Article

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Article

Differential Protective Effect of Zinc and Magnesium for the Hepatic and Renal Toxicity Induced by Acetaminophen and Potentiated with Ciprofloxacin in Rats

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Abstract: **Background:** The purpuse was to investigate the influence induced by magnesium chloride ($MgCl_2$) and zinc gluconate (ZnG) supplementation on liver and kidney injuries experimentally-induced with acetaminophen (AAPh) and potentiated by ciprofloxacin addition in rats. ***Material and methods***: The experiment was performed on 5 animals groups: group 1 – control, treated for 6 weeks with normal saline, 1mL/kg; group 2 – AAPh, treated for 6 weeks with AAPh, 100 mg/ kg/day; group 3 – AAPh+C, treated AAPh 100 mg/kg/ day for 6 weeks and ciprofloxacin, 50mg /kg/day, only in the last 14 days of the experiment; group 4 – AAPh+C+Mg, with the same treatment as group 3, but, and in the last 14 days, $MgCl_2$ 10 mg/ kg/day was added; group 5 – AAPh+C+Zn, with the same treatment as group 3, but, and in the last 14 days, zinc gluconate (ZnG),10 mg/kg/day was added. All administrations were performed by oral gavage. At the end of the experiment, the animals were sacrificed and blood samples were collected for biochemistry examinations. ***Results***: Treatment with AAPh for 6 weeks determined an alteration of the liver function (increases in alanine aminotransferase, aspartate aminotransferase, lactic dehydrogenase and, and gamma-glutamyl transferase) and of renal function (increases in serum urea and creatinine) ($P < 0.001$ group 2 *vs*. group 1 for all mentioned parameters). Also, the antioxidant defense capacity was impaired in group 2 *vs*. group 1 (superoxide dismutase and glutathione peroxidase activity decreased in group 2 *vs*. group 1, $0.001 < P < 0.01$, respectively $0.01 < P < 0.05$). The addition of ciprofloxacin, 50 mg/kg/day during the last 14 days resulted in further increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea and creatinine ($0.01 < P < 0.05$, group 3 *vs*. group 2). $MgCl_2$ provided a slight protection against liver enzymes increase and a more pronounced protection against the increase in serum urea and creatinine ($0.001 < P < 0.01$ group 4 *vs*. group 3). $MgCl_2$ provided a slight protection against the decrease in superoxide dismutase ($0.01 < P < 0.05$ group 4 *vs*. group 3), but not against decrease of glutathione peroxidase. The improvement of mentioned parameters could also be seen in case of ZnG, to a higher extent especially in the case of alanine aminotransferase and lactic dehydrogenase ($0.01 < P < 0.05$ group 5 *vs*. group 4). ***Conclusions***: This study represents a furthermore proof for beneficial effect of magnesium and zinc salts against toxicity induced by different agents, including antibacterials added to the analgesic and antipyretic acetaminophen; the protection is proven on the liver and kidney's function; the antioxidant profile improvement has a key role, especially in case of zinc gluconate.

Keywords: magnesium chloride (MgCl2); zinc gluconate (ZnG); liver diseases; kidney diseases

1. Introduction

Antibacterial drugs represent an important arsenal in fighting against infections. Among the greatest problems related to their utilization, bacterial resistance development is particularly severe, especially in case of injudicious use; but another very serious concern related to antibacterials use of is their potential to induce adverse effects, as their action in the patient's body is not limited to the pathogenic microbial agent. Even though these drugs are regularly bio-transformed in the human body and eliminated, and their toxicity is generally selective, it is often that they induce serious adverse effects or significant toxicity (nephrotoxicity, hepatotoxicity, cardiotoxicity) [1].

The toxicity of antibacterials often overlaps with that of some other drugs that they are concomitantly recommended with or with that of recreational toxics (eg, ethanol). Acetaminophen is commonly used as analgesic and antipyretic, it is strongly nephrotoxic and hepatotoxic, especially through its active metabolites. Fever is a symptom that frequently accompanies infections, including bacterial ones. Acetaminophen association with antibacterials is common in the therapy of bacterial infections, the liver or kidney toxicity of acetaminophen often being cumulative with that of antibacterial medication.

