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Local Traumatization with Heat—Cutaneous Reactions and Early Effects on Serum Zinc Concentration in Rats with Alloxan Diabetes of Very Short Duration

Göran Hallmans, Folke Lithner and Erik Hägg

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

ABSTRACT

Local heat trauma was induced in rats with alloxan diabetes of 3 days' duration. The cutaneous reaction, water content of the skin and serum zinc concentration were estimated.

Petechiae within the area of traumatization were observed more often and were more abundant in the controls than in the diabetic animals.

There was no difference between diabetic animals and controls with regard to the increase of water content of traumatized skin or to the water content of non-traumatized skin.

There was no difference in serum zinc levels between non-traumatized diabetic animals and controls. After traumatization there was a decrease of serum zinc levels in both animal groups, but to a significantly lower level in the diabetic animals compared to the controls.

INTRODUCTION

The skin of the lower extremities in diabetics has an altered reaction to local traumatization with heat and cold (9). This phenomenon is related to the occurrence of late diabetic lesions, such as microangiopathy and neuropathy, but not to the diabetic metabolic derangement per se (9, 11, 12). A similar traumatization with heat has been performed in alloxan diabetic rats of varying age and duration of diabetes (10). 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 to controls, but not until two weeks after the traumatization. In a similar study, traumatization with local heat was performed on rats with 5-7 weeks duration of diabetes in which Evans blue was injected intravenously (6). Concerning the early reaction, there was no difference between diabetic animals and controls. Neither histological nor histochemical studies revealed any differences. However, there was an increased water content of the non-traumatized skin of the diabetic animals to controls.

An increased water content in diabetic tissue has been described earlier (3, 4).

To determine if the increased water content of diabetic tissue is directly related to the diabetic metabolic derangement per se, we have, in the present study, estimated the water content of tissue in rats with alloxan diabetes of very short duration (3 days).

Decreased levels of zinc in plasma, leucocytes and erythrocytes and increased levels in urine have been demonstrated in newly diagnosed diabetics (8). In non-diabetic rats there is a pronounced decrease of plasma zinc levels in different inflammatory conditions already after eight hours (1). We therefore have considered it of interest to determine the serum zinc levels in traumatized and non-traumatized animals with or without diabetes.

MATERIAL AND METHODS

Thirty-two male albino rats of the highly inbred R-strain were used (5). Diabetes was induced in 16 animals at the age of 3 months with an intravenous injection of alloxan, 0.34 mmol/kg, as described earlier (7). No insulin was given after the injection of alloxan. All animals had blood glucose values above 13.9 mmol/l, polyuria and glucosuria.

Methods of depilation, anesthesia and determination of water content were described in a previous article (6). Traumatization was performed three days after the alloxan injection, the temperature used for traumatization was 60 ^{o}C for 5, 10 and 15 seconds. Local traumatization was induced in the anesthetized animals by placing the end surface of an electrically heated cylindrical brass rod, 18 mm in diameter, against the skin, as described previously (6, 9). The traumatized areas were inspected after 0.5, 4 and 8 hours and then the animals were killed. Both induction and inspection of the skin injury were performed without knowledge of the presence or absence of diabetes. The extent of cutaneous hemorrhages was assessed using four grades: 0, 1+, 2+ and 3+.

Serum was collected in weighed, acid-rinsed glass tubes (Jena). The samples were dried for 72 hours at 110 ^{o}C, ashed for 24 hours at 500 ^{o}C and the ash dissolved in 0.5 ml 3 mmol/l HCL overnight. All samples were then diluted with 2 ml deionized water. The serum zinc concentrations were determined using a Varian atomic absorption spectrophotometer at 213.9 nm. Reference samples of zinc in 0.6 mmol/l HCL were used (2).

The significance of the difference between means was calculated using the Student's t-test. Differences of extent of petechiae 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 level at the beginning of the traumatization was 17.4 ± 0.9 mmol/l for the diabetic animals and 5.2 ± 0.4 mmol/l for the controls (Mean ± S.E.). No animal had ketonuria.

Macroscopic assessment of purpura within the area of traumatization.

At all the three degrees of traumatization the controls had an increased extent of purpura 8 hours after the traumatization as compared with the diabetic animals (Fig. 1).

