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Early Cutaneous Reactions to Local Traumatization with Heat in Alloxan Diabetic Rats

GÖRAN HALLMANS, FOLKE LITHNER AND ERIK HÄGG

From the Departments of Pathology and Medicine, University of Umeå, Umeå, Sweden

ABSTRACT

The effect of cutaneous traumatization with heat in connection with intravenous injection of Evans blue was studied in short-term alloxan diabetic rats. The effect of traumatization was dependent on the degree of traumatization. There was no difference between diabetic animals and controls. The water content of traumatized and non-traumatized skin was determined. There was no difference between diabetic animals and controls with regard to the increase in water content of traumatized skin. Histological and histochemical studies on the effect of surgical cutaneous traumatization did not reveal any differences between diabetic and non-diabetic animals. The results are compared with earlier observations in alloxan diabetic animals with and without ketosis and in long-term diabetes.

INTRODUCTION

One of us (F. L.) has previously shown that the skin of the lower extremities in certain diabetic patients has an altered reaction to local traumatization with heat as well as with cold, compared with that of non-diabetics. This phenomenon is related to the occurrence of late diabetic lesions, such as microangiopathy and neuropathy (8, 10, 11) but not to the diabetic metabolic derangement per se. Diabetics with these lesions reacted characteristically with small cutaneous hemorrhages within the area of traumatization.

A similar traumatization with heat has been performed in alloxan diabetic rats (9). Within the area of traumatization there was a pronounced reaction consisting of an increased erythema in animals with long duration of diabetes. In short-term alloxan diabetic rats there was only a slight increase of erythema compared with controls, but not within 2 weeks of post-traumatization. No diabetic rat had ketosis. A similar local traumatization with heat was also performed in alloxan diabetic rabbits, some of which had a pronounced diabetic metabolic derangement with ketosis (12). The minimum temperatures and periods of thermal traumatization which caused visible blue spots within the cutaneous area of traumatization upon intravenous injection of Evans blue were determined. Alloxan diabetic rabbits without ketosis did not differ from controls, whereas in the diabetic rabbits with ketosis there was a significantly decreased cutaneous reaction.

Kiss et al. (6) also performed local cutaneous traumatization with heat in short-term alloxan diabetic rats.They studied the occurrence of blue colour within the traumatized skin areas in connection with intravenous injection of trypan blue. The skin was traumatized for 30 seconds by a heated solid metallic cylinder. There was a reduced leakage of trypan blue in diabetic animals, compared with controls. Information is lacking, however, as to whether the rats had ketosis or if the temperature of the cylinder was constant during the period of traumatization.

The methods used in our studies (12) are similar to those of Kiss et al. (6), but our results are not in agreement with theirs. We have therefore further investigated whether the diabetic metabolic derangement without ketosis is of importance for the cutaneous reaction to local traumatization with heat. We have studied the early cutaneous reaction in short-term alloxan diabetic rats after local traumatization with heat, using a similar technique to that of Kiss and co-workers (6) and to that we previously used in our studies on rabbits (12). However, in the present study we have used a different method of depilation to that of Kiss and co-workers and also varied the intensity of traumatization. We have extended the study to include determination of the water content of traumatized and non-traumatized skin. Furthermore, we have

