RESEARCH REGARDING THE DIVALENT CATIONS CONCENTRATIONS IN PEDIATRIC ACUTE NEPHROPATHIES AND THEIR PHARMACOLOGICAL INFLUENCE

Summary of PhD Thesis

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The numbering of the chapters, subchapters, tables and figures presented in this summary is identical to those of the thesis.

The thesis contains: 166 pages, 118 figures, 102 tables, 434 references.

Keywords: acute renal failure, divalent cations, nephrotic syndrome, child, magnesium
JUSTIFICATION FOR CHOOSING THE THEME

Literature data show that using high-dose therapy for long term in infectious diseases may be associated with impaired renal function, which requires special attention especially in patients with extreme age (newborns, prematures and elderly), in which, due to existing physiological particularities, the side effects are important even at therapeutic doses.

In the same way can be influenced the balance of divalent cations (Ca, Mg, Cu, Zn), with implications for the entire organism, knowing that the functionality of all systems and organs depends on their homeostasis. Regarding Mg, the glomeruli filter the most important quantity of total Mg, and then filtered Mg is absorbed at different levels of renal tubules. A tubulointerstitial alteration induces a Mg imbalance, and can lead to further cardiovascular, muscular, neurological, respiratory complications, since Mg is involved in almost all metabolic and biochemical cellular processes.

It is particularly useful to know how different substances, interfering pathogenic pathways involved in maintaining the integrity and proper functions of renal filter, may increase its resistance to chemical aggression, preventing or reducing the damage of excretory function.

In this context, it is justified the choice of the thesis theme, aiming to research the effects of drugs on renal function impairment and on divalent cations concentrations.

Experimental research conducted in this thesis consisted of investigating the influence of zinc, montelukast and azithromycin on the biochemical and histological changes, which appear in the experimental renal failure induced by gentamicin, cisplatin and glycerol, in Wistar rats, as well as on the cationic imbalances associated with renal failure.

Due to the fact that zinc and montelukast are involved in the modulation of pathogenic pathways providing renal functionality, it was considered useful the idea of investigating the influence of these substances on laboratory animals, on various kidney failure models, internationally used and standardized.

To highlight the implications in medical practice of divalent cations in renal impairment, we conducted a clinical research on
children with idiopathic nephrotic syndrome, a relatively common nephropathy in the pediatric age, responsible for changes in tubulointerstitial function, and thus impaired renal filtration of Mg. The motivation for the clinical study is based on the fact that changes in Mg levels may reflect the degree of tubulointerstitial damage, because the kidney has a major role in the homeostasis of this divalent cation. Thus, the variation of Mg concentration can be used for assessing the severity of acute renal disease, evaluating the functionality of renal filtration, and even for early detection of the cases not responsive to corticotherapy.

**INTRODUCTION**

Acute renal failure (ARF) is a global health issue, affecting a large number of hospitalized patients, with major economic and social implications. ARF evolution varies depending on the onset and on the duration and severity of the acute episode, affecting the survival rate, the need for dialysis and the recover of renal function (Uwaezuoke, 2015).

Concerning the etiology of ARF, the most common causes encountered in the pediatric age group include: hemolytic uremic syndrome, severe dehydration, sepsis, glomerulonephritis, nephritic syndrome, obstructive uropathy (Uwaezuoke, 2015, Jenssen et al., 2014). Also, the growing trend to use potential nephrotoxic drugs (aminoglycoside antibiotics, non-steroidal anti-inflammatory, anti-neoplastic agents, inhibitors of angiotensin converting enzyme) and contrast agents is involved in the etiology of ARF (Varrier et al., 2015).

The use of animal models it is necessary to elucidate the the complex pathophysiology and the molecular mechanisms of ARF, as well as for research and development of new therapies with regenerative and protective effect on renal tissue. Experimental studies are useful for determining the type of affected renal cells (proximal/ distal tubular cells) and the extent of the affected area.

Magnesium is an essential cation for the organism, having anti-inflammatory properties and important cellular functions within enzymatic reactions and proteins and polynucleotides synthesis.
In clinical practice, the serum concentration of Mg is not usually determined, although there are data that suggest that up to 60% of critical patients have Mg deficiency (Escuela et al., 2005). Early detection of Mg concentration disorders may be useful for the establishment of effective therapeutic strategies. It is therefore necessary awareness of the importance of an appropriate Mg balance, understanding Mg homeostasis being helpful to provide important clinical data for diagnosis and treatment of diseases.

