Inflamm. res. 48 (1999) 199–204
1023-3830/99/040199-06 $ 1.50+0.20/0

Inflammation Research

Osteogenic protein-1 (OP-1) blocks cartilage damage caused by fibronectin fragments and promotes repair by enhancing proteoglycan synthesis

H. E. Koepp [1], K. T. Sampath [2], K. E. Kuettner [3] and G. A. Homandberg [3]

[1] Department of Orthopaedic Surgery, University of Ulm, Oberer Eselsberg 45, D-89081 Ulm/Donau, Germany

[2] Creative BioMolecules, Hopkinton, MA, USA

[3] Department of Biochemistry, Rush Medical College at Rush-Presbyterian-St. Luke's Medical Center, 1653 West Congress Parkway, Chicago, IL 60612-3864, USA, Fax +1 312 942 3053, e-mail: ghomandb@rush.edu

Received 26 August 1998; returned for revision 10 October 1998; accepted by G. W. Carter 15 January 1999

Abstract. *Objective and Design:* The abilities of osteogenic protein-1 (OP-1) and TGF-β1 to affect cartilage damage caused by fibronectin fragments (Fn-fs) that are known to greatly enhance cartilage proteoglycan (PG) degradation were compared.
Material: Articular cartilage was obtained from 18 month old bovines.
Treatment: To test blocking of damage, cartilage was cultured with or without OP-1 or TGF-β in the presence of 100 nM Fn-fs. To test restoration of PG, cartilage was first cultured with Fn-fs and the cartilage then treated with factors.
Methods: Cartilage PG content was measured in papain digests using the dimethylmethylene blue assay. PG synthesis was measured by incorporation of ^{35}S labeled sulfate.
Results: OP-1 blocked damage and restored PG in damaged cartilage, apparently due to enhanced PG synthesis. However, TGF-β1 alone decreased PG content.
Conclusions: These results clearly demonstrate differences between OP-1 and TGF-β1, both members of the TGF-β superfamily and illustrate the efficacy of OP-1 in blocking Fn-f mediated damage.

Key words: OP-1 – TGF-β – Fibronectin fragments – Cartilage – Cartilage repair

Introduction

Osteogenic proteins, also known as bone morphogenic proteins (BMPs), are growth factors known to induce bone synthesis [1]. These proteins have been characterized as members of the TGF-β-superfamily [2]. Recent studies have shown that BMP-7, also known as osteogenic protein-1 (OP-1), is able to stimulate the synthesis of sulfated proteoglycan (PG) and collagen type II in human articular chondrocytes [3, 4]. It has also been demonstrated that human OP-1 can stimulate matrix synthesis in avian [5] and murine chondrocyte cultures [6, 7]. Since recombinant human OP-1 can induce new bone formation with an activity comparable to bovine OP-1 [2], the reparative properties of OP-1 can now be extensively studied. In comparison, TGF-β has been shown to stabilize the chondrocyte phenotype, block the effects of IL-1 on chondrocytes [8, 9], decrease secretion of proteases (reviewed in ref. [10]) and suppress synthesis of stromelysin-1 [11].

In order to determine if OP-1 and TGF-β1, as members of the TGF-β superfamily, have similar properties, we have utilized an in vitro cartilage matrix damaging culture system to compare the anabolic effects of OP-1 and TGF-β1. In this culture system, proteolytic fragments of fibronectin (Fn-fs) are added to bovine or human articular cartilage cultures, resulting in greatly elevated release of catabolic cytokines [12, 13], release of matrix metalloproteinases (MMPs) [13–15] and suppression of synthesis of proteoglycan (PG) [16, 17]. This causes a rapid decrease in cartilage PG content [17, 18]. The relevance of this model to physiologic cartilage degeneration or catabolic events in osteoarthritis can be supported in many ways: The up-regulation of MMPs, suppression of PG synthesis and involvement of catabolic cytokines, caused by Fn-fs, are also events observed in osteoarthritis. Fn-fs are found at elevated levels in synovial fluids of patients with osteoarthritis [19] and in extracts of knee cartilage from patients with osteoarthritis [20]. Removal of Fn-fs from osteoarthritic synovial fluid reduces the ability of the fluid to cause damage to cultured cartilage explants [20]. Addition of IL-1 to cartilage explants or addition of MMP-3 (stromelysin-1) to synovial fluids generates Fn-fs [20]. Injection of Fn-fs into rabbit knee joints causes a severe loss of PG from knee articular cartilage [21]. Thus, this Fn-f model is likely relevant to cartilage degeneration in arthritis.

