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Temporal production of nitric oxide and Prostaglandin E_2 in media of explant cultures of equine synovial membrane and articular cartilage

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Summary

The local mediators nitric oxide (NO) and prostaglandin E_2 (PGE_2) play a role in the cascade of extracellular matrix breakdown in articular cartilage. In the present study the spontaneous production of NO and PGE_2 in explant cultures of equine synovial membrane and articular cartilage and their concentration over time in culture was investigated and correlated. Synovial membrane and articular cartilage was harvested from metacarpophalangeal joints of 33 horses during arthroscopic surgery (n=4) or after being slaughtered/euthanised for reasons other than infectious or metabolic disease (n=29). Tissues were cultured as explant cultures in the presence of 4 ml Ham's F-12 medium, supplemented with streptomycin (100 µg/ml) and penicillin (100 U/ml) and incubated in a 5% CO_2 and water saturated atmosphere for 8 days. Results showed that generally equine synovial membrane produced less NO than articular cartilage explant cultures with comparable wet weights/ml of media, but significantly more PGE_2 ($P<0.05$). The concentrations of both mediators were relatively stable over the first 4 days of culture, at least in the group of 25 mg wet weight/ml of media. In this group, there was a weak tendency noticed for NO to decrease, and PGE_2 to increase over time with day 4 being the intersection for both. Tissue cultures of articular cartilage with 12.5 mg wet weight/ml of culture media produced less reliable results, especially with NO measurements, which was thought to be associated with the sensitivity of the assay. It is recommended to collect conditioned media for the measurements of NO and PGE_2 within the first 4 days of explant culture, since after this time period culture effects may mask the results at least for NO measurements. Both mediators may be factors that play an important role in the local mediation of joint inflammations.

Keywords: nitric oxide, prostaglandin E_2, synovial membrane, articular cartilage, equine

Die zeitlich bedingte Produktion von Stickoxid (NO) und Prostaglandin E_2 (PGE_2) in Medium von Gewebekulturen aus Synovialmembran und Gelenkknorpel von Pferden

Die lokalen Mediatoren, Stickoxid (nitric oxide = NO) und Prostaglandin E_2 (PGE_2) spielen eine Rolle in der Kaskade des Abbaus der extrazellulären Matrix von Knorpel und Knochen. In der vorliegenden Studie wurde die spontane Produktion und Konzentration von NO und PGE_2 in Gewebekulturen aus Synovialmembran und Gelenkknorpel von Pferden über eine Zeit von 8 Tagen untersucht und korreliert. Bei 33 Pferden wurde während der Arthroskopie des Gelenkes (n=4) oder zum Zeitpunkt der Schlachtung (n=29) Synovialmembran und Gelenkknorpel aus dem Fesselgelenk entnommen. Das Gewebe wurde in der Gewebekultur (12.5 mg/ml Medium und 25 mg/ml Medium) in Ham's F-12 Medium, angereichert mit Streptomycin (100 µg/ml) und Penicillin (100 U/ml), unter Standardbedingungen von 5% CO_2 und mit Wasser gesättigter Atmosphäre für 8 Tage inkubiert. Es wurde gezeigt, dass allgemein die Synovialmembran Gewebekulturen deutlich weniger NO produzierten als die Gelenkknorpel Gewebekulturen mit vergleichbarem Gewicht (25 mg/ ml. Hingegen produzierten sie deutlich mehr PGE_2 ($P<0.05$). Die Konzentrationen beider Mediatoren waren relativ stabil in den ersten 4 Tagen in der Gewbekultur, zumindest in der Gruppe mit 25 mg/ml Nassgewicht. In dieser Gruppe wurde eine schwache Tendenz beobachtet, dass NO Konzentrationen niedriger, jedoch PGE_2 Konzentrationen im Verlauf der Zeit höher wurden. Der vierte Tag in der Gewebekultur stellte sich dabei als Schnittpunkt heraus.Messungen der beiden Mediatoren aus Gewebekulturen mit 12.5 mg/ml Nassgewicht zeigten weniger zuverlässige Resultate, speziell für die NO Konzentrationen, was der fraglichen Sensitivität des NO Testes (Griess-Reaktion) zugeschrieben wurde. Es wird empfohlen für derartige Messungen von NO in der Gewebekultur Proben von Kulturmedium innerhalb der ersten 4 Tage der Gewebekultur zu entnehmen, da nach diesem Zeitpunkt künstliche Kultureffekte die Resultate verfälschen könnten. Beide Mediatoren werden als interessante Faktoren für die zukünftige Erforschung von degenerativen Gelenkerkrankungen angesehen.

