

**The role of polyols and amino acids in the maintenance of homeostasis in
the skin**

Ph.D. Thesis

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Szeged, Hungary

2016

INTRODUCTION

The most important function of the skin is to form a barrier which provides protection against environmental physical and chemical challenges so as against causative agents. In this barrier, physical and chemical/biochemical factors can be distinguished. Stratum corneum (SC) is the most significant part of the physical barrier, but the nucleated epidermis and its tight junctions also play a role. Concerning chemical/biochemical factors, antimicrobial peptides, enzymes, macrophages hamper the invasion of pathogens. Different types of contact dermatitis (irritant contact dermatitis – ICD and allergic contact dermatitis – ACD) and atopic dermatitis (AD) are accompanied by impaired barrier function. Special attention shall be paid to ICD, which is a non-immunologic and non-specific inflammatory disorder caused by external challenges, since it is a frequent occupational disease affecting workers in healthcare, food- and cosmetic industry. In the mentioned diseases, barrier disruption is characterized by increased transepidermal water loss (TEWL) and decreased SC hydration. Moreover, signs of inflammation can be detected.

Thus, it is practical to supplement external formulations with agents that can provide protection against irritation and help maintain the skin's homeostasis.

Glycerol is a well-known example of such a compound. This polyol exerts potent antiirritant effect and increases the water content of SC via different mechanisms. It is known that glycerol, as a humectant, is able to retain water in the SC. Glycerol also prevents the phase transition of SC lipids from liquid to solid crystalline structure, reduces the average aqueous pore radius in the SC, hereby decreasing water loss and hampering the penetration of irritants. Glycerol also displays keratolytic effect, stabilizes skin collagen and accelerates wound healing. Considering these advantageous properties of glycerol, it may be assumed that also other polyols may have similar beneficial effects.

Xylitol is a naturally occurring polyol found in the fibers of many fruits and vegetables. It is used as sweetener for diabetic persons, since its energy content is lower than that of most carbohydrates and can be taken up by the cells without insulin. Moreover, xylitol was found to inhibit the proliferation of *Streptococcus mutans* in the oral cavity thus can contribute to the prevention of caries. Since xylitol has considerable humectant effect, it seems to be able to hydrate the skin *in vivo*.

Mannitol is a sugar alcohol similar to xylitol. Its main clinical application is the reduction of acutely increased intracranial pressure. However, neither xylitol nor mannitol has yet been used in local formulations in order to prevent irritation.

Nevertheless, polyols are not the only promising antiirritant candidates, amino acids also contribute to the homeostasis of the skin. Natural moisturizing factor (NMF), which is a cornerstone of skin hydration, contains amino acids at high ratio. Mixtures of amino acids are used for skin revitalization. Less information is available on efficacy of separately applied amino acids. However, some of them seem to be appropriate to ameliorate skin irritation. Taurine, a sulfur-containing amino acid, is an organic osmolyte. In the skin, it exhibits antioxidant effects, protects cells from ultraviolet (UV)-induced stress and acts on cell proliferation, inflammation and collagenogenesis. Keratinocytes express specific taurine transporter (TauT) which facilitates taurine uptake of cells thereby providing protection against hyperosmotic stress and other challenges.

Glycine, another amino acid of importance in dermatologic therapy, is traditionally used for the treatment of scars and has beneficial effects on the skin reparation process and the overall rate of wound healing.

Methionine was reported to provide protection against reactive oxygen species (ROS). Moreover, unpublished *in vitro* data suggest that methionine may be able to decrease the activity of matrix metalloproteinase-1 (MMP-1). This enzyme plays a pivotal role in photoaging.

It shall also be considered that different antiirritant agents may have synergistic and complementary effects. Although glycerol and xylitol have similar chemical structure, these polyols induce different gene expression changes in the keratinocytes. *In vitro* experiments have demonstrated that glycerol decreases the expression of human leukocyte DR (HLA-DR), thereby reducing inflammation, while xylitol increases the expression of filaggrin. As a source of NMF and also in other ways, filaggrin contributes to the hydration and homeostasis of the skin. Induction of filaggrin expression, which leads to the hydration of the skin, can be accompanied by the suppression of MMP-1, thereby contributing to skin rejuvenation. Previous animal experiments of our working group have revealed that both glycerol and xylitol possess anti-inflammatory effects, but they influence the expression of inflammatory cytokines in different ways.

