Antioxidant effect of zinc, selenium and their combination on the liver and kidney of alloxan-induced diabetes in rats

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Introduction

A number of studies have reported an association between diabetes mellitus (DM) and alterations in the metabolism of several trace minerals (e.g., chromium, magnesium, selenium, vanadium and zinc) [1]. Some of these minerals (e.g., zinc, chromium, magnesium) are excreted at higher than normal rates in patients with DM, often leading to excessive urinary mineral wasting [2]. The general characteristic polyuria in patients with DM that results from the glucose-mediated hyperosmotic glomerular filtration may be largely responsible for the urinary mineral losses. A lowered availability of minerals may affect optimal insulin secretion and/or action. Increased dietary intake of these minerals or utilising supplemental sources of the minerals may replenish the losses.

Control of free radical production by selenium has been postulated to be of benefit in preventing glucose intolerance and the complications of DM. The insulin-like properties of selenium (translocation of glucose transporters to the plasma membrane) have been demonstrated in in vitro studies using adipocytes. Selenium also improves polydipsia and polyuria to controllable levels. [5] Moreover, the normally observed cardiac function decrements seen in such animals were prevented by selenium.

Zinc and selenium have been shown to singly act in normalising glycaemia and are also postulated to possess insulin-like functions. Supplementation with their combination in the diets of rats with alloxan-induced diabetic diabetes was investigated with the aim of investigating their effects on glucose homeostasis and their antioxidant properties on the liver and kidney of alloxan-induced diabetic rats. Thirty-five rats were randomly assigned to five groups and four groups were made diabetic by the administration of 150 mg/kg body weight of alloxan monohydrate, after which three diabetic groups were fed with diets supplemented with zinc, selenium and a combination of the two. Zinc, selenium and the combination significantly reduced blood glucose concentration, restored hepatic functions, increased the antioxidant status of the diabetic rats and reduced lipid peroxidation in both the hepatic and renal tissues. It was concluded that supplementation with zinc, selenium and the combination facilitated glucose uptake, prevented oxidative stress, reduced lipid peroxidation and preserved hepatic function in diabetes.

Keywords Zinc · Selenium · Diabetes · Free radicals · Catalase · Glutathione
insulin-like functions. A restored Zn and Se status in people with DM may counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes. Therefore, this study was aimed at investigating the effects of single and combined Zn and Se supplementations on variables associated with glucose metabolism and their antioxidative properties on the liver and kidney of alloxan-induced diabetic rats.

Material and methods

Animals

Thirty-five albino rats (75–95 g) of the Wistar strain were obtained from the disease-free stock of the Animal Unit of the Biological Sciences Department, University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were housed in metabolic cages. They were weighed and acclimatised to animal house condition for seven days. They were fed semi-purified standard diet for normal rats (Table 1) and had free access to water.

After acclimatisation, the rats were reweighed and fasted overnight (deprived of food for 12–14 h but had free access to water). Fasting blood glucose level was determined with the aid of a glucometer (Accu-chek Active, Roche Diagnostics, Indianapolis). Four groups of rats were made diabetic (diabetic groups) by the intraperitoneal administration of alloxan monohydrate (Sigma, St Louis, MO, USA) (dissolved in physiological saline) at a concentration of 150 mg/kg body weight while the fifth group (non-diabetic control (NDC)) were injected with an equivalent amount of saline. The feed intake was recorded daily and the body weight taken weekly. The cages were cleaned daily to maintain a proper hygienic conditions for the rats.

After one week of alloxan administration, the preprandial blood glucose concentration was again determined. The rats with blood glucose concentration 3–5 times the initial blood glucose concentration of the rats prior to the administration of alloxan were considered diabetic. The four groups of diabetic rats were regrouped based on equalised mean blood glucose concentration. The 5 groups of rats were designated as follows: 1. Group 1. Non-diabetic control (NDC): Rats fed normal diet.
2. Group 2. Diabetic control (DC) group: Diabetic rats fed normal diet as the NDC.
3. Group 3. Zinc (Zn) group: Diabetic rats fed normal diet supplemented with 1000 mg/kg of diet of zinc as zinc sulphate.
4. Group 4. Selenium (Se) group: Diabetic rats fed normal diet supplemented with 8 mg/kg of diet of selenium as sodium selenate.
5. Group 5. Zinc+selenium (ZnSe) group: Diabetic rats fed normal diet supplemented with a combination of zinc (1000 mg/kg diet) and sodium selenate (8 mg/kg diet).

The rats were fed for four weeks on a semi-purified diet for experimental rats (Table 1). In the experimental diets, zinc and selenium were included and the percentage of carbohydrate was modified according to the composition of zinc and selenium to establish the same total percentage. Fasting blood glucose concentration was determined at the end of the experiment. The rats were killed after anaesthesia with ether and the livers and kidneys were removed; weights were recorded and any morphological changes were noted. The hepatic and renal tissues were homogenised in normal saline (1 g tissue in 4 ml of saline) and kept at 4°C before use.

Biochemical analysis

Fasting blood glucose concentration was determined with a One Touch Glucometer (Lifescan, Mulpital, CA) after blood was drawn from the tail. Tissue protein concentration was determined by the method of Lowry et al. [6]. Catalase activity was determined according to the method described by Tukahara et al. [7] where the rate of decomposition of hydrogen peroxide (H₂O₂) was measured at 570 nm. Reduced glutathione (GSH) was measured by the method of Sedlak and Lindsay [8] where protein-bound sulphhydryl group in tissues was estimated

Table 1 Composition of diet in g/100 g of diet

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Normal diet</th>
<th>Zinc diet</th>
<th>Selenium diet</th>
<th>Zinc+selenium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>50.00</td>
<td>49.90</td>
<td>49.9992</td>
<td>49.8992</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Soy protein</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Mineral mixa</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
<td>3.70</td>
</tr>
<tr>
<td>Vitamin mixb</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>Zinc</td>
<td>–0.10</td>
<td>–</td>
<td>0.10</td>
<td>0.0008</td>
</tr>
<tr>
<td>Selenium</td>
<td>–</td>
<td>–</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

aMineral mix contained the following in g/100 g: calcium phosphate (49.50), sodium powder (11.80), potassium sulphate (5.20), sodium chloride (7.40) magnesium oxide (2.40), potassium citrate (22.40), ferric citrate (0.60), manganese carbonate (0.35), cupric carbonate (0.03), zinc carbonate (0.16), chromium potassium sulphate (0.055), potassium iodate, (0.001), sodium selenate (0.001), choline chloride (0.50)
bVitamin mix contained the following in g/100 g: thiamine HCl (0.06), riboflavin (0.06), niacin (0.30), calcium pantothenate (0.16), biotin (0.010), vitamin B12 (0.10), vitamin D3 (0.025), vitamin E acetate (1.00), pyridoxine (0.07), folic acid (0.02), vitamin A acetate (0.08)