Michał Stepulak[1], Tomasz Kobiela[2], Małgorzata Lekka[3], Karolina Leleń-Kamińska[1], Magdalena Malejczyk[4], Sławomir Majewski[1,4], Jacek Arct[1], Maja Haczyk[5]

Biological activity of synthetic ceramides

[1]Academy of Cosmetics and Health Care, Warsaw, Poland
[2]Warsaw University of Technology, Faculty of Chemistry, Institute of Biotechnology, Warsaw, Poland
[3]The H. Niewodniczanski Institute of Nuclear Physics, Polish Academy of Sciences, Cracow, Poland
[4]Medical University of Warsaw, Department of Dermatology and Venerology, Warsaw, Poland
[5]Pomeranian Medical University, Szczecin, Poland

SCIENCE & RESEARCH

Introduction

It is well known that ceramides play special role in functioning of human body. Firstly, as a structural components of stratum corneum intercellular cement these lipids are responsible for forming barrier protecting from excess water loss and absorption of unwanted exogenous substances. Secondly, ceramides (and their metabolites) play also crucial roles in maintaining normal structure of cell membranes and as a signal molecules they are part of signal transduction pathways.

Cosmetic ceramides

Because of abilities to restore proper barrier functioning when delivered from outside ceramides are used as a cosmetics ingredients. Unfortunately ceramides belong do ingredients which causes troubles when formulating. Mainly it concerns ceramides of animal and plant origin. Typical ceramides (formed of long chain fatty acid and long chain sphingoid base) have high melting point and therefore are difficult to introduce to cosmetic base – some need to be heated over 120°C. High melting temperature also causes their recrystalization in final cosmetic products. Therefore semisynthetic of fully synthetic derivatives of ceramides have been designed. They are much more convenient when formulating and act similarly as the natural ones by supplementing disrupted intercellular cement.

Biological properties

In literature one can find many evidences regarding biological activity of semi- and synthetic ceramides but majority of conducted studies concern activity of model synthetic ceramides containing short chain fatty acids C2, C6 and C8. These substances are responsible for such effects as downregulation of proliferation, upregulation of differentiation and in some cases induction of apoptosis in various cells. There is little known regarding effects caused by long chain semisynthetic and synthetic ceramides with respect to living part of epidermis.

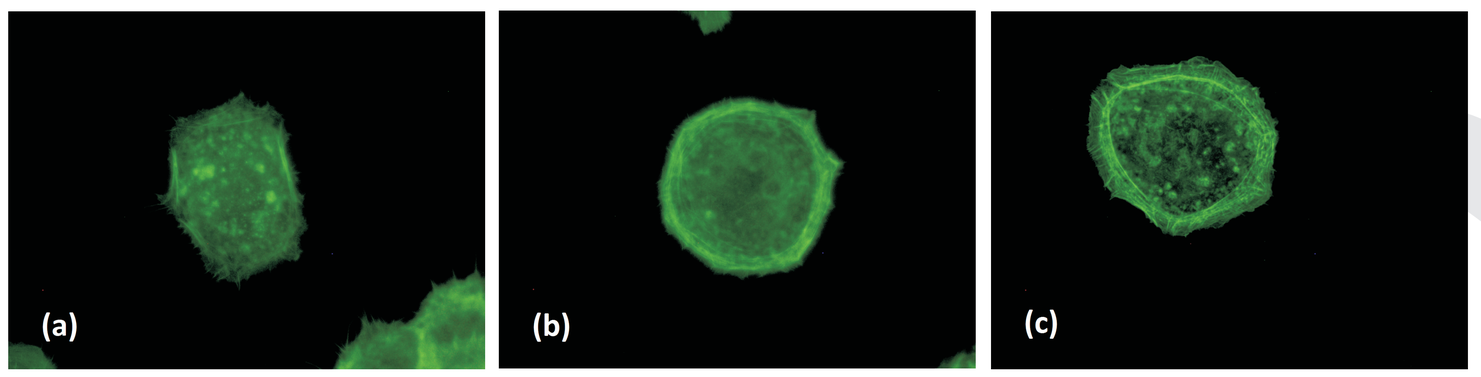

Fig. 7. Actin cytoskeleton (a) control (b) ceramide IIIA after 2 hrs of incubation (c) ceramide Ω-6 after 2 hrs of incubation