On the other hand, certain chemical elements, either essential macronutrients or trace elements (chemicals representing less than a thousandth of the dry tissue composition in the human or animal body) have essential roles in the living world. These roles are often ignored, and the diet does not always manage to provide a necessary supply of certain such elements. Magnesium, for example, induces hepatoprotection against toxicity induced by arsenic trioxide [2] or elevated fructose levels [3], and various clinical studies have demonstrated that an elevated level of magnesium it is associated with longer survival in patients with chronic kidney disease [4]. Beneficial effects of zinc supplementation have been observed in children and adolescents with kidney disease [5]; low zinc levels are associated with various liver pathologies, and supplementation with a zinc-acetate formulation improves liver function [6]. The deficiency of both magnesium and zinc is relatively common: in developed countries, it is estimated that the prevalence of marginal magnesium deficit is 15%–20% of the population [7], while the prevalence of inadequate dietary intake ranges from 7.5% in high-income regions to 30% in South Asia and zinc intake is lower than recommended in more than 75% of pregnant women [8–10].

Based on the above, we justify the current study, in which we aimed to investigate the supposed hepatoprotective and nephroprotective effect of supplementation with magnesium chloride, respectively zinc gluconate in nephro-hepatopathy experimentally induced with acetaminophen and potentiated by the addition of ciprofloxacin.

2. Materials and Method

2.1. Ethical Policies

This study complied with the European Guidelines for Human and Animal Rights, Directive 2010/63/EU and the Romanian Law of Research no. 206/27.05.2004. The research was approved by the Research Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy in Iaşi, Romania (06.VIII.2019).

2.2. Substances

Acetaminophen (AAPh) – powder, ciprofloxacin – neat, magnesium chloride ($MgCl_2$) – anhydrous and zinc gluconate (ZnG) – powder were purchased from Sigma Chemical Co. (St. Louis, MO).

Substances were prepared as solutions.

2.3. Laboratory Animals

Five-week-old male albino Wistar rats (nonconsanguineous; n=50) were used in the experiments. The animals weighed 150 ± 10 g each at the beginning of the experiment and were purchased from the I. C. Cantacuzino National Institute of Research and Development for Microbiology and Immunology, Bucharest, Romania. The animals were individually housed in polypropylene cages according to European standards in the Laboratory for Pharmacology Research in a controlled environment (temperature 23 ± 2ºC, 50–60% relative humidity, central ventilation and artificial light-

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dark cycles of 12 h/12 h). The animals had free access to water and standard food, which were provided *ad libitum*.

The rats were acclimatized for 7 days prior to the beginning of the experimental study.

2.4. Groups and Design of the Experiment

The rats were randomly divided into 5 groups of 10, and the experiment was conducted for 42 days:

Group 1 (control, C): the animals were treated for 6 weeks with normal saline, 1mL/kg.

Group 2 (acetaminophen-induced hepatopathy, AAPh): the rats were treated for 6 weeks with acetaminophen, 100 mg / kg / day;

Group 3 (acetaminophen-induced hepatopathy plus ciprofloxacin, AAPh + C): the rats were treated for 6 weeks with acetaminophen 100 mg / kg / day, by oral gavage. In addition, ciprofloxacin, 50 mg / kg, was added in the last 14 days of the experiment (50mg /kg/day).

Group 4 (acetaminophen-induced hepatopathy plus ciprofloxacin plus magnesium, AAPh + C + Mg): the rats were treated for 6 weeks with acetaminophen 100 mg / kg / day. In addition, in the last two weeks of the experiment, ciprofloxacin, 50 mg / kg / day and magnesium chloride 10 mg / kg / day were added.

Group 5 (acetaminophen-induced hepatopathy plus ciprofloxacin plus zinc, AAPh + C + Zn): the rats were treated for 6 weeks with acetaminophen 100 mg / kg / day. In addition, in the last two weeks of the experiment, ciprofloxacin, 50 mg / kg / day and zinc gluconate 10 mg / kg / day were added.