Correspondence to: G. A. Homandberg

This model is also useful in testing the anabolic and reparative activities of potential therapeutic agents since Fn-f treated 18-month old bovine cartilage does not normally restore lost PG after being subjected to Fn-fs [17]. However, anti-oxidants that presumably block cytokine activity [22, 23], synthetic peptides that presumably block Fn receptor activity [24], and an anabolic growth factor, IGF-1, that stimulates PG synthesis [25], all have the ability to either decrease catabolism or enhance anabolism sufficiently to partially or fully overcome the catabolic activities of the Fn-fs and restore PG in Fn-f damaged cartilage [25]. We do not yet know which of these agents slow the actual catabolic events or which merely allow an enhancement of PG synthesis to compensate for enhanced catabolism. Nonetheless, this model applied to mature bovine cartilage is thus useful for testing the ability of anabolic agents, such as the growth factors, TGF-β and OP-1, in restoring PG in Fn-f damaged bovine cartilage.

We have already reported that IGF-1 has a weak effect on blocking or compensating for Fn-f mediated cartilage depletion in bovine cartilage explants. Our objective was to investigate and compare the abilities of OP-1, with its PG synthesis enhancing activities, and TGF-β1, with its general anabolic activities, to compensate for Fn-f mediated catabolic events.

Materials and methods

Materials

All common chemicals except where noted, were from Sigma Chemical Co. (St. Louis, MO, USA). OP-1 was a gift from Dr. T. Kuber Sampath (Creative BioMolecules, Hopkinton, MA, USA) and was produced in CHO cells as previously described [2]. A well-characterized amino-terminal heparin-binding 29-kDa Fn-f was isolated by sequential cathepsin D and thrombin digests of Fn [18] and is referred to here as the Fn-f.

Explant cultures and tests of OP-1 and TGF-β1

To test blocking effects, articular cartilage slices from adult (18–20 months of age) bovines were cultured as described [17] in Dulbecco's modified Eagle's medium; (DMEM) containing 50 U/ml gentamicin with 10% serum/DMEM with 50 to 80 mg cartilage in 2 ml of media. One day after the start of the culture, the culture was adjusted to 50 ng/ml OP-1 or 200 ng/ml OP-1 or 5 ng/nl TGF-β1 in 10 % serum/DMEM. After 2 h, the culture was adjusted to 100 nM Fn-f and PG content of slices measured at various times. To test effects on restoration of PG, freshly isolated cartilage was allowed to equilibrate in 10 % serum DMEM for 1 day and the culture was then adjusted to 100 nM Fn-f. After 7 days, some of the cartilage slices were removed and total PG quantified to ensure that at least 40% of the PG had been depleted. Other cultures were then adjusted to 50 ng/ml or 200 ng/ml OP-1 in 10% serum/DMEM without the Fn-f, to test for restoration of cartilage PG. Each experiment was performed three times with different bovine cartilage preparations. A typical data set is shown.

Assays of PG content and of PG synthesis rates

The total amount of PG/mg wet weight cartilage was determined by assays with dimethylmethylene blue (DMB) reagent after treatment of 50–80 mg of cartilage in 1.0 ml of 50 mM phosphate buffer, pH 6, containing 10 mM EDTA and 10 mM cysteine, with 50 µg/ml papain for 16 h at 65 °C as originally described [16, 17]. To assay PG, a volume of 0.8 ml DMB reagent, prepared as described [26], was mixed with 190 µl of 1.37 M GuHCl and 10 µl of test sample and after 2 min, a spectrophotometer used to determine absorbances at 525 and 595 nm using water as a blank. The ratios of 525 nm absorbance over 595 nM absorbance were used to establish a standard curve with purified PG and a standard curve of 0.5 to 3 µg/ml purified bovine PG used to estimate PG contents in the papain digests. The data are reported as µg PG/mg wet weight cartilage based on a mean and S.D. of at least three cartilage samples. The overall variability between similar cartilage culture wells was typically less than 12%. Data were subjected to two-sided Student's t-test analysis where the experimental value was typically compared to non-treated control values at the same time points. A p value of < 0.05 was considered to be significant.

Sulfate incorporation was used as an index of synthesis of sulfated PG. Rates were measured using a 2 h pulse with 10 µCi/ml of ^{35}S-sodium sulfate in 1.5 ml of 10% serum/DMEM, followed by a 2 h cold chase with 10% serum/DMEM. After removal of the medium and three washes with DMEM, the slices were removed, placed in a 1.5 ml Eppendorf tube and 1 ml of 4 M guanidine-HCl, 0.1% Triton X-100, 10 mM EDTA, 100 mM sodium acetate, pH 5.5, added. After 16 h of end over end mixing at 9 °C, the extracts were exhaustively dialyzed against water and label quantified by scintillation counting. The amount of label was expressed as dpm/mg wet weight cartilage and converted into % of control values in order to normalize for radioisotope decay over the several months of experiments required for this study. The mean and S.D. values of the rate were based on 3 similar culture wells. Data were subjected to two-sided Student's t-test analysis.

