Study of Labeled Carbonate Metabolism in Healthy Organisms during Reparative Osteogenesis and Denervation by the Bone/Plasma Index

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We studied the effect of mandibular fracture and denervation on [^{14}C]carbonate metabolism. A new index (bone/plasma relative radioactivity) reflecting the ratio between ^{14}C incorporation into bone and plasma was proposed. The percentage of label incorporation and bone/plasma relative radioactivity were measured from the 5th minute to the 192nd hour after intraperitoneal injection of labeled carbonate to 1-2-month-old albino rats. We revealed a biphasic reaction: rapid accumulation and elimination of the isotope form the bone; and slow accumulation followed by slow accumulation and elimination. Changes in carbonate metabolism after bone fracture corresponded to phases of reparative osteogenesis. At the stage of cellular-and-fibrous callus accumulation of labeled carbonate dominated over its elimination. At the stage of chondroid callus elimination dominated over accumulation. At the stage of primary osseous callus elimination dominated over accumulation, but did not surpass the control level. After fracture the index of bone/plasma relative radioactivity underwent general changes that were most pronounced in the zone of trauma. After denervation this index also decreased.

Key Words: *jaw fracture; [^{14}C]carbonate metabolism; bone/plasma index*

Successful therapy of fractures, prevention and treatment of osteoporosis, periodontitis, and genetic bone defects, bone transplantation, distraction osteosynthesis, and integration of implants and bones require profound knowledge on the molecular mechanisms underlying reparative osteogenesis (RO).

Changes in the content and metabolism of Ca and P during fractures were studied in details. In mineralized tissues these elements are mainly presented by hydroxyapatite crystals (HAP, $Ca_n(PO_4)_6(OH)_2$, n=8-12). Calcium and phosphorus constitute 84% HAP.

Changes in the content of carbonate-apatite (CAP) and carbonate during fractures are poorly understood. It should be emphasized that CAP constitutes 4% weight of bones, dentine, and dental root. Vitamin D promotes incorporation of [^{14}C]carbonate into the bone. Substitution of one or several phosphate residues in HAP with carbonate (CO_3^{2-}) leads to the formation of CAP.

Mineralized tissues contain crystals of HAP, *i. e.* CAP, where Ca^{2+} is substituted with Sr^{2+}, Na^{+}, and K^{+} and hydroxyl is substituted with F^{-} or Cl^{-}. The relationship between citrate and apatite remains unknown. Along with the formation of citric apatite, citrate can be absorbed on the surface of HAP crystals.

Here we studied [^{14}C]carbonate metabolism during RO and denervation of the mandible.

MATERIALS AND METHODS

The main object of study was the mandible. Mandibular fractures occur more frequently than fractures of skull bones. These fractures are accompanied by dam-

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age to branch III of the trigeminal nerve and sympathetic nerve. Experiments were performed on young albino rats aging 1-2 months. The animals were kept in a vivarium under standard conditions. [^{14}C]carbonate was injected intraperitoneally in a dose of 0.5 μCi/g.

Radioactivity was determined by calculating the percent of label incorporation into jawbones, femoral bones, and plasma: the counts (cpm) per 1 g bone or 1 ml plasma were divided by count injected per 1 g body weight. A new index (the ratio between relative radioactivities of the bone and plasma, B/P RRA) was proposed for evaluation of the metabolism and transport of [^{14}C]carbonate. This index reflected the ratio between label incorporation into the bone or bone regenerate and blood plasma. β-Radiation was recorded on a gas-discharge and scintillation counter [2]. Fracture on the right side of the mandible was produced under hexenal anesthesia. RO of the bone was studied as described previously [1].

We performed 5 series of experiments. In series I the dynamics of label incorporation into the mandible, maxilla, femoral diaphysis (FD), and femoral metaepiphysis (FME) of intact rats was evaluated 5, 10, 20, 45, and 90 min and 3, 6, 12, 24, 96, and 192 h after intraperitoneal injection of [^{14}C]carbonate. In each stage we examined 4-10 rats.

In series II (n=10), III (n=9), and IV (n=12) we determined label incorporation into the regenerate on the right side of the mandible 20 min after injection of [^{14}C]carbonate. The measurements were performed on days 7, 14, and 28, respectively, which corresponded to stages of RO. Soft cellular-and-fibrous, chondroid, and primary osseous calluses were formed 1, 2, and 4 weeks after treatment, respectively.

Series V was performed on 6 rats without mandibular fracture 2 weeks after transection of the right lower alveolar nerve [1].

The results were analyzed by Student's t test. The data are presented as $M \pm m$.

