The effect of carprofen on selected markers of bone metabolism in dogs with chronic Osteoarthritis*

A. Liesegang[1], S. Limacher[1], A. Sobek[2]

[1]Institute of Animal Nutrition, University of Zurich, Zurich, Switzerland, [2]Pfizer GmbH, Karlsruhe, Germany

Summary

The purpose of this study was to investigate the effect of the nonsteroidal anti-inflammatory drug carprofen on bone turnover and to monitor the progress of chronic osteoarthritic dogs by measuring different bone markers and radiographic evaluation of the corresponding joints. For this purpose 20 dogs of different ages and weight were devided into 2 groups. Ten dogs were assigned to Group R, treated with carprofen, and ten dogs to Group C, which had no treatment. Radiographs of the affected joints were reviewed initially and six months later at the end of the experiment. Blood was taken 8 times from each dog. Four bone markers (Osteocalcin (OC), bone-specific alkaline phosphatase (bAP), carboxyterminal telopeptide of type I collagen (ICTP), serum CrossLaps (CTX) as well as 1,25-$(OH)_2$-Vitamin D and parathyroid hormone (PTH) were monitored for 6 months. No significant group effects on bone markers were notied. In Group R a decrease in ICTP concentrations during the first three months and a significant decrease in CTX concentrations in the first two months of the study were observed. The bone formation marker bAP revealed a significant decrease throughout the experiment. Three dogs of Group C and one dog of Group R showed osteoarthritic progression in the radiographs. The significant decrease of CTX indicates that carprofentreatment could have a retarding effect on the progression of osteoarthritis. Radiological findings suggest that carprofen may delay osteophyte formation. The monitoring of focal metabolic processes as in bone of a osteoarthrotic joint is difficult, since the bone mass is very active and metabolic processes may have an influence on the monitoring.

Keywords: dog, osteoarthritis, bone markers

Die Wirkung von Carprofen auf ausgewählte Marker des Knochenstoffwechsels bei Hunden mit chronischer Osteoarthritis

Ziel dieser Arbeit war es, den Effekt des nicht-steroidalen Entzündungshemmers Carprofen auf den Knochenstoffwechsel und das Fortschreiten von chronischer Gelenksarthrose bei Hunden durch Messung verschiedener Knochenmarker und radiologischer Auswertung der betroffenen Gelenke zu untersuchen. Zu diesem Zweck wurden 20 Hunde unterschiedlichen Alters und Gewichts in zwei Gruppen eingeteilt: 10 Hunde in die Rimadyl®-Gruppe (R) und 10 Hunde in die unbehandelte Kontroll-Gruppe (C). Die osteoarthrotisch veränderten Gelenke wurden am Anfang und am Ende des Versuchs geröntgt. Von jedem Hund wurden insgesamt acht Blutproben entnommen. In jeder Blutprobe wurden die Knochenaufbaumarker Osteocalcin (OC) und knochenspezifische alkalische Phosphatase, die Knochenabbaumarker carboxyterminales Telopeptid von Typ I Kollagen (ICTP) und Serum CrossLaps (CTX) sowie 1,25-$(OH)_2$-Vitamin D und Parathormon (PTH) untersucht. Es wurde kein signifikanter Gruppeneffekt weder für die Knochenaufbau- noch für die Knochenabbaumarker gefunden. Die ICTP-Konzentrationen der Gruppe R zeigten einen deutlichen Abfall in den ersten drei Monaten des Versuchs stiegen aber bis zum Ende des Versuchs wieder an. Die CTX-Konzentrationen der Gruppe R zeigten einen signifikanten Abfall in den ersten beiden Monaten des Versuchs. Der Knochenaufbaumarker bAP zeigte einen signifikanten Abfall vom Anfang bis zum Ende des Versuchs. In der Gruppe R zeigte nur 1 Hund eine radiologische Verschlechterung des betroffenen Gelenks während in der Kontroll-Gruppe 3 Hunde von einer Verschlechterung betroffen waren. Die Resultate dieser Studie zeigen, dass Carprofen den Knochenstoffwechsel von Hunden mit chronischer Gelenksarthrose möglicherweise bremsen könnten. Das Monitoring eines fokalen metabolischen Prozesses wie es im Knochen eines osteoarthrotischen Gelenks stattfindet ist jedoch schwierig, da die ganze Knochenmasse sehr aktiv ist und andere metabolische Prozesse das Monitoring beeinflussen.

Schlüsselwörter: Hund, Osteoarthritis, Knochenmarker

**This work is part of the Dissertation by Sandra Limacher (2005) submitted to the University of Zurich*

©2007 by Verlag Hans Huber, Hogrefe AG, Bern DOI 10.1024/0036-7281.149.08.353

Introduction

Canine osteoarthritis (OA) is a common disease and might be caused by joint trauma, inflammation, congenital and developmental abnormalities (e.g. hip dysplasia) or by metabolic, endocrine, neuro¬pathic, neoplastic and iatrogenic disorders (McLaughlin, 2000). Structural changes of affected joints include erosions of articular cartilage, formation of osteophytes and at the clinical stage of the disease, a variable degree of synovial inflammation (Johnston, 1997). In the early stages of the disease excessive bone resorption predominates causing decreased subchondral bone plate thickness, followed by excessive bone formation leading to sclerosis and increased subchondral bone plate thickness (Brandt, 1991, 1994; Dedrick et al., 1993, 1997; Pelletier et al., 2000). Osteoarthritis can be treated surgically or medically with anti-inflammatory drugs. Common medication includes the administration of anti-inflammatory drugs and/or chondroprotective substances and should be accompanied by weight reduction, exercise control, physical therapy and proper nutrition (Manley et al., 1995; Todhunter et al., 1995; Johnston and Budsberg, 1997; Johnston and Fox, 1997; McLaughlin, 2000).