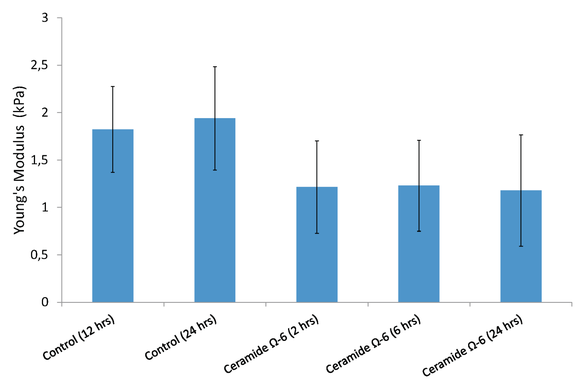

Fig. 8. Young's Modulus after (a) 2 hrs (b) 6 hrs (c) 24 hrs of preincubation with ceramide IIIA

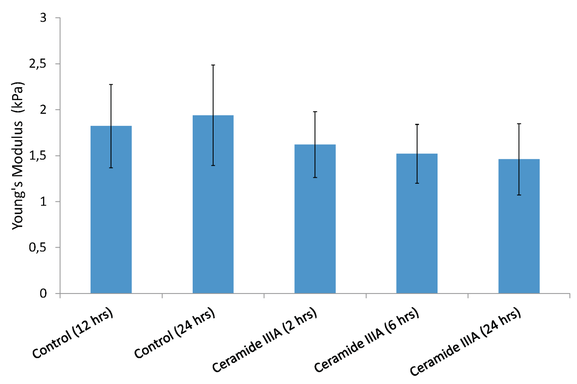

Fig. 9. Young's Modulus after (a) 2 hrs (b) 6 hrs (c) 24 hrs of preincubation with ceramide Ω-6

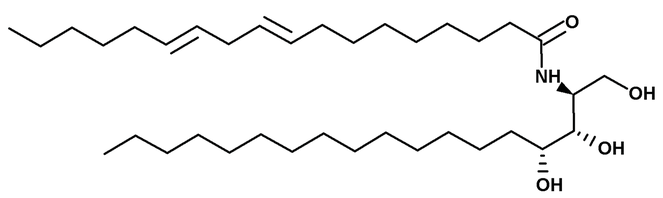

Fig. 1. Structure of Ceramide IIIA

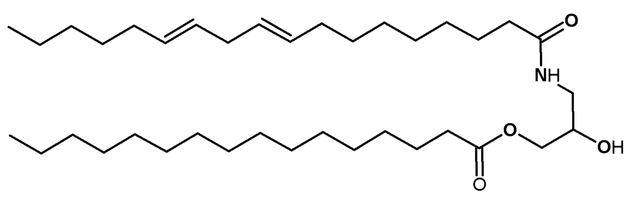

Fig. 2. Structure of Ceramide Ω-6

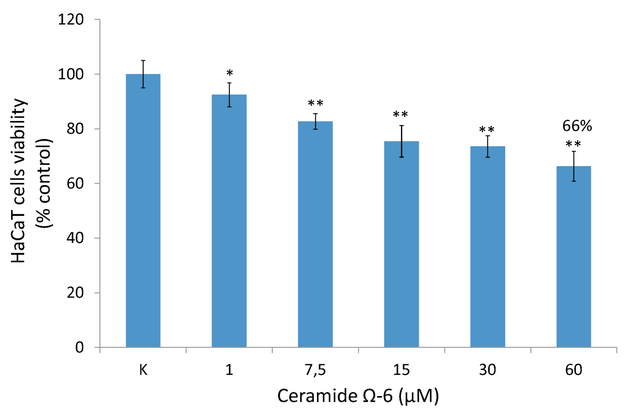

Fig. 3. HaCaT cells viability after 48 hrs preincubation with Ceramide IIIA (* p < 0,05; **p < 0,01)

