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Original Research Article

Role of soluble epoxide hydrolase in age-related vascular cognitive decline

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ABSTRACT

P450 eicosanoids are important regulators of the cerebral microcirculation, but their role in cerebral small vessel disease is unclear. We tested the hypothesis that vascular cognitive impairment (VCI) is linked to reduced cerebral microvascular eicosanoid signaling. We analyzed human brain tissue from individuals formerly enrolled in the Oregon Brain Aging Study, who had a history of cognitive impairment histopathological evidence of microvascular disease. VCI subjects had significantly higher lesion burden both on premortem MRI and postmortem histopathology compared to age- and sex-matched controls. Mass spectrometry-based eicosanoid analysis revealed that 14,15-dihydroxyeicosatrienoic acid (DHET) was elevated in cortical brain tissue from VCI subjects. Immunoreactivity of soluble epoxide hydrolase (sEH), the enzyme responsible for 14,15-DHET formation, was localized to cerebral microvascular endothelium, and was enhanced in microvessels of affected tissue. Finally, we evaluated the genotype frequency of two functional single nucleotide polymorphisms of sEH gene *EPHX2* in VCI and control groups. Our findings support a role for sEH and a potential benefit from sEH inhibitors in age-related VCI.

1. Introduction

The World Health Organization estimates that 35.6 million people currently live with dementia, and that number is expected to double by 2030 and more than triple by 2050 [1]. Alzheimer's disease (AD) remains the most common cause of dementia, but the contribution of cerebrovascular pathology to AD and dementia in general is becoming more appreciated [2]. Indeed, recent autopsy studies have shown that nearly a third of individuals with dementia have co-morbid cerebrovascular pathologies [3,4]. Cerebrovascular pathology can be isolated or combined with AD pathology in the case of mixed-dementia. Recent efforts to create a set of criteria to delineate cognitive impairment, both mild and advanced dementia, which are attributable to cerebrovascular disease have led to the introduction of the term vascular cognitive impairment (VCI) [5,6].

The most common type of VCI is that due to small vessel ischemic disease (also referred to as subcortical VCI) [7]. Evidence of small vessel ischemic disease can be observed on T_2-weighted magnetic resonance imaging (MRI) as hyperintensities primarily localized in white matter, both around the ventricles and as isolated foci. T_2-white matter hyperintensity (WMH) is thought to reflect white matter lesions [8]. Consistent with a central role for small vessel disease in the development of dementia, a recent prospective study showed that acceleration in WMH volume changes was an early predictor of conversion to mild cognitive impairment [9].

Despite recent advances in characterizing VCI, mechanisms underlying the development of cerebral small vessel disease in VCI remain poorly understood. Arachidonic acid derivatives, collectively referred to as eicosanoids, play an important role in control of the cerebral microcirculation [10]. We, therefore, hypothesized that VCI is linked to impaired eicosanoid signaling. In particular, we were interested in the role of cytochrome P450-derived

Abbreviations: OBAS, Oregon brain aging study; WMH, T_2 White matter hyperintensity; MRI, Magnetic resonance imaging; MMSE, Mini-mental state exam; CDR, Clinical dementia rating; VCI, Vascular cognitive impairment; EETs, Epoxyeicosatrienoic acids; DHETs, Dihydroxyeicosatrienoic acids; HETEs, hydroxyeicosatetraenoic acids; AD, Alzheimer's disease; SNP, Single nucleotide polymorphism.

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eicosanoids, since they have previously been linked to cerebrovascular function and disease [11]. For instance, elevated levels of 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor, have been linked to both ischemic and hemorrhagic strokes [12,13]. Conversely, elevated levels of 14,15-epoxyeicosatrienoic acids (14,15-EET), a vasodilator and neuroprotectant, have been shown to limit ischemic injury [14,15].

To test this hypothesis, we identified individuals with VCI and age- and sex-matched controls from the Oregon Brain Aging Study and quantified eicosanoid levels in cortical brain tissue biopsies using liquid chromatography-tandem mass spectroscopy (LC–MS/MS) [16]. We found higher levels of 14,15-dihydroxyeicosatrienoic acids (14,15-DHET), the inactive metabolic conversion product of 14,15-EET via the enzyme soluble epoxide hydrolase (sEH), in the VCI group. Consistent with a role in small vessel function and disease, sEH immunoreactivity was localized in cerebral microvascular endothelium, and was higher close to cerebrovascular lesions. Finally, we determined the frequency of functional single-polymorphisms polymorphisms (SNPs) in the sEH gene *EPHX2* in both VCI and control subjects, which also correlated with WHM volume. While there have been some recent advances in our understanding of dementia, there are currently no effective treatments to limit or reverse the onset of dementia [17]. This work contributes to the understanding of mechanisms underlying VCI and suggests a novel therapeutic strategy to treat VCI.

2. Materials and methods

2.1. Ethics statement

The sources, collection, storage and distribution of information and biological specimens were in accordance with guidelines established by the Layton Center/ORCATECH Research Repository in compliance with Federal regulations and Oregon Health & Science University (OHSU) policies. For autopsy tissue, specific consent for research purposes was obtained from the next of kin as part of the consent for autopsy.

