Upsala J Med Sci 80: 99–102, 1975

Cutaneous Reactions of Alloxan Diabetic Rats to Local Thermal Trauma

FOLKE LITHNER and ERIK HÄGG

From the Department of Medicine, University of Umeå, Umeå, Sweden

ABSTRACT

Thermal injury was induced on the external ears of nondiabetic and untreated alloxan diabetic rats of various ages. The skin reaction (erythema and necrosis) was assessed by naked eye inspection, 1, 7, 14 and 21 days after injury. Erythema was found to be more intense in young than in old controls after 1 and 7 days. The late erythematous reaction was more pronounced in short-term diabetic animals than in controls of the same age, indicating that the diabetic metabolic derangement *per se* alters the reaction. In addition, long-term diabetic rats had a markedly increased skin redness after 1, 7, 14 and 21 days when compared with controls of the same age. Thus, long-term diabetes enhances the erythematous reaction. As to the extent of necrosis, there were no significant differences between the experimental groups. There was, however, a tendency for an increased amount in the long-term when compared with the short-term diabetic rats.

INTRODUCTION

Certain skin lesions, i.e. atrophic circumscribed skin lesions (9) and erythema with or without necrosis (7), on the legs of human diabetics have been described previously from this Department of Medicine. The cause of these lesions has been discussed (7, 9) and it has been suggested that the skin of diabetic individuals, especially old and long-term diabetics, reacts in an altered way to various trauma when compared with that of nondiabetic individuals (7). This suggestion of an altered reaction was confirmed by observation made when one of us (Lithner) directly traumatized the skin of the forearms and the legs of diabetics and nondiabetic controls by local application of heat and cold and registered the reactions. (8).

There are, however, difficulties in selecting a group of diabetic individuals with pure metabolic abnormalities without any signs of diabetic microangiopathy. Furthermore, it is not possible to determine the exact duration of the disease in human diabetics, especially in those with maturity-onset diabetes. Therefore, it was considered valuable to perform similar tests on the skin of rats with alloxan diabetes of various durations. Such a study might make it possible to determine the influence of the diabetic metabolic derangement *per se* on the skin reaction and also to avaluate the role of diabetes duration on the results. A further advantage with experimental diabetes is that the assessment of the induced lesions can be performed without any knowledge of the presence or absence of diabetes or the age of the animal.

MATERIAL AND METHODS

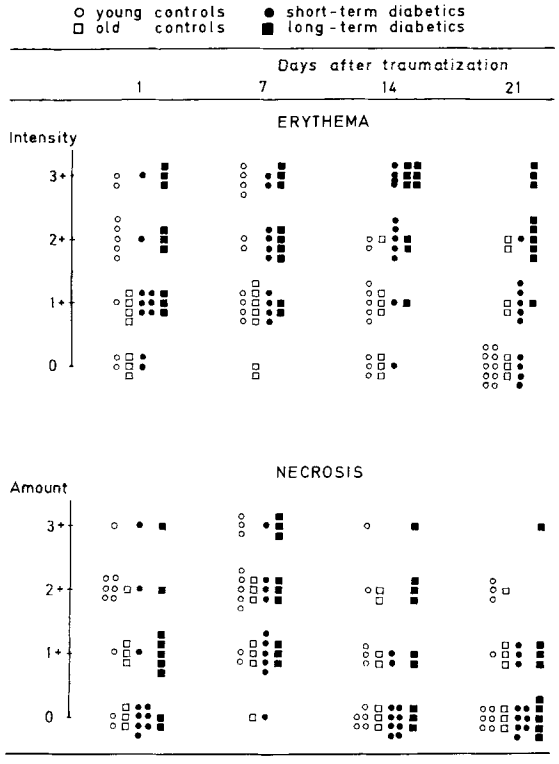
Thirty-six male albino rats of the highly inbred R-strain were used (cf. 2). Diabetes was induced in 19 animals at the age of 3 months with an intravenous injection of alloxan as described previously (2). No insulin was given afterwards. Only animals with a permanent polyuria and glucosuria (controlled once a month) and a nonfasting blood glucose value (controlled every third month) of at least 200 mg/100 ml were used (cf. 2). Seventeen nondiabetic noninjected rats served as controls.

Skin injury was induced in 10, $3^1/_2$-month-old controls and in 7, 13-month-old controls, in 10 diabetic rats 2 weeks after alloxan injection ($3^1/_2$ months old) and in 9 long-term diabetic rats (11–14 months old, 8–11 months after alloxanization).