RESULTS

In intact rats (series I) the dynamics of label incorporation into 4 types of bones and plasma was similar from the 5th minute to the 192nd hour after intraperitoneal injection of [^{14}C]carbonate.

The degree of label incorporation into various tissues peaked 5-20 min after treatment (p<0.001). This parameter did not increase only in FME from the 10th to 20th minute. In series II-V the label was administered on the 20th minute. In series I the percent of label incorporation sharply decreased 20-45 min after treatment (by 1.5-2 times, p<0.001). From the 45th to 90th minute the percent of label incorporation progressively decreased in FME, tended to decrease in the plasma (p>0.05), and remained practically unchanged in the maxilla and FD. The decrease in label incorporation into bones 90 min after treatment was 2 times less pronounced than that observed from the 20th to 45th minute. The degree of [^{14}C]carbonate incorporation over this 25-min period decreased by 35-45%. It was probably related to the existence of rapid (2-fold increase over 15 min and 1.5-2-fold decrease over 25 min) and slow pools of [^{14}C]carbonate. Further decrease in the rapid pool coincided with activation of the slow pool 45 min after treatment: after sharp decrease observed from the 4th minute to the 3rd hour we recorded a slow decrease from the 3rd to the 192nd hour. Elimination of [^{14}C]carbonate in the plasma was more pronounced than in the bone.

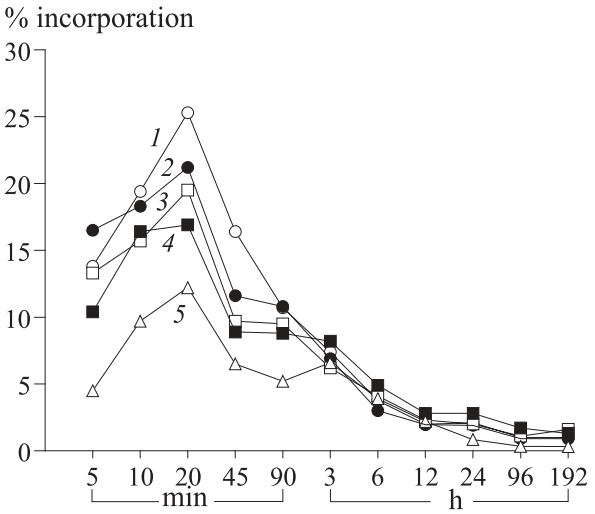
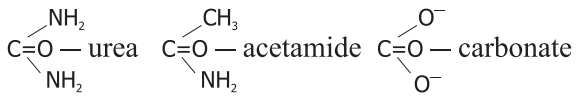


Fig. 1. Dynamics of ^{14}C incorporation into bone and plasma of young intact rats after intraperitoneal injection of [^{14}C]carbonate. Here and in Fig. 2: mandible (*1*), maxilla (*2*), diaphysis (*3*), femoral metaepiphysis (*4*), and blood plasma (*5*).

Therefore, the dynamics of [^{14}C]carbonate incorporation into the bone differed from that described for ^{45}Ca, ^{32}P, and labeled amino acids. These agents were found only in trace concentrations 20 min after treatment. Then the degree of their incorporation progressively increased and peaked after 24-48 h. Interestingly, [^{14}C]urea and [^{14}C]acetamide penetrated a 3.5-mm dentine layer over 20 min [14]. A comparative study of urea, acetamide, and carbonate suggests that high rate of incorporation into mineralized tissue is associated with their structural characteristics.



The B/P RRA index in 4 examined bones decreased by 1.5-2 times from the 5th to 10th minute (p<0.001), but remained unchanged 10-45 min after treatment. This index slightly increased in bones after 45-90 min

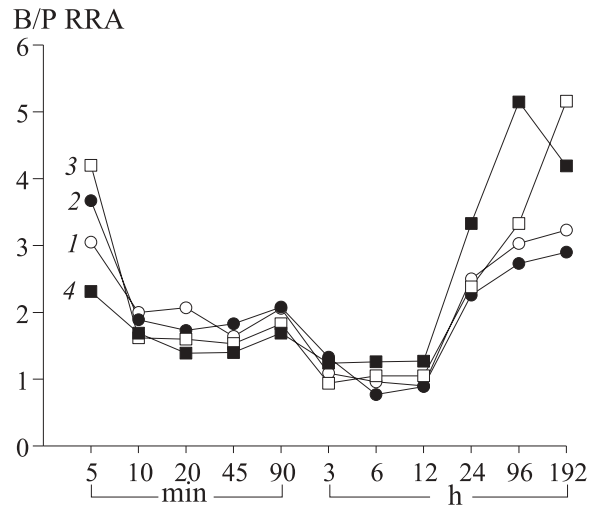


Fig. 2. Dynamics of relative radioactivities in the bone and plasma (B/P RRA) of young intact rats receiving intraperitoneal injection of [^{14}C]carbonate.