All solutions / substances were administered by oral gavage, in unique daily dose, at 7.30. The solutions for each of the pharmacologically active substances administered had the concentration calculated so that the volume of each administration was 1 mL of solution / kg.

2.5. Biochemical Determination

Blood samples were collected from the carotid artery of sacrificed animals and stored in standard biochemistry vacutainers.

The following parameters related mainly to the hepatic function were determined: alanine aminotransferase or glutamic-pyruvic transaminase (ALT / GTP), aspartate aminotransferase or glutamic oxaloacetic transaminase (AST / GOP), lactic dehydrogenase (LDH), and gamma-glutamyl transferase, γ-GT (GGT), alkaline phosphatase (ALP) and their values were expressed in international unites per liter (IU / L), with a precision of 1 IU / L (except for GGT, where the precision was of 0.01 IU / L).

The following parameters related mainly to the kidney function were determined: urea and creatinine and their values were expressed in mg per deciliter (mg / dL), with a precision of 1 mg / dL.

Biochemical determinations were performed using kits supplied by BD Biosciences Co. (Heidelberg, Germany) and a VITROS® 350 Chemistry System biochemical analyzer (Johnson & Johnson Ortho-Clinical Diagnostics) (colorimetric methods).

The following parameters related mainly to the antioxidant status were determined: superoxide dismutase 1 (SOD1) and Glutathione peroxidase 1 (GPX 1). The determinations were performed using enzyme-linked immunosorbent assay (ELISA) kits from Cloud-Clone Corp and an optical density reader by Perkin EnVision. Values were expressed in mg per deciliter (pg / mL), with a precision of 1 pg / mL.

2.6. Statistical Data Analysis

Statistical analysis was performed using SPSS software version 20 for Windows (IBM, Chicago, Illinois, United States of America). Data were first checked for normality in each group and for each tested parameter using the Shapiro-Wilk test. As the data showed a normal distribution, the results were expressed in graphs and tables as average ± standard and one-way ANOVA was used to test for differences among groups; the paired samples t-test was used for same-group comparisons at different time points.

3. Results

3.1. Body Weight, Growth, Food and Water Intake

During the acclimatization period and the 42 days thereafter, the body weight of the animals in all groups increased constantly regardless of their treatments.

3.2. Biochemical Determinations

Treatment with acetaminophen for 6 weeks determined an alteration of:

• liver function: increases in ALT, AST, LDH, GGT and ALP ($P < 0.001$, group 2 *vs*. control group 1);

• renal function: increases in serum urea and serum creatinine ($P < 0.001$, group 2 *vs*. control group 1);

• antioxidant defense capacity (decrease in SOD and GPX activity, $0.001 < P < 0.01$ and $0.01 < P < 0.05$ respectively, group 2 *vs*. control group 1).

The addition of ciprofloxacin, 50 mg/kg/day during the last 14 days of AAph administration resulted in further increase of the following serum biochemical parameters: ALT, AST, serum creatinine and urea ($0.01 < P < 0.05$, group 3 *vs*. group 2), while ALP was even more seriously increased ($0.001 < P < 0.01$, group 3 *vs*. group 2) and SOD was increased ($0.01 < P < 0.05$, group 3 *vs*. group 2). The serum concentration of GGT, LDH and GpX were not modified by the addition of ciprofloxacin.

Magnesium chloride oral treatment, 10 mg/kg/day addition during the 14 days of ciprofloxacin administration on the background of acetaminophen-induced toxicity provided a certain degree of protection against the alteration of certain assessed biochemical parameters:

- pronounced protection against the increase in serum concentration of serum urea and creatinine ($0.001 < P < 0.01$, group 4 *vs*. group 3);
- slight, yet significant protection against the increase in the serum concentration of liver enzymes ALT, AST, LDH, GGT and ALP and against the decrease in the serum concentration of SOD ($0.01 < P < 0.05$, group 4 *vs*. group 3);
- insignificant protection against the decrease in the serum concentration of GPx.

