Relation of CuZnSOD activity with renal insufficiency in hypertensive diabetic patients

Ana Stancic1, Zorica Rasic-Milutinovic2, Gordana Perunicic-Pekovic3, Biljana Buzadzic1, Aleksandra Korac4, Vesna Otasevic1, Aleksandra Jankovic1, Milica Vucetic1 and Bato Korac1*

1University of Belgrade, Institute for Biological Research “Sinisa Stankovic”, Department of Physiology, Serbia
Zemun Clinical Hospital, Departments of 2Endocrinology and 3Clinical Nephrology, Belgrade, Serbia
4University of Belgrade, Faculty of Biology, Institute of Zoology and Center for Electron Microscopy, Serbia

Received 17 November 2011; revised 14 March 2012

Diabetes and renal insufficiency are interrelated metabolic disorders closely associated with redox homeostasis disturbances. The aim of this study was to compare the activity of copper zinc superoxide dismutase (CuZnSOD) in the erythrocytes of hypertensive diabetic patients with or without renal insufficiency with normal healthy control subjects. In both groups of diabetic patients, blood glucose level and the content of glycosylated hemoglobin (HbA1c) were higher than in the control group. However, CuZnSOD activity was significantly higher than control only in hypertensive diabetic patients with renal insufficiency. Our results suggest that disturbances in superoxide homeostasis do correlate with long-term complication in diabetes, i.e. diabetic renal insufficiency and hypertension.

Keywords: Diabetes, Renal insufficiency, CuZnSOD activity

Renal insufficiency and diabetes mellitus, the two metabolic disorders represent the important health problem worldwide, especially due to the fact that they commonly co-exist. Renal damage is a serious complication of diabetes mellitus. It is estimated that death due to renal disease is 17-times more common in diabetes that in non-diabetics1. Also, about 30-45% of the patients with diabetic nephropathy eventually develop end-stage renal failure2,3.

There are plenty of reports in both diabetics and experimental animal models, showing oxidative/antioxidative equilibrium disturbances in many tissues in diabetes. Whereas some investigations have suggested that reactive oxygen species play a causative role in the etiology and pathogenesis of this disease, other have proposed that oxidative stress may merely be a common consequence of diabetic complications4-6. The same holds true for antioxidative defense, which organization has been reported to be compromised in diabetes7,8.

Earlier, we have shown that diabetes-induced hyperglycemia increases copper, zinc superoxide dismutase (CuZnSOD) activity, the only isoform of $O_2^-$ removing enzyme in the erythrocytes, reflecting adaptive response to enhanced superoxide production9. Also, we have observed that changes in redox status are linked with conspicuous morphological changes of erythrocytes9. Erythrocytes may be more sensitive to oxidative pressure compared to other tissues, owing to their high content of iron and polyunsaturated fatty acids, as well as their role in oxygen transport. Besides, Brown et al.10 have shown that impaired erythrocytes deformability positively correlates with renal failure and diabetes.

Previous studies also suggest that oxidative/antioxidative status may be in correlation with severity of diabetes as well as with diabetes-related long-term metabolic complications, including diabetes nephropathy. However, the precise relation is not defined completely and the data obtained are highly controversial11,12.

Thus, in this study, we have compared CuZnSOD activities in the erythrocytes of hypertensive diabetic patients with or without renal insufficiency with normal healthy control subjects.

*Author for correspondence:
Tel: (381-11)-2078-307
Fax: (381-11)-2761-433
E-mail: koracb@ibiss.bg.ac.rs

Abbreviations: CuZnSOD, copper zinc superoxide dismutase; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ROS, reactive oxygen species; TC, total cholesterol; TG, triglyceride.
Materials and Methods

Subjects and sample collection

Thirty-seven patients (21 women and 16 men), non-diabetic and diabetic were recruited from Zemun Clinical Hospital. Both populations of patients were additionally organized in two subjects groups: i) with renal insufficiency, and ii) without renal insufficiency. The criteria for definition of renal insufficiency and diabetic nephropathy in patients were: i) glomerular filtration rate lower than 60 ml/min/1.73 m$^2$ according to MDRD (modification of diet in renal disease) formula, and ii) proteinuria, defined as protein to creatinine ratio higher than 200 mg protein/g creatinine and/or microglobulin to creatinine ratio higher that 30 mg microglobulin/g creatinine.

All patients were hypertensive and treated with angiotensin converting enzyme (ACE) inhibitors. Diagnosis and standard therapy for diabetes were assessed by World Health Organization criteria. Accordingly, all diabetic patients were treated with insulin therapy and those without renal insufficiency were receiving metformin as well. The control group consisted of eight healthy non-smoking individuals without chronic disorders and any therapy. The Institutional Ethical Committee approved the study protocol and the study was performed in accordance with the standards of Declaration of Helsinki. Written consent was obtained from each subject.

Blood samples were collected after an overnight fast. After plasma separation, the erythrocytes were washed 3-times with physiological saline, centrifuged (3020 g, 10 min) and lysed with cold distilled water$^{13}$. For the measurement of CuZnSOD activity in erythrocytes, hemoglobin was removed by the method of Tsuchihashi$^{14}$. Erythrocyte lysates were stored at -70°C until assayed.

Biochemical parameters

Serum levels of fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were determined using commercial enzyme tests with an automated chemical analyzer (Instrumentation Laboratory-“IL 650”, Werfen group, Austria). Low-density lipoprotein cholesterol (LDL-C) level was calculated using the Friedewald equation. Urea levels were determined by glutamate dehydrogenase method. Glycosylated hemoglobin (HbA$_{1c}$) was measured by immunoassay test.