Bone remodelling requires two counteracting processes, bone formation and bone resorption, in which osteoblasts are responsible for bone formation and osteoclasts for bone resorption. Under normal physiological conditions, these two processes are coupled and in balance. Monitoring of bone metabolism is possible with different proteins or enzymes released during bone formation or resorption. Indices of osteoblast activity during bone formation and therefore bone formation markers are osteocalcin (OC) and bone-specific alkaline phosphatase (bAP) whereas markers of bone resorption released into the blood during bone collagen digestion are different epitopes of the carboxyterminal telopeptide of type I collagen (crosslinked carboxyterminal teleopeptide of type I collagen (ICTP), and crosslaps (CTX)) (Epstein, 1988, Liesegang, 2000). OC is a small protein which is synthezised by mature osteoblasts. The protein is characterized by the presence of three gamma-carboxyglutamic acids. These residues are responsible for facilitating the binding of the protein to hydroxyapatite and maintaining its secondary structure. BAP catalyses the hydrolysis of phosphate ester at the osteoblast cell surface to provide a high phosphate concentration for the bone mineralization process as part of the osteoblast cell role in bone remodelling. (Liesegang et al., 2005). The type I collagen represents more than 90% of the organic matrix of bone. The degradation products of mature collagen type I CTX and ICTP recognize different domains of the C-terminus of type I collagen. These markers have been used in several studies in several animal species (Liesegang et al., 2002, 2003). It has been shown that ICTP and CTX are collagen breakdown products derived from different enzymatic processes occurring during bone resorption. CTX is derived directly from cathepsin K-mediated type I collagen turnover, whereas ICTP is mainly derived from matrix metalloproteinases (MMP-9) digestion of the type I collagen (Sassi et al., 2000). The concentration of ICTP and CTX in serum correlates well with bone mineral density and bone mineral content in pigs, goats and sheep (Liesegang et al., 2002, 2006).

Philipov et al. (1995) demonstrated that the concentrations of the bone markers bAP, OC and ICTP were significantly elevated in dogs with experimentally induced osteomyelitis. Arican et al. (1996) assessed the bone formation marker OC in serum of dogs with naturally occurring osteoarthritis caused by osteochondrosis or by rupture of the cranial cruciate ligament. Compared to healthy dogs significantly higher serum OC levels were demonstrated in 60% of the dogs with osteoarthritis caused by osteochondrosis and in 44% of the dogs with rupture of the cranial cruciate ligament. These increases might be associated with osteophyte formation as Campion et al. (1989) have already demonstrated. Pelletier et al. (2000) examined the effect of the nonsteroidal anti-inflammatory drug, carprofen, on the structure and metabolism of articular cartilage and subchondral bone plate in dogs with experimentally induced osteoarthritis. They observed that treatment with carprofen under therapeutic conditions reduced the progression of early structural changes in articular cartilage and delayed or prevented the abnormal metabolism of subchondral osteoblasts (Pelletier et al., 2000). The metabolism of the subchondral bone plate was assessed by preparing cultures of osteoblasts of that region and measuring the activity of bAP and concentrations of OC. Interestingly, under carprofen treatment activities of bAP and concentrations of OC reverted to normal levels. The purpose of this study was to investigate bone turnover of privately-owned osteoarthritic dogs treated with carprofen for six months by measuring different bone markers and compare those dogs to untreated ones. The hypothesis was, that treated dogs would have a lower bone turnover and with that the development of osteoarthritis could be slowed down.

Animals, Material and Methods

Twenty adult privately-owned dogs of different breeds, 6.4 ± 1.7 years of age (mean ± standard deviation (SD)), weighing 28.4 ± 10.3 kg (mean ± SD), were used. All dogs suffered from osteoarthritis in different joints. The animals were not treated before this trial. The clinical diagnosis was confirmed radiographically (Tab 1). Dogs were assigned (according to

Table 1: Distribution of breed and affected joints in 20 dogs with osteoarthritis.

	Control group C; Rimadyl© group R			
Group	breed	age in years	weight in kg	localization of OA
C	Berger des Pyrenées	8	10	Hip (both sides)
C	German Shephard	6	27	Hip (both sides)
C	Labrador Retriever	6	38	Hip (both sides)
C	Flat-coated Retriever	7	30	Ellbow (both sides)
C	Golden Retriever	6	26	Hip (both sides)
C	Golden Retriever	6	32	Hip (both sides)
C	Flat-coated Retriever	5	30	Hip (both sides)
C	Rottweiler	5	30	Hip (both sides)
C	Entlebucher Sennenhund	5	20	Hip (both sides)
C	Mix breed (German shep.Xcollie)	9	20	Tarsal joints
R	Pumi	6	10	Hip (both sides)
R	German Shephard	5	28	Hip (both sides)
R	Labrador Retriever	5	42	Hip (both sides)
R	German Shephard	9	32	Ellbow (both sides)
R	German Shephard	6	39	Hip (both sides)
R	German Shephard	6	36	Hip (both sides)
R	German Shephard	5	35	Hip (both sides)
R	German Shephard	5	40	Hip (both sides)
R	Boxer	6	36	Hip (both sides)
R	Dachshound	11	7	left shoulder
Mean		6.4	28.4	
Standard Deviation			1.7	10.3

weight and form of osteoarthritis) in two groups: Group C (Control-Group) consisted of 10 dogs receiving no treatment while Group R (Rimadyl®-Group) consisted of 10 dogs and was treated with the nonsteroidal anti-inflammatory drug Carprofen (2 mg/kg body weight twice daily for six months). The dogs lived at their owner's home during the whole trial. The owners were instructed to administer the tablets (Rimadyl® 50mg; Pfizer GmbH, Karlsruhe, Germany) with food. No other supplements or nutraceuticals were allowed. In addition owners were asked to report possible adverse reactions associated with the use of this drug such as vomiting, diarrhoea, polyuria and polydipsia.

Radiographs of the affected joints were made at the beginning of the trial and six months later. The radiographs were examined for lesions such as osteophyte formation and sclerosis of the subchondral bone plate by a trained observer who was blinded to the group assignment and clinical findings.