Results

Effect of OP-1 on blocking Fn-f mediated cartilage damage or PG depletion and on promoting restoration of PG in Fn-f treated cartilage

We first tested the effects of two different concentrations of OP-1, 50 and 200 ng/ml, on blocking Fn-f mediated depletion of PG from explant cultures, which we define as cartilage damage. Figure 1 A shows that by day 7, OP-1 alone at 200 ng/ml caused a statistically significant ($p = 0.04$) higher PG content (32.5 ± 1.5 µg/mg cartilage) than control cartilage (25.4 ± 2.2 µg/mg cartilage). By day 14, OP-1 treated cartilage still had a PG content significantly ($p = 0.018$) greater (35.4 ± 2.6 µg/mg cartilage) than control cartilage (26.1 ± 1.9 µg/mg cartilage). The difference was even greater by day 21. The overall effects of 50 ng/ml OP-1 at each time point appeared to be smaller.

Addition of the Fn-f to cultures of bovine cartilage caused, by day 7, a significant ($p = 0.009$) decrease in PG content from control values of 26.5 ± 3.0 µg/mg cartilage to 16.9 ± 1.5 µg/mg cartilage (41% decrease). The PG content of Fn-f treated cartilage did not increase significantly from day 7 to day 14 or day 21. However, when 200 ng/ml OP-1 was included in the medium with Fn-f and blocking tested, the PG content remained at levels similar to that of cartilage treated with OP-1 alone. By day 14, the PG content in OP-1/Fn-f treated cartilage was significantly ($p = 0.028$) greater (29.8 ± 2.9 µg/mg cartilage) than in control cartilage (26.1 ± 1.9 µg/mg cartilage). By day 21, the effect of OP-1 was even greater. We conclude that OP-1 blocked the ability of the Fn-f to decrease the cartilage PG content.

We next tested for reparative activity of OP-1, by first damaging the cartilage with Fn-f. When cartilage with a PG content of 26.5 ± 3 µg/mg cartilage was first treated with Fn-f to damage and enhance PG depletion, the PG content

