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SYNOVIAL FLUID MMP-2 AND MMP-9 LEVELS IN INTERNAL OR EXTERNAL FIXATION FOR INTRA-ARTICULAR FRACTURES

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Abstract

The purpose of this study was to evaluate whether internal fixation or external skeletal fixation (ESF) results in better "joint health" following traumatic injury to the stifle by assessing lameness and measuring matrix metalloproteinase MMP-2 and MMP-9. Dogs with skin grafts and transarticular ESFs were included in group A. Dogs with intra-articular fractures of the distal femur were randomly divided into groups B and C, and treated with either internal or ESF, respectively. Dogs in group D had diaphyseal tibial fractures treated with ESF. Synovial fluid samples were collected pre-operatively and again 7 days and 30 days postoperatively to measure MMP-2 and -9 levels via zymography. Preoperative MMP-9 levels were higher in groups B and C than A and D. Over time, MMP-2 levels increased in groups A–C, and MMP-9 levels significantly decreased in groups B and C by 30 days postoperatively. ESF appears superior to internal fixation for repair of intra-articular fractures of the distal femur, and MMP-2 and MMP-9 could serve as markers of either fracture healing or overall joint health, particularly in the setting of PTOA.

Key words: intra-articular fracture, internal fixation, external skeletal fixation, osteoarthritis, metalloproteinase

Posttraumatic osteoarthritis (PTOA) can develop following various joint traumas (Pardy et al., 2004; Lotz, 2010). It is estimated that 20% of the canine population currently has osteoarthritis (OA), and approximately 50% of patients with traumatic joint injury ultimately develop OA (Johnston, 1997; Lotz, 2010). PTOA is a chronic, debilitating condition that results in reduced physical activity, quality of life, and deconditioning of the musculoskeletal system (Lotz, 2010). There is currently no cure for OA (Aragon et al., 2007). In cases of joint trauma, early restoration of the joint to limit acute damage to the joint, delay onset, and minimize the severity of OA is indicated (Lotz, 2010).

Although the exact chain of events leading to PTOA remain unclear, it is currently thought that both degenerative and regenerative changes in the joint begin immediately following a traumatic event (e.g., transection of the anterior cruciate ligament). Within 4 weeks of the traumatic event, cartilage thickening and osteophytes are notable (Pardy et al., 2004). Within 3–12 months, subchondral bone thickening, more extensive osteophytes, and erosion of the articular cartilage is noted. Within a matter of only a few years, full-thickness loss of the articular cartilage occurs (Pardy et al., 2004). Progressive bone and cartilage degeneration and destruction is accompanied by alterations in the expression and activity of matrix metalloproteinases (MMPs), proinflammatory cytokines, and other proteases (Pardy et al., 2004). Previous studies have shown that various MMPs such as MMP-1, -3, -8, and -9 are produced in higher quantities by chondrocytes following mechanical impact injury in cartilage explant cultures (Patwari et al., 2003; Lee et al., 2005; Lotz, 2010). A number of other studies reported that the gelatinases MMP-2 and MMP-9 are elevated in synovial fluid (SF) collected from patients, including humans, horses, and dogs with either OA or intra-articular fractures (Davidson et al., 2006; Fietz et al., 2008; Alam et al., 2011; Galasso et al., 2012). Elevations in such enzymes contribute to the erosion of articular cartilage, which is the hallmark of OA (Davidson et al., 2006). Together, the findings in those aforementioned studies spurred the suggestion that the gelatinases could potentially play an important role in identifying the early phases of OA as well as monitoring the status of joint degeneration (Alam et al., 2011; Galasso et al., 2012).

Intra-articular fractures of the distal femur are not uncommon in veterinary medicine (Newton and Nunamaker, 1985). Various treatment options exist, including internal and external skeletal fixation (ESF). Typically, the surgeon uses their own personal preference when deciding which surgical technique to apply. To the best of the authors' knowledge, there is currently no consensus that dictates what technique is preferable for a given fracture type. Complications can occur with both internal and ESF (described by Newton and Nunamaker, 1985), and studies describing the outcomes of dogs that were treated via various approaches do not appear to have not been conducted to determine which approach results in optimal fracture repair with minimal PTOA. One study by Gordon et al. (2003) found that PTOA developed in all dogs (n=13) with humeral condylar fractures, and that the fracture reduction score did not correlate with the follow-up OA score (P=0.07).

