Pharmacological inhibition of soluble epoxide hydrolase as a new therapy for Alzheimer's Disease

Christian Griñán-Ferré†*, Sandra Codony‡, Eugènia Pujol‡, Jun Yang§, Rosana Leiva‡, Carmen Escolano‡, Dolors Puigoriol-Illamola†, Júlia Companys-Alemany†, Rubén Corpas¶, Coral Sanfeliu¶, Belen Pérez‖, M. Isabel Loza||, José Brea||, Christophe Morisseau§, Bruce D. Hammock§, Santiago Vázquez‡*, Mercè Pallàs†*, Carles Galdeano#*

Affiliations:

†Pharmacology Section. Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institut de Neurociències, University of Barcelona, Av. Joan XXIII, 27-31, E-08028 Barcelona, Spain.

‡Laboratori de Química Farmacèutica (Unitat Associada al CSIC), Department de Farmacologia, Toxicologia i Química Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació, and Institute of Biomedicine (IBUB), Universitat de Barcelona, Av. Joan XXIII, 27-31, E-08028 Barcelona, Spain.

§Department of Entomology and Nematology and Comprehensive Cancer Center, University of California, Davis, California, USA.

¶Institute of Biomedical Research of Barcelona (IBB), CSIC and IDIBAPS, Barcelona, Spain. And CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain.

‖Department of Pharmacology, Therapeutic and Toxicology. Autonomous University of Barcelona, E-08193 Barcelona, Spain.

||Innopharma screening platform. Biofarma research group. Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS). Universidad de Santiago de Compostela. Spain.

#Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, E-08028 Barcelona, Spain.

*To whom correspondence should be addressed:

Mercè Pallàs

Pharmacology Section. Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institut de Neurociències, University of Barcelona, Av. Joan XXIII, 27-31, E-08028 Barcelona, Spain.

Phone: + 3434024531

e-mail:pallas@ub.edu.

Highlights

- sEH levels are increased in AD human brain and in murine models
- Inhibition of sEH reduces oxidative stress and inflammation in murine AD models
- AD hallmarks in AD mice models are reduced after treatment with sEH inhibitors
- sEH inhibitors improve cognition in AD mice models
- sEH can be proposed as a new pharmacological target for AD therapy

Abstract

The inhibition of the enzyme soluble epoxide hydrolase (sEH) has demonstrated clinical therapeutic effects in several peripheral inflammatory-related diseases, with three compounds in clinical trials. However, the role of this enzyme in the neuroinflammation process has been largely neglected. Herein, we disclose the pharmacological validation of sEH as a novel target for the treatment of Alzheimer's Disease (AD). Evaluation of cognitive impairment and pathological hallmarks were used in two models of age-related cognitive decline and AD using three structurally different and potent sEH inhibitors as chemical probes. sEH is upregulated in brains from AD patients. Our findings supported the beneficial effects of central sEH inhibition, regarding reducing cognitive impairment, neuroinflammation, tau hyperphosphorylation pathology and the number of amyloid plaques. This study suggests that inhibition of inflammation in the brain by targeting sEH is a relevant therapeutic strategy for AD.

Keywords: Soluble epoxide hydrolase, inflammation, Alzheimer's disease, tau, β-amyloid, target engagement, druggability

1.Introduction

Chronic inflammation is recognized as a key player in both the onset and progression of Alzheimer's Disease (AD) (*1-3*). Indeed, 16% of the investment in ongoing clinical trials for AD is related to inflammation (*4*). Neuroinflammation is intimately linked to the oxidative stress associated with AD (*5,6*), controlling the interactions between the immune system and the nervous system (*7,8*). However, several antioxidant therapies and non-steroidal anti-inflammatory drugs have failed in clinical trials. Therefore, it is of vital importance to expand the scope towards novel targets, preferably related to several pathophysiological pathways of the disease (*9*).

Epoxyeicosatrienoic acids (EETs) mediate vasodilatation, reduce inflammation, attenuate oxidative stress and block the pathological endoplasmic reticulum (ER) stress response (*10,11*). The soluble epoxide hydrolase enzyme (sEH, EC 3.3.2.10, EPHX2), widely expressed in relatively high abundance in the murine and human brains (*12,13*), converts EETs and other epoxyfatty acids (EpFA) to their corresponding dihydroxyeicosatrienoic acids (DHETs), whereby diminishing, eliminating, or altering the beneficial effects of EETs (*14*) (Fig. 1).