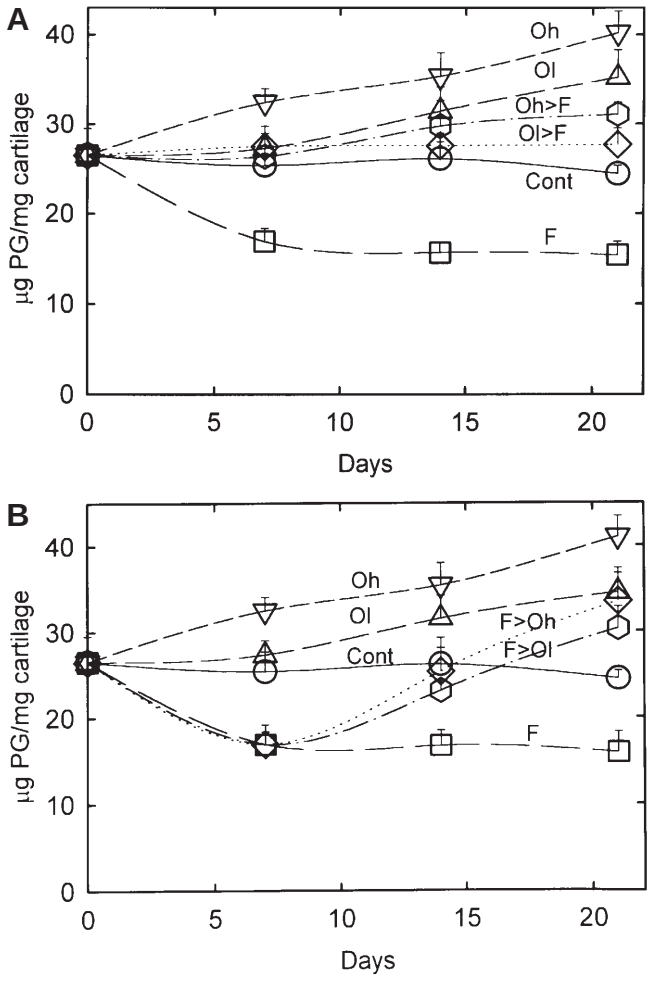

Fig. 1. Effect of OP-1 on blocking Fn-f mediated decreases in cartilage PG content (A) and on promoting restoration of PG in Fn-f treated cartilage (B). For A, bovine articular cartilage cultures in 10% serum/DMEM were adjusted to 100 nM Fn-f (□) alone, or 50 ng/ml OP-1 alone (△) or to 200 ng/ml OP-1 alone (▽) or to 50 ng/ml OP-1 with 100 nM Fn-f (◇) or to 200 ng/ml OP-1 with 100 nM Fn-f (◇). Control with nothing added except 10% serum is shown (○). Media were changed every other day with fresh reagents added. The cartilage was continually incubated with these compounds. Cartilage was assayed for total PG using the DMB assay. For B, cartilage cultures were prepared in 10% serum/DMEM and adjusted to 100 nM Fn-f for day 0–7 only (□) or 50 ng/ml OP-1 alone (△) or to 200 ng/ml OP-1 alone (▽) or to 50 ng/ml OP-1 with 100 nM Fn-f (◇) or to 200 ng/ml OP-1 with 100 nM Fn-f (◇). Control with nothing added except 10% serum is shown (○). Media were changed every other day with fresh Fn-f added. Cartilage was assayed for total PG using the DMB assay.

was significantly ($p = 0.016$) reduced (16.9 ± 1.5 μg/mg cartilage). When the Fn-f was removed, and medium replaced with 10% serum/DMEM alone, the PG content did not increase significantly by day 14 or day 21 (Fig. 1B). However, when the damaged cartilage was cultured with 200 ng/ml OP-1 in 10% serum/DMEM beginning at day 7, the PG content increased significantly ($p = 0.025$) from 16.9 ± 1.5 μg/mg cartilage to 25.3 ± 3.9 μg/mg cartilage by day 14 and significantly ($p = 0.002$) increased to 30.3 ± 2.5 μg/mg cartilage by day 21. The lower concentration of 50 ng/ml OP-1 was also effective on Fn-f damaged cartilage and significantly ($p = 0.006$) increased the PG content (25.3 ± 3.9 μg/mg cartilage) by day 14. By day 21, the PG content of 50 ng/ml OP-1/Fn-f treated cartilage was still significantly ($p = 0.002$) elevated (30.3 ± 2.5 μg/mg cartilage). We conclude that OP-1 was effective at restoring PG.

Effect of OP-1 on PG synthesis and on altering the degradation half-life of labeled PG

In order to determine how OP-1 might be causing the blocking and reparative effects, we investigated the effect of OP-1 on PG synthesis rates (Fig. 2A). OP-1 at 200 ng/ml (O in Fig. 2A) significantly ($p = 0.0002$) increased the rates (206 ± 24% of control) by day 1. By day 2, the rates of OP-1 treated cartilage remained significantly ($p = 0.001$) elevated (210 ± 21% of control rates). The rates were still significantly ($p = 0.001$) enhanced (154 ± 14% of control rates) by day 7 and remained at levels significantly ($p = 0.041$) higher (150–134% of control rates) until at least day 21. Addition of both Fn-f and OP-1 to cultures (O + F) did not significantly change the profile over that of OP-1 alone at any time point.

In order to determine if the enhanced PG synthesis rates altered the rate of degradation, cartilage was radiolabeled with ^{35}S sulfate, treated with 200 ng/ml OP-1, and the kinetics of release of label into the medium measured as shown in Figure 2B. The rates were fit into a first-order decay to determine the respective half-lives. The half-life for control cartilage was 11.3 days based on a slope with a 1.7% standard error, while that for the OP-1 treated cartilage was 29.7 days based on a slope with a 5.3% standard error. The effect of the OP-1 was to increase the apparent half-life of PG by 2.6 fold.

Effect of TGF-β on blocking Fn-f mediated cartilage damage or PG depletion and on promoting restoration of PG in Fn-f or treated cartilage

Figure 3A shows the effect of 5 ng/nl TGF-β on blocking cartilage PG depletion. Control cartilage had a PG content of 32.8 ± 3.6 μg/mg cartilage. Addition of Fn-f (curve F) significantly ($p = 0.016$) decreased the PG content (21.7 ± 3.2 μg/mg cartilage) by day 7. Treatment of cartilage with TGF-β alone caused a statistically significant ($p = 0.04$) increase in PG content (40.2 ± 2.8 μg/mg cartilage) by day 2 and the increase was still statistically significant through day 7 ($p = 0.02$). However, by day 14, TGF-β caused a statistically significant ($p = 0.016$) decrease in PG content (25.2 ± 2.9 μg/mg cartilage) compared to control levels at day 14 (32.2 ± 3.2 μg/mg cartilage). By day 21, the PG contents of TGF-β treated cartilage were still significantly ($p = 0.034$) lower than control levels. When Fn-f and TGF-β were added together, the combination (TGF-β/F) caused a significant ($p = 0.024$) decrease (22.6 ± 2.7 μg/mg) as compared to control levels (32.2 ± 3.2 μg/mg cartilage) at day 14. This decreased PG content of TGF-β and Fn-f treated cartilage was still apparent by day 21. Similar effects of TGF-β on decreasing PG content by 7 days were observed with 1 ng/ml and 20 ng/ml; neither of these concentrations blocked the effect of the Fn-fs (data not shown).

