Inhibition of 12/15-LOX activity and ceramide pattern in the murine brain

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ABSTRACT

Purpose: The 12/15-lipoxygenase (12/15-LOX) activity and the ceramide content are often elevated in neurodegenerative disorders; however their relationship in the brain is not established. To verify whether blocking of 12/15-LOX activity has an impact on ceramide pattern in the brain we inhibited the 12/15-LOX expression by administration of baicalein in mice.

Materials and methods: The ceramides containing fatty acid methyl esters were analyzed using gas–liquid chromatography (GLC) technique.

Results: The total ceramide content increased in baicalein-treated animals comparing to controls and the levels of most ceramide-fatty acids of SAFA, MUFA and PUFA tended to increase in relation to control. Baicalein treatment up-regulated significantly only the ceramide-lignoceric fatty acid in relation to controls.

Conclusion: We have shown in this study that 12/15-LOX inhibition slightly alters the pattern of ceramides in the mouse brain.

Key words: 12/15-lipoxygenase; ceramide; baicalein; mice

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INTRODUCTION

The lipid mediators produced by the 12/15-lipoxygenase (12/15-LOX) or those connected with sphingolipid metabolism are important factors contributing to brain pathology emergence. There are several families of LOX, encoded by Alox genes, catalyzing oxidation at different sites of arachidonic acid. LOX isoforms vary also in substrates specificities and products, which they form [1] and they are characterized by diverse tissue distribution. Among lipoxygenases, 12/15-LOX seems to play a particular role in the brain and it is also called a "neuronal isoform" [2]. 12/15-LOX was identified mainly in neurons and glial cells in canine brain [3]. In humans, the term 12/15-LOX refers to leukocyte-type 12-LOX and reticulocyte-type 15-LOX-1 since they produce the similar lipid mediators such as 12- and 15-hydroperoxyeicosatetraenoic acids (HPETEs) and –hydroxyeicosatetraenoic acids (HETEs). 15-LOX is absent in mice and murine leukocyte-type 12-LOX is regarded as a human 12/15-LOX equivalent. However, lack of unambiguous human LOXs counterparts in other mammals makes, that studies carried on mice must be translated to humans with caution [4]. The engagement of 12/15-LOX in brain damage was noted under neurodegenerative conditions such as Alzheimer disease (AD) [5] or ischemia [6, 7]. The important role of 12/15-LOX in the Central Nervous System (CNS) disorders was proven by using mice with Alox15 gene disruption [6, 8] and animals treated with baicalein [6, 9], an inhibitor of 12/15-LOX [7].

Apart from the 12/15-LOX pathway products the sphingolipids, especially ceramides, are crucial lipid mediators of CNS diseases. The structural components of ceramides are represented by sphingosine and fatty acids of chain length ranges from C14 to C26 [10].

Ceramides can be produced by *de novo* synthesis, sphingomyelin catabolism or on salvage pathway [10]. The up-regulation of these lipids often accompanied the processes leading to CNS injury.

The ceramide content was elevated in brains of patients with HIV-associated dementia [11] and AD [12]. The levels of ceramides increased as well in brains of diabetic rats [13] and the white matter of rats after chronic cerebral ischemia [14].

The 12/15-LOX expression and ceramides are both up-regulated in the brain regions affected by similar pathologies. So far the obvious direct connection between these two important lipid pathways is unknown. The main goal of our study was to verify if blockage of 12/15-LOX activity by using the inhibitor, baicalein, influences the ceramides content and their pattern in murine brain.

MATERIALS AND METHODS

Animal procedures

The experimental protocol was approved by the Ethical Committee for Animal Experiments at the Medical University of Bialystok (nr of approval: 31/2010). The study was performed on 8 week- old, female, C57BL6 mice. The animals were kept in the Center of Experimental Medicine, Medical University of Bialystok in individually ventilated cages in pathogen free conditions with a 12-hour light-dark cycle and were allowed to consume water and food without restrictions. The C57BL6 mice were given 300 mg of baicalein/ kg of body weight in single intraperitoneal (i.p.) injection or were administrated with the vehicle (controls). Baicalein (Cayman Chemical, Ann Arbor, MI, USA) was originally dissolved in dimethyl formamide to produce a stock solution of 50 mg/ml. The stock solution was subsequently diluted in phosphate buffered saline (pH=7.2) in a 1:1 ratio [7]. The final dose of baicalein injected i.p. was 3mg/10g. The control mice received i.p. injections of phosphate buffered saline containing corresponding amounts of dimethyl formamide without baicalein. Mice treated with baicalein or vehicle were sacrificed 24h after injection. Brains were removed and immediately frozen in liquid nitrogen and stored at - 80°C until use. In experiments, 4-5 animals were used per group.