The external ears of the animals were found to be suitable for the skin test. An electrically heated cylindrical brass rod, 8 mm in diameter, was used as described previously (8). The rod was held against the center of the ventral aspect of the ear and on the back side of the ear a piece of cardboard was applied. The pressure against the skin was the weight of the rod. The temperature was 55°C (right ear) and 60°C (left ear) and the duration of skin contact 5 sec. Animals were anaesthetized with ether during the traumatization.

The ears were inspected 1, 7, 14 and 21 days subsequent to the traumatization. Both the procedures, i.e. inducing and inspecting the skin injury, were performed blind, i.e. without knowledge of the presence or not of diabetes or of the age of the animals.

Table I. *Semiquantitatively assessed intensity of skin erythema and amount of necrosis after thermal skin injury (60°C) in alloxan diabetic and nondiabetic rats*



The following parameters within the skin lesions were semiquantitatively assessed by gross inspection: 1) intensity of erythema: 0, 1+, 2+ and 3+, 2) extent of necrosis: 0, 1+, 2+ and 3+.

Differences between groups were tested with the nonparametric rank sum test of Wilcoxon (13). $P<0.05$ was chosen as the level for statistical significance.

RESULTS

The temperature of 55°C of the rod during 5 sec was found to be insufficient in producing enough skin lesions in the rats. The results presented here are therefore confined to 60°C (Table I).

The erythema noted was restricted to the injured skin area; there was no red marginal zone around the lesions. The results of comparing the intensity of erythema between different experimental groups are presented in the text, only when statistically significant differences were present. Concerning the experimental conditions, the compared animal groups differed from each other in one or more of the following three factors: age, diabetes of short duration and diabetes of long duration.

As to differences in age, young control rats exhibited a significantly more intense redness than old control animals after one day ($p<0.05$) and 7 days ($p<0.02$). Thus, young controls have an enhanced "early" reaction.

With reference to the factor, diabetes of short duration, young diabetic rats had a significantly more intense erythematous reaction than age-matched controls after 14 ($p<0.02$) and 21 days ($p<0.05$). It is evident that short-term diabetes accentuates the "late" skin reaction.

When the difference was assigned to the presence or absence of diabetes of long duration it was found that the intensity of the erythematous reaction was significantly more pronounced in old diabetic rats than in old control animals at all times; after one day $p<0.01$, after 7 days $p<0.01$, after 14 days $p<0.01$ and after 21 days $p=0.02$. This means that long-term diabetes increases the reaction.

When comparing groups, differing in two of the aforementioned factors, the following results were obtained. Young diabetic rats had a more intense skin erythema than old control animals after 7 and 14 days ($p<0.02$). Long-term diabetics had an increased erythematous reaction when compared with young controls after 14 and 21 days ($p<0.01$).

Furthermore, old diabetic rats had a significantly more intense erythema than young diabetic rats after 21 days ($p<0.01$). These two groups differed from each other concerning all three factors.

When performing the same comparisons as to the degree of necrosis, no significant differences between the groups could be demonstrated. There was, however, a tendency for an increased amount of necrosis in the long-term when compared with the short-term diabetic rats (after 14 days $p=0.05$).

There were no obvious qualitative differences in the skin reaction between diabetic and nondiabetic animals. No petechiae were noted.

The mean blood glucose level of the young diabetic animals was 399 mg/100 ml±25 (S.E.M.) and old diabetic rats 375 mg/100 ml±17 (S.E.M.). The difference was not significant (Student's *t*-test).

DISCUSSION

The pathogenic mechanisms behind the erythema, induced by thermal trauma, are not known. The mechanisms may not necessarily be the same for

the redness noted one day after injury and for that observed after 21 days.

If the compared experimental groups of the present study differed in two or more of the above mentioned factors, i.e. age, diabetes of short duration and diabetes of long duration, it is not possible to determine the influence of a particular factor for the result. The discussion will therefore be concerned with comparisons, where only one of the factors differed between the groups.

Concerning the intensity of erythema, certain results of interest were noted. Firstly, young controls had a significantly more intense erythematous reaction after 1 and 7 days as compared with old controls. Thus, it can be stated that the age of the animal is of importance for the reaction. Secondly, short-term diabetic rats had more intense erythema after 14 and 21 days than control rats of the same age. This result indicates that the diabetic metabolic derangement *per se* alters the late erythematous reaction. Thirdly, the pronounced increase of redness of the skin of long-term diabetic rats when compared with old controls after 1, 7, 14 and 21 days proves that the factor, long-term diabetes, is of importance for the cutaneous reaction. Similar results were obtained in a preliminary study performed in this laboratory with rats, using solid carbon dioxide instead of heat.

