

Penetration and Enrichment of Flufenamic Acid in Calf Skin from Patients with Stasis Dermatitis

FRIEDRICH A. BAHMER,¹ HELMUT LOTH² and THOMAS WILD²

The ¹Department of Dermatology and the ²Institute for Pharmaceutical Technology, University of the Saarland, Saarbrücken, FRG

To investigate the hypothesis that the application of medicaments to skin from the lower leg of patients with stasis dermatitis might lead to their enhanced enrichment, compared with uninvolved skin from the same region, a penetration study was performed with flufenamic acid. In 5 patients with pronounced changes of chronic venous insufficiency and in 5 control patients without chronic venous insufficiency the flufenamic acid content in skin sections parallel to the surface was determined by HPLC. In chronic venous insufficiency-skin, the flufenamic acid concentration was higher in all skin levels compared to control skin. This enrichment of the substance could lead to a prolonged and more intense contact with antigen-presenting cells in this region, thus promoting the development of contact allergies observed so frequently in this pathologic condition. *Key words: Skin penetration; Chronic venous insufficiency; Drug enrichment; HPLC*

(Accepted February 19, 1990.)

Acta Derm Venereol (Stockh) 1990; 70: 445-448.

* Dedicated to Prof. Dr. P. H. List (Marburg) on the occasion of his 65th birthday.

F. Bahmer, Universitäts-Hautklinik, D-6650 Homburg/Saar, FRG.

Stasis dermatitis (SD) of the lower extremities, with or without leg ulcers, is a fairly common condition and frequently the sequel of deep vein thrombosis. The extent of SD can be assessed based on a clinical scale or by technical methods such as the ultrasound Doppler (1).

The prevalence rate of SD is estimated to about 15% (2). Interestingly, the majority of this large patient group suffer from multiple contact allergies to topically applied medicaments and their vehicles (3).

Previous studies have, indirectly, provided evidence that topically applied substances might enrich in the epidermis of SD-skin (4). To investigate this hypothesis, penetration studies were performed with flufenamic acid in normal and diseased skin from the lower leg. This substance was chosen because of good local tolerance and excellent analytical properties. As vehicle, a mixture of triglycerides was used, because this was known from previous studies to be suitable (5).

MATERIALS AND METHODS

All our patients with SD were suffering from stage 3 disease (1) with extensive areas of discoloration (sideroderma) and induration, but without eczematous changes such as oozing, vesicle formation, crusting or scaling. All had leg ulcers on distal parts of the lower leg, confined to the perimalleolar region. This patient group consisted of 4 men and 1 woman between 43 and 74 years of age (Table I).

As a control group, 4 male and 1 female patient, with no sign of CVI, were chosen. They were between 40 and 77 years old (Table I) and hospitalized for other localized skin diseases not affecting the extremities.

After informed consent had been obtained from both groups, flufenamic acid 6% in Softisan 378 (Dynamit Nobel, Witten, FRG), a mixture of various triglycerides, was placed in a Teflon well with an inner diameter of 15 mm and a capacity of 2.5 ml, thus containing 142 mg of drug substance (5). The well was applied to the medial aspect of the calf, in group 1 always within areas of induration and hyperpigmentation. In all patients, the same batch of the preparation was used. The wells were fixed with Mellolintape (Mölnlycke, Sweden) to the skin for 16 h. After removing the well, the remaining ointment was removed with swabs in an identical manner in all cases. From the centre of the application area, clearly marked up the rim of the well, an 8-mm punch biopsy was taken under local anaesthesia.

The punch biopsies were frozen with solid carbon

dioxide to a cryostat tissue holder (HR Slee, Heidelberg, FRG). After alignment of the knife to the specimen surface, 25- μ m thick sections were cut parallel to the epidermal surface. Since the first cuts are often incomplete, they were weighted and their mean thickness was calculated based on the weight of subsequent, complete cuts. Flufenamic acid was analysed by a method described elsewhere (5). Briefly, the individual cuts were extracted with 0.5 ml 0.05 N sodium hydroxide at room temperature and continuous shaking for 2 h. After centrifugation at 6500 rpm, 100 μ l of the supernatant was analysed by HPLC with photometric detection.

The content of the individual cuts was determined from the heights of the symmetric peaks. Chromatography conditions: stationary phase RP18, 10 μ m, 25 cm. Eluent: buffer pH 2.2 (according to McIlvaine)/methanol 15+85 (v/v); flow 2.0 ml/min (Pump: Waters 6000A, Waters Associates, Milford, Mass., USA). Retention time for flufenamic acid 4.4 min, retention time of the solvent 1.2 min. Photometric detection at 283 nm, medium detection range (Perkin-Elmer photometer LC 85, Perkin-Elmer, Überlingen, FRG).

