29-th EADV Congress, 28.10 – 01.11.2020, Vienna

Medical device for topical application in eczema patients - biocompatibility, safety assessment and *St. aureus* biofilm formation *in vitro*

Renata Dębowska, Katarzyna Śmigielska, Beata Ostrowska, Monika Toporkiewicz, Grzegorz Chodaczek, Adam Junka, Katarzyna Rogiewicz, Irena Eris.
Dr Irena Eris Cosmetic Laboratories, Piaseczno, Poland and Łukasiewicz Research Network - PORT Polish Center for Technology Development, Wroclaw, Poland

Introduction

Eczema is a chronic inflammatory skin disease with considerable impact on quality of life. It is characterized by dry skin, intense itching, and skin lesions. The therapy requires a manifold approach with the focus on restoration of the epidermal barrier and reduction of skin inflammation. Applying topical moisturizing and anti-inflammatory agents is an integral part of the treatment. Bacterial colonization, in particular *Staphylococcus aureus*, can contribute to eczematous flares and overt infection. Use of systemic antibiotics on infected lesions is warranted, however empiric antibiotics use on uninfected changes is controversial. Local antiseptic measures and topical antimicrobial therapies can be considered in patients with high bacterial colonization.
The aim of this study was to develop a medical device (MD) in form of emulsion to be used as a topical treatment for eczema patients. The formula of the MD contained: 43,5% emollients (Canola oil, *Cannabis Sativa* Seed Oil, shea butter, *Orbignya Oleifera* Seed Oil, ceramides: NP, EOP, AP), pre-biotic inulin from *Cichorium intbus* root and antibacterial Piroctone Olamine.

Methods

In order to evaluate safety and efficacy of the medical device (MD emulsion no. 16920) we performed following *in vitro* tests: cytotoxicity on L929 cells according to ISO 10993-5:2009, 10993-12:2012 and skin irritation on EpiDerm skin model according to OECD test guideline 439 and ISO 10993-10:2013. Safety assessment of all MD components was done by a toxicologist.
Human epidermis model (HaCaT cells) and bacterial biofilm (*Staphylococcus aureus*) were grown on cellulose-collagen discs and were treated with various concentrations of the MD to assess bacteriostatic properties. The viability of bacteria and keratinocytes was measured by LIVE/DEAD BacLight Bacterial Viability Kit (containing SYTO-9 dyes and propidium iodide for labeling live and dead cells, respectively). Microscopic images were analyzed in Image software (NIH).

Conclusion

Topical emollients are widely used as an integral part of eczema patients treatment, however their effectiveness remains unclear. We proved that tested emulsion, as a topical medical device, is biocompatible with the skin and can protect epidermal cells against *Staphylococcus* aggression.

Results

An emulsion is considered non-cytotoxic when cell viability is >70% of control in one or more tested concentrations. The tested MD emulsion was **non-cytotoxic** at the concentration less than or equal to 0,1% (Fig. 1). Moreover, MD no. 16920 was confirmed as **non-irritant** on EpiDerm skin model, resulting in the mean tissue viability of 107,9% (Fig. 2).
Toxicological assessment revealed **proper selection of MD components, especially essential oils, ceramides, prebiotics and anti-bacterial compounds** (data not shown).
Viability of keratinocytes *in vitro*, in the presence of bacteria, was significantly reduced (Fig. 3, 5). *St. aureus* biofilm study in the presence of the product showed increase in keratinocytes and bacteria viability (Fig. 3). **This result may suggest a protective effect for keratinocytes in the presence of *Staphylococcus*.** At the same time, the lack of biocidal properties of the product was demonstrated (Fig. 4).

