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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of the novel tetrahydroisoquinoline EDL-155 in rats

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Abstract
Purpose Tetrahydroisoquinolines (THIs) have demonstrated anti-cancer activity in rodent models of glioma, a form of brain cancer refractory to therapeutic intervention. In this study, peripheral and cerebrospinal fluid (CSF) pharmacokinetics in rats were determined to assess the drug developability of the novel THI EDL-155 for the treatment of glioma.
Methods Serial blood and CSF samples were collected from rats following intravenous bolus administration of EDL-155 (10–20 mg/kg). Samples were analyzed by LC/MS/MS. Pharmacokinetic analyses using compartmental and noncompartmental methods were performed using the computer program WinNonlin. Plasma protein binding was measured using the charcoal adsorption method. The in vivo efficacy of EDL-155 (i.p. 20 mg/kg twice daily for 7 days) was assessed in rats with stereotactically implanted C6 glioma cells into the caudate.
Results EDL-155 plasma concentration data were described by a one-compartment model. EDL-155 demonstrated rapid clearance (342.5 ± 49.9 ml/min/kg), high volume of distribution (13.0 ± 1.2 l/kg) and a terminal half-life of 23.7 ± 1.5 min. Dose-normalized CSF area under the curve (AUC_{CSF}) as a percentage of peripheral exposure (AUC_{Plasma}) was 1.4%. EDL-155 was highly bound to plasma proteins (>93%). Intracranial tumor volume at 7 days post-implantation was approximately 30% smaller in animals treated with EDL-155 when compared to vehicle control animals (13.2 ± 5.3 mm^3 vs. 18.7 ± 6.3 mm^3; $P = 0.04$).
Conclusion High clearance and extensive protein binding limit the brain availability of EDL-155 following systemic administration. EDL-155 treatment resulted in reduced tumor size despite limited blood brain barrier penetrability, which suggests that analogs with increased metabolic stability and brain penetrability may provide a therapeutic option for primary central nervous system tumors such as glioma. On-going studies are focused on the design, synthesis, and testing of novel analogs based upon these findings.

Keywords Cerebrospinal fluid · Pharmacokinetics · Tetrahydroisoquinoline · Glioma

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Introduction

Brain tumors are one of the most lethal forms of cancer. The incidence of brain tumors is 10–20 per 100,000, leading to 2% of deaths in Western countries[1–3]. Each year, there are 200,000 new brain tumor patients in the

USA alone. Among more than 120 different types of malignant or non-malignant brain tumors, gliomas account for 42% of all brain tumors and 77% of malignant brain tumors with a <10% 2-year survival rate despite great advances in surgery, radiotherapy, and chemotherapy over the last several decades (www.tbts.org). The diffuse nature of gliomas contributes to their refractoriness to surgical and chemotherapeutic intervention. For example, wide tumor dissemination means that surgical resection of the entire tumor is impractical and sustained central nervous system (CNS) distribution of systemically or cerebrally administered chemotherapy is required to kill the tumor. Unfortunately, the achievement of sustained distribution of CNS tumor therapeutics has proved elusive due to the blood brain barrier (BBB) and blood cerebrospinal fluid (CSF) barrier (BCSFB), the combination of which excludes ~100% of macromolecules and more than 98% of all small-molecules from the brain [4].

We have identified EDL-155, a novel isoquinoline derivative, with potent in vitro anti-tumor activity against the rat C6 glioma cell line (IC_{50} = 2.6 ± 0.6 μM) [5]. In addition, EDL-155 exhibited cytotoxicity against the human glioblastoma cell lines T98G and U87 (IC_{50} = 10.2 ± 1.8 μM and 25.9 ± 3.3 μM). The objective of the present study was to determine the peripheral and CSF pharmacokinetics of EDL-155 in rats for an early insight into the drug developability for brain tumors. Systemically administered drugs can reach the CSF either directly via passage across the choroid plexus, or indirectly by passage across the BBB followed by diffusion/convection transport from the interstitial fluid (ISF) to CSF [6]. Thus, CSF was chosen as a surrogate measure of CNS drug availability.

Materials and methods

Materials

EDL-155 and its analog EDL-115 (internal standard) were synthesized in Dr Miller's laboratory [5]. The purity of both compounds was verified to be greater than 95% by NMR. All HPLC reagents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Heparinized normal rat plasma was obtained from Biomeda (Foster City, CA, USA). Dextran-coated charcoal was purchased from Sigma (St. Louis, MO, USA).

