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Histological evaluation of hair follicle due to papain's depilatory effect

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Abstract

Histological alterations in the skin and hair follicle of mice were evaluated as a result of the application of gel and cream formulas containing papain as a harmless treatment for hirsutism. Papain is a proteolytic enzyme and it has been used in pharmaceutical, cosmetic and nutrition areas. The purpose of this study was to evaluate the efficacy of a depilatory product, through histological analysis using light microscopy. Gel and cream formulas containing papain were developed and daily applied on the back of two groups of mice for 31 days. The depilatory effect of the gel formula applied on the first group was less evident. The second group treated with the cream formula presented an intensive depilatory effect; the morphometrical analysis showed dilation of about 55% of the hair follicle lumen and an increase of the thickness of epidermis. Papain cream had a significantly higher depilatory effect than the papain gel.

Keywords: Papain; Topical drug delivery; Hair follicle; Hirsutism; Mice

1. Introduction

Hirsutism is the presence of excess hair growth in women and affects 5–8% of the total female population of fertile age (Falsetti and Gamberra, 1999; Müderris et al., 2000). The sample probably included only the Caucasian ocidental population. Although the condition may indicate an underlying disorder of androgen production, in most cases hirsutism results from a combination of mildly increased androgen production (compared with that in nonhirsute women) and increased skin sensitivity to androgens.

In this research, the effect of the topical preparations with papain on the decrease of the hair in an animal model was studied. Papain is a purified proteolytic substance derived from *Carica papaya* Linné (Caricaceae) (US Pharmacopoeia 25, 2002). The enzyme consists of a single polypeptide chain with 212 residues with a thiol group in the active site (Zhuang and Butterfield, 1991). Papain is an amorphous, white to light tan powder, slightly soluble in water. Papain is used as a topical debriding agent in open lesions, used for removal of protein deposits from the surface of soft contact lenses (Martindale, 1999). It is widely used in the food industry as a meat tenderizer, a beer clarification agent and in many pharmaceutical products (Chambers et al., 1998). Some proteases, including papain, can hydrolyze the peptide bonds of collagen and keratin in the stratum corneum of the skin (Sim et al., 2000). Therefore, papain is frequently used in cosmetics for exfoliation.

The purpose of this study was to confirm the efficacy of the depilatory formulation, through histological analysis using light microscopy. This is a routine classical technique very suitable for the current purposes. It has been employed by other authors (Brenda et al., 1995; Sun et al., 1999; Sintov et al., 2000).

2. Materials and methods

2.1. Formulations

Gel and cream preparations containing 0.8% (w/w) of papain 450 Wallerstein Papain Activity (W.P.A., Wallerstein) were produced as follows. The drug was dissolved in distilled water. The solution were added gradually into the appropriate vehicle: gel (carbomer 940, methylparaben, disodium edetate, propylene glycol, distilled water, stabilizer) or cream

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(nonionic emulsifying wax, methylparaben, propylparaben, disodium edetate, propylene glycol, mineral oil, distilled water, stabilizer).

2.2. Animals

Twenty 60-day-old male Swiss Webster mice (*Mus domesticus domesticus*), weighing a mean of 35 g, were split into 2 groups (gel group and cream group) containing 10 mice each. The mice were grown in a pathogen-free animal facility. The experiments reported herein were conducted according to the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (COBEA) and were reviewed and approved by the Biomedical Sciences Institute/USP—Ethical Committee for Animal Research (CEEA).

2.3. Treatment

Specimens of approximately 1 g of each topical preparation were spread gently over the right side of lower dorsal region (2 cm^2). The left side was used as control. The application was performed once daily for 31 days. Depilatory effect was monitored during the treatment by macroscopic evaluation.

2.4. Histology

On day 32, the mice were sacrificed using carbon dioxide. Skin fragments were taken, fixed in formaldehyde solution and embedded in paraffin. The fragments were sectioned horizontally and vertically. The sections were stained with hematoxylin and eosin, and examined under light microscope (Bancroft and Stevens, 1996). Qualitative evaluation was performed on all sections.