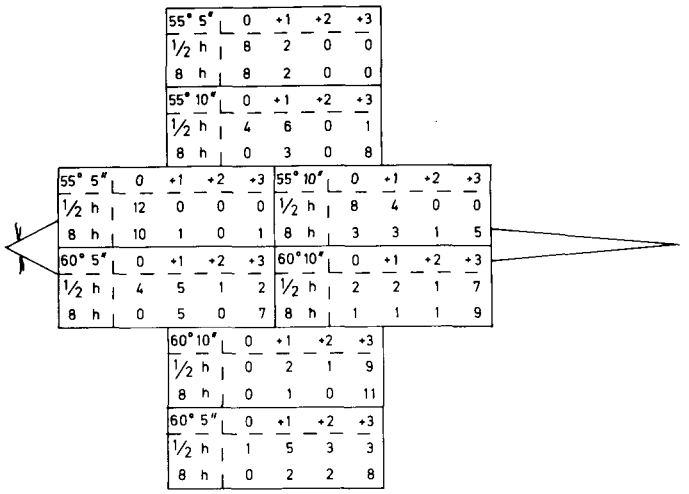

Fig. 1. Controls ($n=12$). Data on the effect of local cutaneous traumatization with heat in connection with intravenous injection of Evans blue. A schematic picture of the animals viewed from below. Information of temperatures used and periods of traumatization within eight different skin areas. The effect of traumatization registered at ½ and 8 hours after the traumatization. The effect is described as the extent of blue colour within the area of traumatization assessed by four grades: 0, 1+, 2+ and 3+. The figures within the right lower part of each of the eight areas indicate the numbers of animals having the extent of the blue colour at the time of registration. For further particulars, see text.

studied the inflammatory response microscopically and histochemically.

MATERIAL AND METHODS

Twenty-four male albino rats of the highly inbred R-strain were used (4). Diabetes was induced in 12 animals at the age of 3 months with an intravenous injection of alloxan, 55 mg/kg, as described earlier (7). No insulin was given after the injection of alloxan. All animals had blood glucose values above 175 mg/100 ml, permanent polyuria and glucosuria. Blood glucose was determined by a modification of the glucose oxidase method (5) and ketonuria was determined by Ketostix® (Ames Company). Twelve non-diabetic, non-injected rats of the same age served as controls.

The abdominal hair and that of the flanks was clipped away 5–7 weeks after the injection of alloxan and the skin was depilated using barium sulphide (13). One day later, cutaneous traumatization was performed. Both induction and inspection of the skin injury were performed without knowledge of the presence or absence of diabetes. After eight hours the animals were killed.

Intravenous injection of Evans blue to determine the presence of blue colour within the area of traumatization

The depilated areas were divided into eight areas (Figs. 1 and 2). Local traumatizations were induced in the anesthetized animals by placing the end surface of an electrically heated cylindrical brass rod, 8 mm in diameter, against the skin on each of these smaller areas as described previously (8, 9, 10, 11). As in earlier studies (8, 9, 10), the temperatures used for local traumatization were 60°C and 55°C for 5 and 10 seconds, thus four different degrees of intensity of traumatization were attained. The cutaneous traumatization was performed identically on each animal.

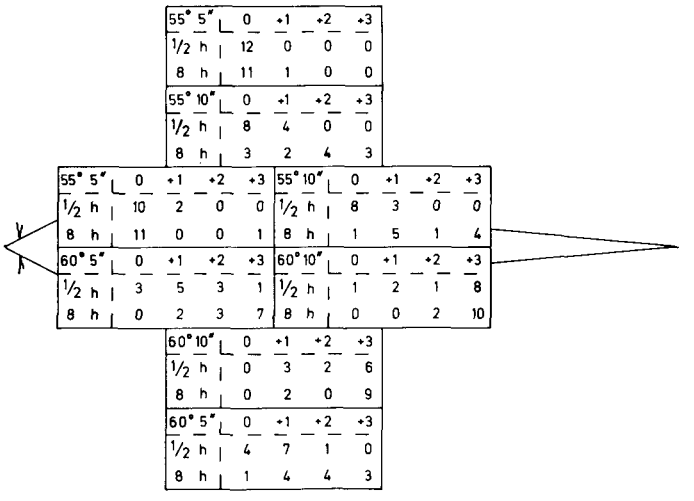

Fig. 2. Alloxan diabetic rats ($n=12$). Text, see Fig. 1.

