DERMATOLOGICAL AND VENEROLOGICAL REVIEW·2014·VOL. 90 4 152-155 10.7188/bvsz. 2014.90.4.4

For the tenth anniversary of Professor Dr. Lajos Kemény as head of department

Antibacterial and skin hydrating effects of Xylinep® gel containing glycerol- and xylitol

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Abstract:
Locally applied glycerol known to exert beneficial effects on the skin, while xylitol is known to inhibit bacterial proliferation. Currently, no information is available on the efficacy of their combination. Our goal was to study the effects of Xylinep® gel, containing glycerol and xylitol.

Antibacterial properties of the gel were examined *in vitro*, using *Streptococcus pyogenes* and *Staphylococcus aureus* cultures. A human trial was also designed to study the effects of the gel on skin bacterial colonization.

Xylinep® gel significantly decreased bacterial colonization both *in vitro* and *in vivo*. Moreover, *in vivo* application of the gel is moisturizing the skin for 24 hours without having any occlusive effect. It has also barrier repair property.

Xylinep® gel moisturizers the skin, provides protection against bacterial colonization and helps barrier repair, thus therapeutic applications have to be beneficial.

Keywords: glycerol, xylitol, skin hydration, skin flora, skin barrier repair

Introduction:

The polyols are widely used in the medical practice. Mostly, their osmotic activity is exploited, for example to reduce the cerebral oedema, as a component of laxatives, or to improve the mucociliary clearance, respectively [1]. In the dermatology, the glycerol (earlier known as glycerine) is the polyol of highest importance which because of its beneficial properties is a component of numerous topically used preparations. Through a multiple process, the glycerol increases the water content of the stratum corneum [2]. On the one hand, it forms a reserve between the lipid double-layer of the cells and entering into interaction with the proteins and lipids it changes their water-binding capacity. Besides, entering into interaction with the polar head groups of the lipid double-layer, it maintains the optimal ratio of the fluid and solid phases of the stratum corneum lipids [3, 4]. On the other hand, the glycerol reduces the density of the water pores and the mean diameter of the water pores, respectively, in the stratum corneum and through this it inhibits not only the loss of water, but it has an effect on the penetration of the irritants with different amphoteric features forming micellas as well [5]. As a result of the above mentioned effects, it has a moisturizing, anti-irritant and barrier-repairing effect and its topical use improves the mechanical features of the

skin as well [6-8]. On the basis of the beneficial features of the glycerol, it has come up that other polyols may also exert positive effect on the skin. The xylitol is a pentahydoxy alcohol which is most frequently used as a sweetener in the industry and households, because its energy content is lower than the same of the most sugars and it is able to enter into the cells without insulin as well. The xylitol taken up with food has a therapeutic effect as well: it has been proved in animal models that it reduces the resorption of the bone substance [9]. Using it as an auxiliary material, the xylitol promotes the release of the antibiotics from the polymer-based delivery systems and through this it makes the treatment of the osteomyelitis more efficacious [10]. However, the xylitol has been proved to be of antibacterial effect in itself as well: the presence of the polyol inhibits the proliferation of the *Streptococcus mutans* bacterium in the oral cavity, thus its regular consumption can contribute to the prevention of the dental caries [11, 12]. As the xylitol has a considerable hygroscopic (humectant) effect, it is able to hydrate (moisturize) the skin [13, 14]. It has been proved in *in vivo* studies that the topical use of the xylitol (with farnesol together) in atopic dermatitis diminishes the colonisation and ratio of the *Staphylococcus aureus* on the skin, as well as – similarly to the glycerol – its moisturising effect is also considerable [15]. However, we do not have any information on whether the concurrent use of the xylitol and other polyols has any impact on the hydrating, barrier-repairing and antibacterial effects already known earlier. Although, the chemical structure of the two introduced polyols is similar, it has been proved in *in vitro* studies that they produce different gene expression changes in the keratinocytes: the glycerol is of anti-irritant effect (it reduces the expression of the HLA-DR), while through enhancing the expression of the filaggrin the xylitol may contribute to the repair of the barrier function [16]. On the basis of all these, we supposed that from the combination of the two polyols more beneficial therapeutic effects can be expected as if those were applied separately. Consequently, our goal was to study the effect of the Xylinep® gel containing the glycerol and xylitol together exerted on the antibacterial and skin physiological parameters (transepidermal water loss – TEWL, pH, hydration) in the course of *in in vitro* and *in vivo* studies.