Schlüsselwörter: Stickoxid, Prostaglandin E_2, Synovialmembran, Gelenkknorpel, Pferd

Introduction

Inflammatory mediators, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), as well as enzymes, such as Matrix metalloproteinases (MMPs), have been shown to be involved in degrading extracellular matrix in osteoarthritis (*Martel-Pelletier* et al., 1985; *May* et al., 1991; *Palmer* and *Bertone*, 1994; *Evans* et al., 1995). The exact role of each of these mediators and MMPs, the sequence of their natural appearance in normal and osteoarthritic joints, and their correlation with each other has been subject of earlier investigations (*McCarty* and *Koopman*, 1993; *Palmer* and *Bertone*, 1994) but remains uncertain.

Inducible NO production was detected in cell cultures of human, lapine, bovine and equine origin soon after stimulation with interleukin-1 (IL-1) and lipopolysaccarides (LPS) of bacterial wall origin (*Palmer* et al., 1993; *Evans* et al., 1995; *Frean* et al., 1997). It has been shown that NO is capable of inducing PGE_2 production in rodent synovial membrane (*Stefanovic-Racic* et al., 1993a) and articular cartilage cell cultures (*Evans*, 1992; *Maier* et al., 1992; *Häuselmann* et al., 1995). It also has been implicated in the activation of MMP production by chondrocytes (*Murrell* et al., 1995). Low concentrations of NO seem to enhance, whereas high

concentrations seem to inhibit PGE_2 synthesis in cell cultures of articular cartilage (*Stadler* et al., 1991).
Elevated concentrations of PGE_2 are known to be involved in joint disease (*Bertone* et al., 1993; *Hawkins* et al., 1993; *Gibson* et al., 1996) as well as in cases of increased bone resorption (*Raisz*, 1993). Even though PGE_2 in association with cartilage matrix breakdown was thought to be upregulating the production and increasing the release of MMP in synoviocytes and in chondrocytes, this role was questioned by more recent investigations (*Bandara* and *Evans*, 1992).
NO and PGE_2 were shown to be produced by synoviocytes and chondrocytes of different species as an immediate reaction to a variety of stimuli (*Stadler* et al., 1991; *Evans*, 1992; *May* et al., 1992; *Mort* et al., 1993; *Palmer* et al., 1993; *Frean* et al., 1997). Most of these studies were carried out with normal synovial membrane and articular cartilage specimens that were subjected to stimulation with cytokines, such as interleukin-1 (IL-1) (*Bandara* et al., 1989; *May* et al., 1992; *Stefanovic-Racic* et al., 1993b; *Frean* et al., 1997), tumor necrosis factor β (TNF-β) (*Stadler* et al., 1991) and lipopolysaccharides from bacterial wall fragments (LPS) (*Maier* et al., 1992; *May* et al., 1992; *Birkedal-Hansen*, 1993; *MacDonald* et al., 1994; *Frean* et al., 1997).
Measurements of NO production by monolayer cell cultures of equine synovial membrane and articular cartilage after stimulation with IL-1 β and LPS showed that both cell types were capable of releasing this mediator, but that synoviocytes produced less than chondrocytes (*Evans* et al., 1996; *Frean* et al., 1997). In the horse, PGE_2 concentrations have been measured in synovial fluid and media of explant cultures of cartilage and synovial membranes in both normal and osteoarthritic joints with increased levels of concentrations in disease (*May* et al., 1988; May et al., 1991; *Bertone* et al., 1993).
Measurement of the spontaneous production of NO (9) and PGE_2 in culture media of equine cartilage and synovial membrane have been performed, however comparisons of concentrations and correlation over time of these two mediators have not been investigated to this date, neither in synovial fluid, nor in explant cultures of equine tissue.
In previous cell culture studies, the time of conditioned media collection ranged widely from 2 to 6 and even up to 14 days after the tissue was harvested and placed in culture (*Dayer* et al., 1986; *May* et al., 1990; *May* et al., 1992; *MacDonald* et al., 1994). However these investigations did not assess the most accurate time of media collection for measurement of spontaneous production of these mediators and enzymes.
Therefore this study was designed to determine the best time to collect media samples from explant cultures of equine synovial membrane and articular cartilage. Apart from the ideal time for media collection, two different amounts of wet weight of tissue samples in relation to the cell culture media to measure NO and PGE_2 concentrations were studied. It seemed important to have some guidelines about tissue samples available, if future studies would be performed in clinical cases, where articular cartilage samples would not readily be obtained without harming the patients. It was felt, that scoring of joint disease was not necessary for the goals of this study. This was supported by separate investigations by the same authors in horses (*Rechenberg* et al., 1999a), dogs[1] and sheep (*Akens* et al., 1998). Development of NO and PGE_2 over time in culture was not influenced by the stage of disease of the tissues where samples were taken from. It was hoped that this study would serve as a basis for future investigations of the spontaneous production of other important substances involved in articular cartilage degradation in an in vitro explant culture system.

