

ALKALINE PHOSPHATASE ENZYME AND LACTATE DEHYDROGENASE ACTIVITY IN URINE OF PATIENTS TREATED WITH METHOTREXATE

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Abstract: In order study methotrexate nephrotoxicity, the activities of proximal tubule epithelial cell membrane enzymes: alkaline phosphatase (AP) and lactate dehydrogenase (LDH) were determined in 12-h-urine samples of 30 patients with lymphoblastomous leukemia. The patients were *i.v.* receiving 4 individual methotrexate doses of 2000 mg/m² every 15 days followed by leucovorin as a protector. Control and methotrexate-treated group, each consisting of 30 examinees, included 4-10 years old children of both sexes.

Statistically significant increase of AP and LDH activities, expressed as unuts/mmol creatinine was observed after the second therapy ($p < 0.05$) in relation to the control. Based on these results it can be concluded that nephrotoxic methotrexate action is irreversible during the time period after the second applications at the level of proximal tubule epithelial cell.

Keywords: methotrexate, nephrotoxicity, urine, alkaline phosphatase, lactate-dehydrogenase.

1. INTRODUCTION

Methotrexate is a folate synthesis inhibitor and one of the first and most commonly applied medicaments in carcinoma chemotherapy. It is efficient against osteosarcoma and breast, head and lung tumor [1-5]. Methotrexate is applied in high, medium and low doses most frequently intravenously [6]. High doses of 500 or more mg/m² are applied in cases of leukemia, lymphoma, leptomeningeal metastases and osteosarcoma. Medium methotrexate doses from 50 to 500 mg/m² are applied in cases of gestational trophoblastic diseases, while the low doses of 50 or less mg/m² are administered in anti-inflammatory therapy of rheumatoid arthritis and psoriasis [7-8].

Intravenous doses methotrexate doses higher than 1000 g/m frequently cause a series of system toxicities. Besides skin, mucosa, liver and brain, they affect the kidney tissue too [9]. In kidney tissue

methotrexate can cause glomerular and tubular toxicity, however the clinical trials show that this is not a general occurrence [10]. Low methotrexate doses already deteriorate the existing glomerular and tubular cell necrosis. The risk of occurrence of nephrotoxicity of methotrexate can be increased by genetic polymorphism included in folate metabolism [11].

In previous trials nephrotoxicity of methotrexate was most frequently determined for applications of low doses of drug for anti-inflammatory treatment of rheumatoid arthritis and psoriasis. Glomerular function was monitored by determining the clearance of inuline, creatinine, ethylene diamine tetra acetate (EDTA) and by increasing the concentration of albumin in urine [12-13]. Tubular function was monitored by determining the concentration of electrolyte in blood, 1-or 2-microglobuline in urine and tubular enzymuria [14-15].

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The purpose of this paper was to determine, on a representative number of subjects suffering from lymphoblastic leukemia and treated with high methotrexate doses, possible nephrotoxicity by monitoring enzymuria of tubular alkali phosphatase enzymes, AP (EC. 3.1.3.1) and lactate-dehydrogenase, LDH (EC. 1.1.1.27.).

2. MATERIAL AND METHODS

60 children of age from 4 to 10 years were included in the study. The children were the patients of the Clinic for Children Diseases of University-Clinical Centre in Banja Luka. The experimental group comprised 30 subjects with acute lymphoblast leukemia and they were treated intravenously with individual methotrexate doses of 2000 mg/ m². Methotrexate was applied in 4 individual doses at 15-day interval and with leucovorin rescue which was applied in doses of 15 mg/m² at 42, 48 and 54 hours after administration of methotrexate. In control group there were 30 subjects who did not have lymphoblast leukemia or any kidney or urinary tract-related disease.

The morning 12-hour urine was taken from the subjects in triplets, 24 hours before and 24 hours after each individual therapy of the experimental group subjects and was kept at -25° C until analysis. The data on the age, sex, height, body mass and health condition of the subjects were collected based on the questionnaire during the first taking of urine.

