Evaluation of (5R,6R)-5-Bromo-6-Ethoxy-5-Ethyl-5,6-Dihydro-2'-Deoxyuridine as a Brain-Targeted Prodrug of 5-Ethyl-2'-Deoxyuridine

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ABSTRACT

(+)-Trans-(5R,6R)-5-bromo-6-ethoxy-5-ethyl-5,6-dihydro-2'-deoxyuridine [(5R,6R)-BEEDU], a potential brain-targeted prodrug of 5-ethyl-2'-deoxyuridine (EDU), was synthesized by the regiospecific addition of BrOEt to the 5,6-olefinic bond of EDU. (5R,6R)-BEEDU is more lipophilic (log P = 0.04) than EDU (log P = -1.09). In vitro incubation of (5R,6R)-BEEDU with rat whole blood and brain homogenate resulted in a 53% and 16% conversion, respectively, to EDU. In contrast, (5R,6R)-BEEDU was not converted to EDU upon incubation with glutathione (GSH) at 37°C for 36 hours. After i.v. injection into rats, (5R,6R)-BEEDU was rapidly converted to EDU, which was then further metabolized like EDU. However, (5R,6R)-BEEDU provided a substantially higher concentration of EDU in blood, relative to that when EDU was injected. A biodistribution study of [4-14C]-(5R,6R)-BEEDU in Balb/c mice showed that (5R,6R)-BEEDU provided significantly higher (P < 0.05) radioactivity levels in brain samples at 8, 18 and 30 min post injection than observed after injection of [4-14C]-EDU. The higher radioactivity levels in liver samples after injection of [4-14C]-(5R,6R)-BEEDU, relative to those after [4-14C]-EDU, indicates that the 5,6-dihydro derivative undergoes a higher hepatic extraction than EDU. Clearance of radioactivity from blood and excretion into urine, after injection of [4-14C]-EDU, was much faster than that after injection of [4-14C]-(5R,6R)-BEEDU. Iran. Biomed. J. 2: 105-113, 1998

Keywords: 5-Ethyl-2-deoxyuridine, Dihydro prodrugs, Herpes Simplex Virus, Brain-targeted

INTRODUCTION

Herpes simplex virus type 1 and type 2 cause a variety of diseases in humans. The usual manifestations of herpes simplex virus infection are orolabial, genital, and anorectal mucocutaneous disease, esophagitis, and less commonly, encephalitis. Although the majority of manifestations caused by herpes simplex virus are subclinical, these diseases are significant causes of mortality and morbidity in human immunodeficiency virus-infected patients [1]. Herpes simplex encephalitis is a severe brain infection that can result in mortality and morbidity when untreated. Estimates of the incidence of herpes simplex encephalitis range from two to four per million populations per year, with approximately 30% of these occurring in childhood [2-5].

Although antitherpetic drugs are now available for the treatment of severe cases of herpes simplex virus infection, and new drugs are being tested, most do not attain therapeutic concentrations in the brain. The pyrimidine nucleoside, 5-ethyl-2'-deoxyuridine (EDU), has shown promising activity against several strains of herpes simplex virus [6-7]. EDU is an effective, nontoxic [8], antiviral agent for the topical treatment of herpes simplex virus infections. It is reported to increase the survival time of herpes simplex virus-encephalitic mice [7, 9-10], although this is not an approved indication for clinical use. Despite its efficacy, EDU possesses some undesirable properties, such as rapid elimination after i.v. administration of the drug [11-13], rapid enzymatic degradation to the nonvirostatic metabolites 5-ethyluracil (EU) and 5-(1-hydroxyethyl)uracil (HEU), and low lipophilicity that hampers its passage across the blood-brain-barrier.

The ability of a drug to traverse the blood-brain-barrier and enter the brain is dependent, at least in

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part, upon its lipid solubility. It has been demonstrated that the lipid/water partition coefficient of a compound is generally predictive of its blood-brain-barrier penetration characteristics [13-15]. Thus, application of the lipophilic-prodrug concept to the design of antiviral nucleoside analogs has been an attractive approach to improve their in vivo biodistributions.