Considering that several lines of evidence underline a broad involvement of signaling by EETs and other EpFA in the central nervous system (CNS) function and disease (*15,16*) and that lack of sEH by genetic deletion improve the signs of AD in a mouse model (*17*), we hypothesized that brain penetrant sEH inhibitors (sEHI) would stabilize EETs in the brain, resulting in a reduction of reactive oxygen species (ROS) and diminished neuroinflammation and neurodegeneration, leading to a positive outcome in AD. To this end, we studied the neuroprotectant role mediated by sEHI in two models ofL AD; the Senescence-accelerated

mouse prone 8 (SAMP8) and 5XFAD mice model. Because SAMP8, a paradigm of late-onset AD and cognitive impairment in age, is characterized by oxidative stress, neuroinflammation, tau hyperphosphorylation and proamilodogenic APP processing, but lacks of β-amyloid (βA) plaques (*18-22)*, we studied plaque load and cognitive impairment in 5XFAD, a mouse model of early-onset AD, to unveil the effect of sEHI on this AD hallmark. (*23-24*)

2. Methods

Details of the experimental protocols, including chemicals, animals, novel object recognition test (NORT), biochemical and molecular methods, target engagement, drug properties characterization and statistical analysis, are given in Supplemental information.

3. Results

3.1. Changes in sEH expression in hippocampus from AD patients, SAMP8 and 5XFAD

The key question was to determine whether sEH expression differs from healthy to pathological conditions. Results demonstrated that sEH levels were higher in AD patients brain (Braak III and V) in comparison with healthy individuals (Table S1 and Fig. 2A). Moreover, sEH expression was also elevated in SAMP8 and 5XFAD hippocampus in reference to their respective controls (Fig. 2B).

3.2. On-target drug inhibition of sEH

We evaluated three structurally different sEH inhibitors as chemical probes (*25*): TPPU (UC1770, IC_{50} for human sEH = 3.7 nM) (*26*), AS-2586114 (IC_{50} for human sEH = 0.4 nM) (*27*), and UB-EV-52 (IC_{50} for human sEH = 9 nM) (*28*) (Fig. 2C). Previous pharmacokinetic data suggest that TPPU,

a very well characterized sEH inhibitor, can enter into the brain (*29*). It is known that AS-2586114 has a prolonged action *in vivo* and an ability to cross the blood-brain barrier (BBB) (*30-31*). UB-EV-52 is a new inhibitor somewhat related with previously reported adamantane-derived sEH inhibitors such as *t*-AUCB (3*2*) and the clinically studied AR9281 (UC1153) (33). To determine whether UB-EV-52 possesses drug-like characteristics, we performed *in vitro* ADMET assays. We found that UB-EV-52 has excellent solubility (>100 μM at 37 °C in 5% DMSO: 95% PBS buffer), good microsomal stability (Table S2), and does not inhibit drug metabolizing cytochromes or the hERG channel (Table S3). Of relevance, some cytochromes are potential *off*-target effects of sEH (Fig. 1), since they are situated upstream in the arachidonic acid cascade. UB-EV-52 showed less than 5% inhibition of the studied cytochromes (CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6) at 10 μM (Table S3). As a preliminary assessment of brain permeability, UB-EV-52 was subjected to the parallel artificial membrane permeation assay-BBB (PAMPA-BBB), a well-established *in vitro* model of passive transcellular permeation (*34)*. UB-EV-52 was predicted to be able to cross the BBB (Table S4), which anticipates its ability to enter the brain. In order to characterize the toxicity of UB-EV-52, we evaluated cell viability in human neuroblastoma SH-SY5Y cells, using an MTT assay for cell metabolic activity and a propidium iodide stain assay for cell death. In both assays, UB-EV-52 showed no cytotoxicity at 1, 10, 50 and 100 μM (Table 1).

To evaluate whether sEH is the direct binding target of the inhibitors in brain tissue, we performed an *in vivo* thermal shift assay (CETSA) (*35*). The results showed a significant shift in the sEH melting curve in the hippocampus of CD-1 mice orally treated with TPPU, AS-2586114 and UB-EV-52, demonstrating *in vivo* compound-induced target stabilization, providing also evidence of central action (Fig. 2D).

