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ASSESSMENT OF ACUTE SKIN IRRITATION IN RABBITS USING ELECTRICAL IMPEDANCE MODEL

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Abstract—**This paper describes the use of an electrical impedance model to assess acute skin reactions to irritant over time. The applied method is noninvasive and quantitative and can detect the irritation before the visual signs. The results showed that the signs of acute irritation (oedema) were present until the second day after irritant application. The method is able to detect the initial phase of irritation and the assessment of regeneration time could be attained by a combination of more than one bioengineering methods.**

Keywords—**Skin irritation, electrical impedance model, SLS.**

I. INTRODUCTION

The reaction of skin during irritation is of concern to dermatological clinicians and cosmetic scientists since the understanding of these phenomena is important to describe a treatment to decrease the effects of irritant surfactant on human skin. Although the induction of skin irritation has been extensively studied [1, 2], little is known about the duration of acute phase of irritation. The signs of acute irritation are oedema, erythema and heat, and the subsequent signs include epidermal hyperproliferation, thickening of stratum corneum (SC) and viable epidermis, and scaling and dryness of the external layers of the SC [3, 4].

In vivo exposure of human skin to irritant can be assessed by a number of methods. The most used of these is the visual score, but such scales are qualitative and introduce inter-observer variability. Kligman [5] has emphasized the need for quantitative methods, such as some bioengineering tools, which are able to measure skin reactions non-invasively and in invisible range. Several bioengineering methods are applied to study skin irritation and each of these evaluates different aspects of the irritate response. Electric impedance has been proposed as feasible quantitative method to assess skin irritation. Ollmar and coworkers [6] developed an instrument (SCIM) that measures the bioelectrical impedance of the skin at multiple frequencies and derives an index that can detect skin changes due to irritation, which are below the limit of visual observation.

The objective of this study was to assess the skin irritation over time in rabbits using an electrical impedance spectroscopy method based on a current response to a voltage step excitation. As such method implies in use of a model to the skin, interesting electric parameters were obtained and a novel index was proposed to assess the irritation.

II. METHODOLOGY

Electrical impedance

The parameters of impedance were obtained by an electrical impedance prototype system based on the current response to a voltage step excitation [7]. The principal advantage of this method is the use of a smaller number of signals to characterize the impedance, since only one excitation signal scans all frequency's components. The model of skin used to calculate the theoretical response to the step excitation (Fig. 1) was based on the one proposed by Grimnes & Martinsen [8], after the adequate adaptation for this study. Such model is in general better suited for tissue and cell suspensions, and actually this model often has been implicitly used [8].

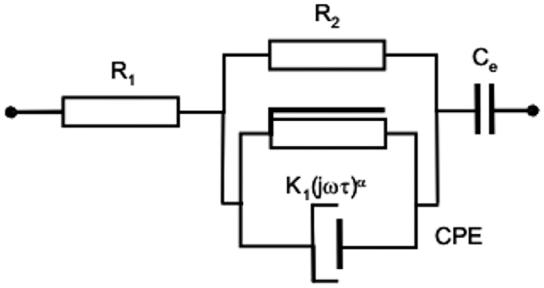

Figure 1. The equivalent circuit model to calculate the current response to a voltage step excitation and to derive the electrical impedance parameters of skin (see the text for more details). *Ce*: electrode capacitance; R_1: internal resistance; R_2: external resistance; *CPE*: constant phase element.

