Relation between Zinc and Magnesium Content in Liver of Rats with Adjuvant Arthritis and Treatment with the Tincture of *Filipendula ulmaria* (L.) Maxim.

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The aim of the study was to investigate the influence of treatment with the tincture of *Filipendula ulmaria* (L.) Maxim. (FU) on the content of Zn and Mg in the liver of 60 rats with adjuvant arthritis (AA). Three different doses of FU (2, 4 and 8 mg/kg^{-1}, calculated to dry weight of tincture) were given as intragastric injections. One of the control groups received acetylsalicylic acid (ASA) at a dose of 14 mg/kg^{-1} for salicylic compounds, and the rest 10 control animals were healthy rats. The content of Zn and Mg in the liver was investigated by atomic absorption spectrometry.

The development of acute inflammatory process for 20 days increased twice the Zn and Mg content in liver. All three doses of FU diminished the content of Zn and Mg to normal values. Zn and Mg in the liver of rats with AA was decreased after treatment with ASA; the reduction was most prominent in the case of Mg. Treatment with FU tincture tended to normalize all the indices of inflammation studied. The content of Zn and Mg in the liver was reduced to normal values in response to therapy of AA with FU tincture.

Key words: zinc, magnesium, adjuvant arthritis, acidum acetylsalicylicum, *Filipendula ulmaria* (L.) Maxim. tincture

INTRODUCTION

Many reports have pointed out that development of acute and chronic inflammatory process induces substantial changes in the metabolism of various minerals (including zinc and magnesium) in laboratory animals and in man. Prevailing opinion considers these changes to be part of the defense mechanism evoked by the organism to bring the inflammatory reaction under physiologic control (1). In this case recent research draws attention to the endogenous mediators and functional proteins that may be involved in both inflammation and trace metal metabolism. Zinc absorption and metabolism are strictly regulated by metallothioneins and the synthesis of these inducible proteins typically promoted in many different tissues such as liver, kidney (2, 3). Nevertheless, proinfammatory cytokines such as interleukin-6 (IL-6) (4) and interleukin-1 (IL-1) are potent inducers of metallothioneins synthesis in liver, bone marrow and thymus, causing marked accumulation of zinc (5). These results have shown that the liver is one of the most suitable body tissues to illustrate the status of elements during inflammation process.

Also, the concentration of zinc in the serum of rheumatoid arthritis patients has been extensively studied and a decrease in zinc content has been demonstrated (6, 7). 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.

As a catalyst of more than 300 enzymes involved in numerous biochemical and physiological functions, magnesium plays a critical role in the regulation of protein synthesis, energy and nutrition metabolism. It is known that magnesium is closely related to calcium metabolism. 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 (7–9) as well as in laboratory animals (10, 11). However, plasma zinc has not been found to be affected by the use of either gold salts or D-penicilamine (12, 13). According to Kishore (14), treatment of adjuvant arthritic rats with ASA reversely increased the content of Zn, Cu and Fe in the liver.

Symptomatic slow-acting drugs for the treatment of AA are herbal medicines (15, 16). *Filipendula ulmaria* (FU) was used historically for a wide variety of conditions, including treatment of rheumatic complaints in the joints and muscles and even arthritis (16). Flowers of FU have are rich in flavonoids, tannines, minerals, vit. C, moreover, their antiinflammatory activity is mostly predetermined by active salicylic acid derivates: salicilin, salicylaldehide and methyl salicylate (17, 18). Salicilin is a phenolic glycoside found in botanic agents that are converted into salicylic acid in the body. FU tincture is gentler to the stomach than ASA, its tannin and mucilage content appears to buffer the adverse effects of isolated salicylates. Salicylic acid has a significant affinity for hepatic tissue (19).

The aim of this study was to investigate the relation of zinc and magnesium content in liver of rats with adjuvant arthritis to the treatment with the tincture of *Filipendula ulmaria* (L.) Maxim.

MATERIAL AND METHODS

Sixty male Wistar rats obtained from Bioreglament (Vilnius, Lithuania), weighing 150–200 g were used in the study. Rats were housed in groups of 10 in large plastic cages at room temperature and under standard light condition. They received standard rat chow and water *ad libitum*. All animals were allowed to acclimate for at least 3 days before the experiments started. Approval of the Lithuanian Ethic Committee for Laboratory Animal Use was obtained prior to commencement of the experiments.

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

FU tincture was used for the treatment. This agent was produced from the wild plant growing in Lithuania (A. Keturkienė et al. Apply for patent N 2001 036). The treatment with FU (in three different doses – 2, 4 and 8 mg/kg^{-1}, calculated to dry weight of tincture) was started the next day after AA was induced. One of the control groups received ASA at a dose of 14 mg/kg^{-1} for salicylic compounds. 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, seven times a week. Body weight and clinical signs of arthritis, including the severity of joint swelling, were assessed three times a week. Experiments were completed after 20 days by decapitation preceded by ether narcosis. Liver tissue was stored at –20 °C for the analysis of Zn and Mg content. The leucocyte and erythrocyte count was determined by Picoscale (Hungary). ESR and macroscopic changes of organs and joints were studied.