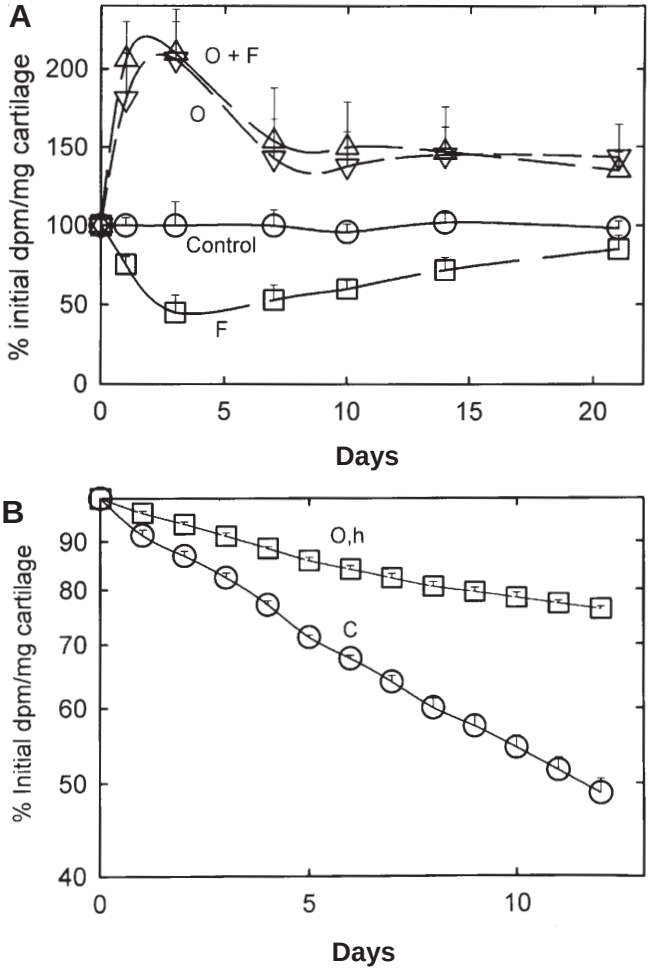

Fig. 2. Effect of OP-1 on PG synthesis rates (A) and on degradation rate of labeled PG (B) ■ For A, cartilage was cultured with (O, h) 200 ng/ml OP-1 or without (control) in 10% serum/DMEM and at various times, adjusted to 5 µCi/ml ^{35}S sulfate for a 2 h pulse, followed by a 2 h chase. The tissue was then subjected to extraction with GuHCl, the extracts dialyzed and label quantified. For B, cartilage was labeled with ^{35}S sulfate (5 µCi/ml) for 48 h and after an additional 1 day equilibration in the absence of label was either treated with (O, h) or without (control) 200 ng/ml OP-1 in 10% serum/DMEM. Label released into the media was quantified periodically and converted into % of initial dpm.

