

Influence of development and joint pathology on stromelysin enzyme activity in equine synovial fluid

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Abstract

Objective—To investigate the role of stromelysin (MMP-3) activity in synovial fluid (SF) at different stages of development and in common joint disorders in the horse.

Methods—Stromelysin activity was determined with a fluorogenic enzyme activity assay in SF of normal joints of fetal, juvenile and adult horses, and in SF of horses suffering from the developmental orthopaedic disease osteochondrosis (OC) or osteoarthritis (OA). Additionally, MMP-3 activity was expressed as a ratio of previously reported general MMP activity in the same SF samples.

Results—The levels of active stromelysin were 30-fold to 80-fold higher in SF from fetal horses than in SF from juvenile and mature animals ($p < 0.001$). Juvenile horses (5 and 11 months of age) showed a twofold to threefold higher stromelysin activity than adult horses ($p < 0.05$). In OC joints, stromelysin activity was not significantly different from the activity in normal, age matched, control joints. In OA joints the activity was about four times higher than in normal joints ($p < 0.001$). The ratio MMP-3 activity/general MMP activity did not change with age in normal, healthy joints. This ratio was more than twofold increased in OA joints compared with normal joints, indicating selective up-regulation of gene expression or activation of proMMP-3, or both, in OA pathology.

Conclusions—The significantly higher stromelysin activity in young individuals parallels the higher metabolic activity occurring at rapid growth and differentiation at early age. In OC, MMP-3 mediated matrix degradation appears to be not different from normal joints. The increased stromelysin activity in OA joints is in agreement with pathological matrix degradation. In these joints MMP-3 activity is selectively increased compared with normal joints.

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participate in the activation of proMMP-1, -8, -9 and -13. Membrane-type MMPs (MMP-14, -15, -16 and -17) are able to activate proMMP-2.¹⁻³ MMPs are present mainly in inactive form (> 95% of total), which is either the latent proform, or an inhibited complex with tissue inhibitor of metalloproteinases (TIMPs) or alpha-2-macroglobulin.¹⁻⁴

During normal development and growth controlled production and activation of MMPs is critical. While in adult cartilage turnover is extremely slow, it may be assumed that metabolism in young individuals has to be maintained at a substantially higher level to allow for growth and remodelling. We recently showed in the horse that general MMP activity (especially of the MMPs with gelatinase-like properties; catalytic efficiencies k_{cat}/K_m general MMP substrate in $10^3/M/s$ at $25^\circ C$; MMP-1=19, MMP-2=230, MMP-3=4, MMP-7=9, MMP-8=29, MMP-9=139, MMP-13=550, and MMP-14=147) in SF of immature joints was significantly higher than in mature joints. This was interpreted as a higher metabolic activity (that is, a higher matrix turnover) in juvenile individuals.⁵

MMP-3 has been implicated to play a pivotal part in joint pathology.^{6,7} Its synthesis by chondrocytes and synoviocytes can be induced by inflammatory mediators like interleukin 1.^{6,8} Highly increased activities of MMP-3 have been found in arthritic cartilage,⁹ synovium,^{7,9} and synovial fluid (SF),¹⁰ suggesting a role for this enzyme in matrix degradation. The other stromelysins, MMP-10 and -11, were shown to be absent or present in low levels only in joint tissues.⁷

In this study a selective fluorogenic assay is used to measure MMP-3 activity in the SF of fetal, juvenile and adult horses, and in the SF of joints affected by osteochondrosis (OC) or osteoarthritis (OA). The objective of this study was to focus specifically on MMP-3 activity during development, in two common equine joint disorders and to relate presented findings with previously reported general MMP activity in the same samples.

Methods

EXPERIMENTAL DESIGN

Stage of development

SF from normal metacarpophalangeal joints of equine fetuses ($n=10$), 5 month old foals ($n=10$), 11 month old foals ($n=10$), and adult horses ($n=10$, age (mean (SD)) 7.3 (1.8) years, range 4–10 years) was obtained (2–4 ml) within four hours after death, centrifuged

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Matrix metalloproteinases (MMPs) have an integral role in connective tissue turnover. Collagenases (MMP-1, -8 and 13) cleave interstitial collagen triple helices. Gelatinases (MMP-2, and -9) act mainly on unwound collagen and gelatin. Stromelysins (MMP-3, -10 and -11) can degrade proteoglycans, collagen types IX, XI and type II N-telopeptides, and