Zinc gluconate, oral treatment, 10 mg/kg/day addition during the 14 days of ciprofloxacin administration on the background of acetaminophen-induced toxicity provided a certain degree of protection against the alteration of certain assessed biochemical parameters:

- particularly pronounced protection against the increase in the serum concentration of liver enzymes ALT and LDH and serum creatinine ($P < 0.001$, group 5 *vs*. group 3);
- pronounced protection against the increase in the serum concentration of liver enzyme GGT and serum urea ($0.001 < P < 0.01$, group 5 *vs*. group 3);
- slight, yet significant protection against the increase in the serum concentration of liver enzymes AST, ALP and the decrease of SOD and GPx ($0.001 < P < 0.01$, group 5 *vs*. group 3).

Only for ALT and LDH the improvement was superior in the case of zinc gluconate compared to magnesium chloride ($0.01 < P < 0.05$ in group 4 *vs*. group 5), while the opposite situation was not recorded for any parameter.

The complete list of values for the assessed biochemical parameters in rats can be found in table 1.

Table 1. The values for the assessed biochemical parameters in rats.

Assessed biochemical	Control	AAPh	AAPh + C	AAPh + C + Mg	AAPh + C + Zn

parameter / Group					
ALT (UI / L)	**69.57 ± 2.57**	**2475.14 ± 284.38** $P < 0.001$ *vs.* control	**2964.57 ± 343.91** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh	**2558.43 ± 251.97** $P < 0.001$ *vs.* control NS *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**2284.14 ± 205.55** $P < 0.001$ *vs.* control NS *vs.* AAPh $P < 0.001$ *vs.* AAPh + C $0.01 < P < 0.05$ *vs.* AAPh + C + Mg
AST (UI / L)	**154.43 ± 13.20**	**578.86 ± 61.56** $P < 0.001$ *vs.* control	**650.43 ± 59.33** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh	**585.71 ± 28.61** $P < 0.001$ *vs.* control NS *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**583.57 ± 29.31** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
LDH (UI / L)	**456.43 ± 117.74**	**3309 ± 287.64** $P < 0.001$ *vs.* control	**3463.86 ± 228.73** $P < 0.001$ *vs.* control NS *vs.* AAPh	**3139.57 ± 302.39** $P < 0.001$ *vs.* control NS *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**2806.14 ± 201.28** $P < 0.001$ *vs.* control $P < 0.001$ *vs.* AAPh $P < 0.001$ *vs.* AAPh + C $0.01 < P < 0.05$ *vs.* AAPh + C + Mg
GGT (x 100UI / L)	**33.57 ± 5.97**	**67.14 ± 6.12** $P < 0.001$ *vs.* control	**69.85 ± 5.73** $P < 0.001$ *vs.* control NS *vs.* AAPh	**60.86 ± 7.10** $P < 0.001$ *vs.* control NS *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**59.71 ± 4.79** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh $0.001 < P < 0.01$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
ALP (x 100UI / L)	**105 ± 6.66**	**166.57 ± 19.04** $P < 0.001$ *vs.* control	**205 ± 15.21** $P < 0.001$ *vs.* control $0.001 < P < 0.01$ *vs.* AAPh	**189.57 ± 9.24** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**188.29 ± 12.75** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
Ureea (mg / dL)	**31.43 ± 2.82**	**63.14 ± 6.15** $P < 0.001$ *vs.* control	**71.57 ± 7.00** $0.01 < P < 0.05$ *vs.* AAPh	**61.57 ± 4.72** $P < 0.001$ *vs.* control NS *vs.* AAPh $0.001 < P < 0.01$ *vs.* AAPh + C	**60.28 ± 4.31** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh $0.001 < P < 0.01$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
Creatinine (mg / dL)	**5.91 ± 1.17**	**8.42 ± 0.80** $P < 0.001$ *vs.* control	**9.33 ± 0.59** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh	**7.66 ± 1.11** $0.01 < P < 0.05$ vs. control NS *vs.* AAPh $0.001 < P < 0.01$ *vs.* AAPh + C	**60.28 ± 4.31** $0.01 < P < 0.05$ vs. control $0.01 < P < 0.05$ *vs.* AAPh $0.001 < P < 0.01$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
SOD (pg / mL)	**24.71 ± 3.45**	**19.29 ± 1.50** $0.001 < P < 0.01$ *vs.* control	**17.42 ± 1.27** $P < 0.001$ *vs.* control $0.01 < P < 0.05$ *vs.* AAPh	**19.57 ± 1.90** $0.001 < P < 0.01$ vs. control NS *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C	**20.43 ± 2.30** $0.01 < P < 0.05$ vs. control $0.01 < P < 0.05$ *vs.* AAPh $0.01 < P < 0.05$ *vs.* AAPh + C NS *vs.* AAPh + C + Mg
GPX (pg / mL)	**31.57 ± 3.60**	**26 ± 4.08** $0.01 < P < 0.05$ vs. control	**23,42 ± 3.46** $0.001 < P < 0.01$ *vs.* control NS vs. AAPh	**26.43 ± 5.71** NS *vs.* control NS vs. AAPh NS vs. AAPh + C	**27.71 ± 3.77** NS *vs.* control NS vs. AAPh $0.01 < P < 0.05$ *vs.* AAPh + C NS vs. AAPh + C + Mg