For assessment of skin barrier function and for testing the efficacy of barrier-improving agents, TEWL and skin hydration are widely used. TEWL represents the diffusion of condensed water through the SC. Alterations of TEWL and skin hydration may be accompanied by changes in skin's mechanical properties, as well. Thus, monitoring of biomechanical parameters of the skin (e.g. viscoelasticity, skin smoothness, etc.) may provide useful information on the efficacy of the applied treatments.

For investigation of ICD and skin barrier recovery, sodium lauryl sulfate (SLS)-induced irritation is a frequent method. SLS, an anionic detergent, is a common surfactant accepted as a reference irritant. Exposure to SLS results in an increased TEWL and in epidermal hyperproliferation. A linear relationship between the dose of SLS and the skin's response has been demonstrated both by visual scoring and by bioengineering techniques. These data drew our attention to the potential antiirritant effects of polyols and amino acids and served as a guideline for the design of our studies.

AIMS

Our principal goal was to study the effects of locally applied polyols and amino acids on the barrier function, the hydration, the biomechanical-, the morphological- and the microbiological parameters of the human skin. For this aim, human studies and *in vitro* experiments were designed. The entire study was divided into 3 consecutive parts (mentioned as Study I, Study II and Study III, respectively).

In Study I, the major objectives were:

- to create a model of mild (subclinical) irritation using SLS as irritant,
- to examine the antiirritant properties of glycerol, xylitol, mannitol, taurine and glycine by measurement of TEWL,
- and to find the effective concentrations of the above mentioned agents.

In Study II, it was set out:

- to study the antibacterial effects of a glycerol- and xylitol-containing combination product *in vitro* and *in vivo*,
- and to monitor its impact on TEWL, skin hydration and skin pH.

The goals of Study III were:

- to observe the effects of the mentioned glycerol- and xylitol-containing formulation on TEWL, skin hydration, biomechanical parameters and morphology after a longer period of application (14 days),
- to study the effects of locally applied methionine on these parameters,
- and to identify the protein quantities of filaggrin and MMP-1 after these treatments.

METHODS

SKIN PHYSIOLOGICAL MEASUREMENTS

The investigations were performed in the Cosmetological and Skin-Physiological Research Laboratory of the Department of Dermatology and Allergology, University of Szeged, under controlled room conditions. Hydration, TEWL, pH, skin friction, elasticity were measured with special devices (Courage + Khazaka electronic GmbH, Cologne, Germany). Furthermore, the skin was examined with a DUB-USB high-frequency, high-resolution ultrasound system (Taberna pro Medicum GmbH, Luneburg, Germany). Epidermal and dermal thicknesses and the echogenicity of the papillary dermis were measured by means of DUB-SkinScanner software.

IN VITRO AND IN VIVO MICROBIOLOGICAL EXAMINATIONS

For the *in vitro* experiments, different suspensions of *S. aureus* and *S. pyogenes* were chosen for test bacteria. Bacteria suspensions were added to Xylinep® gel, and after incubation, germ numbers were estimated to assess the antimicrobial effect. The *in vivo* investigation was performed on the arms of volunteers. A germ number baseline value was determined and the examination was then performed again after a 15 minutes treatment with Xylinep® gel. After that, the subject continued treatments on the marked areas: the gel was applied in the morning and in the evening. The treatment was carried out 6 times in total. 2 hours after the 6th treatment, i.e. 74 hours after the beginning of the study, samples were taken, as described.

STUDY DESIGN

Study I:

The ventral side of the forearm was used as test region. In closed patch tests using extra-large Finn Chambers and corresponding filter discs, 200µL of a 0.1% aqueous solution of SLS was applied to the test chamber and left for 24 hours on one forearm. The test chamber on the corresponding site on the other forearm contained 0.1% SLS solution supplemented with one or another of the tested study agents. Test chambers were removed after 24 hours. TEWL was measured before patch application and 30 minutes after removal of the test chambers and the rinsing and drying of the test areas.

Study II:

This part of our investigation involved *in vitro* experiments and *in vivo* examinations in order to assess the antibacterial effect of glycerol and xylitol (Xylinep® gel). Furthermore, effects of the gel on different skin physiological parameters were also detected. Skin hydration, TEWL and skin pH were measured after 2, 8, 12 and 24 hours.