The present thesis contains an experimental part in which we investigated the effects of some drugs on experimentally induced acute renal failure, the modification of renal parameters and divalent cations concentrations (calcium, magnesium, copper, zinc), the effect on some antioxidant parameters, and histopathological changes that occur in acute kidney injury.

To this purpose we conducted three experimental models of ARF on Wistar rats, using gentamicin, cisplatin and glycerol as nephrotoxic agents. Gentamicin was given intraperitoneally at a dose of 80mg /kgbw/day for 7 days. Cisplatin was used in a single dose of 5 mg /kg, intraperitonealy and glycerol in a single dose of 10 ml/kg (50% v/v) intramuscularly.

On these experimental models we investigated the influence of montelukast, zinc and azithromycin.

We chose the use of montelukast (Mk), a leukotriene receptor antagonist CysLTR1, due to its anti-inflammatory properties demonstrated in other systems, to investigate whether it exerts this effect in the kidney. Zinc (Zn) is a trace element involved in many functions of the organism, being considered a part of the antioxidant tissue defense system. Zn affects cytokine production and stabilizes the cell membrane, preventing inflammatory lesions (Kaur et al., 2014), thus being an antioxidant and anti-inflammatory agent. Azithromycin (Az) is a macrolide antibiotic of second generation, with broad bacterial spectrum. Recent studies have shown that Az exerts beneficial effects in chronic inflammatory diseases, these effects being attributed to immunomodulatory properties on the innate and acquired immune response (Culic et al., 2001). Az inhibits the production of acute phase proteins by reducing the release of cytokines at inflammatory sites.
The personal section of the thesis includes, in addition to the experimental research, a retrospective clinical study of a group of patients admitted in the Nephrology Department of "Sf. Maria " Hospital with idiopathic nephrotic syndrome. We investigated the variation in serum Mg concentrations both in patients who were in the acute phase of the disease, and in those in remission, in order to clarify the relation between Mg concentration and kidney damage.

PERSONAL CONTRIBUTIONS

CHAPTER VI. EXPERIMENTAL RESEARCHES

VI.1. Purpose. Experimental studies regarding the influence of drugs on experimentally induced acute renal failure in rats.

VI.2. Objectives

1. Performing of 3 experimental models of acute renal failure using gentamicin, cisplatin and glycerol as nephrotoxic substances.
2. Investigating on the 3 models of ARF the effects of montelukast, zinc and azithromycin.
3. Highlighting the changes of renal impairment parameters, oxidative stress parameters and histopathological aspects arising in the context of experimental acute kidney damage, and how they are influenced by the administration of the three studied substances.
4. Investigating the variations of divalent cations concentrations in acute renal damage and their pharmacological influence by montelukast, zinc and azithromycin.

The current project was in accordance with the European legislation and the ethical approval of the "Gr. T. Popa" University Ethics Committee of Research on 11/22/2013, under the Law of Research nr. 206/27.05.2004, regarding the scientific research, technological development and innovation.

For the experimental study we used Wistar rats, young males, weighing between 150 - 200g, with health certificate, from the Bucharest National Institute of Research and Development Cantacuzino (INCDMI), Baneasa resort.
Blood samples were collected before the start of the experiment ($I_0$) and 3, 7 and 10 days after the first administration of the nephrotoxic agent, for the experimental models with gentamicin and cisplatin, respectively at 24 and 48 hours for the glycerol model. Urine samples were collected at the same intervals as the blood samples. We determined the serum concentrations of urea, creatinine, calcium, magnesium, copper and zinc, urinary levels of proteins, calcium and magnesium, levels of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx) and plasma levels of total capacity antioxidant (TAS).

At time intervals as specified above, we sacrificed 3 animals per group, under anesthesia, and both kidneys were removed. We analyzed for histological quantification following changes: degeneration, necrosis of the proximal, distal and collecting tubules, interstitial inflammation, accumulation of myoglobin, regeneration.

The results were statistically analyzed using SPSS 13.0 program. All data were presented as mean ± standard deviation (SD). p-values <0.05 were considered statistically significant. For the interpretation of biochemistry data was used ANOVA one-way test, and for multiple comparisons Tukey test was used. Histopathology data were analyzed using the Kruskal-Wallis test.

