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***In vivo* skin effects of a dimethylaminoethanol (DMAE) based formulation**

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Received May 13, 2009, accepted June 22, 2009

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Pharmazie 64: 818–822 (2009)

doi: 10.1691/ph.2009.9636

Dimethylaminoethanol (DMAE) has been used in anti-aging formulations but few scientifically based data address its efficacy. The aim of this study was to evaluate the effects of DMAE-based formulations on hairless mice and human skin. Formulations containing with or without DMAE were applied to the dorsum of hairless mice. Histopathological and histometric evaluations were carried out after seven days. Formulations were also applied to the ventral forearm and the lateral periocular area of human volunteers. Stratum corneum water content and skin mechanical properties were analyzed using Corneometer and Cutometer, before and after a single and repeated application. Histometric evaluations showed that formulations with or without DMAE increased the viable epidermis thickness, but only the DMAE-supplemented formulation led to increased dermal thickness. DMAE also induced increase in collagen fiber thickness, which was observed in the histopathological study. After the single and the 8-week period application on human skin, formulations with and without DMAE enhanced the stratum corneum water content in the forearm skin. Mechanical properties were not significantly modified. So, we can suggest that DMAE action is related to its effects on the dermis as observed in the histopathological and histometric studies and showed hydration effects on skin.

1. Introduction

Skin-care ingredients to improve skin aging are a constant search in the cosmetic field and the driving force for the discovery of topical benefits of substances that have been traditionally used as food supplements or medicines.

Dimethylaminoethanol (DMAE) was first reported as a therapeutic agent in the oral treatment of central nervous system disorders associated with the hypofunction of cholinergic neurons and it has been used for the improvement of children's learning (Lewis and Young 1974). However, some instant anti-aging lifting effects clinically observed in patients during the treatments led to a fairly large use of DMAE in cosmetic and dermatologist-prescribed anti-aging formulations (Perricone 2000). Clinical observations have reported the safety and the efficacy of DMAE in improving the overall appearance of aged skin, in reducing forehead lines, periorbital fine wrinkles and under-eye dark circles and in provoking a lifting effect (Grossman 2005). Furthermore, in a study performed in human volunteers, Uhoda et al. (2002) demonstrated a decrease in the mechanical anisotropy of the skin after a single application of a DMAE containing gel.

However, there are no histological evaluations and objective studies on the effects of this active substance after long-term application. Moreover, the results reported in the literature have poorly understood mechanisms of action suggesting that knowledge about the use of DMAE as a topical agent is just beginning and much remains to be learned.

Histopathological and histometric analysis are able to show alterations produced on the skin by a specific treatment and may play a vital role in the understanding of the effects of top-

ical substances. Since these are invasive techniques, the use of hairless mice as experimental animals could be an appropriate model for preliminary studies on anti-aging properties of topical formulations (Fodil-Bourahla et al. 2003; Fujimura et al. 2000). On the other hand, objective clinical studies are essential to evaluate the efficacy of topical formulations and to elucidate their action on human skin. For this, non-invasive skin biophysical techniques are often used since they allow evaluation of cosmetic products under actual conditions of use (Oba et al. 2002; Laugier and Maibach 1999).

The active substance should reach deeper layers of skin, i.e. the dermis, to provide an anti-aging action. Moreover, it is known that the vehicle affects the skin penetration. So, in the present study, we used a suitable vehicle for optimizing the penetration and consequently the effectiveness of the product.

Thus, the aim of the present study was to evaluate the *in vivo* effects of DMAE-based formulations on hairless mice and human skin. In addition, this study may lead to an improved understanding of the effects of DMAE-based formulations on skin by using histological and non-invasive biophysical techniques.