EDL-155 pharmacokinetic studies

Male Sprague-Dawley rats (250–275 g) pre-cannulated with jugular and femoral vein catheters were obtained from Charles River Laboratories (Wilmington, MA, USA). Animals obtained for use in the CSF pharmacokinetic study bore an additional cisterna magna cannula for continuous sampling of the CSF. The patency of the jugular and femoral vein catheters was maintained every other day with heparinized glycerol solution (500 IU heparin/ml final solution in 50% glycerol) according to vendor's instructions. Animals were fed a normal laboratory diet, allowed *ad libitum* access to water and maintained on a 12-h light–dark cycle (25°C). Rats (n = 5) used in the peripheral pharmacokinetic study were individually housed in the metabolism cages for the separate collection of urine and feces over the 0 to 24-h post-dose period. CSF pharmacokinetic study rats (n = 6) were individually housed in regular micro-isolator cages.

EDL-155 was dissolved in propylene glycol, diluted with twofold normal saline and centrifuged (10,000 *g*, 25°C for 5 min). Drug concentration of the dosing solution was measured by the LC/MS/MS in order to calculate the actual dose administered. For peripheral pharmacokinetics, fasted rats were given a single intravenous (i.v.) bolus dose of EDL-155 (10 mg/kg) via the femoral vein catheter, and then fed 4 h after dosing. Blood (100 μl) was collected from the jugular vein catheter at timed intervals 5, 15, 30, 45, 60, 75, 90, 120, 180, 240, 480, 600, 1,440 min, into Microtainer® brand tubes with lithium heparin (BD, Franklin Lakes, NJ, USA). Samples were centrifuged (10,000 *g*, 25°C for 2 min) and the plasma was stored at –80°C until analysis. Drug-free urine and feces were collected the day before the drug administration. At 24 h post-dose, feces were collected and extracted by adding deionized water (30 ml) containing 1% formic acid for 1 h. For urine collection, the metabolism cage was thoroughly flushed with deionized water to a collection bottle and the total volume of the urine sample was recorded. All feces and urine samples were centrifuged (25°C, 3,200 *g* for 5 min) and the supernatant was stored at –80°C until analysis. Sample aliquots (10 μl) were mixed with 40 μl of the IS working solution (23.4 ng/ml in acetonitrile containing 1% formic acid) to facilitate plasma protein precipitation. The resultant supernatant was diluted with an equal volume of HPLC-grade water before injection.

For CNS pharmacokinetics, fasted rats were given a single i.v. bolus dose (20 mg/kg) of EDL-155 solution via the jugular vein catheter, and then fed 4 h after dosing. CSF samples (15 μl) were collected into flanged polypropylene vial insert (0.25 ml) from the intracisternal cannulae at timed intervals (20, 40, 60, 80, 100, 120, 150, 180, and 240 min). Immediately after sample collection, 10 μl of each sample was mixed with 5 μl of internal standard solution (11.7 ng/ml of EDL-155 in acetonitrile containing 0.1% formic acid) and then injected onto the LC/MS/MS.

Studies were approved by the Institutional of Animal Care and Use Committee (IACUC) of University of

Tennessee, Memphis. Animal treatment was in accordance with regulations outlined in the Animal Welfare Act (9CFR, Parts 1, 2, and 3) and the conditions specified in the Guide for the Care and Use of Laboratory Animals.

LC/MS/MS method

The LC/MS/MS system comprised an Applied Biosystems Sciex (Foster city, CA, USA) API 3000 tandem mass spectrometer, and Shimadzu (Columbia, MD, USA) LC-10ADvp pumps with a Leap (Carrboro, NC, USA) HTS PAL autosampler. Data were processed using Analyst software 1.4. Chromatographic separation of EDL-155 and IS was performed using a Symmetry® C8 analytical column (3.5 µm, 2.1 mm × 50 mm) and guard column Symmetry Sentry™ (3.5µm, 2.1 mm× 10 mm,) purchased from Waters Corporation (Milford, MA, USA). Mobile phase consisting of A (water containing 0.1% formic acid) and B (methanol containing 0.1% formic acid) was delivered at 0.3 ml/min. The total run time was 9.7 min. Gradient elution began at 10% B and was held for 2 min, then ramped to 40% B over 0.2 min and held for 1 min, then ramped to 90% B over 4 min and finished at 10% B for 2.5 min.