**Gentamicin + Montelukast Model**
- Group I (control): distilled water, in a volume of 0.5ml/100g rat/day, p.o., 10 days
  - Group II (Ge): Gentamicin, 80mg/kg/day, i.p., 7 days
  - Group III (Ge+Mk): Gentamicin, 80mg/kg/day, i.p., 7 days, and Montelukast 10mg/kg/day, p.o. (through endogastric tube), 10 days, simultaneously with gentamicin
  - Group IV (Mk): Montelukast (Mk) 10mg/kg/day, p.o., 10 days

**Cisplatin + Montelukast Model**
- Group I (control): distilled water, in a volume of 0.5ml/100g rat/day, p.o., 10 days
  - Group II (Cis): Cisplatin 5mg /kg single dose i.p.
  - Group III (Cis+Mk): Cisplatin 5mg/kg single dose i.p. and Montelukast 2mg/kg, p.o. (through endogastric tube), 10 days
  - Group IV (Mk): Montelukast 2mg/kg, p.o., 10 days
**Gentamicin + Zinc Model**
- Group I (control): distilled water, in a volume of 0.5ml/100g rat/day, p.o., 10 days
- Group II (Ge): Gentamicin, 80mg/kg/day, i.p., 7 days
- Group III (Ge+Zn): Gentamicin 80mg/kg/day, i.p., 7 days and ZnCl2 5 mg/kg/day, i.p. 10 days prior to administration of Ge and then another 7 days at the same time with the administration of Ge
- Group IV (Zn): ZnCl2 5 mg/kg/day, i.p., 17 days

**Cisplatin + Zinc Model**
- Group I (control): distilled water, in a volume of 0.5ml/100g rat/day, p.o., 10 days
- Group II (Cis): Cisplatin 5mg/kg single dose i.p.
- Group III (Cis+Zn): Cisplatin 5mg/kg single dose i.p. and ZnCl2 5mg/kg/day i.p. 10 days prior to administration of Cisplatin, and then 7 days after the cisplatin administration
- Group IV (Zn): ZnCl2 5 mg/kg/day, i.p., 17 days

**Glycerol + Zinc Model**
- Group I (control): Distilled, 0.5ml/100g rat/day i.p., 2 days
- Group II (Glic): Glycerol (50%, v/v), 10ml/kg, i.m. single dose administered in equal volumes in both back limbs
- Group III (Glic+Zn): Glycerol (50%, v/v), 10ml/kg, i.m. single dose, administered in equal volumes in both back limbs and ZnCl2 5 mg/kg/day, i.p. 10 days prior Glic administration and then 2 days after the administration of glycerol
- Group IV (Zn): ZnCl2 5 mg/kg/day, i.p., 17 days

**Glycerol + Azithromycin Model**
- Group I (control): Distilled, 0.5ml/100g rat/day p.o., 2 days
- Group II (Glic): Glycerol (50%, v/v), 10ml/kg, i.m. single dose administered in equal volumes in both back limbs
- Group III (Glic+Az): Glycerol (50%, v/v), 10ml/kg, i.m., single dose, administered in equal volumes in both back limbs and Azithromycin 40mg/kgbw/day, p.o. (endogastric tube) in a volume of 0.5ml/100g rat, 2 days
- Lot IV (Az): Azithromycin 40mg/kgbw/day, p.o. (endogastric tube) in a volume of 0.5ml/100g rat, 2 days
CHAPTER VII. CLINICAL STUDY

VII.1. Purpose. Investigating magnesium concentration in pediatric nephrotic syndrome.

VII.2. Objectives

1. Assessment of biochemical parameters which are typically modified in nephrotic syndrome.
2. Evaluation of serum magnesium concentration in children with acute phase of nephrotic syndrome and during the remission phase.

We conducted a retrospective study in which were followed 27 patients aged 2 to 17 years, hospitalized in the Nephrology Department of "Sf. Maria" Hospital, Iasi, between 2011-2015, with a diagnosis of idiopathic nephrotic syndrome, first episode or relapse.

Inclusion criteria:
- age 0 – 18 years
- diagnosis of idiopathic nephrotic syndrome

Exclusion criteria:
- diagnosis of congenital nephrotic syndrome
- diagnosis of secondary nephrotic syndrome
- simultaneous diagnosis of other systemic diseases such as systemic lupus erythematosus, Henoch-Schönlein purpura
- existence of a disease that could cause magnesium loss (gastrointestinal illnesses, burns)
- administration of nephrotoxic substances (loop diuretics, gentamicin, contrast agents)

For each patient we followed:
- age
- sex
- biochemical parameters: urea, creatinine, total cholesterol, total serum proteins, serum magnesium, urinary proteins
- creatinine clearance
- renal histology for steroid non-responsive patients
Measurements for all investigated parameters were performed using standard methods of biochemistry and histopathology laboratories of "St. Maria" Children Hospital.

We also used a control group of 14 children with normal renal function, in which we followed the magnesium serum concentrations of magnesium, the values being compared to those in the study group.