One potential method to increase the brain uptake of a pyrimidine nucleoside is reduction of the 5,6-double bond, which substantially increases the lipophilicity of the nucleoside [12]. In an attempt to prevent the problems associated with the delivery and enzymatic deactivation of EDU, we previously reported the synthesis and biotransformation of a group of 5-halo-5-ethyl-6-methoxy-5,6-dihydro derivatives of EDU [12]. Although these 5,6-dihydro derivatives improved the pharmacokinetic parameters of EDU, they did not provide significantly higher brain concentrations than did EDU [15].

We now report the synthesis, radiolabeling, biodistribution of (5R,6R)-5-bromo-5-ethyl-6-ethoxy-5,6-dihydro-2'-deoxyuridine (BEEDU), a potential brain-targeted prodrug for EDU.

**MATERIAL AND METHODS**

**Chemistry.** Melting points were determined with a Buchi capillary apparatus and are 1 uncorrected. Nuclear Magnetic Resonance spectra (\( ^1 \text{H NMR}, ^{13} \text{C NMR} \)) were determined on a Bruker AM-300 spectrometer using Me4Si as an internal standard (\(^1\)H NMR). The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D\(_2\)O.

\(^{13} \text{C NMR spectra were acquired using the J modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbons appear as negative peaks. Preparative thin layer chromatography (PTLC) was performed using Whatman PLK5F plates (1.0 mm in thickness) and silica gel column chromatography was carried out using Merck 7734 silica gel (100-200 \( \mu \) particle size). 5-Ethyl-2'-deoxyuridine and 5-ethyluracil were purchased from the Sigma Chemical Co. 5-(1-Hydroxyethyl)uracil was prepared using a literature procedure [16].**

\((+)-\text{Trans-} (5R,6R)-5\text{-bromo-5-ethyl-6-ethoxy-5,6-dihydro-2'-deoxyuridine} \ [ (5R,6R)-\text{BEEDU},1 ] \) and \((-)-\text{Trans-} (5S,6S)-5\text{-bromo-5-ethyl-6-ethoxy-5,6-dihydro-2'-deoxyuridine} \ [ (5S,6S)-\text{BEEDU},2 ] \). A freshly prepared solution of ethyl hypobromite was added dropwise to a solution of EDU (0.51 g, 2.0 mmol) in ethanol (50 mL) at 5°C with stirring until the yellow color persisted. The reaction was allowed to proceed with stirring at 25°C for 15 min prior to neutralization with ethanolic sodium hydroxide. Removal of the solvent in vacuo, and purification of the residue obtained by separation on a silica gel column using chloroform-methanol (95:5, v/v) as eluent, afforded 2 (76 mg, 10%) as a viscous syrup. Further elution with the same solvent yielded 1 (418 mg, 55%) as a viscous syrup.

**Radiochemistry.** [\(^{14} \text{C}\)]-5-Ethyl-2'-deoxyuridine (EDU) was synthesized in high specific activity [2GBq (54 mCi)/mmol] from [\(^{14} \text{C}\)]-BaCO\(_3\) according to the procedure previously reported [16]. The identity of each [\(^{14} \text{C}\)]-diastereomer 1 and 2 was determined by comparison of its thin layer chromatographic (TLC) R\(_f\) value and high performance liquid chromatography (HPLC) retention time on a reverse-phase column, with that of the corresponding authentic unlabelled compound. HPLC separations were performed using a preparative Whatman Partisil M9 10/25 ODS reverse phase column. Radioactivity was determined by liquid scintillation counting in Aquasol fluor (New England Nuclear) using a Beckman LS 9000 liquid scintillation counter. The specific activity of [\(^{14} \text{C}\)]-EDU and each [\(^{14} \text{C}\)]-diastereomer 1 and 2 was determined by counting a known amount of each radiolabelled compound, after purification by HPLC, in a liquid scintillation counter.
yield, > 96% radiochemical purity, specific activity = 2 GBq (54 mCi)/mmol and [4-14C]-5(5S,6S)-BEEDU, eluting at 18.0 minutes [1.0 MBq (27 µCi)], 11% chemical and radiochemical yield, > 92% radiochemical purity, specific activity = 2 GBq (54 mCi)/mmol.

