EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effects of Selenium-Containing Compounds and Their Metabolism in Intact Rats and in Animals with Bone Fractures

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Blood content of MDA in rats increased 1 and 2 weeks after mandibular bone fracture at stages of cellular fibrous and chondroid callus and decreased 4 weeks after fracture at the stage of primary bone callus. Treatment with Se (intragastrically and electrophoretically) reduced this increase by activating selenium-containing glutathione peroxidase. In order to clear out the relationship between Se and carbohydrate metabolism in different ages, the distribution of Se between the blood and mandibular bone, diaphysis and metaepiphyseal zone of the femoral bone was studied using the bone/blood relative radioactivity coefficient after intraperitoneal injection of [^{75}Se]selenate. In control 1-month-old rats the radioactivity had 2 peaks corresponding to 6 and 48 h. The first peak was presumably caused by Se adsorption on hydroxyapatite, the second by chemosorption on hydroxyapatite and protein binding. Only one peak of relative radioactivity (after 12-48 h) was observed in 3-month-old control rats, and it could be increased by sucrose diet. The relative radioactivity was higher in rats receiving sucrose ration for 2 months starting from the age of 1 month in comparison with the control.

Key Words: *[^{75}Se]selenate metabolism; bone fracture; bone/blood coefficient; age; carbohydrate-rich diet*

Alimentary selenium deficit is harmful for mandibular mineralization in rats and humans [14], which can be attributed to changed carbohydrate metabolism [12,13]. Reparative bone regeneration is decelerated after mandibular fractures in indigenous population of selenium-deficient areas of the Chita region [2]. It is also known that bone regeneration after fractures runs a more rapid course and is more effective in children than in adults. Usually bone fracture is associated with inflammation and ischemia, stimulating free-radical oxidation (FRO) [6], while Se (particularly in glutathione peroxidase) participates in antioxidant defense [1,3,7]. The mechanisms of Se binding to mineralized tissues are little studied, though better knowledge of the mechanism of the impact of Se deficiency and Se therapy in bone diseases can be useful for theoretical and clinical medicine.

We studied FRO markers (MDA and selenium-containing glutathione peroxidase) after intragastric or electrophoretic administration of Se preparations to rats with bone fractures and investigated Se distribution between bone and blood in rats of different age or after long carbohydrate-rich diets.

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MATERIALS AND METHODS

Three-month-old rats (n=60) were divided into 4 groups: control, bone fracture, bone fracture+selenomethionine intragastrically, and bone fracture+Se electrophoresis. The animals were decapitated 1, 2, and 4 weeks after injury. At least 4 animals were examined per term in each group. Bone fracture and decapitation were carried out under narcosis. Mandibular bone (MB) fragments were isolated from the fracture zone, purified from soft tissues and blood, frozen in liquid nitrogen, and homogenized. MDA (intermediate FRO product) was measured in supernatant after centrifugation in phosphate buffer (pH 7.3) on a spectrophotometer, glutathione peroxidase activity was measured as described previously [6].

Another series of experiments with ^{75}Se was carried out on 1- and 3-month-old albino rats (n=37 and 29, respectively) fed standard rations, and 20 animals receiving sucrose-casein ration (54% sucrose, 18.5% casein, 18.5% dry wheat bread, 5% sunflower oil, polyvitamins, and mineral salts) for 2 months (from 1 to 3 months of age).

The rats were intraperitoneally injected with [^{75}Se]selenate solution (20,000 cpm/g). Isolated bones were purified from soft tissues and blood and pulverized. The percentage of ^{75}Se incorporation in MB, diaphysis and metaepiphyseal zone of the femoral bone, and blood was evaluated by the percent ratio of cpm per g bone or ml blood to cpm injected per g. In order to evaluate the distribution of ^{75}Se between blood and bones, a new coefficient (relative radioactivity — RRA) was estimated by dividing the percentage of ^{75}Se incorporation in bones by the same parameter for the blood. One, 3, 6, 12, 24, 48, and 192 h after injection γ-radiation of ^{75}Se was scintillated on a radiometer with a scintillation pickup and KJ crystal activated with Tl. The results were statistically processed using $M\pm m$ parameters and Student's t test.

