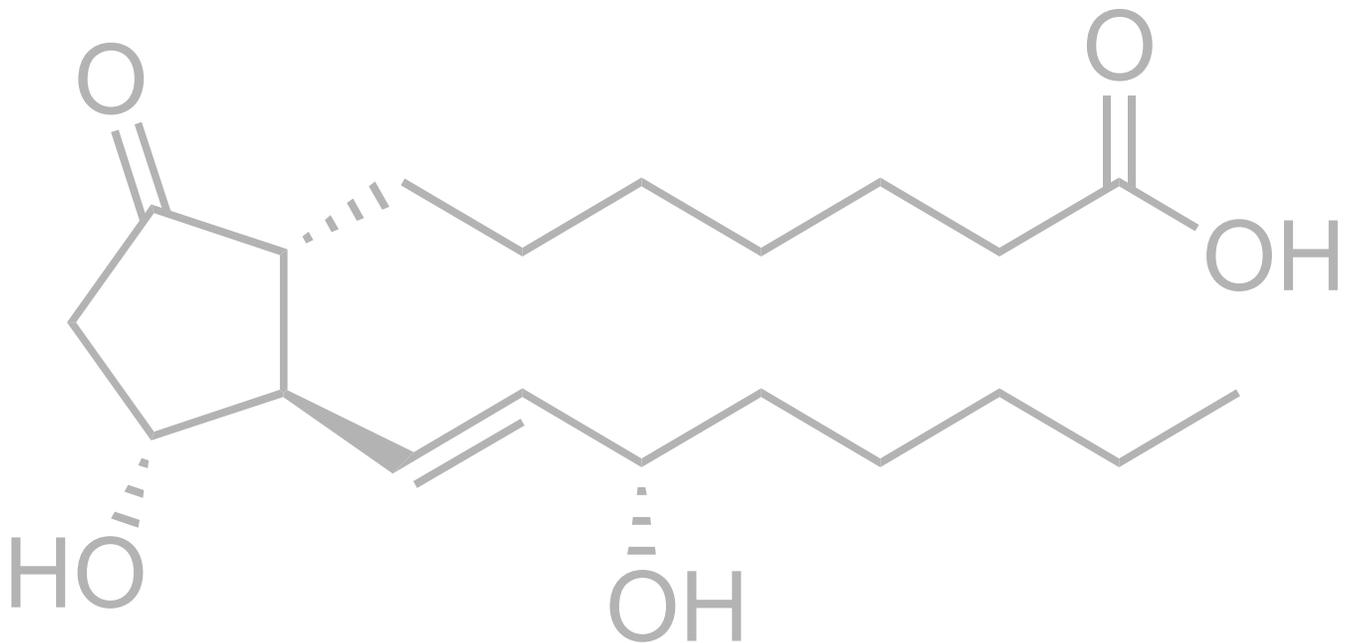


# EFFECTS OF MISOPROSTOL AND PROSTAGLANDIN E2 ON PROTEOGLYCAN BIOSYNTHESIS AND LOSS IN UNLOADED AND LOADED ARTICULAR CARTILAGE EXPLANTS

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# EFFECTS OF MISOPROSTOL AND PROSTAGLANDIN E2 ON PROTEOGLYCAN BIOSYNTHESIS AND LOSS IN UNLOADED AND LOADED ARTICULAR CARTILAGE EXPLANTS

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The effects of misoprostol, a prostaglandin E1 analog, and prostaglandin E2 on proteoglycan biosynthesis and loss were studied in unloaded and mechanically loaded mature bovine articular cartilage explants. The prostaglandins were administered daily at dosages of 0, 10, 100 and 1000 ng/ml for up to seven days, and proteoglycan biosynthesis determined by measurement of radiolabelled sulfate incorporation. The presence of misoprostol lead to a significant ( $p < 0.001$ ) dose-dependent inhibition (30%-50%) in proteoglycan biosynthesis which was also dependent on exposure time ( $p < 0.05$ ). A significant decrease in biosynthesis (34%) was also found for prostaglandin E2, but only at the highest dose (1000 ng/ml). Proteoglycan catabolism rates were not affected by either substance as assessed by loss of newly synthesized proteoglycan. The application of a continuous cyclic mechanical compressive load (stress of 1.0 MPa at 1 hertz for 24 hours) resulted in a significant inhibition of proteoglycan biosynthesis (up to 50%) as compared to unloaded explants. However, there was no additive effect when mechanical load and misoprostol or prostaglandin E2 were combined. These results suggest that prostaglandins may have a role in the degenerative and repair process in various forms of arthritis where elevated intra-articular levels of prostaglandin E2 are present.

## INTRODUCTION

A significant amount of prostaglandins (PGEs) are produced by cells and released into the joint during inflammation. Prostaglandins ability to enhance plasma extravasation and reduce edema is well recognized, as well as its ability to reduce swelling in inflamed joints<sup>1, 2</sup>. Ever since the discovery of an Eseries prostaglandin in inflammatory exudates<sup>3</sup>, the study of the specific effects of prostaglandins on cartilage tissue has expanded. Various studies have shown that production of PGEs by synovial cells occurs under conditions that simulate rheumatoid or antigen-induced, autoimmune arthritis<sup>4, 5</sup>. In addition, increased levels of interleukin-1 (IL-1) have been shown to significantly increase PGE levels in synovial fluid<sup>6</sup> and cartilage (2- 10 times)<sup>7</sup>. Therefore, elevated intra-articular prostaglandin levels may be associated with a number of different inflammatory conditions, such as trauma, meniscal injury and the various forms of arthritis, and may have a role in the degradative and repair processes.

