Serum magnesium, copper and zinc concentration changes in lower limb ischemia and postoperative treatment

M. Iskra¹, D. Barałkiewicz², W. Majewski³, M. Pioruńska-Stolzmann¹

¹ Department of General Chemistry, Poznań University of Medical Sciences, Poland; ² Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland; ³ Department of General and Vascular Surgery, Poznań University of Medical Sciences, Poland

Correspondence: Dr. M. Iskra, Department of General Chemistry, University of Medical Sciences, 6 Grunwaldzka Street, 60-780 Poznań, Poland. Tel./fax: (+00 48 61) 854 65 89. <iskra@amp.edu.pl>

Abstract. A prolonged state of ischemia of the lower limbs may affect the balance of some metal ion concentrations in blood. Ischemia of a critical degree, surgery and complicated postoperative periods invoke an inflammatory response, increased production of interleukin-6 (IL-6) and acute phase proteins (APhPs) as a response to tissue destruction. The aim of the present study was to investigate the modification of Mg, Cu and Zn concentrations in the serum of patients with atherosclerosis obliterans (AO) before surgery and during the postoperative treatment, and the effect of chronic ischemia of the lower limbs on the relationship between the elements. The group studied consisted of 54 men with chronic ischemia of the lower limbs due to AO. The mean value of serum Mg concentration in men with AO was found to be significantly lower, and that of Cu to be higher, in comparison with the control group. In critical ischemia, the mean serum Cu concentration and the ratio Cu/Zn were found higher than in moderate ischemia. The postoperative treatment results in changes in Cu and Zn concentrations in the AO group that are inversely related to the levels before surgery.

Keywords: magnesium, copper, zinc, serum, lower limb ischemia

Chronic ischemia of the lower limbs results from a decreased supply of oxygen and nutrients due to atherosclerosis obliterans. Ischemia of a critical degree invokes an inflammatory response, activation of immune cells, increased production of interleukin-6 (IL-6) and acute phase proteins (APhPs) as a response to tissue destruction. Surgery and complicated postoperative treatment may also result in an increase in the concentration of cytokines and APhPs. Ischemia affects the integrity of the cell membrane, the permeability of blood microvessels, concentrations of intracellular and extracellular electrolytes and free radical generation. Changes in electrolyte concentrations in blood plasma and tissues have been observed in cardiovascular diseases, atherosclerosis, arterial hypertension and diabetes mellitus. Inflammatory changes, occurring in patients with chronic ischemia and undergoing surgical treatment, may alter macro- and trace element concentrations in blood. Serum magnesium concentration was found decreased in some patients with myocardial infarction [1], after heart surgery [2], and traumatic injury [3]. Hypomagnesemia, with total plasma Mg concentration below 0.75 mmol/L, was shown in one third of the critically ill postoperative patients [4]. Dietary magnesium deficiency associated with oxidative stress was recognized as a pathogenic factor in inflammatory processes, cardiovascular pathology, and ageing [5, 6]. Copper is essential for main-
taining the structure and function of some proteins and antioxidants, and seems to be involved in the progress of atherosclerosis. Although deficiency or marginal intake of Cu has been proposed as a risk factor for cardiovascular diseases, serum Cu concentration was found to be increased in atherosclerosis obliterans (AO) [7]. Severe loss of serum Zn and a significant increase in Cu were observed during inflammation [8-10] and after cardiopulmonary bypass [11]. Zinc involvement in the inflammatory processes may result from its indispensability for the activity of Cu,Zn-SOD [12], the induction of metallothionein synthesis, and the stabilization of cell membrane [13]. The mechanism of Zn antioxidant properties remains unclear, but the stimulation of reactive oxygen species (ROS) generation was observed in Zn deprivation [14].

The aim of the present study was to investigate the modification of Mg, Cu and Zn concentrations in serum of inpatients with AO before surgery and during the postoperative treatment, and the effect of chronic ischemia of lower limbs on the relationship between elements.