In this model, R_1 represents the resistance of inner tissue, R_2 models the resistance of outer tissue, C_e represents the electrode/tissue interface and the CPE (Constant Phase Element) models the dielectric relaxation processes of the epidermal stratum corneum [9]. As some other representation for the distributed processes, CPE can be interpreted as a composition of an infinite number of lumped components that models the layers of stratum corneum. The impedance of CPE is defined by equation 1:

$$Z_{cpeF} = \frac{K_1}{(j\omega\tau)^{\alpha}}, \qquad (1)$$

where, K_1 is the resistance of CPE at the characteristic frequency, τ is the characteristic time constant of the system corresponding to a characteristic angular frequency $\omega=1/\tau$, and α is the phase constant of the frequency-dependent component, and is related with the half-angle of the circular-arc at the center in the complex impedance plane

The applying of the model illustrated in Figure 1 in the spectroscopy impedance method based on the current response to the voltage-step excitation leads to the theoretical estimate of the current response express by equation 2:

$$i(t)=\left[k_0+\left(k_1e^{\omega_1 t}\right)+\left(k_2e^{\omega_2 t}\right)\right], \qquad (2)$$

where k_0, k_1, k_2, ω_1 and ω_2 are constants associated with the impedance parameters.

With the expected equation for the current *i(t)* and an experimental analogue version of it, the electric parameters of impedance, i.e., R_1 (inner resistance), R_2 (outer resistance), C_e (electrode capacitance), α and K_1 are estimated using a multiparametric optimization procedure. The implemented algorithm is based on a steepest descend gradient method to obtain the best parameters that adjust the theoretic expectation to the experimental data.

The experimental impedance measures have been made by a prototype instrument based on the principle described above and developed in the Biomedical Instrumentation Laboratory of Biomedical Engineering Program at the Federal University of Rio de Janeiro, Brazil. The current response acquisition and the applied step voltage are generated through a battery-operated circuit specifically designed to this purpose and that is connected to an acquisition card (National® PCM-CIA, DAQCard AI-16E-4 model) mounted in a laptop framework. The data acquisition is performed through a specific program that has been developed in LabVIEW® 6i (National Instruments).

The prototype instrument is equipped with a designed probe to allow the measurement of the impedance at two depths inside the skin. The depth set 1 reflects mainly the properties of the outer epithelium (stratum corneum) and the depth set 2 reflects the superimposed properties of all layers down to. The depth set in the system measures the impedance down to approximately 2.0 mm that is the region of interest for studies of irritation in living skin.

Since electric parameters of impedance show a great individual variation, the measured values were converted into an index that entails normalization and enables the interpretation of the general behavior of the system. The index (IX) was defined by the higher correlation with visual score (unpublished data) and is described by equation 3:

$$IX=\frac{R_1}{R_2}\cdot\alpha, \qquad (3)$$

where *IX* is the irritation index, R_1 is the resistance of inner tissue at depth 2, R_2 is the resistance of outer tissue at depth 1 and α is the phase constant of the frequency-dependent component at depth 1 that models the dispersion phenomena of stratum corneum.

The index was development based on assumptions that at depth 1 the main contribution to the model comes from R_2 since it represents the outer resistance. At depth 2 the R_1 is the major contributor to the model and represents the inner resistance. The parameter α was included in the index equation because it can describe the dispersion characteristics of the horny layer that occur with irritation.

Subjects

The skin irritation test was performed on 15 albino rabbits with body weight between 2.0-3.0 kg without skin disease. These animals were used because the skin of such animals is considered more sensitive than the skin of human's beings, particularly to mild and moderate irritants. The study was approved by local ethics committee for animals care at Federal University of Rio de Janeiro.

Test substance

Irritant reactions were produced by using SLS (sodium lauryl sulphate) dissolved in distilled water in patch tests. The purity of SLS was 93.3 weight per cent. Fifty microlitres of dissolved SLS on a paper disc was applied in 12 mm Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) according to Pirilä [10]. The concentrations used were 0.5, 1.0, 2.0 and 5.0% in single exposure. As a reference, another chamber was filled with distilled water. Ink marks on the skin at a small distance from each site permitted correct placement of the patches and the instrument probes.

