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Alterations in serum tartrate-resistant acid phosphatase and C-terminal telopeptide of type I collagen in experimental canine osteotomies fixed using 2 different techniques

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Abstract: The aim of the present study was to monitor serum tartrate-resistant acid phosphatase (TRAP) and C-terminal telopeptide fragments of type I collagen (CTX) over time in canine experimental osteotomies fixed using 2 osteosynthesis techniques, to determine the relevance of both markers in monitoring bone healing, and to investigate the influence of the 2 osteosynthesis techniques on their concentrations. Transperiosteal osteotomy of the diaphyses of the right tibia and fibula was performed in 12 dogs. The dogs were then randomly assigned to 1 of 2 groups: group 1 (6 dogs) underwent osteotomy fixed with intramedullary osteosynthesis (IMO) and group 2 (6 dogs) underwent osteotomy fixed with a plate (plate osteosynthesis [PLO]). Craniocaudal radiographs were obtained immediately after osteosynthesis, 2 weeks post-surgery and 1, 2, 3, 4, 5, and 6 months post-surgery. The evaluation of bone resorption was performed visually using a 4-grade scoring system. At the same time points, venous blood was sampled for determination of tartrate-resistant acid phosphatase and the concentration of C-terminal telopeptide fragments of type I collagen.

Radiologically visible bone resorption was characterized by 2 peaks. The 1st peak occurred by the end of the 2nd postoperative week with both osteosynthesis methods ($P < 0.001$). The 2nd peak began by the end of the 4th postoperative month and persisted until the end of the experiment.

Serum TRAP was not a reliable marker of bone resorption during the study period. CTX concentrations increased considerably in both groups by the end of the 1st postoperative month ($P < 0.05$), decreased by the 3rd postoperative month, and increased again by the end of the 5th postoperative month. CTX, therefore, could be used to monitor normal bone healing.

Key words: Dogs, osteotomy, osteosynthesis, serum bone markers

Introduction

Bone metabolism is characterized by bone formation and bone resorption. Modeling and remodeling are 2 key features of bone healing (1). In modeling, bone formation and resorption are independent with regard to space and time, whereas remodeling is effectuated by alternating bone removal and transposition from specified places via the coordinated activity of osteoclasts and osteoblasts (2).

Bone remodeling alters the internal bone architecture and is dependent on such conditions as loading, micro-trauma, nutritive and hormonal

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effects, post-fracture conditions, and inflammation (3). In healthy mature individuals (humans and animals) the processes are balanced and there is no change in bone mass.

Complete assessment of the cellular basis of bone pathology requires methods and techniques for separately monitoring bone formation and bone resorption. A good approach is the utilization of blood and urine biochemical bone markers. Ideally, bone markers are specific for bone cell activity in the clinical setting and should not be influenced by alterations in non-osseous metabolism.

Degradation fragments of collagen type I from the N- and C-regions of the molecule (known as NTX and CTX) can be quantified in serum and urine. A variant of CTX (ICTP) can be measured in blood serum (3) and is not dependent on renal clearance rates (4). NTX and CTX urinary levels are dependent on renal clearance and thus need to be corrected based on urinary creatinine excretion. These 3 markers can be used in animals.

Circadian rhythm was reported to be a bone marker in rats (5), mice (6), rabbits, and horses (7). Osteocalcin and ICTP activity in dogs is higher in the morning than in the afternoon. In both dogs and humans the variations are lower in serum than in urine samples (8,9).

The current veterinary medical literature includes reports of bone markers being used to detect differences in bone formation and bone resorption in horses (10,11), dogs (12), and cats (13,14). Lammens et al. (15) and Theyse et al. (16) followed up distraction osteogenesis of the tibia using these markers and growth factors; however, both teams obtained conflicting results.

The bone markers in dogs were studied on a rather theoretical level with regard to age- and breed-related differences (9,12,17). A few reports exist on their application in clinical practice with regard to osteomyelitis (18), radial ostectomy (19), and monitoring normal healing of long bone fractures (20).

The aim of the present study was to monitor serum levels of tartrate-resistant acid phosphatase (TRAP) and C-terminal telopeptide fragments of type I collagen (CTX) over time in experimental osteotomies in dogs fixed using 2 osteosynthesis techniques, to determine the relevance of both bone markers in monitoring bone healing and the influence of each osteosynthesis technique on their concentrations as bone resorption markers.

Materials and methods

Animals

The study included 12 mixed-breed male dogs aged 2-5 years and weighing 12-20 kg. The animals were obtained from a licensed kennel and at the end of the study were returned. The dogs were housed in individual cages, fed a commercial dry food for adult dogs (Jambo-dog, Gallisman-94 S.A., Bulgaria), and received water ad libitum. Prior to the study all dogs were treated for ecto- and endoparasites. The study was approved by the Trakia University Committee on Animal Experimentation, Stara Zagora, Bulgaria, and was performed according to the Animal Welfare Act No. 25/10.06.05 and the Veterinary and Medical Activities Law.