The mass spectrometer was operated in the positive electrospray ionization MRM mode using a transition of 332.4→167.2 for EDL-155 and 360.5→167.2 for internal standard. The specificity, linearity, precision, and accuracy, were evaluated with six replicates at concentrations of 6, 150, 2,500, and 5,000 ng/mi. In addition, matrix effect (ME), recovery rate (RR), process efficiency (PE) in rat plasma were evaluated by preparing samples in three different set samples [7]. For set 1, drug was spiked into neat mobile phase. Set 2 samples were prepared in the supernatant following IS working solution precipitation of plasma proteins. Set 3 samples were prepared by spiking drug into plasma. Peak areas of EDL-155 and IS obtained from set 1(A), set 2 (B) and set 3(C) were used for evaluation as follows:

$$\text{Absolute ME}\% = (\text{B}/\text{A}) \times 100$$
$$\text{RR}\% = (\text{C}/\text{B}) \times 100$$
$$\text{PE}\% = (\text{C}/\text{A}) \times 100$$

EDL-155 plasma protein binding

In preliminary experiments, it was discovered that EDL-155 underwent extensive non-specific adsorption to ultrafiltration devices and membranes. Therefore, the plasma protein binding of EDL-155 was obtained using a non-membrane charcoal adsorption method [8, 9]. The charcoal suspension was prepared by mixing 2 mg of dry dextran-coated charcoal powder into 1 ml drug-free normal rat plasma. An aliquot (20 µl) of EDL-155 working standard solution was spiked into plasma (980 µl) to obtain drug solutions of 300, 1,300, and 5,000 ng/ml and allowed to equilibrate for 10 min at room temperature. The ratio of binding matrix and charcoal suspension was 1:1, leading to the final drug concentrations of 150, 650, and 2,500 ng/ml. Charcoal suspension in plasma (1.5 ml) was transferred to a 10-ml glass vial and mounted on a magnetic stirrer. With continuous stirring, 1.5 ml of EDL-155 spiked plasma was added to the glass vial. Serial samples (80 µl) were transferred to 0.5-ml polypropylene micro-centrifuge tubes at 20, 40 s, and 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 25, and 30 min. Immediate centrifugation at 10,000 *g*, 25°C for 5 s terminated the adsorption by separating charcoal from the plasma. Each sample was divided into three aliquots of 20 µl and subjected to protein precipitation and LC/MS/MS analysis.

Pharmacokinetic data analysis

EDL-155 plasma concentration-time data were analyzed by noncompartmental and compartmental methods using WinNonlin 4.0 (Pharsight, Mountain View, CA, USA). The area under the plasma and CSF concentration-time curves from time 0 to infinity (area under the curve, $AUC_{0-\infty}$) was calculated by the trapezoidal rule with extrapolation to time infinity. Half-life ($t_{1/2}$) was calculated as $0.693/k_{10}$, where k_{10} was the elimination rate constant. The plasma clearance (CL) was calculated as $CL = dose_{i.v.}/AUC_{i.v.,\ 0-\infty}$, where $dose_{i.v.}$ and $AUC_{i.v.,\ 0-\infty}$ were the i.v. dose and corresponding AUC from time 0 to infinity, respectively. The apparent volume of distribution at equilibrium (Vd_{ss}) was calculated as $Vd_{ss} = CL \cdot MRT$, where MRT was the mean residence time after the i.v. bolus dose. MRT was calculated as $MRT = (AUMC_{i.v.,\ 0-\infty})/(AUC_{i.v.,\ 0-\infty})$, where $AUMC_{i.v.,\ 0-\infty}$ was the area under the first moment of the plasma concentration-time curve extrapolated to infinity. The CSF elimination rate constant K_{10} was estimated using a one-compartment model weighted by $1/C_{predicted}$. The CSF clearance of EDL-155 was subsequently calculated as $CL_{CSF} = K_{10} \times V_{CSF}$, where V_{CSF} represents the typical CSF volume found in rats [10]

Plasma protein binding was estimated by fitting the percentage of drug remaining in the plasma versus time profile as described by Yuan et al., in accordance with the model $B(t) = A_1 \times e^{-\alpha t} + A_2 \times e^{-\beta t}$, where $B(t)$ was percent drug bound at time t [9]. At time $t = 0$, the fraction of bound drug in plasma can be estimated as $B_{(0)} = A_1 + A_2$.