Personal data of the patients was not used. Informed consent was obtained from each patient parent. Institution's consent was obtained for the use of medical data.

Statistical analysis of data was performed using unpaired t-test and Fisher's test. The results were expressed as mean ± standard deviation. Coefficient values p <0.05 were considered statistically significant.

CHAPTER VIII. RESULTS

VIII.1. Gentamicin + Montelukast model

*Serum urea and creatinine* concentrations in the group treated with Ge increased significantly at 7 days (p <0.01) compared to $I_0$ values and to the rest of groups. There were no significant differences between groups Ge+Mk, Mk and control (Fig. VIII.4).

In animals that received Ge+Mk, the *activity of SOD* was significantly higher compared to the group treated only with Ge, both at 7 and 10 days (p <0.01) (Fig. VIII.5).

![Fig. VIII.4. Serum creatinine (mg /dl) in Ge+Mk model](image)

*Fig. VIII.4. Serum creatinine (mg /dl) in Ge+Mk model
*p<0,01 vs. $I_0$, Ge+Mk, Mk and control*
In Ge+Mk group, the increase of total serum Mg was lower than in Ge group (p <0.01). At 10 days, Mg concentrations returned to baseline values in both Ge and Ge+Mk groups (fig. VIII.8).

**In group treated with Ge**, examination of kidney sections collected at 10 days revealed extensive cortical necrosis (degree 2-3), fragmented tubular basement lamina and severely altered renal cortical architecture (fig. VIII.16). In **Ge+Mk group**, at 10 days, processes necrosis (degree 2) and degeneration (degree 2) and inflammatory phenomena were of lower intensity compared to Ge group (fig. VIII.20).
VIII.2. Cisplatin + Montelukast Model

*Urinary proteins* concentration showed a significant increase at 3 and 7 days (p <0.01) both in Cis and Cis+Mk groups compared to I<sub>0</sub> values and to Mk and control groups. There were no significant differences between Cis and Cis+Mk groups (table VIII.2.IV).

**Table VIII.2.IV.** Urinary proteins concentration (g/l) (x ± SD) in Cis+Mk experimental model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I&lt;sub&gt;0&lt;/sub&gt;)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>0.41±0.043</td>
<td>*2.49±0.31</td>
<td>*, # 1.04±0.16</td>
<td>0.59±0.10</td>
</tr>
<tr>
<td>Cis+Mk</td>
<td>0.35±0.06</td>
<td>*2.26±0.35</td>
<td>*, # 0.98±0.15</td>
<td>0.62±0.10</td>
</tr>
<tr>
<td>Mk</td>
<td>0.45±0.06</td>
<td>0.51±0.13</td>
<td>0.44±0.11</td>
<td>0.47±0.01</td>
</tr>
<tr>
<td>Control</td>
<td>0.41±0.03</td>
<td>0.43±0.07</td>
<td>0.44±0.08</td>
<td>0.42±0.09</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I<sub>0</sub>, Mk and control; #p<0.01 vs. 3 days

The values of *erythrocyte SOD* in Cis and Cis+Mk groups significantly decreased at 3, 7 and 10 days compared to I<sub>0</sub>, Mk and control (p <0.05), the lowest value being that of 3 days. There were no significant differences between Cis and Cis+Mk groups (table VIII.2.VI).

**Table VIII.2.VI.** Variation of erythrocyte SOD (U/ml blood) (x ± SD) in Cis+Mk experimental model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I&lt;sub&gt;0&lt;/sub&gt;)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>287±17,65</td>
<td>*182.66±21.66</td>
<td>*194.83±26.68</td>
<td>*, # 235.66±16.12</td>
</tr>
<tr>
<td>Cis+Mk</td>
<td>276±19,65</td>
<td>*196.4±28.97</td>
<td>*206.6±20.45</td>
<td>*, # 231.8±24.0</td>
</tr>
<tr>
<td>Mk</td>
<td>287±29,02</td>
<td>286±25.42</td>
<td>284±27.28</td>
<td>297±36.42</td>
</tr>
<tr>
<td>Control</td>
<td>274.6±23.19</td>
<td>264.3±22.01</td>
<td>271.66±21.88</td>
<td>281.33±15.33</td>
</tr>
</tbody>
</table>