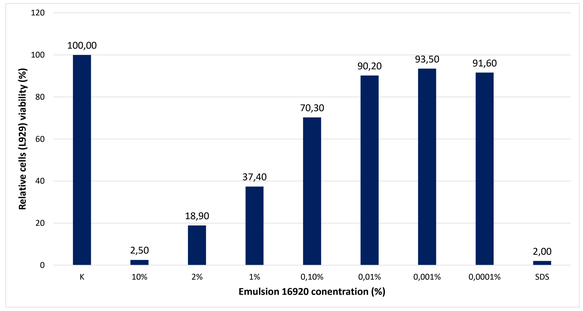


Figure 1. Cytotoxicity of MD 16920 on L929 cells. Viability <70% of the control - cytotoxic potential. Ref – 5% SDS. **The tested MD emulsion was non-cytotoxic at the concentration less than or equal to 0,1% (70,3% cells viability).**

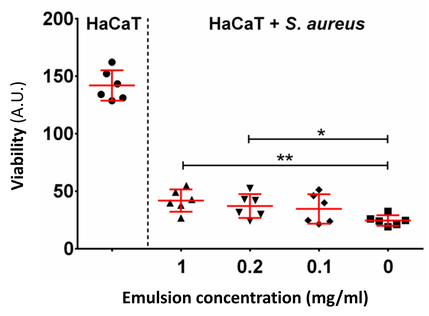


Figure 3. Viability of keratinocytes covered with *S. aureus* biofilm depending on the cream concentration based on microscopic measurement of SYTO-9 fluorescence intensity.

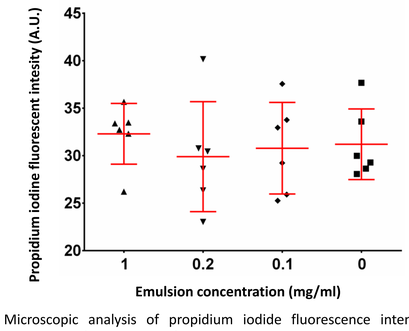
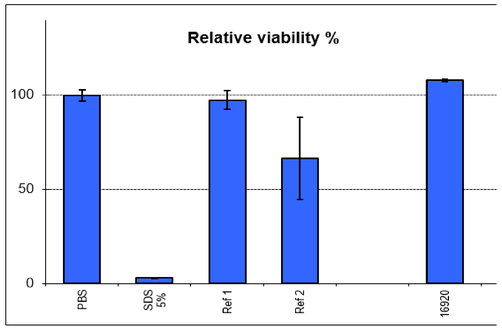


Figure 4. Microscopic analysis of propidium iodide fluorescence intensity in bacteria depending on the emulsion concentration.



	mean of OD	SD of OD	mean of viabilities [%]	SD of viabilities	CV % [%]
PBS	1,720	0,052	100,0	3,02	3,02
SDS 5%	0,050	0,006	2,9	0,32	10,99
Ref 1	1,677	0,083	97,5	4,81	4,93
Ref 2	1,143	0,374	66,4	21,73	32,70
16920	1,855	0,011	107,9	0,63	0,59

PBS	NI	qualified
SDS 5%	I	qualified
Ref 1	NI	qualified
Ref 2	NI	SD>18
16920	NI	qualified

NI – non-irritant, I – irritant

Figure 2. Skin irritation potential of medical device emulsion 16920 tested on EpiDerm model. Ref 1- naphthalene acetic acid (CAS 86-87-3) – non classified (non irritant). Ref 2 - cyclamen aldehyde (CAS 103-95-7) – classified (irritant, Cat. 2). Correlation of in vitro and in vivo results: Tissue viability ≤ 50% of the control (PBS) – irritant (R38). Tissue viability ≥ 50% of the control – non-irritant. **MD 16920 was confirmed as non-irritant on EpiDerm skin model, resulting in the mean tissue viability of 107,9%.**

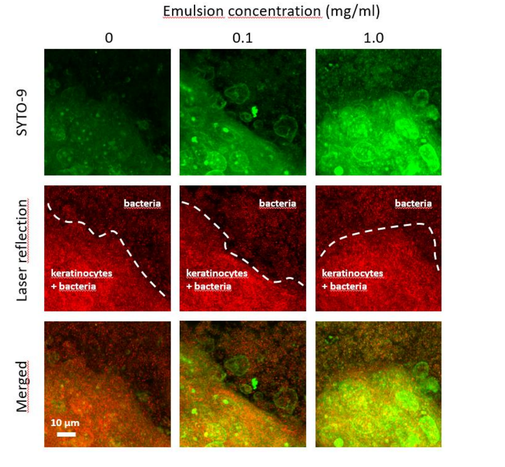


Figure 5. Microscopic analysis of SYTO-9 fluorescence intensity in keratinocytes and bacteria depending on the emulsion concentration.