Variations of serum bone alkaline phosphatase activities and osteocalcin concentrations in dogs with experimental osteotomy fixed by two different osteosynthesis techniques

M. PASKALEV*, S. KRASTEV2 and L. SOTIROV3

1Department of veterinary surgery, Faculty of Veterinary Medicine, 6000 Stara Zagora, BULGARIA. 2Department of veterinary surgery, Faculty of Veterinary Medicine, 6000 Stara Zagora, BULGARIA. 3Department of animal genetics, Faculty of Veterinary Medicine, 6000 Stara Zagora, BULGARIA.

*Corresponding author: Assoc. Prof. M. Paskalev, Email: paskalev@uni-sz.bg

SUMMARY

In 12 dogs, transperiosteal osteotomies of the right tibia and fibula diaphyses were performed followed by intramedullar osteosynthesis (IMO group, n = 6) or plate osteosynthesis (PLO group, n = 6). Cranio-caudal radiographs were obtained immediately after surgery then on week 2 and every month during 6 months for evaluating bone formation (BF) and bone union (BU). Before surgery and at the same time intervals, blood serum samples were collected for determination of bone alkaline phosphatase (ALP) activities and osteocalcin (OC) concentrations. BF was maximal since the 1st month and stopped at the 4th month in the IMO group whereas it appeared delayed in the PLO group: maximal values were recorded since the 2nd month and BF lasted until the 5th month. The bone union (BU) scores were significantly elevated on month 1 (IMO group: p<0.001 and PLO group: p<0.05) and then markedly increased during the whole period (p<0.001) in the 2 groups. Although not significant, decreases of bone ALP activities compared to pre-surgery values were more pronounced in the PLO group particularly during the 1st month. By contrast, dramatic increases of serum OC concentrations were observed after intramedullar osteosynthesis during the 1st month whereas this marker was weakly affected by plate osteosynthesis. Consequently, a more intense bone reaction evidenced by radiological signs and by elevation of serum OC concentrations was induced by the intramedullar osteosynthesis.

Keywords: dogs, tibia osteotomy, intramedullar osteosynthesis, plate osteosynthesis, osteogenesis, bone alkaline phosphatase, osteocalcin.

Introduction

Bone metabolism is directly related to the activity of osteoblasts and osteoclasts. In pathological states, it is important to investigate the bone cell markers that are specific and sensitive, and not influenced by changes other than bone metabolism.

Alkaline phosphatase (ALP) is a membrane-bound protein, synthesized by the cells of various tissues. In animals, 4 ALP variants (intestinal, hepatic, bone and corticosteroid-induced in dogs) are known. Bone and hepatic ALP are considered as isoforms, not as isoenzymes [33] and there are some laboratory assays and kits able to determine only the bone ALP [2].

Osteocalcin (OC), also known as the vitamin K-dependent bone protein, is synthesized only by osteoblasts and megakaryocytes, then deposited in the bone matrix and plays an important role in its mineralization. Despite being one of the
most studied bone non-collagenous proteins, its role in bone formation is not yet clear. As osteocalcin is not released during the resorption of bone, its serum concentrations are indicative only for the osteoblast activity. As established histomorphometrically, serum OC concentrations highly correlated with bone formation, as well as with other biochemical markers of osteogenesis [11, 18].

The circadian rythm of some bone markers has been reported in rats [27], mice [36], and horses [6]. The activity of OC in dogs was higher in the morning than in the afternoon [22, 25]. The bone ALP also exhibits circadian variations with highest activities in the early afternoon [22]. Similar to men, the variability of bone markers was lower in serum than in urine samples [22, 25].

In the available literature, bone markers were used to detect the differences in bone formation and bone resorption in horses at various ages [24, 32], in dogs [1, 3] and in cats [8, 9]. FRANCIS and MILLIS [15] monitored the alterations in bone ALP and OC concentrations after osteotomy of the radius in dogs. In the same animal species, LAMMENS et al. [23] and THEYSE et al. [35] monitored distraction osteogenesis of the tibia using these markers and growth factors. However, both teams obtained conflicting results about plasma osteocalcin concentrations: the first team observed increased OC concentrations whereas the second one reported a reduction in concentrations during distraction osteogenesis. KAMNENOU et al. [20] observed a positive correlation between the serum total ALP activity and bone healing of long bone fractures. Besides, the bone ALP was reported to be useful in monitoring and prognosis of osteosarcomas, rheumatoid arthritis and osteoarthritis in dogs [12, 14, 16].

