasma concentration ratio

Protein binding was determined in duplicate by equilibrium dialysis (Boudinot et al., 1984) over an E-EPSeU concentration range of 0.5–100 μg/ml. To determine the distribution of E-EPSeU into red blood cells, the compound was added to heparinized whole blood to yield concentrations of 0.1, 10, 100 and 200 μg/ml. Samples were equilibrated in a 37°C shaking water bath for 2 h. Half of each sample was centrifuged immediately and plasma was frozen at −70°C until analysis. Red blood cells in the remaining whole blood samples were hemolyzed by freezing and thawing three times. The samples were then centrifuged and the supernatant was separated and frozen at −70°C for E-EPSeU determination.

2.4. Assay methodology

Concentrations of E-EPSeU in blood, plasma and urine were determined by high-performance liquid chromatography (HPLC). CDU served as the internal standard. To measure E-EPSeU concentrations in blood and plasma, 100 μl sample with 50 μl CDU (10 μg/ml) as internal standard were placed in tubes followed by 3 ml methylene

chloride. The extraction tubes were shaken for 15 min and then centrifuged at 8000 g for 5 min. The organic phase was transferred to a clean tube and dried under a stream of nitrogen gas at ambient temperature. The residue was reconstituted in 250 μl mobile phase and 20–100 μl sample was injected onto the HPLC. Preliminary studies had indicated that the stability of the compound was not affected by the extraction procedure. The presence of E-EPSeU in urine was determined by diluting urine samples 1 : 20 with water and 50 μl internal standard was mixed with 100 μl diluted urine sample. An aliquot of 100 μl of sample was injected onto the column. The HPLC separation was achieved with a Hypersil ODS (C_{18}) reversed-phase column (4.6 mm $\times$ 15 cm; 5 μm particle size; Alltech Associates, Deerfield, IL) using a mobile phase of 40% acetonitrile in 0.03 M sodium acetate, pH 6.8, at a flow rate of 2.0 ml/min. E-EPSeU was quantitated at a UV wavelength of 264 nm with a detector range setting of 0.005 absorbance units, full scale. The retention times for internal standard, CDU, and E-EPSeU were 4.7 and 6.7 min, respectively.

Calibration standards ranging from 0.1 to 200 μg/ml for E-EPSeU were prepared in blank rat plasma and urine. Concentrations of E-EPSeU were determined from the standard curve of peak area ratio of the drug : internal standard versus standard E-EPSeU concentrations. Least-squares regression was used to generate the slopes and intercepts using a weighing factor of $1/x^2$. Standard curves were linear in the range of 0.1–200 μg/ml of the compound and the lower limit of quantitation was 0.1 μg/ml (10 ng). The intra- and interday coefficients of variation for the assay were less than 10% for all concentrations. The extraction recovery of E-EPSeU was 88 $\pm$ 5%.

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using non-compartmental analysis. Area under plasma concentration–time curves (AUC) and first non-normalized moment (AUMC) were determined by Lagrange polynomial interpolation and integration from time zero to the last sample time (Rocci et al., 1983) with extrapolation to time infinity using the NONLIN (Metzler et al., 1974) least-squares terminal slope (λ_z). Reciprocal plasma concentration values (1/C) were found to be appropriate as weighing factors for generation of a normal distribution of residuals in NONLIN. Systemic clearance (CL_T) was calculated from dose/AUC, mean residence time (MRT) from AUMC/AUC, steady-state volume of distribution (V_{ss}) from $CL_T \times MRT$, and half-life ($t_{\frac{1}{2}}$) was calculated as $0.693/\lambda_z$. Statistical analysis comparing pharmacokinetic parameters of E-EPSeU after the 10 and 15 mg/kg intravenous doses was performed using the t-test. A probability level of less than 0.05 was considered statistically significant.

3. Results and discussion

Plasma concentration profiles of E-EPSeU for the three doses are depicted in Fig. 1. As evident, the disposition of E-EPSeU following the 5 mg/kg dose could not be reliably described since plasma concentrations in the terminal phase fell below the limitation of quantitation of the assay before complete characterization. Thus, the