2. Investigations, results and discussion

The efficacy of DMAE in oral treatments of central nervous system disorders is well established (Cai 1985; George et al. 1981), but recently the compound has gained popularity as an anti-aging agent (Perricone 2000). In spite of its large use in cosmetic products and dermatologist-prescribed formulations, studies on the effects of DMAE application on skin, as well as, on

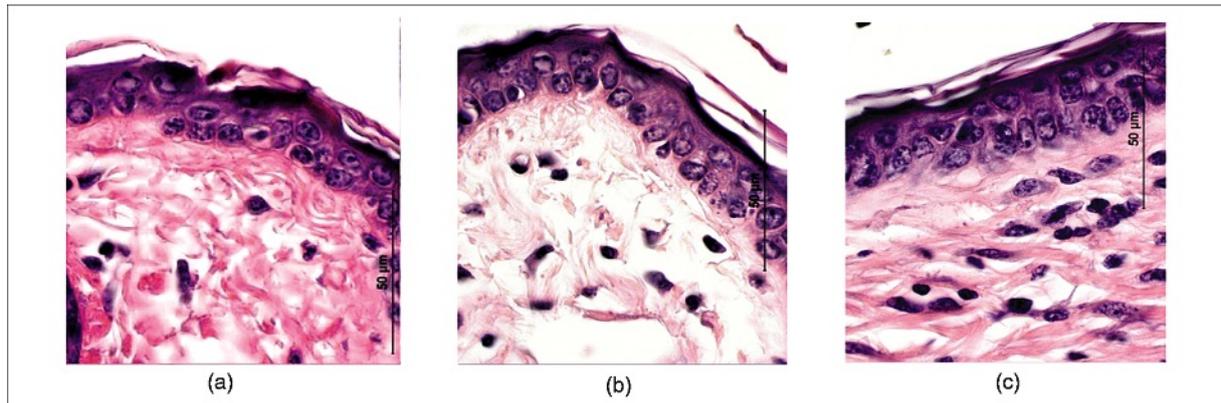


Fig. 1: Photomicrographs of hairless mice epidermis, HE. (a) Control (no treatment), (b) Vehicle (V), (c) V + DMAE

its mechanism of action are missing. In the present investigation, the efficacy of dermocosmetic DMAE-based formulations was evaluated using histopathological and histometric analysis and skin biophysical techniques.

The viable epidermal thickness of hairless mice was increased in all areas receiving both formulations studied (containing or not DMAE) when compared to the control area ($p < 0.001$). However, there were no statistically significant differences when the formulations were compared to each other (Figs. 1 and 2). It is suggested that this could be due to an improvement of skin hydration caused by the vehicle formulation since this laboratory had already observed an enhancement of epidermis thickness caused by intra and extracellular hydration (Silva GM and Maia Campos 2000). Similarly, Sator et al. (2001) observed an improvement in epidermal skin moisture accompanied by an increase in skin thickness at the end of treatment in patients having hormone replacement therapy. Thus, the maintenance of skin hydration is very important not only for aesthetics purposes, but also to keep skin normal conditions and to prevent dry skin alteration (Rawlings and Harding 2004). Moreover an increase in epidermis thickness can be considered a beneficial effect since there is evidence demonstrating that this parameter is decreased during the course of aging (Branchet et al. 1990; Beitner 2003).

When the dermis was evaluated, its thickness was only increased by formulations containing DMAE ($p < 0.001$) (Figs. 3 and 4). Considering that total dermal thickness decreases in the aging process (6% per decade) (Branchet et al. 1990), an increase in dermis thickness would be an improvement of skin conditions. Thus, DMAE seems to be an effective ingredient in anti-aging cosmetic formulations.

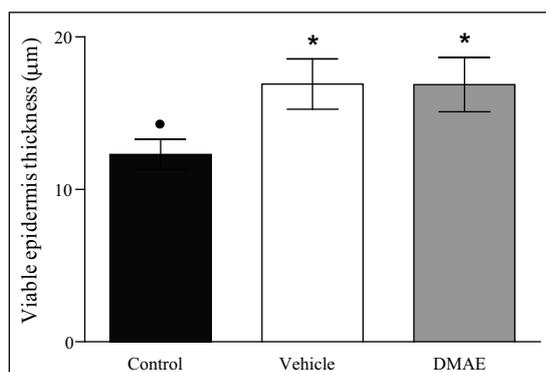


Fig. 2: Mean values for viable epidermis thickness in mice after treatment with formulations containing or not DMAE and the values obtained for the control area (Kruskal-Wallis test, $n = 8$ hairless mice, means \pm confidence interval, $p < 0.001$). Different symbols indicate statistically significant different values