To demonstrate that the tested compounds reduced sEH activity, we measured levels of regulatory lipid mediators in the cortex of treated and control SAMP8 mice. As shown in Fig. S1, the level of pro-inflammatory lipid mediators in cortex such as PGD2 and TXB2 are higher in the control group. At the same time, the anti-inflammatory epoxy fatty acids, including those from Y-linoleic acid (EpODE) and other polyunsaturated fatty acids are all higher in treated groups, especially in TPPU treated group. These differences also verified the target engagement of inhibition of sEH *in vivo*.

Once demonstrated that the compounds tested were able to inhibit sEH at the brain level, we evaluated the pathological hallmarks and the cognitive impairment associated with AD in SAMP8 and 5XFAD.

3.3. sEH inhibitors reduce biomarkers of inflammation, oxidative stress and endoplasmic reticulum stress

Indicators of brain neuroinflammation were determined after oral treatment with TPPU (5 mg/kg/day), AS-2586114 (7.5 mg/kg/day), and UB-EV-52 (5 mg/kg/day) (Fig. 3A and Fig. S2). The three inhibitors reduced gene expression and brain protein levels of the pro-inflammatory cytokines IL-1β (Interleukin-β), CCL3 (C-C motif ligand 3) and, importantly, TNF-α (Tumor necrosis factor-α) in SAMP8 (Fig. 3B) and in 5XFAD (data not shown). IL-1β is intimately involved in neuroinflammatory processes in the CNS (34) and its activity is thought to be closely tied to the process of memory consolidation (34, 35). Chemokines play a critical role in phagocytic activity. Concretely, CCL3 is expressed in astrocytes and is described as a component of the inflammasome complex (36). Of note, TNF-α is not only involved in AD-related brain

neuroinflammation (37, 38), but also contributes to amyloidogenesis via β-secretase regulation (*39, 40*). Additionally, these results suggest an involvement of the two main inflammasome-signalling pathways, NF-kβ and NLRP3 (*41*).

To investigate the influence of the sEH inhibitors in the oxidative stress process, we determined the concentration of hydrogen peroxide in the brain of SAMP8 mice. The three inhibitors significantly reduce hydrogen peroxide (Fig. 3C). Moreover, determination of the brain oxidative machinery was addressed by evaluating gene expression of *Hmox1*, *Aox1* and protein levels of SOD1 (Fig. 3C). *Hmox1* (antioxidant activity) (*42*) was significantly increased by UB-EV-52 and TPPU, but not by AS-2586114 (Fig. 3C). qPCR analysis also demonstrated that treated SAMP8 mice had lower *Aox1* expression (Fig. 3C). *Aox1* controls the production of hydrogen peroxide and, under certain conditions, can catalyze the formation of superoxide (*43*). Furthermore, SOD1 (antioxidant activity) protein levels were significantly increased in all treated groups (Fig. 3C), indicating a reinforcement of the antioxidant response after treatment with sEHI (*44*). By contrast, in 5XFAD, a model with reduced oxidative stress, no significant changes were determined in oxidative stress parameters evaluated (data not shown).

It is known that the ER stress plays a role in the pathogenesis of neurodegenerative diseases, including AD (*45*), and sEHI attenuate activation of the ER stress response (*10*). Therefore, we measured the levels of the ER stress sensors ATF-6 and IRE1α (Fig. 3D). Especially, UB-EV-52 was able to reduce the levels of either protein. Furthermore, we evaluated XBP1, a major regulator of the unfolded protein response, which is induced by ER stress (*46*). XBP1 was significantly reduced by UB-EV-52 and slightly decreased by AS-2586114, but not by TPPU (Fig. 3D). Altogether, these results suggest that the inhibition of sEH protects against oxidative stress and the associated ER stress in the brain.