4. Discussions

The current study demonstrated that both zinc gluconate and magnesium chloride offered a considerable protection against renal and hepatic toxicity induced with acetaminophen and enhanced by ciprofloxacin in rats. The protection is more evident for the zinc-based preparation, especially in the case of liver function.

Both compounds offered a slight level of protection against the alteration of anti-oxidant capacity induced by mentioned toxics (they protected against the decrease in the serum concentration of superoxide dismutase, but just $ZnCl_2$ protects against the decrease in the serum concentration of glutathione peroxidase). This supports the hypothesis that magnesium or zinc cations kidney and liver protection is, at least partially, due to improving the antioxidant defense.

In the medical literature, there are important evidences from studies of different researchers regarding the possible beneficial effects of zinc and magnesium compounds in liver and kidney damages of various causes. There are clinical studies (both observational studies that could prove a possible association of zinc or magnesium deficiency with liver or kidney pathology and interventional studies documenting a possible benefit of the administration of zinc or magnesium-containing compounds in liver or kidney pathology). Also, there are plenty of experimental studies proving benefits of zinc or magnesium compounds in liver or kidney pathology or the potentiation of liver or kidney damage by the deficiency of the two cations.

Some of those studies also manage to reveal certain aspects regarding mechanisms involved in hepatoprotection and nephroprotection induced by zinc and magnesium ions; *in vitro* studies contribute to the understanding of the protective mechanisms as well.

Magnesium – Roles in Hepatoprotection and Nephroprotection

The role of magnesium in hepatoprotection

Several clinical studies (both *observational* and *interventional*) support the idea of supplementing the diet with magnesium in liver diseases.

The relationship between magnesium levels in the human body and liver diseases is complex: *observational* studies have shown that many liver diseases are associated with magnesium deficiency, while insufficient magnesium aggravates, in turn, these diseases [11]. Patients with liver cirrhosis usually have low magnesium body levels [12]. Cirrhosis is often due to alcohol consumption, and magnesium deficiency is common among alcohol-consumers [13].

Several mechanisms can explain this situation. Impaired liver function causes some patients to develop portal hypertension, which favors gastroesophageal varices and intestinal edema. Magnesium intake may be reduced due to malnutrition and lower absorption in the distal jejunum. In addition, the liver synthetizes less albumin, which is an important magnesium carrier in the circulation. Inactivation of many hormones also occurs in the liver, and if this organ is affected, serum levels of aldosterone, growth hormone, and glucagon are increased and therefore urinary magnesium excretion also increases. In addition, diuretics with magnesium-excretion properties (such as furosemide) are often recommended to cirrhotic patients.