Materials and methods:

The Xylinep® gel used in the study contained 5% glycerol and 5% xylitol. The gel used for the microbiological tests did not contain any preservative, the gel was manufactured under sterile conditions. The Xylinep® gel is manufactured by PannonPharma Kft.

In vitro antibacterial studies:

For the study we selected *Streptococcus pyogenes (S. pyogenes* ATCC 19615) and *Staphylococcus aureus (S. aureus,* ATCC 6536) strains. From the test strains, we produced fresh cultures, from which we made series of 10-fold dilutions and then with germ number assessment by spreading we determined the germ concentration of the suspensions. In the case of the S. pyogenes, the initial germ number concentration was 5.9×10^5 CFU/ml; the two selected members of the series of dilutions were 59 CFU/100 μl and 590 CFU/100 μl. When the germ number of the S. aureus was adjusted, our aim was to make a test bacterium suspension of the order of approximately 100,000 CFU. We took 3 samples from the suspension the germ concentrations of which were 121,000 CFU/ 100 μl, 125,000 CFU/100 μl and 54,000 CFU/100 μl. We inoculated the Xylinep® gel with the test bacteria. For this, under sterile conditions, we weighed out and added 1 g of gel into well closable plastic tubes of 5 ml in volume into which 100 μl of bacterium suspension was mixed. We incubated the gels inoculated with bacteria at 25 °C for 24 and 48 hours. After this, we dissolved the gel in 9 ml of sterile distilled water and from this we made a series of dilutions and then we inoculated 100 μl of each member of the series of dilutions onto culture medium. In the case of the *S. pyogenes*, the culture medium was PPLA (Bacto-Beef Heart for Infusions) agar plate

which we incubated at 37 °C for 72 hours, while in the case of the *S. aurues* it was TSA (Trypticase soy agar), the plates were incubated at 35 °C for 24 hours. After incubation, we assessed the germ number. As a control, with both strains, the initial germ number of the gels inoculated with the test bacteria was used (the determination of this happened according to those mentioned afore as well).

In vivo studies:
We studied the antibacterial effect of the Xylinep® gel and its effect on the skin physiology on 15-15 healthy volunteers (of the age between 18 and 65 years). Before the study, detailed information was provided to them and they signed an informed consent. Exclusion criteria were the persistence of any dermatological, endocrinological or immunological disease, systemic, steroid or cytostatic treatment or any topical dermatological treatment, respectively, within 30 days before the study as well as pregnancy and lactation. The applied procedures were in accordance with the Helsinki Declaration, the study was approved by the Regional Ethics Committee of the Szeged University of Sciences (Authorization No. 135/2012).

In vivo antibacterial tests:
A sterile glass ring of 3 cm in diameter was placed onto the arm of each subject involved in the study. 500 µl of sterile PBS containing 0.1% Triton-X was measured out and added to the control sample in the ring and then from this 100 µl was spread on blood agar medium. The area of 3 cm in diameter was marked and was treated with Xylinep® gel. After 15 minutes, following the absorption of the applied gel, we took sample again. On the subsequent 3 days, the studied subjects treated the marked area with Xylinep® gel in the morning and in the evening, after getting themselves clean, 6 times in total. Subsequent to the last treatment, 74 hours after starting the study, we took sample again, then after inoculation we incubated the culture media at 37 °C or 24 hours and we counted the colonies.