(p<0.05). The B/P RRA index decreased by 1.5-2 times from the 90th minute to the 3rd hour after treatment. From the 3rd to 12th hour this index remained unchanged in 3 bones, but continued to decrease in the maxilla (Fig. 2). The B/P RRA index increased by 3-4 times from the 12th hour to the end of observations (p<0.001).

Changes in the B/P RRA index were probably related to rapid and unstable adsorption of carbonate and metabolic products on the surface of HAP followed by rapid desorption (physical sorption) and slow and steady incorporation into apatite with the formation of CAP (chemisorption). HAP is widely used as a sorbent. Changes in the B/P RRA index depend on

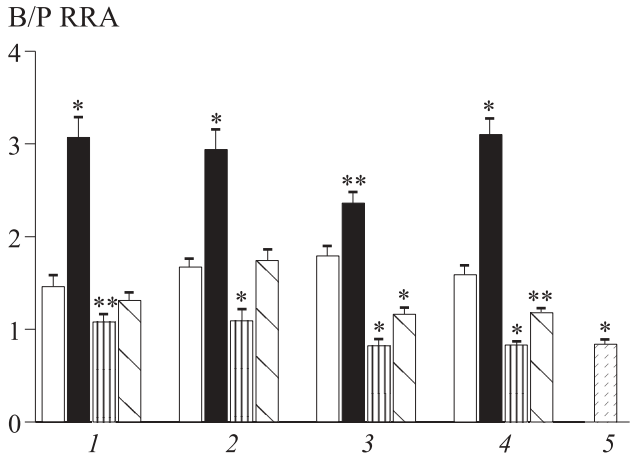


Fig. 3. Changes in the ratio between radioactivities of the bone and plasma after intraperitoneal injection of [^{14}C]carbonate to young rats on days 7 (dark bars), 14 (shaded bars), and 28 after right-sided fracture of the mandible (slant shading) and 14 days after transection of the right alveolar nerve. Light bars: control. Mandible (*1*), maxilla (*2*), diaphysis (*3*), femoral diaphysis (*4*), femoral metaphysis (*4*), and denervation (*5*, interrupted shading). *p<0.001 and **p<0.05 compared to the control.

variations in the degree of label incorporation during slow and rapid pathways of carbonate metabolism. The decrease in the B/P RRA index from the 5th to 10th minute is associated with rapid sorption and desorption of the label in HAP. The B/P RRA index increased 45-90 min after treatment, but decreased from the 90th minute to the 3rd-6th hour. These changes reflect the slow pathway and formation of CAP. Further increase in the B/P RRA index is related to more rapid elimination of ^{14}C from the plasma (compared to bone) and its steady binding in CAP.

In series II the B/P RRA index maximally increased in the bone regenerate and 2-fold surpassed the initial level 1 week after fracture and administration of [^{14}C]carbonate (p<0.001, Fig. 3). The increase in this index in other bones was less pronounced, but statistically significant (p<0.001-p<0.05). In series III the B/P RRA index in 4 examined bones decreased by 3-4 times 2 weeks after treatment (p<0.001 compared to 1 week after treatment). In series IV the B/P RRA index significantly increased in 3 distant bones (p<0.001-p<0.05) and tended to increase in the bone regenerate 4 weeks after fracture (p>0.05). These data indicate that carbonate metabolism after fracture undergoes changes corresponding to the stage of RO.

In series V the B/P RRA index sharply decreased 2 weeks after transection of the alveolar nerve (p<0.05). Our findings are consistent with published data that denervation produces an adverse effect on bone remodeling [3-5,7].

Experiments with labeled atoms showed that carbonate is intensively metabolized in bones under physiological and pathological conditions. This process involves carboanhydrase II, calcitonin and its receptors on osteoclast [6,8,10,12], parathyroid hormone, and 1.25 $(OH)_2D_3$ [11,13]. Active form of vitamin D_3 affecting [^{14}C]carbonate metabolism in bones is used for the therapy and prevention of osteoporosis [13]. RO violates the ratio between the synthesis and resorption of bone structures determined by osteoblasts and osteoclasts [11]. The inhibition of carboanhydrase II and influence of calcitonin via receptors on osteoclasts promote the formation of carbonate pools involved in bone remodeling. Changes in carbonate metabolism during fracture have general manifestations in bone tissue and, particularly, in the zone of trauma. Considerable changes also occur in distant bones. The impairment of innervation can contribute to metabolic disturbances during bone fractures, since denervation of the mandible reduces the B/P RRA index.

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