

Evaluation of cream composed of urea and natural extracts of *R. Officinalis*, *C. Lechleri* and *A. Vera* for humidifying the skin in diabetic foot

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Abstract

Abstract: Topical medications using urea has been useful to reestablish skin physiological conditions. In this prospective, mono-centric clinical trial we apply a formulation based on urea and natural extract of *R. officinalis*, *C. lechlery* and *A. vera* to 40 diabetic patients. The skin clinical response was evaluated after and before the medication. Optimal results were found in patients, the assayed formulation reduced the pathological symptoms characterizing diabetic foot skin. In particular hyperkeratosis, dryness and scaling. Formulation was shown its ability to significantly ($p < 0.05$) improve the bio-physic characteristics of the diabetic skin (pH and moisture). In addition it was significant ($p < 0.05$) improved the barrier function of the skin with the consequent protective effect. The formulation showed its excellent moisturizing, tolerability, fast action, and cosmetic properties.

Keywords: urea, diabetic foot, diabetes, diabetes mellitus, dry skin.

Introduction

The moisturizing of epidermis takes place by different complex mechanisms. However, there are different disturbing factors that induce the disruption of the stratum corneum (SC) and consequently induce the epidermal loss of water. The exposition to some factors as: cool and dry condition, use of soap, hot water and corrosive chemicals can induce skin damage and dried. It's estimated that 20 % of world population have gene involved in lots of skin moisturizing. In addition, peoples whose over cleaning hers hands can eliminate naturals moisturizing present in the skin and consequently can induce a disturbance in skin water retention. The lost of epidermal moisturizing not only are cause of allergy but also can block the main function of this organ: Protective as an anatomical barrier from pathogens and damage between the internal and external environment in bodily defense (Savic *et al.*, 2004).

It has been demonstrated that topical application of Urea can improve the skin water retention. Urea has been applied successfully in different skin diseases as in radio protection, psoriasis, ictus or chronic eczema (Masferrer *et al.*, 2010; Loden, 2003; Petres *et al.*, 1990).

In diabetic patients skin neurological changes may originate foot ulcers or risk of skin damage that can facilitate the skin infection and erosion. Diabetes mellitus modified the skin sweat and lipid secretion composition with a

reduction of ceramides content and loss of amino acids. Those elements are essential for skin moisturizing and also are components of the *acid mantle* with protective function. Those pathological characteristic of diabetic patients induce the so called *diabetic foot*, characterized by foot ulceration and mixed infections in foets (Slovenkai, 1998; Caputo *et al.*, 1994). The aims of this study was to assay an urea 10 % based cream with natural moisturizing agents (pH 5.5) on diabetic (Type 1 and 2) foets with hyperkeratosis and severe dryness in order to improved the skin water retention and avoid de SC damage.

Material

Materials and Methods

The assayed medication (cream) was in its composition: *Rosmarinus officinalis* (Rosemary), *Croton lechleri* (Dragon's blood) and *Aloe vera* (Aloe) extracts, Urea 10%, Linaza oil, sweet almonds oil, vitamin E, propyleneglycol, glycerin and excipients.

Experimental schedule

The study was done in the diabetes consulting room at the Department of Clinical Research (DCR) of the University of Zulia (UZ). The trial was mono centric, prospective and was conducted according to the ethical principles established in the Helsinki declaration (WMA, 2004). The study design was evaluated and approved from the ethical and scientific point of view by the Ethic Committee of DCR/UZ. The declaration of the Ethic Committee was validly all the period in which the study was done.

Inclusion criteria: Both gender subjects without ethnicity distinction which accept to participate in the study aged from 20 to 50 years. Body weight in normal range. Apparently healthy for normal control volunteers and in the group of diabetic: diagnostic of diabetic 1 and 2 and sings of skin foot alteration at epidermal level. In any case, volunteers or patients sing a write consent expressing it approbation to participate in the study.

Exclusion criteria: Subject that no meet the inclusion criteria. Previous clinical record of acute diseases (30 days before inclusion). Chronic disease. In treatment with any drug under research in the last 45 days. Under medication (drug or supplements) in the last 15 days. Phlebotomy or blood lost higher than 400 mL in the last 12 weeks. Hyper sensibility to any drug. Previous history of alcoholism or alcohol consumption in the last 24 h. Smoker (more that 10 unit per day).

