

# Evaluation of the effectiveness of therapy with calcium-containing compounds of experimental osteoporosis.

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## ABSTRACT

The article presents the results of study and a comparative analysis of experimentally reproduced osteoporosis and treatment of osteoporosis with calcium alginate. It was found that bone density increases depending on the duration of reception of medicines and activity of experimental animals, which is possibly due to the quality of assimilation of this drug and the effective regenerative ability of the calcium alginate drugs. The results on the use of effective amounts of calcium chloride, calcium pectate and calcium alginate, dietary supplements in experimental osteoporosis were given, and a differentiated approach to the treatment of osteoporosis taking into account metabolic activity was also proposed.

**Keywords:** osteoporosis, calcium medicines, alginate, pectate, lactate.

## INTRODUCTION

Currently, there are a number of effective drugs for the treatment of osteoporosis that increase bone mineral density and reduce the risk of fractures. However, so far there is no consensus among scientists on the optimal method for assessing the response to treatment with osteoporotic drugs. It is known that the main goal of treating osteoporosis is to prevent the development of bone fractures. But in practice, in a particular patient, it is far from always possible to avoid fractures even when using modern medicines.

This may be due to late treatment, insufficient treatment, the course and the presence of concomitant diseases, hormonal and genetic status, etc. We cannot ignore the commitment to the main drug, as well as the adequacy of using of basic calcium and vitamin D. At the same time, a full assessment of the effectiveness of the

treatment of osteoporosis based on decreasing of the incidence of low-energy bone fractures, which is possible only in the course of long-term randomized placebo-controlled clinical trials.

How to evaluate the effectiveness of osteoporosis therapy in practice? Is it possible to do this according to the so-called secondary artificial endpoints: dynamics of bone mineral density (BMD) and / or biochemical markers of bone metabolism? In this regard, the Committee of Scientific Advisers (CSA) of the International Fund for Osteoporosis believed that for doctors who may face the treatment failure, is necessary to develop practical advice on the correct algorithm for action in such situations. Thereby, the **aim** of the research was to conduct a comparative assessment of metabolic changes and improve the characteristics of organic calcium compounds in experimental osteoporosis.

## MATERIAL AND METHODS

For the study of individual typological characteristics in experimental osteoporosis and the study of biochemical parameters of calcium metabolism, hormonal status and physiological parameters, 180 animals were studied. When studying the effect of treatment with a calcium-containing compound on the biochemical parameters of blood in experimental osteoporosis, 150 rats with experimental pathology were studied. According to the test results, experimental individuals were divided as follows: subgroups Ia, IIa, IIIa, and IVa — active (high activity) (n = 10); Ib, IIb, IIIb, IVb - normal (intermediate activity) (n = 10), Ic, IIc, IIIc, IVc (passive activity) (n = 10).

The concentration of estradiol in blood serum was determined by the method of enzyme-linked immunosorbent assay (ELISA), using test systems for enzyme-linked immunosorbent assay company "Human" (Germany) according to the procedure described in the instructions attached to the test system. The optical density was measured at a wavelength of 450 and 630 nm on a MR96A microplate photometer (Mindray, China). Confirmation of the presence of osteoporotic changes in bone tissue was carried out by morphological examination of sections of skeleton bones by the method of preparation of bone tissue preparations and their microscopy according to the generally accepted method.

## RESULTS

Modeling of experimental osteoporosis led to a decrease in motor activity among all the studied subgroups, however, differences were also revealed here. It was found that the most significant differences in the severity of manifestations of behavioral activity were observed between the most active and passive individuals. So, if in animals of the subgroup with passive activity, only a statistically insignificant decrease in the main activity was found, while much more significant differences were observed in its structure. Among individuals with normal and reduced psycho-behavioral activity in the IIb and IIc subgroups, the main activity did not change significantly, and among some of the IIc subgroups, even a slight increase was observed, also accompanied by significant changes in its structure.

In particular, the frequency of grooming has increased, the number of boluses left has been increased. At the same time, the number of intersected squares decreased, the ratio of intersected central and peripheral squares changed. Animals are increasingly less likely to visit the «Open Field» central areas.

The results of a study of psycho-behavioral activity indicate the increasing of the positive effects of calcium chloride on the behavioral effects studied in the "Open Field" test, in particular, an increase in research activity.

These differences in the severity of changes may be due to the more optimal and physiological pharmacokinetics of ionized calcium, which is formed in calcium alginate. Calcium compounds have a beneficial effect not only to calcium metabolism, but also on homeostasis in general, as well as on the level of the nervous system. However, the pharmacological and pharmacokinetic properties of various combinations lead to significant changes.

A study of the estradiol group among intact rats in group I revealed its significance among animals with an intermediate type of psycho-behavioral activity (normal features) in the group Ib. In the Ib subgroup, the value of this indicator of hormonal status was 31.3% lower than among the active individuals in the Ia subgroup and 39.8% (p <0.05) lower, compared with the passive individuals in the Is subgroup.

