Tr. J. of Medical Sciences
28 (1998) 581-582
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Letter to the Editor

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Protein Sulfhydryl Oxidation in Short-Term Diabetic Rabbit Liver and Kidney Tissues

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Received: March 17, 1997

Nonezymatic glycation of many proteins occurs in diabetes. It has been suggested that this process may play a role in long-term tissue compications (1). In vitro studies in the presence of protein and glucose have shown that glucose can be enolized and thereby made to yield hydrogen peroxide and free radical intermediates (2). In addition to these findings, it has been suggested that glycation of proteins is one of the sources of superoxides (3). The oxidation of proteins by reactive oxygen species has been shown to result in protein modification both at amino acid levels, such as sulfhydryl oxidation, carbonyl formation, bityrosine production, and at structural levels, such as fragmentation and aggregation (4, 5).

In this study we measured sulfhydryl oxidation in the liver and kidney tissues of diabetic male New Zealand rabbits and control animals. Diabetes was induced with a single injection of alloxan (65 mg/kg) via a marginal ear vein. The formation of diabetes was checked on the fourth day by measuring fasting plasma glucose levels (6). At the end of three weeks, animals were dispatched under anesthesia and tissues and blood samples were taken.

Tissues were homogenized by hand-operated homogenizer in cold phosphate buffer (pH 7.4). After centrifugation for removal of debris, protein contents were determined by the modified Lowry method (7) and adjusted to 80-100 μg/ml. Sulfhydryl assay was carried out with cis-dichlorodiammine platinum (II) (cDDP) which binds specifically to methionine and cysteine residues in proteins (8). The unbound cDDP in the deproteinized supernatant was measured and calculated as μmole cDDP/mg protein. The data were expressed as means±SD and the significance was evaluated using the Mann-Whitney U test.

Plasma glucose levels in control (n=10) and diabetic (n=10) rabbits were found to be 101±9 and 373±104 mg/dl, respectively. The unbound cDDP in control group liver (n=10) and kidney (n=10) tissues were 6.10±0.63 and 5.85±0.53, and in diabetic group liver (n=10) and kidney (n=9) tissues 6.01±0.93 and 6.68±0.19 μmole cDDP/mg protein, respectively. There was a significant difference between the control and diabetic kidney unbound cDDP values ($p<0.05$) and no significant difference between the control and diabetic liver unbound cDDP values.

Our data indicated that sulfhydryl groups in protein were oxidized even in short-term diabetic conditions and also that kidney tissue is more suspectible to sulfhydryl oxidation than liver tissue. In a study on streptozotocin-induced diabetic rats, glycated proteins in renal tissues were determied by nitroblue tetrazolium reaction and it was found that the glycation level had increased in 12-week diabetic rats, without histological alteration (9). It has been reported that glycation of proteins contributes to the pathogenesis of diabetic renal complications (10). In conclusion, we state that not only protein glycation but also protein sulfhydryl oxidation are among the biochemical alterations taking place in the pathogenesis of diabetes.

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