Cartilage metabolism involves a continuous turnover of proteoglycan (PG), a vital polysaccharide structural component of cartilage tissue. The influence which E-prostaglandins have on articular cartilage metabolism remains complex and has yet to be fully understood. While prostaglandin levels in normal cartilage are relatively low (< 10 pg/mg wet weight)<sup>8, 9, 10</sup>, the concentration in the synovial fluid of patients with inflammatory diseases are elevated by as much as one-hundred times (~20 ng/ml)<sup>9, 11, 12</sup>. Several in vitro studies have shown an inhibitory effect of PGEs (PGE<sub>1</sub>, PGE<sub>2</sub>) on proteoglycan biosynthesis, but only at high concentrations ( $\geq 5\mu\text{g/ml}$ )<sup>13, 14, 15, 16, 17</sup>. While Lippiello et al.<sup>13</sup> found no change in proteoglycan catabolism after PGE administration, Fulkerson et al.<sup>15</sup> found that PGEs (7&14  $\mu\text{g/ml}$ ) increased proteoglycan loss after 3 hr and 24 hrs. It is well known that a decrease in proteoglycan content will result in diminished mechanical properties, which is detrimental to the functioning of the articular cartilage.

Recent work involving the prostaglandin E1 (PGE<sub>1</sub>) analog misoprostol and prostaglandin E2 (PGE<sub>2</sub>) have challenged the preceding studies. Misoprostol (Cytotec, G.D. Searle) is a more stable, longer-lived analog of prostaglandin E1 which is effective in preventing non-steroidal anti-inflammatory drug (NSAID)-associated gastric ulceration<sup>18</sup>. In explant cultures, Dingle<sup>19, 20</sup> and Shield<sup>2</sup> found that misoprostol had little or no effect on proteoglycan biosynthesis in normal cartilage but was stimulatory in osteoarthritic cartilage. They further showed that misoprostol (1-100 ng/ml) had the capacity to reverse the inhibition of proteoglycan biosynthesis caused by interleukin-1 (IL- 1) and Indomethacin, and to act syn-

ergistically with a variety of anti-inflammatory agents to enhance proteoglycan biosynthesis. Furthermore, Steinberg et al.<sup>21</sup> demonstrated that PGE<sub>2</sub>, inhibited cartilage breakdown in a dose-dependent manner. In their experiments, however, cartilage breakdown was initially stimulated by tumor necrosis factor or lipopolysaccharide endotoxin. Basal levels of cartilage breakdown were not affected by PGE<sub>2</sub> alone over a wide range of concentrations. According to their findings, the effects of misoprostol and PGE<sub>2</sub> on articular cartilage metabolism would appear to be beneficial.

NSAIDs are widely used to control joint inflammation. While their pharmacokinetics are as yet not fully understood, it is well recognized that NSAIDs reduce inflammation by inhibition of prostaglandin production by the synovial membrane. In rheumatoid patients treated with aspirin or indomethacin, prostaglandin concentrations in the synovial fluid were 75% lower compared to untreated patients<sup>11</sup>. Unfortunately, NSAID reduction of joint inflammation via inhibition of prostaglandin production results in a concomitant increase of IL-1 production, which has been shown to inhibit proteoglycan biosynthesis and increase proteoglycan loss<sup>7, 19, 22, 23</sup>. Furthermore, even when an NSAID does not affect proteoglycan biosynthesis it may still suppress prostaglandin biosynthesis, as found for indomethacin<sup>8</sup>. More important, however, is that these responses may be dependent on whether the cartilage is in an area of the joint which is mechanically compressed, that is, a loaded or unloaded region. Pahnoski and Brandt<sup>8</sup> reported that salicylate suppressed proteoglycan biosynthesis, and salicylate and indomethacin suppressed prostaglandin biosynthesis, to a greater extent in unloaded regions compared to loaded regions. They attributed this phenomena to a greater than 50% faster transport rate for the drugs in cartilage from the unloaded regions than the loaded regions (i.e., higher concentration at equivalent transport times), even though the water contents were similar.

These findings suggested that elevated prostaglandin concentrations may be beneficial in combination with NSAID use, and that they might be considered for clinical use via intra-articular injection after an incidence of joint inflammation. As a first step we decided to evaluate the effects of misoprostol and PGE<sub>2</sub> on cartilage proteoglycan biosynthesis under a variety of conditions, including dosage (10, 100 and 1000 ng/ml), exposure time (1 to 7 days) and whether the tissue was unloaded or mechanically loaded. The later condition was considered in order to evaluate the tissue's response to the drugs as would occur during normal joint function. Thus, we simulated the mechanical environment within the joint by imparting a cyclic mechanical compressive

stress (1 MPa at 1 hz for 24 hrs) to the tissue during drug exposure. These three conditions were studied individually and in combination. We also examined the effects of either prostaglandin on the loss of newly synthesized proteoglycans and on the tissue's water content. Finally, the free acid form of misoprostol, that is, the active metabolite circulating in the blood after oral or intramuscular administration, was compared to the form of misoprostol (the neat chemical form) that would most likely be administered intra-articularly.