In vivo glioma model

A total of 21 male Sprague-Dawley rats (250–350 g) were deeply anesthetized with (13 mg/kg, Rompom and 87 mg/kg, Ketalar) for the surgery. The rats were monitored during the surgery to insure that they remained deeply anesthetized and unresponsive to pain. The skull was surgically exposed, a bur hole was placed in the skull and injection cannula was lowered 5 mm below the cerebral cortex into the caudate and approximately 5×10^5 C6 glioma cells were delivered into the brain over a 20-min period. The injection cannula was removed and the incision was closed with surgical staples. The animal was allowed to recover and returned to the animal care facility.

Approximately 12 h after tumor cell implantation, animals began receiving intraperitoneal administration of EDL-155 (20 mg/kg) twice daily for the next 7 days. Animals were placed into one of two treatment groups: 11 rats received vehicle only (10% DMSO in Hanks Balanced Salt Solution, HBSS) and ten rats received EDL-155. Animals were monitored daily during the treatment period. After a survival period of 8 days, the rats were deeply anesthetized with 26 mg/kg Rompom and 174 mg/kg Ketalar and perfused through the heart with saline followed by 4% paraformaldehyde in phosphate buffer (pH 7.4). Brains were removed from the skull, post-fixed for 24 h and then placed in a 30% sucrose solution. The brains were sectioned at 50 μM with a freezing microtome. One 1-in-5 series of sections was mounted on glass slides and stained by the Nissl method.

Tumor size was measured in each animal. The serial section from each case was photographed using a digital camera on a dissecting microscope. A scale was also photographed at the same magnification. The digital images were coded and the codes were kept by one investigator (EEG). The digital images were analyzed to define the volume of the tumor using the program NIH image. This work was conducted in a blinded manner (XW). The codes were released and the data compiled and analyzed using a Student's *t*-test.

Results

LC/MS/MS validation

Assay selectivity was assessed using double blank and samples with only either EDL-155 or IS in the samples. Endogenous peaks at the retention time of the analytes were not observed in double blank samples. No "cross-talk" between MS/MS channels used for monitoring EDL-155 and IS was observed. EDL-155 and IS were baseline separated with retention times of 4.50 and 5.05 min, respectively. Acceptable linearity was obtained for EDL-155 over the concentration range 0.5–5,000 ng/ml in rat plasma ($R^2 = 0.9986$). As shown in Table 1, at concentrations of 6, 150, 2,500, 5,000 ng/ml, intra-day or inter-day precision (RSD) was ≤5.1% and ≤6.9% or less, respectively. The intra-day and inter-day accuracy of EDL-155 were 96.0–112.5% and 93.9–106.8%, respectively. MEs were 90.1–100.6% and 94.5–106.5% for EDL-155 and IS. Thus, endogenous substances in the biological matrix had little effect on the ionization of EDL-155 and IS. RR values of 102.1–112.6% and 92.2–101.1% for EDL-155 and IS suggested that the constant and complete RR by the protein precipitation approach. The overall PE was 95.0–109.1% and 89.8–106.8% for EDL-155 and IS in the linear range. In summary, the partially validated LC/MS/MS method demonstrated good precision, accuracy, linearity, a consistent RR, and PE with little ME.

Table 1 Individual rat CSF and peripheral PK parameters

Sampling compartment	Animal	C_{max} (ng/ml)	V_{SS}^a (l/kg)	k_{10} (min^{-1})	Clearance[b] (ml/min/kg)	Half-life (min)
Plasma	1	665	13.5	0.0315	427	22.0
	2	816	11.3	0.0276	312	25.1
	3	838	11.1	0.0305	336	22.7
	4	1,000	10.7	0.0299	300	23.2
	5	754	12.2	0.0272	337	25.5
CSF	1	18.0		0.0144	3.60	48.1
	2	4.19		0.0113	2.84	61.1
	3	23.0		0.0258	6.46	26.8
	4	7.08		0.0089	2.22	78.2
	5	10.6		0.0136	3.40	51.0
	6	7.84		0.0108	2.70	64.2