RESULTS

MDA level peaked 1 and 2 weeks after bone fracture (Fig. 1, *a*). After 4 weeks the level of MDA approximated the control. After 10-day treatment with selenomethionine (2 μg/kg) intragastrically or by electrophoresis (starting from the negative pole) the 1st peak remained visible, while the 2nd peak leveled and decreased 4 weeks after the injury. Electrophoresis and intragastric treatment with selenomethionine activated glutathione peroxidase and promoted a decrease in the 2nd peak of MDA (Fig. 1, *b*). This corresponded to stages of reparative osteogenesis: cell fibrous (soft) callus forms 1 weeks after bone fracture, chondroid callus after 2 weeks, and primary bone callus forms 4 weeks after the fracture.

Analysis of the dynamics of MB/blood, diaphysis/blood, and metaepiphyseal zone of femoral bone/blood RRA for the period of 1st-192nd hours after isotope injection to 1-month-old rats showed 2 peaks of RRA (6 and 48 h postinjection, p<0.001-0.050), which were more pronounced than the increase in the percentage of incorporation into bones at the same terms (Fig. 2, *a*, *b*). All RRA decreased twice: by hours 12-24 and by 192nd hour. The percentage of incorporation in the blood decreased starting from the first hour till the end of experiment more rapidly than in the bones.

In 3-month rats all RRA had only one maximum 12-48 h postinjection (p<0.001-0.050), delayed in com-

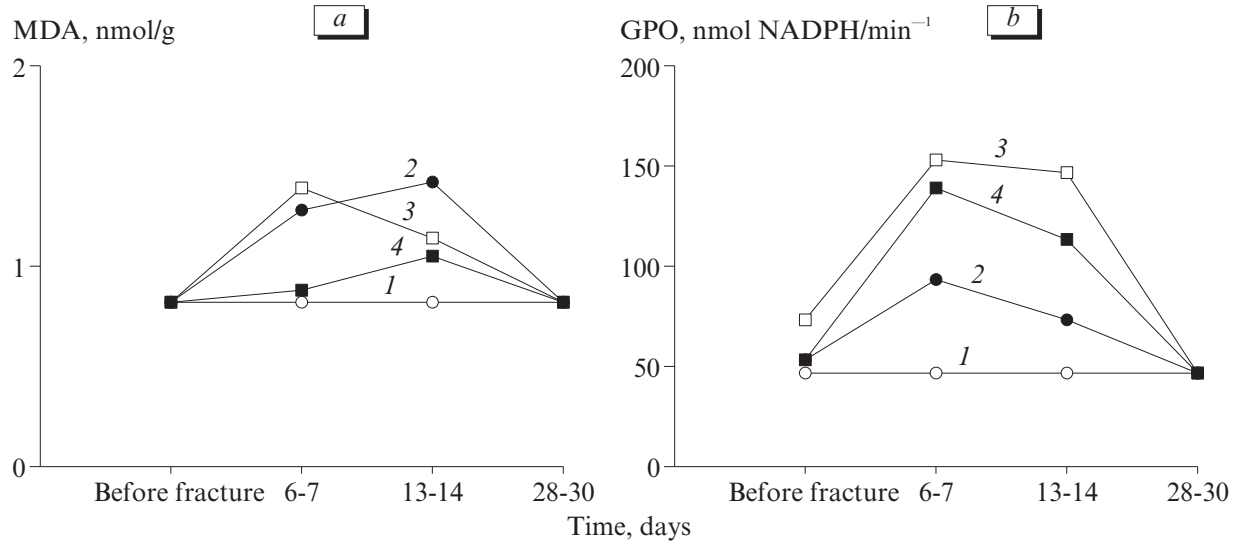

Fig. 1. Changes in MDA content (*a*) in mandibular bone regenerate after fracture and content of selenium-containing glutathione peroxidase (GPO; *b*) in mandibular bone of adult rats before and after fracture. *1*) control; *2*) fracture without Se; *3*) fracture+intragastric Se; *4*) fracture+Se electrophoresis.