3.4. sEH inhibitors modify the two main physio-pathological hallmarks of AD

The brains of patients with AD contain two main physio-pathological hallmarks: tangles of hyperphosphorylated tau protein and βA plaques. As mentioned, considering that SAMP8 has disturbances in tau hyperphosphorylation and APP processing but lacks βA plaques, 5XFAD was used to support the protective effect of sEHI in AD hallmarks studied. On the one hand, after oral treatment of SAMP8 and 5XFAD mice with TPPU (5 mg/kg/day), AS-2586114 (7.5 mg/kg/day), and UB-EV-52 (5 mg/kg/day), sEH inhibition provoked a reduction of the tau hyperphosphorylated species (Ser396 and Ser404), especially Ser404, (Fig. 4A and 4B) in agreement with the idea that oxidative stress can promote tau hyperphosphorylation and aggregation (*47-48*). On the other hand, we examined the ability of the sEHI to modify the amyloid processing cascade. While the 5XFAD transgenic mouse model develops early aggressive hallmarks of amyloid burden and cognitive loss (*49*), SAMP8 is characterized by an abnormal amyloid precursor protein (APP) processing, with a misbalance to the amyloidogenic pathway. Importantly, *C*-terminal fragments (CTF) levels are strongly implicated in neurodegeneration and the cognitive decline process in SAMP8 (*21,22*). We observed a substantial decrease in the ratio of CTFs/APP protein levels in both mice models after treatment with sEHI (Fig. 4C and 4D). In addition, an increase of the sAPPα, and a decrease of sAPPβ supported that sEHI are able to shift the APP processing towards the non-amyloidogenic pathway, thereby reducing the probability of increasing βA aggregation. Finally, treatment of 5XFAD mice with sEHI had a strong effect in reducing the number of βA plaques stained with Thioflavin-S (by an average of 40%) (Fig. 4E), indicating the prevention of amyloid burden in a model characterized by βA plaque formation at early ages as two months.

3.5. sEH inhibitors reduce cognitive impairment

To demonstrate the efficacy on the cognitive decline of the sEH inhibitors, we performed a NORT to obtain a measure of cognition for short- and long-term memory. Treatment of both murine models with the three sEH inhibitors drastically increased the Discrimination Index (DI). The significant increase indicates clear preservation of both memories (Figs. 5A and 5B). In both models, we add a comparator arm of mice treated with donepezil, which is a standard of care in the treatment of AD. As expected, donepezil treatment (5 mg/kg/day) also shifted the DI to values significantly higher than zero. Remarkably, in all the conditions, the sEH inhibitors reduced cognitive decline better than donepezil.

4. Discussion

Our results suggest that the pharmacological stabilization of EETs in the brain has the potential to address multiple etiologies and physio-pathological processes of AD, i.e., neuroinflammation, ER stress and oxidative stress, increasing the chances of success of future therapies based on sEH inhibition. Although the decisive role of sEH inhibition in multiple inflammation-related diseases has been studied, only a few investigations have been conducted about its crucial role in the neuroinflammation process (*15, 16, 30*). Besides, the question of if neuroinflammation is the malicious driver or 'just' a consequence still represents an important conundrum in the AD field. Our findings reinforce the idea that neuroinflammation might drive the pathogenic process in AD. A partial correlation calculation has demonstrated that the anti-inflammatory effects of sEH inhibitors correspond with changes in AD hallmarks, slowing the progression of

the disease and pushing up the cognitive capabilities in the studied animal models (Fig S3-S4 and Table S5-S6).

A characteristic feature of acute inflammatory processes is a general increase in the levels of classic proinflammatory eicosanoids (prostaglandins, leukotrienes and thromboxanes). In neurodegenerative diseases, there is a basal, chronic and silent inflammation that is more related to a disbalance in pro-inflammatory and anti-inflammatory cytokines, such as those studied IL-1β (36), CCL3 (49) and TNFα, as well as by acting on different mechanisms implied in neurodegeneration (e.g., increase of the oxidative stress, increase in the glutamate pathway, among others). Importantly in our landscape, βA activates inflammasomes that in turn, mediate IL-1β maturation in microglial cells (49). This allowed us to anticipate different biological and therapeutic outcomes for sEH inhibition than for the COX and LOX pathway inhibition.

Two structurally different sEH inhibitors have proven to be safe in human clinical trials for other peripheral indications (AR9281 for hypertension and GSK2256294 for diabetes mellitus, chronic pulmonary obstructive disease and subarachnoid hemorrhage)(*26, 50)* A third inhibitor, EC5026, is in human safety trial on a clinical path to chronic pain. This fact, undoubtedly, accelerates the development of new sEHI for the treatment of AD and avoids uncertainties about the possibility of angiogenic effects when inhibiting sEH. Of relevance, for this study, we have employed three structurally different sEH inhibitors, supporting the hypothesis that the biological outcomes observed are not due to *off*-target effects related to a particular inhibitor.

In summary, we have demonstrated that sEH levels are altered in AD mouse models and, more importantly, in the brain of AD patients. We have further shown that the inhibition of sEH has a plethora of beneficial central effects, such as reducing inflammation, ER stress, oxidative stress

markers, p-Tau pathology and the amyloid burden. Consequently, sEHI improve the functional efficacy endpoint for cognitive status in neurodegeneration and AD animal models.