Test procedures

The back of the animals were depilated 24 h before the irritant application and the patches were applied for 24 h on the back of the animals. Readings of the test sites were taken prior to application of test chambers (day 0), 24 h after patch removal (day 2) and on the 3 subsequent days (day 3, 4 and 5). The first reading after patch removal was made on day 2 because the effect of occlusion lasts several hours after removal of the patch [11]. Immediately before impedance measurement the skin was soaked with physiological saline solution for 1 min to reduce the naturally high resistance of the stratum corneum.

Visual assessment

The magnitude of the test reaction in rabbits was scored according to erythema and edema formation as defined by Draize [12]. The sum of these scores gives the skin irritation index and based on this index, the irritant can be classified in different potential degrees of irritation as follows: 0 – 0.9, non-irritant; 1.0 – 1.9, slightly irritant; 2.0 – 4.9, moderately irritant; 5.0 – 8.0, severely irritant.

Statistical Analysis

To perform the comparison between the mean values of the impedance index during all days of measurements,

analysis of variance (ANOVA) was used. The minimum significance level was set at $p \leq 0.05$.

III. RESULTS

Results of visual score for all concentrations of SLS after patches removal (day 2 to 5) are shown in Fig. 2. An increasing number of positive responses were found with the increasing of concentration irritant and over time. Only the concentrations of 2.0 and 5.0% showed positive responses.

The mean values and the standard mean error of the index at each observation time for the reference site and each concentration of SLS are shown in Fig. 3. It can be observed that the index decreased with statistical difference (p<0.05) at all concentrations on day 2, when compared with the reference value. On the subsequent days, it was not observed statistically differences between the reference (distilled water) and all concentrations irritant.

IV. DISCUSSION

The understanding of skin irritation process is important to a better interpretation of the bioengineering results. Several reports tried to detail the mechanism of effect of SLS on the epidermis [3, 4, 13, 14]. Fartasch [13] reported that when the SLS is topically applied on skin it penetrates the living epidermis causing cell damage, although the epidermal lipids in the upper SC remain intact. The impairment of the corneocytes of the basal cell layer is connected with the disruption of barrier lipid synthesis resulting in the exsiccation of the stratum corneum that persists for 1-2 h after the effect of SLS [14]. The damage of the stratum corneum barrier may be responsible for the increased of transepidermal water loss (TEWL) and hyperhydration of SC described in literature [3, 14]. The contact of the SLS (anionic surfactant) with the living epidermis causes the denaturation of SC keratin proteins that is related to oedema [3, 4]. These signs are reported to be the skin reactions during the early acute phase of irritation. After the consecutive evaporation of the excessively bound water through the barrier, SC water content decreases [4] and it is observed the scaliness and roughness of skin.

The recovery of the skin after irritation was followed by some researches [4, 11, 14, 15] using bioengineering methods that measures different skin parameters including stratum corneum capacitance, laser Doppler, transepidermal water loss (TEWL) and skin redness (chomametry). As reported by Gloor *et al.* [14] the course over time showed that parameters returned to the original condition only after 7 days. Löffler & Happle [11] detected irritation by TEWL and laser Doppler that lasted 10 days. Wilhelm *et al.* [4] found signs of skin irritation during 17 days after SLS exposure. Although the healing phase of skin irritation by SLS has been reported [4, 11, 14, 15] little is known about the end of acute phase, that is, the decrease of oedema.

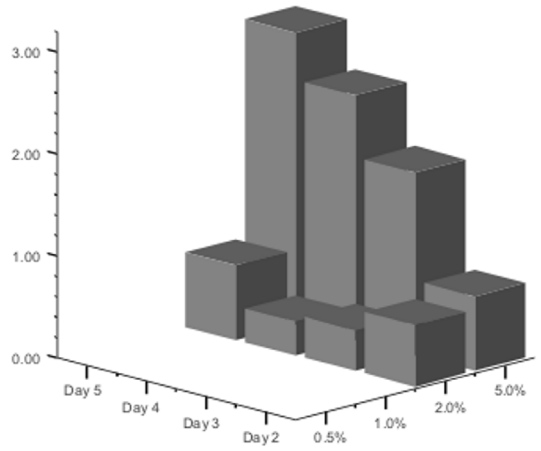

Figure 2. Visual scoring distribution for each concentration of sodium lauryl sulphate on days after patches removal.

