Evaluation of the Effect of Heparin and Tetracycline on the Cohesion of the Dermal–Epidermal Junction

PHILIPPE HUMBERT,[1] ALAIN RENAUD,[2] JOËLLE MILLET,[2] CHRISTIAN TAILLARD,[1] FRANÇOIS MARCHAL,[1] RENÉ LAURENT[1] and PIERRE AGACHE[1]

[1]*Department of Dermatology, Centre Hospitalier Universitaire Saint-Jacques and* [2]*Department of Pharmacology, Faculté de Médecine, Besançon, France*

In an attempt to demonstrate the effect of tetracycline or heparin therapy on the dermo-epidermal junction, the suction blister time was measured at the beginning and at the end of the study in 18 female Sprague-Dawley hairless rats, using the suction blister method. Suction blister time was defined as the time taken for the first sign of a vesicle to appear in the hole in the diaphragm of a suction chamber. 200 IU heparinate calcium was administered daily for 6 days in 6 rats. Six other rats received 27.5 mg tetracycline chlorhydrate daily for 6 days. The others did not receive any drug. Significant statistical differences in suction blister time before and after administration of the drugs were found in the heparin group ($p<0.05$), and in the tetracycline group ($p<0.05$), compared with the control group. Heparin and tetracycline were found to increase suction blister time significantly. These results suggest an increased cohesion in the dermo-epidermal junction due to heparin and tetracycline. ***Key words: Skin pharmacology; Hairless rats.***

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Ph. Humbert, Service de Dermatologie, Hôpital St Jacques, 25030 Besançon cedex, France.

Collagen and glycosaminoglycans are essential components of connective tissue. They are also of major interest in basement membrane changes. Heparin has been shown to interact with collagen (1) and may enhance binding of collagen to fibronectin (2, 3). Moreover, laminin which may be involved in attachment of cells to basement membranes interacts with heparan sulfate (4). Tetracyclines have been shown to inhibit excessive collagenase activity in skin from conventional and germ-free rats (5). As a step towards demonstrating the activity of heparin and tetracycline on the dermal–epidermal junction (DEJ), we studied the effects of these drugs on DEJ using the suction blister method.

MATERIALS AND METHODS

Eighteen female Sprague-Dawley hairless rats weighing 255 ± 15 g were studied. Each animal served as subject in two experiments, the first performed before any drug was administered, the second after drug administration. Suction blisters were raised on the rats' dorsal skin, using a standard suction cup (6) containing a plastic diaphragm perforated by one hole, 4 mm in diameter. The cup was connected to a vacuum pump set to deliver a suction pressure of 140 mBar below atmospheric. Exactly the same previously blistered site was tested again 24 h after the last drug administration. The suction blister time was defined as the time taken for the first sign of a vesicle to appear in the hole in the diaphragm. On completion, the temperature of the skin surface under the suction cup was measured with an electric thermometer. 200 IU calcium heparinate were administered daily for 6 days in 6 rats, in the subcutaneous tissue. Six other rats received daily 27.5 mg oral tetracycline chlorhydrate for 6 days. The others did not receive any drug. In order to determine the level of the separation induced by vacuum suction, we performed skin biopsies in the three groups. These biopsies were examined under the light microscope.

Statistics

From the suction blister time value before and after treatment, a difference variable ΔSBT was computed. This is the difference in the time needed to raise the second blister after the drug administration. We used one-way analysis of variance in the study of ΔSBT. The attribute variable deviation from the mean was calculated for the three categories of treatment as well as the η value which is equivalent to a regression multiple R with the new variables. Concerning skin temperature, the comparison between three independent groups, the non-parametric analogue of one-way analysis of variance, the Kruskal-Wallis test, was used. The Wilcoxon signed rank test was used to estimate differences within skin temperature before and after treatment.

RESULTS (Table I)

Vacuum blisters showed a clear-cut separation between epidermal and dermal tissues. Under the light microscope the basement zone was seen lining the blister floor (Fig. 1).

No differences were found between temperatures within each group. ΔSBT values for the three groups were different ($F=3.96$; $p<0.05$), and statistical differences were observed between heparin group (deviation $=4.94$; $p<0.05$), tetracycline group (deviation $=4.78$; $p<0.05$) and the control group (deviation $=-9.72$) (Fig. 2). Heparin and tetracycline were found to increase suction blister time signifi-

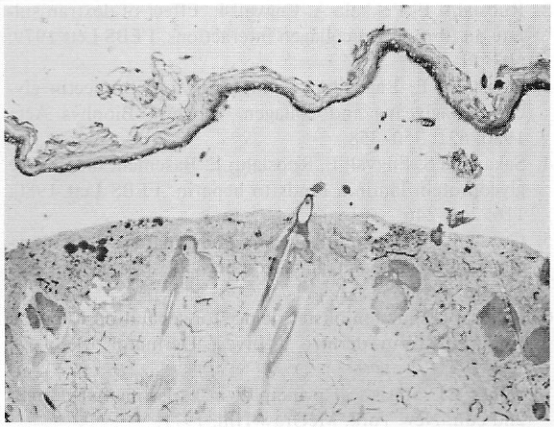

Fig. 1. Biopsy of a suction blister showing separation in the dermal–epidermal junction (HES, ×125).