Hepatic loss of magnesium is associated with greater collagen deposition in the liver [14]. Progression to cirrhosis is very likely particularly in patients with low hepatocytic magnesium concentrations. Decreased intracellular Mg^{2+} content has a negative impact on mitochondrial bioenergetics. Impairment of mitochondrial function implies impairment of oxidation in hepatocytes, associated with a 17% reduction in ATP production and hepatocyte damage. The subsequent repair process of the liver leads to further fibrosis and worsens cirrhosis.

Interventional studies have shown that magnesium supplementation ameliorates various liver diseases. For instance, a 100 mg increase in daily magnesium intake is associated with a 49% decrease in the risk of mortality from all liver diseases [15]. Another study claims that magnesium supplementation can prevent hepatitis C virus replication by binding to the NS3 helicase of the virus [16].

Magnesium hepatoprotective effect has also been demonstrated by numerous *experimental studies*. For example, prophylactic administration of magnesium isoglycyrisinate (9 and 18 mg/kg/day) significantly reduced serum ALT and AST levels in rats treated with 20 mg/kg methotrexate (intravenously) and attenuated methotrexate-induced liver fibrosis, hepatocyte apoptosis and reduced serum malondialdehyde levels more than glutathione, 80 mg/kg/day. Methotrexate-induced cyclooxygenase-2 expression, intestinal permeability, and inflammation were attenuated [17]. Magnesium isoglycyrrhizinate (i.p., 50 mg/kg/day) induces hepatoprotection against arsenic trioxide-induced toxicity in mice (Liu et al., 2021) [2] or elevated fructose levels [18] in rats.

An *in vitro* study showed that magnesium cantharidate has inhibitory effect on the proliferation of SMMC-7721 human hepatoma cells by blocking the MAPK signaling pathway; the phosphorylation levels of C-jun N-terminal kinase (JNK) and extracellular signal-related kinase (ERK) decrease significantly after such treatment [19].

The Role of Magnesium in Nephroprotection

Pathophysiological considerations: The kidney has a vital role in the homeostasis of magnesium; renal Mg excretion is highly adaptive, but it is impaired when renal function declines significantly. In chronic kidney disease (CKD) of moderate severity, the increase in Mg^{2+} excretion largely compensates for the loss of glomerular filtration rate and normal serum magnesium levels are maintained.

However, in more advanced forms of the disease, this compensatory mechanism becomes inadequate, so hypermagnesemia frequently develops in patients with creatinine clearance below 10 mL/minute (as less Mg^{2+} is filtered through the glomeruli to be excreted). The treatment of hyperphosphatemia also contributes to hypermagnesemia (for example, sevelamer is a non-absorbed oral phosphate binder, increases the blood concentration of Mg by increasing fatty acid delivery to the intestines and by sequestering bile salts [20], the prevalence of hypermagnesemia increases as kidney function declines.

In fact, CKD can also be associated with hypomagnesemia [21]. Both patients with polycystic kidney disease and end-stage renal disease on dialysis usually have normal serum Mg sometimes even hypomagnesemia [22]. In CKD, hypomagnesemia may develop due to tubular dysfunction with impaired renal Mg reabsorption. This fact is due to the complexity of magnesium intake, beyond the food source, drugs and the concentration of magnesium in the dialysate. Hypomagnesemia can be an adverse effect of medications such as thiazide diuretics, proton pump inhibitors, cisplatin, aminoglycosides, and calcineurin inhibitors [23]. Proton pump inhibitors may lower the serum Mg by increasing the pH in the intestinal lumen, which decreases decrease Mg absorption by altering the expression of paracellular claudins and the transcellular transporter TRPM 6/7 [24].

Human studies: Observational studies showed that patients with polycystic kidney disease have severely depressed intestinal Mg^{2+} absorption, probably due to a deficiency of active vitamin D, and in end-stage renal disease the adaptive increase in active intestinal absorption of magnesium is impaired [22].

An interventional study showed that a higher level of proteinuria was associated with greater urinary Mg excretion. Mg oxide administration significantly increased serum Mg levels after one year, but only among patients with a urinary protein-to-creatinine ratio of less than 0.3 g/g, while there was no change in serum Mg in those with greater levels of proteinuria, suggesting that hypomagnesemia is a consequence of tubular injury [25].