*p<0.05 vs. I<sub>0</sub>, Mk and control; #p<0.05 vs. 3 days

*Total serum Mg* showed a significant increase in both Cis and Cis+Mk groups compared to I<sub>0</sub> and Mk and control groups, at 3 days (p <0.01). Mk administration induced a minor increase of total Mg in Cis+Mk group compared to Cis (p <0.01) (table VIII.2.XI).
**Tabel VIII.2.XI.** Variation of total serum Mg (mg/dl) (x±SD) in Cis+Mk experimental model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I0)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>2,83±0,06</td>
<td>*4,16±0,34</td>
<td>2,62±0,08</td>
<td>#2,64±0,11</td>
</tr>
<tr>
<td>Cis+Mk</td>
<td>2,55±0,16</td>
<td>*3,75±0,64</td>
<td>2,69±0,38</td>
<td>#2,60±0,38</td>
</tr>
<tr>
<td>Mk</td>
<td>2,25±0,26</td>
<td>2,31±0,07</td>
<td>2,26±0,35</td>
<td>2,31±0,18</td>
</tr>
<tr>
<td>Control</td>
<td>2,39±0,08</td>
<td>2,42±0,21</td>
<td>2,39±0,1</td>
<td>2,46±0,11</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I₀, Mk and control; #p<0.01 vs. 3 days; ^ p<0.01 vs. Cis

In group treated with Cis, at 7 days, there were highlighted intense processes of degeneration (degree 4) and necrosis (degree 3) of proximal and distal tubules, and proteins accumulation (Fig. VIII.24). In Cis+Mk group, it was revealed dilatated collecting tubules in the proximal region, associated with degeneration (degree 4) of distal and proximal tubules and proteic casts in convoluted tubules (Fig. VIII.28).

**VIII.3. Gentamicin + Zinc Model**

**Serum urea and creatinine** concentrations in group Ge increased significantly at 7 days compared I₀ and Zn, Ge+Zn and control groups (p <0.01). Administration of zinc significantly reduced (p <0.01) the increase of urea and creatinine in Ge+Zn group compared to Ge (fig. VIII.33).
Zn administration led to higher values of SOD activity in Ge+Zn group compared to Ge, at 3, 7 and 10 days (p < 0.01) (table VIII.23.III).

Table VIII.3.III. Variation of erythrocyte SOD (U/ml blood) (x ± SD) in Ge+Zn experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I₀)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ge</td>
<td>284±22,1</td>
<td>253,8±23,58</td>
<td>*187±29,25</td>
<td>*191±32,32</td>
</tr>
<tr>
<td>Ge+Zn</td>
<td>271,6±12,89</td>
<td>298±21,13</td>
<td>^285,2±20,21</td>
<td>^287,8±13,7</td>
</tr>
<tr>
<td>Zn</td>
<td>273,6±23,55</td>
<td>305,6±20,94</td>
<td>*328,8±23,26</td>
<td>*315,2±19,26</td>
</tr>
<tr>
<td>Control</td>
<td>274,6±23,19</td>
<td>264,3±22,01</td>
<td>271,66±21,88</td>
<td>281,33±15,33</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I₀; ^p<0.01 vs. Ge

Zn administration significantly diminished the increase of Mg concentration at 7 days in Ge+Zn group compared to Ge (p < 0.01). At 10 days, Mg values significantly decreased (p < 0.01) in both in Ge+Zn and Ge groups, compared to the values of 7 days (table VIII.3.VIII).

Table VIII.3.VIII. Variation of total serum Mg (mg/dl) (x±SD) in Ge+Zn experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I₀)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ge</td>
<td>2,35±0,26</td>
<td>2,31±0,07</td>
<td>*4,31±0,29</td>
<td>#2,30±0,06</td>
</tr>
<tr>
<td>Ge+Zn</td>
<td>2,36±0,17</td>
<td>2,44±0,14</td>
<td>#:2,97±0,18</td>
<td>#:2,45±0,18</td>
</tr>
<tr>
<td>Zn</td>
<td>2,46±0,20</td>
<td>2,52±0,41</td>
<td>*1,86±0,25</td>
<td>2,36±0,15</td>
</tr>
<tr>
<td>Control</td>
<td>2,39±0,08</td>
<td>2,43±0,18</td>
<td>2,40±0,14</td>
<td>2,45±0,13</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I₀ and control; ^p<0.05 vs. 7 days; # p<0.01 vs. Ge
In *group treated with Ge*, histopathological evaluation of the kidneys collected at 7 days revealed necrosis processes affecting > 90% of the proximal tubules (Fig. VIII.38). In *group treated with Ge and Zn*, tubular necrosis was evidenced with a lower severity compared to Ge group, affecting <25% of tubules (Fig. VIII.40).

**VIII.4. Cisplatin + Zinc Model**

*Serum creatinine* in Cis group and in Cis+Zn group, recorded a significant increase at 3 and 7 days (p < 0.01) compared to I₀, Zn and control groups. Zn administration did not significantly affect the variation in creatinine in Cis+Zn group compared to Cis (Fig. VIII.46).