Analysis of ceramide content

Analysis of ceramide contents was described in details in our previous paper [13]. Briefly, the samples (whole brains except cerebellum) were pulverized, then transferred to a tube containing methanol and 0.01% butylated hydroxytoluene (Sigma) as an antioxidant. Lipids were extracted by the method of Folch and co-workers [15] and ceramides were isolated by means of thin-layer chromatography using the methods described by Yano and co-workers [16], and Mahadevappa and Holub [17]. Further analysis was performed as described in details elsewhere [18]. The ceramides containing fatty acid methyl esters were analyzed using gas–liquid chromatography (GLC) technique.

Statistical analysis

All data are presented in median, with percentyles. Statistical comparisons were performed by using the Kruskal–Wallis analysis of variance. $P \leq 0.05$ was considered statistically significant.

RESULTS

The content of total ceramide in the brain was increased, but not statistically significantly, in mice treated with baicalein (Fig. 1). We examined

also the pattern of ceramide species of different fatty acid composition in murine brain (Tab. 1), depending on the 12/15-LOX status. We have found the ceramide –stearic and -palmitic fatty acids as the most highly represented ceramide species in the mouse brain.

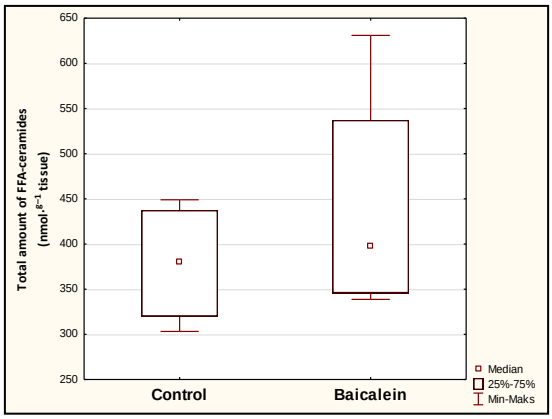

Fig.1. The total ceramide content in brains of mice injected with baicalein.

Both acids belong to the saturated fatty acid (SAFA) group. Less prevalent, but still abundant were ceramides consisting of oleic, nervonic and docosahexaenoic fatty acids.

To establish a connection between 12/15-LOX and ceramide pathways in the brain we used the mice treated with baicalein, an inhibitor of human platelet 12-LOX and 15-LOX-1 [19]. The content of total ceramide in the brain was increased, but not statistically significantly, in mice treated with baicalein. We have reported the levels of most ceramide-fatty acids in brains of baicalein-treated mice tended to increase in relation to control. Baicalein treatment caused significant alteration only in ceramide-lignoceric fatty acid concentration, which was up-regulated in relation to controls.

Table 1 The alteration of individual and total ceramide content and percentage of ceramide species in brains of mice injected with baicalein. All values contained in the table represent median and percentyles. The statistical analyses were performed using the Kruskal–Wallis analysis of variance. *p<0.05 versus control.

Fatty acid		**control**		**baicalein**	
		Content (nmol/g tissue)	%	Content (nmol/g tissue)	%
SAFA	**Myristic acid**	10.60 (9.30 - 13.50)	3.22	11.69 (11.03 - 11.97)	2.56
	Palmitic acid	74.87 (68.75 - 86.02)	21.10	81.99 (80.72 - 86.23)	19.25
	Stearic acid	163.50 (151.55 - 180.60)	44.54	154.07 (133.95 - 180.92)	36.42
	Arachidic acid	10.51 (9.63 - 11.22)	2.73	11.53 (10.52 - 12.02)	2.49
	Behenic acid	10.17 (9.97 - 10.49)	2.72	12.39 (11.40 - 13.70)	2.88
	Lignoceric acid	9.81 (8.77 - 10.40)	2.47	14.18 (12.50 - 18.68) *	3.85
MUFA	**Palmitoleic acid**	6.86 (5.83 - 8.24)	1.90	6.75 (6.69 - 7.20)	1.62
	Nervonic acid	17.69 (16.08 - 19.16)	4.64	26.04 (21.82 - 31.93)	6.28
	Oleic acid	29.68 (24.77 - 35.95)	8.20	39.79 (32.63 - 56.78)	11.24
PUFA	**Linoleic acid**	2.09 (1.67 - 2.89)	0.65	2.98 (2.33 - 3.89)	0.73
	Linolenic acid	3.16 (3.03 - 3.47)	0.88	3.58 (3.17 - 3.91)	0.80
	Arachidonic acid	9.02 (5.59 - 12.31)	2.34	12.31 (7.72 - 22.67)	4.10
	Docosahexaenoic acid	17.11 (10.59 - 23.96)	4.61	22.59 (15.57 - 41.36)	7.78
Total ceramide		381.24 (328.23 - 431.67)		398.02 (349.16 - 490.29)	

DISCUSSION

We have shown in this study that inhibition of the enzyme 12/15-LOX with baicalein led to alterations in the ceramide compositions in murine brain.