Determination of the skin physiological parameters:
We carried out the measurements under controlled circumstances (at 20-22 °C, 40-50% relative humidity) in the Cosmetological and Skin Physiological Laboratory of the Department of Dermatology and Allergology, Szeged University of Sciences. Between the subjects' to be tested arrival in the laboratory and starting the examination 15-20 minutes elapsed for the purpose of acclimatization, then we designated an area of 4x4 cm on their lower arms. After taking the initial values, we treated the area with 0.05 ml of Xylinep® gel. We determined the state of moisturization with Corneometer® CM 825 device (Courage+Khazaka GmbH, Cologne, Germany). We performed the measurements after 2, 8, 12 and 24 hours. We also measured the TEWL and pH values (the applied devices were Tewameter® TM 300 and Skin pH-meter® PH 905, Courage+Khazaka GmbH, Cologne, Germany).

Statistical examination:
For statistical processing of the data we used SigmaSat for Windows (Jandel Scietfica, Ekrtah, Germany) software, variance analysis with repeated measurements and Holm-Sidak test. In the figures, we indicated the arithmetical mean values (m) and the standard deviation (SD), we considered the difference as statistically significant in case of $p<0.05$.

Results:
We did not manage to detect surviving bacteria from the gel inoculated with *S. pyogenes* suspension with the concentration of 590 CFU/100 µl at all; the Xylinep® gel exerted its antibacterial effect already during the preparation of the samples. In the case of a bacterium

concentration of 590 CFU/100 μl, the numbers of the surviving colonies after 24 and 48 hours were 16 and 13.

Its effect exerted on the *S. aurues* suspensions with different germ numbers is demonstrated in the **Figure 1**. The germ numbers in the gel inoculated onto the culture medium inoculated with *S. aureus* and without incubation used as control showed hardly any reduction as compared with the initial suspensions. However, after an incubation time of 24 hours, we observed a considerably lower bacterium concentration and after 48 hours we could not find surviving bacteria at all.

The *in vivo* antibacterial effect of the Xylinep® gel is shown in the **Figure 2**. Before treatment, numerous bacteria were cultivated from the buffer having contact with the skin of the studied volunteers (number of colonies: m=53.27; SD = 100.87). The use of the Xylinep® gel significantly decreased the number of the appearing bacterium colonies (m=7.47, SD=7.73) already after 15 minutes passed and a considerable antibacterial effect could be experienced after 74 hours as well (M=15.27; SD=20.38).

From among the skin physiological parameters, the Xylinep® gel treatment did not influence the TEWL and pH values to an extent statistically significantly different. Its effects exerted on the state of hydration of the skin are demonstrated in the **Figure 3**. As compared to the initial values, already after 2 hours, a considerable growth could be observed (m=45.83; SD=9.86). The moisturization reached the highest value (m=48.45; SD=7.29) 8 hours after the treatment. The moisturization values were significantly higher even12 and 24 hours later (12 hours: m=46.9; SD=7.34; 24 hours: m=42.52; SD=8.69).

Discussions:

It has been proved by our examinations that the Xylinep® gel has a considerable moisturizing and antibacterial effect. However, it is an important question to what an extent the two polyols included the in the gel contribute to the reduction of the vitality of the microorganisms and the moisturization of the epidermis. On the basis of the data published earlier, the glycerol is capable to destroy the bacteria in itself as well, but this effect could be observed only at a very high, 85% concentration and the Gram negative strains proved to be more sensitive to the glycerol than the Gram positive ones [17]. As against to this, the xylitol is capable to reduce the number of the *S. mutans* already in a concentration of 5% even if the bacteria were present in a form having a special organization, showing an enhanced resistance toward the effects from outside, in a so called biofilm form [18]. The xylitol may inhibit the propagation of the bacteria through different kinds of mechanisms. In the case of the *S. mutans* it is known that entering into the cells xylitol-5 phosphate is formed from the xylitol which then is dephosphorilizated; this energy consuming cycle inhibits the proliferation of the bacteria [19]. Besides, the xylitol inhibits the build-up of the polysaccharide envelopment, the so-called glycocalix, in the *S. aureus* bacteria [20]. On the basis of all these we suppose that the xylitol component is responsible for the antibacterial effect of the Xylinep® gel. However, to the hydration of the skin both components may contribute. In the animal model of the irritative contact dermatitis we separately studied the effects of the glycerol and xylitol and on the basis of our results both polyols are capable to counteract the increase in the TEWL and the decrease in the hydration occurred upon the effect of the irritant [21]. Besides, we proved the antiirritant effect of both polyols in the human clinical study [22]. The hydrating effect of the glycerol has been known for a long time, its mechanism of action has been also mapped [2]. Although, on the basis of its hygroscopic (humectant) effect the hydrating effect of the xylitol could be supposed [14], the *in vivo* behaviour of a material can be not always predicted with absolute certainty from its chemical structure, especially in the case if the product contains oil as well [23]. The Xylinep® gel does not contain oil. However, the xylitol contributes not only to the hydration, but to the repair of the barrier function after injuries as well. On the basis of

the results of a human study carried out recently, the 5% aqueous solution of the xylitol (after three treatments a days) accelerated with 15% the repair of the epidermis - on the basis of TEWL measurements – after twentyfold "stripping" (S. Dikstein's not published data). It may be one of the explanations of the hydrating and barrier-repairing effect that the xylitol – in *in vivo* cultured normal keratinocytes – enhances the filaggrin expression. As a contrary to this, the glycerol has no influence on it [16]. On the basis of these results it may be concluded that in spite of the similarity in their chemical structure the two polyols contribute to the treatment of the dry skin through different mechanisms. The regular use of hydrating formulations may help to prevent the irritative contact dermatitis. This kind of inflammations very frequently develops as a consequence of professional harms; its risk is very high among the healthcare- and food industry workers, hairdressers, cosmeticians and metal industry workers [24]. On the irritated skin essentially higher number of microorganisms can be found as well than on the healthy skin [25]. On the basis of our results, consequently, the use of the combination of the two polyols may be beneficial for the reason as well that beside the hydrating effect it may provide protection against the bacterial colonisation as well.

Although, for the purpose of mapping the xylitol's mechanism of action and the better knowledge of the beneficial features which may be observed with the joint use of the two polyols still require further investigations, because of its beneficial skin physiological and microbiological effects considerable results may be expected from the use of the Xylinep® gel.

Acknowledgements:
The authors express their thanks to Prof. Dr. Shabtay Dikstein (School of Pharmacy, The Hebrew University, Jerusalem) for his help rendered in the planning of the experiments. The study was supported by Pannon Pharma Kft. by providing the studied product.

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Received: 28 April 2014
Accepted for publication: 23 July 2014

Table 1: Germ concentration of the bacterium suspensions chosen for the *in vitro* experiments

Bacterium	Germ concentration (CFU / 100 µl)		
	Sample 1	Sample 2	Sample 3
Streptococcus pyogenes	59	590	-
Staphylococcus aureus	121 000	125 000	54 000