Blood was collected from the cephalic vein (10 ml) using 10 ml single-use syringe (Omnifix®, B. Braun, Melsungen, Germany) and single-use 22G needles (Terumo Medical Corporation, Somerset New Jersey, USA). Harvested blood was collected in 10 ml serum tubes with clotting activators (Sarstedt, Sevelen SG, Switzerland). Blood samples were always taken in the morning (8-10 am) before starting the study, after 14 days, after one month and then monthly until the end of the trial (8 samples). Blood was centrifuged (1500 × g for 10 min, 20° C) and serum was harvested. Serum was distributed into three tubes. Two tubes were stored at –20° C until the analyses were performed. Calcium (Ca) and phosphorus (P) were determined by colorimetry using an autoanalyser (COBAS MIRA®; Roche-Autoanalyzer, Basle, Switzerland) and commercial kits. Cross-linked carboxyterminal telopeptide of type I collagen (ICTP) (Orion Diagnostics, Oulunsalu, Finland) and Osteocalcin (OC) (Biomedical Technologies Inc., Stroughton, Minnesota, USA) concentrations were quantified with a radioimmunoassay. Serum CTX and bone-specific alkaline phosphatase (bAP) (Serum CrossLaps®, Nordic Bioscience Diagnostics A/S, Herlev, Denmark; METRA®BAP Eia kit, Quidel, San Diego, USA) were measured using an ELISA with an ELISA plate reader (Multiskan RC, Thermo Labsystem, Helsinki Finland). All tests were previously validated for dogs by the companies (Allen et al. 1998; Liesegang et al. 1999 A and B).

All data are presented as mean ± standard error (SE). A multivariate ANOVA for repeated measures with group as cofactor was performed. Furthermore the group effect at different sample times was tested with the Mann Whitney U-test for independent samples when the results of the ANOVA were significant. Changes of concentrations within time were examined with the two-tailed Wilcoxon's sign rank test for paired comparisons using SYSTAT®l for windows (SYSTAT,Version 7.0; SPSS Inc., Chicago, USA). Differences were considered significant at a value of $p \leq 0.05$.

Results

No clinical signs of adverse reactions associated with Carprofen were observed in the dogs participating in this study.

Mean serum Ca concentrations stayed within reference values (2.3 – 3.5 mmol/l) in both groups during the experimental period (Tab 2). No significant differences between the groups or time of sampling were evident. Also the mean serum P concentrations remained within the reference range (0.8 – 2.4 mmol/l) during the entire investigation and showed no significant difference between group C and R and in the course of time (Tab 2).

Mean serum bAP activities (Fig 1) in group R showed significantly lower activities at some time points and a tendency to lower values at other time points during the whole investigation than activities in group C (Tab 3). A significant difference ($p \leq 0.05$) in the time course in both groups was evident. In group C a significant decrease (p=0.037) in bAP from the beginning (13.8 ± 1.8 IU/L) until week 20 (9.0 ± 1.5 IU/L) and a significant increase (p=0.017) between week 20 and week 24 (12.0 ± 2.0 IU/L) was observed. In group R bAP-activities decreased significantly (p=0.012) from the beginning (11.5 ± 2.7 IU/L) until the end of the trial (6.6 ± 2.0 IU/L). All activities were within the reference range for 3-7 year old dogs of 12.7 ± 6 IU/L (Allen et al., 1998). Mean serum OC (Fig 2) concentrations of the two groups showed no changes during the whole investigation, but were always higher than reference values from the literature (4.9 ± 1.6 ng/ml; Allen et al., 1998; 5.5 ± 3.8 IU/L; Liesegang et al., 1999 A,B). There was neither a significant difference between both groups nor in the time course.

Mean serum ICTP concentrations (Fig 3) of the Carprofen-treated group R decreased during the first 12 weeks (from 10.2 ± 1.7 to 6.4 ± 0.7 µg/l). Then the concentrations increased again until the end of the trial and reached the same concentration level as group C (8.1± 1.1 µg/l; reference 5.5± 1.0 µg/l; Liesegang et al., 1998). There was neither a significant difference between groups during the whole trial nor in the course of time. Mean serum CTX concentrations (Fig 4) of group R decreased significantly (p=0.038) under Carprofen-treatment in the first eight weeks (from 7561 ± 2665 to 3527 ± 712 pmol/ml) and then stayed at the same level. In group C a significant decrease (p=0.013) was obvious from the beginning until week 20 (from 4554 ± 799 to 2189 ± 406 pmol/ml). Then the concentrations significantly (p=0.01) increased again until the end of the trial (4382 ± 1524 pmol/ml). Reference values only exist for humans which are 2304 ± 1110

Table 3. P-Values for bone-specific alkaline phosphatase in the time course and between the groups.

	p-value (time effect)		
week	Group C	Group R	p-value (group effect)
0			0.214
2	0.646	0.575	0.055
4	0.721	0.515	0.086
8	0.386	0.314	0.018
12	0.285	0.086	0.045
16	0.11	0.093	0.079
20	0.093	0.011	0.06
24	0.017	0.012	0.02

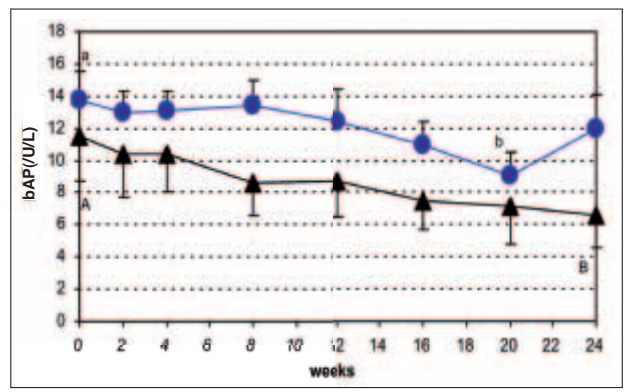

Figure 1. Mean serum activities (± standard error (SE)) of bone-specific alkaline phosphatase (bAP). Values with different letters differ significantly within the group ($p \leq 0.05$) in the time course (Control-Group: a, b; Rimadyl®-Group: A, B). ● = Control-Group. ▲ = Rimadyl®-Group.

Table 2. Ca and P concentrations of group C (control) and R (Rimadyl®).