Study III:

Four 2x2 cm areas were marked out on both lateral upper arms. Area 1 served as untreated control. Area 2 received the vehicle (Carbopol Ultrez 10 0.4%, dissolved in purified water). A gel containing 5% xylitol and 5% glycerol (dissolved in the above-mentioned vehicle) was applied to area 3, while area 4 was exposed to 2% of L-methionine (in the same vehicle). 2 treatments were applied daily for 14 days. Measurements were performed twice: the studied parameters were determined before the first application of the preparations and on day 14, 6 hours after the last treatment. The following parameters were monitored: skin hydration, TEWL, skin friction, skin elasticity and images were also taken by means of high-frequency, high resolution ultrasound system. Finally, full-thickness skin biopsies were taken from each area with a 4-mm circular blade (“punch biopsy”) under local anaesthesia. The wounds were then closed with a single suture.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Tissue samples obtained in Study III were stained with haematoxylin-eosin (H&E) and were subjected to immunohistochemistry (Filaggrin antibody, MMP-1 antibody) as well.

All slides were scanned and analysed with Panoramic Viewer software. In the H&E-stained sections, the interdigitation index was determined. In the filaggrin-stained slides, the percentage of epidermal cells showing positive staining was determined. For the characterization of the quantity of MMP-1 protein, a semi-quantitative scoring system was used: 1: mild, 2: moderate, 3: expressed positivity in the epidermis.

RESULTS

STUDY I

EFFECTS OF POLYOLS ON SKIN IRRITATION

Exposure of the skin to a 0.1% solution of SLS led to a statistically significant increase in TEWL relative to the baseline in all experiments. The addition of glycerol at 2.6% to the SLS did not prevent skin irritation; there was still a significant difference between TEWL values before patch application ($m=7.78$, $SD=3.3$) and TEWL values after patch application ($m=12.06$, $SD=7.71$). However, glycerol at 9% was effective in protection against irritation; TEWL values before patch testing ($m=12.17$, $SD=6.32$) and after patch testing ($m=13.16$, $SD=4.77$) did not differ statistically. Furthermore, this concentration of glycerol considerably reduced TEWL on the treated site ($m=13.16$, $SD=4.77$) as compared to the untreated (but irritated) site ($m=20.39$, $SD=10.0$). Xylitol also was applied in two concentrations: 4.5% and

15%. The lower concentration failed to provide protection against irritation (before application: $m=6.06$, $SD=2.85$; after: $m=11.43$, $SD=4.59$). However, the higher concentration effectively prevented the elevation of TEWL (before: $m=9.01$, $SD=3.51$; after: $m=11.94$, $SD=6.69$). Nevertheless, no significant difference was found between untreated sites ($m=13.14$, $SD=5.09$) and treated sites ($m=11.94$, $SD=6.69$) after the application of SLS. The application of mannitol at concentrations of either 5.4% or 18% was not effective in reducing the SLS-induced increase in TEWL (mannitol at 5.4%: $m=15.71$, $SD=3.22$ before, $m=25.92$, $SD=3.19$ after; mannitol at 18%: $m=15.4$, $SD=4.64$ before, $m=22.99$, $SD=5.09$ after). However, mannitol at 18% led to significantly lower TEWL values after patch testing than SLS alone did (treated site: $m=22.99$, $SD=5.09$; untreated site: $m=35.04$, $SD=9.85$).

EFFECTS OF AMINO ACIDS ON SKIN IRRITATION

Taurine at 3.4% did not lead to a great improvement in TEWL (before: $m=14.29$, $SD=3.38$; after: $m=33.04$, $SD=14.49$), and taurine at 8.4% also failed to inhibit the increase in TEWL (before: $m=12.34$, $SD=2.42$; after: $m=23.53$, $SD=5.57$). However, statistical analysis revealed that the TEWL level after the application of taurine at 8.4% was significantly lower than that observed for the 3.4% solution. Moreover, taurine at 8.4% also decreased TEWL ($m=23.53$, $SD=5.57$) as compared to untreated site ($m=31.18$, $SD=12.56$). Glycine at 5% did not provide protection against TEWL-elevation resulting from exposure to SLS (before: $m=12.47$, $SD=5.19$; after: $m=24.23$, $SD=12.47$). TEWL values at sites not treated with glycine ($m=24.61$, $SD=12.57$) and at treated sites ($m=24.23$, $SD=12.47$) did not differ significantly. Lower concentrations of polyols and amino acids did not result in significant differences in TEWL values at untreated and treated sites after patch testing.