**Biotransformation in rats.** Male Sprague-Dawley rats, 380-400 g in weight, were purchased from the University of Alberta Health Science Laboratory Animal Services Facility. Three animals were used in each experiment. The biotransformations of (5R,6R)-BEEDU and EDU were investigated in rats having an implanted jugular vein catheter. The test compound [(5R,6R)-BEEDU or EDU] was injected (300 µl) into the jugular vein catheter using a dose of 0.55 mmol/kg dissolved in DMSO-water (50:50 v/v). Blood samples (200 µL) were collected via the catheter for up to 4 h post injection of the test compound. The catheter was washed by injection of 0.4 mL of heparinized normal saline into the jugular vein catheter following each dosing and sampling procedure. Immediately after sampling each blood sample was mixed with methanol (2 mL) in a mechanical shaker for 15 min. This mixture was centrifuged for 10 min at 1000 xg and the supernatant fraction was then 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 was dried under a stream of nitrogen gas and the residue obtained was dissolved in 200 µl methanol.

A 20 µL aliquot of this solution was then subjected to quantitative high performance liquid chromatography (HPLC) analysis using an HPLC system comprised of a Waters Baseline 810 computer program running on a 486/33 MHz computer, Waters Model 501 pumps, Waters Model U6K injector and Waters Model 486 variable wavelength absorbance detector. All separations and quantitative analyses were carried out on a Waters Radial-Pak C18 reverse phase cartridge column (10 µ, 8 mm x 10 cm) at 25°C using a gradient of acetonitrile (0% for the first 6 min → 17% for the next 11 min → 0% for the reminder of the HPLC separation) in water (v/v) during a 25 min time interval, with a flow rate gradient of 1.5 mL/min for the first 6 min → 2.5 mL/min for the next 19 min during the separation, with UV detection at 230 nm. The identity of each compound present in the sample was determined by comparison of its retention time to that of an authentic sample. The concentration of each compound in blood, as a function of time, was plotted using the SigmaPlot program (Jandel Scientific). The pharmacokinetic parameters were calculated using the Lagran program (C. Ediss, University of Alberta). The partition coefficient (P) of (5R,6R)-BEEDU and EDU, were determined using methods described previously [12].

**Biodistribution study.** Male Balb-C mice weighing 18-21 g were used for biodistribution studies of (5R,6R)-BEEDU and EDU. The biodistributions of [4-14C]-5(5R,6R)-BEEDU, and [4-14C]-EDU were determined after injection (0.2 mmol/kg) of the test compound [126 kBq (3.4 µCi)] dissolved in 100 µL DMSO-water (50:50 v/v) into the lateral tail vein of mice. Animals were sacrificed and tissues were dissected at 3, 8, 18, 30, 60, and 120 min time periods post injection. The weights of samples collected from each tissue, which included muscle, bone (femur), brain, lung, fat and liver, were limited to a maximum 180 mg of wet tissue, or 100 µL of blood and urine, to ensure complete combustion and quantitative trapping of [14C]-OO2. Samples were air dried at room temperature for at least three days to insure quantitative combustion using an OX-300 Harvey Biological Material Oxidizer. The [14C]-carbon dioxide produced upon combustion of the radioactive tissue samples was trapped in 15 mL of Carbon-14 Cocktail (Harvey Co.). These solutions were then counted using a Beckman LS 9000 liquid scintillation counter.

**In vitro regeneration of the 5,6-olefinic bond.** Regeneration of the 5,6-double bond in vitro in various media was examined by incubation of 100 µl of a 30mM solution (DMSO:water; 50:50, v/v) of (5R,6R)-BEEDU with 0.5 mL of either normal saline, rat plasma, rat whole blood, rat brain homogenate or 2 molar equivalents of a solution of glutathione (GSH). The brain homogenate was prepared by excising the whole brain from an anesthetized male Sprague-Dawley rat. After rinsing with saline, the brain was homogenized in 5 mL of isotonic saline. (5R,6R)-BEEDU was added to the incubation medium (saline, rat plasma, rat whole blood, rat brain homogenate or GSH) and the mixtures were incubated in a shaker-bath at 37°C. Samples were collected after 3, 8, 18, 35, 60 and 120 min of incubation for HPLC analysis.
RESULTS

