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Correction of porphyrin exchange disorders in conditions of the combined effect of lead and fluorine

Korekcja zaburzeń wymiany porfiryn w warunkach jednoczesnego wpływu ołowiu i fluoru

INTRODUCTION

Lead and fluorine are the most common environmental pollutants and they may both get in the organism with water, food, atmospheric air as well as they may adversely affect the adaptation processes and evoke environmentally-dependent pathology. Lead is one of the highly cumulative polytropic substances. Fluorine has a physiological significance, but above the safe level (4 mg/day for adults) it is a polienzymatic poison and affects all organs and systems. The leading pathogenic mechanism of lead is porphyrin and heme biosynthesis disorder. Fluorine income may indirectly disturb the porphyrin metabolism [6]. The use of measures of biological prophylaxis or correction may prevent or reduce the toxic effects of lead and fluorine on the organism.

The aim of this work was to study the dynamics of delta aminolaevulinic acid (δ-ALA) excretion in urine in white rats in conditions of prolonged income of lead alone and in combination with fluorine on the background of bioprotectors.

MATERIAL AND METHODS

This research was carried out on sexually mature rats of the Wistar line by the orthogonal planning scheme 2^2 in compliance with general ethical principles of conducting experiments on animals. Aqueous solutions of $Pb(NO_3)_2$ (lead) in the dose 36 mg/kg and NaF (fluorine) in the dose 10 mg/kg separately and in combination (in the following order: lead-fluorine with interval 1.5–2 hours) were administered to animals in the stomach through a tube for 30 days. The control group of animals received drinking water. Laboratory animals received a conventional cooked diet and water *ab libitum*. Pectin (1 g/kg of body weight), calcium (calcium gluconate, 225 mg/kg of body weight), "Triovit" manufactured by KRKA, Slovenia (1 capsule per 1 kg of body weight; its composition: Vitamin C 100 mg, β-Carotene 10 mg, Vitamin E 15 mg, selenium 50 mcg) were used as bioprotectors. Pectin, a combination of pectin and calcium, and a complex of pectin, calcium, and triovit were successively added to the experimental animals' meal. On the 7^{th}, 15^{th}, and 30^{th} experimental days the

concentration of δ-ALA in urine was measured [7]. The mathematical-statistical analysis of results was performed by the method of least squares. The combined effect was estimated on the basis of the obtained regression equations: $y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2$, where y is the effect, the concentration of δ-ALA in urine, mcmol/g of creatinine, b_0, b_1, b_2, b_{12} – regression coefficients; x_1, x_2 – codes for doses of lead and fluorine, respectively.

RESULTS

Evolution of changes of δ-ALA excretion in urine under the influence of lead and a simultaneous effect of lead and fluorine demonstrated the increase of δ-ALA excretion in urine up to 30 days of the experiment. On the 7th experimental day of lead effect the δ-ALA concentration increased 5.2 times, on the 15th day – 6.5 times, and on the 30th day – 10.6 times compared to control (the level of control varied within 6.05±0.54 ... 7.95±0.35 mcmol/g of creatinine); in the conditions of combined effect of the substances, δ-ALA concentration was slightly higher than with the lead effect alone. Fluorine caused an insignificant, but reliable increase of δ-ALA in urine on the 15th and 30th days of the experiment by 1.6 and 2.2 times, respectively. Supplementation of pectin in the diet insignificantly contributed to reducing the concentration of δ-ALA in urine, which on the 30th day was increased under the action of lead 8 times, with a simultaneous effect of lead and fluorine – 9.1 times compared with control. Pectin did not affect the excretion of δ-ALA in urine of the animals that received fluorine. Pectin and calcium significantly reduced the concentration of δ-ALA in urine. Undulating dynamics was observed during it: on the seventh day lead caused an increase of δ-ALA concentrations in urine by 4.2 times, on 15th – 2.9 times, on 30th day – 4.1 times compared with control. Practically similar quantitative changes of δ-ALA concentration in urine occurred with a simultaneous action of the substances. The effect of fluorine increased the δ-ALA levels in urine 1.6 times only on the 30th day of the experiment. The complex of pectin, calcium and triovit positively effected the porphyrins exchange: on the 7th, 15th, and 30th days of the experiment in the conditions of lead effect, the concentration of δ-ALA increased 3.4, 2.3 and 1.8 times, respectively, with the combined action of lead and fluorine – 3.3, 2.5 and 2.1 times, respectively. With the effect of fluorine δ-ALA concentration was found at the level of control.

Regression equation 2^2 on the 30th day of the experiment is the following one: $y=51.33+38.48x_1+5.08x_2+0.17x_1x_2$ (without correction), $y=41.49+29.40x_1+4.24x_2+0.46x_1x_2$ (with pectin), $y=22.85+12.15x_1+2.05x_2-0.53x_1x_2$ (with pectin and calcium)

$y=8.88+2.82x_1+0,47x_2+0.48x_1x_2$ (with pectin, calcium and triovit).

