CHANGES IN SOME DIVALENT CATIONS CONCENTRATIONS IN AN EXPERIMENTAL RAT MODEL OF GENTAMICIN-INDUCED ACUTE RENAL FAILURE

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CHANGES IN SOME DIVALENT CATION CONCENTRATION IN AN EXPERIMENTAL RAT MODEL OF GENTAMICIN-INDUCED ACUTE RENAL FAILURE (Abstract) Aim: The changes in divalent cations concentration were assessed in an experimentally gentamicin-induced renal failure in white Wistar rats. Material and methods: The white male Wistar rats were distributed into 4 groups of 7 animals each and were treated intraperitoneally as follows: Group I (Control): distilled water in a volume of 0.5ml/100g rat/day for 10 days; Group II (Ge): gentamicin 80 mg/kbw/day for 7 days; Group III (Ge+Zn): gentamicin 80 mg/kbw/day for 7 days and ZnCl₂ 5 mg/kbw/day for 10 days prior to administration of Ge and then another 7 days simultaneously with Ge administration; Group IV (Zn): ZnCl₂ 5 mg/kbw/day for 17 days. Before starting the experiment (I₀) and at 3, 7 and 10 days after the first Ge administration, magnesium, copper and zinc plasma concentrations and urinary magnesium levels were determined. Results: Zn administration significantly decreased (p<0.001) plasma Mg concentrations in Ge+Zn group compared to Ge group after 7 days in the experiment, and induced a lower urinary elimination of Mg in Ge+Zn group (p<0.05) than in Ge group (p<0.01). Also, Zn induced a slight augmentation of Cu concentration in Ge+Zn group (p<0.05) compared to Ge group after 7 and 10 days. Conclusions: The variation in divalent cation concentrations in the context of renal diseases may be helpful for an early diagnosis and effective alternative therapeutic measures. Keywords: DIVALENT CATIONS, RAT MODEL, ACUTE RENAL FAILURE, GENTAMICIN.

The kidney plays an important role in the homeostasis of divalent cations (1). This adjustment is processed at different levels of the nephron involving the participation of ion channels and some transporters.

Zinc (Zn) is the second most abundant metal in the body, after iron, and is the only metal that can be found in all types of enzymes. Zn, being found in all tissues of the body, plays a role in immune system function, tissue repair processes, protein production, DNA synthesis and cell division. Severe Zn deficiency can induce immune system disturbances, alteration of macrophage and neutrophil functions, apoptosis and impairment of complement activity (2, 3).
Magnesium (Mg), the second most common cation in the extracellular space after potassium (K), plays an essential role in physiological cellular processes due to its ability to form complexes with the important intracellular anionic ligands (especially with adenosine triphosphate – ATP), and its capability to compete with calcium for membrane sites. It is known that over 300 enzymatic reactions are dependent on the presence of Mg, those involved in ATP generation and utilization (4).

Copper (Cu) is an essential trace element, being stored primarily in the liver, with small amounts also present in the brain, heart, kidney and muscles. Cu is involved in the functioning of some essential enzymes. Cu maintains the resistance of the skin tissue, blood vessels, epithelial and connective tissues. It also has a major role in the synthesis of hemoglobin, myelin, melanin, and in maintaining the thyroid gland normal functions (5).

In the present study, we aimed at evaluating the changes in divalent cation concentration, in experimentally gentamicin-induced renal failure in white Wistar rats.

**MATERIAL AND METHODS**

The following substances were used in the experiment: gentamicin solution for injection 80mg/2ml (Krka, Slovenia), zinc chloride (ZnCl2) ≥98% (Sigma-Aldrich Corporation), distilled water (Sicomed Romania).

Male white Wistar rats (weighting 210-320g), housed in rooms with controlled temperature (22-24°C) and constant 12 hours’ light-dark cycle, and kept in metabolic cages with free access to water and standard food, were monitored for 24-hour water intake and urine output.

The animals were distributed into 4 groups of 7 animals each, treated intraperitoneally (i.p.) as follows: Group I (Control): distilled water in a volume of 0.5ml/100g rat/day for 10 days; Group II (Ge): gentamicin 80mg/kbw/day for 7 days; Group III (Ge+Zn): gentamicin 80 mg/kbw/day for 7 days and ZnCl2 5 mg/kbw/day for 10 days prior to administration of Ge and then another 7 days simultaneously with Ge administration; Group IV (Zn): ZnCl2 5 mg/kbw/day for 17 days.

Blood samples were collected from retro-orbital plexus of anaesthetized animals, before starting the experiment (I0) and at 3, 7 and 10 days after the first administration of the nephrotoxic agent to determine the magnesium, copper and zinc plasma concentrations. Urine samples were collected at the same intervals as the blood samples, to measure the calcium and magnesium levels.