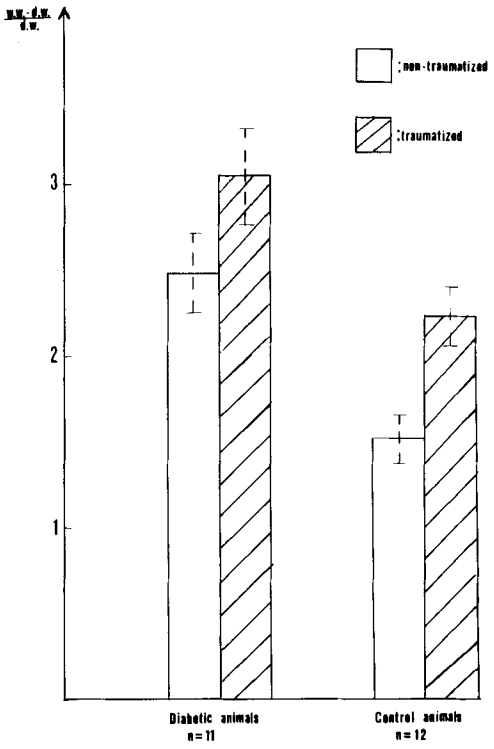

Fig. 3. Water content of skin determined as the wet weight (ww) and dry weight (dw) using the relationship (ww−dw)/dw. The determinations were performed on excised pieces from non-traumatized skin and skin traumatized with heat. Mean ±S.E.

0.5 ml Evans blue (2% solution) was injected intravenously immediately before the traumatization. This dye is bound by the plasma proteins in the blood stream (1, 15) when it is injected intravenously. When there is increased capillary permeability, coloured patches can be seen. The traumatized areas were inspected ½ and 8 hours after the traumatization. The extent of the blue colour within the traumatized area was assessed using four grades: 0, 1+, 2+ and 3+.

Determination of water content of traumatized and non-traumatized skin

Thermal injury was induced on the left side of the thorax with the end surface of an electrically heated cylindrical brass rod, as described above, but now with a diameter of 18 mm. The degree of traumatization was 60°C for 10 seconds. After the animals were killed, the skin surrounding the traumatized skin was cut exactly along a line made by a circular marking device 25 mm in diameter. The excision was performed through the panniculus carnosus down to the underlying fascia. Non-traumatized pieces of skin were removed in a corresponding manner from the equivalent site of the right side of the same rat (17). The wet weight was then determined within 5 minutes after the excision. For dry weight determination, samples were dried in an oven at 110°C for 72 hours. The water content was then determined from the wet weight (ww) and dry weight (dw) using the relationship (ww−dw)/dw.

Microscopical and histochemical methods

On the back of each animal the skin was excised within a circular area, 10 mm in diameter, down to the underlying fascia. After the animals were killed, pieces of a standard size were excised from the margins of the wounds for microscopical as well as histochemical studies. For microscopical investigation the skin was fixed in 10% neutral formaldehyde, embedded in paraffin, sectioned and stained with hematoxyline–eosin. For histochemical investigation the skin was fixed in a liquid propane/propene mixture, frozen with liquid nitrogen to −196°C, sectioned in a cryostat (10 μm), then incubated and stained with an azo-dye for demonstration of alkaline phosphatase activity (2). These investigations were performed without knowledge of the presence or absence of diabetes in the animals.

The significance of the difference between means was calculated using Student's *t*-test. Differences of graded blue colour were tested with the non-parametric rank sum test of Wilcoxon. $P<0.05$ was chosen as the level for statistical significance.

RESULTS

The blood glucose at the beginning of the traumatization was 301±26 mg/100 ml for the diabetic animals and 78±4 for the controls (Mean ±S.E.). No animal had ketonuria.

Presence of blue colour within the area of traumatization after intravenous injection of Evans blue

Local cutaneous traumatization was not performed if the area intended for traumatization was already injured spontaneously or as a result of depilation. The numbers of traumatizations not performed for these reasons were about the same for the diabetic animals and for the controls.