Material and methods

Materials
The material was obtained from the following commercial suppliers: culture media, antibiotics, Gey's balanced salt solution from GIBCO, Life technology (Basel, Switzerland), tissue culture plastic ware and chemicals of analytical grade were purchased from Sigma (Buchs, Switzerland).

Methods
Sample collection and culture: Equine synovial membrane and articular cartilage tissue was harvested aseptically from metacarpophalangeal joints of 33 horses during arthroscopic surgery (n=4) or after being slaughtered/euthanised for reasons other than infectious or metabolic disease (n=29). In arthroscopic surgeries, articular cartilage samples were harvested from the articular surface of the chips removed. In the post mortem cases, articular cartilage samples were removed from the weightbearing area of the distal metacarpus close to the sagittal ridge. Synovial membrane samples were always taken from the anterior aspect of the fetlock joint. The duration and treatment of the horses for OA before taking the samples was variable, although they all were off medication for at least 24 hours in case of surgery and longer than a few days before slaughter. Samples for explant cultures were randomly assembled independent of age, sex or state of joint disease. From each joint tissue samples were assigned to three groups: synovial membrane of 100 mg wet weight/4 ml; articular cartilage of 50 mg wet weight/4 ml; and articular cartilage of 100 mg wet weight/4 ml of culture media. Tissue samples were trimmed, weighed, washed in Gey's Balanced Salt Solution and the specimens placed into 25cm^2 plastic culture flasks in the presence of 4 ml Ham's F-12 medium, supplemented with streptomycin (100 µg/ml) and penicillin (100 U/ml). Cultures were incubated in a 5% CO_2 and water saturated atmosphere for 8 days. From each flask 2 ml of conditioned media were removed on day 1, 2, 4, 6 and 8 and replaced with fresh media as listed above. Media samples were stored at –20°C until used for the assays.

Nitric oxide assay: NO determinations were based on the Griess reaction *(Green* et al., 1982; *Kawcak* et al., 1997; *Hardy* et al., 1998), a spectrophotometric method where nitrite (NO_2-) is determined as a stable endproduct of NO.

Nitrite represents 51.5 ± 3.5% of the total amount of the nitric oxides produced (29), as controlled by the reduction of NO_3- to NO_2- using a cadmium column in a modified HPLC system (11). Optical densities were measured at 550 nm with a microplate reader[2]. Duplicates were averaged and concentrations were calculated from optical densities according to the standard row (0, 1, 2, 4, 8, 16, 32, 64 and 128 µM concentrations) using a linear regression with R^2 ~ 1.000. The detection limit of this assay was 1 µM.

PGE_2 assay: PGE_2 concentrations were determined using a competitive enzyme immunoassay EIA-kit[3] designed for measurement of $PPGE_2$ in biological fluids that had been used in horses before (12, 15). The test was used according to the manufacturer's instructions. Absorbency was read at 405 nm in a microplate reader[1]. The duplicates were averaged and the optical densities were transformed to concentrations by using a linear scale for absorbency (y-axis) plotted versus a log scale for the PGE_2 standard concentrations (x-axis) in pg/ml (sigmoid curve). Standard concentrations of PGE_2 were run with a range of 0–5000 pg/ml. If samples were not within the range of the standard curve, they were diluted and samples were assayed again. The dilution factor was corrected when calculations for concentrations were performed. The detection limit of this assay is 1.5 pg/ml.