	Ca (2.3 – 3.5 mmol/l)		P (0.8 – 2.4 mmol/l)	
week	Group C	Group R	Group C	Group R
0	2.53 ± 0.07	2.60 ± 0.05	1.24 ± 0.09	1.24 ± 0.10
2	2.41 ± 0.05	2.56 ± 0.04	1.25 ± 0.09	1.38 ± 0.04
4	2.53 ± 0.08	2.66 ± 0.04	1.36 ± 0.13	1.49 ± 0.10
8	2.55 ± 0.07	2.62 ± 0.04	1.41 ± 0.13	1.47 ± 0.11
12	2.47 ± 0.06	2.54 ± 0.06	1.28 ± 0.14	1.42 ± 0.08
16	2.55 ± 0.05	2.52 ± 0.06	1.28 ± 0.11	1.53 ± 0.09
20	2.48 ± 0.05	2.47 ± 0.05	1.68 ± 0.30	1.46 ± 0.04
24	2.32 ± 0.05	2.42 ± 0.05	1.15 ± 0.09	1.28 ± 0.06

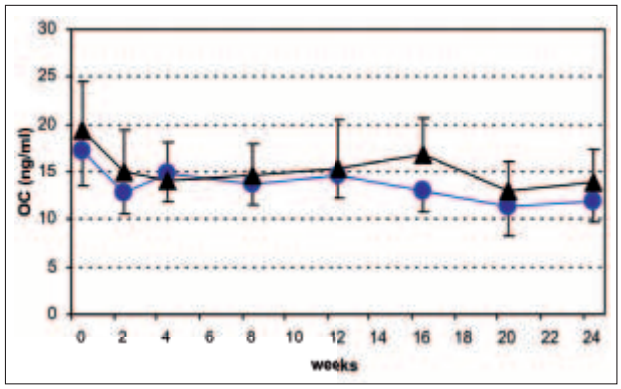

Figure 2. Mean serum concentrations (± SE) of Osteocalcin (OC). ● = Control-Group. ▲ = Rimadyl®-Group.

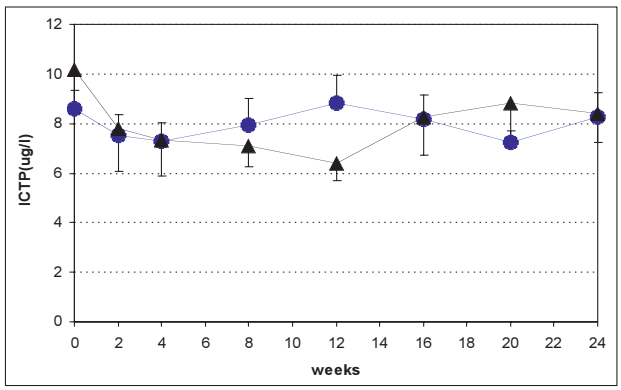

Figure 3. Mean serum concentrations mean (± SE) of carboxyterminal telopeptide of type I collagen (ICTP). ● = Control-Group. ▲ = Rimadyl®-Group. ⋆ indicates significant differenced between the groups at this time point.

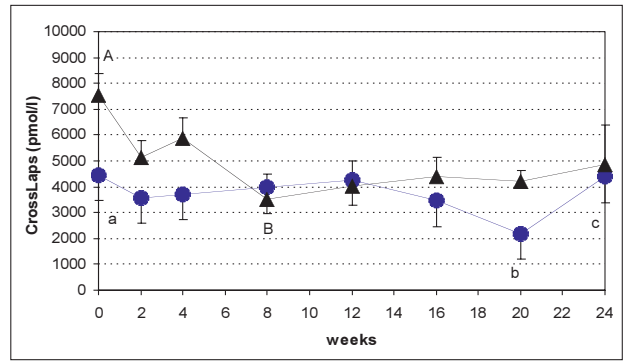

Figure 4. Mean serum concentrations (± SE) of serum CrossLaps. Values with different letters within the group differ significantly ($p \leq 0.05$) with time (Control-Group C: a, b, c; Rimadyl®-Group: A, B). ● = Control-Group. ▲ = Rimadyl®-Group.

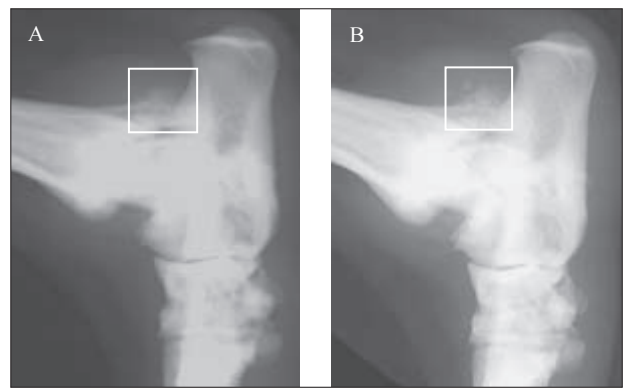

Figure 5. A. Initial radiograph of the left tarsus of a crossbred dog with lameness. There is pronounced soft tissue swelling of the tibiotarsal joint. Osteophytes are noted at the caudal aspect of the tibiotarsal and at the dorsal aspect of the proximal intertarsal joint. B. Radiograph of the left tarsus six months later. The articular/ periarticular mineralisation in the tibiotarsal joint has slightly progressed.

pmol/ml. For dogs unpublished data from our laboratory in healthy dogs confirm, that the reference values are within the reference range published for humans. No significant differences between the two groups were observed during the trial.

In group C three dogs, two with coxarthritis and one with ankle arthritis showed increased proliferation of periarticular osteophytes compared to the initial radiographs. In group R one dog showed progressive mineralisation of the soft tissue around the hip joint compared to the initial radiograph (Fig 5).