![Graph showing variation in serum creatinine (mg/dl) in Cis + Zn model](image)
Zn administration in Cis+Zn group significantly attenuated the decrease of *SOD activity* (p <0.05) compared to Cis group at 3 days (table VIII.4.IV).

**Tabel VIII.4.IV.** Variation of erythrocyte SOD (U/ml blood) (x ± SD) in Cis+Zn experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I0)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>287±17,65</td>
<td>*182,66±21,66</td>
<td>*194,83±26,68</td>
<td>*235,66±16,12</td>
</tr>
<tr>
<td>Cis+Zn</td>
<td>281,16±8,79</td>
<td>*211,5±8,96</td>
<td>*190,33±17,95</td>
<td>246,8±9,02</td>
</tr>
<tr>
<td>Zn</td>
<td>273,6±23,55</td>
<td>305,6±20,94</td>
<td>**328,8±23,26</td>
<td>**315,2±19,26</td>
</tr>
<tr>
<td>Control</td>
<td>274,6±23,19</td>
<td>264,3±22,01</td>
<td>271,66±21,88</td>
<td>281,33±15,33</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I0; **p<0.05 vs. I0; ^p<0.05 vs. Cis

**Tabel VIII.4.IX.** Variation of total serum Mg (mg/dl) (x±SD) in Cis+Zn experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I0)</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis</td>
<td>2,84±0,06</td>
<td>*4,16±0,34</td>
<td>*2,44±0,08</td>
<td>2,62±0,10</td>
</tr>
<tr>
<td>Cis+Zn</td>
<td>2,36±0,21</td>
<td>^2,62±0,55</td>
<td>#2,36±0,35</td>
<td>#2,44±0,27</td>
</tr>
<tr>
<td>Zn</td>
<td>2,46±0,20</td>
<td>2,52±0,41</td>
<td>*1,86±0,25</td>
<td>2,36±0,15</td>
</tr>
<tr>
<td>Control</td>
<td>2,39±0,08</td>
<td>2,43±0,18</td>
<td>2,40±0,14</td>
<td>2,45±0,13</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I0; #p<0.01 vs. 3 days; ^p<0.01 vs. Cis

Zn supplementation decreased the level of *serum Mg* in Cis+Zn group (p<0.01) at 3 days versus Cis (table VIII.4.IX).

In *group treated with Cisplatin* at 7 days there were observed processes of degeneration (degree 4) and necrosis (degree 3) of the proximal tubules, suggesting a severe toxic effect, and proteic casts (Fig. VIII.54).

In *Cis+Zn group* it was highlighted expansion of collecting tubules, associated with degenerative processes (degree 4) in the distal and proximal tubules, as well as accumulation of protein material. It has also been observed necrosis processes (degree 1) and important nefrociytes apoptosis (Fig. VIII.60).
VIII.5. Glicerol + Zinc Model

*SO* *D activity* at 24 and 48 hours showed in Glic and Glic+Zn a decrease compared to $I_0$ values ($p < 0.05$). Zn loading generated no significant changes between Glic and Glic+Zn groups (table VIII.5.II).

Treatment with Zn, 10 days before Glic administration, resulted in a higher increase of *serum creatinine* in Glic+Zn group compared to Glic, at 24 and 48 hours ($p<0.01$) (Fig. VIII.64).

The *total serum Mg* concentration increased at 24 and 48 hours in Glic group compared to $I_0$, while in Glic+Zn group the increase was registered only at 48 hours ($p < 0.01$) (Fig. VIII.67).

**Table VIII.5.II.** Variation of erythrocyte SOD (U/ml blood) (x ± SD) in Glic+Zn model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial ($I_0$)</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glic</td>
<td>283.8±17.22</td>
<td>*212.8±18.74</td>
<td>*179.2±16.84</td>
</tr>
<tr>
<td>Glic+Zn</td>
<td>271.8±18.36</td>
<td>*194.8±20.14</td>
<td>*165.5±17.24</td>
</tr>
<tr>
<td>Zn</td>
<td>273.8±12.21</td>
<td>298.8±21.07</td>
<td>*308.2±19.58</td>
</tr>
<tr>
<td>Control</td>
<td>281.6±12.42</td>
<td>279.2±12.27</td>
<td>282.10±10.52</td>
</tr>
</tbody>
</table>

*p<0.05 vs. $I_0$
Histological examination of the kidneys at 24 hours in Glic group showed severe tubular necrosis (degree 3), myoglobin cylinders (degree 3-4), which obstruct the tubular lumens (fig. VIII.71). Histological appearance in Glic+Zn group maintains significant deposits of myoglobin (degree 3-4), tubular necrosis (degree 3-4) and severe epithelial degeneration (grade 3-4) (fig. VIII.75).