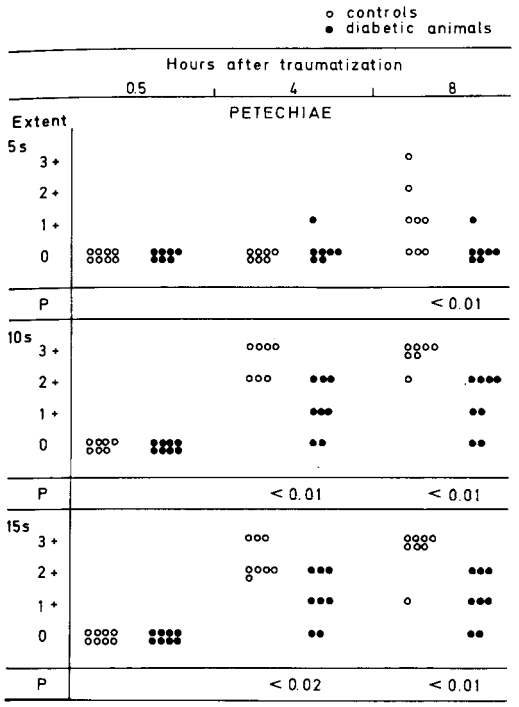

Fig. 1. Data on the effect of local cutaneous traumatization with heat 60 °C and different periods of traumatization (5, 10 and 15 seconds). The effect of traumatization registered at 0.5, 4 and 8 hours after the traumatization. The extent of the petechiae within the traumatized area was assessed using four grades: 0, 1+, 2+ and 3+. The $p \geq 0.05$ values are omitted.
No. of diabetic animals = 8, controls = 8.

Determination of the water content of traumatized and non-traumatized skin.

The water content of traumatized skin was increased compared to that of non-traumatized skin both in diabetic animals and in controls. There was no significant differences between diabetic animals and controls with regard to the increase of water content of traumatized skin, nor with regard to the water content of the non-traumatized skin (Fig. 2).

Determination of serum zinc concentration.

There was no difference in serum zinc concentration between non-traumatized diabetic animals and controls (Fig. 3). After traumatization the serum zinc levels were decreased both in the controls ($p < 0.05$) and in the diabetic animals ($p < 0.001$). Serum zinc concentration after traumatization was significantly lower in the diabetic animals compared to that of the controls ($p < 0.01$).

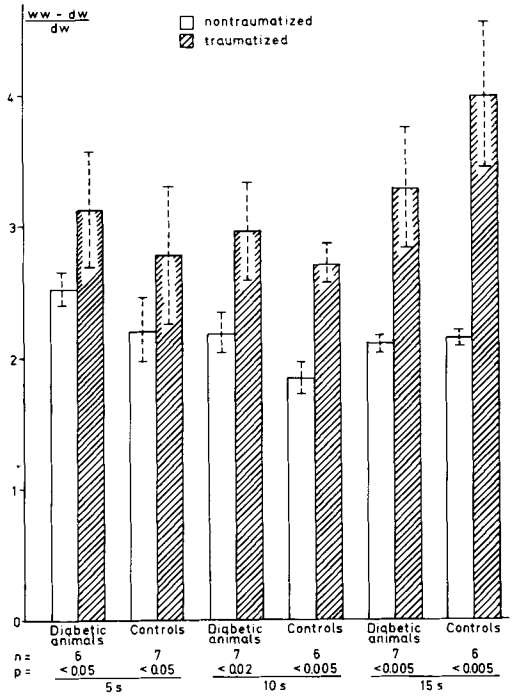

Fig. 2. Data on local cutaneous traumatization with heat 60 °C and different periods of traumatization (5, 10 and 15 seconds). Water content of skin determined as the wet weight (ww) and dry weight (dw) using the relationship $\frac{ww - dw}{dw}$. The determinations were performed on excised pieces from non-traumatized skin and skin traumatized with heat. Number of animals = n. Mean ± S.E.