Discussion

The subchondral bone plate in osteoarthritic joints of humans is metabolically more active when compared to healthy joints (Hunter et al., 2003 B). Its metabolic activity can be quantified by measuring biochemical bone markers in serum or urine (Hunter et al. 2003 B). In histological examinations, Pelletier et al. (2000) also found a thinner subchondral bone plate full of remodeling units in dogs with early OA compared to the subchondral bone plate of healthy dogs. Bettica et al. (2002) measured high urinary CTX-concentrations in humans with knee OA. Pelletier et al. (2000) showed increased bAP-activities and OC-concentrations in cultured osteoblasts of the subchondral bone plate of dogs with early osteoarthritis. Arican et al. (1996) observed high OC-concentrations in osteoarthritic joints of dogs compared to healthy dogs. High OC-concentrations might be associated with osteophyte formation as Campion et al. (1989) already demonstrated. From these observations, it can be assumed that the bone in osteoarthritic affected joints has an increased turnover (Hunter et al., 2003 B). Therefore, measuring bone markers may be useful to monitor progression of OA, but further results from dogs with osteoarthritis and without osteoarthritis would be very useful to make a proper statement.

Pelletier et al. (2000) observed that activities of bAP and concentrations of OC under Carprofen-treatment reverted to levels compared to those of healthy dogs. They concluded that Carprofen reduced the activity of the osteoblasts of the subchondral bone plate.

In the present study only bAP showed a significant decrease under Carprofen-treatment from the beginning until the end of the trial whereas OC-concentrations remained unchanged during the whole investigation. Whether the decrease under Carpofen-treatment is biologically significant should be further investigated. In addition, bAP activities increased in the control group, which could be due to the fact that some of the dogs in this group were in a phase of acute osteoarthritis, since also CTX concentrations rose again.

Although both OC and bAP are considered specific markers of bone formation, they differ largely with respect to their function and origin (Parthemore et al., 1993; Seibel and Woitge, 1999). OC and bAP reflect different stages of osteoblast differentiation and function (Parthemore et al., 1993). OC is synthesized in late osteoblast differentiation and induced following bAP increase (Parthemore et al., 1993). Thus, an inhibition of anabolic osteoblast activity under carprofen-treatment may not necessarily be accompanied by simultaneously decreased bAP- and OC-release. In the present study OC has been assessed in serum and not in cultured osteoblasts, which may explain the discrepant findings for OC-levels compared to the study of Pelletier et al. (2000). Serum OC concentrations may also decrease under carprofen-treatment but as OC response may be delayed, the present study should have lasted longer than six months to approve this hypothesis. It may be concluded that bAP measurements should be preferred to OC measurement to assess therapeutic effect of Carprofen on periarticular bone turnover because bAP is distributed during early osteoblast differentation and induced before OC (Parthemore et al., 1993). In the present study results of the bone formation marker bAP suggest that Carprofen significantly reduced bone formation from the beginning until the end of the trial. Although, no significant difference between the groups was obvious, a tendancy towards lower bAP activities was observed in group R. Since this study lasted only for 6 months, this may have been too short to show a significant group effect. Another important fact is that this study was carried out under field conditions, meaning no homogenous group was investigated.

Pelletier et al. (2000) showed that after treatment with Carprofen the subchondral bone plate in their histological examinations resembled that of healthy dogs. They assumed that there was reduced catabolic activity of the subchondral bone plate under Carprofen-treatment. In the present study serum CTX decreased significantly in the first two months of the Carprofen-treatment and ICTP concentrations decreased in the first three months of the trial and then increased again. ICTP and CTX reacted similarly to the Carprofen treatment, although CTX seemed to react faster to Carprofen than ICTP. ICTP and serum CTX are both degradation products of type I collagen of the organic bone matrix. While the release of ICTP is mediated by different matrix metalloproteinases (MMP-2, -9, -13, -14), the release of serum CTX is mediated by cathepsin K (Garnero et al., 2003B). The Cox-2 inhibitor Carprofen influences the formation of prostaglandins, especially PGE2, which itself influences the activity of MMPs and also of osteoclasts. If the MMPs activities are reduced, this may lead to decreased ICTP concentrations. Garnero et al. (2003B) observed that ICTP concentrations are strongly influenced by metastatic bone diseases while CTX is the prevailing bone marker in metabolic bone diseases. Remodelling of osteoarthritic subchondral bone plate is considered as a local metabolic bone disease (Hunter et al, 2003 B). Rovetta et al. (2003) observed that serum CTX concentrations were much higher in patients with erosive osteoarthritis of the hands than those of patients with non-erosive osteoarthritis. In erosive osteoarthritis involvement of the subchondral bone plate occurs very early as a consequence of the rapid destruction of articular cartilage. From their study it can be concluded that CTX is a sensitive marker to monitor bone resorption in erosive osteoarthritis. Canine osteoarthritis is generally non erosive, and therefore these results from humans may not be true for dogs. Guillemant et al. (2003) observed that in healthy young men serum CTX concentrations were affected stronger and faster than ICTP-concentrations to acute inhibition of bone resorption induced by repeated ingestion of high calcium doses. They concluded serum CTX to be more sensitive than ICTP during acute changes of bone turnover. In the present study CTX also seems to be more sensitive than ICTP to monitor bone resorption in osteoarthritis as Carprofen significantly reduced CTX in the first two months of the treatment whereas ICTP was not significantly decreased, although also a tendency towards lower concentrations was observed. The different forms of osteoarthritis in the groups may have played a role that bone resorption was not influenced the way it was expected from the literature.

Allen et al. (1998) established reference values for the bone markers OC, AP, bAP and ICTP in healthy Beagle dogs of various ages. Breur et al. (2004) assessed bAP and ICTP in two breeds of vastly different sizes, Irish Wolfhounds and Pomeranians. They found that all concentrations measured were within the reference range reported for Beagle dogs by Allen et al. (1998). Burki (2000) assessed bAP and OC in healthy standard poodles and miniature poodles for two years. At the age of two years, OC-concentrations were similar in standard poodles and miniature poodles. The adult standard poodles showed similar bAP-activities compared to the adult miniature poodle. From these two studies it can be concluded that bAP, OC and ICTP do not depend on the size of the dogs. This finding is important because in this study dogs of different

breeds and sizes were present, although pairs for weight and disease were formed.