## MATERIALS

### *Chemicals and Culture Media*

All materials were purchased from Gibco (Grand Island, NY) unless otherwise specified. Explants were incubated in culture media composed of Dulbecco's Modified Eagle's Medium (DMEM, 4.5g/liter glucose and 0.58g/liter L-glutamine), 10% fetal bovine serum, 50 µg/ml fresh ascorbic acid (Sigma), 1% HEPES Buffer solution (2.383 g/l), 1% antibiotic-antimycotic solution (100 units/ml penicillin G sodium, 100 µg/ml streptomycin sulfate, 0.25 µg/ml amphotericin B as Fungizone in 85% saline) and 1% L-glutamine solution (292.3 µg/ml). Aquasol scintillation fluid and radiolabelled sulfate ( $\text{Na}^{35}\text{SO}_4$ , 500 mCi/mg) were purchased from NEN Research Products (Boston, MA). Papain (25 mg/ml) was purchased from Sigma (St. Louis, MO). Misoprostol neat chemical and free acid forms were provided by G.D. Searle (Chicago, IL), while  $\text{PGE}_2$  was purchased from Ciba-Geigy (Somerset, NJ). Unless otherwise stated, the neat form of misoprostol was used and all drugs were mixed with media immediately prior to administration.

### *Cartilage Explant Cultures*

The occipital joints from mature, fully grown bovine steers (18-24 months old) were removed at the time of death. Using sterile instruments, the articular cartilage from the weight-bearing area of the joint was immediately dissected free from the underlying subchondral bone (strips approximately 8 mm long, 3 mm wide and 1 mm thick) and placed in serum-free DMEM containing 10% antibiotic-antimycotic solution for transportation to the laboratory (within 2 hours of death). Once in the laboratory, four millimeter diameter, fullthickness cartilage explants were removed using a biopsy punch and washed three times for 30 minutes each in DMEM containing a 10% antibiotic-antimycotic solution. Thereafter, each explant was incubated individually in 24-well culture plates containing 1 ml of culture media at 37°C, 95% humidity and 5%  $\text{CO}_2$ . The explants were incubat-

ed for a minimum of seven days postharvest prior to treatment to allow for stabilization of the proteoglycan biosynthesis<sup>24</sup>. The medium was changed every other day throughout the stabilization period.

## METHODS

Dose-response curves for proteoglycan biosynthesis were determined for misoprostol and  $\text{PGE}_2$  for concentrations of 10, 100 and 1000 ng/ml. At each concentration a time-response curve for PG biosynthesis was determined after 1, 2, 3, and 7 days of incubation. For incubation periods longer than a day the culture medium, containing fresh drug, was changed daily.

Control explants were defined as explants maintained in culture media without either drug, while the experimental samples consisted of explants in culture media containing the appropriate drug, concentration and time period. At the time of testing and for each treatment effect, the controls and experimental explants were animal and age-matched, that is, from the same harvest and at the same post-harvest time. A minimum of 6 explants were used for each treatment protocol.

### *Proteoglycan Biosynthesis*

Proteoglycan biosynthesis was determined from the incorporation of  $^{35}\text{S}$ -labelled sulfate (10 µCi/ml), which is proportionate to sulfated proteoglycan biosynthesis. Radiolabelled sulfate was added to the culture media 24-hours before the end of the experiment. In independent measurements sulfate incorporation was found to be linearly correlated with incubation time for up to 24 hours. After labelling the explants were washed twice for a total of 90 minutes to remove free tracer. Using freeze-thawed (killed) explants as controls, less than 1% of the radiolabelled sulfate remained in the tissue after washing. The explants were then digested overnight with papain (0.2 mg/ml) at 68°C, and the digest counted 24 hours after scintillate addition using a liquid scintillation counter (Beckman, Irvine, CA). Counts were corrected for radioactive decay and quench, and recorded in disintegrations per minute (dpm). Proteoglycan biosynthesis was normalized by wet weight, labelling time and media sulfate concentration, and reported in µmoles  $\text{SO}_4$  incorporation per milligram wet weight per hour.

### *Water Content*

Immediately after free  $^{35}\text{S}$ -sulfate desorption, each explant's wet weight was measured on an electrobalance (resolution 1 µg) (Cahn Instruments, Cerritos, CA), the explant lyophilized for 24-hours, and a dry weight

measured. Explant water content was calculated from the difference between the wet and dry weights. In mechanically loaded explants, wet weights were also measured after desorption, at which time the explants had fully swollen and reached their equilibrium water content (verified by independent measurements).