A few reports have appeared in the literature on physical skin injury in experimental diabetes (1, 5). Kiss et al. (5) applied a 60°C heat stimulus for 30 sec on the skin of short-term alloxan diabetic rats. This was followed by an intravenous injection of trypane blue. The intensity of the dye colour in the injured skin area, recorded within 30 min of heat application, was found to be impaired in most of the diabetic rats. Gunn et al. (1) reported increased skin lesions in response to prolonged local radiation (during 1 to 6 weeks) in alloxan diabetic rats with about one to two months of diabetes. Direct comparison of the previous observations (1, 5) with those of the present study is not possible since the experimental conditions were quite different. Furthermore, the above reports only dealt with diabetes of short duration.

Lithner (8) induced skin lesions in human diabetics and nondiabetic controls by heat and cold (solid carbon dioxide). When comparing his results with those of the present investigation, certain differences can be noted. In both nondiabetic and diabetic patients dermal vesicles and petechiae occurred. Such changes were not seen in the rats. A marginal zone of intense redness was often observed in human long-term diabetics but not in the animals. Erythema within the area of traumatization was not evaluated in humans because of the frequent occurrence of non-transparent blisters. A direct comparison of the results of Lithner's investigation with those of the present one is therefore not possible. In both studies, however, the age of the individual/animal is of significance with regard to the reaction after thermal injury. Furthermore, the factor long-term diabetes is of great importance for the intensity of the dermal reaction in both studies.

The altered skin reaction in diabetic rats might be explained by an increased sensitivity of skin tissue to injury (cf. 11) and/or impaired reparative processes after injury caused by the metabolic derangement associated with the disease. There is, in fact, well-documented evidence showing that alloxan diabetic rats have a decreased ability to form granulation tissue (6, 10, 12). Furthermore, in long-term diabetic rats there might be other factors superimposed on pure metabolic abnormalities, e.g. the presence of microvascular lesions, which could modify the skin reaction. It has been demonstrated in this laboratory that alloxan diabetic rats with a diabetes duration of 12 months have clearcut glomerular lesions similar to human diabetic glomerulosclerosis (2, 3). On the other hand, rats with diabetes for only one month did not show any light microscopical glomerular lesions. It may also be added that alloxan diabetic rats have a decreased skin capillary resistence after 12 months of diabetes but not after 3 months (4). No histological study of the skin was performed in the present investigation. Consequently, no definite statements can be made concerning the presence or absence of dermal microangiopathy in the diabetic rats.

ACKNOWLEDGEMENT

This investigation was supported by grants from the Medical Faculty, University of Umeå.

REFERENCES

1. Gunn, W. G., Slager, U. & Koukol, D. C.: Radiation sensitivity in diabetes: Preliminary studies with the alloxan diabetic rat. Radiology *88:* 349, 1967.
2. 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, 1974.

3. — Glomerular basement membrane thickening in rats with long-term alloxan diabetes. A quantitative electron microscopic study. Acta Pathol Microbiol Scand *82 A:* 211, 1974.
4. — Unpublished observations.
5. Kiss, A., Csaba, B., Damjanovich, S., Veress, O. & Szilágyi, T.: Diabetes and anaphylaxis. Acta Phys Acad Sci Hung *23:* 275, 1963.
6. Kraus, S. D.: Effect of alloxan diabetes, glucose feeding and insulin on true anaphylaxis and granuloma formation. Acta Endocr *47:* 149, 1964.
7. Lithner, F.: Cutaneous erythema, with or without necrosis, localized to the legs and feet—a lesion in elderly diabetics. Acta Med Scand *196:* 333, 1974.
8. — Cutaneous reactions of the extremities of diabetics to local thermal trauma. Accepted for publ. in Acta Med Scand.
9. Melin, H.: An atrophic circumscribed skin lesion in the lower extremities of diabetics. Acta Med Scand, Suppl. 423, 1964.
10. Nagy, S., Rédei, A. & Karády, S.: Studies on granulation tissue production in alloxan diabetic rats. J Endocr *22:* 143, 1961.
11. Vracko, R. & Benditt, E. P.: Manifestations of diabetes mellitus—their possible relationships to an underlying cell defect. Am J Pathol *75:* 204, 1974.
12. Wagner, H., Junge-Hülsing, G., Otto, H., Adler, J. & Hauss, W. H.: Animal experiments concerning the disturbed wound-healing in diabetes mellitus. Diabetologia *6:* 412, 1970.
13. Wilcoxon, F.: Individual comparisons by ranking methods. Biometrics Bull *1:* 80, 1945.

Received March 12, 1975

Address for reprints:

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