The bone markers in dogs were studied on a rather theoretical level with regard to age- and breed-related differences [1, 3, 7, 22]. There are few publications about their application in the clinical practice: in osteomyelitis [31], radial osteotomies [15], osteosarcomas and osteoarthritis [12, 14, 16] and in monitoring of normal, delayed bone healing and non-healing of long bone fractures [20, 30].

The aims of the present investigation were to determine the serum levels of some biochemical bone metabolism markers (bone alkaline phosphatase activity and osteocalcin concentration) prior to and after long bone osteotomy in dogs and to investigate the influence of the osteosynthesis technique upon the concentrations of bone formation markers.

Materials and Methods

The experiment was approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria and was performed according to Animal Welfare Act N° 25/10.06.05 and the Law on Veterinary and Medical Activities.

ANIMALS

A total of 12 mixed-breeds, 2–5 years old, male dogs, weighing 12–20 kg were used and divided into 2 equal groups: group 1: 12.50–20.00 kg, mean 16.25 kg and group 2: 12.00–19.50 kg, mean 15.50 kg. The animals were obtained from a licensed kennel and at the end of the experiment that lasted for 8 months, they were returned back to it. Prior to the experiment, all dogs were treated against endoparasites with praziquantel/pyrantel pamoate (Azipyrin®, Balkanpharma, Bulgaria) and ectoparasites with Fipronil (Frontline®, Merial, France).

OPERATIVE PROTOCOL

After pre-treatment with 0.02 mg/kg s.c. atropine sulphate (Sorpharma, Bulgaria) and 0.05 mg/kg i.m. acepromazine maleate (Neurotranq, Alfasan, Woerden, Holland), anaesthesia was induced with 6 mg/kg i.v. 2.5% thiopentone sodium (Thiopental, Biochemie GmbH, Kudl, Austria) and maintained after intubation with 2.5% halothane (Narcotan, Spofa, Czech Republic).

In all 12 dogs, after aseptical preparation and medial approach, transperiosteal osteotomies of diaphyses of the right tibia and fibula were performed. After that, 2 different osteosynthesis techniques were performed:

- In the group 1 (n = 6), also called IMO group, the osteotomies were fixed with a normograde insertion of a Kuntscher nail in the medullar canal (intramedullar osteosynthesis). Three of the Kuntscher nails had a diameter of 6 mm and the 3 other 8 mm.

- In the group 1 (n = 6), also called PLO group, the osteotomies were fixed with a plate and 6 cortical screws (3 in the distal and 3 in the proximal bone fragment) (plate osteosynthesis). The used plates had a thickness of 3.5 mm and width of 10 mm; the screws were cortical, with a diameter of 3.5 mm.

The soft tissues were routinely sutured and protective bandages were placed on operated areas.

Just before the surgery and during 3 consecutive post-operative days, the animals were managed for pain with butorphanol tartrate (Torbutil, Fort Dodge, USA, 0.2 mg/kg s.c., every 6 hours). An intramuscular antibiotic combination of lincomycin and spectinomycin (Linco-Spectin®, Pharmacia N.V./S.A., Puurs, Belgium) was administered at 1 mL (50 mg lincomycin and 100 mg spectinomycin) / 5 kg for four days following surgery. The dogs were housed in individual boxes and fed with a commercial dry food for adult dogs (Jambo-dog, Gallissman-94 S.A., Bulgaria) and received water ad libitum.

CLINICAL EXAMINATIONS AND BIOCHEMICAL ANALYSES

A general physical examination of each dog with emphasis on the condition of the wound, the type and degree of lameness was daily performed during the first ten post operative days, then weekly until the end of the experiment.

Cranio-caudal radiographs of the operated limbs were done immediately after the osteosynthesis, 2 weeks after the surgery and monthly for 6 months with X-ray equipment (TUR 800-1, Germany). All radiographs were taken in a standardized manner under identical exposition (60 kV, 8 mAs)
then radiographs were processed automatically (Compact-35, Swissray, Switzerland). The evaluation of bone formation (BF) and bone union (BU) was done using the scoring system presented in Table I. All radiographs were independently interpreted by 2 radiologists that were not aware about the post-osteosynthesis period. The means of scores of both observers were retained.