The anti-inflammatory effect of sEHi has been demonstrated in different pathologies [16, 17, 30]. Nevertheless, considering the results obtained in this work, we cannot rule out the beneficial effects of inhibition of sEH by regulating the processes of proteostasis and OS. Those effects should be implicated in the β-amyloid plaque removal and tau hyperphosphorylation reduction. Based on the results presented in this work, we firmly believe that inhibitors of sEH could represent an entirely new stand-alone treatment for the treatment of AD. However herein, we do not demonstrate, since is it beyond the scope of this study, if inhibition of sEH could also represent an add-on therapy together with more symptomatic-like drugs, i.e. donepezil.

Acknowledgements

This study was supported by the *Ministerio de Economía, Industria y Competitividad* (Agencia Estatal de Investigación, AEI) and *Fondo Europeo de Desarrollo Regional* (MINECO-FEDER) (Projects SAF2017-82771, SAF2016-77703 and SAF2015-68749) and Generalitat de Catalunya (2017 SGR 106). S.C. and E.P. thank the Universitat de Barcelona and the Institute of Biomedicine of the Universitat de Barcelona (IBUB), respectively, for PhD grants. R.L. and D.P.-I. thank the *Ministerio de Educación, Cultura y Deporte* for PhD grants (FPU program). We would also like to thank *Xunta de Galicia (ED431C 2018/21)* and *Ministerio de Economía, Industria y Competitividad (Innopharma Project) and Fondo Europeo de Desarrollo Regional (*MINECO-FEDER*)*. This study was also supported, in part, by a grant from the *National Institute of Environmental Health Sciences* (NIEHS) Grant R01 ES002710, the RIVER Award (WIEHS grant) and *NIEHS Superfund Research Program* P42 ES004699. The content is solely the responsibility of the authors and does not necessarily represent the official views of the *National Institutes of Health*.

Supplementary Data

References

1. D. Krstic, I. Knuesel, Deciphering the mechanism underlying late-onset Alzheimer disease. Nat. Rev. Neurol. **9**, 25–34 (2012).

2. V. Calsolaro, P. Edison, Neuroinflammation in Alzheimer's disease: current evidence and future directions. Alzheimer's Dementia **12**, 719–732 (2016).

3. W.-Y. Fu, X. Wang, N. Y. Ip, Targeting neuroinflammation as a therapeutic strategy for Alzheimer's disease: mechanisms, drug candidates, and new opportunities. ACS Chem. Neurosci. **10**, 872-879 (2019).

4. A. T. Kodamullil, F. Zekri, M. Sood et al. Tracing investment in drug development for Alzheimer disease. Nat. Rev. Drug Discovery **16**, 819 (2017).

5. E. Tönnies, E. Trushina, Oxidative stress, synaptic dysfunction, and Alzheimer's disease. J. Alzheimer's Dis. **57**, 1105–1121 (2017).

6. K. J. Barnham, C. L. Masters, A. I. Bush, Neurodegenerative Diseases and Oxidative Stress. Nat. Rev. Drug Discovery **3**, 205-214 (2004).

7. M. T. Heneka, M. J. Carson, J. El Khoury et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. **14**, 388–405 (2015).

8. F. L. Heppner, R. M. Ransohoff, B. Becher, Immune attack: the role of inflammation in Alzheimer disease. Nat. Rev. Neurosci. **16**, 358–372 (2015).