VIII.6. Glycerol + Azithromycin Model
Administration of glycerol induced significant decrease in antioxidant activity of SOD, GPx and TAS (p <0.01) compared to I₀.
and control group. In Glic+Az group antioxidant enzymes activity was lower than in Glic group (table VIII.6.IV).

**Tabel VIII.6.IV.** Variation of erythrocyte SOD (U/ml blood) in Glic+Az experimental model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I0)</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glic</td>
<td>283,8±17,22</td>
<td>*179,2±15,12</td>
</tr>
<tr>
<td>Glic+Az</td>
<td>274,6±10,57</td>
<td>*165,8±12,21</td>
</tr>
<tr>
<td>Az</td>
<td>278,6±14,28</td>
<td>*172,2±15,08</td>
</tr>
<tr>
<td>Control</td>
<td>281,6±12,42</td>
<td>282,2±10,52</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I0 and control

Animals treated with Glic+Az had serum creatinine levels at 24 and 48 hours significantly higher (p<0.01) compared to group treated with Glic (table VIII.6.II).

**Tabel VIII.6.II.** Variation in serum creatinine (mg/dl) in Glic+Az experimental model

<table>
<thead>
<tr>
<th>Group</th>
<th>initial (I0)</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glic</td>
<td>0,53±0,05</td>
<td>*3,59±0,78</td>
<td>*4,48±0,86</td>
</tr>
<tr>
<td>Glic+Az</td>
<td>0,5±0,07</td>
<td>*^4,46±0,33</td>
<td>*^6,15±0,93</td>
</tr>
<tr>
<td>Az</td>
<td>0,53±0,06</td>
<td>0,67±0,07</td>
<td>0,54±0,10</td>
</tr>
<tr>
<td>Control</td>
<td>0,53±0,06</td>
<td>0,54±0,05</td>
<td>0,52±0,08</td>
</tr>
</tbody>
</table>

*p<0.01 vs. I0, Az and control; ^p<0.01 vs. Glic

![Graph](image)

**Fig. VIII.81.** Variation of total serum Mg (mg/dl) in Glic+Az model

*p<0.01 vs. I0; ^p<0.01 vs. Glic
**Total serum Mg** in Glic and Glic+Az groups presented at 24 and 48 hours significant increases (p<0.01) compared with controls and $I_0$. Rats that received Glic+Az had serum Mg values significantly higher (p<0.01) compared to Glic group throughout the experiment (fig. VIII.81).

Histopathological evaluation at 24 hours highlighted in **Ge group** the presence of myoglobin cylinders, processes of degeneration (degree 3) and necrosis (degree 2) (fig. VIII.86). Glic+Az group maintains important accumulation of myoglobin, tubules degeneration (degree 2) and extremely aggressive necrosis (degree 2) appear, distinguished by massive lysis of nephrocytes (fig. VIII.93).

VIII.7. **Clinical study**

Based on the criteria of inclusion and exclusion, it was made a group of 27 patients, aged 2 to 17 years, diagnosed with idiopathic nephrotic syndrome (NS), who had at least one hospitalization in the Department of Nephrology, Children Hospital "St. Maria" Iasi, between 2011-2015.

12 patients had idiopathic NS in acute phase, and 15 patients NS in remission phase. Depending on the response to treatment, steroid resistance was detected in 6 patients from the active NS group, and 8 patients with remission phase of NS.

Investigation of serum Mg levels showed low values in SN active group (1.96 ± 0.30 mg/dl) compared to control group (2.23 ±
0.10 mg/dl, with a statistically significant difference (p <0.05) (table VIII.7. VII).

**Table VIII.7.VII.** Serum Mg (mg/dl) in active NS and control groups. Statistical significance – Fischer test

<table>
<thead>
<tr>
<th>Serum Mg (mg/dl)</th>
<th>active SN (n=12)</th>
<th>Control (n=14)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nr.</td>
<td>%</td>
<td>Nr.</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>58</td>
<td>14</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.96 ± 0.30</td>
<td>2.23 ± 0.10</td>
<td>&lt; 0.05*</td>
</tr>
<tr>
<td>Values</td>
<td>1.53 – 2.30</td>
<td>2.10 – 2.43</td>
<td></td>
</tr>
</tbody>
</table>

*p statistically significant

In the present study, serum Mg registered significantly decreased values in patients with acute nephropathy, compared to patients in remission phase. Since the Mg is a divalent cation whose homeostasis depends on the kidney filtration, low levels of magnesium in children with active SN reflects an alteration of renal excretion and reabsorption.