Venous blood samples were collected prior to the surgery, on week 2 and every month after the osteosynthesis by cephalic vein puncture always between 7.30 and 8.00 AM, for avoiding circadian effects. After clotting at room temperature for 1 hour, blood samples were centrifuged (1 000 g, at room temperature for 10 min.) and sera were carefully harvested and stored at -25°C until analysis.

The bone alkaline phosphatase (bone ALP) was assayed spectrophotometrically with a commercial kit (Human Diagnostica, Germany) immediately after serum separation. The osteocalcin (OC) assay was performed using the N-MID® Osteocalcin ELISA (Nordic Bioscience, Denmark): this kit can allow determination of the intact osteocalcin and N-MID fragment concentrations with a precision of 0.5 ng / mL. Each sample was analyzed in duplicate and the mean of both measurements was retained.

STATISTICAL ANALYSIS
The data were statistically processed by the Friedman’s test for two-way repeated measures analysis. In case of significant P-values, the non-parametric Tukey HSD test was performed. Differences were considered as significant when p values were less than 0.05.

Results
CLINICAL SIGNS
In the post operative period, dogs did not exhibit deviations in the general clinical parameters. The rectal body temperature, respiratory and heart rates were within reference ranges. The appetite was good. In the crural region of the operated limb, there was a moderate, slightly painful and warm swelling that disappeared within 4-6 days. During the first day after the osteosynthesis the dogs were predominantly lying down then manifested a grade 2 to 3 weight-bearing lameness (lasting for up to 1 week in PLO dogs and up to 2-3 weeks in IMO dogs). After that period, the full weight-bearing of limbs was resumed.

RADIOLOGICAL FINDINGS
The data of radiological scores are presented in Table II.

DYNAMICS OF SERUM BIOCHEMICAL MARKERS
The evolution of the 2 bone markers (bone ALP activity and osteocalcin (OC) concentration) is shown in Table III.
No significant variation of bone ALP activities according to time was evidenced for the 2 groups: they were close to the initial values for the whole experimental period even if bone ALP activities remained below the baseline values in the PLO group. Furthermore, no statistically significant difference was noticed between the 2 groups at any time.

By contrast, in the IMO group, significantly increased OC concentrations compared to initial values were observed since the 2nd week after surgery (p < 0.01) and OC concentrations remained elevated during the 1st month (p < 0.01). They also tended to increase in the PLO group but not significantly at the same time. The difference in OC concentrations was statistically significant between groups on the 2nd week (p < 0.05). Thereafter, gradual declines in bone ALP activities were observed and values became identical in the 2 groups.

Discussion

The osteogenesis during bone fracture or experimental long bone osteotomy healing is performed by the pathway of endochondral ossification, unlike distraction osteogenesis, where the processes are mediated via intramembranous ossification [5]. During endochondral ossification, a cartilaginous callus is initially formed and then, it is replaced by cancellous bone. These stages depend on the animal species, the type and site of the fracture, the fracture stability after the osteosynthesis, the state of surrounding soft tissues etc. The callus becomes radiologically visible when mineralization (ossification) occurs [38]. In the present experiment, the conditions for callus formation were the same except the osteosynthesis technique. It is known that the osteosynthesis with a plate and screws provides a better stability than intramedullar osteosynthesis [17, 37], and therefore, a more enhanced bone reaction could be expected in fractures or osteotomies fixed by intramedullar nails. In agreement with that, in the present study, the bone formation in the IMO (intramedullar osteosynthesis) was earlier than in the PLO (plate osteosynthesis) group. Maximal bone formation intensities were observed within the 1st – 2nd months and the 2nd – 3rd months for the IMO group and the 2nd – 3rd months for the PLO group.

Table II: Radiological scores of bone formation (BF) and bone union (BU) after osteotomy of the right tibia and fibula fixed by two osteosynthesis techniques (IMO: intramedullar osteosynthesis and PLO: plate osteosynthesis) in dogs. Results are expressed as median and extreme values in parenthesis.