The established diagnosis technique for OA is taking radiographs (Garnero and Delmas, 2003). When investigating bone markers in peripheral fluid it is difficult to analyse whether bone markers reflect bone turnover in the osteoarthritic joint of interest or of another affected joint or even reflect generalized skeletal alterations (Garnero et al., 2001). Thus, combining radiological findings with bone marker measurement is useful to assess rate of osteoarthritic progression (Garnero et al., 2001). In the present study the independent radiologist diagnosed mild worsening of osteoarthritis in three dogs of group C and in one dog of group R. The dog of group R showed mineralisation of the soft tissue around the hip joint. The three dogs of group C showed mild increase in osteophyte size.

Formation of osteophytes is associated with increased OC-concentrations (Campion et al., 1989) as osteophytes contain a core of bone and are covered with hyaline or fibrinous cartilage (Bruyère et al., 2003; Garnero et al., 2003 A). Hunter et al. (2003) measured elevated OC-concentrations and bAP-activities in human knee osteoarthritis with osteophytes compared to human knee osteoarthritis without osteophytes. However, in the present study neither an increase of OC-concentrations nor bAP-activities were measured in the dogs with progressive osteophyte formation probably because osteoblast activity in the osteophytes was not high enough to influence serum levels of bone formation markers. Finally, less radiological worsening of OA could be observed under Carprofen-treatment, but it has to be considered that the groups were small and osteoarthritis was not evident in all the dogs only at the hip. But to obtain a more accurate progression rate of joint damage or its reduction under treatment by radiography, Ravaud et al. (1998) suggested an interval of at least one year preferably two years between initial and control radiographs.

Monitoring progression of human osteoarthritis clinically and radiologically changes are poorly sensitive (Garnero and Delmas, 2003). Alternatively, bone markers reflect quantitative and dynamic changes in periarticular bone turnover (Hunter et al., 2003 B) and may help to assess bone rate of osteoarthritic progression and to monitor the therapeutic efficacy on OA, as in response to treatment is faster and more sensitive than that of radiographic findings (Bruyère et al., 2003; Garnero et al., 2003 A). Berger et al. (2004) found elevated ICTP values in humans with rapidly progressive hip osteoarthritis compared to humans with slowly progressive hip osteoarthritis, whereas they found no significant differences of serum level of the bone formation markers OC and bAP between both groups. Because of their fast response to acute changes in bone metabolism (Guillemant et al., 2003; Rovetta et al., 2003), bone resorption marker may be preferred to bone formation marker to monitor therapeutic effects in patients with OA progression.

Results from the present study may suggest that both serum CTX and bAP are markers which are able to detect an effect of the nonsteroidal anti-inflammatory drug Carprofen on bone turnover of dogs with OA although the bone resorption marker serum CTX may be preferred to the bone formation marker bAP as serum CTX reacted faster to the treatment than bAP.

Some limitations still have to be kept in mind. The dog groups were small with only 10 dogs per group. Due to animal welfare reasons, it is much better to use privately-owned dogs. But owners have difficulties as soon as animals are treated. Another problem is a negative attitude of the owner population in relation to experiments. So, for this reason, only 20 animal owners could be recruited. Although, dogs were paired, it was not possible for all factors like e.g. sex, and type of osteoarthritis in all of the dogs. Since we mainly had hip dysplasia in our groups, the dogs were younger than usually expected from the typical osteoarthritis dog. In a preliminary study (data not published), it was shown, that the bone markers were higher in dogs with different forms of osteoarthritis compared to healthy dogs.

The findings in the present study are not as evident as expected, maybe due to the fact that not all of the animals did show identical forms of osteoarthritis. It may be possible that the different forms of osteoarthritis react differently to Carprofen. Another possible explanation may be that the osteoarthritis forms chosen in this study are not active enough to show an extreme decrease in bone turnover under Carprofen treatment. The results of the present study suggest that in addition to the analgesic and anti-inflammatory effect, long term administration of Carprofen may possibly have a positive influence on progression of clinical OA in the field. The significant decrease of both serum CTX and bone-specific alkaline phosphatase show that Carprofen-treatment could have a retarding effect on periarticular bone turnover and finally, a retarding effect on the progression of osteoarthritis. In addition, radiological findings suggest that Carprofen-treatment probably delays osteophyte formation. However, these results need to be confirmed by further studies under standardized conditions with a large homogeneous group of dogs (e.g. same age, same size, similar form of osteoarthritis) and a treatment with Carprofen longer than six months to evaluate the therapeutic efficacy of Carprofen on long term progression of osteoarthritis.

Acknowledgements

The authors thank Brigitte Küffer and Barbara Schneider for the excellent technical assistance.

Effet du Carprofen sur certains marqueurs du métabolisme osseux chez des chiens souffrant d'ostéoarthrite chronique

Le but de ce travail était d'étudier l'effet de l'anti inflammatoire non stéroïdien Carprofen sur le métabolisme osseux de chiens souffrant d'arthrose chronique au moyen de divers marqueurs osseux et de l'examen radiologique des articulations atteintes. Pour cela, 20 chiens d'âges et de poids divers ont été divisés en deux groupes : 10 chiens dans le groupe Rimadyl&® et 10 chiens dans le groupe de contrôle (C) non traité. Les articulations atteintes d'arthrose ont été radiographiées au début et à la fin de l'essai. Huit prises de sang ont été effectuées sur chaque chien. Dans chaque prise de sang le marqueur d'ostéogenèse osteocalcine (OC), la phosphatase alcaline osseuse, les marqueurs d'ostéolyse ICTP et CTX ainsi que la 1.25 $(OH)_2$ vitamine D et la parathormone (PTH) ont été recherchés. On n'a pas trouvé un effet de groupe significatif pour les marqueurs osseux. La concentration en ICTP du groupe R montrait une diminution nette dans les 3 premiers mois de l'essai mais remontait ensuite jusqu'à la fin du test. La concentration en CTX du groupe R montrait un abaissement significatif dans les deux premiers mois de l'essai. La phosphatase alcaline montrait une baisse significative du début jusqu'à la fin de l'essai. Dans le groupe R, seul un chien montrait une aggravation radiologique sur l'articulation concemée alors que, dans le groupe de contrôle, 3 chiens présentaient une aggravation. Les résultats de cette étude montrent que le Carprofen peut peut être freiner le métabolisme osseux chez des chiens souffrant d'arthrose chronique. Le monitoring d'un processus métabolique focal comme il se produit dans l'os d'une articulation arthrosique est toutefois difficile car l'ensemble du squelette est très actif et d'autres processus métaboliques influencent le monitoring.