2.5. Morphometrical analysis

Quantitative evaluation of the gel and cream groups was performed on the horizontal sections with a microscope (Carl Zeiss) interface via a color camera (Axio Vision) to an image analysis software (Ks 300, Kontron Electronik), by measurement of hair follicles. Three fields/section were used from all animals in each group. The three fields were chosen according to standardized conditions: they had to be clear, not coincident, at the center of the image and at level of the sebaceous glands layer. At this moment the observer did not know which sample was observed. The observer was blinded.

The number of hair follicles were determinated under ×20 magnification (area of 340 μm × 430 μm), by two observers ("A" and "B"). All the structures identified as hair follicle were counted. The data are the average of readings taken from 24 to 30 fields (gel group: 27 samples for control and 30 samples for treated; cream group: 24 samples for control and 30 samples for treated).

The diameter of the hair follicle lumen were determined under ×40 magnification (area of 171 μm × 215 μm), only for the cream group. The data are the average of readings taken from 27 (control group) and 30 fields (treated group). The numbers of hair follicles that had the lumen measured in the control group and treated group were 204 and 190, respectively.

All results are given as means ± standard deviation. For statistical analysis, Student's *t*-test was used.

3. Results

3.1. Macroscopic analysis

Only 2 among the 10 mice treated with gel formulation showed a light depilatory effect, while all 10 mice treated with cream formulation presented a heavy depilatory effect. The hair turned white in all treated areas in both groups.

3.2. Histological and morphometrical analysis

The qualitative analysis of horizontal and vertical sections of the skin and hair follicle treated with gel containing papain did not show any difference in comparison with the control. On the other hand, the group treated with cream showed an increase in

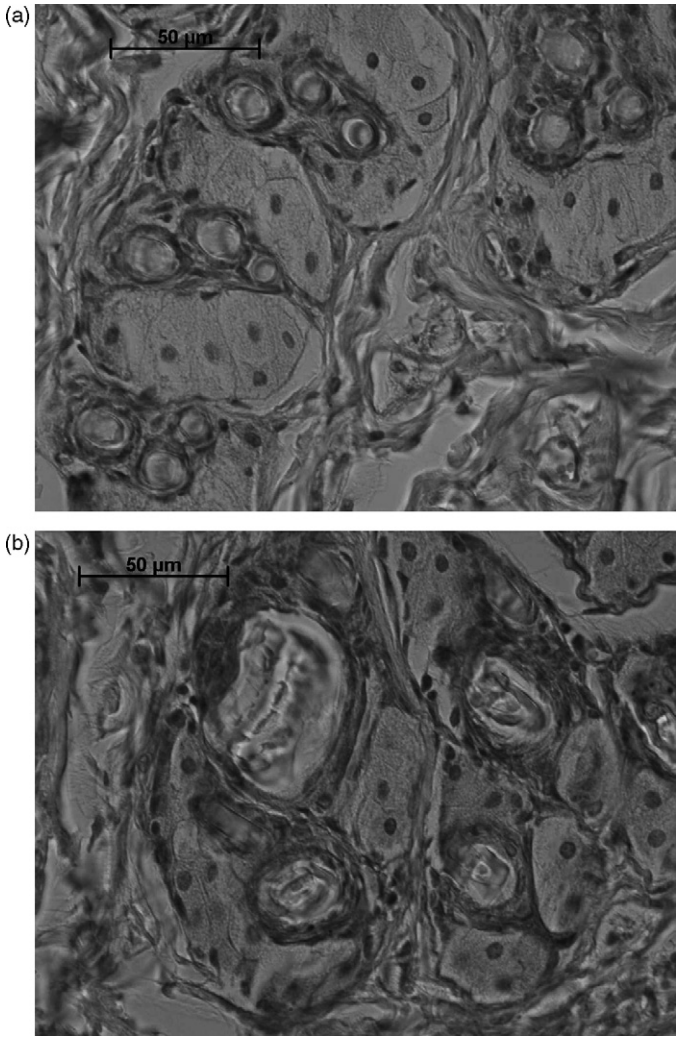

Fig. 1. Dilation of the hair follicle lumen observed in the treated group with cream formulation (b), in comparison to the control group (a).

