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Original Contribution

CHANGES IN SOME SERUM BONE MARKERS AFTER EXPERIMENTAL FRACTURE AND INTRAMEDULLARY OSTEOSYNTHESIS IN DOGS

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ABSTRACT

The aim of the present study was to follow the changes in serum concentrations of some bone markers in experimentally-induced normal healing femoral fracture in dogs. In six dogs, a diaphyseal osteotomy of one femur and then, an intramedullary osteosynthesis with a Kuntcher nail were performed. Prior to the operation and at post operation weeks 1, 2, 3 and 4, blood samples were obtained for analysis of serum concentrations of total and ionized calcium, inorganic phosphate, total and bone alkaline phosphatase, osteocalcin and carboxyterminal telopeptide of collagen type I (ICTP). Significant alterations in the levels of these bone markers occurred as follows: for total calcium – by week 1, for ionized calcium – by weeks 1 and 3, for inorganic phosphate – by week 3, for ICTP – by weeks 1 and 2. In the other parameters, the changes were not significant. In conclusion, it could be stated that within a period of one month, the markers of bone resorption were altered whereas the markers of bone formation showed only a tendency towards decrease.

Key words: dogs, fractures, bone metabolism

INTRODUCTION

In human medicine, bone markers are commonly used for control of various therapeutic protocols and monitoring of cell activity in bone metabolic diseases and other disorders related to bone changes [1]

They are a good alternative in studies on animal models of osteoporosis, bone metastases of tumours and some types of arthritis [2, 3, 4].

Regardless of the fact that in humans there is a close relationship between blood and urine bone markers and histomorphometric parameters of bone remodelling [5], the definitive diagnosis in osteoporosis and Paget's disease is made after densitometry and/or biopsy.

In available literature, bone markers in animals were used for determination of differences in the levels of bone formation and bone resorption in horses at different age [6, 7] and cats [8, 9]. In dogs, bone markers have been studied in general with regard to age and breed-related differences [10, 11, 12, 13, 14]. There are single reports about their clinical applications in osteomyelitis [15], osteosarcomas and osteoarthritis [16, 17, 18], ostectomy of the radius [19], for monitoring of distraction osteogenesis [20], delayed bone union and non-union in tubular bones [21]. With these in view, it became necessary to continue further studies on these markers.

Therefore, the aim of the present investigation was to follow up the changes in serum concentrations of some biochemical markers of bone metabolism in dogs, occurring in the early period of bone healing (within one month).

MATERIALS AND METHODS

Six male mixed-bred dogs, aged 1-3 years, were subjected to unilateral femoral osteotomy under aseptic conditions. Then a Kuntcher nail was introduced through the *fossa intertrochanterica* into the medullar channel. In the post-operative period, clinical (comprising body temperature, heart and respiratory rates), radiological and laboratory

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studies were performed for 30 days. The clinical and radiological survey was done in order to monitor whether the bone healing was normal.

Blood samples were obtained between 7.00 and 8.00 h for elimination of circadian influences prior to the operation and, by the end of post operation weeks 1, 2, 3 and 4, from the *ramus dorsalis* of *v.saphena parva*. The dogs were housed in individual boxes and fed with a commercial dry canine food (*Jambo-dog, Gallisman-94 S.A., Bulgaria*).

The following blood laboratory parameters were determined: concentrations of total and ionised calcium, inorganic phosphate, total and bone alkaline phosphatase (with commercial colorimetric kits, *Biotrol, France*).

Serum carboxyterminal telopeptide of collagen type I (ICTP) was assayed radioimmunologically (*Orion Diagnostica, Finland*, with precision of the method of 0.2 ng/ml). Serum osteocalcin was determined with a RIA kit (*Henning-Berlin GmbH, Germany*). Each sample for both markers was run in duplicate and the average value of the two measurements was retained.

The data were statistically processed by one-way analysis of variance (ANOVA).

RESULTS

The clinical and radiological survey did not show any complications in bone healing.

The data of blood determinations are presented on **Table 1**.

Table 1. Dynamics of some serum bone markers in dogs after osteotomy and intramedullary osteosynthesis (n=6). Data are presented as mean ± SEM

Parameter	***Prior to operation***	***Weeks after the osteosynthesis***			
		1	*2*	*3*	*4*
Total calcium, mmol/L	2.48±0.20	2.02±0.14*	2.24±0.10	2.39±0.11	2.38±0.11
Ionized calcium, mmol/L	1.14±0.18	0.99±0.13*	1.12±0.18	1.09±0.09*	1.15±0.09
Inorganic phos-phate, mmol/L	1.54±0.19	1.80±0.10	1.78±0.12	1.98±0.06*	1.86±0.15
Total AlP, U/L	38.3±2.6	28.5±4.2	33.7±3.7	30.8±7.3	32.2±6.7
Bone AlP,U/L	12.7±3.0	9.3+1.7	9.3±3.0	7.7±3.2	6.2±0.8
Osteocalcin, ng/mL	3.90±0.38	4.50±0.54	4.42±0.51	3.95±0.24	4.38±0.48
ICTP, ng/mL	3.78±0.28	4.62±0.21*	4.61±0.31*	4.10±0.25	3.85±0.19

AlP= alkaline phosphatase; ICTP = C-terminal telopeptide of collagen type I.