Chemistry. Reaction of 5-ethyl-2'-deoxyuridine (EDU) with molecular bromine in ethanol at 25°C afforded the (+)-trans-(5R,6R)-BEEDU (1) and (-)-trans-(5S,6S)-BEEDU (2) diastereomers in 55 and 10% yields, respectively (Scheme 1). The mechanism of formation of these 5-bromo-6-ethoxy-5,6-dihydro derivatives of EDU is thought to proceed via the initial formation of a 5,6-bromonium ion intermediate which is susceptible to regiospecific nucleophilic attack by ethanol at the sterically less hindered C-6 position. The configuration of compounds 1 and 2 at the C-5 and C-6 positions was assigned by comparing the optical rotation and 1H NMR spectral data with that of similar, known compounds [12,17]. The most distinct differences in the 1H NMR spectra for these diastereomers are due to the chemical shifts for the H-1', H-2' and H-2" protons in the sugar ring and the H-6 proton of the base. The methylene protons of the C-5 ethyl substituent are chemically non-equivalent due to the C-5 chiral center. The diastereomers 1 and 2 are stable products that can be separated by silica gel column or HPLC.

Radiochemistry. [4-14C]-(+)-Trans-(5R,6R)-BEEDU and [4-14C]-(−)-trans-(5S,6S)-BEEDU diastereomers of 5-ethyl-2'-deoxyuridine were synthesized by reaction of [4-14C]-EDU with molecular bromine in ethanol at 25°C, in 38 and 11% chemical and radiochemical yield, respectively, with radiochemical purity > 92% and a specific activity = 2 GBq (54 mCi)/mmol. These [4-14C]-5,6-dihydro diastereomers were separated by HPLC using water:acetonitrile (75:25, v/v) as the mobile phase at a flow rate of 2 ml/min.

Biotransformation in rats. The mean concentration-time profiles of (5R,6R)-BEEDU and EDU in rats which received a 0.55 mmol/kg dose of the test compound are summarized in Fig. 1. (5R,6R)-BEEDU showed a very short blood residence time and its concentration in blood samples taken 18 min post i.v. injection was below detection limit (0.4 µg/mL). However, this 5-bromo-6-ethoxy-5,6-dihydro derivative of EDU converted rapidly to EDU after injection and afforded a high concentration of EDU in blood samples (Fig. 1). The area under the blood concentration vs time curve (AUC) for EDU, as a metabolite of (5R,6R)-BEEDU, was much higher (4.25 µmol.h.mL⁻¹) than that observed when an equimolar dose of EDU itself was injected (1.83 µmol.h.mL⁻¹) (Table 1). As expected, the metabolism of (5R,6R)-BEEDU, after conversion to EDU, was similar to that of EDU. 5-Ethyluracil (EU) and 5-(1-hydroxyethyl)uracil (HEU) were detected as secondary metabolites of (5R,6R)-BEEDU (Scheme 2). The AUC of EU, a metabolite of EDU, was also higher after injection of (5R,6R)-BEEDU (8.71 µmol.h.mL⁻¹) than after injection of EDU (4.35 µmol.h.mL⁻¹).

In vitro regeneration of the 5,6-olefinic bond. Regeneration of the 5,6-double bond in (5R,6R)-BEEDU was determined following incubation in several media (Fig. 2). (5R,6R)-BEEDU did not undergo conversion to EDU in normal saline or upon incubation with 2 molar equivalents of glutathione (GSH) (37°C; 36 h), but it was converted to EDU after a 2 hour incubation with rat plasma (8%), rat brain homogenate (16%) and rat whole blood (53%).

Biodistribution in mice. The biodistributions of [4-14C]-(+)-trans-(5R,6R)-5-bromo-5-ethyl-6-ethoxy -5,6-dihydro-2'-deoxyuridine and [4-14C]-EDU were
investigated after injection into the lateral tail vein of mice (Tables 2-3 and Fig. 3). Injection of [4-\textsuperscript{14}C](5R,6R)-BEEDU provided radioactivity levels in brain samples that were higher (P < 0.05) than after injection of [4-\textsuperscript{14}C]-EDU at 8, 18 and 30 min post injection. Although the amount of radioactivity in fat samples after injection of [4-\textsuperscript{14}C](5R,6R)-BEEDU was only slightly different from those after injection of [4-\textsuperscript{14}C]-EDU, liver samples taken from animals injected with [4-\textsuperscript{14}C](5R,6R)-BEEDU showed substantially higher radioactivity levels than after injection of [4-\textsuperscript{14}C]-EDU. This indicates that (5R,6R)-BEEDU undergoes a higher hepatic extraction than EDU. However, clearance of radioactivity from blood and excretion into urine, after injection of [4-\textsuperscript{14}C]-ETRU, was much faster than that after injection of [4-\textsuperscript{14}C](5R,6R)-BEEDU (Tables 2-3). This observation indicates that there are substantial differences between the (5R,6R)-BEEDU and EDU with respect to excretion and/or metabolism.