**Table VIII.7.XI.** Serum Mg (mg/dl) in active NS and remission NS groups. Statistical significance – Fischer test

<table>
<thead>
<tr>
<th>Serum Mg (mg/dl)</th>
<th>active NS (n=12)</th>
<th>Remission NS (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nr.</td>
<td>%</td>
<td>Nr.</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>58</td>
<td>12</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>42</td>
<td>3</td>
</tr>
<tr>
<td>Mean ± DS</td>
<td>1.96 ± 0.30</td>
<td>2.31 ± 0.77</td>
<td>0.39</td>
</tr>
<tr>
<td>Values</td>
<td>1.53 – 2.30</td>
<td>1.61 – 4.81</td>
<td></td>
</tr>
</tbody>
</table>

p statistically insignificant

However, although serum Mg was significantly lower in patients with acute nephropathy compared with controls (p<0.05), divalent cation concentration variation between active NS and remission NS groups showed no statistical significance (table VIII.7. XI).
CONCLUSIONS

- Administration of montelukast 10mg/kgbw had a protective effect on the kidney in gentamicin induced ARF model, but the dose of 2mg/kgbw had no protective effect on cisplatin-induced ARF model
- Administration of zinc 5mg/kg/day induced renal protective effect on gentamicin-induced ARF model and a minor protective effect on cisplatin experimental model, while in the experiment with glycerol did not exert any protective effect on renal function
- Azithromycin 40mg/kgbw p.o. in glycerol-induced ARF model potentiated the aggressive effects of glycerol in the kidney
- Monitoring the concentrations of divalent cations in ARF is important for early diagnosis, but also for the choice of therapeutic alternatives
- serum Mg registered significantly lower values in patients with acute nephropathy compared to those in remission phase, reflecting impaired tubulointerstitial function
- Serum and urinary Mg concentrations must be taken into account when considering the management of nephrotic syndrome and other acute renal disease in children

NOVELTY ELEMENTS AND PERSPECTIVES OF THE THESIS

The theme studied in this thesis is of great interest, acute renal failure being a frequent pathology in medical practice, both in neonates, children and adults.

Understanding the pathogenic mechanisms of renal lesions is particularly important for developing strategies for diagnosis and treatment. Investigating the changes of some divalent cations which occur in ARF could have implications for early diagnosis and monitoring of disease progression. The novelty of the thesis includes:

- systematization of the latest theoretical data about ARF, including classification criteria, pathophysiological mechanisms, therapeutic possibilities
- providing an overview about the most widely used experimental models of acute renal failure in literature, detailing the methods, pathophysiological processes, mechanisms of renal damage
- realization of 3 models of acute renal failure in rats using gentamicin, cisplatin and glycerol, each substance having different mechanisms of action
- investigating on these ARF experimental models the effects of some drugs with anti-inflammatory properties (zinc and montelukast), obtaining valuable data regarding the therapeutic possibilities of renal protection in the context of nephrotoxicity
- investigating the changes of divalent cations (calcium, magnesium, copper, zinc) in the process of acute renal impairment, highlighting their role both in the diagnostic algorithm, and in therapy
- evaluating, in parallel with biochemical parameters, the renal histopathological changes, conducting a thorough analysis and semi-quantitative quantification of the histological aspects induced by nephrotoxicity
- conducting a retrospective study on children diagnosed with nephrotic syndrome and investigating the variation of magnesium concentration

The theoretical information gathered in the study, and the results of the clinical and experimental research, represent valuable items, comparable with data from scientific literature, offering at the same time new perspectives for clinical and experimental research, such as:
  - use of experimental models for a better understanding of the renal failure production mechanisms
  - use of montelukast as a possible treatment option in acute renal failure, in the present study being demonstrated its protective effects in the kidney
  - assessment of serum and urinary magnesium levels in case of acute kidney injury, as a possible early diagnosis marker of ARF
  - the need to investigate zinc concentrations in assessing ARF, since Zn, playing a well known role in antioxidant redox system regulation, can influence the evolution of renal damage,
precisely by altering the antioxidant defense system, and also the immune system.
- The need to monitor serum and urinary concentrations of divalent cations, especially Mg, in children with acute nephropathy,
- because serum Mg correlated with urinary excretion of magnesium, may be a useful early recognition marker of cases with resistance to classic steroid treatment.

SELECTIVE REFERENCES