Modeling of experimental osteoporosis practically did not lead to decrease in the severity of changes between active and normal features, i.e. in the IIa and IIb subgroups, however, it can be noted, that the estradiol values among the normal and passive wings in the IIa and IIc subgroups are not significant differences.

In the III group of animals that received calcium chloride, the content of estradiol in the blood serum did not change significantly, relative to its values in the II group. Thus, in active individuals in the IIIa subgroup, a statistically insignificant tendency to increase this indicator was observed. Among animals with a "normal" degree of activity in the IIIb subgroup, the changes were extremely insignificant and the values were almost at the same level. In "passive" rats, a slight decrease was noted.

In the IVa subgroup, after treatment of experimental osteoporosis with calcium alginate, among individuals with an active type of psycho-

behavioral activity, there was an insignificant tendency to increase the calcium concentration. Regarding animals treated with calcium chloride therapy in the IIIa subgroup, as well as the IVb and IVc subgroups, relative to the IIIb and IIIc subgroups, there were no statistically significant differences in the concentration of estradiol.

In the Va subgroup, no statistically significant differences were found in the serum estradiol content relative to intact animals, control animals, and animals treated with calcium chloride and alginate. There were also no significant differences in the IVb and IVc subgroups, compared with animals of the subgroups Ib and Ic, IIb and IIc, IIIb and IIIc, respectively. Moreover, if in the group of animals after ovariectomy that did not receive treatment, there was a tendency to lower hormonal status, then in treated animals - to its increase. However, these changes were extremely insignificant and statistically insignificant. At the same time, differences in estradiol concentrations could be detected between animals with varying degrees of psycho-behavioral activity. So, if in intact rats with a normal (intermediate) type of psycho-behavioral activity (Ib subgroup), the concentration of estradiol was 31.3% ( $p < 0.05$ ) lower than in the Ia subgroup, then in animals with a passive type (Ic - subgroup) - 39.8% ( $p < 0.05$ ) higher relative to the Ib subgroup.

In animals with experimental osteoporosis and a normal type of psycho-behavioral activity, lower estradiol values were observed in the IIb subgroup in the IIb subgroup, and a 29.0% ( $p < 0.05$ ) higher in the IIc subgroup its concentration.

Calcium alginate intake practically did not affect the severity of differences between animals with increased and intermediate psycho-behavioral activity. A decrease in the statistical significance of these differences between animals with reduced activity (IIIc subgroup) and individuals with increased and intermediate psycho-behavioral activity was observed. So, the level of estradiol in the IVb subgroup was 55.7% lower than in the IIIa subgroup, however, in the IIIc subgroup the concentration of estradiol was not significantly lower than in the IIIa subgroup and very slightly higher compared to the IIIb subgroup.

A similar overall picture could be observed in the study of rats with different psycho-behavioral

activity, both among animals treated with calcium alginate (group IV) and calcium pectate (group V). In this case, it is worth noting the tendency to a slight increase in the estradiol content in experimental individuals. At the same time, in all experimental rats excluding psycho-behavioral activity, in the IV and V group, the concentration of estradiol was 21.6% ( $p < 0.05$ ) and 24.4% ( $p < 0.01$ ) higher than in II group.

The features of changes in serum calcium and alkaline phosphatase levels in experimental osteoporosis depending on psycho-behavioral activity were also investigated. The highest calcium values in the first group were recorded among active animals, and in the group with normal (intermediate) activity and in passive animals, the calcium content was lower by 26.2 ( $p < 0.0001$ ) and 22.4% ( $p < 0.0001$ ), respectively.

In animals with experimental osteoporosis, the highest serum calcium levels were found among animals with increased activity, which were one fifth ( $p < 0.02$ ) and a quarter ( $p < 0.0001$ ) higher than in animals with normal and low activity, which in turn can be associated with a higher intensity of bone decalcification processes with a high degree of metabolic processes in the body of experimental animals.

These changes in alkaline phosphatase among passive individuals may indicate a more effective restoration of the studied remodeling marker, compared with the use of calcium alginate in individuals with increased and medium activity, which may indirectly indicate a more physiological absorption of calcium from this compound with this type of psycho-behavioral and corresponding metabolic activity.