Withdrawal criteria: voluntary abandon 15 days after the end of the study. Appearance of exclusion criteria. Appearance of adverse event. Necessity of consume any drug during the treatment. Decease.

Subject's selection

In a first phase it was selected at random 60 subjects, 70 % healthy volunteers and 30 % diabetic patients. This phase involved the evaluation of the cosmetic properties of the drug under assay at its tolerability at the skin level. The apparition of allergy or irritation was conducted by the (*Repeated insult patch test* (RPIT) using a scale from 0 to 3. In this scale quantitative values correspond with qualitative indices as: 0 bad, 1 light, 2 good and 3 excellent. A second phase involved 40 diabetic patients (Diabetes Mellitus type 1 and 2) with local symptoms of pruritus, hyperkeratosis, dryness, erythema and small ulcer only at dermal or epidermal level. Each symptom was evaluated according to a four point scale (0 assents, 1 minimum, 2 moderate, 3 intense). The cutaneous dryness was assayed using the visual anagologic scale (VAS) from 1 to 10, 0 correspond to the normal status and 10 to maximum dryness. Normal skin was considered a hydrated lightly skin, soft and smooth. To measured the skin hydration was used a Dermo Scan SC-110. According to this method the optimum of hydratation was 13 % (from 10 to 15 %) in correspondence with the mechanical and esthetic condition of the skin. Values under 10 % mean dehydration. Measured was done 10 min after the first application and one month after the treatment. To measure pH was used a device Skin-pH-meter. Measured was done at time cero and one month after the treatment. To all subject enrolled were indicated an initial exfoliative treatment at the SC level, because usually in diabetic the SC had from 40 to 60 slides in contrast to the normal status (from 12 to 15 slides) that avoid the right penetration of the drug components.

Treatments schedule: Drug was applied twice a day (morning and afternoon) before the usual foot hygienic.

Statistic

Initially the OUTLIERS preliminary test for detection of error values was applied as a first step in the statistical analysis. After this, the homogeneity of variance test (Levene) was used and afterward ANOVA. The values of the measured indices before and 1 month after treatment was compared using independent *t* test, two tailed. Data are expressed as means \pm SD. The minimal level of significance was identified at $p < 0.05$. The STATISTICA software package version 6, was used for all statistical analyses.

Results

In the first phase of the study (evaluation of RPIT in healthy volunteers, no symptoms of irritation or sensibilization was detected. Patients enrolled was 40 diabetic aged from 20-50 years, in this group patients between 40-50 was predominant (56.8%). Male was 53.8 %, with diagnostic of diabetes type 1 52.5 % and 47.5 with diabetes type 2. In the initial evaluation of diabetic patients

sing of hyperkeratosis, dryness and alkaline pH was detected (Table 1).

Table 1. Intensity of symptoms measured in diabetic patients after treatment.

Index	No	Results		
		%	Intensity of symptoms according to the scale (%)	
Hyperkeratosis	40	100	2 (45)	3 (55)
Dryness	40	100	(8.5) mean	
Scaling	30	75	2 (35)	3 (40)
Erythema	32	80	2 (40)	3 (40)
Superficial Ulcer	14	35	2 (25)	3 (10)
Erosions	34	85	1 (2)	2 (18)
Pruritus	24	60	2 (45)	3 (15)
pH (7 – 7.5)	40	100	3 (65)	

Legend: Values was measured in 40 diabetic patients. The intensity of symptoms was measured as described in Materials and Methods.

The moisture skin index, assayed in 40 diabetic patients before treatment was between 7 % and 9.5% (mean 8.5 %), 10 min after the first treatment the values have a significant ($p<0.05$) increase (from 10.7 % to 11.4 %) compared to started time values. One month after treatment this index increase (13.7 % to 14.0 %, mean 13.9 %) significantly ($p<0.05$) different from the started time (Fig. 1). All sing and clinical symptoms evaluated was significantly ($p<0.05$) improved. Hyperkeratosis, erosion, dryness and ulceration disappear in all patients.

Scaling reach normal values (absent) in 90.95 %, minimum in 7.2 % and moderate in 1.85 % of patients. Erythema fist present in 80.0 % patients was reduced (absent in 75.0 % of patients) and only 5 % remain in the minimum values of the scale. Pruritus was absent in 86.7 % remaining 13.3 % of patient between the minimum values of the scale (Fig. 2).

