Influence of Treatment with the Tincture of *Aesculus hippocastanum* L. on Zinc and Magnesium Content in Liver and Kidney of Rats with Adjuvant Arthritis

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The aim of the study was to investigate the relation of treatment with the tincture of *Aesculus hippocastanum* L. (AH) on the content of Zn and Mg in the liver and kidney of 70 rats with adjuvant arthritis (AA). Three different doses of AH (2.4, 4.8 and 9.5 mg/kg^{-1} calculated to dry weight of tincture) were given as intragastric injections. Control groups: acetylsalicylic acid (ASA) at a dose of 21 mg/kg^{-1} as an antiinflammatory drug, ethanol 5 mg/kg^{-1} for tincture solvent. The rest 10 control animals were healthy rats. The liver and kidney content of Zn and Mg was investigated by atomic absorption spectrometry.

The development of acute inflammatory process for 20 days significantly increased Mg content and by 6% the Zn content in liver, while in kidney tissue Mg content decreased. The content of Mg in liver remained increased after the treatment with all three doses of AH as well as with ASA, while in kidney tissue it was the same as in the control group of rats. The level of Zn in liver and kidney increased in response to the treatment of AA with AH tincture. Similar results were obtained also after the treatment with ASA.

Key words: zinc, magnesium, adjuvant arthritis, acidum acetylsalicylicum, *Aesculus hippocastanum* L. tincture

INTRODUCTION

The relationship between macro- and trace element content in human and animal tissues and the pathogenesis of rheumatoid arthritis have been investigated in many studies (1–6). It is known that Zn is a cofactor of matrix metalloproteinases that degrade proteoglycans and collagen leading to connective tissue destruction (1). Absorption and metabolism of this trace element are strictly regulated by metallothioneins and the synthesis of these inducible proteins is typically promoted in many different tissues such as liver, kidney (2, 3). Nevertheless, proinflammatory cytokines such as interleukin-6 (IL-6) (4) and interleukin-1 (IL-1) play a role in the expression of the metallothionein gene, wich increases Zn levels in the liver, kidney and other tissues, with a concomitant decrease in serum (5). According to Milanino (1), in the adjuvant arthritis model a decrease of zinc concentration in liver was accompanied by an increase in plasma metal level, therefore experimental evidence suggested that inflammation promotes a redistribution of metals between plasma and liver (6).

The liver appears to play a special role in zinc metabolism. Once absorbed from the gastrointestinal tract into the circulation, zinc is cleared within 3 h. It is taken up by the liver and eventually appears in the kidneys, pancreas, and other tissues, suggesting that the liver may be central to zinc transfer and distribution (7).

Magnesium is the fourth most plentiful cation in the body. As a catalyst of more than 300 enzymes involved in a numerous biochemical and physiological functions, magnesium plays a critical role in the regulation of protein synthesis, energy and nutrition metabolism. About 70% of plasma Mg is ultrafiltered by the kidney; the remainder is bound to protein. The kidney represents the principal patway for magnesium elimination from the body. Apparently, a renal threshold for magnesium exists in humans; only 5% to 6% of the filtered magnesium is actually eliminated in urine. Because the renal capacity to conserve magnesium is so remarkable, urinary magnesium is a useful indicator of total body stories (8). As with calcium, protein binding of magnesium is pH-dependent. Plasma Mg concentration

and either total body magnesium or intracellular magnesium content are not closely related. For this reason, serum magnesium concentration may not accurately reflect total magnesium stories in all compartments (9). The data concerning changes in the status of magnesium during the course of rheumatoid arthritis are scarcely presented.

Patients suffering from rheumatoid arthritis represent a population that often undergoes multiple drug therapy, and some of the agents currently used to treat these subjects are known to be able potentially to interfere with elements (especially with zinc) absorption and metabolism. Much interest focuses on the already available non-steroidal antiinflammatory drugs (NSAID) and steroids. Some studies have shown that steroids and NSAIDs can induce a decrease in plasma zinc levels in rheumatoid arthritis patients (10–12) as well as in laboratory animals (13, 14). However, plasma zinc has not been found to be affected by the use of either gold salts or D-penicilamine (15, 16). According to Fontaine (17), in both normal and adjuvant arthritic rats, glucocorticoid administration causes an initial and transient decrease in serum zinc concentration, promptly followed by a return of this parameter to the levels measured before the beginning of the treatment. It has been observed that treatment of adjuvant arthritis rats with acetylsalicylic acid (ASA) reversely increased the content of Zn, Cu and Fe in the liver (18).