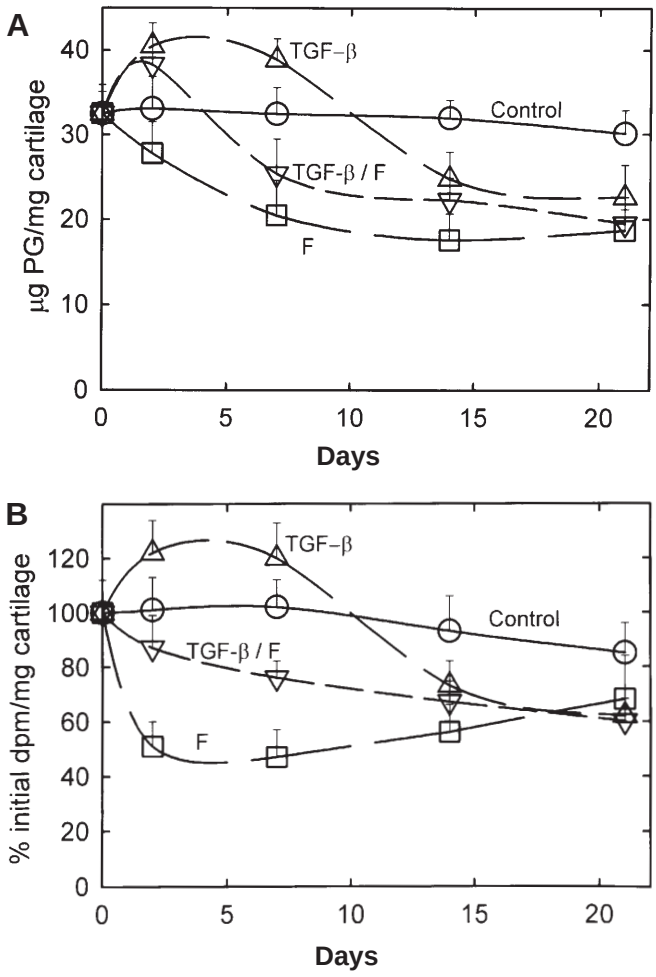

Fig. 3. Effect of TGF-β on blocking Fn-f mediated decreases in cartilage PG content (A) and on Fn-f mediated suppression of PG synthesis (B). For A, bovine articular cartilage cultures in 10% serum/DMEM were adjusted to 100 nM Fn-f (□) alone, or 5 ng/ml TGF-β alone (△) or to TGF-β with Fn-f (▽) or only serum/DMEM control (○). Media were changed every other day with fresh reagents added. The cartilage was continually incubated with these compounds. Cartilage was assayed for total PG using the DMB assay. For B, similar cultures of 100 nM Fn-f (□) alone, or 5 ng/ml TGF-β alone (△) or TGF-β with Fn-f (▽) or only serum/DMEM control (○) were subjected to assays of ^{35}S sulfate incorporation. DPM/mg cartilage was normalized to initial value for control.

Figure 3B shows the effect of TGF-β on PG synthesis rates. Treatment of cartilage with TGF-β alone caused a statistically significant ($p = 0.049$) increase in rates (121 ± 4.4% of control cultures) by day 2. The increase was also obvious at day 7. However, by day 14, there was a statistically significant ($p = 0.017$) decrease (74 ± 8 % of control rates) and by day 21 the rates were still significantly ($p = 0.043$) reduced as compared with control rates at day 21. Treatment with Fn-f alone caused a significant ($p = 0.0007$) decrease (51 ± 7 % of the control rate) by day 2, with rates subsequently increasing. Treatment with a combination of Fn-f and TGF-β (TGF-β/F) caused a significant ($p = 0.008$) decrease in PG synthesis rates (78 ± 4 % of control) by day 7. The rates in Fn-f and TGF-β treated cartilage remained suppressed up to day 21.

Discussion

The objectives were to investigate whether OP-1 was able to both block damage and promote restoration of PG in cartilage initially damaged by Fn-fs and whether TGF-β would have similar effects. Our data clearly showed that OP-1 not only had a protective effect but also a reparative effect. The higher concentration of 200 ng/ml appeared to have a greater effect in both cases than 50 ng/ml OP-1 but both increased the PG content to above control levels at some time point.

The action of OP-1 could be through its ability to promote PG synthesis. While we did demonstrate a dramatic effect of OP-1 on PG synthesis rates, we also found that the turnover of PG was reduced by up to 3-fold. This latter effect may have been due to either suppression of degradation of PG or

due to enhanced PG synthesis, which only caused an apparent decreased degradation. Further work will be required to resolve these possibilities. Nonetheless, the effect of OP-1 on PG synthesis is consistent with the possibility that OP-1 compensates for the ability of the Fn-f to decrease PG content and restores PG by enhancing PG synthesis sufficiently. Since we cannot be sure that OP-1 also slowed PG degradation, we cannot conclude that OP-1 is reversing or blocking the actual damage events, although it apparently maintains the steady state levels of PG. It should also be noted that our studies were performed in the 10% serum and that other serum factors might have regulated its beneficial activity. Since we did not study serum-free conditions, we cannot conclude that the beneficial effects observed upon addition of OP-1 were not due to the interaction between OP-1 and serum factors.