Experimental studies that demonstrated the protective effect of magnesium on the nephrotoxicity of colistin, decreasing serum urea and creatinine, but increasing the concentration of malondialdehyde and improving renal histopathological aspects [26].

A low-magnesium diet exacerbated kidney damage induced by the high-phosphate diet. Impairment of parathyroid hormone (PTH) secretion and down-regulation of renal α-Klotho were likely involved in the reduced urinary phosphate excretion by the low-magnesium diet. Increasing dietary magnesium may be helpful to attenuate phosphate-induced renal injury in mice [27].

Magnesium is known to protect against phosphate-induced tubular cell damage *in vitro* [28].

Zinc – Role in Hepatoprotection and Nephroprotection

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Also, in case of zinc, results of different studies support both the idea of zinc deficiency as an element associated with liver or kidney pathology, and the idea of the opportunity of dietary zinc supplementation in various forms of kidney pathology and hepatic disorders.

The Role of Zinc in Hepatoprotection

The association of zinc deficiency with liver failure is clearly proven in experimental models and in clinical studies. The idea of dietary zinc supplementation appears to be supported by both observational and interventional clinical studies.

Since the 1950s, *observational studies* have demonstrated that serum Zn levels are associated with liver pathology. Vikbladh (1950; 1951) [29,30] reported that serum zinc was low in many chronic diseases, and in 1956, Bartholomay et al. [31] reported that serum Zn concentration was low in patients with liver cirrhosis and suggested that this was a consequence of hyperzincuria (increased urinary Zn excretion in such a condition being somewhat paradoxical).

Interventional Studies have Shown that Zinc Supplementation Improves Various Liver Conditions.

Some cirrhotic patients who experienced night blindness did not respond to vitamin A supplementation, but responded to zinc administration [32]. Zinc supplementation ameliorated liver fibrosis in patients with early cirrhosis [33], and supplementation with a zinc-acetate formulation significantly improved liver function [6]. Zinc therapy has been shown to be beneficial in subjects with hepatic encephalopathy [34].

Their results are reinforced by *experimental studies* that demonstrated the protective effect of zinc on liver damage induced by D-galactosamine or nickel in rats [35,36]. Pretreatment with zinc chloride (50 or 100mg/kg daily, 36 days) reduced hepatic toxicity of glyphosate, ameliorated numerous biochemical parameters (such as increases in serum enzymes associated with hepatobiliary injury), but were ineffective in preventing histological lesions [37].

The ***mechanism*** underlying the association between zinc deficiency and liver dysfunction is insufficiently elucidated. These reports indicate an association between zinc deficiency and organ damage due to fibrosis and oxidative stress. Elevated levels of ammonia in the blood are known to be a factor in the development of hepatic coma. Zinc-deficient rats are known to have a defect in the metabolism of sulfur-containing amino acids. Zinc deficiency also affects urea synthesis, and thus an abnormality related to amino acid and ammonia metabolism may act in concert to produce hepatic coma. Dietary zinc restriction can lead to hyperammonemia. Rabbani and Prasad, 1978 [38] observed a decrease in hepatic ornithine transcarbamoylase activity and an increase in plasma ammonia levels in zinc-deficient rats. In addition, increased activity of the enzyme adenosine monophosphate purine deaminase has been observed as a result of zinc deficiency, which may also contribute to increased ammonia levels.

The Role of Zinc in Nephroprotection

The association of zinc deficiency with renal failure, especially in hemodialyzed patients, is clearly proven, and so is the beneficial effects of zinc in various forms of renal pathology.

Observational studies have shown that serum zinc levels tend to decrease with the progression of CKD [39,40]. Survival analysis showed that zinc deficiency is a risk factor for progression to end-stage renal disease and death [41]. Analysis using competing risk models showed that the low serum zinc group had a high risk of progression to end-stage renal disease.