<table>
<thead>
<tr>
<th>Time after surgery</th>
<th>Bone formation (BF)</th>
<th>Bone union (BU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMO group (n = 6)</td>
<td>PLO group (n = 6)</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1 (0 – 3)</td>
<td>1 (0 – 1)</td>
</tr>
<tr>
<td>1 month</td>
<td>2 (2 – 2)</td>
<td>1.5 (1 – 2)</td>
</tr>
<tr>
<td>2 months</td>
<td>2 (2 – 2)</td>
<td>2 (1 – 2)</td>
</tr>
<tr>
<td>3 months</td>
<td>1 (1 – 1)*</td>
<td>2 (1 – 2)</td>
</tr>
<tr>
<td>4 months</td>
<td>0 (0 – 1)*</td>
<td>1 (0 – 1)**</td>
</tr>
<tr>
<td>5 months</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)*</td>
</tr>
<tr>
<td>6 months</td>
<td>0 (0 – 0)</td>
<td>0 (0 – 0)</td>
</tr>
</tbody>
</table>

Table III: Variations of bone markers (serum bone ALP activities and osteocalcin (OC) concentrations) according to time after osteotomy of the right tibia and fibula fixed by two osteosynthesis techniques (IMO: intramedullar osteosynthesis and PLO: plate osteosynthesis) in dogs. Results are expressed as median and extreme values in parenthesis.

<table>
<thead>
<tr>
<th>Time after surgery</th>
<th>Bone ALP activity (U/L)</th>
<th>OC concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMO group (n = 6)</td>
<td>PLO group (n = 6)</td>
</tr>
<tr>
<td>Prior to surgery</td>
<td>31 (13 – 82)</td>
<td>25 (3 – 44)</td>
</tr>
<tr>
<td>2 weeks</td>
<td>13 (8 – 74)</td>
<td>19 (11 – 28)</td>
</tr>
<tr>
<td>1 month</td>
<td>39 (23 – 82)</td>
<td>15 (13 – 41)</td>
</tr>
<tr>
<td>2 months</td>
<td>31 (13 – 58)</td>
<td>21 (12 – 34)</td>
</tr>
<tr>
<td>3 months</td>
<td>24 (16 – 57)</td>
<td>25 (8 – 55)</td>
</tr>
<tr>
<td>4 months</td>
<td>24 (13 – 50)</td>
<td>27 (11 – 32)</td>
</tr>
<tr>
<td>5 months</td>
<td>21 (6 – 41)</td>
<td>18 (13 – 47)</td>
</tr>
<tr>
<td>6 months</td>
<td>32 (20 – 40)</td>
<td>27 (13 – 48)</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001 vs. baseline values.
Different superscripts a,b in the same line indicate significant difference (p < 0.05 or p < 0.01) between the 2 groups at a given time interval for bone formation and bone union respectively.
IMO group and the PLO group respectively. Besides, the bone formation period stopped on the 4th month in the case of intramedullary osteosynthesis whereas it lasted until the 5th month in the case of plate osteosynthesis. On the other hand, the kinetics of bone union was similar whatever the osteosynthesis technique.

It is currently accepted that bone ALP is a specific marker of bone formation. Considerable increases of blood total and bone ALP activities were reported after operative treatment of fractures in men [13, 21, 28, 29] and fixation of canine osteotomies [23]. In sheep, the opposite tendency was observed during the first 9 weeks in tibia osteotomies fixed by the Ilizarov’s device [34]. Surprisingly, serum bone ALP activities after surgery did not significantly vary in comparison with initial values (before surgery). However, in the PLO group, they tended to be lowered particularly during the 1st month and this observation was in discordance with the study of KAMNENOU et al. [20]. This could be attributed to the different protocol designs (clinical vs. experimental study), and to the fact that the authors have measured total ALP instead of only the bone ALP activities [4, 17]. As it is established both in vitro and in vivo, bone ALP was more closely related to the synthesis of collagen in the newly formed bone [23, 26, 35, 37], the release of the enzyme into the blood flow coincides to the osteoblast activity and the formation of the organic part of the bone matrix and precedes the collagen mineralization which is evidenced on radiography. It is probable that, in the present study, the genesis of the bone callus was too weak for inducing massive release of bone ALP enzyme into the blood with the 2 osteosynthesis techniques and particularly when plate osteosynthesis was performed. In this case, the notably low bone ALP activities were associated with low bone formation scores obtained from radiographs.

