SOLUBLE EPOXIDE HYDROLASE: A NOVEL THERAPEUTIC TARGET IN STROKE

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SUPPLEMENTAL MATERIALS AND METHODS

Studies with animals were conducted in accordance with the National Institute of Health guidelines for the care and use of animals in research and protocols were approved by Animal Care and Use Committee of Oregon Health and Science University, Portland, Oregon.

Analysis of sEH inhibitors in brain tissue extracts

12-(3-Adamantan-1-yl-ureido)-dodecanoic acid butyl ester (AUDA-BE) was dissolved in sesame oil and administered intraperitoneally (*i.p.*, 10mg/kg) to C57Bl/6 mice (20-26 g). A second set of animals was injected with sesame oil alone (vehicle). Mice were decapitated; and brain tissue samples were collected at 1, 3, 6, and 24 hours after administration with two mice analyzed for each time point. The sEH inhibitor AUDA-BE and its equally active metabolite 12-(3-adamantyl-ureido)-dodecanoic acid (AUDA) or the inactive metabolite, 12-(3-adamantyl-ureido)-butyl acid

(AUBA) were measured in brain tissue homogenates. Briefly, brain tissue samples were flash frozen in liquid nitrogen and were ground with a motor and pestle. Tissue homogenates were spiked with a surrogate internal standard (ADU,1-adamantyl-3-decyl urea, 100ng/ml), and the samples were extracted three times with chloroform, methanol and water (2:1:1) (*Folch et al. J Biol Chem. 1951;191:833-41*). After extraction, samples were dried under N_2, and reconstituted with methanol that contained the internal standard (CUDA, 1-cyclohexyl-3-dodecanoic acid urea, 500 ng/ml). The concentration of sEH inhibitors and metabolites were quantified in extracts using HPLC with positive mode electrospray ionization and tandem mass spectrometry (ESI-MS/MS). Analytes were quantified on a 5-point curve with internal standard methods. Surrogate recoveries were evaluated by quantification against the internal standard as described previously (*Watanabe T et al. Anal Chim Acta. 2006;559:37-44*). Surrogate recoveries in brain tissue homogenates were relatively similar but approximately 30% with only two independent experiments from two individual animals in each time point injected with AUDA-BE (10mg/kg) or vehicle alone (sesame oil) . The analysis of reagent blanks, matrix spikes, and analytical replicates were used to document the stability of the method during this study. Molecular ion and transition ions for the analytes were as followed, AUDA-BE (449.2>272.2), AUDA (393.1>135), AUBA (281.2>104), surrogate compound ADU, 1-adamantyl-3-decyl urea (335.1>135) and internal standard CUDA (341.2>216.2).

Pharmacokinetic Parameter Analysis for sEH inhibitors

The pharmacokinetic parameters were obtained by fitting the blood concentration-time data to a non-compartmental model with the WinNonlin software (Pharsight, Mountain View, CA). Parameters estimated included the time of maximum concentration (T_{max}), the maximum concentration (C_{max}), elimination half-life ($T_{1/2}$), area under the concentration-time curve to

terminal time (AUC_t) and the mean residence time (MRT). See Table S1. A semilog plot of the data in Figure 3 is linear supporting a non-compartmental model.

SUPPLEMENTAL RESULTS

In order to determine sEH inhibitory effect in the brain, the level of sEH inhibitors was first examined in brain extracts (Fig. S1). Brain tissues were homogenized and inhibitors were extracted with methanol and chloroform (*Folch et al. J Biol Chem. 1951;191:833-41*). Although that the recovery of the extracted compounds from the brain was low, it is clearly seen that AUDA accumulated in brain tissue within the first six hours after a single *i.p.* injection of 10mg/kg AUDA-BE (Fig. S1). AUDA was first seen after one hour post injection and reached maximum levels at three hours. Slow degradation of AUDA was observed over the following twenty four hours with a mean residence time (MRT) of six hours. Only 3 pmol/g tissue of AUDA were measured at 24 hours. AUDA-BE was not detected in the brain tissue. AUBA was not measured in these samples since the extraction method is not adequate for hydrophilic compounds (Fig. S1). Pharmacokinetic analysis of AUDA in plasma and brain tissue is summarized in Table S1. As shown, the maximum concentration of AUDA in plasma was measured as 52 nM, while brain maximum concentration was 105 nM (Table S1). The time that AUDA reached maximum concentration was calculated as one hour in plasma samples followed by three hours in brain tissue homogenates, and the mean residence time was nine hours in plasma samples and six hours in brain tissue homogenates (Table S1). AUDA accumulates in brain few hours after injection. These data are consistent with the AUDA-BE ester penetrating membranes quickly, being hydrolyzed to AUDA and AUDA only leaving the brain tissue slowly and being slowly metabolized. Alternatively AUDA could be taken up selectively by the brain. With this particular method, hydrophilic compounds as AUBA cannot be extracted and