By the 1st week after the osteotomy, the values of total and ionised calcium decreased whereas those of ICTP were elevated – from 2.48±0.20 mmol/l to 2.02±0.14 mmol/l for total Ca; from 1.14±0.18 mmol/l to 0.99±0.13 mmol/l (ionised Ca) and from 3.78±0.28 ng/ml to 4.62±0.21 ng/ml for ICTP. In all three parameters, the level of significance was $p<0.05$. ICTP remained elevated by the end of the second week too ($p<0.05$), and at the end of week 4, returned to levels close to baseline. During the 3rd week after the osteosynthesis, another significant decrease in ionised calcium levels were observed (from 1.14±0.18 mmol/l at baseline to 1.09±0.09 mmol/l at $p<0.05$), and inorganic phosphate concentrations increased to 1.98±0.06 mmol/l from 1.54±0.19 mmol/l at baseline ($p<0.05$). The activities of total and bone alkaline phosphatase decreased by the end of the first week; serum osteocalcin was elevated, but the differences were not significant. They varied insignificantly up to the end of the experimental period.

DISCUSSION

Bone healing is a local process that has an effect on systemic mineral homeostasis. The latter involves vitamins, hormones, enzymes and other factors. Apart from the radiological survey of bone callus formation, some serum markers are successfully used for this purpose. Since this complex process is characterised by a close interrelationship of bone resorption on one hand and bone formation on the other, we chose biochemical markers that straddle both processes together with the primary macroelements that build the inorganic bone matrix.

Bone metabolism parameters are influenced by factors such as age, nutrition, exercise [22, 23] and time of blood sampling

[13, 24]. The dogs included in our study were adults and were fed with the same food throughout the trial; and blood samples were obtained only in the morning to eliminate circadian influence. Our dogs were not purebred, but the breed and body weight were assumed to have no effect on determined bone makers [14].

Kurdy [25] monitored the role of propeptides of collagen types I and III and of bone-specific alkaline phosphatase (BsAlP) in abnormally healing diaphyseal tibial fractures in men and found that, up to the 10th week after the trauma, no significant decrease in BsAlP and propeptide levels occurred; even the propeptides of collagen type III were considerably elevated. Data for inadequate osteoblastic response were however present after the 20th week. In our study, the reverse situation was observed – although the fractures healed normally, BsAlp activities decreased but not significantly.

On the basis of determinations of bone-specific alkaline phosphatase, osteocalcin and telopeptides, Emami et al. [26] observed that in human tibial fractures with delayed healing the bone resorption was similar to that in fractures healing normally up to the 4th week, whereas bone formation was shifted later (between the 10th-16th week). In our studies on femoral fractures n dogs, the bone resorption marker (ICTP) was significantly increased during the 2nd week and, by the end of the 4th week, returned to the initial values. This corresponded to the processes of osteolysis around the bone ends after the trauma that probably occurred earlier in dogs. The data about the bone-specific alkaline phosphatase and osteocalcin indicating a decrease (bone-specific alkaline phosphatase) or no change (osteocalcin) are confirmed, although the fractures in dogs healed without complications. In view of these, our results are similar to the conclusions of Akesson et al. [27], which showed that in wedge-shaped tibial osteotomies bone metabolism was seriously impaired and that it could confuse seriously the monitoring of osteoporosis in these patients. Using serum and urine bone markers, the authors evidenced that bone resorption exceeded bone formation in the post ostectomy (fracture) period.

According to Herrmann et al. [28] in fractures with delayed healing in men osteocalcin could be an earlier marker, because it remains unchanged after the 4th week whereas in normal healing fractures it increased significantly. In a similar study, Nyman et al. [29] established a significant increase in osteocalcin and bone-specific alkaline phosphatase levels by week 6 in both groups. The facts about the normal healing fractures are confirmed by our results too, because the variations in osteocalcin levels were insignificant, and bone-specific alkaline phosphatase was reduced almost twice but not significantly.

Komnenou et al. [21] established a correlation between the levels of the total serum alkaline phosphatase in dogs and the healing of long bone fractures. According to them, the total alkaline phosphatase increased significantly as early as the 10th day and by day 30, returned to initial values. In our opinion, the bone-specific isoenzyme should also be monitored as it is a more sensitive index of bone metabolism. In our experiment, total alkaline phosphatase activities were maintained but that of the bone isoenzyme decreased as early as the first week and this tendency persisted up to the end of the experiment. Our results agree with those of Akesson et al. [27], who reported that bone resorption exceeded bone formation.

In our previous studies [15], we followed up the dynamics of the same biochemical markers but in experimental osteomyelitis of the femur in dogs. In this model, after the first week, there was a constant trend in elevation of ICTP up to the end of the 4th week, accompanied by enhanced bone formation during the first 2 weeks (significantly higher levels of bone-specific alkaline phosphatase and osteocalcin). Regardless of the changes occurring in osteomyelitis, bone resorption in the present study (assessed through ICTP levels) was the highest during post operation weeks 1 and 2 and was rapidly restored to normal after that period. The differences were probably due to the more serious osteolytic processes in osteomyelitis, accompanied by an extensive periosteal reaction in the beginning while in normal healing fractures, the resorption stage is short on the background of a relatively constant bone formation rate.

In conclusion, significant alterations in the levels of these bone markers occurred as follows: for total calcium – by week 1, for ionised calcium – by weeks 1 and 3, for inorganic phosphate – by week 3, for ICTP – by weeks 1 and 2. In the other parameters, the changes were not significant. It could be stated that within a period of one month, the markers of bone resorption were altered whereas the markers of bone formation showed only a tendency towards decrease.

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