Operative protocol

The anesthetic protocol included premedication with atropine sulphate (0.02 mg/kg s.c.) (Sopharma, Bulgaria) and acepromazine maleate (0.05 mg/kg i.m.) (Neurotranq, Alfasan, Woerden, Holland), induction with 2.5% thiopentone sodium (6 mg/kg i.v.) (Thiopental, Biochemie GmbH, Kudl, Austria), intubation, and maintenance of general anesthesia with 2.5 vol.% halothane (Narcotan, Spofa, Czech Republic).

Following aseptic preparation and medial treatment, transperiosteal osteotomy of the diaphyses of the right tibia and fibula was performed in all the dogs. Then the dogs were randomly assigned to 1 of 2 groups and treated as follows: group 1 (6 dogs): osteotomies were fixed with normograde insertion of a Kuntscher nail in the medullar canal (intramedullary osteosynthesis, IMO); group 2 (6 dogs): osteotomies were fixed with a plate and 6 cortical screws (3 in the distal and 3 in the proximal bone fragment) (plate osteosynthesis [PLO]).

Soft tissues were sutured routinely and protective bandages were placed on the crural area. Postoperatively the animals were treated for pain with butorphanol tartrate (0.2 mg/kg, s.c.) (Torbutrol, Fort

Dodge, USA). An intramuscular antibiotic combination of lincomycin (50 mg/mL) and spectinomycin (100 mg/mL) (Linco-Spectin, Pharmacia N.V./S.A., Puurs, Belgium) was administered at 1 mL/5 kg for 4 days following surgery.

Clinical observations

After surgery the following clinical parameters were monitored: rectal body temperature, heart and respiratory rates, appetite, and state of the operated limb.

Radiography

Craniocaudal radiographs of the operated limbs were obtained immediately after osteosynthesis, at postoperative week 2, and at postoperative months 1, 2, 3, 4, 5, and 6. They were examined to determine bone resorption and remodeling events by means of a scoring system that allowed for conversion of qualitative evaluation into quantitative parameters (Table 1). Scores at each time point were compared to the scores obtained immediately after surgery. All radiographs were interpreted independently by 2 radiologists blinded to the post-osteosynthesis period. Mean scores of both observers were calculated.

Blood serum bone markers assays

Venous blood samples were obtained prior to surgery, and at postoperative week 2 and postoperative months 1, 2, 3, 4, 5, and 6. Blood samples were collected between 0730 and 0800 to eliminate any circadian effects.

Tartrate-resistant acid phosphatase (TRAP) was assayed immediately after separation of sera using a colorimetric kit (Biolabo SA, France). C-terminal telopeptide fragments of collagen type I were quantified using a Serum CrossLaps® ELISA kit (Nordic Bioscience, Denmark), which measures degradation products of C-telopeptide fragments of type I collagen in blood with a precision of 0.02 ng/mL. Each sample was analyzed in duplicate and the mean of both measurements was obtained.

Statistical analysis

Data were statistically analyzed using the non-parametric Friedman's test for 2-way repeated measures. In the case of significant P values the non-parametric Tukey's HSD test was then applied. Differences between the groups at each time point were tested via the Mann-Whitney U test.

Results

Clinical results

During the postoperative period the dogs did not exhibit any deviation in the general clinical parameters. Rectal body temperature, and respiratory and heart rates were within reference ranges, and the dogs' appetites were good.

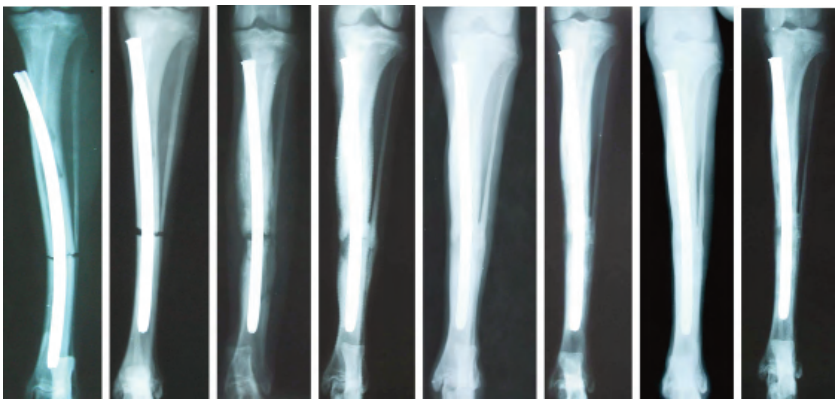
In the crural region of the operated limbs there was moderate, slightly painful warm swelling that disappeared within 4-6 days. During the first post-osteosynthesis day the dogs were predominantly lying down, then manifested grade 2-3 weight-bearing lameness (lasting for up to 1 week in PLO dogs and up to 2-3 weeks in IMO dogs). After that period full weight bearing was resumed.