### Mechanical Load

The affect of misoprostol and PGE<sub>2</sub> on proteoglycan biosynthesis in mechanically loaded explants was studied using a mechanical explant test system (METS)<sup>25</sup>. The METS consisted of two ten-chamber loading fixtures and a load controller. Explants were placed into separate chambers and individually loaded by a 35  $\mu$ m porous filter attached to a pneumatic cylinder. The cylinder was controlled using an electropneumatic valve and signal function generator. A continuous, repetitive stress (sinusoidal waveform) of 1.0 MPa was applied to each explant at a frequency of 1 hertz for 24 hours. The loading fixtures were placed within the incubator, and the same incubation media and explant preparation and handling methods used as described above.

Explants (n=10) were treated with misoprostol or PGE<sub>2</sub> at a concentration of 1000  $\mu$ g/ml, during the 24-hour loading period. Sulfate incorporation was also measured during this time period. For comparison purposes, matched unloaded explants (n=10) with drugs, as well as matched loaded (n=10) and unloaded (n=10) explants without drugs, were also tested.

### Neat Chemical vs Free Acid Form

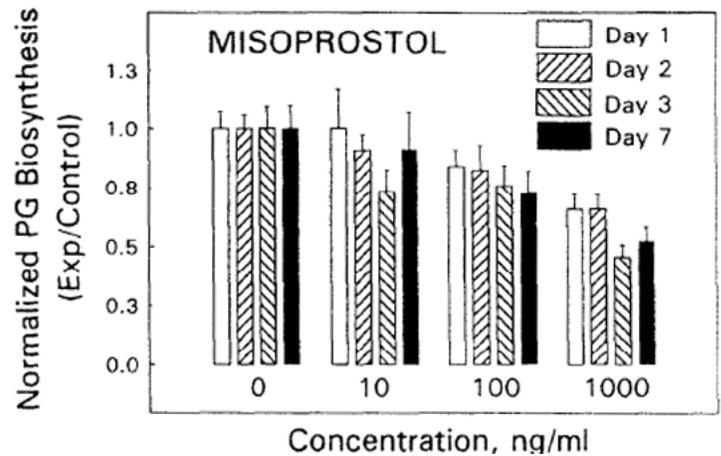
Separate experiments were performed to compare the original form of misoprostol, known as the neat chemical form, to the free acid form, the metabolite formed in-vivo after the drug has been absorbed and entered the bloodstream<sup>26</sup>. Misoprostol (neat) is an ester; however the ester group is almost instantly hydrolyzed to a carboxylic acid group, and this form of misoprostol (free acid) is the active metabolite circulating in the blood.

Explants were treated with 100  $\mu$ g/ml concentrations of the neat chemical or free acid forms of misoprostol, and either unloaded (n=25 each) or mechanically loaded (n=10 each) with 1.0 MPa at 1 Hz, for a 24-hour period during which sulfate incorporation was measured. These explants were compared to unloaded controls (n=25).

### Proteoglycan Loss

Explants were first incubated with <sup>35</sup>S-sulfate for 24 hours and then washed in fresh culture medium for an additional 24 hours (three medium changes, two 60-minute intervals plus 22 hours incubation) to re-

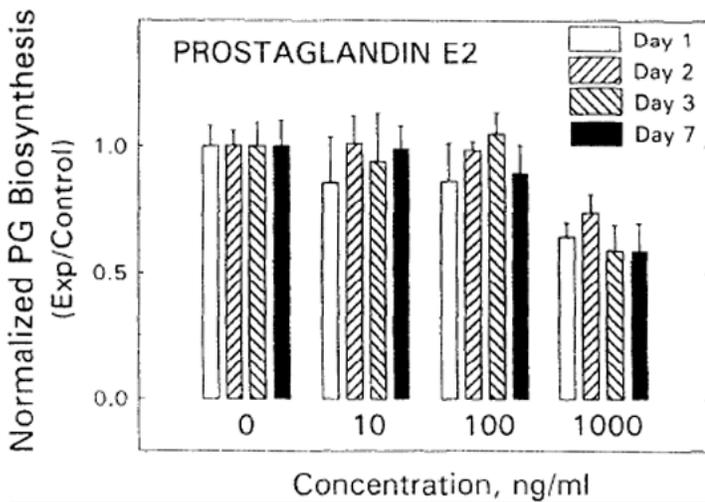
move unincorporated isotope. Media washings were discarded. The explants were then randomly separated into three groups (n=9-11 each), cultured for another 24 hours, and the media from each explant saved for counting. The loss of newly synthesized proteoglycan, measured as <sup>35</sup>S-sulfate labelled proteoglycan in the culture media, was considered proportionate to the products of proteoglycan catabolism. The recovered <sup>35</sup>S-sulfate labelled proteoglycan during this 24-hour period represented the normal release of proteoglycan, and served as a check that all explant groups had an equivalent PG loss prior to introduction of the drugs. The explants were then incubated for 4 days without drugs or with misoprostol (1000  $\mu$ g/ml) or PGE<sub>2</sub> (1000  $\mu$ g/ml). Media was exchanged and saved every 24 hours. The explants and media were counted, and the PG loss per day was calculated as a percentage of the total PG synthesized (sum of explant and 5 days of media).