[a] Cerebrospinal fluid (CSF) volume used to calculate CSF clearance is 250 μl

[b] Cerebrospinal fluid clearance calculated as $k_{10} \times V_{CSF}$

Peripheral pharmacokinetics

The plasma concentration versus time profile of EDL-155 during the sampling period demonstrated rapid elimination (Fig. 2). Estimated with noncompartmental analysis, the AUC_{Plasma} and clearance (CL) were 29,640 ± 3,811 min ng/ml and 342.5 ± 49.9 ml/min/kg, respectively. Clearance of EDL-155 was approximately sixfold higher than liver blood flow in rats (55 ml/min/kg) [11]. In addition, using one-compartment model with weighting scheme $1/C_{predicted}$, the elimination constant k_{10} and half-life $t_{½}$ were estimated as 0.0293 ± 0.0019 min^{-1} and 23.7 ± 1.5 min. EDL-155 was extensively bound to plasma proteins with percent bound values of 93.0 ± 2.4 %, 95.4 ± 2.4 %, and 93.6 ± 1.7% at concentrations of 150, 650, and 2,500 ng/ml, respectively. Approximately 4.1 and 0.35% of the parent compound was found in feces and urine over 24-h post-dose period, respectively. Individual rat PK parameters are reported in Table 1.

CNS pharmacokinetics

As shown in Fig. 2, EDL-155 CSF concentrations were detectable until 180 min with a C_{max} of 11.4 ± 7.4 ng/ml at 10 min (T_{max}). The AUC_{CSF} was calculated as 828 ± 288 min ng/ml using noncompartmental analysis. The dose-normalized ratio of AUC_{CSF}/AUC_{Plasma} was calculated to be 1.4%. In addition, the CSF elimination constant k_{10} was estimated as 0.0144 ± 0.0047 min^{-1} using a one-compartment model weighted by $1/C_{predicted}$. CSF clearance of EDL-155 was determined to be 3.53 ± 1.52 ml/min, which is approximately equal to CSF bulk flow clearance [10]. The CNS and peripheral concentration versus time profiles showed that limited drug penetrated into CSF and slower elimination of EDL-155 from the CSF. Individual rat PK parameters are reported in Table 1.

In vivo analysis of the effects of EDL-155

In control animals treated with the carrier solution (HBSS) relatively large tumors were observed in the brains. The C6 glioma could be observed as a large mass and out of this mass, cells were observed infiltrating the surrounding tissues attached to local blood vessels. This was rather extensive with cells marking blood vessels a considerable distance away from the bulk of the tumor. The tumors appeared to be smaller in EDL-155 treated animals compared to vehicle control animals. To provide a measure of the size of tumors in two groups, we reconstructed the tumors from serial sections to define the total tumor volume in each case. The section was photographed and the area of the tumor was measured using the program NIH image. EDL-155 treatment resulted in a statistically significant reduction in tumor size as compared to vehicle control animals (13.2 ± 5.3 mm^3 vs. 18.7 ± 6.3 mm^3; $P = 0.04$).

Discussion

Brain penetrability has been demonstrated for 1,2,3,4-tetrahydroisoquinoline (THI) derivatives following peripheral administration to rats [12, 13]. Therefore, CNS exposure following systemic administration of EDL-155 was determined to assess druggability for CNS cancers (e.g., gliomas). Ideally, brain availability following systemic drug administration is obtained through the use of microdialysis to determine the free drug concentration in the ISF [6, 14]. However, in preliminary experiments it was determined that EDL-155 bound extensively to the dialysis probe membrane and tubing (data not shown), thus, the CNS penetrability was assessed by direct continuous CSF sampling in rats. Compared to the endothelial cells forming the BBB, epithelial cells forming the BCSFB are more permeable and contain numerous fenestrations. As a result, the BCSFB is considered more "leakier" than the BBB. Despite the complexity of CSF pharmacokinetics, for some drugs a rapid kinetic equilibrium exists between the CSF and biophase. Thus, CSF drug levels have been used to investigate factors affecting the pharmacodynamics of centrally acting drugs [6, 15–20].