Figure 1

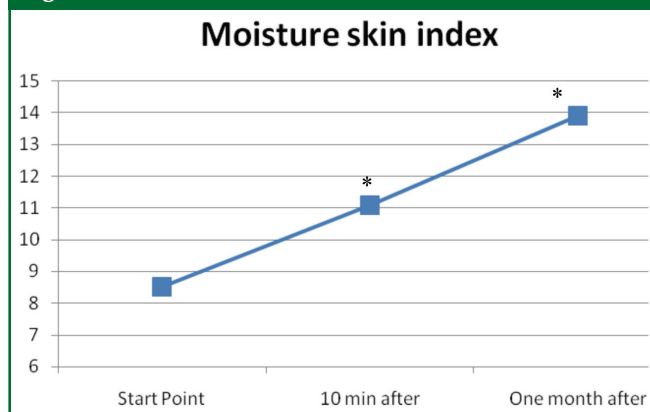


Figure 1. Mean of moisture skin index of 40 diabetic patients, before treatment and 10 min or one month before treatment. * Significant different from start point ($p<0.05$).

Figure 2

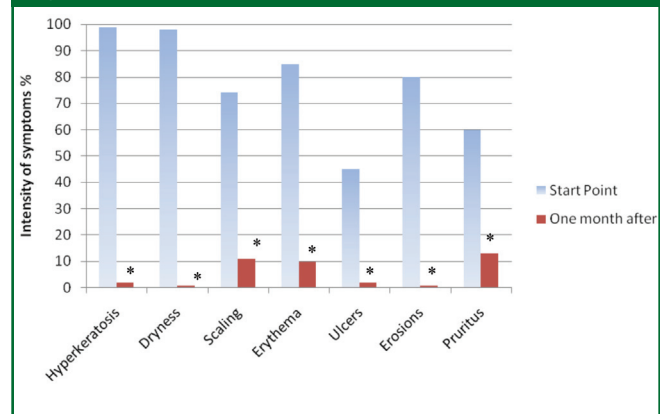


Figure 2. Evolution of sing and symptoms before / one month after treatment. * Significant different from start point ($p<0.05$).

The values of pH at the beginning of the study was high 7.00 in all patients, one month after values get normal ranges (5.5 to 6.5) in all subjects. All patients refereed the efficacy, tolerability, fast action and good cosmetic quality of the drug applied.

Discussion

The deficit of urea at skin level is ones of the main problems associated to the skin water retention. Urea is normal present in epidermis and is part of the Normal Moisturizing Factor (NMF). It was found that in peoples with dry skin there was a 50 % low level of urea compared to normal volunteers. Low levels of urea reduce the water retention capacity of the skin and consequently it was observed the related symptoms (Ferré *et al.*, 2007). A recent research conducted in Westfälische Wilhem University (Germany) shown a significant reduction in pruritus and dryness after 4 week of application of urea 10% formulation (Ferré *et al.*, 2007). This previous observation is in line with our results. Urea preparation are well know by it use in hyperkeratosis or skin dryness and its benefits had been shown in the reversion of this diseases (Raab, 1997).

When a reduction in urea concentration take place in the skin layers, as happen in different skin diseases, the ability of SC to be hydrated is reduced and consequently a reduction in the mechanic and physiological function of the skin is affected.

In diabetic mellitus patients, there was a reduction in urea content and loss of essential fatty acid involved in the skin pathology. I this patients there was observed an irregular ceramides layer (ceramides are part of the lipids barrier of the SC), also involved in the regulation of the water content. Ceramides are also important in the skin regeneration by its content in linoleic acid which

has protective abilities. In addition, its content in linolenic and gamma-linolenic acid stabilized the barrier (Rippke, 2004). The assayed cream contains urea and also fatty acid that probably has also a role to reach the therapeutic effect.

Recent research has been demonstrated that direct application of fatty acid can be incorporated to the skin layers and improve its functionality. This effect is also favorable to facilitate the penetration of moisturizing components of the formulation. Additionally, fatty acid can improve the cohesion of the keratin component of the SC and the barrier permeability (Faulí, 2002). The main inconvenience to add fatty acid to a pharmaceutical is the fact of its oxidation, that's why antioxidant should be present. One of the antioxidants recommended by *American Journal of Clinical Nutrition* (2003) for topical application was vitamin E, by its liposolubility can reach the SC and cellular membranes.