Table I. *Results of suction blister time values in the three groups, before and after treatment*

Group	SBT (min) before therapy	SBT (min) after therapy	ΔSBT (min)
Heparin	44	53	+9
	44	51	+7
	43	36	−7
	51	54	+3
	48	55	+7
	45	36	−9
Tetracycline	45	68	+23
	42	49	+7
	46	37	−9
	45	50	+5
	48	35	−13
	42	38	−4
Control	42	34	−8
	42	31	−11
	52	22	−30
	59	43	−16
	34	32	−2
	43	32	−11

SBT: suction blister time.

cantly ($\eta^2 = 0.35$). η^2 has an intuitive interpretation as the proportion of variance in the dependent variable explained by the independent variable (time) (7).

DISCUSSION

We have examined the effects of heparin or tetracycline on the suction blister time in hairless rats. Our results indicate that heparin and tetracycline may play an important role in increasing the cohesion of the epidermis on the dermis. A number of factors influence suction blister time, including body region, suction pressure and skin temperature (8). These factors had been considered in the present study where skin temperature was controlled. In the second experiment, suction blister time was decreased in each group compared with the initial suction blister time.

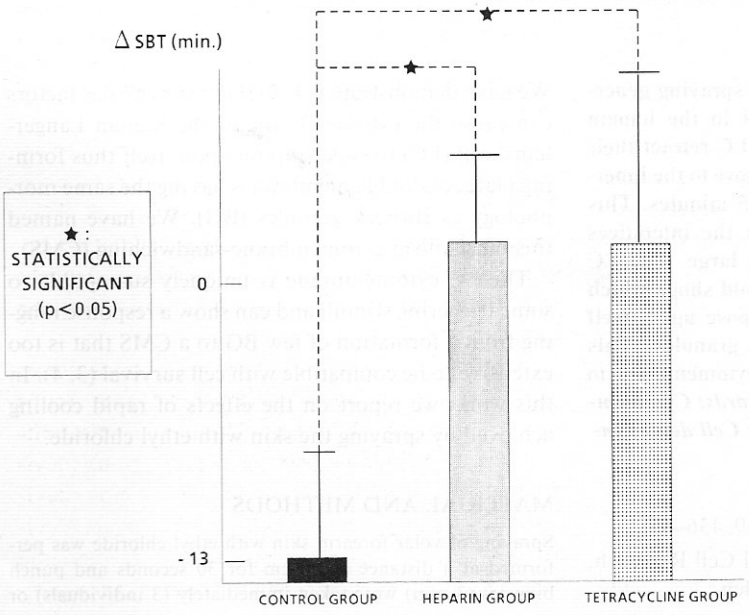

Fig. 2. Histogram showing ΔSBT in the three groups.

This seems to be due to the fact that the second blister was raised on exactly the same blistered site. Activation of collagenase is thought to occur after the first blister, and suggests a potential role of drugs known to inhibit collagenase. The hypothesis that tetracyclines might have an effect on connective tissue breakdown derived from the observation that minocycline inhibited pathologically excessive collagenase activity and collagen breakdown in skin and gingiva in both conventional and germ-free rats (5, 9). The interaction of laminin and heparin could contribute to the structural integrity of basement membranes (4). The measurement of suction blister time on animal skin may prove useful in measuring the effect of drugs on the DEJ. This was demonstrated in the present study, where increased suction blister time was shown after heparin or tetracycline therapy, in hairless rats.

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Human Epidermal Langerhans' Cells are Sensitive to Rapid Cooling by Ethyl Chloride

KARIN WARFVINGE, LARS ANDERSSON and JACEK BARTOSIK

Department of Medical Cell Research, University of Lund, Lund, Sweden

Topical anesthesia with ethyl chloride spraying generates some remarkable reactive events in the human Langerhans' cell (LC) system. Many LC retract their dendrites and a considerable number move to the innermost layers of the epidermis within 15 minutes. This rapid motility supports the view that the interstices between cells in living epidermis are large. The LC cytomembrane is very susceptible to cold shock which causes the cytomembrane to superimpose upon itself forming abnormally shaped Birbeck granules. This process may consume too much of the cytomembrane to be compatible with cell survival. ***Keywords: Cytomembrane; Birbeck granules; Cell motility; Cell death; Intercellular space***

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K Warfvinge, Department of Medical Cell Research, Biskopsgatan 5, S-223 62 Lund, Sweden.

We have demonstrated (1, 2) that extracellular factors can cause the cytomembrane of the human Langerhans' cell (LC) to superimpose upon itself thus forming plates of double membranes having the same morphology as Birbeck granules (BG). We have named this mechanism cytomembrane-sandwiching (CMS).

The LC cytomembrane is uniquely susceptible to some triggering stimuli and can show a response ranging from a formation of few BG to a CMS that is too extensive to be compatible with cell survival (3, 4). In this work, we report on the effects of rapid cooling achieved by spraying the skin with ethyl chloride.

MATERIAL AND METHODS

Spraying of volar forearm skin with ethyl chloride was performed at a distance of 30 cm for 30 seconds and punch biopsies (3 mm) were taken immediately (3 individuals) or