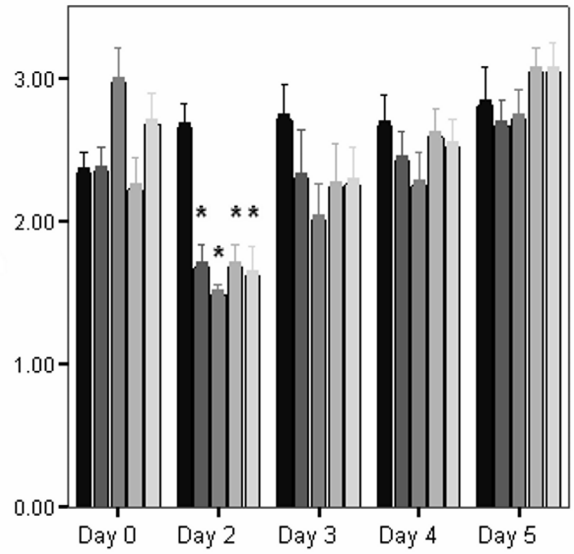

Figure 3. The mean values and standard mean error of impedance index (vertical axis) for the reference (■) and each concentration of SLS: 0.5% (■); 1.0% (■); 2.0% (□); 5.0% (□) on day 0 (before application of the patches), day 2 (24 hours after patches removal) and the subsequent days. The markers (*) show a statistically significant change between the reference and each concentration of SLS (p<0.05) using analysis of variance (ANOVA).

According to histological findings in literature [16] the most changes during acute irritation involves the basal part of the epidermis. These changes are related to oedema, lymphocytic infiltrate and degeneration of keratinocytes. Thus, it is expected a greater change at depth 2 than at depth 1 with irritation. The presence of oedema in epidermis reduces R_1 at depth 2 but the alteration of R_2 at depth 1 is small. So, an increased irritation corresponds to a decreased of the index. Results showed an index decrease at all concentrations of SLS only on day 2. On the subsequent days it was not detect any difference from the reference suggesting the presence of oedema until the second day after

SLS exposure. Similar results were reported by Ollmar *et al.* [17] after the assessment of skin irritation by electrical impedance in humans. When compared with the reference value, differences were statistically significant on day 2, except for the lowest concentration of SLS (0.5%). On day 7 the difference was significant only for the highest concentration (5.0%). Probably they found differences on day 7 due to the purity of SLS of 99.8 weight per cent.

As reported by Koopman *et al.* [15] the increase in TEWL values was observed in humans until day 2. From measurement day 2 on, it was described a decrease in TEWL. This is probably attributable to water loss of the oedema.

The positive response to irritation assessed by visual scoring until the fifth day after SLS exposure indicate the presence of hyperaemia in the sub-papillar vascular plexus as detected by colorimetric and laser Doppler measurements in literature [11, 14]. Since the scales of the method include erythema and oedema, the agreement between visual score and impedance index could be detect only during the presence of oedema. Even though, the electrical impedance method showed the ability to detect the oedema in the absence of positive visual response.

It must be emphasized that the bioelectrical impedance of the skin is a bioengineering method able to detect the oedema that is the principal sign of acute irritation. The others methods, as reported by Ollmar *et al.* [17], detect different physical parameters, which may or may not be relevant in particular test situation. When the aim is to evaluate the course of irritation the use of more than one quantitative method could provide a better interpretation of the phenomena.

V. CONCLUSION

This work suggests that the electrical impedance model should be used to investigate the early acute irritant skin reaction even in invisible signs.

ACKNOWLEDGEMENTS

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