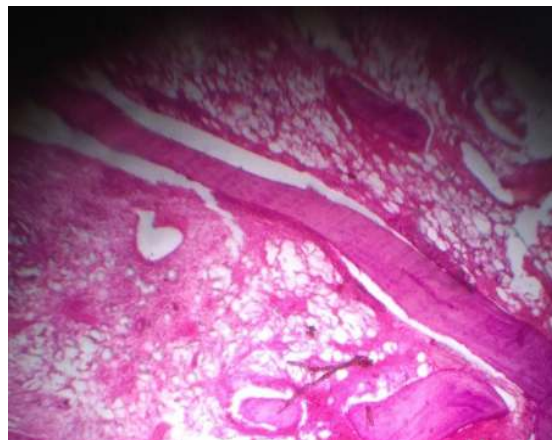
It should be noted that passive individuals after treatment with calcium alginate had the lowest content of total alkaline phosphatase, compared with active and normal animals - by 15.6 and 23.9% ( $p < 0.05$ ), respectively, which may indicate the most pronounced effectiveness of its use in the presence of this type of psycho-behavioral activity, and therefore with a lower level of metabolic activity. Thus, the most effective restoration of alkaline phosphatase activity could be observed among animals with an average and low level of activity, but the most balanced values of this indicator could be observed among passive and normal rats. So among animals with normal activity, one could note the highest values of alkaline phosphatase indicating a high

activity of remodeling processes. Moreover, among passive individuals, the values of this indicator were lower, which indicates lower remodeling processes compared to active and normal individuals and, accordingly, both osteodestruction and osteosynthesis, the alkaline phosphatase is considered to be a marker.

150 rats with experimental pathology were studied to identify the influence of treatment with calcium containing compound on the biochemical parameters of blood in experimental osteoporosis. For histological examination, bone pieces were placed in a 4% neutral formalin solution, then decalcified in 15% nitric acid and then fixed in alcohols. After sufficient fixation, paraffin blocks were prepared from the materials. Finished sections were stained with hematoxylin and eosin. During a morphological study of the intact group I — before the reproduction of experimental osteoporosis, the density of the compact bone plate was preserved (Figs. 1 and 2). In Figure 4, you can see that the bone plate is dense, elements of the red bone marrow are visible. No pathological rarefaction or decrease in bone density was found. Thus, among the loose network and elements of the bone marrow, dense compact bone tissue can be seen.



**Fig.1: Group I - before reproduction of experimental osteoporosis, intact. Compact bone plate is dense, bone marrow elements. Coloring: hematoxylin and eosin.**



**Fig.2: Group I - before reproduction of experimental osteoporosis, intact. Among the loose network and bone marrow elements, dense compact bone tissue. Coloring: hematoxylin and eosin.**

The results of morphological studies of bone tissue of experimental animals were compared with the results of studies of bone of group II (control). As shown by the data of morphological studies of bones in rats in group II (control) - animals with experimental osteoporosis without treatment, in the samples of the epiphyseal part of the tibia taken after ovariectomy, bone resorption, rarefaction areas due to leaching of calcium salts and porosity, softening of bone tissue with the presence of primitive weakly calcified bone beams.

At the same time, processes of bone resorption, foci of lysis, osteomalacia in central areas are noted. With microscopy, it is necessary to note the predominance of shaped elements and weakly calcified primitive bone beams.

Also, the data of morphological changes are supported by the functional changes described above. As is known, according to the researchers, one of the foundations of violation of the bone remodeling process is a decrease in microcirculation associated with endothelial dysfunction, which may be the reason for the development of the changes described above. The daily administration of a preparation containing calcium alginate in the III group for 60 days to the operated animals led to the restoration of bone structure, as evidenced by the absence of obvious morphological signs of osteoporosis.

So, in group IV (main, experimental) - with experimental osteoporosis after the administration of calcium alginate, growth of osteogenic fibrous tissue was noted with subsequent densification of compact bone tissue with repair areas, the disappearance of porosity and osteomalacia due to saturation of calcium salts with calcium alginate therapy. Areas of compact bone tissue lack porosity and have uniform color. The growth of fibrous connective bone tissue is one of the main indicators of the intensity of restoration processes. Also, compaction of bone beams is observed, providing strength characteristics of the bone, which indicates the effectiveness of therapy with calcium alginate. All this indicates the positive effect of therapy with the natural compound of calcium alginate on the structure of bone tissue, which is confirmed by the proliferation of osteogenic connective tissue - the basis of the future bone.

Thus, the presence of areas of compaction of bone flakes can be the result of replenishing calcium deficiency, due to the intake of an ionized calcium source - calcium alginate, as well as foci of proliferation of osteogenic fibrous connective tissue, which indicate osteogenic recovery processes. Moreover, fragments of dense bone tissue and layers of cartilaginous plates alternate with areas of osteogenic fibrous structure growth, which indicates regenerative processes in the bone that occur during calcium alginate therapy. The data obtained are consistent with the literature data indicating a more physiological and optimal recovery of calcium when deficient when taking such organic calcium compounds as calcium alginate.

## CONCLUSION

The serum calcium content in patients receiving calcium alginate is restored in the passive type, and full recovery was found in the normal (intermediate) state of psycho-behavioral activity. The relationships between the variants of psycho-behavioral activity and estradiol in blood serum were established, both in intact animals and in experimental osteoporosis. The most pronounced decrease in hormonal status was revealed to a special extent, depending on the activities and passive individuals. During therapy with experimental osteoporosis, calcium alginate causes an improvement in the basic parameters

of psycho-behavioral activity, as determined in the "Open field" test.

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