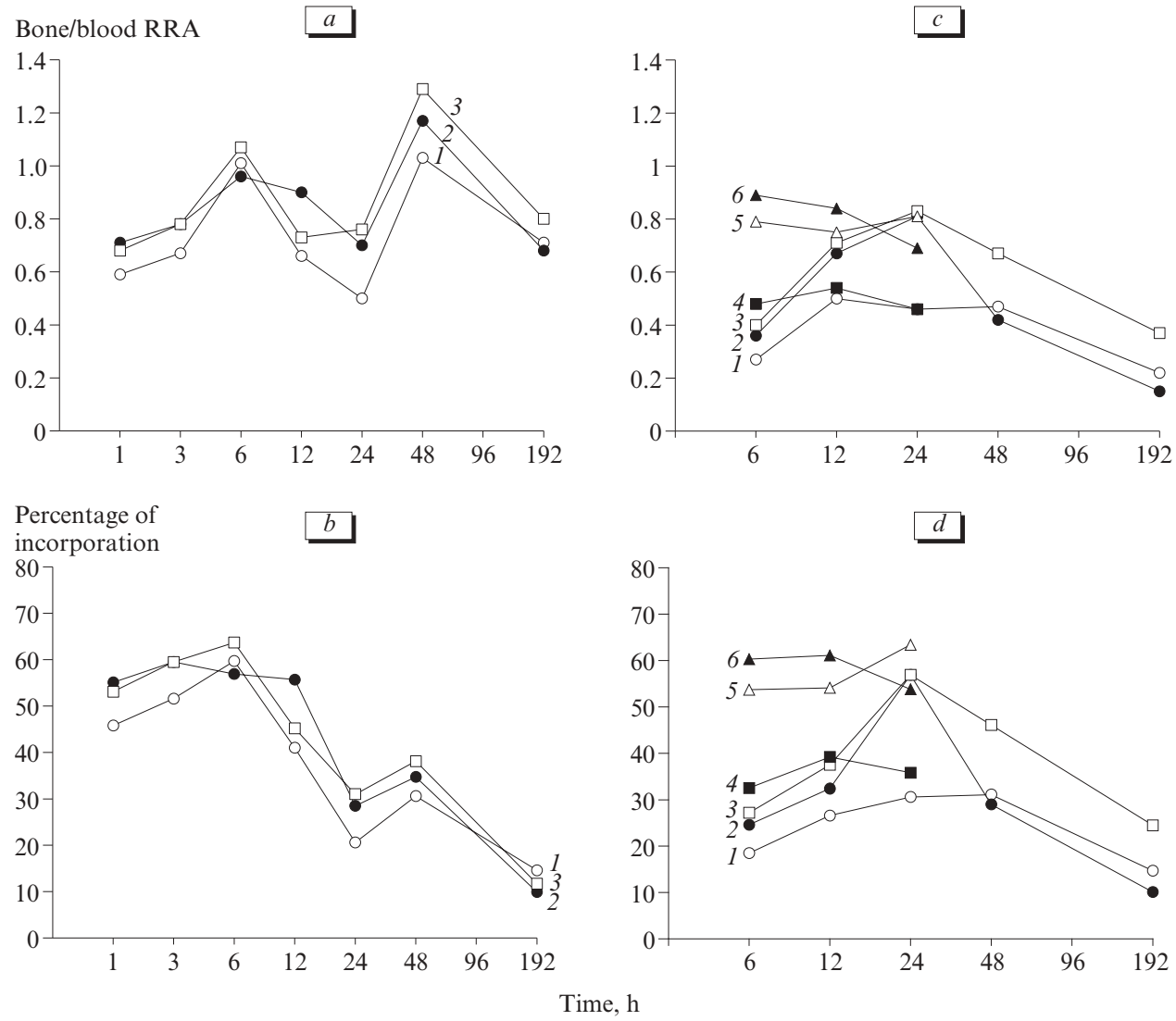

Fig. 2. Time course of bone/blood relative radioactivity (RRA) and percentage of incorporation in the bones after intraperitoneal injection of [^{75}Se]selenate to 1-month rattlings (*a*, *b*) and 3-month rats (*c*, *d*). *1*, *2*, *3*) rats receiving common vivarium ration; *4*, *5*, *6*) rats receiving carbohydrate-rich diet; *1*, *4*) mandible; *2*, *5*) diaphysis; *3*, *6*) metaepiphyseal zone of femoral bone.

parison with the first peak in rat pups and decreasing by the 192nd hour (Fig. 2, *c*). The peak of RRA in adult rats was lower than both peaks in 1-month rats, and the percentage of incorporation in the blood from 6th to 192nd hours changed less markedly.

In rats receiving highly caloric diet RRA after 6 and 12 h was higher for the majority of ratios in comparison with animals receiving common ration; the percentage of incorporation in the bones was also higher (Fig. 2, *d*). Presumably, the excess reflects the generalized metabolic changes caused by carbohydrate-rich diet, which was not confined to oral cavity tissues. The relationship between Se and carbohydrate metabolism in mineralized tissues in rats was reported [12,13].

The differences in RRA in two age groups can be explained by more intensive metabolism of [^{75}Se]selenate in growing organism. Changes in Se metabolism during pre- and postnatal ontogenesis were previously described [8,13].

Biphasic reaction of the bone/blood RRA in young age can be partially attributed to specific features of selenate adsorption by mineralized tissue hydroxyapatite from the blood, which is essential for selenate distribution between free and bound fractions. Phase 1 with a rapid increase and rapid decrease can be regarded as physical adsorption, while phase 2 (slower increase followed by slower decrease) as mainly more stable chemosorption and as a result of Se binding by bone protein.

