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Inhibitors of dihydroceramide desaturase 1: Therapeutic agents and pharmacological tools to decipher the role of dihydroceramides in cell biology

Mireia Casasampere, Yadira F. Ordoñez, Ana Pou, Josefina Casas*

Research Unit on BioActive Molecules, Department of Biomedicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Jordi Girona 18-26, E-08034 Barcelona, Spain

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ABSTRACT

Dihydroceramide desaturase (Des1) is the last enzyme in the *de novo* synthesis of ceramides (Cer). It catalyzes the insertion of a double bond into dihydroceramides (dhCer) to convert them to Cer, both of which are further metabolized to more complex (dihydro) sphingolipids. For many years dhCer have received poor attention, mainly due to their supposed lack of biological activity. It was not until about ten years ago that the concept that dhCer might have regulatory roles in biology emerged for the first time. Since then, multiple publications have established that dhCer are implicated in a wide spectrum of biological processes. Physiological and pathophysiological functions of dhCer have been recently reviewed. In this review we will focus on the biochemical features of Des1 and on its inhibition by different compounds with presumably different modes of action.

1. Introduction

Sphingolipids (SLs) are the second largest class of membrane lipids and thousands of distinct species have been identified (Merrill, 2011). They have a diverse range of functions related to cell survival, membrane integrity, metabolic regulation, and general adaptations to cellular stressors (Siddique et al., 2015). Biosynthesis of SLs consists of a highly conserved sequence of enzymatic reactions that take place in different intracellular compartments (Morad and Cabot, 2013). The biosynthesis *de novo* occurs in the endoplasmic reticulum (ER) and starts with the condensation of L-serine with palmitoyl-CoA to give 3-ketodihydrosphingosine in a reaction catalyzed by serine palmitoyl transferase. By the action of a reductase, 3-ketodihydrosphingosine is reduced to dihydrosphingosine (dhSo), which is *N*-acylated to dihydroceramides (dhCer) by specific ceramide synthases of different chain length specificities. The oxidation of dhCer to ceramide (Cer) by dihydroceramide desaturase 1 (Des1) is the last step of this biosynthetic pathway. Cer, and, to a lesser extent, dhCer are further metabolized to complex SLs, such as (dihydro) sphingomyelins and (dihydro) glycosphingolipids (Fig. 1) (Morad and Cabot, 2013).

The search for the term dihydroceramide in the PubMed database retrieves 541 papers, 56% of them being published in the last 10 years (Fig. 2). Among the above papers, 44 are reviews, although only 3 of them are devoted to dhCer (Fabrias et al., 2012; Rodriguez-Cuenca et al., 2014; Siddique et al., 2015). The growing interest on dhCer is indubitable as shown by the fact that the last two reviews have been published in 2015. Our previous review (Fabrias et al., 2012) dealt with Des1 biochemistry and mode of action and the biological functions of dihydrosphingolipids. In their review, Rodríguez-Cuenca and coworkers describe the function of Des1 and its dysregulation by factors such as oxidative stress, hypoxia and inflammation, and present pathological scenarios characterized by specific increases in dhCer (Rodriguez-Cuenca et al., 2014). On the other hand, the uncovered roles of dhCer in autophagy, hypoxia, and cellular proliferation and its implication in the etiology, treatment, or diagnosis of diabetes, cancer, ischemia/reperfusion injury, and neurodegenerative diseases have been summarized by Siddique et al. 2015. Given that the description of the physiological and pathological roles of dhCer has been extensively discussed the last two outstanding reviews, the

Abbreviations: Cer, ceramide(s); 4-HPR, N-(4-hydroxyphenyl)retinamide or fenretinide; Aβ, amyloid β peptides; DEGS1, drosophila degenerative spermatocyte 1; DEGS2, drosophila degenerative spermatocyte 2; Des1, dihydroceramide desaturase 1; Des2, dihydroceramide desaturase 2; dhCer, dihydroceramide(s); dhSo, dihydrosphingosine; ER, endoplasmic reticulum; HIV-1, human immunodeficiency virus 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; SLs, sphingolipids; THC, Δ9-tetrahydrocannabinol; TRB3, *tribbles*-related protein 3.

* Corresponding author.
E-mail address: fina.casas@iqac.csic.es (J. Casas).

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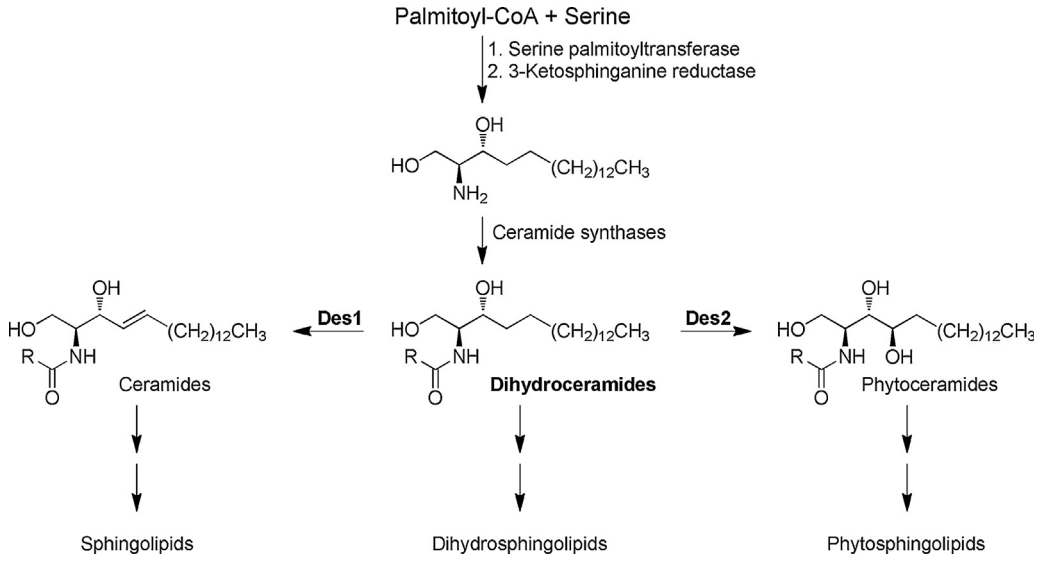