**FIGURE 1.** Dose-response and time-response effects of misoprostol on proteoglycan biosynthesis. There was a significant dose-dependent ( $p < 0.001$ ) and time-dependent ( $p < 0.05$ ) inhibition of proteoglycan biosynthesis. Data expressed as the ratio (mean  $\pm$  sem) of the experimental group (concentration  $> 0$ ) to control group (concentration = 0), for N=6-10 explants per group.

### Misoprostol Half-Life

To determine if the drugs concentration (potency) in the incubation media was significantly decreased as a function of time between media changes (24 hours), the concentration decay rate of misoprostol (free acid) was measured (PGE<sub>2</sub> was assumed similar). Misoprostol in incubation media (0.25  $\mu$ g/ml) was placed into the incubator and duplicate samples extracted daily for up to 20 days. Media without misoprostol was used as a control. The tests were conducted exactly as performed for the explants, that is, sterile conditions and at 37°C and 95% humidity. After extraction the samples were frozen at -70°C until assayed (courtesy of Dr. Charles Jeuell, G.D. Searle).



**FIGURE 2.** Dose-response and time-response effects of prostaglandin E2 on proteoglycan biosynthesis. There was a significant ( $p < 0.001$ ) inhibition of proteoglycan biosynthesis only at the highest concentration tested (1000 ng/ml), independent of treatment time. Data expressed as the ratio (mean  $\pm$  sem) of the experimental group (concentration  $> 0$ ) to control group (concentration = 0), for N= 6-10 explants per group.

The half-life,  $\tau_{1/2}$  was determined from the relationship between the initial concentration  $C_0$  and concentration  $C_t$  at any time  $t$ , given by the equation  $C_t = C_0 e^{-t/\tau}$ , where  $\tau$  is the mean-life (reciprocal of the decay-rate  $\lambda$ ) and  $\tau_{1/2} = 0.69315\tau$ .

#### Data Analysis

Proteoglycan biosynthesis and catabolism were quantified from  $^{35}\text{S}$ -sulfate incorporation and loss into the media, respectively, and expressed as disintegrations/minute (DPM). Water content was expressed as a percent of total wet weight. Treatment groups were averaged and expressed as means and standard deviations. Statistical analysis was performed using analysis of variance (ANOVA) and the Student's t-test, at the  $\alpha = 0.05$  significance level, to compare experimental groups to control groups. In addition, the dose and time responses and half-life determination were analyzed using linear regression analysis. For graphical purposes only, means and standard errors of the means were used, and the dose and time responses normalized by dividing the experimental groups by the mean of the control groups.

## RESULTS

### Proteoglycan Biosynthesis

The presence of misoprostol produced a significant dose-dependent inhibition of proteoglycan biosynthesis, independent of incubation time ( $p < 0.001$ ) (Figure 1). There was also a significant time-dependent inhibition in biosynthesis for each concentration ( $p < 0.05$ ) (Figure 1). At the highest dose there was a 30% depression in

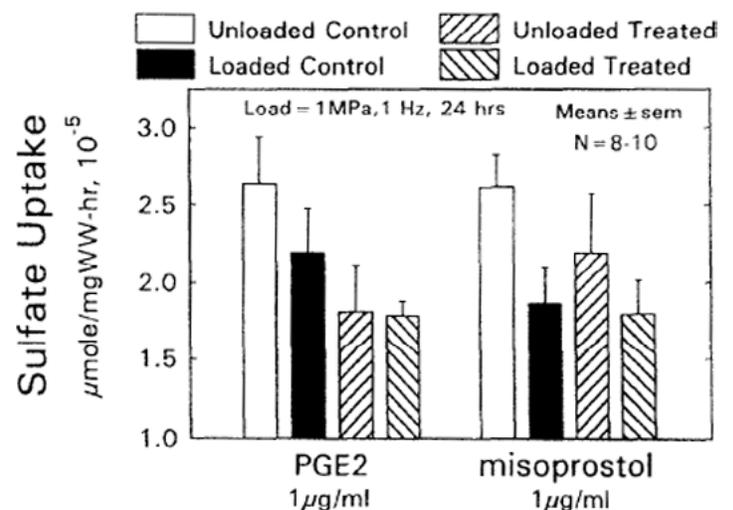
PG biosynthesis after 24 hours, which increased to almost 50% after three days. Inhibition of proteoglycan biosynthesis due to the presence of  $\text{PGE}_2$  was only seen at the highest dose (1000 ng/ml), resulting in a 34% depression after three days (Figure 2). No time-dependent effect was found at any concentration due to the daily administration of  $\text{PGE}_2$ .

### Water Content

There was no significant difference in water content between any treatment group (misoprostol,  $0.753 \pm 0.020$ ;  $\text{PGE}_2$ ,  $0.756 \pm 0.026$ ) and the controls ( $0.761 \pm 0.009$ ). In addition, no significant change was found in the water content as a function of drug concentration or treatment time. Finally, mechanical loading did not effect the equilibrium water content in either the treatment or control groups when compared to unloaded explants.