The purpose of this study was to evaluate whether internal fixation or ESF resulted in better "joint health" following traumatic injury to the stifle by exploring the temporal relationship between MMP-2 and MMP-9 relative to placement of the fixation devices. The hypotheses were that ESF would be a better therapeutic option than internal fixation devices and that SF MMP-2 and -9 could serve as markers for healing and PTOA. Early diagnosis of PTOA could result in earlier interventions to help slow the progression of OA.

Material and methods

Between January 2008 and December 2011, client-owned dogs with intra-articular fractures of the distal femur were included. Dogs requiring skin grafts involving the stifle joint and who were fitted with ESFs to prevent the motion of the grafts and dogs with fractures of a long bone that did not involve the stifle joint treated with an ESF were also included. Only dogs with one closed fracture that were otherwise healthy were included. Dogs weighing <5 kg were excluded due to the anticipated small volume of SF that would be collected.

All included dogs were divided into one of four groups. Dogs that received skin grafts in the stifle area and had transarticular ESFs to minimize movement of the stifle were included in group A. Dogs with intra-articular fractures of the distal femur were alternately included in groups B and C and were treated via either internal fixation or transarticular ESF, respectively. Dogs in group D had diaphyseal fractures of the tibia that were treated with ESFs. All owners provided informed written consent.

Surgical procedures were performed according to established published techniques (Kraus et al., 2003; Piermatei et al., 2006). For internal fixation, a mini-driver (3M, St. Paul, MN, USA) and smooth trocar tip pins (Synthes, Switzerland) measuring 20–30% of the bone diameter (calculated using the preoperative radiographs) were used. Acrylic frames (Lang Dental FMG Co., Chicago, USA) with smooth pins (Synthes, Switzerland) were selected for ESF (all type II). All repairs in dogs with ESFs were closed reductions, and no arthrotomies were performed. Synthetic absorbable polyglycolic acid (Dexon, Davis and Geck, Wayne, NJ, USA) was used in all patients.

SF samples were collected before surgery (day 0) and again 7 days and 30 days postoperatively using sterile, disposable 22 gauge, 1.5 inch hypodermic needles with 3 mL syringes (Houlton and Collinson, 1994). All dogs were anaesthetized during the procedure, and radiographs were taken before the fluid was collected. SF samples were frozen at -20°C and shipped on dry ice for analysis.

Zymography was performed as described in detail elsewhere (Paolocci et al., 2006). Briefly, a 4% stacking gel and an 8% sodium dodecylsulfate polyacrylamide gel containing 10 mg of gelatin were used. Samples were incubated for 2 h in sample buffer prior to being loaded onto the gels, and 10 μL neutrophil extract (MMP-9) (Amersham Pharmacia Biotech, Amersham, UK) was used as a positive control. Samples were run at 4°C with a constant current of 20–30 milliamps and 200 volts. Gels were stained with Coomassie Brilliant Blue for 15–30 min, then destained (with 3% [v/v] glycerol to stabilize the gels during drying).

A molecular analyst densitometer (Amersham Pharmarcia Biotech, Uppsala, Sweden) was used to measure the integrated optical densitometry (IOD) of the 72 kDa (MMP-2) and 95 kDa (MMP-9) bands. The gels were scanned and analysed with Molecular Analyst software. The molecular weights of the bands were calculated from a logarithmic plot of molecular weight standards. Representative gels are shown in Figure 1. All samples were analysed concurrently, and each sample was analysed only once.

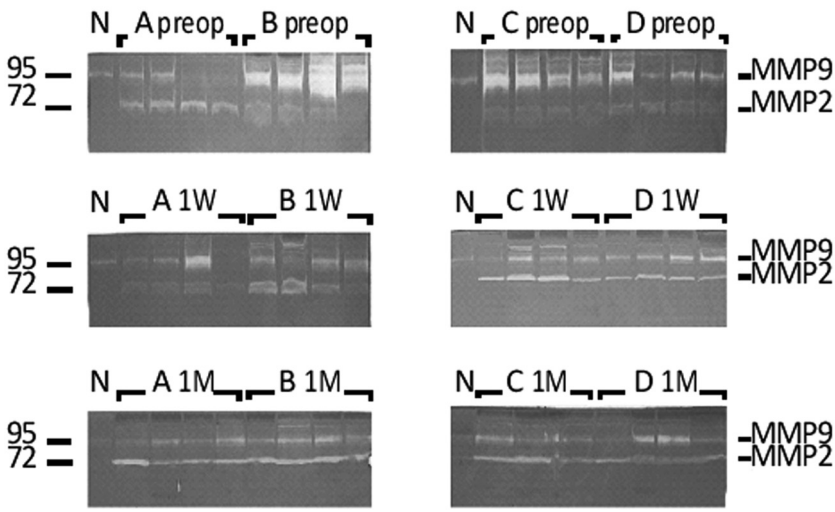

Figure 1. Representative zymography gels showing the 95 kDa (MMP-9) and 72 kDa (MMP-2) bands in the four treatment groups (A–D) preoperatively, and again 7 days (1W) and 30 days (1M) postoperatively. N, neutrophil extract (MMP-9) for the positive control