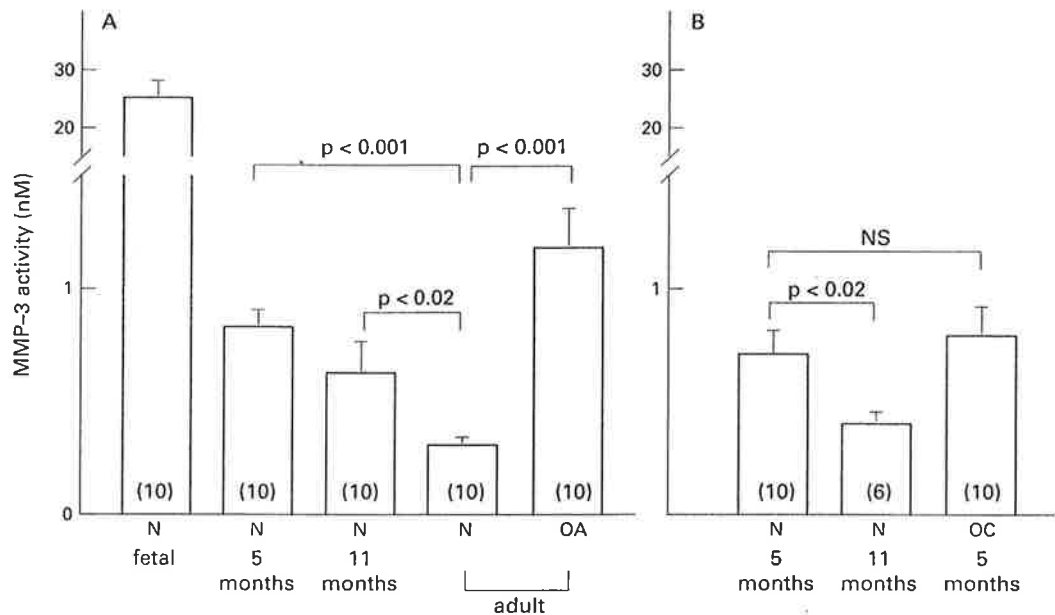


Figure 1 (A) MMP-3 activity (nM; mean (SEM)) in SF of the normal fetal, normal juvenile (5 and 11 months), normal adult (mean age 7.3 years, range 4–10 year), and osteoarthritic (mean age 8.2 years, range 4–11 year) equine metacarpophalangeal joints. (B) MMP-3 activity (nM; mean (SEM)) in SF of the normal juvenile (5 and 11 months), and osteochondrotic (5 months) equine tarsocrural joints. MMP=matrix metalloproteinase; N=normal joints; NS=not significant; OA=osteoarthritic joints; OC=osteochondrotic joints; SF=synovial fluid.

(10 000 × g, 15 minutes), and the resulting cell free supernatant was frozen at -20°C until further analysis. Similar collection procedures were performed for normal tarsocrural joints of 5 month old (n=10) and 11 month old (n=6) foals. No effect of collection time (up to 12 hours) was observed on MMP activity.

Joint disorders

SF was collected from 10 adult horses (age mean (SD) 8.2 (2.3) years, range 4–11 years; age matched with the above mentioned control animals; $p > 0.05$), with evidence of OA of the metacarpophalangeal joint and from 10, 5 month old foals with OC (as reported elsewhere).⁵

MMP-3 ACTIVITY ASSAY

After 10-fold dilution in buffer A (50 mM TRIS (pH 7.5), 150 mM NaCl, 5 mM CaCl_2 , 1 μM ZnCl_2 , 0.01% Brij-35) MMP-3 activity in SF was measured as described elsewhere^{11,12}: 90 μl diluted SF was added to 22.5 μl of the selective MMP inhibitor CP138521 (2.4 μM ; Pfizer Central Research, Groton, CT, USA), 22.5 μl EDTA free Complete (a mixture of non-MMP inhibitors; 2 tablets in 2.5 ml buffer A; Boehringer Mannheim, Germany), and 45 μl of the substrate TNO003-F (4 μM).^{11,12} Incubations were performed in black round bottom 96 well plates (Dynatech, Denkendorf Germany) at 37°C . Increase in fluorescence was followed in a Cytofluor II ($\lambda_{\text{ex}} = 360 \text{ nm}$, $\lambda_{\text{em}} = 490 \text{ nm}$; PerSeptive Biosystems). Incubation time was 3.5 hours and consisted of 20 fluorescence measurements. The initial velocity of substrate turnover (linear increase in fluorescence over time) was used as a measure for enzyme activity. The concentration of active MMP-3 in the synovial fluid was calculated using a calibration curve of MMP-3 (human recombinant

MMP-3, Pfizer, Central Research, Groton, CT, USA) spiked to SF.^{11,12}

Previously reported general MMP activities of the same SF samples were used to present the ratio of MMP-3/general MMP activity calculated individually for each SF sample.⁵

STATISTICAL ANALYSIS

Data are presented as the mean (SEM). Differences between groups were tested by one way analysis of variance. Fisher's least significant difference multiple comparisons test of the means was applied as post hoc test. Differences were considered significant when $p < 0.05$.