Phytotherapy refers to treatment with medications made exclusively of plants, parts of plants or botanical ingredients. Science traces its origins back to the herbal remedies of past centuries. As in many fields of modern medicine, phytotherapy is returning to gentler and more careful forms of therapy. Herbal medicines are symptomatic slow-acting drugs used also for treatment of rheumatic diseases (19, 20). In this case, *Aesculus hippocastanum* L. can be also used as a homeophatic medication. It has an astringent effect, reduces infections, promotes circulation, dehidrates tissues and reduces swelling (21). Flowers, bark, seeds and fruits of AH are rich in flavonoids, saponins, coumarins, tannines, minerals. Flavonoids show anti-inflammatory, anti-allergenic, cardiotonic, hypotensive effects, inhibit cyclo-oxygenase and lipoxygenase products (prostanoids and leucotrienes), also exert a protective effect on gastric mucosa.

The therapeutic benefit of AH is well supported by a number of experimental investigations in different animal models, indicative of clearcut anti-oedematous, anti-inflammatory and venotonic properties (22, 23). According to Chang (24), all these chemicals (saponins, flavonoids, coumarins et al.) act sinergistically in treatment of rheumatic diseases, and AH has been used in folk medicine as an anti-inflammatory analgetic for rheumatism.

The aim of the study was to investigate the influence of treatment with the tincture of *Aesculus hippocastanum* L. on zinc and magnesium content in the liver and kidney of rats with adjuvant arthritis.

MATERIALS AND METHODS

Seventy Wistar rats (thirty male and forty female) obtained from Bioreglament (Vilnius, Lithuania), weighing 200–300 g were used in this study. Rats were housed in groups of 5 (male and female separated) in large plastic cages at room temperature and under standard light conditions. They received standard rat chow and water *ad libitum*. All animals were allowed to acclimate for at least 3 days before experiments started.

AA was induced by a single injection of 0.1 ml complete Freund's adjuvant into left hind paw.

AH tincture was used for the treatment. This agent was produced from wild plants growing in Lithuania. The treatment with AH (in three different doses – 2.4, 4.8 and 9.5 mg/kg^{-1} calculated to dry weight of tincture) was started next day after the AA was induced. The control groups received ASA at a dose of 21 mg/kg^{-1} as an anti-inflammatory drug and ethanol at a dose of 5 mg/kg^{-1} for control of the tincture solvent. The remaining 10 control animals were healthy rats. ASA (Ratiopharm GmbH 89070, Ulm, Reg. No 45624) was prepared *ex tempore* as a fine homogeneous suspension in water. The pharmacological substances were given into stomach volume through a metallic sound daily, 7 times a week. Body weight and clinical signs of arthritis including the severity of join swelling were assessed 3 times a week. Experiments were completed after 20 days by decapitation. Liver and kidney tissues were stored at –20 °C for analysis of Zn and Mg content. Leucocyte and erythrocyte count in blood was determined by using Picoscale (Hungary). ESR and macroscopic changes of organs and joints were studied.

The preparation of liver and kidney samples is described in detail by M. A. Rios et al. (25). The analyses were carried out on the wet liver and kidney samples and the results are also given on a wet weight basis. The dry-to-wet ratio was determined by drying a selected piece of these organs at a 95 °C. The mean value for the dry-to-wet weight ratio of all the liver and kidney samples was 30% (water content 70%).

The concentration of Zn and Mg in liver and kidney was determined by atomic absorption spectrophotometry (A Unicam SP 190/191, UK) and cal-

culated by the standard additions method. The results were statistically analysed by the Statistic for Windows Program.

RESULTS AND DISCUSSION

In the liver and kidney of rats with untreated AA the levels of Zn were increased (1.1 and 1.3 times, respectively) as compared to control group (Table). Our data are in agreement with numerous experimental data (ref. cited in 26). Changes in the level of Mg in the liver were similar to those of Zn (Table) – the content of Mg statistically significantly increased, while in the case of kidney the content decreased by 17%. Relevant references were not found.

These studies showed that treatment of adjuvant arthritis with *Aesculus hippocastanum* L. tincture led to marked changes in metal content in the liver and kidney of rats by using three doses of the tincture.

The level of Mg in the liver remained not significantly increased after the treatment with all three doses of AH. The level of Zn in the liver was slightly decreased in case of a medium (4.8 mg/kg^{-1}) dose of AH (approximately by 4%, as compared to AA group), while a highest dose of the tincture significantly increased the content of Zn ($p < 0.01$).

The investigation of the influence of *Aesculus hippocastanum* L. tincture on the Zn and Mg changes in kidney from rats with AA revealed greater changes of Mg than of Zn level (Table). The level of Zn in kidney remained increased after treatment of AA rats with all three doses of AH, while the content of Mg increased to normal values.