Before urine analysis enzymes were separated by gel filtration [16]. Activities of enzymes AP [17] and LDH [18] and the creatinine concentrations were determined by spectrophotometry. Enzyme activity was expressed in international units of enzyme activity by mmol/l. The results were analyzed using standard statistical methods, expressed as mean values ± standard deviation and graphically presented. Significance of the differences between the results obtained for experimental and control group was determined by Student's t-test.

3. RESULTS

The mean results of AP and LDH enzyme activities with standard deviations were presented in figures 1 and 2. As shown in Figure 1, statistically significant increase of activity of AP in urine of subjects treated with methotrexate compared to control group subjects occurred after the second therapy. The mean values of enzyme activity with standard deviations after the second therapy amounted to $\bar{x} = 2,22 \pm 0,6$ for the experimental group compared to $\bar{x} = 1,48 \pm 0,45$ for the control group ($p < 0,05$). Statistical significance of differences in the activities of experimental compared to the control group was kept even after two remaining therapies.

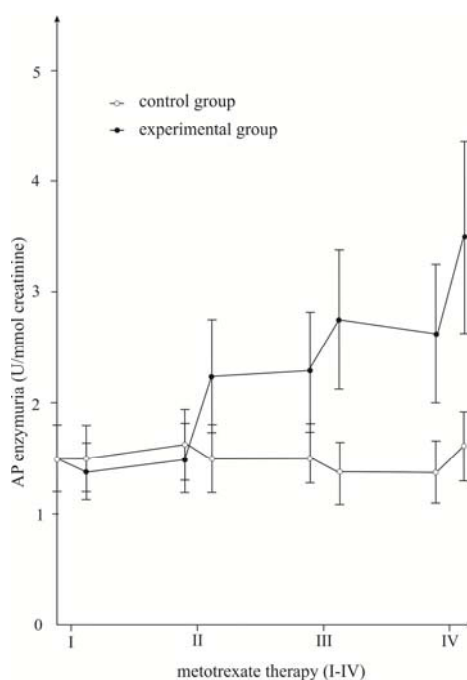


Figure 1. Kinetics of activity changes of alkaline phosphatase (AP) in urine of the patients treated with 4 therapy doses of methotrexate and of appropriate control values expressed as mean values ± S.D

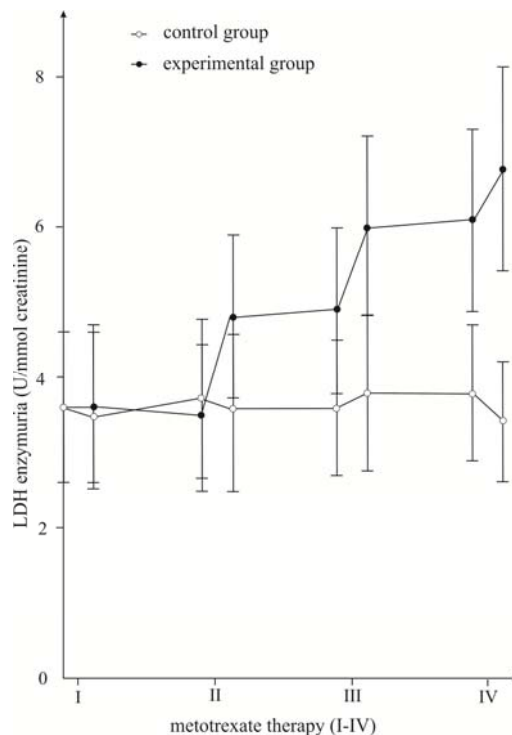


Figure 2. Kinetics of changes of the activity of lactate-dehydrogenase (LDH) in urine of the patients treated with 4 therapy doses of methotrexate and appropriate control values expressed as mean values \pm S.D

The mean values of LDH enzyme activity for the experimental group, the first time after the second therapy, significantly increased too, first time after the second therapy, compared to the control group when they amounted to $\bar{x} = 4,48 \pm 1,61$ /as opposed to $\bar{x} = 3,72 \pm 1,22$ ($p < 0,05$). The level of differences of statistical significance for LDH was kept all the time until the end of the therapy.

