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Iron content in rats' teeth in glucocorticoid-induced osteoporosis

Corticosteroids increase bone resorption in several ways. They decrease calcium excretion, causing secondary hyperparathyroidism and inhibit bone formation, both directly and indirectly by decreasing gonadal steroid secretion (10). It has been shown that prolonged administration of hydrocortisone or prednisolone may result in osteopenia and a decrease in bone strength in the rat (5). In human prolonged corticosteroid therapy increases the risk of osteoporosis and fracture (10). It has to be stressed that osteoporosis is a systemic condition that also has dental implication.

Mineralized tissues of teeth play an important role in mineral metabolism of animal and human organisms. When the level of ions in plasma decreases these ions are mobilized from reserves in bone and teeth. Corticosteroids influence the mineral homeostasis in the human body (1, 8).

Iron exists in ionic form in the biological system. Dietary intake of this mineral in many subpopulations is lower than current recommendation and iron deficiency remains the most prevalent nutritional problem in the world. Symptoms like low hematocrit and hemoglobin levels are the main effects of impaired iron transport from the liver into bone marrow. Symptoms were less severe among female than male rats and could be corrected to some extent by supplementation with iron. Such effect was not observed in humans (2).

Nutritional status, age and gender, the presence of diseases and stress as well as body's adaptive capacity are the main factors in iron metabolism. In the teeth of older people an increase of iron with age was observed but in the teeth with caries the level of iron was higher (3, 4). There were also differences in iron content according to sex: man have less iron in teeth than women (11).

We did not find any reports or papers giving results of iron examination in teeth in the process of osteoporosis after corticoidotherapy, therefore we undertake this study to assess the level of iron in rat teeth after administration of hydrocortisone.

MATERIAL AND METHODS

Young adult male rats were classified for the experiment, after two weeks' adaptation to experimental conditions (7). They were randomized into 6 groups – 20 rats in each. Every group consisted of 2 subgroups (10 rats in each) depending on the duration of the experiment. In the first one the research was carried out after 4 weeks of the experiment and in the other one after 8 weeks.

The control group K consisted of rats to whom 0.9% NaCl was given twice a day, in a dose of 0.5 mg/kg of body weight, subgroup K_1 – during 4 weeks, and subgroup K_2 – during 8 weeks.

The experimental group H consisted of rats taking Hydrocortisonum hemisuccinatum (Polfa) intraperitoneally in a dose of 30 mg/kg of the weight, twice a day, subgroup H_1 – during 4 weeks, and subgroup H_2 – for 8 weeks.

The rats of the Ca experimental group obtained 10% Calcium Polfa intraperitoneally in a dose of 0.01 g/kg of the weight and a solution of vitamin A + D_3 (Terpol), with a gastric probe of over 200 i.u. of vitamin A and 100 i.u. of vitamin D_3 , subgroup Ca_1 – during 4 weeks, and subgroup Ca_2 – for 8 weeks.

The animals of experimental group H + Ca received Hydrocortisonum hemisuccinatum with 10% Calcium Polfa in the same doses and for the same period of time as the rats in groups H and Ca. The animals were also given the solution of vitamin A + D_3 , subgroups H_1 + Ca_1 – during 4 weeks, and subgroups H_2 + Ca_2 for 8 weeks.

The animals of M groups were given salmon calcitonin Miacalcic (Sandoz) in the dose of 5 i.u./kg of the body weight once a day, subgroup M_1 – during 4 weeks, and subgroup M_2 – for 8 weeks.

Both Hydrocortisonum hemisuccinatum intraperitoneally and salmon calcitonin Miacalcic were taken by H + M experimental groups in the same dose and for the same period of time as rats in groups H and M, subgroup H_1 + M_1 for 4 weeks, and subgroup H_2 + M_2 – for 8 weeks.

After 4 weeks, the rats from subgroups K_1 , M_1 , Ca_1 , H_1 + M_1 , H_1 + Ca_1 were anaesthetised using ketamine, decapitated and the mandible incisors were prepared. The samples for each experimental group were carefully labelled and kept separately. After 8 weeks of experiment the remaining rats' teeth were prepared in the same way.

We used rats' incisors in the experiment because they have wide apical foramen, spacious root canal and big pulp. They are good for experimental conditions and ensure regular mineral metabolism (1).

Rat teeth were mineralized in muffle furnace at 450°C (dry method) and the iron level was then estimated with a Pye-Unicam atomic absorption spectrophotometer (9). The iron level per unit teeth tissue amount ($\mu\text{g/g}$ tissue) was calculated.

The data obtained were analysed statistically by calculating mean and standard deviation. Differences between mean values were compared using the Student's *t* – test and $p < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Table 1 and Figure 1 and 2 present the level of iron in rat teeth after 4 and 8 week of experiment and the type of drug administered.