STUDY II

IN VITRO AND IN VIVO ANTIBACTERIAL EFFECTS OF POLYOLS

The effects of the glycerol- and xylitol-containing Xylinep® gel on *S. pyogenes* cultures were studied. When the gel was inoculated with the *S. pyogenes* suspensions, no viable bacteria were detected from the suspension of 59 CFU/100 μ L bacterial concentration after 24 and 48 hours. 16 bacteria colonies were found after 24 hours and 13 colonies were found after 48 hours from that of 590 CFU/100 μ L bacterial concentration.

In case of *S. aureus* suspensions, the initial germ numbers of the applied suspensions (3 suspensions were produced) were found to be 121 000 CFU/100 μ L, 125 000 CFU/100 μ L and 54 000 CFU/100 μ L, respectively. Bacterial concentrations in the control samples (which were taken immediately after the inoculation) came to somewhat lower, but statistically no

difference was found (108 000 CFU/g, 124 000 CFU/g, 53 200 CFU/g, respectively). However, considerable decrease was observed in the germ numbers after incubation of 24 hours (germ numbers of the 2-2 parallel gels: 27 200 CFU/g, 38 500 CFU/g, and 61 800 CFU/g, 39 600 CFU/g, and 29 750 CFU/g, 5000 CFU/g, respectively). After an incubation of 48 hours, no bacterial growth was detected.

As concerns *in vivo* antibacterial effect of Xylinep® gel, several bacteria was found on the skin of the subjects prior to the treatment (colony numbers: $m=53.27$, $SD=100.87$). Local application of Xylinep® gel resulted in a significant fall in colony numbers even after 15 minutes ($m=7.47$, $SD=7.73$). An expressed antibacterial effect was observed after 74 hours ($m=15.27$, $SD=20.38$).

EFFECTS OF POLYOLS ON SKIN PHYSIOLOGICAL PARAMETERS WITHIN 24 HOURS

Application of Xylinep® gel did not influence skin pH and TEWL values significantly during the 24 hours of observation. Regarding the effect of Xylinep® gel on skin hydration, we found that compared to baseline (0h) values ($m=38.24$, $SD=5.84$), a considerable elevation in hydration values was detected after 2 hours ($m=45.83$, $SD=9.86$). The highest hydration values were measured after 8 hours ($m=48.45$, $SD=7.29$). Furthermore, skin hydration values surpassed baseline values after 12 and 24 hours, as well (12h: $m=46.9$, $SD=7.34$; 24h: $m=42.52$, $SD=8.69$).

STUDY III

EFFECTS OF POLYOLS ON SKIN PHYSIOLOGICAL PARAMETERS WITHIN 14 DAYS

Statistical analysis did not reveal any difference between the day 0 values for different areas in terms of any studied skin physiological parameter. The data demonstrate that the skin hydration did not display considerable changes in the control area (control: day 0: $M=20.45$, $25p=17.49$, $75p=24.39$, day 14: $M=22.18$, $25p=18.68$, $75p=27.89$). The vehicle appeared to exert some skin-hydrating effect (vehicle: day 0: $M=17.94$, $25p=16.39$, $75p=23.8$, day 14: $M=25.05$, $25p=17.99$, $75p=32.39$). However, glycerol and xylitol led to more expressed increase in hydration (glycerol + xylitol: day 0: $M=18.9$, $25p=16.18$, $75p=23.4$, day 14: $M=29.44$, $25p=24.9$, $75p=37.51$).

The lack of treatment or exposure to vehicle did not alter the TEWL by the end of the observation period (control: day 0: M=10.5, 25p=8.45, 75p=12.38, day 14: M=8.65, 25p=8.25, 75p=10.13, vehicle: day 0: M=9.55, 25p=6.95, 75p=11.6, day 14: M=7.85, 25p=6.08, 75p=9.2). Application of glycerol and xylitol significantly reduced the TEWL. Furthermore, the TEWL values of the areas exposed to the polyols were found to be considerably lower than those of the control areas on day 14 (glycerol + xylitol: day 0: M=11.3, 25p=8.35, 75p=12.18, day 14: M=5.45, 25p=4.25, 75p=7.9).

Friction values measured after the use of vehicle were higher than day 0 data, but did not differ from the appropriate values of the control area (control: day 0: M=113.0, 25p=94.55, 75p=131.1, day 14: M=126.9, 25p=98.35, 75p=144.3, vehicle: day 0: M=128.8, 25p=93.8, 75p=150.15, day 14: M=149.2, 25p=138.6, 75p=228.7). Treatment with glycerol and xylitol resulted in much more expressed elevations in friction values, which were also higher than those determined in the control area (glycerol + xylitol: day 0: M=138.1, 25p=100.95, 75p=183.3, day 14: M=241.8, 25p=207.05, 75p=742.05).