Cu and Zn plasma concentrations were determined by atomic absorption spectrophotometry using an Analyst 600 Atomic Absorption Spectrophotometer (Perkin Elmer), a AS-800 Graphite Furnace Autosampler, and a copper or zinc HCL (Halow Cathode Lamp). A Randox kit and a Xyliydl blue colorimetric method were used to measure serum and urinary magnesium levels.

All data were presented as mean ± standard deviation (SD) of mean for 7 animals in a group. P-values less than 0.05 were considered statistically significant. Data were processed using one-way ANOVA method and Tukey’s test.

The experimental protocol was implemented in compliance with the instructions of the "Grigore T. Popa" University of Medicine and Pharmacy Iași Committee for Research and Ethical Issues, per the Re-
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search Law 206/27.05.2004 regarding the conduction of scientific research, technological development and innovation and the actual European legislation.

RESULTS

The biochemical analysis revealed a statistically significant increase in serum Mg levels at 7 days compared with I₀ in Ge group (p<0.01) and Ge+Zn group (p<0.05). A reduction in Mg levels was recorded at 7 days (after 17 days of Zn administration), statistically significant compared with I₀ in Zn group (p<0.01). The administration of Zn significantly diminished (p<0.01) the plasma Mg concentrations in Ge+Zn group compared to Ge group at 7 days in the experiment (tab. I).

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Variation in plasma Mg concentration (mg/dl) in Ge, Zn and Ge+Zn groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot</td>
<td>initially (I₀)</td>
</tr>
<tr>
<td>Ge</td>
<td>2,35±0,26</td>
</tr>
<tr>
<td>Ge+Zn</td>
<td>2,36±0,17</td>
</tr>
<tr>
<td>Zn</td>
<td>2,46±0,20</td>
</tr>
<tr>
<td>Control</td>
<td>2,39±0,08</td>
</tr>
</tbody>
</table>

* p<0.01 vs. I₀ and Control; *p<0.05 vs. 7 days; ^ p<0.01 vs. Ge

Significant increases (p<0.01) in serum Cu levels were recorded in Ge+Zn group, but not in Ge group after 3, 7 and 10 days, compared to the initial moment I₀.

The laboratory determinations highlighted a progressive decrease in plasma Cu values, statistically significant (p<0.01) at 7 days, compared with I₀ in Zn group (after 17 days of ZnCl₂ administration). The treatment with Zn induced a significant augmentation of Cu concentration in Ge+Zn group (p<0.01), compared to Ge group at 7 and 10 days in rats with experimentally gentamicin-induced acute renal failure (tab. II).

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Variation in plasma Cu concentration (μg/dl) in Ge, Zn and Ge+Zn groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot</td>
<td>I₀</td>
</tr>
<tr>
<td>Ge</td>
<td>139,30±5,93</td>
</tr>
<tr>
<td>Ge+Zn</td>
<td>127,78±8,09</td>
</tr>
<tr>
<td>Zn</td>
<td>125,28±13,89</td>
</tr>
<tr>
<td>Control</td>
<td>148,06±6,75</td>
</tr>
</tbody>
</table>

* p<0.01 vs. I₀; ^p<0.01 vs. Ge

Our investigation showed a statistically significant elevation (p<0.01) of serum Zn levels at 3, 7 and 10 days compared to I₀ in Ge and Ge+Zn groups. The treatment with Zn was associated with a significant increase of plasma Zn levels in Ge+Zn group compared with Ge group at 3, 7 and 10 days in the experiment (tab. III).

The biochemical analysis detected an elevation of Mg urinary excretion, statistically significant at 7 and 10 days compared to I₀ in Ge group (p<0.01). In Ge+Zn group
it was also registered an increased excretion of urinary Mg at 7 days compared to I₀ (p<0.01). The administration of Zn resulted in a lower urinary elimination of Mg in Ge+Zn group than in Ge group (p<0.01) at 10 days (fig.1).

### TABLE III

<table>
<thead>
<tr>
<th>Lot</th>
<th>I₀</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ge</td>
<td>126,45±5,91</td>
<td>*146,03±6,66</td>
<td>*158,74±8,26</td>
<td>*144,17±8,02</td>
</tr>
<tr>
<td>Ge+Zn</td>
<td>157,41±8,14</td>
<td>.*168,12±13,45</td>
<td>*.^189,49±16,26</td>
<td>*.^196,78±11,96</td>
</tr>
<tr>
<td>Zn</td>
<td>168,04±12,91</td>
<td>*183,67±16,58</td>
<td>*198,64±12,22</td>
<td>*204,27±10,72</td>
</tr>
<tr>
<td>Control</td>
<td>122,50±7,07</td>
<td>124,32±10,90</td>
<td>121,22±15,14</td>
<td>124,94±10,56</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I₀; ^p<0.05 vs. Ge

**DISCUSSION**

Zn is considered an important element necessary for the physiological function of cell signaling, and influences the redox status, enzyme activity, gene transcription, energy metabolism, cell cycle, apoptosis and cell proliferation. Zn deficiency increases the levels of nitric oxide and superoxide radicals by inhibiting the N-methyl-D-aspartate receptor (6, 7).