The data on possible mechanisms of Se-containing compounds binding to mineralized tissues are scanty. For example, the fundamental monograph by V. A. Tutel'yan *et al.* [7] discusses in detail general

Se metabolism, role and metabolism of Se in diseases of many organs and systems, but presents little data on the bones. A large section of the monograph by A. P. Avtsyn *et al.* [1] devoted to Se contains almost no information on mineralized tissues.

Interestingly, after injection of [^{75}Se]selenate and [^{75}Se]selenomethionine to pregnant rats the protein fraction of maturing molar enamel of 13-day-old rat pups contained more ^{75}Se than mature enamel of adult rats [13]. Three forms of bound Se were distinguished: soluble bound Se, protein-bound selenotrisulfide, and protein tightly bound Se. Presumably, phase 1 of biphasic reaction, described above, can be explained by the effect of labile soluble Se, while phase 2 is due to the effect of protein tightly bound Se.

We can hardly agree to the hypothesis [11] on selenate binding to dental protein via sulfur substitution for Se in enamel protein cysteine, as it is known that enamel proteins (enamelines and amelogenines) contain no cysteine and total enamel proteins contain it in just trace amounts [5]. The activity of antioxidant system enzymes and the content of MDA and Fe in the oral fluid increased in atrophied mandibular alveolar process of patients with periodontitis [4].

The coefficient proposed in this report shows changes in the ratio of two selenate fractions: dissolved in the blood and adsorbed by hydroxyapatite or bound by mineralized tissue proteins. Studies with the use of this coefficient can show changes in the Se compounds transport from biological fluids to mineralized tissues and *vice versa*. The method can be also used for the analysis of changes in the distribution of other compounds between biological fluids and mineralized tissues.

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