From Figs. 1 and 2 it is evident that the result of traumatization was directly dependent upon the degree of traumatization. At slight traumatization (55°C, 5 sec) as well as pronounced traumatization (60°C, 10 sec), the effect of traumatization was not dependent upon the interval of time between traumatization and registration of the effect. However, at moderate traumatization (55°C, 10 sec and 60°C, 5 sec), the effect of the traumatization increased with the interval between traumatization and registration.

Diabetic animals and controls were compared at

corresponding temperature, period of traumatization, area of traumatization and time of registration (Figs. 1 and 2). In none of these comparisons was there any significant difference between the diabetic animals and the controls.

Determination of water content in traumatized and non-traumatized skin (Fig. 3)

No significant difference between the two groups of animals could be demonstrated with regard to the water content of traumatized skin, compared with non-traumatized skin. There was, however, a significantly increased water content of the non-traumatized skin of the diabetic animals compared with that of the controls ($p<0.01$).

Microscopical and histochemical study

In the sections, collections of inflammatory cells, mostly granulocytes, were found near the margins of the wounds and beneath the panniculus carnosus. No difference was observed between diabetic animals and controls. An increase in alkaline phosphatase activity was observed near the margins of the wounds. Pronounced enzymatic activity was found in connective tissue cells, mainly fibroblasts, and in the infiltrates of inflammatory cells. A central zone of decreased anzymatic activity, as described previously (14), was seen in some wounds. There was no difference of enzymatic activity between the two animal groups.

DISCUSSION

In short-term experimental diabetes we have previously studied reactions to local cutaneous traumatization with heat (9, 12). These studies were performed on alloxan diabetic rats and rabbits. The occurrence of erythema and extent of blue colour after intravenous injection of Evans blue was estimated. Short-term alloxan diabetic rats demonstrated a slightly greater erythema than controls, but not until 2 weeks after the traumatization. The rats had no ketosis. In alloxan diabetic rabbits a decreased reaction to traumatization was found, but only when the animals had ketosis. The reaction was registered after ½ and 4 hours.

In the present study, diabetic rats and controls reacted identically to cutaneous traumatization with heat as regards the occurrence of blue colour within the traumatized areas. The reaction was dependent upon the degree of traumatization. At moderate traumatization, the traumatization effect increased with the period of time between traumatization and registration. This latter circumstance has been described previously by Sevitt (16).

In similar studies Kiss et al. (6) described a reduced cutaneous reaction in alloxan diabetic rats compared with controls. In spite of conformity concerning the species used, there are differences between the results of Kiss et al. and ours. The cause of these divergencies is not clear. There are, however, several differences concerning the method used. Kiss and co-workers used trypan blue, while we used Evans blue. Trypan blue is bound to the plasma proteins in a different manner and has a faster elimination rate from the blood stream than Evans blue (15). More important is perhaps the fact that there is an increased water content in the skin of diabetic rats compared with controls (see Results). This, in connection with Kiss and co-workers using a longer period of traumatization vis-à-vis that used in our study, and that they then apparently did not have control over possible changes in temperature of the traumatization model used, gives rise to the question whether the biological degree of traumatization in the investigation of Kiss et al. was identical in the diabetic rats and the controls.

The fact that there is an increased water content in diabetic tissue has also been described earlier (3). Concerning the increase in water content of skin in connection with traumatization in the present investigation, there was no difference between the two groups. Nor did microscopical and histochemical investigations of the effect of cutaneous traumatization caused by the surgical method demonstrate any differences between the tissue reactions in diabetic rats and controls.

In conclusion, the methods of investigation used by us in the present and in previous studies (8, 9, 10, 11, 12) have demonstrated that the diabetic metabolic derangement per se, contrasted with the late diabetic state, is of no, or only of slight importance in the cutaneous reactions to local traumatization with heat, unless the degree of diabetes is so pronounced that ketosis is also present.

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Address for reprints:

Folke Lithner, M. D.
Department of Medicine
University of Umeå
S-901 85 Umeå
Sweden