Radiological studies

The results of radiological examinations are shown in Figures 1 and 2, and Table 2. Radiologically visible bone resorption was characterized by 2 peaks in both

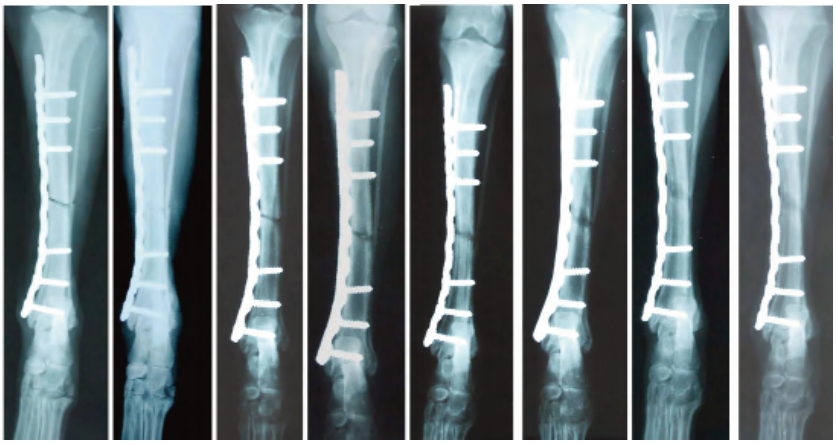
Table 1. Scoring system for bone resorption evaluation.

Description	Score
No signs of bone resorption	0
Decreased bone density near the site of osteotomy	1
Decreased bone density near and far from the site of osteotomy	2
Bone callus remodeling	3



A B C D E F G H

Figure 1. Craniocaudal radiographs of the tibia of a dog from the IMO group immediately after the osteosynthesis (A) and at postoperative week 2 (B) and months 1, 2, 3, 4, 5, and 6 (from C to H, respectively).



A B C D E F G H

Figure 2. Craniocaudal radiographs of the tibia of a dog from the PLO group immediately after the osteosynthesis (A) and at postoperative week 2 (B) and months 1, 2, 3, 4, 5, and 6 (from C to H, respectively).

Table 2. Mean radiological scores of bone resorption and remodeling in dogs after osteotomy fixed by 2 osteosynthesis techniques.

Parameter	Osteo-synthesis	Time after surgery						
		2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
Bone resorption and remodeling	IMO (n = 6)	1.00 ± 0.00***	1.16 ± 0.30**	1.16 ± 0.40	0.83 ± 0.40	3.00 ± 0.00***	3.00 ± 0.00***	3.00 ± 0.00***
	PLO (n = 6)	1.33 ± 0.21***	1.50 ± 0.34**	0.50 ± 0.22	0.83 ± 054	2.33 ± 0.42***	3.00 ± 0.00**	3.00 ± 0.00***

IMO – intramedullary osteosynthesis; PLO – plate osteosynthesis; *P < 0.05; ** P < 0.01; *** P < 0.001 vs. the preceding period.

groups. The first occurred by the end of the second postoperative week in both groups ($P < 0.001$). The second peak was observed by the end of the fourth postoperative month and persisted until the end of the study.

Dynamics of bone markers

Changes in the blood concentration of the studied biochemical markers are presented in Table 3. Tartrate-resistant acid phosphatase (TRAP) did not change significantly during the study in either group. There were, however, alterations in serum C-terminal telopeptide fragments of type I collagen (CTX). CTX increased considerably in the IMO group by the end of the 1st postoperative month (from 1.05 ± 0.11 ng/mL at baseline to 1.85 ± 0.13 ng/mL [$P < 0.05$]), decreased by the 3rd postoperative month, and increased again by the end of the 5th postoperative month. In the PLO group the values also increased by the end of the 1st postoperative month (from 1.05 ± 0.11 ng/mL to 1.86 ± 0.10 ng/mL [$P < 0.05$]), but returned to baseline, and then increased significantly again by the end of the 6th postoperative month (1.69 ± 0.19 ng/mL [$P < 0.05$]).

Discussion

Type I collagen is composed of collagen molecules bound by their amino- and carboxy-terminal groups. The bridges between them are made of pyridine, deoxypyridinoline, and pyridinoline. Degradation of type I collagen during bone resorption is performed by an osteoclastic protease (acid phosphatase), and telopeptides and disrupted bonds are released as end products.