Statistics: Mean concentrations were calculated. Concentration levels of NO and PGE_2 in culture media over time within groups were compared using nonparametric Friedman-tests. Differences in concentrations of NO and PGE_2 between groups were assessed with multiple Wilcoxon-rank-sum-tests. Non parametrical tests were used due to the assumption that samples were not evenly distributed, since stage of disease was not considered. Correlation matrices were calculated to investigate the relationship between NO and PGE_2 and their consistency over time. $P<0.05$ was considered to be statistically significant. Statistics were performed using a commercial software package[4].

Results

NO concentrations

Synovial membrane explants produced low levels of NO with mean concentrations of 3.735 µMol on day 1, decreasing to 3.361 µMol on day 4, and dropping further to 2.626 µMol on day 6 and 2.466 µMol on day 8 (Fig. 1). The overall decrease, however, was not statistically significant ($P > .05$) (Tab. 1).

The NO production of the 50 mg articular cartilage explants was somewhat irregular and did not reveal any significant pattern over time. Mean concentrations of NO varied from 3.371 µMol on day 1, decreasing to 2.833 µMol on day 2, back up to 3.025 µMol on day 4, undulating again to 2.963 µMol on day 6 and 3.276 µMol on day 8 ($P>0.05$).

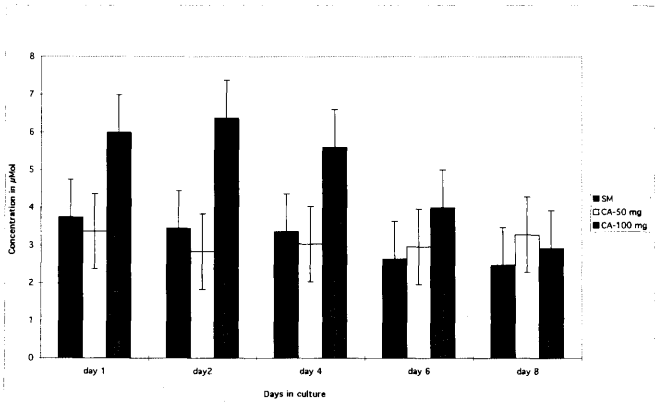

Fig. 1 Mean concentrations of NO production in synovial membrane (SM), 50 mg articular cartilage (CA-50 mg), and 100 mg articular cartilage explants (CA-100 mg). Concentrations of NO are given in µMol. Interaction bar plots represent standard deviations. Note that NO concentrations are higher in articular cartilage (CA-100mg) compared to synovial membrane. In addition, concentrations are relatively stable in the first 4 days of culture.

Mittlere NO-Konzentration in Gewebekulturen von Synovial-membran (SM), 50 mg (CA-50 mg), und 100 mg (CA-100 mg) Gelenkknorpel. Die NO-Konzentration ist in µMol mit Standardabweichungen angegeben. Die NO Konzentrationen im Gelenkknorpel sind höher im Vergleich zu Gewebekulturen von Synovialmembran. Allgemein sind die Konzentrationen in den ersten 4 Tagen der Kultur relativ stabil.

Higher levels of NO were produced by the 100 mg articular cartilage explants, where mean concentrations on day 1 were 5.991 µMol, with a slight tendency to increase on day 2 (6.373 µMol), and slightly decreasing on day 4 (5.601). The concentrations dropped more pronounced on day 6 (3.995 µMol) and day 8 (2.920 µMol). The change of concentration of NO over time was statistically significant for the 100 mg articular cartilage explants ($P < 0.05$).

Comparisons of NO production between groups showed significant differences for the synovial membrane and the 100 mg articular cartilage explants (Tab. 1) up to day 6 ($P< 0.05$), whereas on day 8 a significant difference ceased to exist ($P> 0.05$). Articular cartilage explants with 100 mg wet weight/4 ml of medium produced almost the double amount of NO within the first 4 days of culture compared to synovial membrane explants of the same weight. Differences between the 50 mg and 100 mg articular cartilage explants were highly significant from day 1–2 ($P< 0.005$), significant on day 4 ($P< 0.05$) and insignificant on day 6–8 ($P> 0.05$).