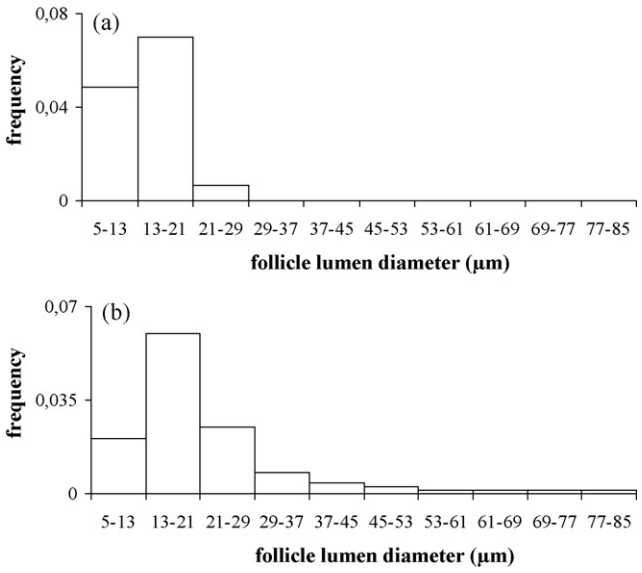

Fig. 2. Histograms illustrate the hair follicle lumen measured within the control group (a) and treated group (b). The measures of the hair follicle lumen within 190 follicles of the control group showed a media of 14.1 ± 3.7 μm, and the measures of lumen within 204 follicles of the treated group showed a media of 22 ± 13 μm (into an area of 171 μm × 215 μm).

the thickness of the epidermis, a reduction of hair follicles and a dilation of the hair follicle lumen (Fig. 1).

The numbers of hair follicles of the gel group were 24 ± 9 (control) and 25 ± 7 (treated) into an area of 340 μm × 430 μm, counted by the two observers ("A" and "B"). There was no statistically significant difference between control and treated within the gel group ($P < 0.001$). The numbers of hair follicles of the cream group were 24 ± 8 (control) and 20 ± 5 (treated) into an area of 340 μm × 430 μm (counted by "A" and "B"). The difference of numbers of hair follicles between control and treated within the cream group was statistically significant ($P < 0.05$).

Morphometrical analysis of the cream group showed a statistically significant dilation of the hair follicle. The measures of the hair follicle lumen within 190 follicles of the control group showed a media of 14.1 ± 3.7 μm, and the measures of lumen within 204 follicles of the treated group showed a media of 22 ± 13 μm (into an area of 171 μm × 215 μm). The difference was statistically significant ($P < 0.001$). There was an increase of about 55% in the hair follicle lumen. Histograms were used to illustrate the distribution of the measurements of the hair follicle lumen (Fig. 2).

4. Discussion

Macroscopic and histological alterations in the skin and hair of mice were evaluated as a result of the application of gel and cream formulas containing papain 0.8% (w/w) as a treatment for hirsutism. Papain was chosen as the drug to compose the formulations for being a natural substance, easily available, inexpensive and it can be well accepted as harmless treatment for hirsutism.

Both the formulas gel and cream containing papain led to a visible depilatory effect (more intensive in the cream group) and, moreover, whitened the hair. The changing of the color and the depilatory effect may be explained by the action of the papain. As papain is a proteolytic substance which hydrolyses polypeptides, keratin may have been eliminated by the enzyme. A cosmetic lotion containing papain 1% conjugated to a biopolymer produced by *Schizophyllum commune* was more effective in exfoliating stratum corneum than a lotion containing lactic acid 5%, one of the popular exfoliating agents (Sim et al., 2000).

After the macroscopic evaluation, the mice were sacrificed and skin fragments were analysed through histological techniques. While there were no histological alterations in the group treated with gel, the group treated with cream showed an increase in the thickness of the epidermis, a reduction of hair follicles, and furthermore, a dilation of the hair follicle lumen. Measurements of hair follicle lumen diameter was performed and histograms (Fig. 2) showed that in the control group 38.7% of all measurements were from 5 to 13 μm and 55.9% from 13 to 21 μm, and in the treated group 47.9% of all measurements were from 13 to 21 μm followed by 20.0% from 21 to 29 μm. The intensive depilatory effect observed in the cream group was accompanied by histological alterations too.