Limb function was assessed using a "yes" or "no" principle regarding full weight bearing, and full weight bearing without evidence of lameness was considered normal limb function. Limb function was assessed either 1 month following ESF removal or 2 months postsurgically for group B.

Statistical analysis

Due to the small sample size, data were presented as median and interquartile range (25th to 75th percentile). Differences over time within groups were tested with the Friedman test, and post-hoc tests for comparisons between two specific time points were measured using the Wilcoxon signed-rank test when the Friedman test yielded a significant difference. Differences between groups were tested with the Kruskal-Wallis test, and post-hoc tests for comparisons between two groups were performed using the Mann-Whitney test when the Kruskal-Wallis result was significant. All post-hoc tests were performed using Bonferroni adjustment. The statistical tests were two-sided, and a significance level of 0.05 was used. All analyses were performed using a statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

One hundred nineteen dogs were prospectively enrolled, including 29 in group A, 30 in group B, 32 in group C, and 28 in group D. No differences in age, sex, or body weight existed at baseline between the four study groups (Table 1).

Table 1. Baseline demographics of the dogs included in each of the four study groups

	Group A (n=29)	Group B (n=30)	Group C (n=32)	Group D (n=28)	P
Age (months)	24.0 (12.0, 48.0)	36.0 (24.0, 60.0)	24.0 (12.0, 51.0)	24.0 (12.0, 60.0)	0.199
Sex					
female	18 (62.1%)	12 (40.0%)	17 (53.1%)	19 (67.9%)	0.164
male	11 (37.9%)	18 (60.0%)	15 (46.9%)	9 (32.1%)	–
Weight (kg)	12.0 (10.0, 15.0)	11.7 (9.4, 16.0)	10.1 (7.1, 12.8)	10.2 (7.4, 12.3)	0.028*

Data are presented as mean (interquartile range) or count (percentage).
*Post-hoc testing revealed no significant differences between any two groups.

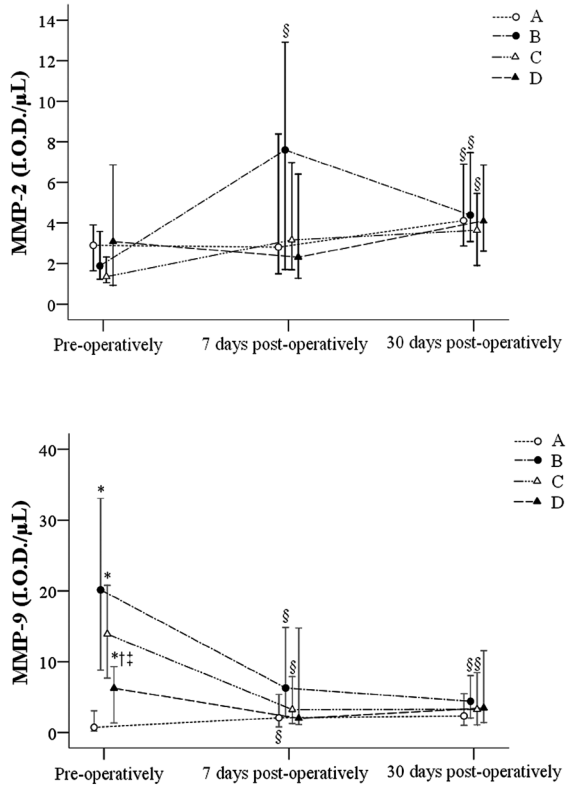

Figure 2. Expression of MMP-2 (A) and MMP-9 (B) measured by zymography in synovial samples collected before surgery (pre-operation) and again 7 days (post-operation 1 week) and 30 days (post-operation 1 month) after application of either internal fixation or external skeletal fixation. Group A, dogs with skin grafts; group B, intra-articular fractures treated via internal fixation; group C, intra-articular fractures treated via ESF; group D, tibial diaphyseal fractures treated with ESF not crossing a joint. Data have been presented as median and the inter-quartile range. * indicates a significant difference compared to group A; † indicates a significant difference compared to group B; ‡ indicates a significant difference compared to group C; § indicates a significant difference compared to samples collected preoperatively

No significant differences in MMP-2 SF levels between any two of the four groups were observed at any time point (Figure 2A). Significantly higher MMP-9 SF levels were observed in groups B and C compared with group A (median MMP-9 levels were 20.15 IOD/μL, 13.90 IOD/μL, and 0.72 IOD/μL, respectively, $P<0.0001$, Figure 2B). MMP-9 levels in group D were significantly lower than groups B and C (median MMP-9 level in group D was 6.26 IOD/μL, $P<0.0001$) and were significantly higher than group A ($P=0.00013$).