Moreover, histopathological evaluation showed that collagen fiber thicknesses were improved by DMAE. This fact can explain the enhancement observed in dermis thickness, confirming reports that a decrease in collagen is usually accompanied by a decrease in skin thickness (Brinchat et al. 1987). Furthermore, enhancement of the collagen fiber thickness may be considered an improvement in aged skin since observations of age-related changes in the dermis using light and electron microscopy demonstrated a severe disorganization of the elastic fiber network (Braverman IM and Fonferko 1982) together with a decrease in the number of collagen fiber bundles (Lovell et al. 1987).

Age-related cutaneous changes, such as wrinkles and skin laxity are especially prominent on facial skin, so several studies have used this site to evaluate the efficacy of cosmetic products (Uhoda et al. 2002; Robert et al. 2005). On the other hand, the ventral forearm, which has limited exposure to sunlight and is an easier site for measurements, has also been used to evaluate age-related changes to characterize chronological aging (Sumino et al. 2004). In this study, the effects of DMAE-based formulations on human facial and ventral forearm skin were evaluated using biophysical techniques.

The statistically analyzed results showed that both formulations (with and without DMAE) enhanced stratum corneum water content after a single application only in the forearm skin when compared to baseline values ($p < 0.001$) (Fig. 5). These results are in line with the data obtained by Uhoda et al. (2002), who analysed the hydration effect of a gel containing DMAE by impedance measurements, using the Dermal Phase Meter DPM[®] 9103. After the 2-week period application, formulations with and without DMAE enhanced water content of the stratum corneum only in the forearm skin ($p < 0.01$), which remained constant until the end of the study (8 weeks) (Fig. 6). One week after the last application these values showed a decreasing tendency, not statistically significant, when compared to baseline values. The stratum corneum water content in the lateral periorcular area was not altered probably because this area had high baseline values. Skin hydration is a valuable parameter when evaluating the anti-aging efficacy of a substance, since moisturizing products can prevent cutaneous early aging and may be used as support in the treatment of several skin alterations (Rawlings and Harding 2004).

The stratum corneum water contents were similar after a single and an 8-week period application, suggesting that long-term results can be predicted by the single application data. This is in agreement with the results reported by Dal'Bel et al. (2006) who also suggested that a single-application of a moisturizing formulation could accurately predict results of long-term (2-week) studies with multiple applications.

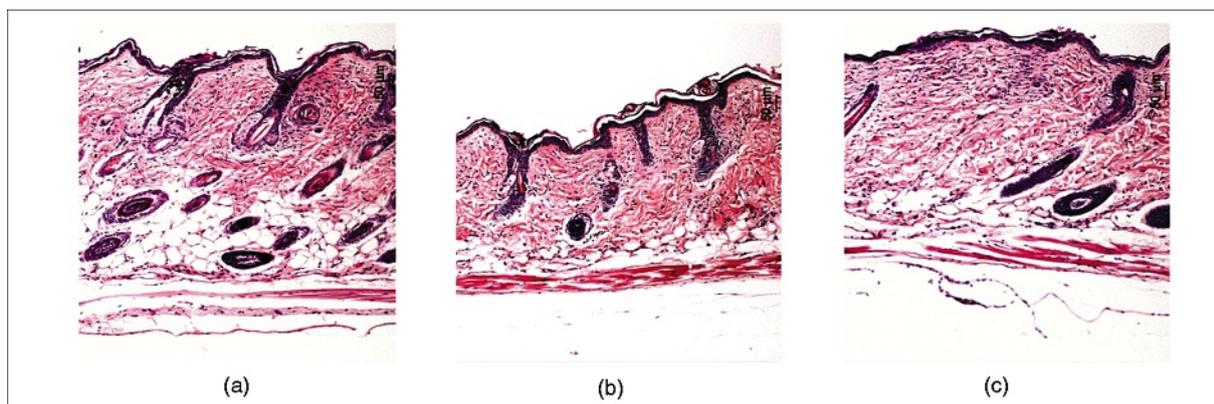


Fig. 3: Photomicrographs of hairless mice dermis, HE. (a) Control (no treatment), (b) Vehicle (V), (c) V + DMAE

In the study of mechanical properties, which have been determined using the Cutometer all the parameters studied, U_a/U_f , U_r/U_e , U_v/U_e and U_r/U_f , were not altered after a single or an 8-week period of daily application of the formulations on both skin regions (data not shown). Similarly, Uhoda et al. (2002) did not observe differences in threshold suction to perceive skin traction, provoked by the Cutometer, when comparing the DMAE-based gel treated area to the vehicle treated area.