The preparation of liver samples is described in detail by S. Luterotti et al. (20). The analyses were carried out on wet liver samples, and the results are also given on a wet weight basis. The dry-to-wet ratio was determined by drying selected pieces of the livers at 95 °C. The mean value for the dry-to-wet weight ratio of all liver samples was 30% (water content 70%).

The concentration of Zn and Mg in liver was investigated by atomic absorption spectrophotometry (A Unicam SP 190/191, UK) and calculated by the standard method of additions. The results were expressed as mean values ± S.E.M. The obtained data were processed by the method of variation statistics and their validity calculated according to the Student's t test. Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Data of the experiment revealed the quantity of Zn to be statistically significantly (twice) increased in the liver of rats with AA as compared to control group (Table). Our data are in agreement with numerous experimental works (ref. cited in 21). Changes in the level of Mg in liver were similar to that of Zn (Table) – the content of Mg significantly increased (1.8 times). Relevant references were not found.

These studies showed that treatment of adjuvant arthritis with *Filipendula ulmaria* (L.) Maxim. tincture led to marked changes in metal content in rat liver by using all three of doses of the tincture. The content of Zn after treatment of AA with FU diminished to normal values. A small dose of FU had a great effect on the changes of Zn quantity ($p < 0.05$), the medium dose of the tincture also significantly diminished the content of Zn ($p < 0.001$). A lowered increase of Mg content in liver during AA was observed after ad-

Table. **Effect of Filipendula ulmaria (L.) Maxim. tincture on the changes of Zn and Mg content in the liver of rats with AA**

No	Group	Doses, mg/kg^{-1}dry weight	N	mg/kg dry wt	
				Zn	Mg
I	AA	–	9	140.2 ± 11.86$^{\lozenge}$	77.75 ± 4.24 $^{\lozenge}$
II	AA+ Filipendula ulmaria tincture	2	10	68.08 ± 6.71**	52.52 ± 6.17*
III	AA + Filipendula ulmaria tincture	4	6	66.2 ± 6.38***	80.67 ± 8.74
IV	AA + Filipendula ulmaria tincture	8	8	85.79 ± 8.74*	54.41 ± 13.66
V	AA+ acidum acetylsalicylicum	14	9	111.38 ± 9.19	50.94 ± 3.53***
VI	Control + water	4	8	70.69 ± 7.6	43.09 ± 2.71

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

ministration of a small dose (4 mg/kg^{-1}) of FU ($p < 0.01$).

Treatment of AA rats with ASA resulted in a marked lowering of the increased quantity of Mg in liver ($p < 0.001$), however, differences in Zn levels between AA group and group treated with ASA were not significant. Administration of ASA diminished the content of Zn approximately by 30% as compared to AA group.

Zn and Mg in liver were in a strong positive relation ($r = 0.98$, $r = 0.5$, respectively) to ESR in healthy rats. A positive correlation, though lower, was calculated between Zn and Mg content in liver and blood indices of rats with untreated AA. A strong positive relation of Zn in liver to ESR remained after treatment with ASA ($r = 0.5$) and with the small and medium doses of FU ($r = 0.5$, $r = 0.6$, respectively). A strong positive relation of Zn in liver to the count of leucocytes was found in the group of rats treated with the smallest dose of FU ($r = 0.69$), while in case of Mg it was strongly negative ($r = –0.5$). The Zn content in liver was also in a negative relation to the adrenal weight after treatment with the tincture at a dose of 8 mg/kg^{-1} ($r = –0.42$). A strong negative relation of Mg content in liver to erythrocyte count was calculated in the group of rats treated with the greatest dose of FU ($r = –0.54$). There was a certain relationship of Zn and Mg content in liver to inflammation activity markers such as a strong positive correlation of Zn in liver to ESR, leucocytes count, and to adrenal weight after treatment with FU as well as with ASA. These results confirmed the antiarthritic properties of *Filipendula ulmaria* (L) Maxim. tincture. They could be determined by a wide variety of chemical compounds in this plant, such as flavonoids, tannines, and especially active salicylic acid derivates (17).