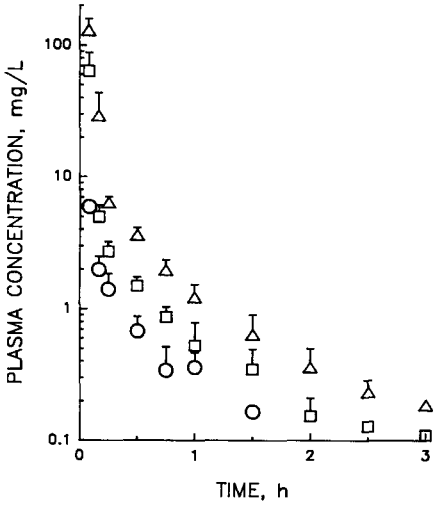

Fig. 1. Mean (±S.D.) plasma concentrations of 1-(ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil (E-EPSeU) after intravenous administration of 5 (○), 10 (□) and 15 (△) mg/kg to rats.

pharmacokinetic parameters for the 5 mg/kg dose are not presented. At doses of 10 and 15 mg/kg, E-EPSeU plasma concentrations declined in a bi-exponential manner with a short terminal half-life. Plasma protein binding of E-EPSeU was independent of concentration averaging 86 ± 4%. In vitro studies demonstrated that whole blood and plasma concentrations were virtually identical, indicating distribution into red blood cells by passive diffusion.

Pharmacokinetic parameters of E-EPSeU following intravenous administration of 10 and 15 mg/kg E-EPSeU are presented in Table 1. The AUC increased proportionally with dose. The terminal half-life was similar, averaging 0.37 ± 0.083 (mean ± SD) and 0.52 ± 0.11 h after 10 mg/kg and 15 mg/kg, respectively. Systemic clearance (CL_T) values following the 10 and 15 mg/kg doses were moderate, averaging 0.70 ± 0.12 and 0.56 ± 0.10 l/h/kg, respectively. Unbound clearance of E-EPSeU averaged 4.5 ± 0.93

Table 1
Pharmacokinetic parameters of 1-(ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil following single-dose intravenous administration of 10 and 15 mg/kg to rats

Parameter	AUC (mg/h/l)	$t_{\frac{1}{2}}$ (h)	MRT (h)	CL_T (l/h/kg)	V_{ss} (l/kg)
10 mg/kg	14.80 ± 2.79 [a]	0.37 ± 0.083	0.13 ± 0.03	0.70 ± 0.12	0.091 ± 0.027
15 mg/kg	27.50 ± 4.20 [a]	0.52 ± 0.11	0.16 ± 0.04	0.56 ± 0.10	0.092 ± 0.038
Mean		0.45 ± 0.12	0.15 ± 0.034	0.63 ± 0.13	0.092 ± 0.032

Note: AUC, area under the plasma concentration–time curve; $t_{\frac{1}{2}}$, half-life; MRT, mean residence time; CL_T, systemic clearance; V_{ss}, steady-state volume of distribution; Values are mean ± S.D. for 5 rats.
[a] Statistically significant difference ($P < 0.05$).

l/h/kg for the two doses. These clearance values suggest that E-EPSeU can be considered a moderate extraction drug whose clearance was limited, in part, by plasma protein binding. No unchanged compound was detected in urine 24 h after intravenous administration of the three doses. In a further study, rat bile was collected through a bile duct cannula after intravenous administration of 10 mg/kg E-EPSeU. The biliary excretion rate paralleled plasma concentrations; however, only 0.04% of the dose was excreted in the bile over 8 h following E-EPSeU administration. The steady-state volume of distribution values after administration of 10 and 15 mg/kg were virtually identical, averaging 0.091 ± 0.027 and 0.092 ± 0.038 l/kg, respectively. This small V_{ss} suggests that the compound was confined primarily in the blood with only minimal distribution to extravascular spaces. No statistically significant differences were found in pharmacokinetic parameter values between the doses. Therefore, the disposition of E-EPSeU was independent of dose over the range of 10–15 mg/kg. Mean pharmacokinetic parameters for the two doses are also shown in Table 1.

The half-life values (0.45 h) for E-EPSeU were similar to those reported for other non-nucleoside reverse transcriptase inhibitors in preclinical studies. Half-lives of a series of HEPT derivatives administered orally to rats ranged from 0.14 to 0.94 h (Baba et al., 1990). Following intravenous administration of [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methyl-thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (TSAO-T) to rats, a half-life value of 0.37 h was reported (Balzarini et al., 1993b). Although half-life values for these non-nucleoside reverse transcriptase inhibitors were similar, differences were noted in other pharmacokinetic parameters. The volume of distribution of E-EPSeU was low compared to that of HEPT derivatives and TSAO-T. The distribution of E-EPSeU was limited primarily to vascular spaces. Total clearance of E-EPSeU was also lower than the clearance of the other compounds. However, clearance of E-EPSeU based on unbound plasma concentrations of the compound was within the range of total clearance values for HEPT derivatives and TSAO-T. The distribution and elimination of E-EPSeU were restricted, in part, by a relatively high degree of plasma protein binding. The protein binding of the other non-nucleoside reverse transcriptase inhibitors was not reported.

