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Evaluation of skin hydration after exposition to the aqueous and hydroalcoholic bases and silicone emulsion

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ABSTRACT

Several dermocosmetic bases even without active substances, can increase the cutaneous hydration, resulting in a beneficial effect to the skin. The evidence and interpretation of possible hydration effect of formulations in the skin can be carried through by means of histopathological and histomorphometrical evaluation, a time that allows the analysis of the epithelial tissue, of dermis and also of the cellular characteristics. The objective of this research was to evaluate the skin hydration after exposition to the aqueous and hydroalcoholic bases and silicone emulsion. Swines had areas submitted to treatments during 15 days with three different formulations (F1 - aqueous gel, F2 - hydroalcoholic gel and F3 - silicone emulsion). By means of histometric and histopathological techniques were gotten the thickness of the epidermis and stratum corneum. Comparison of means was done using ANOVA followed by the Tukey test. The F1 provoked significant increase in the thickness of the epidermis. The formulaton F2 provoked significant reduction in the thickness of the epidermis and stratum corneum. F3 not presented significant difference in this structures. According to the study, the type of base chosen intervenes with the skin hydration.

INTRODUCTION

Technological advances in the industry of cosmetic raw materials, combined with market needs, has allowed the study and use increasingly more considerable, from different dermocosmetic bases. This study becomes even greater due to the fact that this bases without active substances can increase skin hydration, resulting in a beneficial effect on the skin Leonardi *et al*., 2000; Hamed *et al*., 2012). The appropriate choice of the base to which the active substances will be incorporated is of fundamental importance for the stability and skin penetration and, consequently, to obtain pharmacodynamic effects expected. In addition, the requirements of each type of skin should be considered in this choice (Maia Campos, 1994; Rim *et al*., 2005). Among the various types of preparations used as dermocosmetic bases we have: oil/water (O/W), W/A, silicone/W and W/silicone emulsions, aqueous gels, hydro-alcoholic gels, among others. The gels, semi-solid preparations consisting of colloidal particles dispersed, have been widely used because they are easy spreading, non oily and can carry active substances soluble in water and liposomes, being more used on oily and mixed skin (Maia Campos, 1994). Furthermore, gels have been used extensively in the techniques of phonophoresis because, generally, provide good transmission of ultrasound waves (Williams *et al*., 1990; Cameron and Monroe, 1992). Alberti *et al*., 2001, report that the effectiveness of the formulation may depend of the vehicle's ability to release the active substance and of the penetration in the stratum corneum. Thus, many formulators often use chemical enhancers in the formulations with the purpose of modifying the solvency of the active in the vehicle and improve your penetration in the skin (Yilmaz and Borchert, 2006). Among the various components used for this purpose has been employed ethanol (Schueller and Romanowski, 2000). However, chemicals used as permeation enhancers must be harmless to skin tissue for that your use in both cosmetic and pharmaceutical field is perfectly feasible (Yilmaz and Borchert, 2006). The evidence and interpretation of possible side effects in the skin tissue can be performed by histometric and histopathology-cal evaluation, since they allow the analysis of epithelial tissue, of the dermis and also

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of cellular characteristics. In addition, can assist the experimental protocol for studies of effectiveness of the dermis and in the development of new cosmetic products (Maia Campos *et al*., 1999; Silva *et al*.,2000). Histopathologic analysis consists of visual observation or biopsy to an optical microscope that allows the qualitative assessment of the various structures present in skin tissue. Histopathology, as a qualitative analysis must be complemented by histometric analysis, determining the thickness of the epidermis and dermis, as well as quantifying the various cell types, mainly in the dermis (Tadini *et al*., 2005).

The objective of this research was to evaluate the skin hydration after exposition to the aqueous and hydroalcoholic bases and silicone emulsion.

METHODOLOGY

Formulations

For this research was employed three dermocosmetic bases: an aqueous gel, a hydroalcoholic gel and a silicone emulsion (Table 1).

Table. 1: Dermocosmetic bases.

Components	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
Polyacrylic acid	1	1	-
Propylene glycol	10	10	-
Methyldibromo glutaronitrile (and) phenoxyethanol	0.2	0.2	0.2
Triethanolamine	qs pH 6.5	qs pH 65	-
Ethanol	-	25	-
Deionized water	qsp 100%	qsp 100%	qsp 100%
Cetoestearlic alcohol 20 EO / sorbitol monostearate (and) berreniltrimonium methosulfate and cetoestearilic alcohol (and) isopropyl palmitate (and) cyclpentasiloxane (and) dimethicone crosspolymer	-	-	80

Animals

During the experiment, the animals were maintained according to principles established by Olfert *et al*. 1993, and the Statement of Principles, which has been adopted by the FASEB Board. Five male hybrid swine (Landrace x Large White), weaned and not castrated, with 40 days of life and approximately 15 kg of weight were utilized. The animals had access to food and water *ad libitum*. Twenty-four hours before to treatment start, four areas were delimitated on the back of each animal and divided into the following experimental groups: C (control); F1 (3g); F2 (3g) and F3 (3g). With the exception of the control area, the others areas were submitted to a daily treatment for 15 days, always at the same time, accompanied by the use of frictional circular massage until hyperemia was evidenced.