Literature data suggest that, besides the action of highly reactive radicals, another mechanism of Ge nephrotoxicity is represented by the changes of trace elements in the kidney (8). In our study, Zn loading resulted in a significantly lower increase of serum Mg in Ge+Zn group compared to Ge group (2.97 ± 0.18 mg/dl vs. 4.31 ± 0.29 mg/dl). Similarly, after Ge administration, on day 7 we recorded elevated serum Mg levels as compared with I₀. However, Zn administration did not significantly affect
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serum Ca concentration in Ge+Zn group compared to Ge group.

The values showed no significant change in plasma Cu following Ge administration, but Zn treatment generated increased Cu concentration in Ge+Zn group compared to Ge group (175.37 ± 9.03 μg/dl vs. 144.37 ± 6.1 μg/dl at 7 days). Plasma Cu values showed an increase in the group treated with Ge compared to I_0, and Zn charge resulted in higher plasma Zn levels in Ge+ Zn group compared to Ge group (189.49±16.26 μg/dl vs. 158.74±8.26 μg/dl at 7 days).

Mg modulates the contractility and electrical activity of myocardial cells, and the conduction system that influences the transport of ions (sodium, potassium, calcium) in the sarcolemma. Mg can also influence vascular smooth muscle tone. The exact physiological mechanism that regulates plasma Mg concentration is not completely elucidated. It seems that serum Mg is regulated through a dynamic mechanism and interaction between the transport in the kidney, intestine and bones (9).

Approximately 95% of ultra-filtered Mg is reabsorbed in the kidney: 25-30% in the proximal tubules, about 60-65% is reabsorbed in the ascending loop of Henle, and 5% in the distal segment. Mg excretion is controlled primarily by serum Mg concentration (10).

Hypermagnesemia is associated with an increase in Mg excretion which can be up to 100% of the filtered amount. Literature data confirm that Ge can generate renal Mg loss through a furosemide-like mechanism, by inhibiting the paracellular transport in the ascending loop of Henle (11).

Copper can act both as an antioxidant and as a pro-oxidant. As an antioxidant, it removes or neutralizes free radicals and reduces the damage caused by these agents, which can promote pathological processes through interaction with genetic material. When acting as a pro-oxidant, Cu can induce the generation of free radicals and contribute to the development of such diseases as Alzheimer's disease. Normal Cu homeostasis, as well as other mineral balance (Zn, Mg), is important for maintaining health (12).

Some studies investigated the Cu/Zn imbalance in Cu deficiency in rats. In case of Cu deficiency, decreased activity of superoxide dismutase and increased malondialdehyde levels compared to normal values were recorded (13).

CONCLUSIONS

Divalent cations homeostasis is essential for the proper functioning of the entire organism. Our study suggested the importance of taking in consideration the variation in concentrations of these cations in the context of renal diseases, being possibly helpful for early diagnosis and effective alternative therapeutic measures.

REFERENCES


NEWS

CALCIUM SUPPLEMENTS MAY AFFECT THE CORONARY ARTERIES

This is the conclusion of a recent article published in Journal of American Heart Association. This result represents part of a bigger study conducted in six research American universities, the Multi-Ethnic Study of Atherosclerosis. The researchers used dietary questionnaires and CT scans spanning 10 years apart to assess the daily calcium intake (either as supplements or food) and the calcium score. The results showed that supplement users have a 22% higher risk of developing coronary artery disease (defined as a rise of the calcium score over the 10 years) than the subjects with a high dietary intake of calcium. It is still unclear why taking the same amount of calcium as supplement is more harmful, but the hypothesis takes into account the form of calcium that can be differently absorbed (calcium salt in supplements) and the inability of the body of processing a large dose of calcium all at once (John J.B. Anderson, PhD; Bridget Kruszka, MPH et al. Calcium Intake From Diet and Supplements and the Risk of Coronary Artery Calcification and its Progression Among Older Adults: 10-Year Follow-up of the Multi-Ethnic Study of Atherosclerosis (MESA). J Am Heart Assoc. 2016; 5:e003751).

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