PGE_2 concentrations: The PGE_2 concentrations of synovial membrane explants were 3.312 ng/ml on day 1 and increased to 6.235 ng/ml on day 8 (Fig. 2). The increase over time, however, was statistically insignificant ($P>0.05$)

[1] *Urs Abbuehl:* Biochemical changes at the bone cement interface associated with aseptic loosening of the implant, using canine hip prosthesis as a model, Dissertation, University of Zurich, Switzerland,1999

[2] Microplate Reader, MR 7000, Dynatech Produkte AG, 8423 Embrach, Switzerland, Filter: 550nm for NO, 405 nm for PGE_2 measurements

[3] PGE_2 EIA Kit, PerSeptive Diagnostics, Cambridge, MA 02138, USA

[4] StatView®, Version 4.5, Abacus concepts Inc., Berkeley, CA

(Tab. 2). The 50 mg and 100 mg articular cartilage explants showed considerably lower concentrations of PGE_2 than synovial membranes overall, with no significant variation over time and differences between 50 and 100 mg explants. Both groups had the highest concentrations on day 1 and 6 (50 mg articular cartilage explants with 1.227 ng/ml; 100 mg articular cartilage explants with 1.281 ng/ml on day 1; 50 mg articular cartilage explants with 1.877 ng/ml and 100 mg articular cartilage explants with 1.079 ng/ml on day 6) with lower levels on day 2 and 4, more pronounced in the 100 mg articular cartilage explants. The total PGE_2 concentrations of the 50 mg were higher compared to the 100 mg articular cartilage explants, despite the fact that the wet weight of tissue /ml of media was higher.
The differences of PGE_2 concentrations between synovial membrane and the 100 mg articular cartilage explants were not significant on day 1 ($P > 0.05$), but highly significant on day 2, 4 and 8 ($P < 0.01$) and significant on day 6 ($P<0.05$). Comparisons between the 50 mg and 100 mg articular cartilage explants were significant on day 4 ($P < 0.05$), just missed significance on day 1 and 8, and were insignificant on day 2 and 6 ($P > 0.05$).

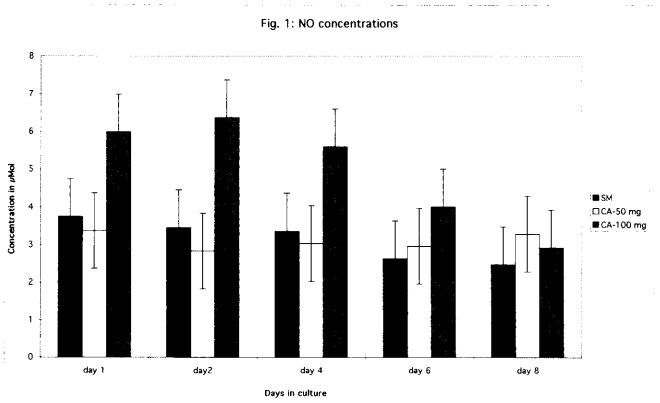

Fig. 2: Mean concentrations of PGE_2 production in synovial membrane (SM), 50 mg articular cartilage (CA-50 mg), and 100 mg articular cartilage explants (CA-100 mg). Concentrations of PGE_2 are given in ng/ml. Interaction bar plots represent standard deviations. Note that synovial membrane produces higher PGE_2 concentrations compared to articular cartilage.

Mittlere PGE_2 Konzentration in Gewebekulturen von Synovial-membran (SM), 50 mg (CA-50 mg), und 100 mg (CA-100 mg) Gelenkknorpel. Die PGE_2-Konzentration ist in ng/ml mit Standardabweichungen angegeben. Die PGE_2 Konzentrationen in Synovialmembranen sind höher im Vergleich zu Gewebekulturen von Gelenkknorpel.

The correlation between NO and PGE_2 concentrations of all groups were calculated within groups and did not reach statistical significance in any of them ($P > 0.05$) (Tab. 3). However, there was a tendency for weak negative correlations between NO and PGE_2, such that high levels of NO were parallel to low PGE_2 concentrations for the 100 mg tissue samples of synovial membrane and articular cartilage. This was not true for the correlation within the 50 mg articular cartilage explants. The 50 mg and 100 mg articular cartilage explants had a very high correlation for PGE_2 concentrations, but not for NO.

Tab. 1: Results of the Wilcoxon-rank-test comparing NO concentrations between groups. (SM: synovial membrane; CA-50 mg: 50 mg articular cartilage; CA-100 mg: 100 mg articular cartilage explants).

