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The efficacy of the bone markers osteocalcin and the carboxyterminal cross-linked telopeptide of type-I collagen in evaluating osteogenesis in a canine crural lengthening model

Lars F.H. Theyse [a,*], Jan A. Mol [a], George Voorhout [b], Maarten Terlou [c], Herman A.W. Hazewinkel [a]

[a] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[b] Division of Diagnostic Imaging, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
[c] Department of Image Processing and Design, Faculty of Biology, Utrecht University, Utrecht, The Netherlands

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Abstract

The aim of the present study was to determine the efficacy of the bone markers osteocalcin (OC) and carboxyterminal cross-linked telopeptide of type-I collagen (ICTP) in evaluating new bone formation in the dog, using commercially available immunoassay kits. Dogs were randomly divided into three groups and a circular external skeletal fixation system (CESF) was mounted on the tibia. In the first group a distraction osteogenesis procedure of the crus was performed. The second group received an osteotomy without crural lengthening, whereas the third group served as a sham-operated control. Bone formation was assessed using densitometric image analysis of crural radiographs. Despite significant differences in the amount of newly formed bone, this finding was not reflected in the plasma levels of OC and ICTP. In conclusion, OC and ICTP were not efficacious as markers of bone formation and resorption during osteogenesis in this canine model.

Keywords: Bone markers; Osteocalcin; ICTP; Distraction osteogenesis; Dog

1. Introduction

Bone metabolism can be monitored non-invasively using markers of bone formation and resorption. A variety of commercially available bone marker assays has been developed. Although designed initially for use in humans, several of these assays are also validated to monitor bone metabolism in laboratory animals, dogs and horses (Breur et al., 2004; Carstanjen et al., 2003, 2004; Ladlow et al., 2002).

Bone markers can be subdivided into enzymatic markers and metabolic products of bone formation and resorption. The enzymatic markers include bone-specific alkaline phosphatase (BAP), an osteoblast related marker of bone formation, and tartrate-resistant acid phosphatase (TRAP), an osteoclast-related marker of bone resorption (de Vernejoul, 1998; Garnero and Delmas, 1997; Halleen et al., 2000). Serum or plasma markers of metabolic products of bone metabolism in companion animals include osteocalcin (OC), the carboxyterminal propeptide of type-I procollagen (PICP), the aminoterminal propeptide of type-I procollagen (PINP), the cross-linked carboxyterminal telopeptide of type-I collagen (ICTP), and the C-terminal

* Corresponding author. Tel.: +31 30 2539411; fax: +31 30 2518126.
E-mail address: l.f.h.theyse@vet.uu.nl (L.F.H. Theyse).

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cross-linked telopeptide of type-I collagen (CTX). OC is an osteoblast-related marker of bone formation, but its precise function is unknown (Ducy et al., 1996; Szulc et al., 2000). PICP and PINP are markers of type-I collagen synthesis and hence bone formation (Cortet et al., 2001; Polak-Jonkisz et al., 2003).

ICTP and CTX are markers of type-I collagen breakdown and hence bone resorption (Loviselli et al., 1997). ICTP is released through the actions of matrix metalloproteinases and is therefore also known as CTX-MMP. CTX is released through the actions of cysteine proteinases, including cathepsin K (Garnero et al., 2003).

Bone markers have been used successfully in humans to monitor metabolic bone disease, including chronic renal disease, osteoporosis, hyperthyroidism, rheumatoid arthritis and growth hormone deficiency (Al Awadhi et al., 1999; Garnero, 2000; Loviselli et al., 1997; Polak-Jonkisz et al., 2003; Sartorio et al., 2001; Sneppen et al., 2002). In the dog, reports concerning bone markers to monitor bone pathology or osteogenesis are limited to OC, ICTP, and BAP (Ehrhart et al., 1998; Fink et al., 2002; Lammens et al., 1998; Philipov et al., 1995).

In both man and dog, distraction osteogenesis is used to treat a variety of skeletal conditions, including bone length deficits, growth deformities, bone loss after trauma or radical resection, and cosmetic craniofacial surgery (Aronson, 1994a; Maffulli et al., 1996; Sabharwal et al., 2000; Stanitski et al., 1996). This principle allows the production of large quantities of new bone from the osteotomy sites under controlled mechanical distraction. In the dog, there is extensive experience with distraction osteogenesis, both clinically and as an animal model, although insights in the fundamentals of this type of bone formation are still insufficient. Both in research and clinical settings evaluation of the distraction bone regenerate is mainly performed by plain radiography, sonography, and the histomorphometric assessment of bone biopsies (Aronson et al., 1989, 1990; Delloye et al., 1990; Garnero, 2000; Orbay et al., 1992; Richards et al., 1998; Rowe et al., 1998). Application of advanced imaging techniques, including magnetic resonance imaging and computed tomography, is often impeded by the presence of the external skeletal fixation system, used for the distraction procedure.

Bone markers could provide us with another means to evaluate bone formation and resorption during both clinical and experimental lengthening procedures and other types of bone healing. The objective of the present study was to determine using a canine crural lengthening model whether the bone markers OC and ICTP could be of value in evaluating osteogenesis following either a distraction procedure or bone healing after an osteotomy.

2. Materials and methods

2.1. Animals

The Utrecht University Ethical Committee for Animal Care and Use approved all procedures in this study. The data presented in this manuscript were collected during a study, assess the fundamentals of distraction osteogenesis, including the local expression of bone growth factors and systemic induction of growth factors.

Twelve mature Labrador retriever dogs were used, with a mean age of 20 months (range 12–31 months), and a mean body weight of 27 kg (range 21–32 kg). The animals were allocated to three groups. Dogs were fed a balanced commercial dog food twice a day at a set time (i.e., 0900 and 1800 h) and water was available ad libitum.

2.2. Surgery and distraction

Circular external skeletal fixation (CESF) systems were prepared prior to surgery and steam sterilized (Imex Veterinary Inc.). All frames were identical and consisted of two proximal and two distal full rings with a 100 mm diameter, connected by three treaded rods with a 1 mm pitch.

The dogs received medetomidine (Domitor, Pfizer Animal Health B.V.) as a pre-anaesthetic sedative and anaesthesia was induced IV with propofol (Rapinovet, Schering-Plough Animal Health N.V.). General inhalation anaesthesia was accomplished with nitrous oxide, oxygen, and isoflurane. Amoxicillin with clavulanic acid (Augmentin, SmithKline Beecham Farma B.V.) was administered IV (20 mg/kg b.w.) prior to surgery.

The right hind limb was prepared in a standard sterile fashion. The CESF was placed on the right tibia with the distal ring 3 cm proximal of the tarsocrural joint. The most proximal and distal rings were mounted on the tibia, using two 1.5 mm diameter transosseus wires each. The two inner rings were both mounted to the tibia with one 1.5 mm diameter transosseus wire. All wires were tensioned, using a dynamometric wire tensioner (Hofmann SaS), to an equivalent of 60 kg. An anteromedial approach to the tibia and fibula was used. The periosteum of the tibia was preserved by making a longitudinal incision of 3 cm and by carefully elevating the periosteum circumferentially from the bone. In the dogs of the distraction ($n = 4$) and osteotomy ($n = 4$) groups the tibia and fibula were osteotomized in the diaphysis, while protecting the periosteum, at two-thirds of the tibial length from the proximal, using an oscillating saw and ample lavage for cooling. In the dogs of the control group ($n = 4$), after placement of the CES, the periosteum of the tibia was also elevated. In all three groups, the periosteum was closed with an absorbable suture