Acid values of pH (5.5) are also important to maintain skin moisturizing because correlate with the content of water in the skin. It has been shown some years ago the presence in the skin of a physico-chemical complex named "acid-mantel" composed by the aerial mantel and the epi-cutaneous emulsion (Pillsbury y Rebell 1952). The aerial mantel is formed by metabolic CO₂ and water vapor. Epi-cutaneous emulsion is formed by skin metabolites and cells. Normal skin pH values are 4.5-5.9 and essentially depend of the content in lactic acid and urocanic acid (that came from the sweat), dicarboxylic amino acid (glutamic-aspartic), and low molecular weight fatty acid (propionic, butyric and pentanoic) in low proportion by its low water solubility (Tsai y Maibach, 2000). As shown in Table 1, the assayed medication restarts the normal pH values facilitating the skin equilibrium.

Acid values of pH in the skin has a paramount role in the functionality of SC because stimulate enzymes involved in lipid synthesis. Those lipids are evolved in the cohesion of CV. In general the use of cosmetics induce increased values of pH (Rieger, 2000; Klein *et al.*, 1990). It is well known that washing the skin with cosmetic with neutral pH (7) or alkaline pH (>7) can modify the "acid mantel". A cosmetic cream or lotion remain in the skin for hours, thus can modify the composition of the acid mantel and can induce pruritus, skin irritation or dermatitis. Soaps are basically salts of strong alkalis (sodium or potassium hydroxide) with saturated fatty acids. The regular use of those soaps can damage the lipid layer and induce dryness. The modification of pH is also expected. The combination of alkaline soap with hot water can potentiate the skin lipid extraction, and consequently the damage. Moreover an acid pH facilitates the barrier regeneration after a mechanic or chemical damage. In addition values of pH near to 3.8 are bacteriostatic. According to different authors assaying the presence of *Staphylococcus aureus* or *albus* after

of a pH modification from 7 to 5 will find a reduction in the microbial content. In general a pH near to 7 is optimal for the bacterial growth.

Under physiological condition the SC has a 10-20% content of water in equilibrium with the environmental humidity. To avoid damage originated by loss of flexibility, the critical level of water of the skin is 10 % (Klein *et al.*, 1990; Wiechers, 2000 Triebkorn y Gloor, 1993). To maintain the moisturizing and flexibility epidermis produce NMF. NMF are formed by groups of components able to get 3-4 times its own weight in water. The content of water decrease proportionally when the content of NMF decrease. Some diseases can modify normal physiology and the levels of NMF and urea (even in 50 % of reduction) (Ferré *et al.*, 2007).

In diabetic patients, physiological modification of the moisturizing factor is present, as consequence dryness, scarring, skin sclerosis and loss of elasticity are common symptoms (Sakai *et al.*, 2005; Slovenkai, 1998). Those alterations were evident in the group of patients under clinical trials (Table 1). In addition, neuropathy are present in those patients associated with the diabetic foot syndrome (Caputo *et al.*, 1994; Yoon *et al.*, 2002). The arthropathy of Charcot has a main role in the foot infective condition in those patients. In consequence glandular and vasomotor responses are disturbed, modifying the thermal regulation of the skin and its water content. This complex scenario induces ulcer and foot wounds (Bridges y Deitch, 1994). The restitution of the water content in the skin and physiological pH in this disease is essential to avoid lesions and chronic ulcer.

Conclusion

The applied formulation was tolerable and reduces clinical symptoms frequently present in diabetic. In particular a reduction in hyperkeratosis, dryness and scarring was noted after one month of treatment. A significant ($p < 0.05$) improvement of the protective function of the skin reduce the risk of ulcer and wounds.

This particular formulation demonstrated its clinical effect with a significant ($p < 0.05$) improvement of the bio-physic properties of the skin (water content and pH) in diabetic patients. The increment in the moisturizing index even after 10 min of application demonstrated the ability of the formulation to recuperate the physiological status of the skin. This moisturizing natural cream is well tolerated, had a fast action and high cosmetic qualities.

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