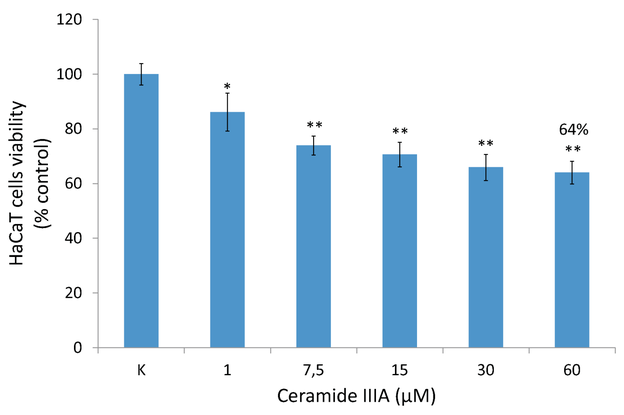

Fig. 4. HaCaT cells viability after 48 hrs preincubation with Ceramide Ω-6 (* p < 0,05; **p < 0,01)

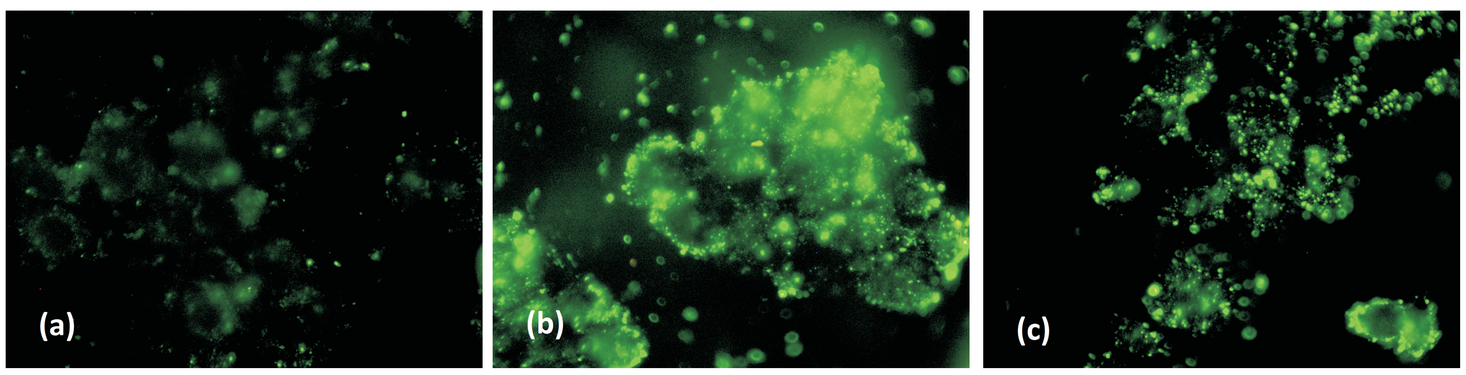

Fig. 5. Apoptosis in HaCaT cells induced by Ceramide IIIA. Images taken after (a) 2 hrs (b) 6 hrs (c) 24 hrs of preincubation with ceramide