As concerns the R-parameters determined with the Cutometer MPA 580, only R0 exhibited noteworthy changes. R0 values were significantly higher after the application of glycerol and xylitol for 14 days, the other preparation did not influence this parameter (control: day 0: M=0.199, 25p=0.174, 75p=0.255, day 14: M=0.205, 25p=0.15, 75p=0.224, vehicle: day 0: M=0.174, 25p=0.156, 75p=0.187, day 14: M=0.18, 25p=0.15, 75p=0.2, glycerol + xylitol: day 0: M=0.177, 25p=0.165, 75p=0.196, day 14: M=0.205, 25p=0.18, 75p=0.235).

EFFECTS OF POLYOLS ON MORPHOLOGICAL PARAMETERS

Exposure to glycerol and xylitol led to a significant increase in epidermal thickness (day 0: M=187.67, 25p=175.33, 75p=218.33, day 14: M=235.83, 25p=210.33, 75p=257.33). The vehicle alone did not induce changes in this parameter that differed statistically from the day 0 values (vehicle: day 0: M=212.33, 25p=192.92, 75p=223.92, day 14: M=223.83, 25p=196.0, 75p=231.33).

The dermal thickness was also enhanced by glycerol and xylitol. However, such changes were not found in the other areas (control: day 0: M=1564.0, 25p=1317.17, 75p=1846.67, day 14: M=1678.33, 25p=1319.25, 75p=1939.58, vehicle: day 0: M=1448.0, 25p=1201.08, 75p=1871.17, day 14: M=1586.17, 25p=1248.75, 75p=1836.08, glycerol + xylitol: day 0:

M=1539.33, 25p=1406.33, 75p=1690.33, day 14: M=1776.17, 25p=1538.33, 75p=1942.75). Both the vehicle and the preparation with glycerol and xylitol decreased the echogenicity of the papillary dermis. However, the polyols led to a more considerable reduction in this parameter and a difference was also found compared with the control area (control: day 0: M=14.0, 25p=10.5, 75p=20.25, day 14: M=14.0, 25p=10.25, 75p=18.88, vehicle: day 0: M=14.75, 25p=13.25, 75p=17.25, day 14: M=14.0, 25p=11.25, 75p=16.56, glycerol + xylitol: day 0: M=14.0, 25p=12.25, 75p=15.38, day 14: M=10.0, 25p=9.13, 75p=11.13).

The interdigitation index displayed relatively low values in the control area (control: M=1.11, 25p=1.06, 75p=1.19). 14 days of use of glycerol and xylitol resulted in a more expressed interdigitation between the epidermis and the dermis compared with the untreated control (glycerol + xylitol: M=1.26, 25p=1.18, 75p=1.32). The vehicle did not appear to influence the interdigitation (vehicle: M=1.11, 25p=1.06, 75p=1.18). In the control areas, approximately 25% of the epidermal cells showed positivity to filaggrin. Exposure to glycerol and xylitol increased this ratio considerably (control: M=19.25, 25p=15.13, 75p=33.88, glycerol + xylitol: M=37.9, 25p=23.87, 75p=47.53). Application of the vehicle was not accompanied by changes in the expression of filaggrin (vehicle: M=24.5, 25p=15.44, 75p=31.0). MMP-1 protein was also found to be present in the epidermis. However, the applied formulations did not change the quantity of MMP-1. No significant difference was found between the control and the treated areas in terms of MMP-1 (data not shown).

Locally applied L-methionine for a 14 day period had no considerable effect on the studied parameters (hydration, TEWL, friction, elasticity, epidermal/dermal thickness, echogenicity, interdigitation index, ratio of filaggrin positive cells).

DISCUSSION

Antiirritants, as a group, include many different compounds that are added to topical products to reduce the irritative effects of other components. An important goal of our study was to evaluate the effects of various polyols and amino acids on SLS-induced skin irritation.

4.5% and 15% solutions of xylitol and 5.4% and 18% solutions of mannitol contained as much active agent as 2.6% and 9% solutions of glycerol, respectively. Concerning taurine, it was not possible to use a concentration higher than 8.4% because of the solubility of the material. Similarly, 5% was the highest concentration of glycine that could be used in the study.