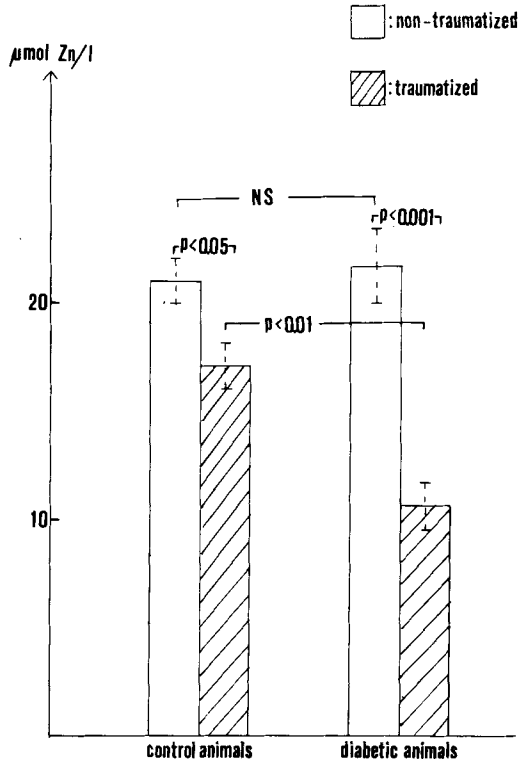

Fig. 3. Zinc concentration in serum (µmol Zn/1) of controls and diabetic animals with or without local cutaneous traumatization with heat 60 °C (5, 10 and 15 seconds). Eight animals in each group. Mean ± S.E.
NS = not statistically significant.

DISCUSSION

In the present study, the controls had an increased extent of purpura within the area of thermal traumatization compared to diabetic animals. In a similar study we have previously estimated the extent of visible blue spots within the cutaneous area of thermal traumatization upon intravenous injection of Evans blue, there being no difference in this respect between diabetic rats and controls (6) or between diabetic rabbits without ketosis and controls (13). It is possible that, in demonstrating plasma leakage with Evans blue we might have concealed an increased extent of purpura.

In the present study there was no difference with regard to the water content of non-traumatized skin between the controls and the animals with diabetes of very short duration. Thus, the early diabetic state does not seem to alter the water content of skin. An increased water content of different tissues in diabetic animals (3, 6) and man (4) is probably caused by a long-standing diabetic metabolic derangement.

The demonstrated decrease of the serum zinc levels after traumatization in our study is in accordance with earlier reports of induced acute inflammatory reactions in non-diabetic rats (1). The more pronounced decrease of serum zinc levels observed in the animals with diabetes of short duration might be explained by a difference in function of phagocytic cells. Phagocytic cells release a leucocytic endogenous mediator (LEM). LEM initiates a variety of effects including a decrease in the serum zinc levels and an increase in the zinc concentration in the liver (1).

REFERENCES

1. Beisel, W.R., Pekarek, R.S. & Wannemacher, R.W.: Homeostatic mechanisms affecting plasma zinc levels in acute stress. In: Trace Elements in Human Health and Disease, Academic Press, 1:87-106, 1976.
2. Bergman, B., Sjöström, R. & Wing, K.R.: The variation with age of tissue zinc concentrations in albino rats determined by atomic spectrophotometry. Acta Physiol Scand 92:440-450, 1974.
3. Forscher, B.K. & Cecil, H.C.: Some effects of alloxan diabetes on acute inflammation. J Applied Physiol 13:278-282, 1958.
4. Fuchs, U.: Elektronmikroskopische Untersuchungen menschlichen Muskelkapillaren bei Diabetes mellitus. Frankfurter Zeitschr Pathol 73:318-327, 1964.
5. Hägg, E.: Renal lesions in rats with long-term alloxan diabetes. A semiquantitative light microscopic study with particular reference to the glomeruli. Acta Pathol Microbiol Scand 82 A:199-210, 1974.

6. Hallmans, G., Lithner, F. & Hägg, E.: Early cutaneous reactions to local traumatization with heat in alloxan diabetic rats. Upsala J Med Sci 83: 17-21, 1978.
7. Korec, R.: Experimental diabetes mellitus in rat. Publishing House of the Slovak Academy of Sciences, Bratislava 1967.
8. Kumar, S. & Rao, K.S.J.: Blood and urinary zinc levels in diabetes mellitus. Nutr Metabol 17:231-235, 1974.
9. Lithner, F.: Cutaneous reactions of the extremities to local thermal trauma. Acta Med Scand 198:319-325, 1975.
10. Lithner, F. & Hägg, E.: Cutaneous reactions of alloxan diabetic rats to local thermal trauma. Upsala J Med Sci 80:99-102, 1975.
11. Lithner, F.: Skin lesions of the legs and feet and skeletal lesions of the feet in familial amyloidosis with polyneuropathy. Acta Med Scand 199:197-202, 1976.
12. Lithner, F.: Lesions of the legs in diabetics and in patients with familial amyloidosis and polyneuropathy. Acta Med Scand Suppl 589, 1976.
13. Lithner, F. & Hallmans, G.: Cutaneous reactions to local traumatization with heat in alloxan diabetic rabbits with and without ketosis. Upsala J Med Sci 83:23-28, 1978.

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Address for reprints:

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