Materials and methods

Subjects

The studied group consisted of 54 men with chronic ischemia of the lower limbs due to AO. Patients were admitted to the Department of General and Vascular Surgery at University of Medical Sciences in Poznań, Poland. The age of patients ranged from 40 to 83 years (58.5 ± 11.0). In all patients aortography and ultrasound measurement of ankle blood pressure was performed before surgery (vascular reconstruction). Patients were divided into two subgroups according to the degree of ischemia of lower limbs: moderate ischemia (MI) – intermittent claudication and ankle systolic blood pressure above 50 mmHg (25 patients), and critical ischemia (CI) – rest pain and/or ulceration or necrosis, and ankle systolic pressure below 50 mmHg (29 patients). The classification was done according to the ankle systolic pressure index following the European Report of Critical Limb Ischemia [15, 16]. Patients were not supplemented with Mg, Cu and Zn before or after surgery, and those with hypertension, cancer and kidney diseases were excluded from the study. The control group comprised 24 healthy male blood donors, aged 20-59. They underwent a routine medical checkup before the blood collection was done.

Blood samples were collected in the fasting state from the brachial vein in the group of men with AO and in the control group. In AO group, blood samples were collected before surgery and in the postoperative period of 1-4, 7-12 and after 12 days of treatment. The group of patients with AO studied during the postoperative treatment was 50, i.e. 4 patients less than before surgery. In order to analyse the results obtained during the postoperative treatment for a more consistent group of patients, 4 patients (one with MI and three with CI) were excluded by reason of complicated postoperative treatment (second surgery, myocardial infarction, cancer). The study was approved by the Ethical Committee at the University of Medical Sciences in Poznań, Poland.

Methods

The concentration of Mg, Cu and Zn in serum was determined by using Varian SpectrAA Plus atomic absorption spectrometer with deuterium background correction and a GTA-96 graphite furnace. Certified reference material (Trace elements in serum, level 1) from LGC Promochem, UK, was used.

Concentration of interleukin-6 (IL-6) was measured by ELISA (Quantikinine, R&D Systems, Minneapolis, USA), and those of C-reactive protein (CRP), α1-glycoprotein (AGP) and ceruloplasmin (Cp) with rocket immunoelectrophoresis by using rabbit antibodies (Dakopats, Copenhagen, Denmark) and human standard serum (Behringwere AG Marburg, Germany).

Oxidase activity of Cp in serum was measured spectrophotometrically after incubation with o-dianiside as a substrate according to Schosinsky [17].

Element and Cp concentrations and the activity of Cp (expressed as mean ± standard deviation) were compared by Student’s t-test. The results for IL-6, CRP and AGP (presented as medians) were compared between groups using Mann-Whitney unpaired test.

Results

The mean values from baseline (0 days) determinations of Mg, Cu and Zn, namely before surgery, for the whole AO group, MI group separately from CI group, and for the control group are presented in Table 1. The mean values of serum Mg and Cu concentrations in men with AO were found significantly different in comparison with the control group. Before surgery, increased Cu concentration and decreased Mg were determined, followed by a subsequently lower concentration ratio Mg/Cu and higher Cu/Zn in AO than in the control group. Ischemia of the lower
limbs affects the concentration of Cu, but not that of either Mg or Zn. In men with critical ischemia the mean values of serum Cu concentration and the ratio Cu/Zn were found higher, when compared with the group of moderate ischemia.

Levels of inflammation markers and APhePs respond to a chronic state of ischemia of the lower limbs. IL-6, CRP and AGP concentrations (expressed as medians) before surgery were observed to be higher in CI than in MI patients. The mean Cp concentration and its oxidase activity were found significantly increased in CI as compared with MI (table 2).

During the postoperative treatment a significant increase in the mean serum Cu concentration after 12 days (C3), and in the mean Zn concentration after the period of 7-12 days (C2), were observed (table 3) when compared with the values found before surgery (C0).

The changes in Mg, Cu and Zn concentrations in the postoperative treatment were calculated for three periods of 1-4 (Δ1), 7-12 (Δ2) and more than 12 days (Δ3) after surgery according to the formula: Δx = Cx - C0. Respective concentration changes (Δx) and the negative correlation coefficients r (C0 vs Δx) with the concentration of the element before surgery (C0) were calculated (table 3).