Antioxidative defence in erythrocytes

CuZnSOD activity was examined by a modified method of Misra and Fridovich$^{15}$. Enzymatic activity was expressed in units/g of Hg. SOD units were defined as the amount of the enzyme inhibiting epinephrine autooxidation by 50% under appropriate reaction conditions. Catalase was assayed as suggested by the supplier (Sigma-Aldrich, St. Louis, Mo.) and the activity expressed in micromoles of H$_2$O$_2$/min/g of Hb.

Results

Clinical determinants as well as hematological and biochemical parameters in the study subjects are summarized in Table 1. Significantly higher urea level was noted in diabetic and non-diabetic patients with renal insufficiency as compared to that in the control subjects (P< 0.001). However, no significant differences in total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride between the patients and healthy subjects were observed.

Table 1—Clinical and biochemical data of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Non-diabetic hypertensive</th>
<th>Diabetic hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>With renal insufficiency</td>
<td>Without renal insufficiency</td>
</tr>
<tr>
<td>Sex, F/M</td>
<td>1/7</td>
<td>5/2</td>
<td>1/5</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>51 ± 2.45</td>
<td>55 ± 3.1</td>
<td>54 ± 2.62</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>27.31 ± 0.24</td>
<td>23.4 ± 0.29</td>
<td>25.72 ± 0.31</td>
</tr>
<tr>
<td>History of diabetes (Yrs)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>5.78 ± 0.27</td>
<td>22.52 ± 7.40***</td>
<td>7.8 ± 0.26</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.6 ± 0.45</td>
<td>5.5 ± 0.65</td>
<td>5.1 ± 0.85</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.22 ± 0.14</td>
<td>1.07 ± 0.14</td>
<td>1.21 ± 0.03</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.47 ± 0.09</td>
<td>3.56 ± 0.54</td>
<td>3.37 ± 0.84</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.14 ± 0.36</td>
<td>1.91 ± 0.21</td>
<td>1.15 ± 0.07</td>
</tr>
</tbody>
</table>

*Compared to control, *** P< 0.001
As can be seen from Fig. 1, blood levels of glucose and HbA\textsubscript{1c} were higher in both groups of diabetic patients with or without renal insufficiency than in healthy subjects.

The activities of CuZnSOD and catalase in erythrocytes are given in Fig. 2. As seen, CuZnSOD activity was significantly elevated in diabetic patients with renal insufficiency (P<0.01) in comparison with the control subjects. In contrast, no significant differences in catalase activity between the examined groups were observed.

**Discussion**

In this study, we found that blood glucose and HbA\textsubscript{1c} levels were higher than control in both groups of hypertensive diabetic patients, while erythrocytes CuZnSOD activity was increased only in the patients with co-existing diabetes, hypertension and renal insufficiency, but not in hypertensive diabetics without nephropathy. These results suggest that disturbed superoxide equilibrium in diabetes do correlate with functional disturbances in the kidney.

The effect of diabetes on antioxidative defense, especially the activity of CuZnSOD was erratic with no discernable pattern based on sex, duration of diabetes, its severity and tissue studied. Erythrocytes CuZnSOD activity is reported to be increased\textsuperscript{16}, decreased\textsuperscript{17} and not affected by diabetes\textsuperscript{18}. Our recent study in diabetic patients\textsuperscript{9} has shown the increased CuZnSOD activity in erythrocytes and its relation with increased plasma lipid peroxidation and erythrocytes morphological abnormalities. Additionally, the present study showed that increased activity of this enzyme accompanied diabetes-related disturbances in kidney function. Furthermore, CuZnSOD activity was increased only in diabetic patients with coexisting hypertension and renal insufficiency. It has been shown previously that coupled hypertension and diabetes is a state, where the increase in superoxide production in platelet is greater that in hypertension alone\textsuperscript{19}, and that hypertension exacerbates pro-oxidant generation in the diabetic kidney\textsuperscript{20}.

It is still less certain whether oxidative stress contributes to the devolvement of long-term complications or merely reflects associated processes that are affected by diabetes\textsuperscript{21,22}. Although we can not categorically refute that the increased oxidative pressure may be secondary to renal
insufficiency, this scenario seems unlikely, because erythrocytes from non-diabetic subjects with renal insufficiency showed similar CuZnSOD activity to that of both control and diabetic subject without nephropathy. Thus, it is proposed that renal impairment is the consequence of high oxidative pressure in diabetes, and not its cause.

Chronic hyperglycemia in diabetes is usually considered as the main source of reactive oxygen species. The observation that despite hyperglycemia in both groups of diabetic patients, only a portion of the patients population will progress to diabetic nephropathy indicated that there was individual diversity in cell response to high glucose concentrations. Besides, our results suggested that metabolic disturbances in kidney could not be linked to the lipid status, since no differences were observed in the lipid profile between groups. So, mechanisms that determine the nephropathy-prone phenotype in diabetic population remain to be elucidated. It has been suggested previously that clinical manifestations of diabetes, including kidney dysfunction are complex multi-factorial phenomena that are affected by an interaction of environmental, familiar and genetic influences.

In conclusion, our results suggest that disturbances in superoxide homeostasis correlate with renal insufficiency and hypertension accompanying diabetes. Further studies aimed to elucidate redox-related molecular mechanisms underlying etiopathology of diabetic metabolic complications, especially diabetic nephropathy, are in progress.

Acknowledgments

This work was supported by the Ministry of Education and Science of the Republic of Serbia, Grant No. 173055.

References

14. Tsuchihashi M (1923) Biochem Z 140, 65-74