Advances in Medical Sciences 61 (2016) 72–77

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Reduction of ceramide de novo synthesis in solid tissues changes sphingolipid levels in rat plasma, erythrocytes and platelets

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ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form 3 July 2015
Accepted 17 September 2015
Available online 1 October 2015

Keywords:
Sphingolipids
Plasma
Erythrocytes
Platelets
Myriocin

ABSTRACT

Purpose: De novo sphingolipid synthesis does not occur in plasma, erythrocytes and platelets. The purpose of the study was to examine the effect of inhibition of sphingolipid synthesis in solid tissues on the level of the following bioactive sphingolipids: sphinganine, ceramide, sphingosine and sphingosine 1-phosphate in plasma, erythrocytes and platelets.
Material/methods: The experiments were carried out on male Wistar rats. Myriocin was used to inhibit serine palmitoyltransferase activity (the enzyme catalyzes the first step of ceramide de novo synthesis) and nicotinic acid was used to reduce the concentration of plasma free fatty acids (a substrate for the de novo ceramide synthesis). The sphingolipids were quantified by means of liquid chromatography/mass spectrometry.
Results: Myriocin reduced the level of each compound in plasma. It reduced the level of sphinganine, sphingosine-1-phosphate and total ceramide and elevated the level of sphingosine in erythrocytes. In platelets, myriocin reduced the total level of ceramide. Nicotinic acid reduced the plasma level of sphinganine, sphingosine and total ceramide. It increased the level of sphingosine-1-phosphate in erythrocytes. In platelets, nicotinioc acid increased the level of sphinganine and sphingosine and reduced the level of sphingosine-1-phosphate and total ceramide.
Conclusions: Inhibition of serine palmitoyltransferase activity in solid tissues and reduction in plasma free fatty acids concentration affects sphingolipid level in plasma, erythrocytes and platelets. The changes in erythrocytes and platelets depend both on the cell type and the sphingolipid studied and only partially follow the changes in the plasma.

1. Introduction

Metabolism of bioactive sphingolipids in solid tissues is quite well recognized. The key compound is ceramide. It is formed on two major pathways: de novo synthesis pathway and from sphingomyelin. The first reaction of the former pathway is condensation of serine and palmitoyl-CoA to form 3-ketosphinganine (Fig. 1). Next, 3-ketosphinganine is converted to sphinganine and then, sphinganine is converted to dihydroceramide. The latter step involves addition of a long chain fatty acid residue. Dihydroceramide is converted to ceramide. Ceramide is catabolized to sphingosine which can be phosphorylated to sphingosine-1-phosphate (Fig. 1). Hydrolysis of sphingomyelin is catalyzed by the enzyme sphingomyelinase [1,2]. Ceramide, sphingosine and sphingosine-1-phosphate (S1P) play variety of important biological functions [3–6]. The compounds are present in the plasma, erythrocytes and platelets of different mammalian species [7–12]. However, contrary to the solid tissues, metabolism of bioactive sphingolipids in different blood compartments is only poorly recognized. Plasma sphingolipid level, with the exception of sphingosine-1-phosphate, seems to depend mostly on the rate of the de novo synthesis in solid tissues. Plasma sphingosine-1-phosphate is secreted by erythrocytes [7,9,13,14], platelets and vascular endothelium [15–17]. Partial inhibition of serine-palmitoyl transferase activity by myriocin in lean mice was shown to reduce the plasma ceramide and elevate plasma sphingosine level whereas the level of sphingosine-1-phosphate remained unchanged [18]. On the other hand, in apoE-deficient mice, myriocin reduced plasma level of not only ceramide but also sphinganine, sphinganine-1-phosphate, sphingosine and sphingosine-1-phosphate [19]. Moreover, it was shown in mice that deficiency of serine-palmitoyl transferase resulted in a reduction of

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http://dx.doi.org/10.1016/j.advms.2015.09.006
1896-1126/

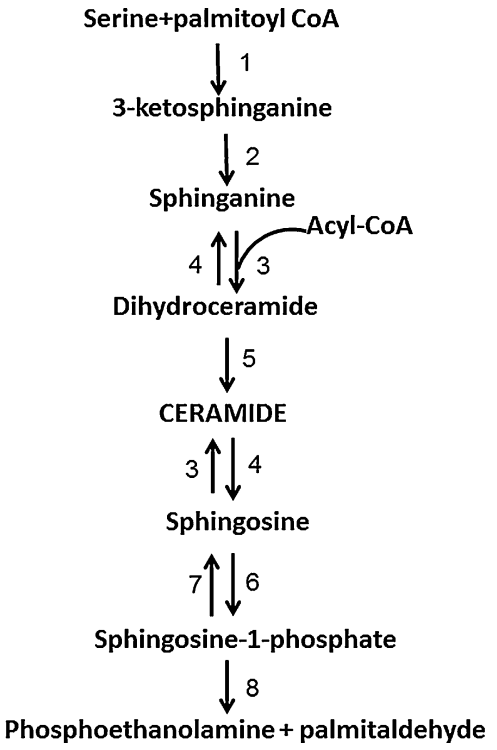

Fig. 1. Simplified scheme of the de novo ceramide synthesis pathway. Numbers denote the following enzymes: 1 – serine palmitoyltransferase, 2 – 3-keto-sphinganine reductase, 3 – ceramide synthase, 4 – dihydroceramide desaturase, 5 – ceramidase, 6 – sphingosine kinase, 7 – sphingosine-1-phosphate lyase. Sphingosine-1-phosphate can be also dephosphorylated by phosphohydrolase – 8.

the plasma levels of the above compounds, with the exception of sphinganine [20]. Oversupply of fatty acids increases plasma ceramide concentration [21,22]. On the other hand, reduction in the level of plasma free fatty acids (FFA) did not affect the plasma ceramide level [22]. No data are available on effect of reduction in plasma long chain fatty acid supply on plasma concentration of other bioactive sphingolipids. Erythrocytes and platelets are not able to synthetize ceramide de novo. However, there are no data on the effect of changes in the concentration in the plasma sphingolipid on the level of the compounds in the anucleated blood cells.