2.2. Study subjects

88 individuals were used for measurements of white matter hyperintensity (WMH) volume and for the genetic analysis were selected from the Oregon Brain Aging Study (OBAS) [16] based on the availability of DNA, T_2-Weighted MRI measurements and neurocognitive assessment. All individuals in this study were Caucasian, except one, whose ethnicity is unknown. WMH volume measurements, mini mental state exams (MMSE), and clinical dementia ratings (CDR) were quantified as previously described [18].

Brains tissue used for immunohistochemistry and mass spectrometry-based analysis of eicosanoids was obtained from the Oregon Brain Bank and selected based on histopathological assessment performed by The Neuropathology Core of the Oregon Aging Alzheimer's Disease Center. VCI cases were selected based on previously described criteria of the Honolulu-Asia Aging Study [4], while controls were age-matched subjects that lacked microvascular ischemic injury as defined in that study. Specifically, VCI subjects were defined as those who had a high burden of microvascular lesions in either the neocortex or the basal ganglia and thalamus, or at both sites, while control subjects contained negligible microvascular lesions at both sites. Alzheimer's disease-related lesions (neuritic plaques and neurofibrillary tangles) were variable in subjects in both groups but were statistically matched. Cases containing Lewy bodies or other significant neurodegenerative disease-associated pathologies were excluded. Demographic data for individuals used for eicosanoid quantification are presented in Table 1. For one VCI individual, the post-mortem interval is unknown. Data for individuals used for immunohistochemistry are presented in Table 2.

Table 1
Demographic data of subjects used for mass spectrometry-based eicosanoid analysis.

Group	*N*	Mean age (range)	% Male	Postmortem interval (range)
Controls	5	95.8 (91–104)	80%	0.2 to 1.5 days
VCI	5	94.6 (88–108)	20%	0.1 to 1 day

Table 2
Demographic data of subjects used for immunohistochemistry.

Group	*N*	Mean age (range)	% Male	Postmortem interval (range)
Controls	11	89.1 (79–96)	45%	0.2 to 1 day
VCI	16	92.6 (87–106)	44%	0.2 to 8 days

2.3. LC–MS/MS analysis for eicosanoid metabolites

Post-mortem human brain samples were kept on dry ice until homogenization. Each sample was placed into 2.0 ml of PBS and then homogenized on ice using a polytron, at setting 2–3 for 20–30 s until homogenous. Samples were then diluted 1:1 with PBS. A 1 ml aliquot of the sample was mixed with 20 μl of an anti-oxidant mix consisting of 0.2 mg/ml BHT, 2 mg/ml triphenyl phosphine, and 2 mg/ml indomethacin. Samples were then spiked with an internal standard mix consisting of 1 ng of each of the following, d8-15 HETE, d6-20 HETE, d8 14,15 EET, and d11-14,15 DHET. Samples were kept on dry ice prior to homogenization and wet ice at all times thereafter until hydrolysis. 1 ml of 15% KOH was added to each tube containing 1 ml of the homogenized sample. The tube was briefly vortexed, capped tightly, and then hydrolyzed at 40 °C for 1 h. Samples were cooled briefly (<=5 min) and then acidified with 200 μl of glacial acetic acid, and then pH checked using pH paper for a desired range of 3–4. Samples were extracted with 3 ml of ethyl acetate, followed by 3 ml of hexane:ethyl acetate 1:1, followed by 2 ml of hexane. The extracts were combined and dried under vacuum for 35 min at 35 °C. 150 μl of 0.1 N HCl was added to residue in each tube, followed by the addition of 1 ml of hexane. Samples were vortexed for 2 × 20 s, spun at 2000 × *g* for 5 min and then hexane was transferred to a fresh tube. Samples were then dried under vacuum for approximately 7 min till dry and immediately brought up in 100 μl of start solvent which consisted of 45:55 (vol:vol) acetonitrile:water with 0.2 mg/ml TPP, 0.01% BHT and 0.01% formic acid and filtered through 0.22 μm placed in sample vials with inserts and analyzed immediately by LC–MS/MS. The injection volume was 20 μl. An un-extracted standard curve was used for these studies.

Levels of DHETs, HETEs and EETs were analyzed using a 5500 Q-TRAP hybrid/triple quadrupole linear ion trap mass spectrometer (Applied Biosystems) with electrospray ionization (ESI) in negative mode as described previously [19]. The mass spectrometer was interfaced to a Shimadzu (Columbia, MD) SIL-20AC XR autosampler followed by 2 LC-20AD XR LC pumps and analysis on an Applied Biosystems/SCIEX Q5500 instrument (Foster City, CA). The instrument was operated with the following settings: source voltage −4000 kV, GS1 40, GS2 40, CUR 35, TEM 450 and CAD gas HIGH. The scheduled MRM transitions monitored with a 1.5 min window are presented in Supplemental Table 1. Compounds were infused individually, and instrument parameters optimized for each multiple reaction monitoring transition. The gradient mobile phase was delivered at a flow rate of 0.5 ml per minute and consisted of two solvents, solution A, which consists of 0.05% acetic acid in