4. DISCUSSION

Nephrotoxic activity of methotrexate, administered intravenously to patients of 4 to 10 years of age, was observed in our study. The patients suffered from lymphoblast leukemia. Nephrotoxicity was determined by monitoring enzymuria of two enzymes of proximal tubule, alkaline phosphatase (AP) and lactate-dehydrogenase (LDH). A statistically significant increase of the activity of both enzymes was registered after the second therapy (Figures 1 and 2) with statistical significance of 0.05. The obtained values of enzyme activities of AP and LDH are not in conformity with the results obtained by Miedany et al. [20] and Minaur et al. [21] most probably because they observed enzymuria while applying the therapy of low dose

methotrexate. The obtained results are in conformity with the results of Westhuyzen-a cap. trial [22]. Their conditions of conducting the trial were similar to ours.

As AP is a lysosomal enzyme, and LDH cytosolic enzyme, an increase in their activity after implementing the second therapy means that nephrotoxicity occurred due to changes within proximal tubule epithelium cells, at the level of organelle, lysosome and cytosol. After statistically significant increase of the activity of both enzymes after implemented second therapy and before the beginning of the third, i.e. of the fourth therapy, enzyme activities were not normalized. This points out at a conclusion that the nephrotoxic effect of high methotrexate doses is cumulative and that it is accompanied by irreversible changes within the cells of affected tissue resulting in cell apoptosis and necrosis of proximal tubule epithelium necrosis.

5. CONCLUSION

Nephrotoxic activity of methotrexate applied to the patients in high doses in four therapies at 15-day interval and with leucovorin rescue was confirmed in this trial. Nephrotoxicity was determined by significant increase of enzymes AP

and LDH activity in urine. Nephrotoxic activity of methotrexate is cumulative and the damage occurs at the level of cell organelles. Administration of high methotrexate doses over a long period of time results in the necrosis of proximal tubule epithelium tissue. Therefore, whenever possible, high doses of methotrexate in therapy should be avoided.

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АКТИВНОСТИ ЕНЗИМА АЛКАЛНЕ ФОСФАТАЗЕ И ЛАКТАТ-ДЕХИДРОГЕНАЗЕ У УРИНУ ПАЦИЈЕНАТА ТРЕТИРАНИХ МЕТОТРЕКСАТОМ

Сажетак: Ради утврђивања нефротоксичности метотрексата, одређиване су активности ензима проксималних тубула, алкалне фосфатазе (АП) и лактат-дехидрогеназе (ЛДХ) у 12-часовном урину 30 пацијената који су били обољели од лимфобластне леукемије. Њима је интравенски аплициран метотрексат у четири појединачне дозе од 2 000 мг/м² са размаком од 15 дана и уз заштиту са леуковорином. Иста одређивања су вршена и у 12-часовном урину 30 испитаника контролне групе. Обје групе су се састојале од испитаника оба пола, старости од четири до десет година.

Статистички значајна повећања активности АП и ЛДХ, изражених у интернационалним јединицама ензимске активности по милимоловима креатинина, експерименталне у односу на контролну групу, регистрована су након прве двије терапије ($p < 0,05$) и повећања са истом статистичком значајношћу су задржана све до краја терапије. На основу добијених резултата се може закључити да је нефротоксично дјеловање метотрексата изражено, да је на нивоу ћелија епитела проксималних тубула и да је кумулативно.

Кључне ријечи: метотрексат, нефротоксичност, алкална фосфатаза, лактат-дехидрогеназа.