Median MMP-2 in group A significantly increased from 2.91 IOD/μL presurgically to 4.12 IOD/μL by 30 days postoperatively (Figure 2A). In group B, MMP-2 increased from 1.88 IOD/μL presurgically to 7.6 IOD/μL 7 days postoperatively ($P=0.0004$) and 4.39 IOD/μL 30 days postoperatively ($P=0.0001$). Similarly, median MMP-2 in group C increased from 1.36 IOD/μL presurgically to 3.64 IOD/μL by 30 days postoperatively ($P=0.0037$).

Significant within-group changes in MMP-9 levels were also observed (Figure 2B). MMP-9 increased in group A from 0.72 IOD/μL presurgically to 2.08 IOD/μL by 7 days postoperatively ($P=0.0087$). In group B, MMP-9 decreased from 20.15 IOD/μL presurgically to 6.29 IOD/μL and 4.40 IOD/μL by 7 days and 30 days postoperatively, respectively ($P=0.0118$ and $P<0.0001$, respectively). Finally, MMP-9 decreased in group C from 13.90 IOD/μL presurgically to 3.24 IOD/μL and 3.27 IOD/μL by 7 days and 30 days, respectively (both $P<0.0001$).

None of the dogs included in group A had any evidence of lameness 1 month following skin grafting, but 5/28 dogs in group B (53.6%) were lame 2 months following fracture repair. In groups C and D, 26/32 (81.25%) and 29/30 (96.7%) dogs had no evidence of lameness (χ^2 test, $P<0.05$).

Discussion

The group of dogs diagnosed with a single, closed, intra-articular fracture of the distal femur treated via ESF had better limb function than dogs treated via internal fixation. Almost 100% of the dogs in groups A and D were completely sound by the end of the study period; however, 18.75% and 46.4% of dogs in the ESF and internal fixation groups, respectively, were lame at the end of the study period. The fact that some dogs in groups B and C remained lame at the time of follow-up is not surprising as PTOA may already have begun to develop, causing lameness. Alternatively, or perhaps in addition to PTOA, either arthrodesis or joint stiffness could also have contributed to the observed postoperative lameness. Dogs treated via internal fixation may have flexed their stifles and not bear weight during the recovery period. In the ESF group, the dogs' paws were able to touch the floor and those dogs could bear weight postoperatively.

Persistent lameness following fracture repair in dogs has been described previously (Muir and Norris, 1997). In dogs with condylar fractures of the humerus treated via different fixation methods, only 46% of the 133 included dogs regained full limb function, 36% had a slight or occasional lameness, and 18% had moderate

to severe lameness (Denny, 1983). Further, the frequency of PTOA in dogs following humeral condylar fracture repair developed in all 15 fractures in 13 dogs in another study (Gordon et al., 2003). Similar statistics do not appear to have been published regarding fractures of the distal femur; however, there is no indication that dogs with fractures involving the stifle joint would be saved from either lameness or PTOA. This theory is supported by a case report describing a dog that underwent total knee replacement due to severe osteoarthritis that had developed subsequent to a femoral fracture (Eskelinen et al., 2012). That said, not all dogs have persistent lameness. One report describes two farm dogs that returned to work following repair of medial unicondylar, intra-articular fractures of the distal femur with no evidence of lameness 6 months following surgery (Davis and Worth, 2009).

In addition, (some) dogs in this study might not have been administered canine rehabilitation postoperatively (Johnson et al., 2005). Physical therapy, when applied properly, can decrease healing time, prevent disability, and facilitates normal function. Techniques such as heat and cold therapy, massage, and therapeutic exercise can be instituted as soon as the fracture is healed and reportedly reduce edema and pain (Doyle, 2004; Johnson et al., 2005).