### Discussion

Increased levels of chosen biomarkers of acute phase reaction, found in patients with CI as compared with the MI group (table 2), indicate inflammation while experiencing severe ischemic changes in the lower limbs. Ischemia-reperfusion events and inflammatory responses cause increased production of reactive oxygen species and alterations of macro- and trace element concentrations [18]. The impact of chronic lower limb ischemia and the postoperative treatment on Mg, Cu and Zn in human serum has not been considered efficiently. The modulatory effect of Mg status on immune cell function has been observed in vitro [19] and on reperfusion injury in patients with acute myocardial infarction pretreated with Mg sulfate [20]. The nutritional status plays an important role in modification of diseases related to inflammation [21]. An early consequence of Mg deficiency recently observed in Mg-deficient rats was a significant increase in IL-6 in plasma, suggesting that reduced extracellular Mg might be responsible for the activated state of immune cells [22]. Serum Mg concentration in humans is often observed normal

### Table 1. Mg, Cu and Zn concentrations and their ratios in serum of men with atherosclerosis obliterans (AO), with critical (CI) and moderate ischemia (MI) of lower limbs due to AO, and in control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mg (mmol/L)</th>
<th>Cu (μmol/L)</th>
<th>Zn (μmol/L)</th>
<th>Mg/Cu</th>
<th>Mg/Zn</th>
<th>Cu/Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>AO</td>
<td>N = 54</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>CI</td>
<td>N = 29</td>
<td>0.84 ± 0.26</td>
<td>22.3 ± 4.7</td>
<td>13.8 ± 4.1</td>
<td>41.8 ± 10.2</td>
<td>64.2 ± 20.1</td>
</tr>
<tr>
<td>MI</td>
<td>N = 25</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a,c</td>
</tr>
<tr>
<td>Control</td>
<td>N = 24</td>
<td>0.89 ± 0.13</td>
<td>16.1 ± 2.2</td>
<td>14.6 ± 2.1</td>
<td>53.6 ± 12.8</td>
<td>52.7 ± 14.0</td>
</tr>
</tbody>
</table>

*a – significant difference versus control, b – versus AO, c – CI versus MI, p ≤ 0.05.

### Table 2. Concentration of IL-6, CRP, AGP (median), Cp and the oxidase activity of Cp (mean ± SD) in serum of men with lower limb critical (CI) and moderate ischemia (MI).

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-6 (ng/L)</th>
<th>CRP (mg/L)</th>
<th>AGP (mg/L)</th>
<th>Cp (mg/L)</th>
<th>Cp (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 25</td>
<td>24</td>
<td>1</td>
<td>720</td>
<td>442 ± 186</td>
<td>123.1 ± 38.3</td>
</tr>
<tr>
<td>CI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 29</td>
<td>62*</td>
<td>44*</td>
<td>1045*</td>
<td>655 ± 175*</td>
<td>177.8 ± 63.1*</td>
</tr>
</tbody>
</table>

*p ≤ 0.05.
despite depletion of its total level in the human organism [23]. Lower Mg found in serum of the group of patients studied with AO, in comparison with the control subjects, may influence numerous regulatory functions of Mg²⁺ ions in hormonal, cardiovascular and immune systems. Depletion of Mg in plasma induces higher susceptibility of the lipoproteins to the oxidative stress, and a possible pro-oxidant effect [24, 25]. Mg plays a critical role in the function of mitochondrial ATP and its decrease in ischemia may suggest inadequate ATP synthesis, and lower level of ATP for the metabolic processes. There are contradictory observations of the impact of ischemia on serum Mg concentration [10, 26]. In the present study Mg concentration in serum was not significantly influenced by ischemia and was maintained at a similar level during the postoperative treatment. It may only be concluded that the measurement of intracellular Mg concentration in the erythrocytes and the estimation of the ratio of ionized/total Mg plasma concentrations in patients with ischemia would better reflect Mg changes in the inflammatory response.