Resultate des Wilcoxon-Rang Testes, wo Unterschiede der NO-Konzentration zwischen den verschiedenen Gruppen verglichen wurden. (SM: Synovialmembran; CA-50 mg: 50 mg, CA-100 mg: 100 mg Gelenkknorpel Gewebekulturen).

Group	Variable	Day	Significance
Synovial membrane 100mg / 100 mg cartilage	NO	1	P=0.026*
		2	P=0.005**
		4	P=0.036*
		6	P=0.001**
		8	P=0.102
50 mg / 100 mg cartilage explant	NO	1	P=0.003**
		2	P=0.001**
		4	P=0.002**
		6	P=0.021**
		8	P=0.499

Tab. 2: Results of the Wilcoxon-rank-test comparing PGE_2 concentrations between groups. (SM: synovial membrane; CA-50 mg: 50 mg articular cartilage; CA-100 mg: 100 mg articular cartilage explants).

Resultate des Wilcoxon-Rang Testes, wo Unterschiede der PGE_2-Konzentration zwischen den verschiedenen Gruppen verglichen wurden. (SM: Synovialmembran; CA-50 mg: 50 mg, CA-100 mg: 100 mg Gelenkknorpel Gewebekulturen).

Group	Variable	Day	Significance
Synovial membrane 100mg / 100 mg cartilage	PGE2	1	P=0.053
		2	P=0.006**
		4	P=0.013*
		6	P=0.021*
		8	P=0.007**
50 mg / 100 mg cartilage explant	PGE2	1	P=0.053*
		2	P=0.075
		4	P=0.023**
		6	P=0.148**
		8	P=0.052

Tab. 3: Correlations between NO and PGE_2 for synovial membrane, 50 mg and 100 mg articular cartilage explants on day 1, 2, 4, 6 and 8 of measurements.

Korrelationen zwischen NO und PGE_2 Konzentrationen der Synovialmembran und Gelenkknorpel Gewebekulturen am Tag 1, 2, 4, 6 und 8 der Messungen.

Correlations Pearson's (r)	Corr. coeff. day 1	Corr. coeff. day 2	Corr. coeff. day 4	Corr.coeff. day 6	Corr.coeff. day 8
Synovial membrane	-.404	-.093	-.430	-.261	-.063
50 mg cartilage	-.004	.068	-. 017	.130	.045
100 mg cartilage	.127	-.053	-.017	.134	-.147

Discussion

Our study showed that equine synovial membrane and articular explant cultures spontaneously release NO and PGE_2 into culture media confirming earlier published results (*May* et al., 1988; *Frean* et al., 1997). Due to the limited collec-

tion of media samples from the cultures, samples using specific inhibitors for NO, PGE_2 and NMPs were not run parallel to the explant cultures of synovial membrane and articular cartilage for this study. However, previous tests performed with equine, bovine and canine tissue verified that the initial addition of NG-monomethyl-L-arginine (L-NMA) almost completely abolished the production of NO, the addition of indomethacin the synthesis of PGE_2 (data not shown).

The heterogenicity of the selection of joints and their stage of disease where the samples were taken from was not optimal in this study; however, later similar investigations in equine synovial membrane and articular cartilage, where all tissues were taken from the same joints and where differences were made between stage of disease, confirmed the results of this study in all points (*Rechenberg* et al., 1999a; *Rechenberg* et al., 1999b)

Comparing the groups, the wet weight of tissue/ml of medium was judged to be a factor to consider. An amount of 25 mg wet weigh /ml of medium seemed desirable to obtain reliable results, at least for measuring NO concentrations in articular cartilage explants. These results could be different, if media changes were less frequent and concentrations of both substances allowed to accumulate over 2–4 days. However, a compromise between optimal culture conditions for the tissue and measurable concentration of substances within the culture media had to be found. Tissue samples of 25 mg wet weight/ml of media seemed to fulfill these requirements.