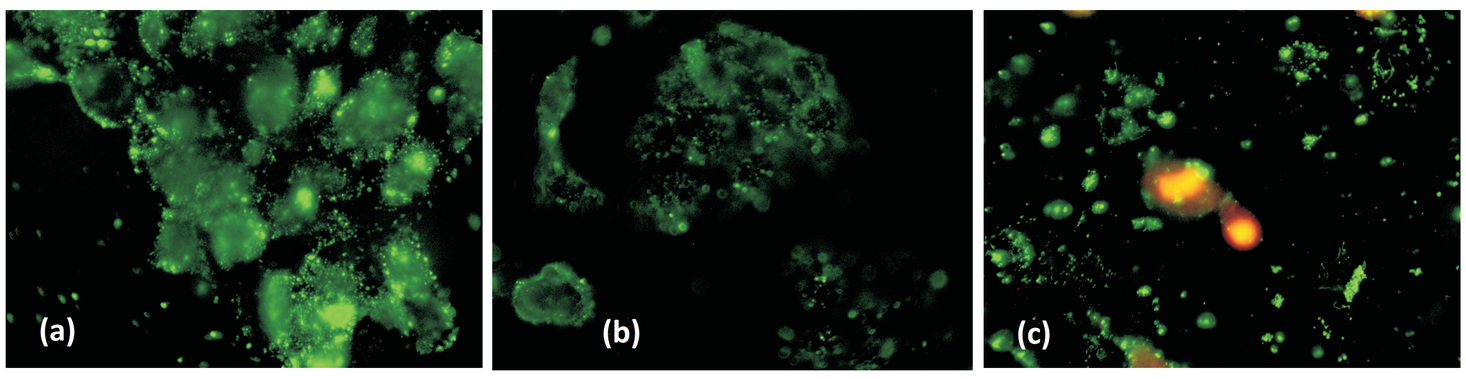

Fig .6. Apoptosis in HaCaT cells induced by Ceramide Ω-6. Images taken after (a) 2 hrs (b) 6 hrs (c) 24 hrs of preincubation with ceramide

Our studies

To assess if the long chain pseudoceramides are also able to induce biological effects we have selected two commercially available compounds: Ceramide IIIA (Fig. 1) and Ceramide Ω-6 (Fig. 2). Their biological properties were tested in cell culture (HaCaT keratinocytes) by utilizing following tests and methods:

- MTT test for evaluation of keratinocytes proliferation (cell viability)
- Annexin-V-FLUOS Staining Kit for detection of apoptosis and necrosis
- Fluorescent microscope to visualize the organization of actin filaments and AFM (Atomic Force Microscope) to measure cell stiffness

Results and Conclusions

Conducted studies revealed biological activity of tested pseudoceramides. Similarly like the short chain ceramides C2 and C6, used pseudoceramides decreased cell viability of HaCaT keratinocytes (Fig. 3 and 4). Moreover both molecules were able to induce apoptosis (programmed cell death) as early as 2 hours after preincubation with the highest concentration of tested ceramides (60 μM), (Fig. 5 and 6). AFM revealed morphological changes in the structure of cytoskeleton in samples preincubated with studied pseudoceramides. Both ceramides were able to induce actin reorganization causing cell rounding and forming so called actin ring (Fig. 7). Measurements of Young's modulus also confirmed changes of mechanical properties in keratinocytes preincubated with tested pseudoceramides (Fig. 8 and 9).

We have demonstrated that not only short chain synthetic ceramides are able to exert biological effects in cells. Despite different structures (especially with respect to the structure sphingoid base hydrophilic group) both pseudoceramides show similar properties. Taking under consideration all obtained results and information found in literature we assume that these two pseudoceramides could be valuable ingredients of products dedicated to consumers suffering from hyperproliferative diseases.

References

1. Coderch L., López O., Maza A., Parra J.L. (2003) Ceramides and skin function. Am. J. Clin. Dermatol. 4 (2): 107-129
2. Goñi F.M., Alonso A. (2006) Biophysics of sphingolipids I. Membrane properties of sphingosine, ceramides and other simple sphingolipids. Biochimica et Biophysica Acta. 1758 (12): 1902-21.
3. Holleran W.M., Takagi Y., Uchida Y. (2006) Epidermal sphingolipids: Metabolism, function, and roles in skin disorders. FEBS Letters. 580: 5456-5466

Corresponding Author: Michał Stepulak, m.stepulak@wszkipz.pl

ACADEMY OF COSMETICS AND HEALTH CARE (Warsaw, POLAND)
ul. Podwale 13, 00-252 Warszawa, POLAND; phone: +48 22 635 14 47, fax: +48 22 635 79 23, e-mail: rektorat@wszkipz.pl, **www.kosmetyka.edu.pl**
Academy o 10 years Cosmetics and Health Care