Ionized Mg (i-Mg) should be considered as more relevant clinical marker of Mg metabolism, and i-Mg in serum has been suggested by other authors to reflect more adequately the status of Mg in blood. However, the insufficient quality and the lack of standardized procedure for i-Mg measurement with the selective electrodes means that i-Mg is currently not used in clinical practice [27]. Moreover, there is strong evidence that total-Mg (t-Mg) and i-Mg are very closely related. Therefore, serum t-Mg is the most practical and commonly used parameter for assessing disorders of Mg metabolism in clinical practice [28]. In the present study, the t-Mg in serum was determined because we intended to follow the total Mg changes in the postoperative treatment, and find possible relationships with levels of other elements (Cu, Zn). Dramatic changes in Mg after surgery, and differences between the AO and the control groups were not observed. Even under such deleterious conditions as ischemia of different degrees may cause, no statistically significant difference was found in the total Mg concentration in serum between MI and CI groups.

It may only be suggested that t-Mg in serum represents quite a stable value, regulated homeostatically, and its level should not be recognized as a marker of atherosclerotic and ischemic alterations to the whole organism. On the other side, the elucidation of the mechanism of Mg homeostasis in atherosclerosis and ischemia needs further study, including the ionized and total Mg determinations. It is suggested that a speciation of Mg in blood should be performed, i.e. find and determine free and bound forms of Mg(II) ions and follow their changes during the postoperative treatment.

Dietary deficiency or marginal intake of Cu was recognized as a risk factor for cardiovascular diseases [29]. However, in serum of patients with critical ischemia of the lower limbs due to atherosclerosis.

### Table 3. Concentrations of Mg, Cu and Zn in serum of men with AO before surgery and in the postoperative treatment (n = 50).

<table>
<thead>
<tr>
<th>Element</th>
<th>Days after surgery</th>
<th>C₀ (mean ± SD)</th>
<th>Δₓ</th>
<th>r (C₀ vs Δₓ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>0</td>
<td>C₀ 0.80 ± 0.20</td>
<td>Δ₁ -0.030</td>
<td>r₁ = -0.2407</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>C₁ 0.83 ± 0.25</td>
<td>Δ₂ 0.003</td>
<td>r₂ = -0.2670</td>
</tr>
<tr>
<td></td>
<td>&gt; 12</td>
<td>C₃ 0.83 ± 0.29</td>
<td>Δ₃ 0.049</td>
<td>r₃ = 0.3197</td>
</tr>
<tr>
<td>Cu</td>
<td>0</td>
<td>C₀ 21.7 ± 3.8</td>
<td>Δ₁ -0.451</td>
<td>r₁ = -0.5768 **</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>C₁ 21.3 ± 6.2</td>
<td>Δ₂ 0.928</td>
<td>r₂ = -0.6759 **</td>
</tr>
<tr>
<td></td>
<td>&gt; 12</td>
<td>C₃ 25.1 ± 5.0</td>
<td>Δ₃ 5.238</td>
<td>r₃ = -0.7048 **</td>
</tr>
<tr>
<td>Zn</td>
<td>0</td>
<td>C₀ 13.8 ± 2.9</td>
<td>Δ₁ 0.050</td>
<td>r₁ = -0.1477</td>
</tr>
<tr>
<td></td>
<td>1–4</td>
<td>C₁ 13.8 ± 4.3</td>
<td>Δ₂ 1.466</td>
<td>r₂ = -0.5324 **</td>
</tr>
<tr>
<td></td>
<td>&gt; 12</td>
<td>C₃ 13.9 ± 2.3</td>
<td>Δ₃ 0.671</td>
<td>r₃ = -0.8223 **</td>
</tr>
</tbody>
</table>