**Scheme 2.** Proposed metabolic pathway for (5R,6R)-BEEDU.

**Scheme 1.** Synthesis of the (5R,6R)-1 and (5S,6S)-2 5-bromo-6-ethoxy-5,6-dihydro derivatives of 5-ethyl-2'-deoxyuridine (EDU).
DISCUSSION

5-Ethyl-2'-deoxyuridine (EDU) is an anti-herpes simplex virus infections which is rapidly inactivated \textit{in vivo}, undergoing rapid metabolism to inactive 5-ethyluracil (EU) in the presence of pyrimidine phosphorylases, followed by hydroxylation of the 5-ethyl group to produce 5-(1-hydroxyethyl)uracil (HEU) (Scheme 2). Due to its low lipophilicity and apparent absence of a high capacity nucleoside transporter in the brain, EDU does not cross the blood-brain-barrier effectively.

It has recently been shown that 5,6-dihydro derivatives of other pyrimidine nucleosides exhibit interesting characteristics as prodrugs to their 5,6-olefinic parent nucleosides. For example, it is reported that 5-bromo-5-fluoro-6-methoxy-5,6-dihydro-2'-deoxyuridine acts as a slow releaser of 5-fluoro-2'-deoxyuridine (FUDR) \textit{in vivo} [17]. It has also been shown that 5-halo-6-methoxy-5,6-dihydro derivatives of EDU exhibit improved pharmacokinetic parameters relative to EDU [12]. Based on previous results [12, 15], (5R,6R)-BEEDU is now being investigated as a brain-targeted prodrug of EDU. (5R,6R)-BEEDU is chemically stable, but it converts to the parent drug (EDU) upon incubation with rat brain homogenate. This conversion is essential for trapping the active drug (EDU) in the brain, since without conversion, (5R,6R)-BEEDU would simply egress from the brain through equilibrative diffusion.

In contrast to incubation in whole blood, conversion of (5R,6R)-BEEDU to EDU following incubation with rat plasma was minimal. This observation underlines the crucial role of blood cells in this conversion, and perhaps in further metabolism, of (5R,6R)-BEEDU to EDU.

It is reported that 5-bromo-5-fluoro-5,6-dihydro-2'-deoxyuridine underwent up to 18% conversion to FUDR upon incubation with GSH after 24 hours at 35°C [17] and that some degree of regeneration of the 5,6-olefinic bond for 5-bromo-6-methoxy-5,6-dihydro derivatives of 3'-azido-3'-deoxythyridine (AZT) occurred under similar conditions. However, no conversion of (5R,6R)-BEEDU to EDU was observed following incubation with GSH after 36 hours at 37°C. The results of this study and others [17] indicate that the nature of substrates on C-5 of the pyrimidine ring play a crucial role in \textit{in vitro} conversion of 5,6-dihydro prodrugs to their 5,6-olefinic parent drugs upon incubation with GSH and further, that mechanisms other than the thiol-based mechanism exist for elimination of the 5,6-dihydro substrates.

(5R,6R)-BEEDU was rapidly cleared from blood after i.v. injection into rats, and no (5R,6R) BEEDU was detected in blood samples taken 18 min post injection. However, as expected from \textit{in vitro} studies, (5R,6R)-BEEDU was converted to EDU, providing high concentrations of EDU in blood. The AUC (0 \rightarrow last sample) of EDU after injection of (5R,6R)-BEEDU, was significantly higher (4.25 µmol.l.ml$^{-1}$) than after injection of EDU (1.83 µmol.l.ml$^{-1}$). In fact, the concentration of EDU in blood samples taken 3 and 4 h post injection of the prodrug was much higher than after injection of EDU (Fig. 1). AUC(0 \rightarrow last sample) of EU, a metabolite of EDU, was also higher after injection of (5R,6R)-BEEDU compared to that after injection of EDU (Table 1). These observations indicate that although the concentration of pro drug in blood samples taken >18 min post injection was
below the HPLC detection limit (0.4 µg/mL), the prodrug is present in other tissues and acts as slow releaser of EDU into the vascular compartment.