9. S. Makin, The amyloid hypothesis on trial. Nature **559**, S4-S7 (2018).

10. B. Inceoglu, A. Bettaieb, F. G. Haj, A. V. Gomes, B. D. Hammock, Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors. Prostaglandins Other Lipid Mediators **133**, 68–78 (2017).
11. X. Liu, C. M. Davis, N. J. Alkayed, P450 eicosanoids and reactive oxygen species interplay in brain injury and neuroprotection. Antioxid. Redox Signaling **28**, 987–1007 (2018).
12. A. Marowsky, J. Burgener, J. R. Falck, J. M. Fritschy, M. Arand, Distribution of soluble and microsomal epoxide hydrolase in the mouse brain and its contribution to cerebral epoxyeicosatrienoic acid metabolism. Neuroscience **163**, 646–661 (2009).
13. P. Sura, R. Sura, A. E. EnayetAllah, D. F. Grant, Distribution and expression of soluble epoxide hydrolase in human brain. J. Histochem. Cytochem. **56**, 551–559 (2007).
14. C. Morisseau, B. D. Hammock, Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. Annu. Rev. Pharmacol. Toxicol. **53**, 37–58 (2013).
15. L. Wang, G. Luo, L. F. Zhang, H. X. Geng, Neuroprotective effects of epoxyeicosatrienoic acids. Prostaglandins Other Lipid Mediators **138**, 9–14 (2018).
16. K. M. Wagner, C. B. McReynolds, W. K. Schmidt, B. D. Hammock, Soluble epoxide hydrolase as a therapeutic target for pain, inflammatory and neurodegenerative diseases. Pharmacol. Ther. **180**, 62–76 (2017).
17. H.T. Lee, K.I. Lee, C.H. Chen, T.S. Lee. Genetic deletion of soluble epoxide hydrolase delays the progression of Alzheimer's disease. J. Neuroinflammation **16**, 267 (2019).
18. J. E. Morley, S. A. Farr, J. F. Flood, Antibody to amyloid β protein alleviates impaired acquisition, retention, and memory processing in SAMP8 mice. Neurobiol. Learn. Mem. **78**, 125–138 (2002).

19. T. Takeda, Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. Neurochem. Res. **34**, 639–659 (2009).

20. I. Akiguchi, M. Pallàs, H. Budka et al. SAMP8 mice as a neuropathological model of accelerated aging and dementia: Toshio Takeda's legacy and future directions. Neuropathology **37**, 293–05 (2017).

21. M. Pallàs, Senescence-accelerated mice P8: a tool to study brain aging and Alzheimer's disease in a mouse model. ISRN Cell Biol. **2012**, 917167 (2012).

22. C. Griñan-Ferré, V. Palomera-Ávalos, D. Puigoriol-Illamola et al. Behaviour and cognitive changes correlated with hippocampal neuroinflammaging and neuronal markers in female SAMP8, a model of accelerated senescence. Exp. Gerontol. **80**, 57–69 (2016).

23. H. Oakley, S. L. Cole, S. Logan et al. Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J. Neurosci. **26**, 10129–10140 (2006).

24. R. Kimura, M. Ohno, Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5xFAD Alzheimer mouse model. Neurobiol Dis. **33**, 229–235 (2009).

25. C. H. Arrowsmith, J. E. Audia, C. Austin, et al. The promise and peril of chemical probes. Nat. Chem. Biol. **11**, 536–541 (2015).

26. T. E. Rose, C. Morisseau, J. Y. Liu et al. 1-Aryl-3-(1-acylpiperidin-4-yl)urea inhibitors of human and murine soluble epoxide hydrolase: structure-activity relationships,

pharmacokinetics and reduction of inflammatory pain. J. Med. Chem. **53**, 7067–7075 (2010).

27. M. Miura, I. Sato, H. Kiyohara, et al. Cyclic amino compound, or salt thereof. JP2011016743 (2011).
28. S. Vázquez, E. Valverde, R. Leiva, M. Vázquez-Carrera, S. Codony, Analogs of adamantylureas as soluble epoxide hydrolase inhibitors. WO 2017017048 (2017).
29. A. I. Ostermann, J. Herbers, I. Willenberg et al. Oral treatment of rodents with soluble epoxide hydrolase inhibitor 1-(1-propanoylpiperidin-4-yl-)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU): resulting drug levels and modulation of oxylipin pattern. Prostaglandins Other Lipid Mediators **121**, 131–137 (2015).
30. N. Taguchi, S. Nakayama, M. Tanaka. Single administration of soluble epoxide hydrolase inhibitor suppresses neuroinflammation and improves neuronal damage after cardiac arrest in mice. Neurosci. Res. **111**, 56–63 (2016).
31. M. Ma, Q. Ren, Y. Fujita, T. Ishima, J. C. Zhang, K. Hashimoto, Effects of AS2586114, a soluble epoxide hydrolase inhibitor, on hyperlocomotion and prepulse inhibition deficits in mice after administration of phencyclidine. Pharmacol. Biochem. Behav. **110**, 98–103 (2013).
32. S. H. Hwang, H. J. Tsai, J. Y. Liu, C. Morisseau, B. D. Hammock, Orally bioavailable potent soluble epoxide hydrolase inhibitors. J. Med. Chem. **50**, 3825-3840 (2007).
33. R.Chen, E.Whitcomb, V. MacIntyre, et al. Pharmacokinetics and pharmacodynamics of AR9281, an inhibitor of soluble epoxide hydrolase, in single- and multiple-dose studies in

healthy human subjects. J. Clin. Pharmacol. **52**, 319–328 (2013).