After 4 weeks the mean teeth iron level in the control group was 257.79 $\mu\text{g/g}$ (mean \pm SD). In the group of rats receiving hydrocortisone the iron level showed a significantly marked decrease (175.33 $\mu\text{g/g}$) – $p < 0.001$. The teeth iron level had also decreased substantially after administration of Miacalcic (group M) – $p < 0.05$ and Miacalcic and Hydrocortisone (group H + M) – $p < 0.001$. No significant differences between the K group and Ca and H + Ca animal groups were observed in the iron level.

The mean results in the animal groups examined after 8 weeks of the experiment compared with the results after 4 weeks were similar. Only significant differences were observed between control and hydrocortisone group ($p < 0.01$). After steroid therapy a decrease in the level of iron in the teeth was noticed.

In the literature available, the authors have not come across any papers giving results of examinations of iron content in rat teeth in drug-induced osteoporosis. In the present examinations there were statistically significant differences in teeth of rats after steroid therapy. These findings suggest that when the teeth iron level is affected by the hydrocortisone metabolism – the concentration of iron is decreased.

Deficiency of iron could cause bone demineralization. Also the deficit of this element induced inhibition of growth and structure change in bone tissues and teeth (6). The accumulation and mobilization of minerals from bones and teeth are controlled by number of regulatory mechanisms including physicochemical, endocrine and other factors, that is why providing full explanation to our results is very difficult.

Table 1. The level of iron in the rat teeth

Examined group	Examining period (week)	Number of rats	Fe $\mu\text{g/g}$ (mean)	Standard deviation	The level of significance $p <$
Control (K)	4	10	257.79	9.38	ns
	8	10	259.59	31.79	ns
Hydrocortisone (H)	4	10	175.33	3.15	0.001
	8	10	175.42	12.34	0.01
Calcium (Ca)	4	10	278.79	52.54	ns
	8	10	339.97	66.25	ns
Hydrocortisone + Calcium (H + Ca)	4	10	216.27	68.52	ns
	8	10	218.82	23.28	ns
Miacalcic (M)	4	10	222.43	23.59	0.05
	8	10	224.35	5.03	ns
Hydrocortisone + Miacalcic (H+M)	4	10	181.34	5.15	0.001
	8	10	235.27	42.33	ns

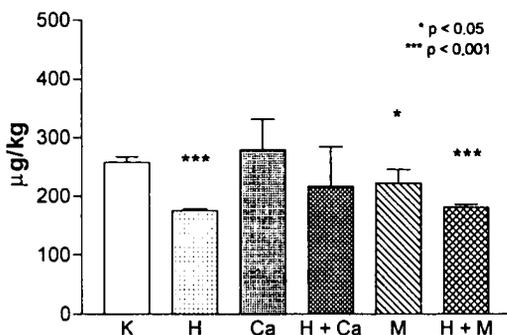


Fig. 1. The level of iron in rat teeth after 4 weeks' examination

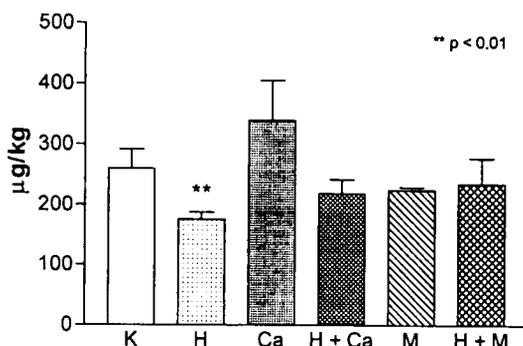


Fig. 2. The level of iron in rat teeth after 8 weeks' examination

CONCLUSIONS

1. A decrease in the level of iron in the teeth after steroid therapy was observed.
2. Lower iron concentration in rat teeth in drug-induced osteoporosis could suggest intracellular deficiency of this ion.

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SUMMARY

On the basis of atomic absorption spectrophotometry rat teeth iron levels in glucocorticoid-induced osteoporosis were measured. Hydrocortisone caused decreasing of the iron level in rat teeth after both 4 and 8 weeks of the duration of the experiment. The other drugs used in osteoporosis treatment (calcium and calcitonin) administered together with hydrocortisone or separately also influenced the content of this ion in rats' teeth.

Zawartość żelaza w zębach szczurów w doświadczalnej osteoporozie posteroïdowej

Na podstawie badań z użyciem atomowej spektrometrii absorpcyjnej określono poziom żelaza w zębach szczurów w doświadczalnej osteoporozie posteroïdowej. Hydrokortyzon powodował obniżenie poziomu żelaza w twardych tkankach zęba zarówno po 4, jak i po 8 tygodniach trwania eksperymentu. Określono również zawartość żelaza w zębach zwierząt eksperymentalnych, którym podawano inne leki stosowane w leczeniu osteoporozy. Zauważono, że leki te użyte razem z hydrokortyzonem lub osobno mają wpływ na poziom żelaza w zębach szczura.

