Percutaneous Penetration of Hydroxyethylrutosides

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Summary. Hydroxyethylrutosides (HR) were applied topically as a 2% gel to the skin of hairless albino mice and human volunteers. After various penetration times methanol-extractable constituents of HR were estimated by TL chromatography in separated samples of epidermis, connective tissue and subcutaneous fat. Tetrahydroxyethylrutoside accumulated in the epidermis. Trihydroxyethylrutoside, the major constituent of HR, passed quickly through the epidermal layers and appeared early and in relatively large quantities in dermal and subcutaneous tissues. Identical results were obtained in mouse and human skin.

Key words: Hydroxyethylrutosides, percutaneous penetration, thin layer-chromatography, fluorescence.

The semi-synthetic bioflavonoids hydroxyethylrutosides (HR) are considered to have an effect on varicose disorders. They protect against oedema formation [4, 8], increase oxygenation of the blood of varicose veins [10], improve capillary and venule function [1], enhance capillary resistance and protect against X-ray-induced depolymerization of mucopolysaccharides in the connective tissue [2, 7]. The precise mode of action of HR is still uncertain.

Several authors [1, 3, 4, 10] have concluded that the experimentally demonstrated effects of HR after oral or parenteral administration can be correlated with diminished venous insufficiency. Wasilewski [11] and MacTaggert-Kopecka [9] demonstrated in human volunteers, that topically applied HR also affected the pathological reactions of cutaneous blood vessels. This implies penetration of the substance or an active fraction of it through the skin and this has been investigated in hairless albino mice and in 5 human volunteers.

Material and Methods

0-β-hydroxyethylrutosides (HR) were used as a 2% preparation in a hydrophilic gel¹ for topical application. Hairless albino mice (Chester Beatty hairless and Rentschler) 12—14 weeks of age, were kept at room temperature and were given water and dry food (Altromin) ad libitum.

A thin layer of HR gel (0.5 g) was applied to the backs of mice after cleaning the skin area with 70% ethanol. After various times (1 min to 8 h) the remaining gel was removed by repeated cleaning with 70% ethanol. After this procedure the mice were killed and a 4 cm² area of the treated skin of the back was excised.

The epidermis was removed from this specimen by the stretch method and was collected with the aid of a scalpel. The subcutaneous fatty tissue was separated from the remaining material, so giving 3 samples in all:

a) epidermis
b) connective tissue (dermis)
c) subcutaneous fat

Analytical procedure

Each specimen was methanol-extracted after mechanical stirring for 12 h at room temperature. The supernatant was used for the identification of HR and its components by thin layer chromatography (Silicagel F₅₄, Merck AG, Darmstadt, Germany). Solvent: Butanol: glacial acetic acid: water as 4 : 1 : 5 upper phase. HR and its components were identified from their fluorescence colours (Universal UV lamp Camag/Berlin, 350 nm) and Rf values.

In 5 human volunteers skin specimens from non-pigmented areas on the thigh were treated as described for the mice experiments. HR gel was applied on a test tape for 1 and 4 h. After cleaning the epidermis with 70% ethanol, an area of 3 cm² was scraped with a scalpel and the epidermal material collected.

Connective and fatty tissue was obtained together by punch biopsy (cylinder 3 mm ø, 7 mm of length, weight 0.8 g). It was then processed in the same way as described for the mice experiments.

Results

HR contains several constituents which can be differentiated by their Rf values and their fluorescence colours. The main components are:

<table>
<thead>
<tr>
<th>No.</th>
<th>Component</th>
<th>Fluorescence</th>
<th>Rf. (350 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5,7,3',4' tetrahydroxyethyl-rutoside blue</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>7,3',4' trihydroxyethyl-rutoside yellow</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>7,4' dihydroxyethyl-rutoside yellow</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

¹ Venoruton Gel®, Zyma SA Nyon, Switzerland.

a After treatment with 0.1 m AlCl₃.
With the same Rf of 0.32 as component 2, there is a small amount of 5,7,3' trihydroxyethylrutoside which has a blue fluorescence.