34. L. Di, E.H. Kerns, K. Fan, O. J. McConnell, G.T. Carter. High throughput artificial membrane permeability assay for blood-brain barrier. Eur. J. Med. Chem. **38**, 223-232 (2003).

35. R. Martínez Molina, M. Jafari, M. Ignatushchenko, et al. Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay. Science **341**, 84–87 (2013).

36. S.S. Shaftel, S. Kyrkanides, J.A. Olschowka, J.H. Miller, R.E. Johnson, M.K. O'Banion. Sustained hippocampal IL-1β overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J. Clin. Invest. **117**(6), 1595–1604 (2007).

37. H. Schneider, F. Pitossi, D. Balschun, A. Wagner, A. del Rey, H.O.Besedovsky HO. A neuromodulatory role of interleukin-1beta in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. **95,** 7778-83 (1998).

38. A.V. Andjelkovic, D. Kerkovich, J. Shanley, L. Pulliam, J.S. Pachter. Expression of binding sites for beta chemokines on human astrocytes. Glia. **28,** 225-35 (1999).

39. A. Singh, O.D. Jones, B.G. Mockett, S.M. Ohline, W.C. Abraham. Tumor Necrosis Factor-α-Mediated Metaplastic Inhibition of LTP Is Constitutively Engaged in an Alzheimer's Disease Model. J. Neurosci. **13,** 9083-9097 (2019).

40. J. O. Ekert, R. L. Gould, G. Reynolds, R. J. Howard, TNF alpha inhibitors in Alzheimer's disease: a systematic review. Int. J. Geriatr. Psychiatry **33**, 688–694 (2018).

41. M. T. Heneka, R. M. McManus, E. Latz, Inflammasome signalling in brain function and neurodegenerative disease. Nat. Rev. Neurosci. **19**, 610–621 (2018).

42. Z. Si, X. Wang, Z. Zhang et al. Heme oxygenase 1 induces tau oligomer formation and synapse aberrations in hippocampal neurons. J. Alzheimers Dis. **65**, 409–419 (2018).

43. R. M. Wright, L. K. Weigel, M. Varella-Garcia, G. Vaitaitis, J. E. Repine, Molecular cloning, refined chromosomal mapping and structural analysis of the human gene encoding aldehyde oxidase (AOX1), a candidate for the ALS2 gene. Redox Rep. **3**, 135–144 (2016).

44. K. Murakami, N. Murata, Y. Noda, et al. SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid β protein oligomerization and memory loss in mouse model of Alzheimer disease. J. Biol. Chem. **286**, 44557–44568 (2011).

45. C. Hetz, S. Saxena, ER stress and the unfolded protein response in neurodegeneration. Nat. Rev. Neurol. **13**, 477–491 (2017).

46. C. Corona, A. Pensalfini, V. Frazzini, S. L. Sensi, New therapeutic targets in Alzheimer's disease: brain deregulation of calcium and zinc. Cell Death Dis. **2**, e176 (2011).

47. H. Zempel, E. Thies, E. Mandelkow, E. M. Mandelkow, Abeta oligomers cause localized $Ca(^{2+})$ elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J. Neurosci. **30**, 11938–11950 (2010).

48. S. D. Girard, K. Baranger, C. Gauthier, M. Jacquet et al. Evidence for early cognitive impairment related to frontal cortex in the 5XFAD mouse model of Alzheimer's disease. J. Alzheimers Dis. **33**, 781–796 (2013).

49. J .Couturier, I.C. Stancu, O. Schakman et al. Activation of phagocytic activity in astrocytes by reduced expression of the inflammasome component ASC and its implication in a mouse

model of Alzheimer disease. J Neuroinflammation. **27**, 13-20 (2016).

50. A. L. Lazaar, L. Yang, R. L. Boardley, N. S. Goyal et al. Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor. Br. J. Clin. Pharmacol. **81**, 971–979 (2016).