Osteogenesis during bone fracture or experimental long bone osteotomy healing is performed via endochondral ossification, unlike distraction osteogenesis in which the process is mediated via intramembranous ossification (21). This means that the first cases involve stages of fibrous, cartilaginous, and bone callus that gradually evolve from one into the other. Duration of the stages varies by species, the type and site of the fracture, fracture stability after osteosynthesis, status of the surrounding soft tissues, etc. The bone callus becomes radiologically visible only when mineralization (ossification) islets begin to appear (22). In the present study all conditions were the same in both groups, with the exception of the osteosynthesis technique used. It is known that plate osteosynthesis provides better stability than intramedullary osteosynthesis; therefore, more extensive bone reaction in intramedullary fixed osteotomies could be expected. This was also confirmed in the present study. There was a statistically significant difference between the groups in bone formation rates by the end of the 1st month (higher in the IMO group) and the 3rd month (higher in the PLO group) (23). The callus was, however,

Table 3. Changes in blood concentrations of studied biochemical resorption markers in dogs after osteotomy fixed by 2 osteosynthesis techniques.

Parameter	Prior to surgery	Osteo-synthesis	Time after surgery						
			2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
Tartrate-resistant acid phosphatase, U/L	2.60 ± 0.53 (n=12)	IMO (n=6)	3.48 ± 0.45	2.11 ± 0.27	2.31 ± 0.43	1.94 ± 0.44	3.59 ± 0.96	2.92 ± 0.40	2.58 ± 0.35
		PLO (n=6)	1.72 ± 0.27	2.02 ± 0.34	3.00 ± 0.53	3.45 ± 0.69	3.95 ± 0.68	3.14 ± 0.45	3.71 ± 0.68
CTX, ng/mL	1.05 ± 0.11 (n=12)	IMO (n=6)	1.33 ± 0.09	1.85 ± 0.13*	1.74 ± 0.14*	1.47 ± 0.26	1.50 ± 0.15*	1.52 ± 0.20*	1.21 ± 0.14
		PLO (n=6)	1.28 ± 0.11	1.86 ± 0.10*	1.51 ± 0.23	1.26 ± 0.11	1.36 ± 0.08	1.38 ± 0.20	1.69 ± 0.19*

IMO – intramedullary osteosynthesis; PLO – plate osteosynthesis; *$P < 0.05$ vs. baseline (prior to surgery).

visualized as early as the end of the 2nd postoperative week, indicating the beginning of the mineralization of newly formed bone tissue.

By the end of the first month of fracture healing bone resorption was the result of inflammation, whereas by the end of the study it was directed towards remodeling and smoothing of bone callus (24). In the present study bone resorption was clearer by the end of the study when the callus was undergoing remodeling, but the osteosynthesis technique did not significantly affect this process.

In the present study we monitored 2 markers of bone resorption: tartrate-resistant acid phosphatase (TRAP) and the carboxy-terminal telopeptide fragments of collagen type I (CTX). The first marker did not exhibit significant differences, although there were radiographic signs of bone resorption, both at the beginning and by the end of the study. This might have been due to the fact that we monitored total TRAP and that mammalian serum is known to contain 2 forms (5a and 5b); however, only 5b-TRAP was released by osteoclasts and was a marker of their activity. The 5a form is of non-osseous origin (25). Our results confirm the opinion that total serum TRAP is not a specific marker of bone resorption. The other marker we used is a metabolite(s) of collagen type I degradation. Theyse et al. (16) reported that even the insertion of a fixation device without osteotomy provoked a bone reaction and resulted in early (by the 2nd week) elevation of carboxy-terminal telopeptide of collagen type I (ICTP) concentrations.

In our previous studies on intramedullary fixed canine femoral osteotomy, we observed an increase in serum ICTP levels by the 1st and the 2nd postoperative week, and then a subsequent decrease (21). In the same experimental design, but complicated by experimental osteomyelitis, high ICTP levels persisted until the end of the 4th postoperative week (19). The present study confirms that intramedullary nailing is more invasive to the bone. CTX concentrations in the present study increased significantly, as compared to baseline, in both groups by the 1st postoperative month; in the IMO group by the 2nd, 4th, and 5th months, and in the PLO group by the 6th month, and the difference between the groups was not statistically significant. It could be concluded that CTX was reliable for monitoring normal bone healing because alterations in its concentration corresponded with resorption of bone ends immediately after trauma (osteotomy or fracture), and then decreased and increased again at a later stage when newly formed bone callus underwent remodeling. This event occurred at different time points when different osteosynthesis techniques were used.

In conclusion, total serum tartrate-resistant acid phosphatase was not a reliable marker of bone resorption during the study period. Serum levels of C-terminal telopeptide fragments of type I collagen (CTX), however, could be used to monitor normal bone healing.

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