Our data confirmed that glycerol effectively suppressed SLS-induced irritation of the skin when pre- and postpatch TEWL values and untreated and treated sites, respectively, were

compared. Xylitol, mannitol and taurine also demonstrated antiirritant effects whereas glycine failed to do so.

However, glycerol is not only an antiirritant compound but also a potent moisturizer (although these categories show some overlap). Moisturizers of different types are cornerstones for the treatment of dry skin since they can promote barrier repair, reduce TEWL or contribute to aesthetic improvement of irritated skin. Glycerol, together with other polyols, lactic acid, urea and amino acids, belongs to the humectants which is one of the three main classes of moisturizers. Humectants hydrate the skin mainly by attracting and binding water from the deep dermis and environment. Compared to other formulations, they are absorbed faster and therefore are aesthetically better, promoting patient compliance.

We presumed that other compounds, possibly those of humectant group, may also positively affect skin barrier function. The cosmetologic applicability of urea is well-known and it has also been studied, but the polyols xylitol and mannitol have not been tested under *in vivo* conditions. Although *in vitro* data have been reported on their water-binding properties, *in vitro* and *in vivo* effects may differ. According to our results, both mannitol and xylitol can reduce TEWL, i.e. provide protection against SLS-induced skin irritation. However, these polyols differently affect other skin physiological parameters. Mannitol hydrates the skin when it is injected together with hyaluronic acid, while xylitol considerably increases hydration values alone when applied topically.

Considering the beneficial properties of glycerol and xylitol and a possible synergistic effect, which may originate in their different effect of gene expression, examination of their combined application seemed to be useful.

Study II has revealed that the glycerol- and xylitol-containing Xylinep® gel is able to decrease bacterial colonization both *in vitro* and *in vivo*. Furthermore, this preparation hydrates the skin for 24 hours. Xylitol is responsible for the antibacterial effect of the formulation. However, both polyols contribute to the hydration of the skin. According to recent results of our working group, both glycerol and xylitol provide protection against increase of TEWL and decrease of hydration in an animal model of ICD, and xylitol is also able to hamper penetration of SLS into the deeper layers of the skin. Due to these properties, a glycerol- and xylitol-containing formulation may contribute to the prevention of ICD. In terms of this disease, the antibacterial effect of the combined formulation shall also be underlined, because reduction of contamination in irritated skin is more difficult than that in healthy skin.

Nevertheless, Study II characterized the effects of polyols only in a relatively short period. Study III has shown that a longer application of glycerol and xylitol increases not only the water content of SC, but also that of the deeper layers. Ultrasound imaging has revealed an elevation in both epidermal and dermal thickness after exposure to the polyols. Thickening of the skin can be regarded as a marker of hydration. Moreover, the echogenicity of the papillary dermis was measured to be lower and can be explained in terms of the binding of a larger amount of water due to the moisturizing effect.

Improvement of the studied biomechanical parameters may also originate in the hydrating effect of glycerol and xylitol. Since the friction coefficient correlates positively with the hydration of the SC, the higher friction values, i.e. the smoother skin, detected in our investigation may be explained in terms of a higher water content of the skin. The parameter R0, which reflects the passive behavior of the skin to force, was also found to be higher after the application of polyols. Nevertheless, we have confirmed by means of different methods that glycerol and xylitol considerably increase the water content in the superficial and deeper layers of the skin.

The water-binding capacity of the polyols, originating in their chemical structure is a possible, but not the only, explanation of the hydrating effect. An important new finding of our study is that the application of these polyols increases the quantity of filaggrin at the protein level in the skin. The preliminary *in vitro* data suggest that xylitol leads to an elevated protein expression of filaggrin. Since the application of glycerol alone does not seem to influence the expression of filaggrin *in vivo*, it can be assumed that xylitol is responsible for the increased quantity of filaggrin. However, the exact mechanism via which this polyol elevates the expression of filaggrin demands further investigation. Although the vehicle alone (as an aqueous solution) influences few parameters, which may suggest some hydrating effect of this preparation, the application of the glycerol- and xylitol-containing gel was accompanied by a more expressed hydration of the skin, the filaggrin expression was not altered by the vehicle. The polyols therefore seem to be responsible for the real hydrating ability of the preparation. Besides hydration, glycerol and xylitol effectively improve barrier function of the skin, as indicated by reduced TEWL values. As Study II revealed, exposure to glycerol and xylitol for 24 hours increased SC hydration, which was not accompanied by a change in TEWL. Thus, a longer application of polyols appears to be needed for an improvement of the barrier function. This finding is in accordance with those of previous studies, which conclude that the use of glycerol for at least 3 days reduces TEWL significantly. Xylitol tends to decrease TEWL in patients with AD after 7 days of use, but this change is not significant. The hydrating effect of