Dobrev (2000) related that the Cutometer equipped with a 2-mm diameter suction probe is a device suitable for assessment of epidermal mechanical properties; when large diameter probes are used (8-mm diameter) the parameters analyzed reflect the dermal conditions. The histological analysis in this study evaluated on hairless mouse skin indicated that DMAE action is related to its effects on the dermis. It was not possible to observe any alteration of human dermis with this technique because we used a 2-mm diameter suction probe. Moreover, hairless mouse skin being thinner than human skin allows a larger permeation of active substances. Thus, considering the differences between hairless mouse and human skins caution is needed in interpreting the results. Nevertheless, the data obtained in this study may be an important guide in the use and understanding of the effects of topical DMAE-based formulations.

Considering the DMAE effects on the dermis, the development of formulations containing this substance must be accompanied by permeation studies, since DMAE must reach the dermis to be effective. In conclusion, it is considered that DMAE-based formulations can be used for skin hydration and anti-aging treatments since dermal conditions are altered in the aging process and compound DMAE seems to be beneficial to this layer.

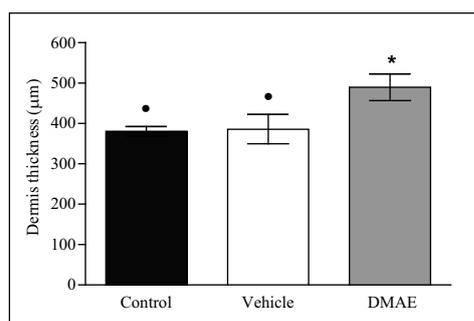


Fig. 4: Mean values for dermis thickness in mice after treatment with formulations containing or not DMAE and the values obtained for the control area (Kruskal-Wallis test, $n = 8$ hairless mice, means \pm confidence interval, $p < 0.001$). Different symbols indicate statistically significant different values

3. Experimental

3.1. Test formulations

The formulation studied contained 3.5% (w/w) self emulsifying wax (batyl alcohol, stearic acid, caprylic/capric triglyceride and lecithin), Nikkolipid®81S (Nikko Chemicals, Tokyo, Japan), 0.9% (w/w) hydroxyethylcellulose, 2.5% (w/w) coco-caprylate/caprate, 2.0% (w/w) propylene glycol, 2.0% (w/w) glycerin, 2.0% (w/w) dimethicone DC 200/50CS and 0.8% (w/w) blend of parabens and phenoxyethanol, Phenonip® (Nipa Labs, Wilmington, USA). The formulation was supplemented or not (vehicle) with 9% (w/w) DMAE acetamidobenzoate (corresponding to 3% (w/w) of DMAE). DMAE was kindly provided by Galena Química e Farmacêutica Ltda, Campinas, Brazil.

3.2. In vivo studies

3.2.1. Biological assays in experimental animals

Adult male hairless mice (HRS/J-hairless, Jackson, Bar Harbor, ME) weighing 30 g on average were kept in individual cages and received commercial ration (Nuvilab CR-1), as well as water *ad libitum*. This study was carried out in accordance with the "Principles of Laboratory Animal Care" (NIH). The protocols for all of the animal experiments were approved by the Committee of Ethics in Animal Experiments at the University of São Paulo (Campus Ribeirão Preto).

The formulations were applied (2 mg/cm^2) on the dorsum of the animals ($n = 8$) in three groups once a day for seven days, as follows: a) no treatment (control); b) application of the vehicle; c) application of the formulation containing 9% of DMAE acetamidobenzoate.