Osteocalcin is another biochemical marker, thought to play a role in bone formation. The data about its dynamic after fractures, osteotomies and some methods of osteosynthesis are conflicting: in sheep [34] and dogs [35], OC concentrations were lowered up to the 9th and the 6th week respectively, after tibia osteotomies and osteosynthesis with the Ilizarov’s device [34]. It is currently accepted that bone ALP is a specific marker of bone formation. Considerable increases of blood total and bone ALP activities were reported after operative treatment of fractures in men [13, 21, 28, 29] and fixation of canine osteotomies [23]. In sheep, the opposite tendency was observed during the first 9 weeks in tibia osteotomies fixed by the Ilizarov’s device [34]. Surprisingly, serum bone ALP activities after surgery did not significantly vary in comparison with initial values (before surgery). However, in the PLO group, they tended to be lowered particularly during the 1st month and this observation was in discordance with the study of KAMNENOU et al. [20]. This could be attributed to the different protocol designs (clinical vs. experimental study), and to the fact that the authors have measured total ALP instead of only the bone ALP activities [4, 17]. As it is established both in vitro and in vivo, bone ALP was more closely related to the synthesis of collagen in the newly formed bone [23, 26, 35, 37], the release of the enzyme into the blood flow coincides to the osteoblast activity and the formation of the organic part of the bone matrix and precedes the collagen mineralization which is evidenced on radiography. It is probable that, in the present study, the genesis of the bone callus was too weak for inducing massive release of bone ALP enzyme into the blood with the 2 osteosynthesis techniques and particularly when plate osteosynthesis was performed. In this case, the notably low bone ALP activities were associated with low bone formation scores obtained from radiographs.

Osteocalcin is another biochemical marker, thought to play a role in bone formation. The data about its dynamic after fractures, osteotomies and some methods of osteosynthesis are conflicting: in sheep [34] and dogs [35], OC concentrations were lowered up to the 9th and the 6th week respectively, after tibia osteotomies and osteosynthesis with the Ilizarov’s device. However, another study [17] reported significant increases of OC concentrations up to the 7th week. In the opinion of HERRMANN et al. [17], OC is probably an earlier marker of delayed unions in men, because it remains unaltered after the 4th week whereas in normally healing fractures, is reported to increase significantly. In a similar study, NYMAN et al. [29] observed significant elevations of OC concentrations and bone ALP activities on the 6th week in normal and delayed healing fractures, but whereas these markers decrease in normally healing fractures, they persisted elevated in delayed unions, although not significantly. In the present experimental model, the variations of the OC concentrations were similar to those reported by NYMAN et al. [29] and LAMMENS et al. [23], particularly for the IMO group. Early variations of the OC concentrations (within the 1st month) in the PLO group were not significantly enhanced compared to the initial values contrary to those observed in the IMO group. The higher concentrations of this marker would correlate with the higher amount of bone callus formed after IMO because a new bone is also formed in the medullar canal triggered by the fixation devices. However, DUCY et al. [10] observed an enhanced bone formation in mice with low OC concentrations and presume that OC may negatively control osteogenesis probably by osteopontin inhibition [10, 19]. Some OC metabolites have been identified and may also be involved in osteogenesis, questioning in this way the role of osteocalcin in bone formation in the dog. Furthermore, some species-related particularities, osteosynthesis technique and bone healing process are also implicated for osteogenesis because the placement of fixation devices without osteotomy or fracture was found to stimulate bone metabolism [5].

As a conclusion, the techniques of osteosynthesis used after diaphyseal osteotomies of the tibia in dogs influenced the bone formation biochemical markers as followed:

1. Serum bone ALP activities did not significantly vary compared to initial values according to time and whatever the osteosynthesis technique, although they tended to be more lowered after plate osteosynthesis and were in agreement with the radiographic bone formation scores.

2. Intramedullar osteosynthesis induced significant increases of OC concentrations within the 1st month after surgery compared to baseline values and to those obtained with plate osteosynthesis. The variations of this marker may be associated to the formation of a greater bone callus.

References

CANINE SERUM BONE MARKERS IN TWO OSTEOSYNTHESIS TECHNIQUES

449