Previously, we described the percentage distribution of ceramide species in the rat brain [13]. We have obtained the similar results for the mouse brain tissue. The murine brain tissue was enriched particularly in ceramides containing palmitoleic, oleic and docosahexaenoic acids when compared with the rat brain, but conversely the mice had a diminished amount of ceramide-lignoceric fatty acid. Thus, even closely related species demonstrate some differences in brain ceramide pattern.

A direct connection between two important inflammatory mediators in the brain, 12/15-LOX and ceramides, is not established yet. The present experiment was performed on healthy animals and there are solid reasons to hypothesize that 12/15-LOX might influence the ceramide pattern. Arachidonic acid, one of PUFA acids, is a major substrate for the 12/15-LOX. In our study the content of ceramide-arachidonic acid species is subtly up-regulated in baicalein-treated mice (however not statistically significantly). This observation suggests that non-metabolized arachidonic acid may be, to some extent, incorporated into membrane ceramides and alter the membrane structure. However, a role of 12/15-LOX is not restricted to free arachidonic acid metabolism. This enzyme oxygenates a set of PUFA acids, more complex lipid esters and lipoproteins, also those anchored in the cell membrane [20]. Ceramides constitute an important part of cell membrane, being localized mainly in lipid rafts [21]. Thus, 12/15-LOX, by changing the membrane organization, may influence the composition of sphingolipids as ceramides and sphingomyelins. The only statistically significant changes were found in ceramide-lignoceric fatty acid concentration, which was quit unexpected observation. Probably lignoceric fatty acid is another one substrate for the 12/15-LOX. This hypothesis suggests involvement of such enzyme in creation of lipid rafts thus an indirect role in integration of extracellular signaling.

Additional studies are needed to explain this modification.

Both 12/15-LOX and ceramides were found to be involved in neurodegeneration. The augmented levels of 12/15-LOX were detected in vulnerable brain structures in AD, which was correlated with their stronger peroxidation [5]. Additionally, Cutler and co-workers [22] have shown the alterations in ceramide-stearic and –lignoceric acids content in AD brains. The changes in 12/15-LOX and ceramides levels were also found in ischemia. The enhanced expression of 12/15-LOX was detected in penumbra, mainly in neurons [7] and the enrichment in ceramide-stearic and palmitic acids was observed in ischemic human brains [23].

Moreover, the 12/15-LOX was engaged in pathological processes occurring under ischemic condition since Alox15(-/-) knockout mice displayed a significant attenuation of brain edema and blood brain barrier (BBB) leakage [6]. The similar results were obtained using baicalein [6, 9]. Moreover, administration of baicalein to Alox15 (-/-) knockout mice was no additionally neuroprotective to neurons in ischemic brain [7], which suggests that the whole beneficial effect exerted by baicalein in ischemia comes from 12/15-LOX blockage. Taking into account that ceramides containing different fatty acids vary in their biological actions [24], changed ceramide composition which results from blocking 12/15-LOX activity may lead to different involvement of those lipids in the neurodegenerative processes. Probably over expression of 12/15-LOX as well as inhibition of that enzyme activity in pathological conditions may have different effects on ceramide species. This hypothesis needs detailed further study and we plan the experiments with Alox15(-/-) knockout mice in some models of pathology of brain.

It seems that the pattern of ceramide species may be unique for each brain disorder (and not only), hence ceramide augmentation may produce differential outcomes since each ceramide species may differ significantly in mechanism of action. Baicalein used in the present experiment presents many additional than blockade 12/15LOX activities being anti-virus, anti-tumor or anti-inflammatory agent, thus it affects also other regulatory systems in the cell [9], which can have even stronger impact on the ceramide content than 12/15-LOX inhibition.

However, it clearly shows that 12/15LOX may participate in creation of ceramide species pattern in physiology. Based on our studies, we propose that the improvement of brain function in pathology by blocking 12/15-LOX may be also gained by modulation of ceramide content.

CONCLUSION

We have shown in this study that the inhibition of 12/15-LOX slightly alters the pattern of ceramide species in the mouse brain. The only ceramide-lignoceric fatty acid concentration probably is connected with 12/15-LOX activity.

Conflicts of interest

The authors declare no conflict of interest.

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