DISCUSSION

It is a well known fact that lead suppresses the activity of enzymes responsible for heme synthesis, in particular the activity of dehydrogenase δ-ALA and hemesynthetase by blocking their sulfhydryl groups. Transformation of δ-ALA into porphobilinogen and coproporphyrinogen into protoporphyrinogen is suppressed accordingly; binding of iron to protoporphyrin is also impaired. Abnormality of chain of reactions of heme biosynthesis leads to accumulation of intermediate products

of heme metabolism (mostly of δ-ALA which is excreted with urine) in the bone marrow, and blood. The increase of δ-ALA concentration in urine under the influence of fluorine is an unspecific reaction, and, probably, an indirect effect on the porphyrins metabolism. Porphyrinogenesis is a complex process and there are a lot of factors that can impair this process. Porphyrins play a role in many biochemical processes. In particular, ferroprotoporphyrins are the constituents of such enzymes as catalase and cytochromeoxidase. In our previous research [1,2] we established that the activity of catalase decreases after administration of lead, fluorine and their combination. Besides, we also determined that with the combined action of both substances fluorine contributed to the increase of lead concentration in the blood and, probably, effected the exit of lead from the bone depot and its redistribution in tissues of the organism. Such suppositions coincide with the results of other authors [3, 5]. Therefore, the increase of δ-ALA with the effect of fluorine is also caused by the lead release from the bone system in the blood. The progressive increase of the concentration of δ-ALA in urine under the effect of lead and the combined effect of lead and fluorine can be explained by cumulative properties of lead, increase of lead concentrations in biosubstrate of animals and increase of oxidizing stress to the end of the experiment. Some decline of δ-ALA excretion with urine after correction by pectin relates to its known properties of heavy metals sorption in the bowels and the formation of insoluble pectinates, stimulation of peristalsis and elimination from the organism. The use of pectin with calcium contributed to a considerable reduction of lead alone and lead with fluorine effect. These bioprotectors prevent absorption and deposition of lead and promote its elimination. With the concurrent income of lead and calcium, calmodulin binds calcium and lead ions equally [4]. The formation of hyposoluble compounds of lead gluconate and calcium fluoride is not excluded. The created compounds due to pectin supplementation are excreted from the organism. An addition of triovit with pectin and calcium contributed to the maximal decline of δ-ALA excretion with urine. Triovit, due to its four constituents, renews and normalizes the processes of free radical oxidation and antioxidant defense, which also promoted renewal of processes in porphyrins metabolism and accordingly – a decline of δ-ALA concentration in urine. All constituents of bioprotectors complex demonstrated synergic action concerning the negative influence of lead alone and in combination with fluorine. The efficiency of successive application of bioprotectors for the correction of porphyrins metabolism is confirmed by the following aliquot rows of exceeding of δ-ALA concentration in urine on the 30th day of the experiment comparatively with the control with the effect of lead alone: 10.6 (without bioprotectors) > 8.0 (with pectin) >4.1 (with pectin and calcium) >1.8 (with pectin, calcium and triovit), at the combined effect of lead and fluorine – 12.0 > 9.1 > 4.5 > 2.1, respectively.

The combined action of lead and fluorine on the 7th day of the experiment without and with correction with bioprotectors in all groups of animals was characterized by an independent effect of lead. On 15th and 30th days of the experiment the combined effect without correction demonstrated a tendency to more than additive and additive effects, respectively. The leading role in the development of the effect is played by lead – 81.4% and 88.3%. On the 15th day sensitization of lead effect by fluorine, on 30th day – additive effect developed on addition of pectin. The other groups of animals with an addition of bioprotectors were marked for an independent effect of lead. The values of coefficients b_0 in regression equations certify that the effect of both factors (lead and fluorine) goes down in the process of correction, and coefficients b_1, b_2 – excretion of δ-ALA with urine at action of

lead and fluorine, respectively. The decline of δ-ALA concentration in urine was due to the reduction of lead effect.

CONCLUSIONS

The correction of porphyrins metabolism disorders with pectin did not remove the negative influence of lead alone and in combination with fluorine and did not practically affect the excretion of δ-ALA with urine in animals that received fluorine. Pectin with calcium considerably weakened the action of lead due to the prevention of lead and fluorine absorption and deposition. The addition of triovit strengthened the action of pectin and calcium. The correction of porphyrins metabolism disorders with the complex of pectin, calcium and triovit is directed on toxicodynamics and toxicokinetics of the lead and fluorine.

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SUMMARY

Protracted influence of $Pb(NO_3)_2$ in the dose 36 mg/kg of body weight, NaF in the dose 10 mg/kg of body weight and their combined action impairs heme biosynthesis and is accompanied by increased excretion of δ-ALA with urine. A combined effect of lead and fluorine by the explored effect depends on the term of the factors effect, applied bioprotectors and is due to the independent lead effect, additive and more than additive effect. The leading role in the development of the effect belongs to lead. The simultaneous influence of lead and fluorine can be optimally corrected by the complex of pectin, calcium and triovit, which manifest a synergic action.

Key words: lead, fluorine, combined effect, delta aminolaevulinic acid, bioprotectors, correction

STRESZCZENIE

Przedłużony wpływ $Pb(NO_3)_2$ w dawce 36 mg/kg masy ciała, NaF w dawce 10 mg/kg m.c. i ich jednoczesne oddziaływanie zaburzają biosyntezę hemu i towarzyszy im wzrost wydalania δ-ALA z moczem. Jednoczesny wpływ ołowiu i fluoru w badanym aspekcie zależy od efektu pojedynczych czynników oraz stosowanych bioprotektorów. Główną rolę w rozwoju efektu odgrywa ołów. Jednoczesny wpływ ołowiu i fluoru może być optymalnie skorygowany poprzez stosowanie kompleksu pektyn, wapnia i triovitu, które wykazują działanie synergistyczne.

Słowa kluczowe: ołów, fluor, jednoczesny efekt, kwas delta-aminolewulinowy, bioprotektory, korekcja