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Original Research Article

Myriocin treatment affects lipid metabolism in skeletal muscles of rats with streptozotocin-induced type 1 diabetes

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ABSTRACT

Purpose: The aim of this work was to assess the effect(s) of de novo ceramide synthesis inhibition on lipid metabolism in skeletal muscle tissue of type 1 diabetic rats. The latter seems to be of vital importance, since previous works have shown its positive influence on lipid metabolism and glucose homeostasis in the case of its counterpart – type 2 diabetes.

Materials/methods: The animals were randomly assigned to one of the following groups: C – control, M – myriocin (ceramide de novo synthesis inhibitor), D – diabetes (induced by streptozotocin injections); D + M – diabetes + myriocin. We have evaluated intracellular concentration of key sphingolipid species, via chromatography (GC and HPLC), and the activity of their most important enzymes, using radiometric approach. The aforementioned assessments were evaluated in respect to the three different types of muscle tissue representing different spectra of muscle metabolism (soleus – oxidative, red gastrocnemious – oxidative-glycolytic, white gastrocnemious – glycolytic).

Results: Interestingly, our therapeutic intervention not only lowered the level of ceramide, its precursors (sphinganine) and derivatives (sphingosine and sphingosine-1-phosphate), but also reduced other lipid species (triacylglycerols, diacylglycerols and free fatty acids) content, thus improving glucose homeostasis in type 1 diabetic animals.

Conclusions: In the light of the results ensuing from this study, it seems conceivable that the reduction of intramuscular ceramide production and accumulation could bestow an insulin-sensitizing effect. If so, then SPT inhibition could find potential future applications as a therapeutic intervention aimed to mitigate the effects of insulin resistance.

1. Introduction

Diabetes mellitus is currently one of the most widespread diseases. According to the International Diabetes Federation it affected approximately 400 million people globally (as for the year 2014), accounting for more than 5% of the Earth's human population [1]. Moreover, in the year 2012 the World Health Organization (WHO) classified diabetes as the 7th leading cause of death worldwide [2], whereas its (diabetes) estimated annual costs (just for the US economy) reached $245 billion [3]. Therefore, nowadays the pursuit for a new effective diabetic drug is a pressing economical and medical issue.

The relationship between bioactive lipid species accumulation and the subsequent development of diabetes seems to be well established since it has been confirmed in human, animal and cell culture models [4–6]. This class of biological molecules is highly implicated in the onset and progress of insulin resistance in skeletal muscle. The background for its development seems to be an imbalance between bioactive lipids supply and their intracellular oxidation occurring secondarily to an increased lipids uptake and/or decreased mitochondrial oxidative capacity [7]. The subclasses of lipids responsible for the pathogenesis of insulin resistance are intracellularly accumulated free fatty acids (FFAs), diacylglycerol (DAG) and triacylglycerol (TG) moieties. The accretion of intramuscular DAG possibly occurs as a result of prolonged, intensified free fatty acids release from the insulin

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resistant white adipose tissue [8]. Interestingly, formerly published data indicate an existence of a strong positive correlation between the increased intramuscular triacylglycerol content and skeletal muscle insulin resistance origin/development. Moreover, the aforementioned increased intramuscular TG quantity is currently recognized as one of the markers, although not a direct cause, of skeletal muscle insulin resistance [9,10].

It seems that also, and perhaps above all, ceramide tissue overaccumulation makes individuals more prone to diabetes development. In a previously conducted study the authors demonstrated that the addition of ceramide analogs to cell cultures (derived from liver, skeletal muscle or fat tissue) results in the inhibition of glucose uptake and glycogen synthesis, i.e. one of the early symptoms of insulin resistance build-up [11]. An increase in the intracellular ceramide concentration activates PP2A (protein phosphatase 2A), which in turn dephosphorylates and thereby deactivates PKB/Akt (protein kinase B) molecules in skeletal muscles [12–14]. In result the incorporation of so called glucose storage vesicles (GSV) into a plasma membrane decreases. This leads to a drop in the plasmalemmal GLUT-4 transporters number and therefore a reduced response to insulin signaling (smaller glucose uptake) [13–15]. Moreover, ceramide appears to be also entangled in the onset of type 1 diabetes, since many studies have confirmed its involvement in the development of pancreatic β-cells dysfunction [16]. It seems that ceramide, produced in response to inflammatory signaling molecules (i.e. cytokines, like: TNFα and IL-1), can suppress insulin gene expression process, arrest β-cell proliferation and/or promote cellular apoptosis [17,18]. The above-mentioned literature data give strong rationale for considering ceramide as an important physiological and pathophysiological factor involved in the genesis of type 1 diabetes.