E-EPSeU showed potent activities against HIV-1 without cytotoxicity in primary human lymphocytes in vitro (Goudgaon et al., 1992) with a relatively low EC_{50} of approximately 10 μg/l. In this study, no apparent toxicities were observed in rats following the 5 and 10 mg/kg doses. However, after 15 mg/kg E-EPSeU, shortness of breath was noticed in two rats, and one of the rats died 4 h after the dose was administered. In a preliminary study, rats died within 5 min to 1 h following the administration of 20 and 25 mg/kg E-EPSeU. Secretions containing blood from both the nose and mouth were observed at the time of death. These results suggest that the compound was highly toxic to the rats at doses greater than 15 mg/kg when administered as an intravenous bolus. The mechanism of this toxicity was not elucidated; however, it may be related to the high initial plasma E-EPSeU concentrations observed in the distribution phase after intravenous bolus administration (Fig. 1). Plasma concentrations of E-EPSeU after intravenous administration of 10 and 15 mg/kg were significantly greater than the EC_{50} (10 μg/l) of the compound in vitro. In fact, after the dose of 5 mg/kg of E-EPSeU, plasma concentrations were greater than 100 μg/l for

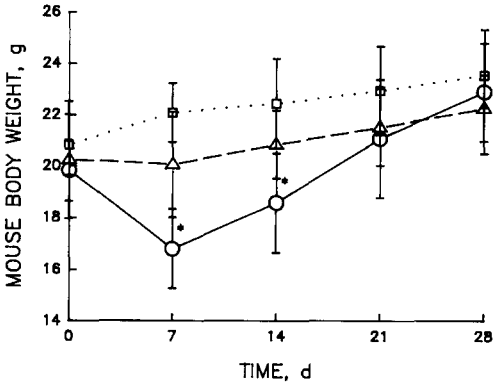

Fig. 2. Mean (±S.D.) mouse body weight after intraperitoneal administration of 30 mg/kg/day of 1-(ethoxymethyl)-6-(phenylselenenyl)-5-ethyluracil (E-EPSeU) (○), 100 μl/day DMSO (△) and 100 μl/day PBS (□) for 6 days. * Statistically significant difference ($P < 0.05$).

1.5 h (Fig. 1), suggesting that HIV inhibitory plasma concentrations of E-EPSeU could be accomplished in vivo by administering non-toxic lower doses.

Further studies were conducted in mice to assess the toxicity of E-EPSeU after multiple-dose administration. Mouse body weight before and after intraperitoneal administration of 30 mg/kg/day of E-EPSeU for 6 days is delineated in Fig. 2. Control groups for the vehicle used are also included. No statistically significant differences in body weight between the mice receiving either DMSO or PBS were noted. Two of the mice receiving E-EPSeU died (on day 4 and day 9) during the experiment. Significant loss of body weight on day 7 and day 14 was observed in surviving mice treating with E-EPSeU. However, after E-EPSeU treatment was terminated, the mice recovered, and their mean body weight increased to a similar weight as the control groups. These precursory results suggest that the E-EPSeU toxicity is acute and was reversible in surviving mice.

Another common problem associated with non-nucleoside reverse transcriptase inhibitors is a rapid emergence of drug-resistant HIV-1 variants both in cell culture and in clinical trials (Merluzzi et al., 1990; Buckheit et al., 1993; Balzarini et al., 1993a; Byrnes et al., 1993; Klein et al., 1993; Maass et al., 1993; Richman, 1994). Indeed, HIV-1 resistance to E-EPSeU has recently been reported to occur rapidly in vitro (Nguyen et al., 1994). The clinical therapeutic efficacy of E-EPSeU, thus, is likely to be limited if used as monotherapy. However, E-EPSeU is highly active against HIV-1 and may potentially be useful in combination with other reverse transcriptase inhibitors, possibly at reduced dosage regimens.

In summary, the pharmacokinetics of E-EPSeU in rats were characterized by a moderate clearance, limited in part by plasma protein binding, and a small volume of distribution. While toxicities were observed after high doses of E-EPSeU, effective anti-HIV E-EPSeU plasma concentrations were achieved without toxicities by administrating lower doses. Further studies assessing the in vivo antiviral activity and toxicity of E-EPSeU in combination with other antiviral agents are warranted.

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