The observations that TGF-β not only did not block Fn-f mediated PG depletion, but by itself promoted a decrease in cartilage PG content may seem surprising in view of reports on the anabolic effects of TGF-β. However, more recent studies show that the actions of TGF-β are dependent upon the context in which it acts (reviewed in ref. [27]). For example, TGF-β has been shown to inhibit PG synthesis in intact murine patellae cartilage [28]. TGF-β stimulates PG synthesis under serum-free conditions, but inhibits PG synthesis under serum conditions with chondrocytes encapsulated in a three-dimensional fibrin matrix [29]. Serum and serum-free conditions also cause opposite effects in terms of TGF-β activities toward cell proliferation cell and DNA synthesis [30]. Some of the variant effects of TGF-β have been proposed to be due to time-dependent differential expression of different TGF-β receptors [33]. For example, TGF-β has been shown to stimulate PG synthesis in freshly isolated chondrocytes [31] and in cultured chick limb bud mesodermal cells [32], but in both cases, to inhibit at later time points. Whether TGF-β1 should be considered an anabolic factor or not was not our focus, but rather whether it altered the effects of Fn-fs or had different effects than OP-1 in our culture system. Our work should not be used to suggest that TGF-β1 is catabolic *per se* since its activity may have been affected by the presence of serum factors.

Nonetheless, these data demonstrate that although OP-1 is a member of the TGF-β superfamily, OP-1 and TGF-β1 have quite different effects on Fn-f mediated cartilage metabolism under our culture conditions. The data further illustrate that OP-1 is a very powerful blocker of Fn-f mediated cartilage PG depletion, a very powerful promoter of reparative responses and is more potent than IGF-1 and certainly more potent than TGF-β in this activity. If we consider that Fn-f mediated cartilage degradation may have physiologic significance and be involved in osteoarthritis as discussed earlier, then OP-1 which has great therapeutic potential in general, would certainly be effective as a therapeutic agent in suppressing in vivo activities of Fn-fs.

Acknowledgements. This work was supported by the Greater Chicago Chapter of the Arthritis Foundation and the National Institute of Arthritis, Musculoskeletal and Skin Diseases SCOR Grant AR39239-03. HK was a recipient of a scholarship from the Deutscher Akademischer Austauschdienst. GH gratefully acknowledges the support of the Dr. Ralph and Marian C. Falk Medical Trust Fund.

References

[1] Urist MR. Bone: formation by autoinduction. Science 1965; 150: 893–9.
[2] Kuber Sampath T, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RW, et al. Recombinant human osteogenic protein-1 (hop-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. J Biol Chem 1992; 267: 20352–62.
[3] Flechtenmacher J, Huch K, Thonar EJ-MA, Mollenhauer JA, Davies SR, Schmid TM, et al. Recombinant human osteogenic protein 1 is a potent stimulator of the synthesis of cartilage proteoglycans and collagens by human articular chondrocytes. Arthritis Rheum 1996; 39: 1896–904.
[4] Huch K, Wilbrink B, Flechtenmacher J, Koepp HE, Aydelotte MB, Kuber Sampath T, et al. Effects of recombinant human osteogenic protein-1 on the production of proteoglycan, prostaglandin E_2 and interleukin-1 receptor antagonist by human articular chondrocytes cultured in the presence of interleukin-1. Arthritis Rheum 1997; 40: 2157–61.
[5] Chen P, Vukicevic S, Kuber Sampath T, Luyten FP. Osteogenic protein-1 promotes growth and maturation of chick sternal chondrocytes in serum-free cultures J Cell Sci 1995; 108: 105–14.
[6] Asahina I, Kuber Sampath T, Hauschka PV. Human osteogenic protein-1 induces chondroblastic, osteoblastic, and/or adipocytic differentiantion of clonal murine target cells. Exp Cell Res 1996; 222: 38–47.
[7] Dieudonné SC, Semeins CM, Goei SW, Vukicevic S, Klein-Nulend J, Sampath TK, et al. Opposite effects of osteogenic protein and transforming growth factor β on chondrogenesis in clutured long bone rudiments. J Bone Miner Res 1994; 9: 771–80.
[8] Chandrasekhar S, Harvey AK. Transforming growth factor B is a potent inhibitor of IL-1 induced protease activity and cartilage proteoglycan degradation. Biochem Biophys Res Commun 1988; 157: 1352–9.
[9] Andrews HJ, Edwards TA, Cawston TE, Hazleman BL. Transforming growth factor-beta causes partial inhibition of interleukin 1-stimulated cartilage degradation in vitro. Biochem Biophys Res Commun 1989;162: 144–50.
[10] Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol 1987; 105: 1039–45.
[11] Kerr LD, Miller DB, Matrisian LM. TGF-β1 inhibition of transin/stromelysin gene expression is mediated through a fos-binding sequence. Cell 1990; 61: 267–78.
[12] Homandberg GA, Hui F, Wen C, Purple C, Bewsey K, Koepp H, et al. Fibronectin fragment induced cartilage chondrolysis is associated with release of catabolic cytokines. Biochem J 1997; 321: 751–7.
[13] Homandberg GA, Hui F, Wen C. Association of proteoglycan degradation with catabolic cytokine and stromelysin release from cartilage cultured with fibronectin fragments. Arch Biochem Biophys 1996; 334: 325–31.
[14] Xie DL, Hui F, Homandberg GA. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases; stromelysin plays a major role in chondrolysis. Arch Biochem Biophys 1994; 311: 205–12.
[15] Bewsey K, Wen C, Purple C, Homandberg GA. Fibronectin fragments induce the expression of stromelysin-1 mRNA and protein in bovine chondrocytes in monolayer culture. Biochim Biophys Acta 1996; 1317: 55–64.
[16] Xie DL, Hui F, Homandberg GA. Fibronectin fragments reversibly alter matrix protein synthesis in cartilage in vitro. Arch Biochem Biophys 1993; 307: 110–8.
[17] Homandberg GA, Hui F. High concentrations of fibronectin fragments cause short term catabolic effects in cartilage tissue while lower concentrations cause continuous anabolic effects. Arch Biochem Biophys 1994; 311: 213–8.