One week after starting the treatment, mice were euthanized by CO_2 inhalation and skin fragments were obtained and immediately immersed in a fixing solution consisting of 85 mL of 80% ethanol, 10 mL formaldehyde, and 5 mL acetic acid. After 24 h the fixed fragments were dehydrated, cleared, and embedded in paraffin. Semi serial $6 \mu\text{m}$ -thick sections were then obtained and each section corresponded to an interval of fifty sections, i.e., ten sections were obtained from the 2 mm biopsy. The sections were stained with haematoxylin and eosin for general histopathological and histometric analysis (Silva and Maia Campos 2000; Tadini et al. 2006).

Viable epidermis and dermis thicknesses were analysed by using a light microscope Leica DMLB, coupled with a digital camera DC 300, in 100-fold magnification and through an image analysis program (Image J). Thicknesses were measured at ten randomly selected locations per slide and averaged as described previously (Tadini et al. 2006; Lu et al. 1999), and the means \pm confidence intervals calculated. Dermal thickness was measured from the dermal-epidermal junction down to the underlying subcutaneous tissue.

3.2.2. In vivo studies on human skin

The study was approved by the Faculty of Pharmaceutical Sciences of Ribeirão Preto - USP Ethics Committee (CEP/FCFRP 30/2003).

Thirty six healthy female subjects 38–63 years old having skin Fitzpatrick types II, III and IV participated in this study after having given their written informed consent. Exclusion criteria were the presence of any dermatitis and/or other skin or allergic diseases and smoking. Volunteers were instructed not to apply any topical products to the test sites for 2 weeks before and during the study. The subjects were allowed to wash normally, but not to use other skin care products on their arms and face. They were asked to avoid getting a sun tan, although the formulations applied had sunscreens in their composition to avoid the effects of daily exposure to UV radiation.

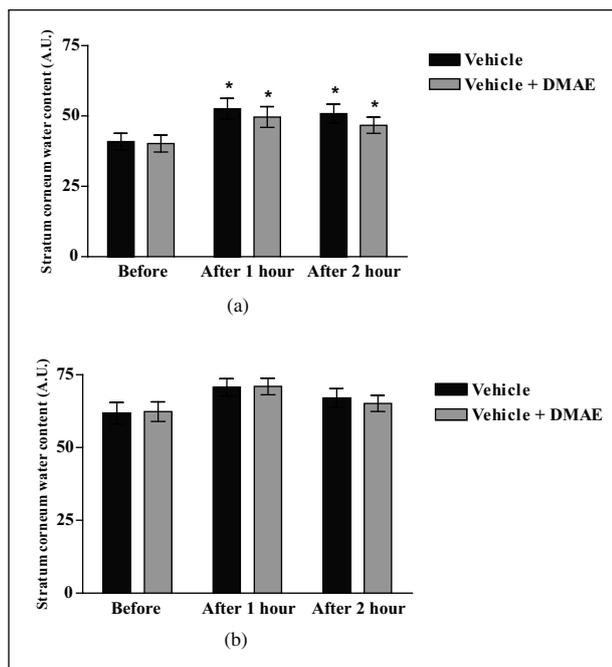


Fig. 5: Stratum corneum water content before and 1 and 2 hours after a single application of the formulations (vehicle, vehicle + DMAE) on the forearm (a) and lateral periocular area (b) of volunteers

Prior to all measurements, subjects remained in the room for at least 30 min in order to allow full skin adaptation to room temperature ($20 \pm 2^\circ\text{C}$) and humidity (45–60%) (Wu et al. 2003). The formulations studied and the measurement sites were randomized between subjects. Stratum corneum water content (capacitance method) and skin mechanical properties were examined using a standardized study protocol.

3.2.2.1. Effects after single applications. Twenty five female subjects were included in this study. After the baseline measurements, 0.2 g of the formulation containing DMAE and of the formulation vehicle were applied on each of the two halves of the face and the ventral forearm skin. One and two hours after application new measurements were carried out on the forearms and lateral periocular area.