the polyols may be a potential explanation for the improvement of TEWL. An inverse relationship between TEWL and SC hydration is well-known and moisturizers often improve barrier function. The mechanism of this interplay has not yet been fully clarified. However, the filaggrin expression and NMF level appear to contribute to the skin barrier integrity. An age-related decline in barrier function has been described and it may be connected with a lower NMF level in aged skin. A recent study revealed that exposure to irritants, which results in barrier disruption, decreases the levels of NMF. Thus, influencing the NMF level via the filaggrin expression may contribute to the antiirritant effect of the polyols.

Histological analysis has revealed that the morphology of the dermal-epidermal junction (DEJ) is also influenced by the polyol treatment. It is known that skin ageing is accompanied by the flattening of the DEJ, and the rate of ridge height increase decreases with age. The interdigitation index is an appropriate indicator for the characterization of this alteration. Morphological changes of ageing may originate in dermal atrophy, decreased collagen biogenesis and loss of elastic fibers. An elevation in interdigitation index after exposure to polyols might indicate some rejuvenation effect of glycerol and xylitol. Since ultrasound images have shown that polyol-induced hydration affects not only the epidermis, but also the dermis, these compounds may interfere with age-related dermal alterations. However, it should be mentioned that polyols influenced neither the gross elasticity (parameter R2 of the Cutometer) nor the net elasticity (parameter R5 of the Cutometer) in the present study. Hence, no considerable restoration of the elastic fibers is to be expected. Moreover, glycerol and xylitol do not seem to decrease the quantity of MMP-1, which might have contributed to their potential anti-aging effect. Thus, a rejuvenation effect of the polyols and its mechanism requires further examination. Accordingly, the combined application of glycerol and xylitol exerts several beneficial effects on the skin.

On the other hand, the advantageous impact of the studied amino acids was less expressed. Taurine did not significantly reduce TEWL after exposure to SLS. However, TEWL after application of taurine at 8.4% was lower than that after application of taurine at 3.4%. Further, treatment with taurine at 8.4% decreased TEWL as compared to treatment with SLS alone. Glycine was not found to have antiirritant effect and methionine also failed to influence the studied parameters in Study III.

In conclusion, our work has revealed the antiirritant effects of glycerol, xylitol, mannitol and taurine and underlined the advantages originating in the combination of glycerol and xylitol. The explanation of their efficacy is that they induce direct physical and/or chemical changes in the skin, influence expression of different genes (not only expression of filaggrin but also

that of inflammatory cytokines is affected by polyols) and have microbiological effects, too. These results may broaden the possibilities for antiirritant protection, lead to development of new cosmetic products, and contribute to the therapy of certain dermatologic diseases.

SUMMARY AND NEW FINDINGS

Our *in vivo* investigation and *in vitro* experiments were focused on the applicability of polyols and amino acids for the maintenance of homeostasis in the skin. We have demonstrated that many of these compounds are effective.

- In a model of subclinical irritation, it has been shown that, in addition to glycerol, xylitol, mannitol and – in a limited way – taurine possess antiirritant effect.
- Combination of glycerol and xylitol decreases the number of viable bacteria in *S. pyogenes* and *S. aureus* cultures.
- Application of this combination considerably reduces germ number *in vivo*.
- Glycerol and xylitol together increase skin hydration, their long-term application improves epidermal barrier function and lead to better mechanical properties of the skin.
- The mentioned combination positively influences morphological parameters of the skin (rejuvenation effect).
- Glycerol and xylitol increase the protein quantity of filaggrin. This may be an explanation of many above mentioned beneficial effects.

Hence, these agents can be utilized in cosmetic industry in order to prevent irritation and the combination of glycerol and xylitol may be useful additional therapy for dry skin and may also soothe the age-associated changes in the skin.

ACKNOWLEDGMENTS

Firstly, I wish to express my sincere gratitude to Professor Lajos Kemény for initiating my scientific career and for providing me with the opportunity to perform my scientific work at the Department of Dermatology and Allergology and for his valuable scientific guidance and help.

I am indebted to Professor Shabtay Dikstein for the continuous support of the studies and for the fruitful discussions.