Previous (6) and other own investigations (unpublished) have shown that there is no significant difference between normal and SD-skin from the lower leg concerning the thickness of the epidermis. In cryostat sections, the epidermis is between 50 and 150 μ m thick. Since in horizontal sections epidermis and dermis blend in the zone where dermal papillae and rete ridges are present, the mean epidermal thickness was considered to be about 100 μ m in the present study. The specimens were cut to a total depth of 500 μ m, thus yielding an average of 400 μ m for the dermal part.

Statistical analysis of the data was performed using the Mann-Whitney U-test because of the small number of cases and their unknown distribution (7).

Table I. Concentration of flufenamic acid in the skin of the lower leg from patients with stasis dermatitis and in control skin

| Case/sex/age | Mean concentration (μ mol/ml) | |
|----------------------|------------------------------------|------------------------------|
| | Epidermis ($<100 \mu$ m) | Dermis ($100-500 \mu$ m) |
| <i>CVI skin</i> | | |
| A/F/68 | 4.08 | 0.28 |
| B/M/70 | 2.42 | 0.26 |
| C/M/43 | 2.18 | 0.22 |
| D/M/74 | 2.07 | 0.40 |
| E/M/57 | 5.33 | 0.28 |
| Mean | 3.22 (cv 45%) | 0.29 (cv 23%) |
| <i>Control skin</i> | | |
| F/F/60 | 1.23 | 0.18 |
| G/M/40 | 1.78 | 0.11 |
| H/M/45 | 1.43 | 0.23 |
| I ^a /M/63 | 1.62 | 0.25 |
| K ^b /M/77 | 2.27 | 0.23 |
| Mean | 1.67 (cv 24%) | 0.20 (cv 28%) |

^aSlight leg edema due to cardiac insufficiency. ^bTest induced slight dermatitis.

RESULTS

The concentration profiles of flufenamic acid in two individuals with and without SD are shown in Fig. 1. In both cases, the concentration profile displays the typical curve (8), with a steep concentration gradient in the upper epidermal levels and with a gradual flattening of the profile in the lower parts of the skin. These profiles are principally identical in all probands, but differ with respect to the individual concentration levels.

The individual values of the measurements are shown in Table I. They clearly demonstrate that the concentration of the flufenamic acid in SD-skin was substantially higher (mean plus 93%) than in the uninvolved skin specimens from controls. Thus, the concentrations within the epidermis of SD-skin were between 2.07 and 5.33 μ mol/ml (mean 3.22), in the unaltered skin from the controls between 1.23 and 2.27 μ mol/ml (mean 1.67), however. In the lower

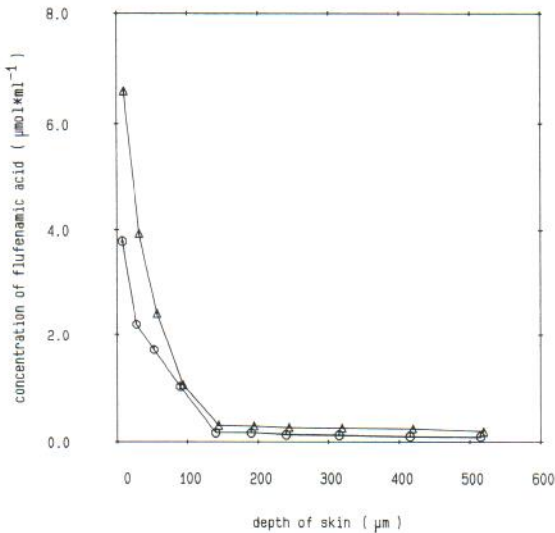


Fig. 1. Typical concentration profiles of flufenamic acid in unchanged skin (O) and in skin of lower leg altered by stasis dermatitis (□) (1 subject each; for experimental conditions, see text).

skin levels the difference is similar, although not as impressive. In the dermis of involved skin the concentrations of the substance were (proband A to E) between 0.22 and 0.40 $\mu\text{mol/ml}$ (mean 0.29), which is 45% higher than in the uninvolved skin (proband F to K). In the controls, the concentrations were between 0.11 and 0.25 $\mu\text{mol/ml}$ (mean 0.20). Additionally, the values of flufenamic acid in the epidermis of SD-skin also showed a considerably higher coefficient of variation than those in uninvolved skin (Table I).

Interestingly, in one control case (K) slightly eczematous changes were seen after removal of the well, confined to the application area.

The difference between the content of flufenamic acid in SD-skin and in control skin was statistically significant, with a p -value of 0.028 for the epidermal concentrations, and a p -value of 0.047 for the dermal concentrations.