measured, while it is most likely that AUDA is degraded by β-oxidation to less active compounds that do not reach biological effective levels in plasma. In plasma samples, the maximum concentration of AUDA is lower than the brain extracts but the half time of elimination and mean residence time both are longer in plasma tissue extracts that likely is a result of the degradation of AUDA-BE to AUDA by esterases.

Table S1. Pharmacokinetic parameters of AUDA in plasma and brain tissue samples.

AUDA	Plasma Samples	Brain tissue Samples
C_{max} (pmol/mL)[a]	52	105
T_{max} (hr) [b]	1.0	3.0
$T_{1/2}$ (hr) [c]	7.2	4.2
AUC (hr*pmol/mL)[d]	582	84
MRT (hr) [e]	9.1	6.0

PK parameters calculated by WinNonlin 5.1 software (Pharsight, Mountain View, CA) with 0-24 hour data points. R^2=0.996 and adjusted R^2=0.995 for $T_{1/2}$ calculations.

[a]Maximum concentration.

[b]Time of maximum concentration.

[c]Elimination half-time.

[d]Area under the concentration (0-24).

[e]Mean residence time.

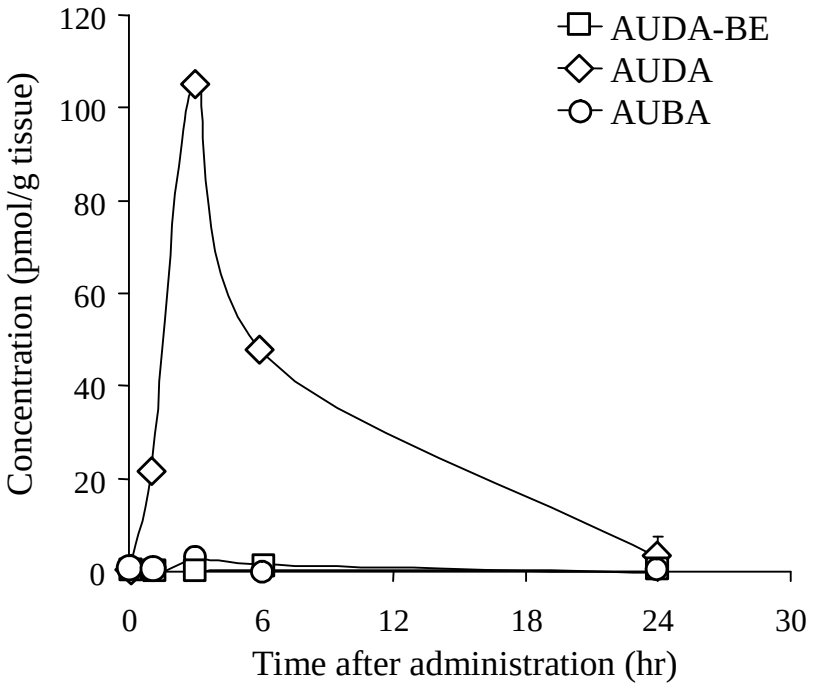

Fig. S1. AUDA concentration in brain tissue extracts. Mice, injected with 10mg/kg AUDA-BE have clearly measurable levels of AUDA but not of AUDA-BE in brain extracts. **AUBA was not detected since the extraction method is not appropriate for hydrophilic compounds**. Results are mean ±SEM of two mice from each treatment and time point.