Sphingolipids constitute a class of important, biologically active lipids that are involved in numerous cellular processes. These encompass proliferation and differentiation of the cells, inflammatory responses and programmed cell death (apoptosis). Ceramide is a central molecule of sphingolipid metabolism (Fig. 1). It is a sphingosine-based lipid moiety, which acts as a second messenger in a sphingomyelin (SM) signal transduction pathway activating many kinases, phosphatases and transcription factors [19]. Ceramide can be generated as a result of plasma membrane sphingomyelin hydrolysis occurring in response to the activation of neutral or acidic isoform of the enzymes sphingomyelinases (nSMase and aSMase). However, its major synthesis rout takes place within an endoplasmic reticulum and is called ceramide de novo synthesis pathway. It encompasses many tightly regulated enzymatic processes beginning with a condensation reaction (serine + palmitoyl-CoA) that leads to the formation of 3-ketosphinganine, which is then rapidly reduced to sphinganine (SFA). This first and rate limiting step (the condensation reaction) is governed by the enzyme serine palmitoyltransferase (SPT). Afterwards, sphinganine is acylated to form dihydroceramide, which is converted into ceramide by the addition of trans 4,5-double bond. Importantly, ceramide can be converted into other sphingolipids by its degradation processes catalyzed by the enzymes ceramidases. Three main isoforms of these enzymes, neutral, alkaline and acidic ceramidase, have been previously described. Moreover, it seems that also ceramide derivatives, bioactive lipids: sphingosine (SFO) and sphingosine-1-phosphate (S1P), can exert divergent biological effects. These moieties, *nota bene*, influence the processes of cellular growth, differentiation and apoptotosis, and for these reasons they may be involved in the pathogenesis of type 1 and 2 diabetes mellitus [20,21].

Myriocin ($C_{21}H_{39}NO_6$) is a drug isolated from certain fungi species (*Isaria sinclairi* and *Mycelia sterilia*) characterized by its potent and highly selective SPT inhibitory properties [22]. Interestingly, some of these fungi have found applications in a branch of traditional Asian medicine attempting to achieve eternal youth [23]. Moreover, some of the previously published studies provided evidence that myriocin may be of potential importance also as a drug targeted for the treatment of selected cardiovascular diseases, such as atherosclerosis [24,25]. Moreover, there are also some reports, based on animal models of obesity and both type 1 and 2 diabetes, suggesting that inhibition of ceramide de novo synthesis with myriocin ameliorates glucose homeostasis and improves whole-body insulin responsiveness [26,27]. Furthermore,

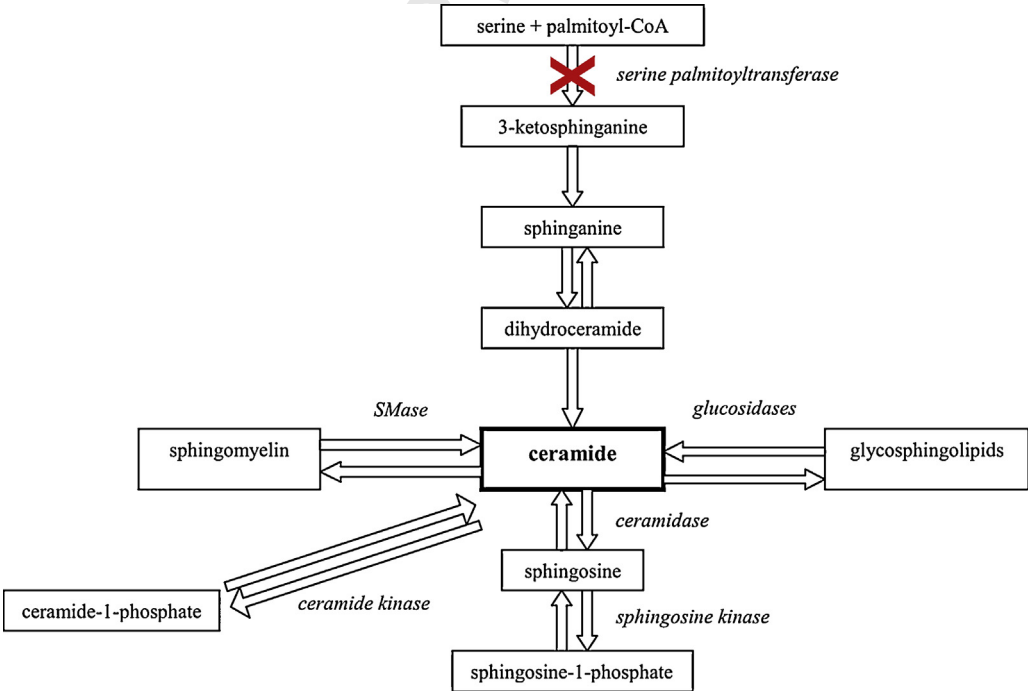

Fig. 1. Ceramide as a central link in sphingolipids metabolism. Myriocin inhibits serine palmitoyltransferase (SPT) – marked with red "X".

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