Results

STAGE OF DEVELOPMENT

Extremely high stromelysin activity (30-fold to 80-fold higher than in normal juvenile and adult joints) was observed in fetal metacarpophalangeal joints. After birth, stromelysin activity gradually declined in SF from normal joints (5 months > 11 months > adult; fig 1A). Consistent with these findings, MMP-3 activity in SF of normal tarsocrural joints also gradually decreased during maturation (fig 1B). Stromelysin activities in SF from the tarsocrural joints and from the metacarpophalangeal joints of foals were identical ($p > 0.05$ for each age group).

JOINT DISORDERS

With respect to OC, MMP-3 activity in SF from tarsocrural joints with OC did not differ significantly from activity in SF from normal age matched tarsocrural joints (fig 1B). MMP-3 activity in SF of osteoarthritic adult metacarpophalangeal joints was almost four-fold higher than in the SF of normal adult metacarpophalangeal joints (fig 1A).

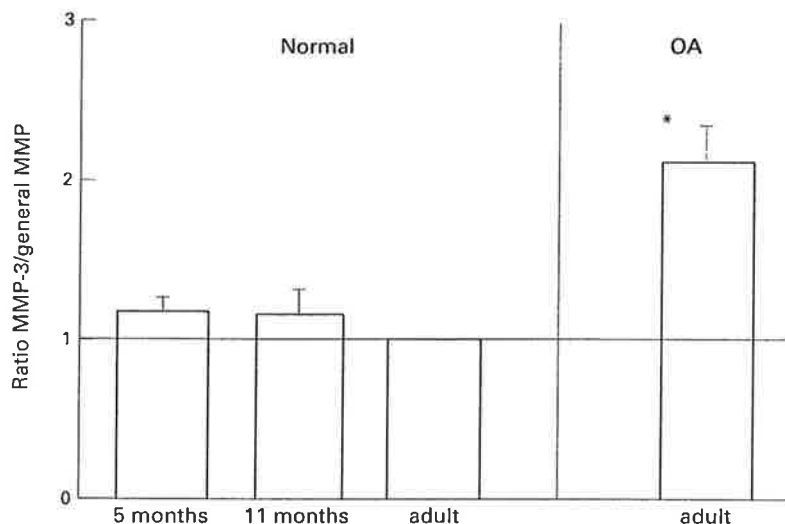


Figure 2 The ratio of MMP-3 activity/general MMP activity in SF of the metacarpophalangeal joint. Normal adult set at 1. $p^* p < 0.05$ versus normal adult.

MMP-3 ACTIVITY COMPARED WITH ACTIVITY OF OTHER MMPs

Figure 2 gives the ratio of MMP-3 activity and general MMP activity for the metacarpophalangeal joint.⁵ In normal joints, at all ages, a ratio MMP-3/general MMP identical to one was observed. Similar findings were done for normal tarsocrural joints at the age of 5 months (0.99 (0.15)) and 11 months (0.86 (0.15)). This indicates that MMP-3 gene expression, activation of proenzyme, or inhibition by TIMP is not different from general MMP activity during development. In OA joints the ratio MMP-3/general MMP is twofold higher than in normal joints ($p < 0.01$).

Discussion

This study shows a gradual decrease in stromelysin activity during maturation starting with very high activities in the fetal joint. The amounts of active MMP-3 in both the metacarpophalangeal and tarsocrural joint fluid were similar, which suggests that the age related decline in MMP-3 activity can be considered a systemic effect—that is, the same for all synovial joints in an individual animal. In fetal metacarpophalangeal joints MMP-3 activity was as much as 80-fold higher than in the adult horse. These very high activities are consistent with the rapid tissue turnover in the fetal joint. The influence of developmental stage on MMP activity emphasises the need for age matched controls when studying pathological conditions, especially when growing individuals are concerned such as in OC or other developmental orthopaedic diseases.

Early diagnosis of OA is a major problem, both in human and veterinary medicine.^{4 10 13-15} In the horse, Clegg *et al* showed that MMP-2 and MMP-9 activities are significantly increased in both aseptic and septic joint diseases.¹³ Balkman *et al* showed that MMP-3 mRNA expression is low in normal cartilage and synovial membrane, but increased in arthritic cartilage.¹⁴ Recently, we found a twofold higher general MMP activity in osteoarthritic SF than in controls.⁵ In this

study we show that the activity of stromelysin, is even more increased (fourfold higher).

This study showed that specific MMP-3 activity was not significantly higher in OC tarsocrural joints when compared with age matched control joints. Also, general MMP activity was not increased as shown previously in the same SF samples.⁵ This again is an indication that metalloproteinase mediated matrix destruction is not of major importance in OC.

During growth, development and maturation, the ratio MMP-3/general MMP activity did not change in normal, healthy joints. In contrast, this ratio was more than twofold increased in OA joints compared with normal joints, indicating selective upregulation of gene expression and/or activation of proMMP-3 and/or inhibition by TIMP in OA pathology.

It is concluded that stromelysin activity in equine SF reflects physiological and pathophysiological tissue turnover of cartilage. The activity of MMP-3 is more outspoken in pathological tissue degradation as occurs in OA than in physiological turnover during maturation.

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