Clinical Research

Overproduction of nitrate and S-nitrosothiols in diabetic patients

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Objective The present study was designed to investigate changes in serum or plasma concentrations of nitric oxide and its derivatives in diabetic patients. Methods Serum nitrate concentration of 84 diabetic patients was measured by using an enzyme kinetic method, and the plasma S-nitrosothiols concentration of 10 cases was measured by using HPLC technique. Results Serum nitrate concentration and plasma S-nitrosothiols concentration in the diabetics were significantly higher than in control group (P<0.01 and P<0.05, respectively). The serum nitrate concentration in diabetics also had a significant positive correlation with the serum glucose concentration (R=0.7256, P<0.05), but this correlation was not found in control group. Conclusion These data showed that NO and its derivatives are overproduced in the diabetic patients. (J Geriatr Cardiol 2008; 5:25-27)

Key Words S-nitrosothiols; nitric oxide; diabetics

Introduction

Nitric oxide (NO) in biological system is synthesized by nitric oxide synthase (NOS) which at least has three distinct isozymes. All of these three isoforms are implicated in physiological or pathophysiological process of the cardiovascular system. NO plays important roles in the control of the balance of cardiovascular system, keeping the vascular lature dilation, protecting the vascular intima from platelet aggregates and leukocyte adhesion, and inhibiting smooth muscle proliferation. Recent studies have confirmed that cardiovascular disease is often associated with hypercholesterolemia and diabetes. Oxidative stress and the inactivation of NO by superoxide anions play an important role in these disease states. Furthermore, several studies have showed that abnormal metabolism of NO may contribute to diabetes. To investigate the relationship between abnormal metabolizing of NO and diabetes, the concentration of human serum nitrate and the plasma NO complexes S-nitrosothiols in diabetes has been studied in the present work.

Subjects and methods

Subjects

For the measurement of serum nitrate concentration, 84 diabetic patients (48 males and 36 females), with an mean age of 47.8 years (25-71 years) were enrolled as the diabetic group. The control group consisted of 50 healthy blood donors (29 males and 21 females, mean age, 47.2 years).

Measurement of serum nitrate concentration

Subjects were deprived of food overnight. Blood was drawn by venipuncture into serum separation tubes, allowed to clot for 45 min at 37°C water bath and centrifuged at 3000g for 5 min. The supernatant was collected and stored at -40°C. NO3 concentration was determined by using an enzyme kinetic method. Nitrate can be reduced to nitrite by nitrate reductase, consuming reductive coenzyme NADPH. Nitrate concentration can be calculated from the decrease in OD340 of NADPH.

Measurement of plasma S-nitrosothiols

Subjects were deprived of food overnight. 2.0 ml of blood was collected into tubes containing 0.5ml of anticoagulate (0.1M EDTA in 1M pH 3.8 phosphate buffered saline) and centrifuged at 1300g for 10min. The plasma was separated and kept under -40°C for future use. The detection of S-nitrosothiols was performed according to modified Goldman method. Twenty three wveiliter aliquots of 20% HgCl2 (in 1M HCl), 454 ml of 10% p-aminobenzene sulfonic acid (in 15% ethylene glycol and 85% 2.2M HCl) and
108 ml of 0.36% \( \text{HgCl}_2 \) were added into 650 ml of plasma, while 20% \( \text{HgCl}_2 \) was substituted with 23 ml of 1M \( \text{HCl} \) in control tubes. The mixture was incubated in dark at 25 \( ^\circ \text{C} \) for 150 min before the addition of 225 ml of HPLC grade acetonitrile. After another 30 min incubation, protein in the mixture was eliminated by filtration. Forty willilliters of supernatant were measured by HPLC on HP1100. The stationary phase consisted of a column(150mm \( \times 4.6\)mm) packed with ODS-3, 5 mm particles size. The mobile phase was 24% solution A (acetonitrile/ water, 50/50, v/v), 20% solution B (0.1M \( \text{H}_3\text{PO}_4 \)) and 56% water, the pH of which was adjusted to 3.0 by \( \text{NH}_3 \cdot \text{H}_2\text{O} \). The mobile phase was gradually changed into 80% solution A and 20% solution B within 30 min. The effluent was monitored at 350nm and 532nm.

**Statistical analysis**

Results were expressed as mean \( \pm \text{SD} \). Statistical analyses of significance were performed using unpaired Student’s \( t \) test. The correlation of serum nitrate concentration and blood glucose concentration was analyzed by linear regression.

### Results

**Changes in serum nitrate concentration in diabetic patients**

Serum nitrate concentration was significantly higher in diabetic patients than in control group (\( P < 0.01 \)) (Table 1), which suggested that there was an abnormal metabolism of NO and oxygen free radical in diabetics patients. Since NO and S-nitrosothiols concentration in diabetic patients was also higher than in control group (\( P < 0.05 \)) (Table 2).

**Table 1. Serum nitrate concentration in diabetic patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Serum nitrate concentration (mean ( \pm \text{SD} ), ( \mu \text{M} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics</td>
<td>84</td>
<td>62.70 ( \pm )16.33**</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>33.56 ( \pm )10.24</td>
</tr>
</tbody>
</table>

**Correlation between serum nitrate concentration and blood glucose concentration**

The correlation between serum nitrate concentration and blood glucose concentration was statistically significant (\( r=0.7256, P<0.05 \)), suggesting that there was a high level of NO metabolizing in diabetic patients. NO and its complexes oxidize or combine with biomacromolecules, making obstacle to the production and utilization of insulin. However, the increase in blood glucose in the healthy resulting from transient high-sugar diet did not lead to the increase in serum nitrate concentration. The changes in serum nitrate concentration were poorly correlated with the changes in blood glucose concentration in healthy blood donor. These results suggested that the high level of NO and its derivatives in diabetics is one of the important factors resulting in disorders in glucose metabolism.

**Discussion**

Studies have been shown that the development of diabetes mellitus is associated with abnormal metabolism of NO,1,2,9 Yamada et al10 found that the expression of NO synthase mRNA in rat pancreatic islet cells can be induced by IFN-\( \gamma \) and TNF. It is also found that the development of diabetes could be induced in animal by STZ with nitro-group, but not by STZ without nitro-group. The high level of NO in body may result in the methylation and the split of nuclear and mitochondrial DNA in pancreatic islet \( \beta \)cells, which leads to the dysfunction in pancreatic islet cells and the decrease in the secretion of insulin.

As a small molecular free radical with extensively biological activity, NO is prone to be oxidized in oxygenic circumstances so that it is difficult to be transferred in authentic NO gas.2 NO is usually combined with other molecules, such as reduced thiols6,7, to form the stable complexes or be oxidized. The end oxidation product of NO in biological systems is nitrate. The changes of nitrate and S-nitrosothiols in blood could reflect the metabolism of NO in vivo. The results in this study showed that there was a high level NO and its derivatives in diabetics, which further confirmed that overproduced NO is a major pathological reason of diabetes mellitus.

**References**

2. Tare M, Parkington HC, Coleman HA, EDHF, NO and a prostanoid: hyperpolarization-dependent and -independent re-