These components were taken as qualitative evidence of the passage of HR through the skin of mouse and man, and were also used for the semiquantitative evaluation of penetration. Other substances, which only appeared in trace amounts, were not analysed because they could not be demonstrated reliably. Tetrahydroxyethylrutoside (1) is a good tracer for investigation of the epidermis. It appeared to be stored in this layer and penetrated only in small amounts into the subjacent dermis. Trihydroxyethylrutoside (2) quickly passed through the epidermal layers and appeared early and in large amounts in the dermis and even in the subcutaneous fatty tissue. Comparative application of purified tetrahydroxyethylrutoside alone and HR to mouse skin showed that in the gel experiments there was true selective penetration and not degradation of tetrahydroxyethylrutoside to trihydroxyethylrutoside by epidermal or dermal enzymes. After percutaneous penetration, therefore, the major constituents of HR gel were unchanged and could be demonstrated in large amounts in dermal and subcutaneous tissues where they should be able to act on blood vessels.

There is evidence of a definite metabolic effect on HR of passage through the skin as two new substances were found, one with an Rf of 0.48 and blue fluorescence, and the other with an Rf of 0.55 and yellow fluorescence. Both were found after penetration of HR gel through mouse skin and also as traces after passage through human skin. They were never observed in chromatograms of HR. HR gel or gel excipients alone without passage through living skin. They are probably metabolites of constituents of HR formed during passage through the skin. They were not characterised further.

In order to evaluate the speed of penetration and the duration of availability of HR in the various layers of mouse skin the experiments were repeated whilst varying the application time of HR gel to the skin surface from 1 min to 8 h. It was also possible then to estimate tetrahydroxyethylrutoside and trihydroxyethylrutoside semiquantitatively from the area of the spot and the intensity of its fluorescence in relation to test spots of pure substances. After different periods of application to mouse skin tetrahydroxyethylrutoside was found mainly in the epidermal layers and trihydroxyethylrutoside in connective and fatty tissues (Fig. 1). Tetrahydroxyethylrutoside appears to become rapidly and strongly bound to the epidermis and only small amounts of it could penetrate into deeper tissues, where it could exert pharmacological effects on dermal blood vessels. As experiments using pure tetrahydroxyethylrutoside in the gel showed no evidence of any appreciable metabolic degradation, it was concluded that this substance accumulated in the epidermis forming a deposit there.

Trihydroxyethylrutoside, the principal constituent of HR, gave very different results as it passed quickly through the epidermis without forming a deposit there. After exposing mouse skin to HR gel for 30 to 70 min the largest amount of trihydroxyethylrutoside was always found in the dermal connective tissue; whilst in subcutaneous fat the peak concentration appeared after 2 to 5 h.

![Fig. 1. Penetration of hydroxyethylrutosides (HR) in mouse skin.](image)

**Fig. 1.** Penetration of hydroxyethylrutosides (HR) in mouse skin. ——— Tetrahydroxyethylrutoside (epidermal deposit). ——— Trihydroxyethylrutoside (dermal connective tissue). ——— Dihydroxyethylrutoside (subcutaneous fatty tissue).

Ordinate: Fluorescence intensity in arbitrary units. Abscissa: Duration of gel contact with skin surface in hours.

This is the time interval during which trihydroxyethylrutoside can be expected to act on dermal and subcutaneous blood vessels. There is also evidence from the mouse experiments that the accumulated trihydroxyethylrutoside in dermal and subcutaneous tissues has disappeared 3 to 5 h after a single application of HR gel (Fig. 1).

In 5 human volunteers the assays were repeated to find out whether there was a difference in the penetration of HR in mouse and human skin. 1 and 4 h after a single application of HR gel with the aid of a test tape an accumulation of tetrahydroxyethylrutoside could be demonstrated in human epidermis as in mouse skin. Trihydroxyethylrutoside also showed similar penetration of mouse and human skin: The substance was identified and found to accumulate in dermal connective and subcutaneous fatty tissues of both species.

**Discussion**

These investigations show that a single topical application of HR gel results in an extensive penetration of active constituents through unaltered skin. The use of different application times in hairless albino mice showed that tetrahydroxyethylrutoside accumulated in the epidermis and only smaller amounts reached the dermal and subcutaneous tissues, whereas