Histological Procedures

After the experimental period, the animals were sacrificed by exsanguinations realized by a qualified professional (Andrade, Pinto,Oliveira, 2002), and transverse skin segments of each area were fixed in formaldehyde solution buffered 10% for 48 hours and routinely processed for semi-serial decalcified sections. Five non serial histological sections of 5 μm of depth for each blade were obtained, being three blades per treatment area. The sections were processed for coloration in hematoxylin and eosin (HE).

For the histometric analysis was measured the thickness of the epidermis and stratum corneum in 30 randomly selected areas for each histological section, using a millimeter ocular lens (Zeiss) attached to a light microscope. For the epidermis, we obtained measures in plain areas, without many dermal papillae, across of the distance between the basal membrane to the outer edge of the stratum corneum. The stratum corneum was measured in areas where it was well adhered to the epidermis and was considered the distance between the upper portion of the granular layer to the surface (Polacow *et al*., 2004). From these measurements, it was realized the correction of the micrometer coefficient, whereas we used a objective of 40x, and obtained the mean and standard deviation for each experimental group.

Statystical analysis

Based on these measurements, mean values and standard deviation for each experimental group was obtained, as well as variance analysis (ANOVA) and the Tukey test in order to assess differences between treatments. A comparison analysis for paired measurements was conducted by the Tukey test, in which values of $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Dry skin and other skin disorders are characterized by impaired stratum corneum (SC) barrier function and by an increase in transepidermal water loss (TEWL) leading to a decrease in skin hydration (Yilmaz and Borchert, 2006).

For the development of new cosmetic products, important criteria are their increase effects on skin hydration and viscoelasticity. These effects can be influenced by adequate vehicles. The water content of the skin is 10–20% and it hydration can be increased by occlusive topicals. Many topical preparations have an undesirable esthetic appearance (e.g. petrolatum), and the need for novel occlusives has been growing (Wissing and Muller, 2003).

The main objective in carrying out the histometric analysis of the epidermis was to determine whether the formulations could be used to increase skin hydration, which would be evidenced by an increase in interstitial spaces (Leonardi *et al*., 2000).

Weaned swine have been utilized as animal model for experiments related to cutaneous permeation, due to the fact that their epidermis and stratum corneum present similarities with the human skin (Bronaugh, 1989). In the present study, the results point to values of 72.55±10.02 μm for epidermis thickness in control group, values which are similar to those obtained by Bronaugh *et al*., 1989, 65.8±1.8 μm, indicating that the methodology here utilized was adequate. Linear and morphometric

measurements are important in histopathological studies, since besides being more objective and easily reproducible, they also permit detection of alterations which could be easily neglected by visual observations (Hamilton, 1995; Oriá *et al*., 2003).

According to the variance analysis and Tukey test can verify that the aqueous gel (F1) did not cause significant chance in the stratum corneum, but increased the thickness of the dermis. Looking at Figure 1b can be seen that the aqueous gel caused an increase in the interstitial spaces, indicating hydration of the skin tissue. Probably this is due to the occlusive film formed by the gel, which impedes the evaporation of water from the skin to the environment, increasing water retention. Similar results were obtained by Maia Campos *et al*. 1999, and Bradley *et al*. 1990, who also noted a significant increase in skin hydration provided by aqueous gel.

Already gel with ethanol caused a significant reduction in the thickness of the epidermis and the stratum corneum (Table 2 and Figure 1c). According to Rieger, 1993, the stratum corneum is composed of corneocytes embedded in a lipid environment. Alcohol present in the gel to penetrate the skin, causing removal of the stratum corneum, leading to a reduction of the barrier properties lipophilic (Kitagawa and Li, 1999; Levang *et al*., 1999). As can be seen in Figure 1, the epidermis treated with this gel showed the stratum corneum significantly reduced, while the other layers were very compacted, not observing the interstitial spaces. With the loss of the lipid mantle is most likely a reduction in the rate of skin hydration.