### Mechanical Load

Mechanically compressing the explants with a stress of 1.0 MPa for 24 hours without drug presence significantly inhibited PG biosynthesis by as much as 50% ( $p < 0.05$ ), while in unloaded explants a similar depression in biosynthesis, as noted above, was again found with either misoprostol or  $\text{PGE}_2$  ( $p < 0.05$ ) (Figure 3). However, there was no significant effect on proteoglycan biosynthesis when misoprostol or  $\text{PGE}_2$  treatment was combined with mechanical load (Figure 3). The combined effects of compression and addition of either prostaglandin was not additive; the response did not differ significantly from the response due to the mechanical load or drug administration alone. This lack of synergy was found at any of the drug concentrations used.



**FIGURE 3.** Combined effect of prostaglandins (misoprostol and  $\text{PGE}_2$ ) and repetitive mechanical load on proteoglycan biosynthesis. Explants treated for 24 hours with either misoprostol (1000 ng/ml),  $\text{PGE}_2$  (1000 ng/ml) or a compressive stress (1.0 MPa), resulted in a significant inhibition of PG biosynthesis ( $p < 0.05$ ). There was no synergistic effect of combined compression and prostaglandin presence.

### Neat Chemical vs Free Acid Form

In unloaded explants, there was no significant difference in proteoglycan biosynthesis between the neat chemical form ( $3.56 \pm 1.21$   $\mu\text{mole sulfate/mg wet weight-hr}$ ) and free acid form ( $3.91 \pm 1.71$   $\mu\text{mole sulfate/mg WW-hr}$ ) of misoprostol when explants were treated with a 100  $\text{ng/ml}$  dose for 24 hours. Neither form produced a significant change in biosynthesis compared to unloaded control explants ( $4.02 \pm 1.79$   $\mu\text{mole sulfate/mg WW-hr}$ ). While the biosynthesis was significantly reduced in loaded explants ( $p < 0.001$ ), there was no difference in the biosynthesis of loaded explants treated with the two forms of misoprostol ( $0.84 \pm 0.41$  and  $0.93 \pm 0.58$   $\mu\text{mole sulfate/mg WW-hr}$ , respectively).

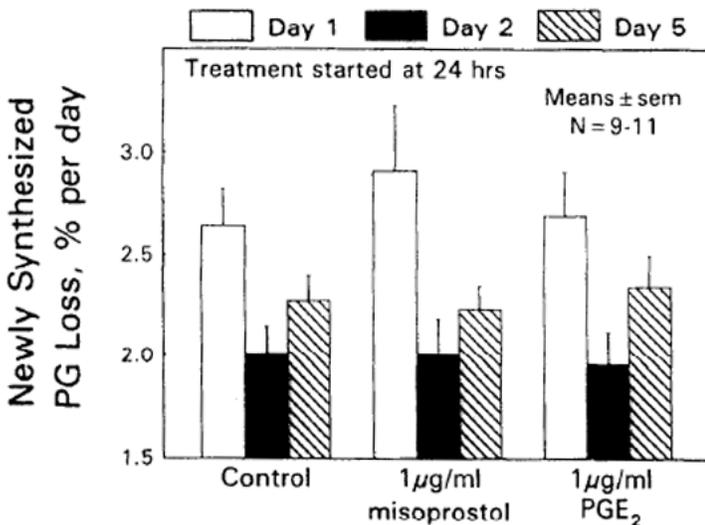


FIGURE 4. Loss of newly synthesized proteoglycans resulting from presence of misoprostol or prostaglandin E2 (1000  $\text{ng/ml}$ ). There was no change in proteoglycan loss after one and four days of drug treatment when compared with controls. Data is expressed as the percent loss per day (mean  $\pm$  sem;  $N=9-11$  explants per group). Drug treatment started after 24 hours.

### Proteoglycan Loss

Cartilage explants treated for 1 and 4 days with 1000  $\text{ng/ml}$  of misoprostol or PGE<sub>2</sub> showed no significant change in the loss of newly synthesized proteoglycans compared between themselves or with controls (Figure 4). Proteoglycan loss ranged from 2-3% per day.

### Misoprostol Half-Life

After 24 hours in solution the concentration of misoprostol decreased to 78.3% of its initial concentration (Figure 5). Based on the measured concentrations of misoprostol in the media over the 20 days sampled, the calculated half-life was  $\tau_{1/2} = 68.0$  hrs (2.8 days). Since the media and drugs were changed daily, the affect of the decrease in concentration over 24 hours was considered negligible.