Regardless of soundness postoperatively, many, if not all dogs with intra-articular fractures are at-risk for developing PTOA (Buckwalter and Brown, 2004). One way to potentially assess whether a dog is either at-risk or is in the process of developing PTOA is to measure markers of joint health, such as degradative enzymes or mediators of inflammation in serum, urine, or SF (Lotz, 2010; Wigner et al., 2012). In this study, MMP-2 and MMP-9, the gelatinases, were measured. As suspected, alterations in MMPs do occur rapidly following joint and/or bone trauma, and not all MMPs are altered in similar ways (Lotz, 2010). As reported previously by Nyman et al. (2011), MMP-2 and -9 have similar substrates, but (in bone) are produced by osteoblasts and osteoclasts, respectively. Temporal changes in MMP expression have also been previously noted. In one study of dogs, MMP-2 serum levels significantly decreased after OA was induced in a surgically-created patellar luxation model. In the SF, MMP-2 was significantly increased during the early phases of OA (Alam et al., 2011). That study assessed dogs at 1.5- and 3-month intervals. In the current study, the longest follow-up period was 30 days, so it is impossible to suggest what the MMP-2 SF levels would be if they had been measured for a longer period of time. In a separate study involving horses, both MMP-2 and -9 were significantly increased in one of the horse's carpal joints (with either osteochondrosis dissecans or intra-articular fractures) compared to the unaffected contralateral carpal joint (Okumura et al., 2011). Finally, a comprehensive review of the gelatinases published by Glasso et al. (2012) also found that MMP-2 and -9 were differentially expressed by various tissues and in various conditions. For example, cyclic compression of osteoarthritic subchondral bone results in the increased expression of MMP-9, whereas MMP-2 did not appear to be a mechanosensitive gene. Together, the above-described data, including the study presented herein, suggest that the gelatinases, not just collagenases, could serve as markers for bone healing as well as the development of OA. Further, Okumura et al. (2011) suggest that zymography is a simple and easy way to measure those MMPs.

Some limitations of this study include the small number of patients included in each of the four treatment groups and the short follow-up period limiting the temporal evaluation of MMPs in SF and overall lameness. In addition, other markers of joint health and fracture healing, either involving other MMPs, other cartilage breakdown products, or even a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS), using a variety of techniques rather than simply zymography would have been useful. Finally, the lack of information regarding specific complications in groups B and C limits the discussion of the merits of internal and ESF; however, the overall lameness scores still clearly support ESF. Using client-owned dogs limited the use of more objective measures of lameness and follow-up examination and periods.

In conclusion, this study supports the use of ESF for the repair of intra-articular fractures of the distal femur. Surgeons are encouraged to consider the use of ESFs for intra-articular fractures of the distal femur. This study also suggests that MMP-2 and -9 could serve as markers of either fracture healing or overall joint health, particularly in the setting of PTOA.

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Poziom MMP-2 i MMP-9 w mazi stawowej przy wewnętrznym i zewnętrznym zespoleniu po złamaniach wewnątrzstawowych

STRESZCZENIE

Celem badań było stwierdzenie – poprzez ocenę kulawizny i oznaczenie poziomu metaloproteinaz macierzy międzykomórkowej (MMP-2 i MMP-9) – jaki rodzaj zespolenia (wewnętrzne czy zewnętrzne) zapewnia lepsze „zdrowie stawów" po urazowym uszkodzeniu stawu kolanowego. Psy z przeszczepem

skóry i zewnętrznym zespoleniem przezstawowym stanowiły grupę A. Psy z wewnątrzstawowym złamaniem dystalnej części kości udowej, poddane zespoleniu wewnętrznemu i zewnętrznemu, przydzielono losowo odpowiednio do grup B i C. U psów z grupy D, u których stwierdzono złamanie trzonu kości piszczelowej, zastosowano zespolenie zewnętrzne. Próbki mazi stawowej pobierano przed operacją oraz ponownie 7 i 30 dni po operacji celem zymograficznego określenia poziomu MMP-2 i MMP-9. Przedoperacyjny poziom MMP-9 był wyższy w grupach B i C w porównaniu do grup A i D. Z upływem czasu poziom MMP-2 wzrastał w grupach A–C, natomiast poziom MMP-9 zmniejszył się istotnie w grupach B i C w 30 dniu po operacji. Stwierdzono wyższość zespolenia zewnętrznego nad wewnętrznym w przypadku naprawy wewnątrzstawowych złamań dystalnej części kości udowej, natomiast MMP-2 i MMP-9 mogą służyć jako wskaźniki gojenia się złamań lub ogólnego zdrowia stawów, szczególnie w odniesieniu do pourazowego zapalenia kości i stawów.