I would like to express my special appreciation and thanks to my advisor Dr. Gábor Erős, I could not have imagined having a better advisor and mentor. His guidance helped me in all the time of research and writing of this thesis.

I am appreciative to Zoltán Behány for his excellent contribution to my work, and sharing his expertise on dermatophysiological measurements with me.

I thank Dr. Erika Varga and Dr. Edit Szél for helping in histological evaluations and Dr. Kornélia Szabó for her essential contribution to the microbiological examinations. Special thanks to the technical staff of the laboratories for their skillful assistance.

I thank Dr. Gábor Mohos for performing the skin biopsies and Dr. Ágnes Dura for preparing the tested formulations.

LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE DISSERTATION

- I. **Korponyai C**, Kovács RK, Erős G, Dikstein S, Kemény L. Antiirritant properties of polyols and amino acids. *Dermatitis* 2011; 22: 141-146. (IF: 1.211)
- II. Erős G, **Korponyai C**, Szabó K, Behány Z, Szél E, Kemény L. Antibacterial and skin hydrating effects of Xylinep® gel containing glycerol- and xylitol. *Bőrgyógyászati és Venerológiai Szemle* 2014; 90: 152-155. (Hungarian) (IF: 0)
- III. **Korponyai C**, Szél E, Behány Z, Varga E, Mohos G, Dura A, Dikstein S, Kemény L, Erős G. The effects of locally applied glycerol and xylitol on the hydration, barrier function and morphological parameters of the skin. *Acta Dermato-Venereologica* DOI: 10.2340/00015555-2493 (IF: 3.678)

ABSTRACT RELATED TO THE SUBJECT OF THE DISSERTATION

Korponyai C, Szél E, Behány Z, Mohos G, Dikstein S, Nagy K, Kemény L, Erős G. A lokálisan alkalmazott glicerol, xilitol és metionin hatásai a bőr hidratációjára, barrier funkciójára és morfológiájára. *Bőrgyógyászati és Venerológiai Szemle* 2014; 90(6): 275.

LIST OF OTHER FULL PAPERS

- I. Szél E, Polyánka H, Szabó K, Hartmann P, Degovics D, Balázs B, Németh IB, **Korponyai C**, Csányi E, Kaszaki J, Dikstein S, Nagy K, Kemény L, Erős G. Anti-irritant and anti-inflammatory effects of glycerol and xylitol in sodium lauryl sulfate-induced acute irritation. *Journal of the European Academy of Dermatology and Venereology* 2015; 29(12):2333-2341. (IF: 3.029)
- II. Meszes A, **Korponyai C**, Orvos H, Csoma Z. Transient zebra-like hyperpigmentation in a healthy newborn. *The Indian Journal of Pediatrics* 2016; 83(7):758-759. (IF: 0.808)

LIST OF OTHER ABSTRACTS

1. **Korponyai C**, Altmayer A, Varga E, Szőke A, Bata-Csörgő Zs, Kemény L: Myeloma multiplex és amyloidosis együttes előfordulása. *Bőrgyógyászati és Venerológiai Szemle* 2009; 85:248.
2. Degovics D, Erős G, Hartmann P, Gáti K, Németh IB, Czóbel M, **Korponyai C**, Nagy K, Kemény L. The effects of oxygen- and hydroxyproline-containing

solutions on the healing of acute wounds. *Clinical Hemorheology and Microcirculation* 2013; 54(2):193-194.

3. Szél E, Erős G, Hartmann P, Németh IB, Degovics D, **Korponyai C**, Dikstein S, Boros M, Nagy K, Kemény L. Anti-irritant and anti-inflammatory effects of polyols in irritant contact dermatitis. *Clinical Hemorheology and Microcirculation* 2013; 54(2):205.
4. Szél E, Erős G, Hartmann P, Degovics D, **Korponyai C**, Boros M, Dikstein S, Nagy K, Kemény L: Poliolok antiirritáns és gyulladáscsökkentő hatása irritatív kontakt dermatitiszben. *Bőrgyógyászati és Venerológiai Szemle* 2013; 89(6):179.
5. Szél E, Erős G, Hartmann P, Németh I, Degovics D, **Korponyai C**, Kaszaki J, Polyánka H, Szabó K, Dikstein S, Nagy K, Kemény L: Poliolok antiirritáns és gyulladáscsökkentő hatása irritatív kontakt dermatitiszben. *Bőrgyógyászati és Venerológiai Szemle* 2014; 90(6):261.