Gli effetti del carprofene su marker specifici del metabolismo osseo nei cani con osteoartrite cronica

Scopo di questo lavoro è di analizzare l'effetto non steroido dell'antinfiammatorio carprofene nel metabolismo osseo e i progressi dei cani affetti da artrosi cronica delle articolazioni grazie alla misurazione di diversi marker e misurazioni radiologiche dell'articolazione interessata . A questo scopo sono stati suddivisi in due gruppi 20 cani di età e peso differente: 10 cani nel gruppo Rimadyl® Gruppe (R) e 10 cani nel gruppo di controllo non trattato (C). Le articolazioni osteoartritiche modificate sono state radiografate all'inizio e alla fine dell'esperimento. Su ogni cane sono stati presi in totale 8 campioni di sangue. In ogni campione sanguigno sono stati analizzati: il marker della rigenerazione ossea osteocalcina (OC) e la fosfatasi alcalina specifica delle ossa, il marker della degenerazione ossea telopeptide carbossiterminale collagene di tipo 1 (ICPT) e il CrossLaps sierico (CTX) e infine 1,25 $(OH)_2$ vitamina D e il paratormone (PTH). Non è stato rilevato alcun significativo effetto di gruppo né per il marker della rigenerazione ossosa né per quello della degenerazione ossea. Le concentrazioni di ICTP del gruppo R hanno mostrato una nefta caduta nei primi tre mesi dell'esperienza poi aumentata verso la fine dell'esperimento. Le concentrazioni di CTX del gruppo R hanno mostrato una significativa caduta nei primi due mesi dell'esperimento. Il marker della rigenerazione ossea bAP ha mostrato una significativa caduta dall'inizio alla fine dell'esperienza. Nel gruppo R soli 1 cane ha mostrato a livello radiologico un peggioramento dell'articolazione affetta, mentre nel gruppo di controllo, 3 cani sono stati colpiti da un peggioramento. 1 risultati di questo studio mostrano che il carprofrene potrebbe frenare probabilmente il metabolismo osseo nei cani con artrosi cronica alle articolazioni. Il monitoraggio di un processo metabolico focale come si puô rilevare nelle ossa di un'articolazione osteoartritica è perà difficile, poiché la massa ossea è molto attiva e il monitoraggio influenza gli altri processi metabolici.

References

Allen M. J., Hoffmann W. E., Richardson D. C., Breur G. J.: Serum markers of bone metabolism in dogs. Am. J. Vet. Res. 1998, 59: 250–254.

Arican M., Carter S. D., Benett D.: Osteocalcin in canine joint diseases. Br. Vet. J. 1996, 152: 411–423.

Berger C. E., Kroner A., Stiegler H., Leitha T., Engel A.: Elevated levels of serum type I collagen C-telopeptides in patients with rapidly destructive osteoarthritis of the hip. Int. Orthop. 2004, 30: 122–131.

Bettica P., Cline G., Hart D. J., Meyer J., Spector T. D.: Evidence for increased bone resorption in patients with progressive knee osteoarthritis. Arthritis Rheum. 2002, 46: 3175–3184.

Brandt K. D., 1991: Transsection of the anterior cruciate li-

gament in the dogs: a model of osteoarthritis. Semin. Arthritis Rheum. 21, 22–32.

Brandt K. D.: Insights into the natural history of osteoarthritis provided by the cruciate-deficient dog. An animal model of osteoarthritis. Ann. NY Acad. Sci. 1994, 6, 199–205.

Breur G. J., Allen M. J., Carlson S. J., Richardson D. C.: Markers of bone metabolism in dog breeds of different size. Res. Vet. Sci. 2004, 76: 53–55.

Bruyère O., Collette J. H., Ethgen O., Rovati L. C., Giacovelli G., Henroti P., Seidel L., Reginster J.Y: Biochemical markers of bone and cartilage remodeling in prediction of longterm progression of knee osteoarthritis. J. Rheumatol. 2003, 30: 1043–1050.

Burki C. D., 2000: Wachstumshormon (GH) und Insulinähnliche Wachstumsfaktoren (IGF) beim wachsenden Hund in Relation zur Körpergrösse am Modell des Pudels.

Dissertation, Universität Zürich, 2000.

Campion G. V., Delmas P. D., Dieppe P. A., 1989: Serum and synovial fluid osteocalcin (bone gla protein) levels in joint disease. Br. J. Rheumatol. 1989, 28: 393–398.

Chandnani V., Gerharter J., Neumann C., Kursunoglu-Brahme S., Sartoris D. J., Resnick D.: MR findings in asymptomatic shoulders: a blind analysis using symptomatic shoulders as controls. Clin. Imaging. 1992, 16: 25–30.

Dedrick D. K., Goldstein, S. A., Brandt, K. D., O'Connor, B. L., Goulet, R. W., Albrecht M., 1993: A longitudinal study of subchondral plate and trabecular bone in cruciate-deficient dogs with osteoarthritis followed up for 54 months. Arthritis Rheum. 1993, 36: 1460–1467.

Dedrick D. K., Goulet R. W., O'Connor B. L., Brandt K. D.: Preliminary report: increased porosity of the subchondral plate in an accelerated canine model of osteoarthritis. Osteoarthritis Cartilage. 1997, 5: 71–74.

Epstein S., 1988: Serum and urinary markers of bone remodelling: Assessment of bone turnover. Endocr. Rev. 1988, 9: 437–449.

Garnero P., Piperno M., Gineyts E., Christgau S., Delmas P. D., Vignon E.: Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relation with disease activity and joint damage. Ann. Rheum. Dis. 2001, 60: 619–626.

Garnero P., Conrozier T., Christgau S., Mathieu P., Delmas P. D., Vignon E.: Urinary type II collagen C-telopeptide levels are increased in patients with rapidly destructive hip osteoarthritis. Ann. Rheum. Dis. 2003 A, 62: 939–943

Garnero P., Delmas P. D.: Biomarkers in osteoarthritis. Curr. Opin. Rheumatol. 2003, 15: 641–646.