According to Libardi, 1999, which makes the skin stay healthy, soft, with flexibility and elasticity is the maintenance of skin hydration and ability that the organism needs not only to stimulate cell renewal, but also to synthesize substances part of the epidermis. For good functioning of the mechanism of skin hydration, the stratum corneum should be able to retain water, so that the rate of evaporation of water always remains at a normal level. Proksch *et al*., 2005, claim that skin hydration is intrinsically related to the integrity of the stratum corneum.

Therefore, the presence of ethanol in the formulation, despite increasing the skin permeation to remove the stratum corneum, which is the main barrier to permeation, may cause an undesirable effect in cosmetic and / or dermatologic products because the hydration content is one of the most important factors to maintain the optimum conditions of the skin, since the water-deficient skin can become dry and brittle, resulting in surface cracks of the stratum corneum (Jones and Brown, 1992).

For F3, according to the analysis of Table 2 and Figure 1d, it is observed that there was no significant increase in the thickness of the stratum corneum and epidermis after application of the formulations when compared to the control for a period of 15 days, using 3g of formulation per day. The analysis suggests that no have increase in the skin hydration after the use of the formulations. An important question to be commented is that the formulations were applied by a relatively short period to verify significant effects. Maybe, if applied for longer periods, results might have been more expressive.

Table. 2: Thickness of the skin layers (μm) subjected to the different formulations (mean of 20 measurements made at random in the sections). [n=5].

Formulations	Stratum corneum	Epidermis
Control	23.125±7.11	68.500±10.95
	28.875±9.44	77125±10.52
	26.625±6.45	69.625±6.75
	26.500±4.83	69.250±9.14
	34.375±5.06	78.250±12.72
F1	32.250±6.97	112.125±14.08
	28.000±6.37	96.750±14.58
	34.375±7.02	102.500±15.24
	34.125±6.55	94.625±13.60
	31.250±5.82	92.400±11.20
F2	11.250±6.10	60.000±11.15
	14.000±6.09	53.375±13.06
	11.250±5.23	60.500±9.38
	9.125±3.37	55.500±13.19
	12.750±7.07	58.750±10.96
F3	24.138±6.15	69.240±8.93
	29.943±7.48	78.231±9.57
	28.441±4.49	70.114±7.44
	27.473±5.81	69.893±8.32
	36.412±6.61	79.463±9.75

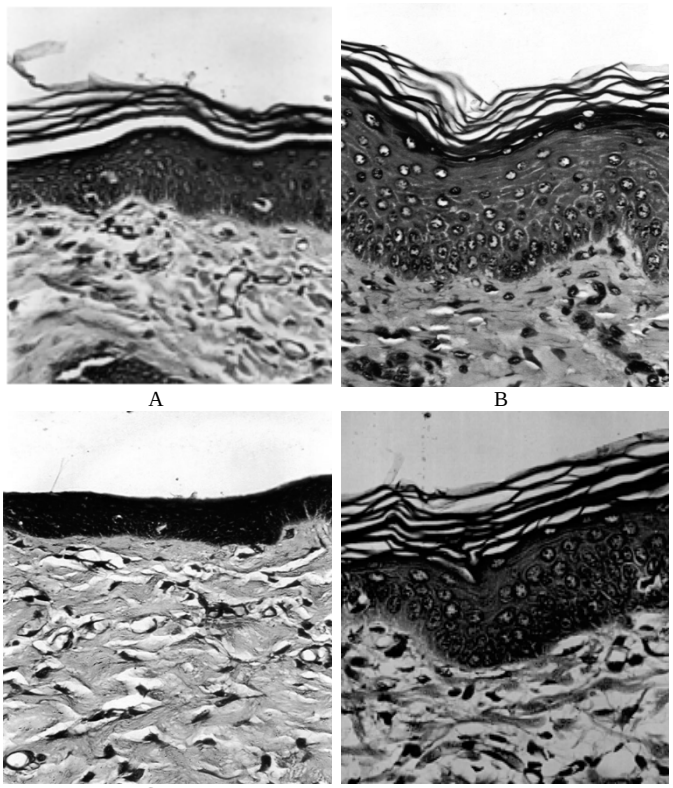

Fig. 1: Photomicrographs showing the stratum corneum and epidermis following treatments: (A) - control, (B) – F1, (C) – F2, (D) – F3 (200x).

CONCLUSIONS

This study concludes that aqueous gel (F1) increased the thickness of the epidermis, evidencing increase in the water retention in the skin. The hydroalcoholic gel (F2) caused to reduction of the thickness of stratum corneum and epidermids, probably by reduction of interstitial liquid provoked by the hydration loss. The values of thickness of the epidermis and

stratum corneum gotten for the groups dealt with silicone emulsion (F3) had not differed statistically from the control group. Thus, the type of base chosen intervenes with the skin hydration.

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