Although gel is a vehicle used frequently in pharmaceuticals and cosmetics due to ease of use and removal, cream should be selected for a depilatory formulation. The higher effectiveness of the cream formulation in comparison to the gel formulation could be owing to various factors. Firstly, the cream is constituted by surfactants, aqueous and lipophilic material. The surfactants (present in the emulsifying wax) may help as a penetration enhancer (Barry, 2001). As the surfactants have potential to solubilise lipids (Williams and Barry, 2004), they may be removing the sebum or the proteolipid membrane that cover the surface of hair, and enhancing the acting of papain. Secondly, the lipophilic material (mineral oil) prevents water loss. Promoting full hydration may enhance drug bioavailability into skin and hair. Thirdly, the cream may help in softening and swelling the hair (Ramos-e-Silva et al., 2001). Lastly, on the outermost or exposed surface of hair cuticle, a thin proteolipidic membrane-like structure forms a hydrophobic barrier (Jones, 2001). Only the cream could enhance papain bioavailability into hair. The gel does not contain an oil phase and it does not have similarity with the material that covers the surface of the skin and hair.

The increase in the thickness of the epidermis due to papain was previously observed (Traversa et al., 2002). In this study, it was verified that the thickness of the epidermis was enhanced by the application of papain solution 2% (w/v). The epidermal thickening reflects an increase in the number of epidermal cell layers.

Proteolytic enzymes applied by iontophoresis to skin of experimental animals have shown depilatory effects (Protopapa et al., 1999). As the depilatory action is long-lasting, the hypothesis had been put forward that hair follicle stem cells could be among the cells affected by the proteolytic enzyme. The increase of the thickness of the epidermis as a result of the papain cream formulation application confirms this hypothesis. We suggest that papain interferes with the stem cells of the follicle, which are required for hair follicle cycling and for epidermal keratinocytes (Taylor et al., 2000; Cotsarelis, 2002). Proliferation versus ter-

minal differentiation of keratinocytes and hair follicle epithelial cells in the skin and its appendages can be viewed as competitive. A precise balance between proliferation and differentiation is necessary to maintain sensitivity to environmental changes. A number of key players in these processes have been identified including growth factors, their receptors, and extracellular matrix or cell adhesion molecules.

The purpose of this study was to confirm the efficacy of the depilatory formulation, through histological analysis using light microscopy. This is a routine classical technique which is very suitable for the current purposes. Quantitative evaluation was performed by measurements of density and diameter of hair follicles. Other authors also determined the density and diameter of hair follicle from horizontal sections (Bronaugh et al., 1982; Sintov et al., 2000). It is interesting to note that the numbers of the hair follicles in the gel group and cream group have the same measurement for the control (24 ± 9 and 24 ± 8), so this helps to indicate that the method of counting used is suitable and precise.

Whole animal systems are the most relevant but also the most difficult to control, quantify, and analyse hair growth. Animals commonly include mice, rats, sheep, monkeys, but studies have been conducted on other mammals including the cat, horse, rabbit, opossum, guinea pig and hamster. The laboratory mouse has been a favorite subject for hair studies (Chase, 1954; Stenn and Paus, 2001). Of course, human studies should be conducted to provide evidence of the clinical efficacy of this product. The adverse effect of thickening of epidermis observed in mice may not appear in humans as human skin is thicker than mouse skin (46.9 ± 2.3 μm versus 12.6 ± 0.8 μm) (Bronaugh et al., 1982). Furthermore, another difference is that the number of terminal hair in mice is higher than in human. According to Bronaugh et al. (1982), $658 \pm 38\ cm^{-2}$ versus $11 \pm 1\ cm^{-2}$ (values are the average of readings taken from three to six sections). So, the skin of the mouse is more permeable than that of the human specie. During human use it is possible that less papain reaches the hair follicle stem cells, that may mean less thickening even less depilation too.

On the basis of our results, the cream formula must be given continuously and for a long time. The purpose of this study was to confirm the efficacy of the depilatory formulation and it is not possible to know if such effect is irreversible. Perhaps with continuous use the weakening of the hair follicle may inhibit hair growth permanently.

In conclusion, it was shown that papain cream had a significantly higher depilatory effect than the papain gel. Further work should be done to elucidate the thickening and penetration mechanism of papain.

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