Garnero P., Ferreras M., Karsdal M. A., Nicamhlaoibh R., Risteli J., Borel O., Qvist P., Delmas P. D., Foged N. T., Delasissé J. M.: The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. J. Bone Miner. Res. 2003 B, 18: 859–867.

Guillemant J. A., Accarie C. M., Gueronniere V., Guiellman S. E.: Different acute responses of serum type I collagen telopeptides, CTX, NTX and ICTP, after repeated ingestion of calcium. Clin. Chim. Acta. 2003, 337: 35–41.

Hunter D. J., Hart D., Snieder H., Bettica P., Swaminathan R., Spector R.: Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. Rheumatology 2003 A, 42: 1311–1316.

Hunter D. J., Spector T. D.: The role of bone metabolism in osteoarthritis. Osteoarthritis 2003 B, 5: 15–19

Johnston S. A.: Osteoarthritis: Joint anatomy, physiology and pathobiology.

Vet. Clin. North Am. Small Anim. Pract. 1997, 27: 699–723.

Johnston S. A., Budsberg S. C.: Nonsteroidal anti-inflammatory drugs and corticosteroids for the management of canine osteoarthritis. Vet. Clin. North Am. Small Anim. Pract. 1997, 27, 841–861.

Johnston S. A., Fox S. M., 1997: Mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis. J. Vet. Med. Assoc. 1997, 210: 1486–1492.

Liesegang A., Reutter R., Sassi M. L., Risteli J., Kraenzlin M., Riond J. L., Wanner M., 1999 A: Diurnal variation in concentrations of various markers on bone metabolism in dogs. Am. J. Vet. Res. 1999 A, 60: 949–953.

Liesegang A., Sassi M. L., Risteli J., Kraenzlin M., Riond J. L., Wanner M.: Influence of low calcium, high calcium and energy-rich diets on bone markers in ovariectomized Beagle dogs. J. Anim. Physiol. a. Anim. Nutr. 1999 B, 81: 223–231.

Liesegang A.: Anwendung von Knochenmarkern in der Veterinärmedizin.

Schweiz. Arch. Tierheilk. 2000, 142: 613–623.

Liesegang A., Burgi E.., Sassi M. L., Risteli J., Wanner M.: Influence of vegetarian diet versus a diet with fishmeal on bone in growing pigs. J. Vet. Med. A. 2002, 49: 230–238.

Liesegang A.: Possibilities of monitoring bone metabolism in ruminants - an overview of the methods in use. Acta Vet. Scand. Suppl. 2003, 97: 23–28.

Liesegang A., Risteli J.: Influence of different calcium concentrations in the diet on bone metabolism in growing dairy goats and sheep. J. Anim. Physiol. Anim. Nutr. 2005, 89: 113–119.

Liesegang A., Risteli J., Wanner M., 2006: The effects of first gestation and lactation on bone metabolism in dairy goats and sheep. Bone 2006, 38: 792–802.

Manley P. A., Vanderby R. J. R., Kohles S., Markel M. D., Heiner, J. P.: Alterations in femoral strain, micromotion, cortical geometry, cortical porosity, and bony in growth in uncemented collared an collarless prostheses in the dog. J. Arthroplasty 1995, 10: 63–73.

McLaughlin R., 2000: Management of chronic osteoarthritic pain. Vet. Clin. North Am. Small Anim. Prac. 2000, 30:933-949.

Moskowitz R. W., 1987: Primary osteoarthritis: epidemiology, clinical aspects, and general management. Am. J. Med. 1987, 83: 5–10.

Parthemore J. G., Burton D. W., Deftos L. J.: Associations and dissociations between serum bone Gla protein and alkaline phosphatase in skeletal metabolism. J. Orthop. Res. 1993, 11: 671–676.

Pelletier J. P., Lajeunesse D., Jovanovic D. V., Lascau-Coman V., Jolicoeur F. C., Hilal G., Fernandes J. C., Martel-Pelletier J.: Carprofen simultaneously reduces progression of morphological changes in cartilage and subchondral bone in experimental dog osteoarthritis. J. Rheumatol. 2000, 27: 2893–2902.

Philipov P., Pascalev M. D., Aminkov B.Y., Grosev C. D., 1995: Changes in serum carboxyterminal telopeptide of type I collagen in experimental model of canine osteomyelitis. Calcif. Tissue Int. 1995, 57: 152–154.

Ravaud P., Giraudeau B., Auleley G. R., Drape J. L., Rousselin B., Paolozzi L.: Variability in knee radiography: Implication for definition of radiological progression of medial knee osteoarthritis. Ann. Rheum. Dis. 1998, 57: 624–629.

Rovetta G., Monteforte P., Grignolo M. C., Frignone A., Buffrini L., 2003: Hematic levels of type I collagen C-telopeptides in erosive versus non-erosive osteoarthritis of the hands. Int. J. Tissue React. 2003, 25: 25–28.

Sassi M. L., Eriksen H., Risteli L., Niemi S., Mansell J., Gowen M., Risteli J.: Immunochemical characterization of the assay for the carboxyterminal telopeptide of human type I collagen (ICTP). Loss of antigenicity by treatment with cathepsin K. Bone 2000, 26: 370–377.

Seibel M. J., Woitge H. W., 1999: Basic principles and clinical applications of biochemical markers of bone metabolism. J. Clin. Densitom. 1999, 2: 299–321.

Todhunter R. J., Yeh L. A., Sheldon A., Grisanio L., Walker S. L., Burton-Wurster N., Lust G.: Effects of stromelysin activity on proteoglycan degradation of canine articular cartilage explants. Am. J. Vet. Res. 1995, 56: 1241–1247.

Address of correspondence

Dr. med. vet. Annette Liesegang, Institute of Animal Nutrition, Winterthurerstr. 260, 8057 Zürich,
Tel. +41-44-635 88 23, Fax +41-44-635 89 32, E-mail: aliese@vetphys.unizh.ch

Received: 25 August 2006
Accepted: 20 October 2006