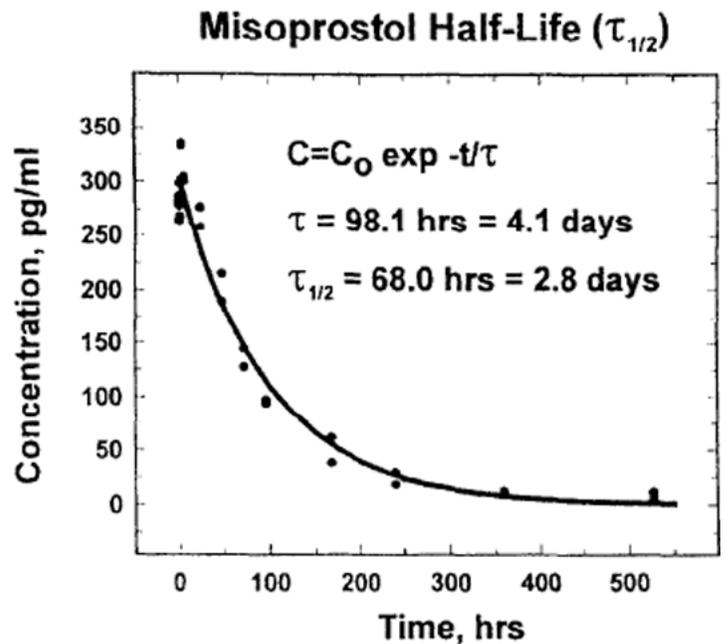


FIGURE 5. Decrease in misoprostol concentration as a function of incubation time in culture medium. The half-life,  $\tau_{1/2} = 68.0$  hrs, was calculated from the least-squares best fit between the experimental data (circles) and the predicted concentration  $C_t$  at time  $t$ , given by  $C_t = C_0 e^{-t/\tau}$ , where  $C_0$  is the initial concentration,  $\tau$  is the mean-life and  $\tau_{1/2} = 0.66315 \tau$ .

### DISCUSSION

Chondrocytes in articular cartilage, while relatively sparse compared to other tissues, are metabolically active<sup>27</sup>. Although the turnover of collagen in articular cartilage is extremely slow, that of its proteoglycan component is significantly more rapid<sup>27</sup>. Healthy tissue is characterized by a continuous and delicate balance between the anabolic biosynthesis of new matrix components and the catabolic loss of matrix components, namely from degradative enzymes<sup>28</sup>. It is the chondrocyte's metabolic activity that regulates constituent production and loss, and controls the balance between healthy and degenerative tissue.

Significantly higher levels of PGEs (PGE<sub>1</sub> and PGE<sub>2</sub>) are found in the joints of individuals with inflammatory diseases. Clearly the PGEs act as mediators of inflammation. In addition, they have the ability to regulate synoviocyte and chondrocyte anabolic and catabolic activity and their response to pharmacologic agents. While PGE<sub>2</sub> is the more commonly studied prostaglandin, chondrocytes produce a significant proportion of PGE<sub>1</sub> (1: 5)<sup>10</sup>. Both types of PGEs are important in the inflammation process, and as shown here and described else<sup>1, 29</sup> they can produce very similar and very different effects on cellular response. The exact reasons for the differences is not fully understood, but is probably related to the specificity of cell receptors to the different prostaglandins.

In this study we found that daily administration of prostaglandin E<sub>1</sub> (i.e., misoprostol) caused a significant dose and time-dependent inhibition in proteoglycan biosynthesis without a concomitant loss of newly synthesized proteoglycans. A similar result was found for prostaglandin E<sub>2</sub>, but only at the highest concentration (1000 ng/ml). These results were, however, for short-term exposure, only for a maximum of seven days. It is unknown whether longer intervals of exposure would result in greater inhibition of PG biosynthesis, or, more important, cause a significant degradation of the tissue matrix.

The mechanisms that underlie articular cartilage metabolism are not yet well understood. Recently, there has been considerable evidence to suggest that interleukin-1 (IL-1), a cytokine directly associated in inflammation, is intricately involved in cartilage matrix metabolism. Its effect on chondrocyte function is at least twofold; it stimulates the biosynthesis and release of prostaglandins, specifically PGE<sub>2</sub><sup>7, 30</sup>, and it inhibits proteoglycan biosynthesis<sup>7, 19, 20</sup>. However, the exact mechanisms behind IL-1 induced release of PGEs and suppression of proteoglycan biosynthesis remains unclear. Zurier<sup>1</sup> suggests that PGEs may initiate the cells immune response via the cyclic AMP pathway, to modify [suppress or enhance] joint inflammation and IL-1 release, and that the response may be different for normal and immunodeficient cells. Dingle<sup>20</sup> has suggested that in osteoarthritis local release of IL-1 may be a transient event brought about by inflammation or mechanical trauma, leading to an imbalance between proteoglycan biosynthesis and degradation. The results from our study suggests that IL-1 may induce suppression of proteoglycan biosynthesis indirectly through its stimulation of chondrocyte prostaglandin production. Prostaglandin-induced inhibition of proteoglycan biosynthesis has been demonstrated by other investigators in chondrocyte cell<sup>14</sup> and cartilage explant cultures<sup>13, 15, 16, 17</sup>. Our data supports these studies, and suggests that prostaglandins may have a role in the degradation and repair processes observed in the pathogenesis of the various forms of arthritis.

Interestingly, Dingle<sup>20</sup> reported that misoprostol (100 ng/ml) stimulated proteoglycan biosynthesis in human osteoarthritic cartilage (68% increase), and reversed the inhibitory effects of IL-1 in OA (182%↑ after 39%↓) and normal (76%↑ after 69%↓) tissue. However, misoprostol did not effect the proteoglycan biosynthesis of normal tissue, nor did it increase the biosynthesis level in OA and IL-1 treated cartilage to that of normal tissue (58%, 38% and 55% of normal, respectively). Why these differences in proteoglycan biosynthesis exist for normal tissue between Dingle's studies and those of others, in-

cluding our own, is unclear. As suggested by Dingle<sup>20</sup>, it may be due to the difference in specie used (human vs animal). In addition, the difference may be attributed to the age of the donor, since OA cartilage is normally from older individuals and animal tissue is either from immature or young animals. It may be that the cell receptors of the different species and older or aged chondrocytes are either different or respond differently to the prostaglandins. The present data would indicate these possibilities.