In the present study, we demonstrate that EDL-155 penetrates the brain and that the CSF half-life is approximately twice that of the plasma half-life (57 min versus 24 min). The increased CSF half-life may reflect a slower CSF elimination and/or relatively slow equilibration between CSF and plasma. In addition, CSF exposure as a percentage of plasma exposure (AUC_{CSF}/AUC_{Plasma}) was determined to be 1.4%. The unbound plasma drug fraction of EDL-155 is approximately 6%, thus the ratio of equilibrated CSF:unbound plasma concentration ratio is significantly less than 1. The ratio of equilibrated CSF:unbound plasma concentration reflects the balance between drug permeability across the blood-CNS barriers and the sink action of CSF turnover. As lipophilicity and membrane permeability increase, the ratio rises from well below 1 toward unity. Lipophilic drugs exhibiting ratios significantly less than unity are often efflux transporter substrates [6]. Therefore, our data suggested that the brain penetration of the lipophilic drug EDL-155 might be limited by one or more efflux transporters (e.g. P-glycoprotein; P-gp) present in the BBB. Isoquinoline derivatives have previously been shown to activate P-gp ATPase activity in

membranes isolated from bovine brain endothelial cells (BCECs) as well as alter rhodamine-123 efflux in cultured BCECs [21, 22]. Consistent with these findings, in vitro experiments demonstrate that EDL-155 alters the intracellular accumulation of the P-gp substrate calcein-AM (data not shown). The finding that CSF clearance of EDL-155 (3.6 ± 1.2 μl/min) is within the normal range of CSF bulk flow in rats (2.1–5.4 μl/min) [10], however, suggest that efflux transporters do not play a significant role in the CSF clearance of EDL-155. Transport experiments using P-gp expressing cell monolayers (e.g., Caco-2 cells) are needed to further clarify the potential interaction between EDL-155 and P-gp.

To design and develop potential CNS therapeutics, one must understand factors that contribute to systemic drug elimination in addition to understanding mechanisms controlling the rate and extent of brain accumulation. Thus, a second aim of this study was to characterize the peripheral pharmacokinetics of EDL-155. Our results indicate that EDL-155 exhibits rapid systemic clearance (≈sixfold greater than liver blood flow), which leads to a relatively short plasma half-life (23.7 min) and low plasma exposure. Extra-hepatic elimination represents one potential mechanism to explain non-physiologic clearance of EDL-155. For example, the catechol (2, 3-dihydroxy) of EDL-155 represents a potential site of metabolism by plasma and tissue catechol O-methyltransferase. Consistent with this hypothesis, the second most abundant metabolite behind C-ring hydroxylation was the A-ring mono-methylated metabolite (Fig. 1, 2). Our findings mirror those of others who report significant O-methylation of 2,3-dihydroxy (catecholic)-1,2,3,4-THIs in rats [23–27]. Moreover, the whole blood itself could play a significant role in the non-physiologic clearance of EDL-155. Following i.v. administration, the red blood cell (RBC) is the first tissue compartment to which the drug may distribute. Drug partitioning between RBCs and plasma thus determines the appropriateness of the biological fluid (whole blood, plasma, or serum) used for pharmacokinetic PK analysis [26]. In addition, RBCs may serve as a site of non-specific drug degradation. Studies evaluating the RBC partitioning and whole blood stability of EDL-155 are on-going.

Noscapine, an opium alkaloid THI with antitussive action, exerts anti-tumor activity by binding to tubulin and altering microtubule assembly [28]. Orally administered noscapine (300 mg/kg) reduces C6 tumor burden by 78% in animals receiving intra-cerebral tumor implantation [13]. Histopathologic examination revealed no signs of toxicity to the duodenum, spleen, liver, or hematopoietic tissues. These data are consistent with in vitro experiments which demonstrate that noscapine preferentially kills C6 glioma cells (IC_{50} = 250 μM) compared to primary glial cells (IC_{50} = 500 μM) [13]. EDL-155 reduces C6 tumor burden by approximately 30% after 7 days of treatment. The in vivo anti-glioma activity of noscapine and EDL-155 cannot be directly compared since the route of administration and dosage were different. However, EDL-155 is more potent against cultured C6 glioma (IC_{50} = 1.5 μM)