3.2.2.2. Effects after 2, 4, 6 and 8-week period of daily applications. Thirty six female subjects participated in this study. They were instructed to apply 0.2 g of the formulations containing DMAE on the ventral region of one forearm and the same amount of vehicle on the other forearm, twice daily. Half the subjects applied the formulation containing DMAE on the full face and the other half the vehicle. Measurements on forearms and lateral periocular area were carried out 2, 4, 6 and 8 weeks after daily application, as well as, 1 week after the last application.

3.3. Instrumentation

The stratum corneum water content was determined with a non-invasive, skin capacitance meter (Corneometer[®] CM 825, Courage + Khazaka, Germany). This device determines the water content of superficial epidermal layers down to a depth of about 0.1 mm and expresses the values obtained in arbitrary units. The averaged values of twenty measurements were used for subsequent calculations (Dal' Belo et al. 2006).

Mechanical properties of the skin were determined using a non-invasive skin-elasticity meter (Cutometer[®] SEM 575, Courage + Khazaka, Germany). The instrument consists of a microprocessor-regulated pneumatic system that applies suction via a 2-mm circular opening in the handheld probe. Evaluation is based on measurements of skin deformation in response to suction. Each measurement consisted of five consecutive cycles of a 2 s suction application period followed by a 2 s relaxation period. The suction load was 450 mbar. The following mechanical parameters were analysed: U_a/U_f , the ratio of total retraction to total distension, called gross elasticity; U_r/U_e , net-elasticity of the skin without viscous deformation; U_v/U_e , the ratio of viscoelastic to elastic distension and U_r/U_f , the ratio of immediate retraction to total distension, called biological elasticity (Dobrev 2000, 2002)

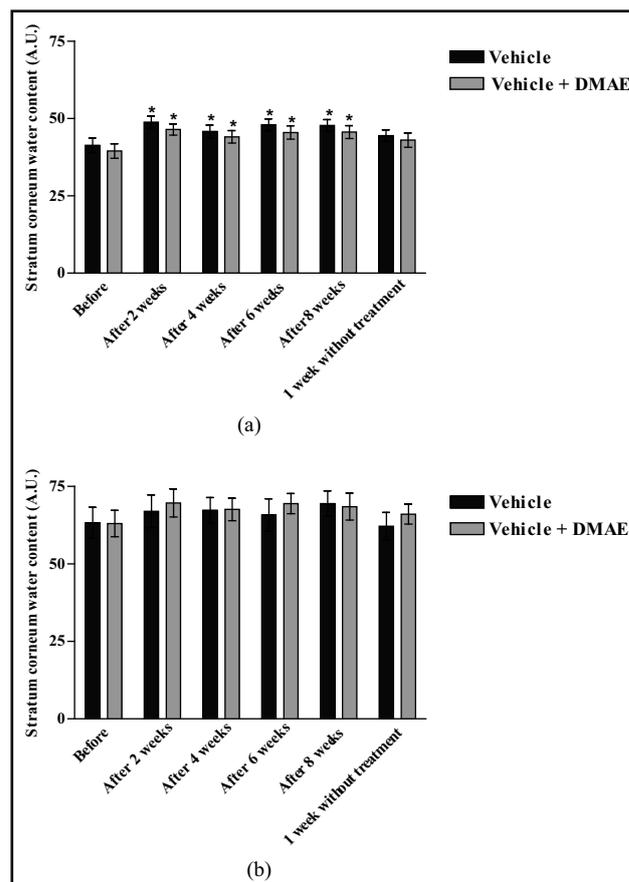


Fig. 6: Stratum corneum water content before and after a 2, 4, 6 and 8-week period of application of the formulations (vehicle, vehicle + DMAE) on the forearm (a) and lateral periocular area (b) of volunteers. Stratum corneum water content was also measured one week after the last treatment for both formulations

3.4. Statistical analysis

Results of biological assays in experimental animals were statistically analysed using the Kruskal-Wallis test. The ANOVA test evaluated the effect on human skin. Differences were accepted as statistically significant at $p < 0.05$.

Acknowledgements: The authors gratefully acknowledge the financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the helpful discussions with Geraldo Maia Campos, PhD, and Miguel Angel Sala Di Matteo, PhD.

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