Hypoalbuminemia may exacerbate the progression of zinc deficiency-induced renal disease. About 80% of serum zinc is bound to albumin, and serum zinc and albumin levels are positively correlated [41]. Malnourished patients, who often have hypoalbuminemia, are prone to zinc deficiency. Insufficient zinc intake is more likely to occur in malnourished older people, especially those with CKD. Gastrointestinal absorption is lower in patients with CKD. Furthermore, hypoalbuminemia reduces the amount of albumin-bound zinc in the blood, leading to increased urinary zinc excretion. In the cited study, the number of patients with nephrotic syndrome was statistically significantly higher in the low zinc group. Thus, higher urinary excretion of albumin lowers zinc levels even more.

The association between hemodialysis and zinc deficiency is probably due to the increased elimination of zinc during such procedure, inadequate dietary intake, and malabsorption. Patients with CKD have higher urinary zinc excretion, which tends to increase as the disease progresses [42].

Interventional studies have shown that zinc supplementation improves the response to erythropoietin therapy in dialysis patients [43], and beneficial effects of zinc supplementation have been observed in children and adolescents with kidney disease [5]. A clinical trial demonstrated that zinc supplementation reduces urinary albumin excretion in type 2 diabetes patients with microalbuminuria [44]. In patients with low Zn levels, the risk of progression of primary kidney disease was lower if zinc-containing compounds were administered during the observation period [41].

Experimental studies that demonstrated that in diabetic rats, zinc supplementation suppresses pathological changes associated with tubulointerstitial and glomerular damage [45].

Pretreatment with zinc chloride (50 or 100mg/kg daily, 36 days) reduced renal and hepatic toxicity of glyphosate, ameliorated numerous biochemical parameters (such as hypercalcemia and metabolic alkalosis, and attenuated serum accumulation of creatinine), but did not alleviate histological damages in rats [37].

Mechanisms Involved in Zinc Hepatoprotection and Nephroprotection

If the protective effect induced by Zn^{2+} has been proven by numerous researches, the mechanism underlying the association between zinc deficiency and renal dysfunction remains unclear. Various fundamental studies have shown that zinc is a regulator of oxidative stress, in its capacity as a cofactor of superoxide dismutase; its deficiency induces oxidative stress and kidney damage through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [46]. Evidences indicates that oxidative stress is the common denominator among major pathways involved in the development and progression of kidney diseases [47], with NADPH oxidase being identified as a major source of oxidative stress in kidney diseases. An intervention study in healthy individuals also demonstrated that zinc supplementation reduced oxidative stress [48]. Zinc deficiency promotes renal fibroblast activation and leads to interstitial fibrosis in diabetic mice [49], and, in line with what has been shown, zinc supplementation improves liver fibrosis in patients with early cirrhosis [33]. These reports indicate an association between zinc deficiency and organ damage due to fibrosis.

Supplementation with zinc and / or magnesium should be recommended in patients undergoing long term antibiotherapy combined with antipyretic administration. The idea is also sustained by the low price and low toxicity of such compounds and by their beneficial effect on the immune system [7,50].

5. Conclusions

The hepatotoxicity of the antipyretic and analgesic drug acetaminophen is potentiated by ciprofloxacin administration in rats, but both zinc gluconate and magnesium chloride slightly alleviate the combined toxicity of the mentioned substances (diminish the elevated level of liver enzymes and creatinine). Especially in case of the zinc compound, where the effect is slightly higher, this protection seems to partially associated with the potentiation of the antioxidant effect.

Administration of dietary supplements with magnesium and especially zinc may represent an easy, yet efficient way to reduce the toxicity of antibacterials associated to cyclo-oxygenase's inhibitors.

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Abbreviations

- ALT: alanine aminotransferase (also called, GTP – glutamate-pyruvate transaminase);
- ALP: alkaline phosphatase;
- AST: aminotransferase (also called, GOP – glutamate- oxaloacetic transaminase) ;
- CKD: Chronic kidney disease;
- gamma-glutamyl transferase, γ-GT;
- GGT: glutathione peroxidase (GPx);
- LDH: lactic dehydrogenase;
- SOD: superoxide dismutase.

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