A plausible metabolic pathway for the biotransformation of (5R,6R)-BEEDU is presented in Scheme 2. After conversion to EDU, EU and HEU are produced as secondary metabolites of (5R,6R)-BEEDU. Although 5-(1-hydroxyethyl)-2’-deoxy uridine (HEDU) was detected as a metabolite of 5-halo-5-ethyl-6-methoxy-5,6-dihydro derivatives of EDU after injection into rats [12], HEDU was not detected in blood samples taken from rats receiving (5R,6R)-BEEDU. It was previously postulated that HEDU most likely arises from oxidative attack at C-1 of the 5-ethyl substituent of 5-halo-5-ethyl-6-methoxy-5,6-dihydro derivatives of EDU after injection into rats [12]. Although this was not observed, the difference in the metabolic pathways of (5R,6R)-BEEDU and the 5-halo-5-ethyl-6-methoxy-5,6-dihydro derivatives of EDU may arise from the fact that in vivo conversion of (5R,6R)-BEEDU to EDU is much faster than that of 5-halo-5-ethyl-6-methoxy-5,6-dihydro derivatives.

(5R,6R)-BEEDU exhibited a higher octanol/water coefficient (Log P = 0.04) than EDU (Log P = -1.09) (Table 1). The enhanced lipophilicity of (5R,6R)-BEEDU should enable it to enter cells and cross the blood-brain-barrier more readily than EDU, by diffusion. This higher lipophilicity could contribute to higher radioactivity levels present in brain samples taken after injection of [4-14C]-(5R,6R)-BEEDU. Radioactivity levels were substantially higher than those after injection of [4-14C]-EDU (Tables 2-3 and Fig. 2). The substantial higher levels of radioactivity in muscle after injection of [4-14C]-(5R,6R)-BEEDU relative to those after injection of [4-14C]-EDU, further indicate that the 5,6-dihydro prodrug can readily penetrate other tissues.

The higher levels of radioactivity present in liver samples after injection [4-14C]-(5R,6R)-BEEDU, relative to that of [4-14C]-EDU, indicate that this 5,6-dihydro prodrug undergoes a higher hepatic extraction than EDU. However, the observations that clearance of radioactivity from blood and excretion of radioactivity into urine after injection of [4-14C]-(5R,6R)-BEEDU were much slower than that of [4-14C]-EDU, indicate that the prodrug is exerting a depot effect. The differences between excretion of radioactivity and higher radioactivity levels in muscle sample

### Table 2. Biodistribution of [4-14C]-EDU at 3, 8, 18, 30, 60, and 120 min post injection of 126 kBq (3.4 into tail vein of Balb-C mice. Data are presented as dpm per gram of wet tissue or mL of blood, as the mean ± SEM (n = 3).

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<th>Tissue</th>
<th>3 min</th>
<th>8 min</th>
<th>18 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
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<sup>a</sup>% of injected dose per gram or mL  
<sup>b</sup>Activity as dpm/100 µL of urine, based on content of bladder at time of sacrificing.  
<sup>c</sup>Not determined.
taken after injection of \([4-^{14}C}]\)-(5R,6R)-BEEDU compared to those of \([4-^{14}C}]\)-EDU indicate that prodrug may stay in tissues such as muscle and act as slow releaser of EDU.

It was previously reported that 5-bromo-6-methoxy-5,6-dihydro derivatives of EDU did not provide a significantly higher brain concentration relative to EDU [12] and that they did undergo a slower \(in\) \(vivo\) conversion to EDU [12] relative to the rapid conversion of (5R,6R)-BEEDU observed in this study. However, the current observation that (5R,6R)-BEEDU provided a significantly higher brain concentration at 8, 18 and 30 min post injection indicates that in contrast to its faster conversion into EDU, the increased lipophilicity of (5R,6R)-BEEDU provided higher concentration of prodrug in brain prior to the regeneration of 5,6-olefinic in peripheral tissues. Once the 5,6-olefinic bond is regenerated, antiviral activity, metabolism and excretion kinetics in brain and all other tissues would be the same for the prodrug as for EDU.

In conclusion, the results of the \(in\) \(vivo\) and \(in\) \(vivo\) comparative studies for (5R,6R)-BEEDU and EDU described in this investigation clearly indicate that this 5,6-dihydro compound could serve as a useful brain-targeted prodrug model for EDU.

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