Δₓ - the difference between the value of element concentration in the postoperative treatment in comparison with the value obtained before surgery (0 day) (Δₓ = Cₓ - C₀). r₁, r₂, r₃ - correlation coefficients calculated between 0 day value and Δ₁, Δ₂, Δ₃ respectively, i.e. C₀ versus Δₓ. * - significant difference in comparison with 0 day, p ≤ 0.05. ** - significant value of the correlation coefficient, p ≤ 0.05.
crosis, the Cu concentration was found to be significantly increased and positively correlated with the oxidase activity of Cp, not only in this study, but also in all previously studied groups of patients with critical ischemia of the lower limbs due to AO [30-32]. The increase in serum Cu and the activity of Cp, an APHP in plasma, was also found in patients with chronic lower limb ischemia [33] and in patients with coronary heart disease undergoing coronary angioplasty [34]. The present results show that the postoperative treatment may affect serum Cu concentration, and also, what is more interesting, the magnitude of changes is in inverse relation to the level before surgery.

Decreased plasma Zn concentration was observed in the inflammatory state and seemed to be a result of the modification to Zn distribution in the organism and a protective mechanism in inflammation [35]. The immune dysfunction in Zn deficiency involves an increase in T lymphocytes and cytokine production followed by enhanced free radical generation. Zinc may be capable to retard oxidative processes through different mechanisms, namely as a component of the antioxidants: Cu,Zn-SOD and metallothioneins, and an antagonist of copper [14]. A deficiency of Zn in rats stimulates the generation of reactive oxygen species by decreased Cu,Zn-SOD activity and results in oxidative damage [19]. The results of the present study showed that AO and ischemia did not affect Zn levels in serum. A rise in Zn observed at 7-12 days after surgery followed by normalization may suggest a shift of Zn from tissues or erythrocytes to blood plasma as a consequence of an increased demand for this element after surgery. Moreover, Zn concentration seems to be homeostatically regulated as the changes in Zn observed during treatment were found inversely correlated with the concentration of Zn before surgery. Speciation of Zn in plasma/blood during the postoperative treatment might help to elucidate this phenomenon. The level of Zn in erythrocytes and concentration of α2-macroglobulin, another acute phase reactant, should be also analyzed.

It was shown previously that the ratio Mg/Cu is affected by ageing, atherosclerosis, and the degree of ischemia, and its value differs between serum and the arterial wall [26]. It was also suggested that serum Mg/Cu might be a marker of distorted equilibrium between Mg and Cu, because it reflects their relationship better than element concentrations. In this study it was shown that the value of the serum Cu/Zn ratio also differs between control and atherosclerotic subjects, and increases in critical ischemia when compared to moderate degree ischemia. It may be postulated that the ratios Mg/Cu and Cu/Zn may be markers of the impaired relationships between those elements in atherosclerosis and ischemia.

The present results showed the inverse relationship between baseline concentration both Cu and Zn in serum and their changes in the postoperative treatment. It may give rise to further questions about the effect of the magnitude of inflammation on the regulation of Cu and Zn concentrations, and also other trace elements, in circulation in patients with lower limb ischemia.

The correlations found for both Cu and Zn before surgery and changes observed during the postoperative treatment may suggest a modulatory mechanism for Cu and Zn serum concentrations after surgery, i.e. the lower value before surgery may result in a rise in Cu and Zn concentrations after surgery (and vice versa).

The importance of some nutrients for the immune system and the antioxidant status of the human body support the opinion that the sequence of events leading to the modification of macro- and trace element concentrations during ischemia and the inflammatory response needs to be elucidated. The relationship between Mg, Cu and Zn concentrations in monitoring the postoperative treatment needs more consideration.

Conclusions

Chronic ischemia of the lower limbs affects serum concentrations of Mg and Cu, and the ratios Mg/Cu and Cu/Zn. Critical ischemia of lower limbs increases serum Cu concentration, and the oxidase activity of Cp, the main protein carrier of Cu in plasma. It may be postulated that the ratios Mg/Cu and Cu/Zn may be markers of the impaired relationships between those elements in atherosclerosis and ischemia. Postoperative treatment results in changes in Cu and Zn concentrations that are inversely related to the levels before surgery.

Acknowledgements

This work was supported by a Grant No 502-2-80-07 from Poznań University of Medical Sciences and Adam Mickiewicz University in Poznań, Poland.

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