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References

1. Milanino R, Rainsford KD, Velo GP (eds.) Copper and Zinc in Inflammation. Kluwer, Dordrecht 1989.
2. Webb M, Cain K. Function of metallothionein. Biochem Pharmacol 1982; 31: 137–42.
3. Cousins RJ. Absorption, transport and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol Rev 1985; 65: 238–309.
4. Schroeder JJ, Cusins RJ. Interleukin-6 regulates metalothionein gene expression and zinc metabolism in hepatocyte monolayer cultures. Proc Natl Acad Sci USA 1990; 87: 3137–41.
5. Cousins RJ, Leinart AS. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 1988; 2: 2884–90.
6. Mussalo-Rauhamaa H, Konttinent YT, Lehto J, Honkanen V. Predictive clinical and laboratory parameters for serum zinc and copper in rheumatoid arthritis. Ann Rheum Dis 1988; 47: 816–9.
7. Peretz A, Neve J, Famaey JP. Effects of chronic and acute corticosteroid therapy on zinc and copper status in rheumatoid arthritic patients. J Trace Element Electrolytes Health Dis 1989; 3: 103–8.
8. Balogh Z, El-Ghobarey AF, Fell GS et al. Plasma zinc and its relationship to clinical symptoms and drug treatment in rheumatoid arthritis. Ann Rheum Dis 1980; 39: 329–32.
9. Elling H, Kiilerich S, Christiansen C, Gylding-Sabroe J. The effect of indomethacin and naproxen on zinc metabolism. Scand J Rheumatol 1978; 7: 145–6.
10. Song HK, Adham NF. Role of prostaglandin E_2 in zinc absorption in the rat. Ann J Physiol 1978; 234: 99–105.
11. Fontaine J, Neve J, Peretz A et al. Comparison of effects of chronic inflammation and long-term prednisolone administration on zinc metabolism in rats. Int J Tissue React 1989; 11: 253–9.
12. Mataran-Perez L, Gonzalez- Dominguez J, Rodriguez-Perez et al. Plasma and intraerythrocytic zinc in rheumatoid arthritis and ankylosing spondylitis. Ann Intern Med 1989; 6: 629–32.
13. Milanino R, Frigo A, Bambara LM et al. Copper and zinc status in rheumatoid arthritis: Studies on plasma,

erythrocytes and urine, and their relationship with disease activity markers and pharmacological treatment. Clin Exp Rheumatol 1993; 11: 271–91.
14. Kishore V. Effects of copper aspirinate and aspirin on tissue copper, zinc and iron concentration following chronic oral treatment in the adjuvantic arthritic rat. Biol Trace Elem Res 1990; 25 (2): 123–35.
15. Li RL, Lin PL, Wu XC. Clinical and experimental study on sustained release tablets of *Tripterygium wilfordii* in treating rheumatoid arthritis. Chung Kuo Chung His I Chic Ho Tsa Chin 1996; 16 (1): 10–3.
16. Tao X, Lipsky PE. The Chinese anti-inflammatory and immunosuppressive herbal remedy *Tripterygium wilfordii* Hook F. Rheum Dis Clin North Am 2000; 26 (1): 29–50.
17. Physicians Desk Reference for Herbal Medicines. New Jersey, 1998: 608, 849, 1104.
18. Duke AJ. Handbook of phytochemical constituents of grass herbs and other economic plants. Boca Raton: FL, CRC Press, 1992.
19. Sahin S, Rowland M. Distribution kinetics of salicylic acid in the dual-perfused rat liver preparation. Drug Metab Dispos 1999; 27 (3): 373–8.
20. Luterotti S, Žanič-Grubišič T, Juretič D. Rapid and simple method for the determination of copper, manganese and zinc in rat liver by direct flame atomic absorbtion spectrometry. Analyst 1992; 117: 141–3.
21. Milanino R, Marella M, Gasperini R et al. Copper and zinc body levels in inflammation: an overview of the data obtained from animal and human studies. Agents and Actions 1993; 39: 195–209.

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CINKO IR MAGNIO KIEKIO ŽIURKIŲ KEPENYSE RYŠYS SU ADJUVANTINIO ARTRITO GYDYMU *FILIPENDULA ULMARIA* (L.) MAXIM. TINKTŪRA

Santrauka

Tirtas Zn ir Mg kiekis 50 žiurkių kepenyse adjuvantinio artrito metu priklausomai nuo gydymo pelkinės vingiorykštės (*Filipendula ulmaria* (L.) Maxim.) tinktūra 2, 4 ir 8 mg/kg^{-1} dozėmis, skaičiuojant sausųjų medžiagų masei tinktūroje. Kontrolei vartota acetilsalicilo rūgštis (14 mg/kg^{-1}) *per os* kasdien. Nustatyta, kad, praėjus 20 d. nuo adjuvantinio artrito pradžios, Zn ir Mg kiekis žiurkių kepenyse padidėjo du kartus. Visos trys pelkinės vingiorykštės tinktūros dozės sumažino tirtųjų metalų kiekį iki sveikų žiurkių reikšmių, o ypač maža ir vidutinė šio preparato dozė. Panašiai veikė ir acetilsalicilo rūgštis. Po gydymo koreliacinis ryšys su ligos aktyvumo rodikliais buvo neigiamas. Pateikti duomenys leidžia manyti, kad Zn ir Mg normalizacija kepenyse žiurkių adjuvantinio artrito metu gali būti sietina su pelkinėje vingiorykštėje esančių aktyvių salicilo darinių poveikiu.