Fig. 1. The arachidonic acid cascade. The arachidonic acid (AA) cascade is a group of metabolic pathways in which AA and other polyunsaturated fatty acids are the central molecules. Metabolism via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways gives rise to largely pro-inflammatory and pro-algesic metabolites. Both pathways have been pharmaceutically targeted. CYP enzymes either hydroxylate or epoxidize AA leading to hydroxyeicosatetranoic acids (HETEs) or epoxyeicosatrienoic acids (EETs), respectively. The latter compounds, which are endowed with potent anti-inflammatory properties, are rapidly subjected to hydrolysis to their corresponding diols by the soluble epoxide hydrolase (sEH) enzyme. Inhibitors of sEH block this degradation and stabilize EETs levels *in vivo* (*14*). They also reduce the corresponding diols which have some inflammatory properties. Major CYPs that oxidize AA are listed in the figure, but many others make a contribution.

Fig. 2. Soluble epoxide inhibition and its relevance in AD. (A) Immunoblot of sEH (EPHX2) of human brains from AD patients (Braak stage III-V). Groups were compared by Student t-test (n = 4-7). *p<0.05 vs. non-demented. **(B)** Immunoblot of sEH (EPHX2) in the hippocampus of SAMP8 mice (groups were compared by Student t-test, n = 12-14, **p<0.01 vs. SAMR1) and 5xFAD mice (groups were compared by Student t-test, n = 12-14, ****p<0.0001 vs. Wt). **(C)** Chemical structure of the sEH inhibitors employed. **(D)** CETSA experiments to monitor brain target engagement. Groups were compared by Student t-test or Two-Way ANOVA and post-hoc Dunnett's, n = 3 per group, *p<0.05, **p<0.01 and ***p<0.001 vs. Control.

Fig. 3. Role of sEH inhibitors in neurodegenerative biomarkers. (A) Scheme of experimental procedures in *in vivo* experiments. **(B)** Gene expression of neuroinflammatory markers (*Il-1β, Tnf-α*, and *Ccl3*) and protein levels of proinflammatory cytokines IL-1β, TNF-α, and CCL3 in the hippocampus of SAMP8 mice after treatment with sEH inhibitors. **(C)** Oxidative stress measured by hydrogen peroxide concentration in homogenates of the hippocampus. Representative gene expression of *Hmox*1 and *Aox*1 and representative Western blot and quantification of protein levels for (antioxidant enzyme) SOD1 in the hippocampus of SAMP8 mice after treatment with sEH inhibitors. **(D)** Representative Western blot and quantification of protein levels for ER stress markers ATF-6, IRE1α and XBP1 in the hippocampus of SAMP8 mice after treatment with sEH inhibitors. Gene expression levels were determined by real-time PCR, cytokine protein levels by ELISA and SOD1 by immunoblotting. Results are expressed as a MEAN ± SEM and were significantly different from the control group. Groups were compared by Student t-test or by One-Way ANOVA and post-hoc Dunnett's, n = 4-6 per group, (*p<0.05, **p<0.01, ***p<0.001 and ****<0.0001) vs. Control. See partial correlations between selected variable in Fig. S2 and Table S7.

Fig. 4. AD hallmarks in both SAMP8 and 5xFAD mice models after treatment with sEH inhibitors. **(A)** and **(B)** Representative Western blot, and quantifications for p-Tau Ser396 and p-Tau Ser404. **(C)** and **(D)** Representative Western blot, and quantifications for CTFs/APP ratio, sAPPα and sAPPβ. **(E)** Histological images, and quantification of

amyloid plaques stained with Thioflavin-S in Wt and 5xFAD. Values in bar graphs are adjusted to 100% for a protein of control group from each strain. Results are expressed as a MEAN ± SEM and were significantly different from the control group. Groups were compared by Student t-test or by One-Way ANOVA and post-hoc Dunnett's, n = 12 per group, (*Significant at $p<0.05$, **Significant at $p<0.01$, ***Significant at $p<0.001$ and ****Significant at $p<0.0001$). See partial correlations between selected variable in Fig. S2, Fig. S3, Table S7 and Table S8.

Fig. 5. **Characterization of the effect of sEH inhibitors and donepezil on cognitive status in both SAMP8 and 5xFAD mice models. (A)** Short-term memory evaluation after 2 h acquisition trial by Discrimination Index and **(B)** Long-term memory evaluation after 24 h acquisition trial by Discrimination Index after exposure to novel objects. Results are expressed as a MEAN ± SEM and were significantly different from the control group. Groups were compared by Student t-test or by One-Way ANOVA and post-hoc Dunnett's, n = 12 per group, (*Significant at $p<0.05$, **Significant at $p<0.01$, ***Significant at $p<0.001$ and ****Significant at $p<0.0001$). See partial correlations between selected variable in Fig. S3, Fig. S4, Table S7 and Table S8.