DISCUSSION

The investigation of stratum corneum permeability with flufenamic acid in an ointment with good spreading properties was carried out under conditions where diffusion of the substance through the stratum corneum determines its velocity. In SD-skin, a significantly higher mean concentration of flufenamic acid, within both epidermis and dermis,

was found, compared with control specimens. As possible explanations for this phenomenon, a higher permeability combined with a higher capacity of the diseased epidermis for this particular substance, together with a reduced removal of flufenamic acid by the superficial blood vessels, may be discussed.

In other skin diseases, an enhanced permeability of the stratum corneum has been demonstrated (9). This is mainly due to an incomplete formation of stratum corneum during the disease. The higher variability of the substance quantities found in our cases with diseased skin could be explained by this fact. The higher values in case K from the control group, where slightly eczematous skin changes were observed by the time the substance-containing cup was removed might also be explained hereby.

The permeability of the stratum corneum can be enhanced by occlusion of the application area. Since the method of ointment application was identical in both groups, the differences observed could not be due to the application procedure. Thus, the lowered diffusion resistance of the stratum corneum, i.e. the decreased tightness of the stratum corneum barrier as a consequence of an altered differentiation of the corneocytes, can be assumed to be the main factor for the increased penetration of flufenamic acid into the epidermis and dermis. Another important factor for the increased substance concentrations in the diseased skin might be the diminished removal of the substance by the impaired peripheral circulation.

Of considerable interest is the enrichment of flufenamic acid in the stratum corneum in the diseased skin, compared with the uninvolved skin from controls. This could be explained by an increased penetration of the liquid phases of the vehicle together with the substance itself into the intercellular spaces of the stratum corneum (5, 8). This, in turn, might explain the phenomenon of sensitization to substances such as petrolatum (10) which do not penetrate readily into the normal stratum corneum. This points to an enhanced permeability of vehicle components in the stratum corneum in SD-skin.

Our investigations demonstrate that topically applied substances might be found in higher concentrations in the leg skin of patients suffering from CVI. The reduced removal of the substance by the peripheral blood flow could thus prolong the time the substance remains in the tissue.

It is well known that patients with SD very often develop contact allergies even to substances with a low sensitizing potential (3, 11). Besides the sub-

stance enrichment due to an increased horny layer permeability and to a diminished removal by blood flow, other factors such as the frequent application of ointments over long periods of time to the diseased skin, which probably contains considerable numbers of antigen presenting cells (4, 6, 12), might also contribute to these sensitizations.

REFERENCES

1. Braun-Falco O, Plewig G, Wolff HH. *Dermatologie und Venerologie*. Berlin: Springer-Verlag, 1984: 576-578.
2. Fischer H. *Venenleiden. Eine repräsentative Untersuchung in der Bevölkerung der Bundesrepublik Deutschland (Tübinger Studie)*. München: Urban & Schwarzenberg, 1981.
3. Dooms-Goossens A, Degreef H, Parijs M, Kerkhofs L. A retrospective study of patch test results from 163 patients with stasis dermatitis or leg ulcers. I. Discussion of the patch test results and the sensitization indices and determination of the relevancy of positive reactions. *Dermatologica* 1979; 159: 93-100.
4. Bahmer FA, Lesch H. Density of Langerhans' cells in ATPase stained epidermal sheet preparations from stasis dermatitis skin of the lower leg. *Acta Derm Venereol (Stockh)* 1987; 67: 301-304.
5. Wild T. Einfluß der physikochemischen Eigenschaften von Arzneistoffen und Vehikeln auf die Permeabilität der menschlichen Hornschicht. Thesis, University of the Saarland, Saarbrücken, 1988.
6. Bahmer FA. Immunhistologische Charakterisierung stauungsdermatotisch veränderter Unterschenkelhaut. *Z Hautkr* 1986; 62: 1056-1063.
7. Armitage P. *Statistical Methods in Medical Research*. Oxford: Blackwell Scientific Publications, 1971; 398-403.
8. Loth H. Skin permeability. *Meth and Find Exptl Clin Pharmacol* 1989; 11: 155-164.
9. Grice KA. Transepidermal water loss. In: Jarret A, ed. *The Physiology and Pathophysiology of the Skin*, vol. 6. London: Academic Press, 1987: 2147-2155.
10. Hannuksela M, Kousa M, Pirilä V. Allergy to ingredients of vehicles. *Contact Dermatitis* 1976; 2: 105-110.
11. Dooms-Goossens A, Degreef H, Parijs M, Kerkhofs L. A retrospective study of patch test results from 163 patients with stasis dermatitis or leg ulcers. II. Retesting of 50 patients. *Dermatologica* 1979; 159: 231-238.
12. Malten KE. Contact allergic investigation in 100 patients with ulcer cruris. *Dermatologica* 1973; 147: 241-254.