Fig.1. Biosynthesis *de novo* of sphingolipids.

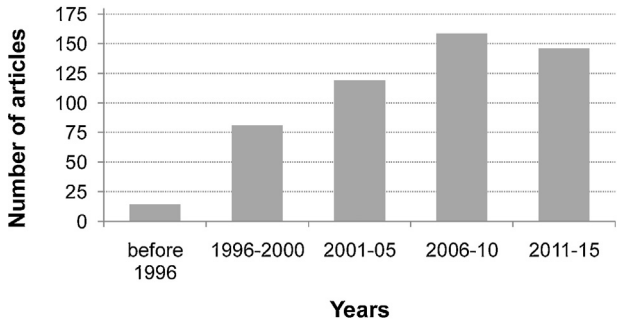

Fig. 2. Number of articles retrieved from PubMed by searching for the term dihydroceramide.

present one will mainly focus on Des 1 inhibitors and the biological consequences of blocking Des1 activity.

2. Dihydroceramide desaturases

The gene encoding for Des1 was first cloned in 1996 from *Drosophila melanogaster*, and it was given the name"drosophila degenerative spermatocyte 1" or DEGS1 (Endo et al., 1996). One year later, Cadena et al. 1997 demonstrated that the DEGS1 gene product was a membrane bound desaturase and found that its overexpression inhibited epidermal growth factor receptor biosynthesis. Despite the importance of this receptor in several malignancies, this finding has not been further investigated. Currently, the dihydroceramide desaturase gene is known as either DES1 or DEGS1. Ternes et al. 2002 identified a Des1 homologue, namely Des2, as a bifunctional enzyme with dihydroceramide C4-desaturase and C-4-hydroxylase activities. This enzyme is responsible for the biosynthesis of glycosphingolipids containing 4-hydroxysphinganine in the small intestine (Omae et al., 2004). Thus, while Des1 exhibits high dihydroceramide C4-desaturase and very low C-4 hydroxylase activities, Des2, the product of the gene DEGS2 or DES2, exhibits bifunctional sphingolipid C-4 hydroxylase and C4-desaturase activities (Ternes et al., 2002). The tissue distribution profile of both enzymes is considerably different. Des1 is ubiquitously distributed, whereas Des2 is preferentially expressed in small intestine, skin and kidney (Fabrias et al., 2012), where the production of phytoceramides is essential.

A few lines of evidence demonstrate that Des1 is regulated by fatty acids. Thus, Rioux and co-workers (Rioux et al., 2011) showed that myristoylation of Des1 increases the enzyme activity (Beauchamp et al., 2007; Ezanno et al., 2012) and alters its subcellular localization, targeting the enzyme from the ER to the mitochondrial outer membrane, wherein causes an increase in Cer levels that in turn leads to apoptosis (Beauchamp et al., 2009). The recombinant non myristoylable mutant form of Des1, on the contrary, is almost completely absent in mitochondrion (Beauchamp et al., 2009). Another fatty acid, palmitic acid, increases mRNA encoding DES1 leading to increased Cer synthesis *de novo*. In contrast, co-treatment with oleate prevented the increase in ceramide, and this occurred through attenuation of the increase in message and activity of Des1. These findings provide insight into the mechanisms of oleate-mediated protection against metabolic disease and provide novel evidence for fatty acid-mediated regulation of Des1 (Hu et al., 2011). From the biochemical point of view, studies carried out in the late 90s demonstrated that Des1 requires NADPH (Geeraert et al., 1997) or NADH (Michel et al., 1997) as electron donor and oxygen as electron acceptor. The electron provided by NAD(P)H is sequentially transported from the cofactor to NADH-cytochrome b5 reductase, cytochrome b5, and the terminal desaturase, which reduces oxygen to water and oxidizes dhCer to Cer (Fig. 3). A similar mechanism was proposed by Enomoto et al. 2006 for the hydroxylation reaction catalyzed by Des2. Oxygen-dependence explains that both Des1 and Des2 are

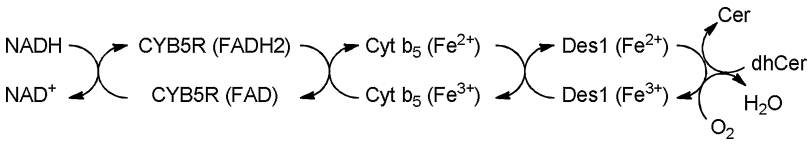

Fig. 3. Dihydroceramide desaturase enzymatic complex.