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SHORT COMMUNICATION

Lipid peroxidation in early experimental diabetes in rats: effects of diabetes and insulin

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The degree of lipid peroxidation was measured in organs from diabetic rats receiving no treatment, and in those from insulin-treated diabetic rats and controls. Lipid peroxidation was measured as organ content of malondialdehyde, a degradation product of polyunsaturated fatty acids. In the kidney, lipid peroxidation was increased after one week of diabetes; insulin treatment reduced the level of lipid peroxidation to levels lower than seen in controls. In the liver, diabetes caused an increased lipid peroxidation, which could be reversed by insulin; no additional effect of insulin was found. In heart and pancreas no effects of diabetes or insulin were demonstrated. The present paper provides evidence that lipid peroxidation is increased in the early stages of experimental diabetes and is reversible by insulin treatment. Hyperinsulinaemia may, in itself, counteract lipid peroxidation in kidney.

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The role of oxidative stress in diabetes remains unsettled. Oxidative stress has been associated particularly with the development of complications in diabetes, as recently reviewed by Baynes (1). A number of changes in free radical-associated parameters, especially scavenging enzymes, have been described in diabetic patients as well as in animal models of diabetes and other endocrinopathies (2–7).

Lipid peroxidation, broadly defined as the oxidative deterioration of polyunsaturated lipids and usually associated with the initial attack on such lipids of free radicals (8), has been associated with the development of angiopathy, also in diabetes (9–10).

We have examined the extent of lipid peroxidation in early experimental diabetes, as well as the effects of insulin.

Materials and methods

Animals

Female Wistar rats, 8 weeks of age (Møllegårds Avlslab., Eiby, Denmark), were studied. They were housed, three per cage, at 21°C and at 55% humidity in a 12:12 (06.00–18.00) hour light/dark cycle, with free access to standard rat chow (Altromin, Lage, Germany) and tap water. The animals were randomized into three groups matched for body weight: (1) controls (N = 6); (2) diabetic animals, no insulin (N = 6); and (3) diabetic animals, insulin-treated (N = 6). Diabetes was induced on day 0 by iv injection of streptozotocin (55 mg/kg body weight) in 0.154 mol/l NaCl (pH 4.0) following 12 h food deprivation. Twenty-four hours after the administration of streptozotocin, and daily thereafter, the animals were weighed, urinanalysis was performed for glucose and ketones using Neostix 4 (Ames Ltd., Stoke Poges, Slough, UK) and tail vein blood glucose was determined by Haemoglucotest 1-44 and Reflolux II reflectance meter (Boehringer Mannheim). Insulin treatment with heat-treated Ultralente Insulin (Novo-Nordisk, Bagsværd, Denmark) was initiated 24 h after streptozotocin administration, when all animals had blood glucose levels above 20 mmol/l. Insulin was given at an initial dose of 4–6 U, followed by 1–3 U daily depending on blood glucose levels. Twenty-four hours' food consumption was measured in all animals on days 2, 4 and 7. Average blood glucose level in the untreated group was 27 mmol/l; after treatment, the insulin-treated group did not differ from controls. Body weight increased in parallel in controls and insulin-treated animals; untreated diabetics lost approximately 10% of initial body weight.

Comparison of food consumptions among the groups revealed no differences at days 2 and 4, whereas untreated diabetic rats significantly increased their intake at day 7 (by approximately 45%). All untreated animals, but no others had glycosuria (> 111 mmol/l); none had ketonuria.

At day 7, all animals were sacrificed, tissues rapidly cleaned, weighed and immediately frozen in liquid nitrogen and stored at −80°C until analysis for MDA, which was performed within three weeks. Initial analysis on kidney tissue was performed on the left kidneys; the contralateral kidneys were processed for analysis 10 weeks after freezing, confirming the significant effects of both diabetes and insulin treatment on MDA contents.

Baseline values were elevated, however, probably due to auto-oxidation during storage.

Estimation of tissue MDA content

The procedure described by Okhawa (11), including modifications, (12) was followed except for the omission of organic phase extraction of MDA. The tissues were homogenized in ice-cold 1.15% KCl (1/10, tissue/KCl, w/v) using a Teflon homogenizer. Aliquots of 0.4 ml were used for further procedures. Within 30 min of homogenization of the tissue 0.4 ml 8.1% sodium dodecylsulphate and 3.0 ml 20% acetic acid (pH 3.5) were added to each aliquot. Finally, 3.0 ml of a 0.67% aqueous thiobarbiturate (TBA) solution was added; tubes were placed in boiling water for 1 h. After cooling, the test tubes were centrifuged at $1810 \times g$ for 10 min. Approximately 0.5 ml of the top layer was used for absorbence spectrophotometry at 532 nm, with distilled water as baseline reference. MDA levels were expressed as nmol/g weight tissue. Standards of 3.125, 6.25, 12.5, 25, 50, 100 and 200 nmol MDA prepared from malondialdehyde-bis-dimethylacetate were processed along with the homogenates at all measuring sessions.

Statistics

Probabilities were calculated using the non-parametric Mann-Whitney test, two-tailed, significance defined as $p<0.01$.

Results

Organ content of MDA appears from Table 1. In kidney tissue, our initial results showed a significant trend towards higher levels of MDA in the untreated diabetic animals than in controls ($p<0.05$, by parametric *t*-test). The trend was confirmed by analyses performed on the contralateral kidneys, the results here being highly significant. In the liver, similar results were seen, with untreated diabetic animals having a significantly higher content of MDA.

Insulin treatment of diabetic animals reverted MDA levels to those of the controls in liver tissue. In kidney, MDA levels were significantly suppressed to values below those found in controls after insulin treatment. This was confirmed by re-analysis of the contralateral kidneys (see above).

Table 1. MDA levels/g organ wet weight in rats one week after induction of diabetes. Median (range). (1) Significantly different from diabetes + insulin and controls; (2) significantly different from controls and untreated diabetes

nmol MDA	Diabetes	Diabetes + insulin	Controls
Kidney	346 (338–375)[1]	211 (196–260)[2]	305 (254–365)
Liver	425 (382–451)[1]	342 (318–392)	357 (315–399)
Heart	265 (238–296)	242 (180–261)	267 (247–305)
Pancreas	271 (236–281)	282 (214–558)	285 (217–360)

In heart, as well as in the pancreas, no differences were detected among the three groups.

Discussion

One of the major consequences of increased oxidative stress is lipid peroxidation, the oxidative degradation of lipids with more than two double bonds (C=C). Cell membrane fluidity, in particular, and consequently the function of cell and intracellular membrane systems, is negatively affected by lipid peroxidation (8). Other consequences of oxidative stress include damage to nucleic acids (13). In theory, increased oxidative stress can be caused by an increased formation of oxygen-derived or other free radicals, or by malfunction of the elaborate intra- and extracellular scavenging systems. A number of reports have suggested alterations in scavenger enzyme function in diabetes (3–6).

Our data suggest that lipid peroxidation is increased at a very early stage in experimental diabetes. This increase was found in both kidney tissue, a primary site for the development of complicated diabetic disease, and liver. Although this suggests a systemic effect, we were unable to demonstrate increased lipid peroxidation in pancreas or heart tissue, thus indicating some organ specificity. Whether the primary defect is to be found in scavenger enzymes or in increased levels of free radicals remains to be determined.

Normalization of the hyperglycaemic state reverted lipid peroxidation to normal. Furthermore, in kidney, insulin depressed MDA levels to subnormal, indicating a direct or indirect effect of insulin (or relative hyperinsulinaemia as found in insulin-treated diabetic rats) itself on the degree of lipid peroxidation in this particular organ.

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