The aim of the present study was to examine effect of inhibition of serine-palmitoyl transferase and reduction in the plasma free fatty acid supply on the level of principal bioactive sphingolipids in different blood compartments, namely plasma, erythrocytes and platelets.

2. Material and methods

The experiments were carried out on male Wistar rats 280–300 g of body weight. The experiment has been approved by the Ethical Commission for Animal Studies at the Medical University of Białystok. 12 h light/dark schedule was maintained in the animal room. The rats were fed ad libitum with a chow diet and had free access to tap water. They were divided into three groups: 1-control, 2-treated with myriocin, 3-treated with nicotinic acid. Myriocin (Sigma) was dissolved in 0.9% NaCl and administered intraperitoneally, daily, for 7 days, in a dose of 0.3 mg/kg [18]. Nicotinic acid (Sigma) was administered subcutaneously in a dose of 250 mg/kg body weight and the blood was taken 3 h later [23]. $N = 10$ in each group. The rats were anaesthetized with thiopental (80 mg/100 g of body weight) and the blood was taken from the abdominal aorta. Sodium citrate was used as an anticoagulant. Immediately after sampling, 4 ml of blood was centrifuged at 1400 × *g* for 10 min at 4° and platelet-rich plasma was transferred to a fresh plastic tube. The leukocyte-rich buffy coat was thoroughly removed. Separated erythrocytes were suspended in 3 ml of cold PBS buffer (pH 7.4), centrifuged at 1400 × *g* for 10 min and the upper layer and the remaining buffy coat was discarded. Erythrocytes were then re-suspended in 2 ml of PBS buffer (pH 7.4) and flash frozen in liquid nitrogen. Platelet-rich plasma was centrifuged at 2000 × *g* for 10 min at 4° to isolate platelets. Isolated platelets were washed with cold platelet wash buffer (5 mM KH_2PO_4, 5 mM Na_2HPO_4, 0.1 M NaCl, 1% glucose, 0.63% sodium citrate, pH 6.6), suspended in 0.3 ml of PBS, and flash frozen in liquid nitrogen. Supernatant was then transferred to a fresh plastic tube and centrifuged at 5000 × *g* for 10 min to obtain platelet-free plasma. All samples were stored at −80 °C until analysis. The plasma FFA level was determined enzymatically with the use of Randox FA 115 kit.

The level of sphingolipids in plasma, erythrocytes and platelets was measured by means of an Agilent 6460 triple quadrupole mass spectrometer using positive ion electrospray ionization (ESI) source with multiple reactions monitoring (MRM) according to [24] with minor modification. Briefly, extraction solution composed of isopropanol:water:ethyl acetate (35:5:60; v:v:v) together with internal standard solution (17C-Shingosine, 17C-Sphingosine-1-phosphate, and C17-Ceramide (Avanti Polar Lipids)) was added to each sample. The mixture was vortexed, sonicated and then centrifuged for 10 min at 4000 rpm (Heraeus Multifuge 3SR+, Thermo Scientific). The supernatant was transferred to a new tube and pellet was re-extracted. After centrifugation supernatants were combined and evaporated under nitrogen. The dried samples were reconstituted in 100 μl of LC Solvent A (2 mM ammonium formate, 0.15% formic acid in methanol) for liquid chromatography–tandem mass spectrometry (LC/MS/MS) analysis. The analytical column was a reverse-phase Zorbax SB-C8 column 2.1 × 150 mm, 1.8 μm. Chromatographic separation of sphingolipids (sphingosine, sphinganine, sphingosine-1-phosphate, ceramides – C14:0, C16:0, C18: 1, C18:0, C20:0, C22:0, C24:1 and C24:0) was conducted in binary gradient using 2 mM ammonium formate, 0.15% formic acid in methanol as Solvent A and 1.5 mM ammonium formate, 0.1% formic acid in water as Solvent B at the flow rate of 0.4 ml/min using an Agilent 1290 Infinity Ultra Performance Liquid Chromatography (UHPLC). The concentration of each sphingolipid species was measured against standard curves. The flow was diverted to waste for the first and the last four minutes to prevent eluting impurities from entering the mass spectrometer.

Statistical analysis: Data were analyzed by ANOVA with Tukey HSD (Honestly Significant Difference) post hoc test. In the presence of unequal variances, Welch ANOVA was used together with Games–Howell post hoc test. $p < 0.05$ is regarded as significant.

3. Results

3.1. Plasma free fatty acids

The plasma level of free fatty acids was (μmol/liter): control – 131.0, after treatment with myriocin – 155.9 ($p > 0.05$) and after treatment with nicotinic acid – 45.6 ($p < 0.001$).

3.2. Sphingolipids

3.2.1. Plasma (Fig. 2 and Table 1)

Myriocin treatment reduced the level of each compound studied (sphinganine, sphingosine and total ceramide, $p < 0.001$), sphingosine-1-phosphate ($p < 0.05$). Nicotinic acid reduced the level of sphinganine ($p < 0.05$), sphingosine and total ceramide ($p < 0.001$). Neither treatment affected the total level of ceramide. Quantitatively, the major ceramide in each group was ceramide containing C24 fatty acid residue (Table 1).