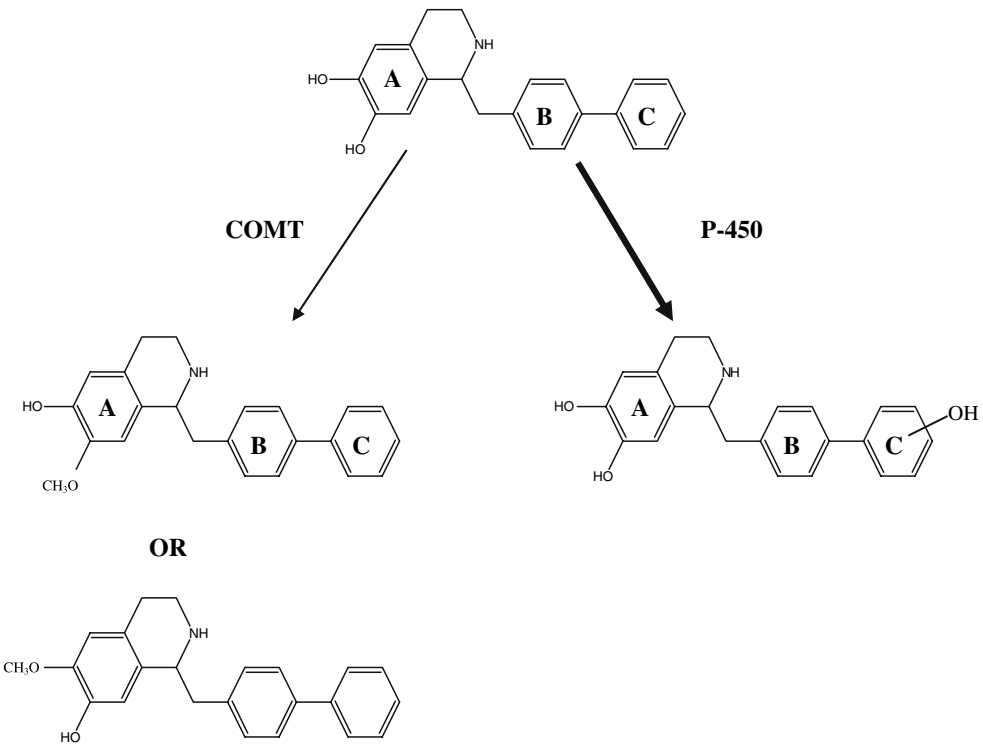

Fig. 1 Proposed metabolism scheme for EDL-155. Following intravenous administration, EDL-155 undergoes P-450 mediated oxidative metabolism of the C-ring. Alternatively, A-ring mono-methylation occurs via enzymatic transformation by catechol-O-methyltransferase (COMT)

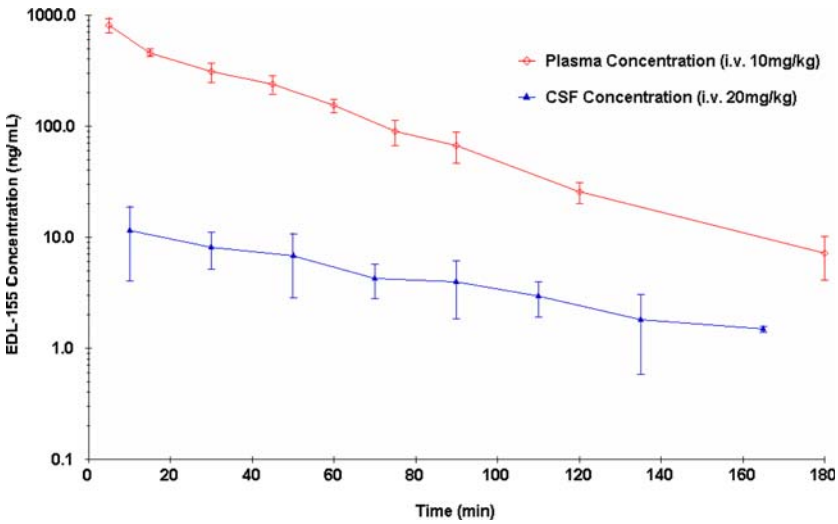

Fig. 2 Time-concentration profiles of EDL-155 in rat plasma and cerebrospinal (CSF) fluid. A single intravenous bolus dose of EDL-155 was used to determine plasma (10 mg/kg; $n = 5$) and CSF (20 mg/kg; $n = 6$) pharmacokinetics

with a wider margin of safety (i.e., IC_{50} = 15.3 μM against primary glial cells) [5, 29].

In summary, we demonstrate that a limited fraction of EDL-155 penetrates the CNS following systemic administration. A number of factors conspire to limit the brain penetration of EDL-155. These include rapid systemic clearance, limited plasma free drug fraction secondary to high plasma protein binding, and the potential for EDL-155 to serve as a substrate for efflux transporters expressed in the BBB. EDL-155 treatment resulted in reduced tumor size despite limited BBB penetrability, which suggests that analogs with increased metabolic stability and brain penetrability may provide a therapeutic option for primary CNS tumors such as glioma. On-going studies are focused on the design, synthesis, and testing of novel analogs based upon these findings.

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