Recent studies have also demonstrated that NSAIDs can also affect cartilage metabolism through the depression of proteoglycan biosynthesis<sup>20, 31</sup>. Dingle found that the adjunctive use of misoprostol reduced the adverse effects of various NSAIDs on proteoglycan biosynthesis<sup>19, 20</sup>. However, a series of experiments by Brandt et al.<sup>32</sup> could not demonstrate a protective effect of misoprostol on the inhibition of proteoglycan biosynthesis induced by acetylsalicylic acid or sodium salicylate. In our study the addition of misoprostol lead to a dose and time-dependent inhibition of proteoglycan biosynthesis. According to these various findings, the supplementation of misoprostol to NSAID therapy for chronic inflammatory arthritis has unclear effects on cartilage tissue and should remain open to debate and further study.

The concentrations of prostaglandin used in our study (10, 100, 1000 ng/ml) were higher than those measured in the blood of individual's taking misoprostol (0.6-1.2 ng/ml)<sup>26</sup> and in the synovial fluid from inflamed joints (1.2-1.4 ng/ml)<sup>11</sup>. While the concentrations were higher, there is evidence that the effective physiological concentrations at the cellular level may be higher than those measured in blood or synovial fluid, as a concentrative phenomenon for prostaglandins has been reported in cellular systems<sup>33</sup>. The objective of our study was to determine the effect of high-dose concentrations of prostaglandins on cartilage metabolism in order to evaluate its potential use for intra-articular injection during joint inflammation. Our results indicate that intra-articular injection of misoprostol or PGE<sub>2</sub>, for protective purposes in conjunction with NSAIDs, may be detrimental to cartilage metabolism. However, in comparison with oral or intramuscular use, intra-articular administration of either prostaglandin may widen the range of therapeutic drug dosages, provide a more rapid effect secondary to decreased transit time to tissues, and significantly decrease systemic side effects. Further studies will be necessary to adequately evaluate its efficacy and safety in this manner.

The etiology of prostaglandin-induced suppression of proteoglycan biosynthesis remains unclear. However,

the effects of prostaglandins on cartilage metabolism are not restricted to proteoglycan biosynthesis. Prostaglandins have been shown to cause a myriad of effects in chondrocyte cultures, including inhibition of DNA, RNA, and protein biosynthesis<sup>14</sup>. In addition, PGE<sub>2</sub> has been shown to lead to an increase in collagenase production by synoviocytes<sup>34</sup> and to increase cyclic adenosine monophosphate (cAMP) levels in chondrocyte culture<sup>35</sup>, while misoprostol and PGE<sub>2</sub> have been shown to markedly increase intracellular cAMP levels in peripheral blood monocytes and peritoneal macrophages<sup>36</sup>. The stimulatory effect of prostaglandins on cAMP levels may somehow lead to an inhibition of proteoglycan biosynthesis, either directly or perhaps through stimulation of collagenase.

To the best of our knowledge the metabolic response of cartilage to combined mechanical stress and pharmacological agents has not been explored. Nor is any information available on prostaglandin production in mechanically loaded cartilage. However, increased PGE production has been reported in mechanically loaded osteoblasts and cancellous bone<sup>37, 38</sup>. Palmoski and Brandt<sup>39</sup> did compare the response of unloaded and loaded areas of adult dog cartilage to sodium salicylate and Indomethacin after explantation, but not while the tissue was being loaded. No difference was found in proteoglycan biosynthesis between the two test sites themselves; however biosynthesis was reduced in loaded areas to a lesser extent than in unloaded areas. Similarly, Farquhar et al.<sup>40</sup> measured an increase and decrease, respectively, in proteoglycan biosynthesis during mechanical loading one day after ending carti-

lage exposure to low (0.01 mg/ml) and high (1 mg/ml) dose methylprednisolone.

We have previously shown that conditions of static and dynamic compression inhibit proteoglycan biosynthesis<sup>24</sup>. While our experiments demonstrated inhibition secondary to mechanical loading or the presence of prostaglandins, no combined effect was seen. One possible explanation for the lack of a synergistic effect of prostaglandin and load may occur at the molecular level. Haynes et al.<sup>36</sup> demonstrated that misoprostol and PGE<sub>2</sub> had similar effects on a range of biological activities and suggested that these two drugs have the same mechanism of action, that is, they bind to the same cell surface receptor. Since the response of the explants to the combination of compression and prostaglandin was similar to the response of mechanical compression alone, it may be possible that the drug receptors may have been affected by the loading process. Performing tests under loaded conditions does provide a better simulation of the true physiologic environment experienced by articular cartilage, which is continuously undergoing mechanical stress during joint motion. Indeed, if mechanical loads do affect cell receptors then perhaps loaded conditions should be considered in future experiments involving the interaction of cartilage biology and physicochemical and pharmacological agents.

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