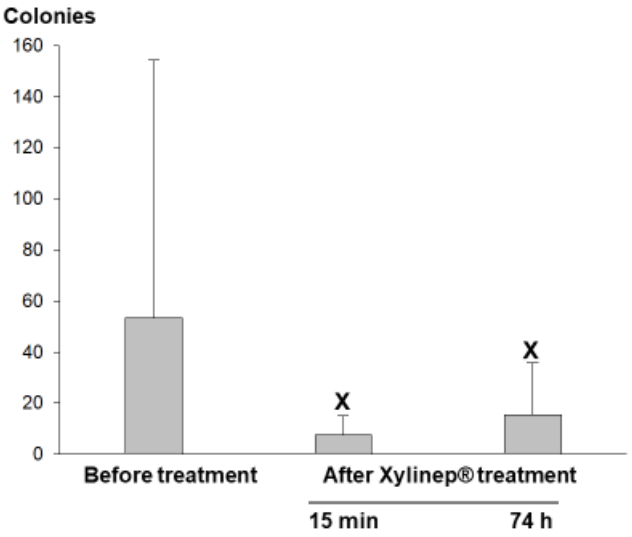
Captions

Figure 1: Effects of the Xylinep® gel on the *Staphylococcus aureus* suspensions of different germ concentration. X: $p < 0.05$ vs initial suspension. After 48 hours, no surviving bacteria were found.

Figure 2: Number of the bacterium colonies culturable from the buffer having contact with the skin of the examined persons before and after Xylinep® gel treatment. X: $p < 0.05$ vs values before treatment

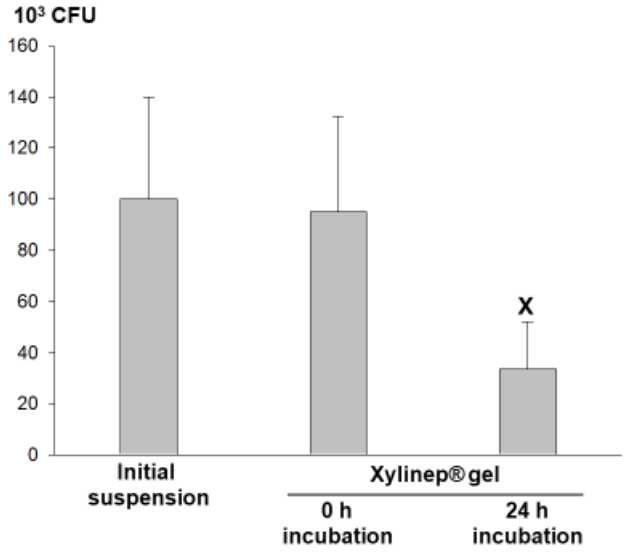
Figure 3: Change in the moisturization of the skin after the treatment with Xylinep® gel. X: $p < 0.05$ vs 0 h value.

Figure 2: Number of the bacterium colonies culturable from the buffer having contact with the skin of the examined persons before and after Xylinep® gel treatment



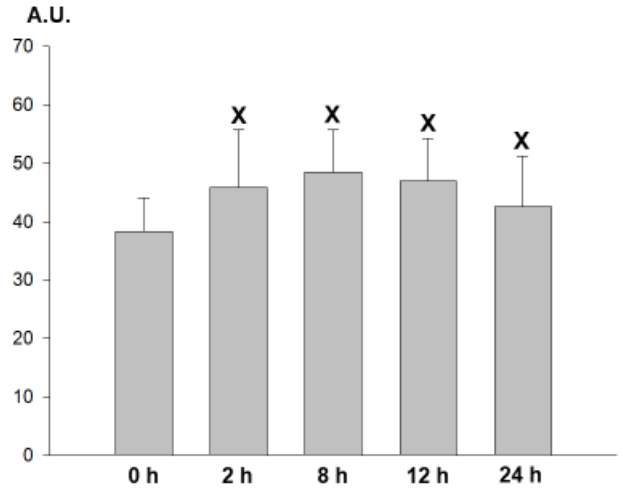
X: p<0,05 vs value before treatment

Figure 1: Effects of the Xylinep® gel on the *Staphylococcus aureus* suspensions of different germ concentration (After 48 hours, no surviving bacteria were found)



X: p<0,05 vs initial suspension; CFU: Colony Forming Unit

Figure 3: Change in the moisturization of the skin after the treatment with Xylinep® gel



X: p<0,05 vs 0 h values, A.U.: a Corneometer® CM825 arbitrary unit