[18] Homandberg GA, Meyers R, Xie DL. Fibronectin fragments cause chondrolysis of bovine articular cartilage slices in culture. J Biol Chem 1992; 267: 3597–604.
[19] Xie DL, Meyers R, Homandberg GA. Fibronectin fragments in osteoarthritic synovial fluid; pathologic implications. J Rheumatol 1992; 19: 1448–52.
[20] Homandberg GA, Hui F, Wen C. Cartilage damaging activities of fibronectin fragments derived from cartilage and synovial Fluids. Osteoarthritis Cartilage 1988; 6: 231–44.
[21] Homandberg GA, Meyers R, Williams J. Intra-articular injection of fibronectin fragments induces cartilage proteoglycan release. J Rheumatol 1993; 20: 1378–82.
[22] Homandberg GA, Hui F, Wen C. Fibronectin fragment mediated cartilage chondrolysis: (I) suppression by anti-oxidants. Biochim Biophys Acta 1996;1317: 134–42.
[23] Homandberg GA, Hui F, Wen C. Fibronectin fragment mediated cartilage chondrolysis: (II) reparative effects of anti-oxidants. Biochim Biophys Acta 1996; 1317: 143–8.
[24] Homandberg GA, Hui F. Arg-Gly-Asp-Ser analogs suppress cartilage chondrolysis activities of integrin-binding and non-binding fibronectin fragments. Arch Biochem Biophys 1994; 310: 40–8.
[25] Homandberg GA, Wen C, Hui F. Agents that block fibronectin fragment-mediated cartilage damage also promote repair. Inflamm Res 1997; 46: 467–71.
[26] Chandrasekhar SA, Esterman MA, Hoffman HA. Microdetermination of proteoglycans and glycosaminoglycans in the presence of guanidine hydrochloride. Anal Biochem 1987; 161: 103–8.
[27] Trippel SB. Growth factor actions on articular cartilage. J Rheumatol 1995; 43: 129–32.
[28] van der Kraan PM, Vitters EL, van den Berg WB. Inhibition of proteoglycan synthesis by transforming growth factor beta in anatomically intact articular cartilage of murine patellae. Ann Rheum Dis 1992; 51: 643–7.
[29] Fortier LA, Nixon AJ, Mohammed HO, Lust G. Altered biological activity of equine chondrocytes cultured in a three-dimensional fibrin matrix and supplemented with transforming growth factor beta-1. Am J Vet Res 1997; 58: 66–70.
[30] Vivien D, Galera P, Loyau G, Pujol JP. Differential response of cultured rabbit articular chondrocytes (RAC) to transforming growth factor beta (TGF-β)-evidence for a role of serum factors. Eur J Cell Biol 1991; 54: 217–23.
[31] Glansbeek HL, van der Kraan PM, Vitters EL, van den Berg WB. Variable TGF-β receptor expression regulates TGF-β responses of articular chondrocytes. Agents Actions 1993; 39: 139–45.
[32] Glansbeek HL, van der Kraan PM, Vitters EL, van den Berg WB. Correlation of the size of type II transforming growth factor beta (TGF-β) receptor with TGF-β responses of isolated bovine articular chondrocytes. Ann Rheum Dis 1993; 52: 812–6.
[33] Carrington JL, Reddi AH. Temporal changes in the response of chick limb bud mesodermal cells to transforming growth factor beta-type 1. Exp Cell Res 1990; 186: 368–73.