Treatment of AA rats with ASA resulted in a marked increase of Zn in liver ($p < 0.05$) and kidney, while the level of Mg remained increased in liver and increased to normal values ($p < 0.05$) in kidney.

Ethanol caused a decrease in the content of both elements in the liver and kidney. Our data are in agreement with the experimental work described in (27).

Zn in liver and kidney was in a strong negative relation ($r = –0.6$; $r = –0.8$, $p < 0.01$ respectively) to adrenal weight in healthy rats. A positive correlation was calculated between Zn and Mg content in these organs and blood indices of rats with untreated AA. A strong positive relation of Zn in kidney to erythrocyte count remained after treatment with ASA ($r = 0.7$) and with all three doses of AH ($r = 0.6$, $p < 0.05$). A strong positive relation of Mg in kidney to ESR was found in the group of rats treated with the highest dose of AH ($r = 0.7$, $p < 0.05$). The same correlation was calculated in this group between Zn content in kidney and leucocyte count.

Zn content in liver showed also a negative relation to the adrenal weight after treatment with the small and highest doses of the tincture ($r = –0.5$). A strong negative relation of Mg content in liver to adrenal weight was calculated in the group of rats treated with ASA ($r = –0.54$). There was a certain relationship of Zn and Mg content in liver and kidney to inflammation activity markers such as a strong positive correlation of Zn in kidney to erythrocyte count and in liver to adrenal weight after treatment with AH as well as ASA. These results confirmed the antiarthritic properties of *Aesculus hippocastanum* L. tincture. Apparently, the tincture studied is able to induce changes in magnesium and zinc me-

Table. **Influence of *Aesculus hippocastanum* L. tincture on the changes of Zn and Mg content in the liver and kidney of rats with AA**

No	Group	Dose mg/kg^{-1} dry weight	N	Liver		Kidney	
				mg/kg dry wt			
				Zn	Mg	Zn	Mg
I	AA	–	10	161.66 ± 7.35	49.34 ± 1.51***	129.93 ± 3.68	19.05 ± 1.81
II	AA+ethanol	5	9	127.48 ± 16.88	46.2 ± 4.38	123.64 ± 4.44	18.67 ± 1.05
III	AA + *Aesculus hippocastanum* tincture	2.4	10	164.92 ± 8.96	46.62 ± 1.61***	137.63 ± 4.99***	24.47 ± 2.39
IV	AA + *Aesculus hippocastanum* tincture	4.8	10	155.29 ± 7.69	41.17 ± 3.32	130.96 ± 4.11	22.15 ± 1.49
V	AA + *Aesculus hippocastanum* tincture	9.5	10	176.89 ± 5.42*	44.26 ± 1.64***	136.34 ± 6.18***	22.33 ± 1.11
VI	AA + acetylsalicylic acid	21	10	173.19 ± 7.66**	48.53 ± 7.98	126.34 ± 5.60	24.38 ± 1.19**
VII	Control	–	10	152.6 ± 6.50	38.7 ± 0	118.01 ± 9.71	23.65 ± 1.54

*$p < 0.01$; **$p < 0.05$, – versus AA group
***$p < 0.001$ – versus Control group

tabolism during the treatment of inflammatory process.

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GYDYMO *AESCULUS HIPPOCASTANUM* L. TINKTŪRA POVEIKIS CINKO IR MAGNIO KIEKIUI ŽIURKIŲ KEPENYSE IR INKSTUOSE ADJUVANTINIO ARTRITO METU

Santrauka

Tirtas Zn ir Mg kiekis 70 žiurkių kepenyse ir inkstuose adjuvantinio artrito metu priklausomai nuo gydymo paprastojo kaštono (*Aesculus hippocastanum* L.) tinktūra 2,4, 4,8 ir 9,5 mg/kg^{-1} dozėmis. Kontrolei vartota acetilsalicilo rūgštis (21 mg/kg^{-1}) ir etanolis (5 mg/kg^{-1}) *per os* kasdien. Praėjus 20 dienų nuo adjuvantinio artrito pradžios žiurkių kepenyse nustatytas padidėjęs Zn ir Mg kiekis, o inkstuose – sumažėjęs Mg kiekis. Visos trys paprastojo kaštono tinktūros dozės padidino tirtųjų metalų kiekį kepenyse bei Zn kiekį inkstuose. Panašiai veikė ir acetilsalicilo rūgštis. Tuo tarpu gydymas tinktūra Mg kiekį inkstuose padidino iki sveikų žiurkių reikšmių. Gauti duomenys rodo, kad paprastojo kaštono tinktūra gydant uždegiminį procesą sukelia Zn ir Mg metabolizmo pokyčius.