Equine synovial membrane explants produced low levels of NO, whereas articular cartilage produced almost double the concentrations of NO within the first 4 days of culture. This observation has to be considered in view of the fact that synovial membrane contains more cells per unit measured compared to articular cartilage where a lower cell density is to be expected per mg of tissue. This could have been verified with measuring DNA content of tissue samples, but was not considered necessary in this study. Furthermore our data is consistent with another recently published study of nitric oxide production in equine articular cells in vitro, where monolayer cell cultures of equine synovial membrane and articular cartilage cells were stimulated with LPS and IL-1β (*Frean* et al., 1997). The same observation was made in monolayer cell cultures of human synoviocytes and chondrocytes, also after stimulation with IL-1 (*Stefanovic-Racic* et al., 1993a; *Evans* et al., 1995). In contrast to this, high levels of NO concentrations were measured in cell cultures of bovine and lapine synovial membrane as well as articular cartilage after stimulation with IL-1 (*Stefanovic-Racic* et al., 1994). Since in some European countries human material for cell cultures is not easily obtained for research purposes (for ethical reasons), it seems that the equine synovial membrane and articular cartilage cultures could serve as an interesting model for studies of NO production relative to human joint disease.

The concentration of NO was relatively consistent within the first 4 days of culture, after which the concentration dropped and returned to basic levels on day 6–8, at least in the articular cartilage samples of 25 mg/ml wet weight. It may be speculated that the irregular pattern observed in the samples with lower wet weights/ml was due to the sensitivity of the assay in low concentrations and not due to a true difference in concentrations. This sharp drop of NO concentrations was also observed in bovine and human cell cultures, where the highest concentration was found within the first 72 hours after stimulation of tissue with IL-1 (*Stadler* et al., 1991) and in other studies carried out by the same authors (*Akens* et al., 1998; *Rechenberg* et al., 1999a).

PGE_2 concentrations were significantly higher in synovial membrane than in articular cartilage explant cultures. The concentration over time showed a tendency to increase until day 6–8. This was in combination with a trend of parallel decreasing NO concentrations. Furthermore, PGE_2 concentrations were lower in the 100 mg cartilage explants compared to the 50 mg cartilage explants. Similar observations were made in lapine cell cultures where low levels of NO enhanced and high levels of NO inhibited PGE_2 concentrations (*Stadler* et al., 1991). Besides the tendency of negative correlations between these mediators, it could be speculated that the increase of PGE_2 concentration until day 8 may be partly influenced by culture effects over time, although the culture media were observed as indicated by the unchanged color (phenol red) of the medium.

The negative correlations between NO and PGE_2 did not reach significance in the synovial membrane and 100 mg articular cartilage explants. However, trends were observed. Missing significance could be due to the fact, that data for both explants were aggregated. As mentioned above, samples were randomly assigned, not considering stages of degenerative joint disease. If groups would have been seperated according to stage of disease. a more significant trend for negative correlations may have been found. It would be reasonable to expect that concentrations of NO as well as PGE_2 may be decreased or increased depending on the inflammatory status of the joints. This assumption is supported by other studies in our laboratory published elsewhere (*Akens* et al., 1998; *Rechenberg* et al., 1999a).

Even though our study was meant for research purposes to assess the best time for media collection and minimally required wet weight of tissue in relation to media, our results could have some clinical implications. If the tendency for the negative correlation between NO and PGE_2 could be verified in future studies, this correlation could be interesting for the clinical therapy of acute or chronic synovitis in the horse.

Conclusions

This study showed that explant cultures of equine synovial membrane and articular cartilage spontaneously release NO and PGE_2 into the culture media. Articular cartilage explants generally release more NO and less PGE_2 than syno-

vial membrane. For both mediators the concentrations are relatively stable within the first 4 days of culture, although there is a tendency for a weak negative correlation between slightly decreasing NO and increasing PGE_2 concentrations over time with day 4 being the point of intersection for both. For the determination of spontaneous production of NO and PGE_2 in culture media, it seems that collection of conditioned media for both parameters on day 4 of explant culture is advisable.

Furthermore it was demonstrated that the ratio of tissue to culture media should not be less than 25 mg wet weight/ml of media at least in articular cartilage samples, since concentrations of released mediators may be too dilute to be measured by the assays. This is especially true for NO determinations with the Griess reaction.

Our results indicated that the horse may be an interesting research model for studies with NO synthesis in human joint disease, since synovial membrane and articular cartilage of equine origin seemed to behave similarly to explant cultures of human origin. In addition, both NO and PGE_2 may be interesting factors to be measured in clinical cases of degenerative joint disease in the future as indicators of the effect of therapeutic interventions. Laboratory assays to determine NO and PGE_2 production in synovial fluid will have to be adapted before clinical application in equine joints. Future studies of this research group will be focused on this problem.

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