

OP-1 Augments Glucocorticoid-inhibited Fracture Healing in a Rat Fracture Model

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Abstract Glucocorticoids inhibit bone remodeling and fracture healing. We sought to determine whether osteogenic protein 1 (OP-1) can overcome this inhibition in a closed fracture model in the rat. Time-released prednisolone or placebo pellets were implanted subcutaneously; closed femoral fractures were created 2 weeks later in rats. Fractures received sham, OP-1 and collagen, or collagen-only implants. Femurs were harvested at 3, 10, 21, 28, and 42 days postfracture. Fractures were examined radiographically for amount of hard callus; mechanically for torque and stiffness (also expressed as a percentage of the contralateral intact femur); and histomorphometrically for amount of cartilaginous and noncartilaginous soft callus,

hard callus, and total callus. Glucocorticoid administration inhibited fracture healing. The application of a devitalized Type I collagen matrix mitigated the inhibitory effects of prednisolone on fracture healing. However, further increases in indices of fracture healing were observed when OP-1 was added to the collagen matrix compared with collagen alone. OP-1 and collagen was more effective than collagen alone.

Introduction

In 1965, Marshall Urist identified an extractable peptide in devitalized, decalcified bone capable of inducing de novo bone formation [49]. Subsequent work by Urist [50] and others [37, 39, 54] has identified up to 20 variants of this protein called “bone morphogenetic protein” (BMP), a member of the transforming growth factor β superfamily [36]. At present, only BMP-2 and BMP-7 are produced commercially as a therapy to enhance bone healing with BMP-7 called osteogenic protein 1 (OP-1). BMP-2 and OP-1 both promote bone formation in a variety of experimental models (rat [8, 13, 25, 46, 47, 56], rabbit [3, 10, 29, 48], sheep [15, 20], dog [9, 19, 32, 35, 40, 43]). Although these proteins are now used clinically, most of the information on their effects is known from these and other experimental studies.

In current human and veterinary orthopaedic practice, one of the most widely accepted means of enhancing the bone healing process is by grafting the defect with cancellous auto- or allograft [45]. However, patient morbidity from graft collection in human orthopaedics is well-documented [17, 40, 41]. Bone graft collection in veterinary orthopaedics also is associated with patient morbidity at the donor site [45]. The increasing availability of BMPs has

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made it possible to explore the use of these proteins to directly enhance bone healing as an alternative to bone grafting.

Glucocorticoids inhibit bone remodeling [18, 28, 30, 51, 52] and inhibit fracture healing in animal models [1, 22–24, 31, 33, 44, 53]. A recent study reported BMP-2 enhances osteotomy healing in glucocorticoid-treated rabbits [29]. If OP-1 enhances fracture healing under circumstances of prednisolone-induced inhibition, it may be used clinically to enhance fracture healing in patients receiving glucocorticoid therapy.

We therefore raised the following questions: (1) Do pharmacologic doses of glucocorticoid inhibit fracture healing in the rat closed femoral fracture model (as measured radiographically for amount of hard callus; mechanically for torque, stiffness, and amount of cartilaginous and noncartilaginous soft callus, hard callus, and total callus)? (2) Does OP-1 and a collagen carrier overcome the inhibition of fracture healing resulting from glucocorticoid administration in this model? (3) Does collagen alone overcome the inhibition of fracture healing resulting from glucocorticoid administration in this model?

Materials and Methods

One hundred seventy-four skeletally mature male Sprague-Dawley rats (Harlan SD, Madison, WI), 3 months of age, were housed two per cage and allowed to acclimate to the housing environment for 3 days. The 174 rats were divided into six groups: no OP-1 or collagen carrier applied to the fracture site with (treatment: Group 1; $n = 34$) or without (placebo: Group 2; $n = 37$) prednisolone; OP-1 and collagen carrier with (treatment: Group 3; $n = 41$) or without (placebo: Group 4; $n = 40$) prednisolone; or collagen

carrier alone with (treatment: Group 5; $n = 11$) or without (placebo: Group 6; $n = 11$) prednisolone. Rats were anesthetized using intraperitoneal ketamine/xylazine at a dose of 40 to 60 mg/kg and 5 to 7.5 mg/kg, respectively, for subcutaneous implantation of 50-mg pellets of 60-day time-released prednisolone or placebo (Innovative Research of America, Sarasota, FL). This equates to 0.83 mg prednisolone released per rat per day. Rats weighed between 350 and 375 g at the initiation of this study; therefore, each rat received approximately 2.2 to 2.3 mg/kg prednisolone daily. This is equivalent to the dose administered clinically. The harvested femurs from the prednisolone treatment and placebo groups were compared at 10, 28, and 42 days for radiographic analysis; at 3, 10, 21, 28, and 42 days for histomorphometric analysis; and at 28 and 42 days for mechanical analysis (Table 1). An earlier study from our laboratory found a two-fold difference in radiographic callus between low and high concentrations of OP-1 in the presence of an acute bacterial infection [7]. In order to detect a 40% difference in radiographic callus area between treatment groups with an 80% power ($\alpha = 0.05$), we determined that six animals would be required per group. However, group numbers varied as a result of unexpected mortality. This study received prior approval of the Institutional Animal Care and Use Committee of the University of Minnesota.

Two weeks after implantation, each rat underwent surgery for intramedullary pin placement and subsequent fracture of either the right or left femur, alternating sides for each consecutive rat. Ketamine/xylazine (40–60 mg/kg and 5–7.5 mg/kg, respectively) was used for rat anesthesia and closed fracture performed as described previously [4]. For OP-1 treatment groups, OP-1 was reconstituted with normal saline; 25 μg was then mixed with 50 mg collagen carrier. In collagen-only groups, we mixed normal saline

Table 1. Experimental animal number: treatment distribution

Analysis	Treatment	Postfracture									
		3 Days		10 Days		21 Days		28 Days		42 Days	
		Placebo	Pred	Placebo	Pred	Placebo	Pred	Placebo	Pred	Placebo	Pred
Radiography	No OP-1 or collagen			6	6			6	6	6	5
	OP-1 and collagen			6	7			6	6	6	6
	Collagen only							6	5		
Histomorphometry	No OP-1 or collagen	6	5	6	6	6	6	6	6	5	5
	OP-1 and collagen	6	6	6	7	4	5	6	6	6	4
	Collagen only							6	5		
Mechanics	No OP-1 or collagen							7	6		
	OP-1 and collagen							6	5	6	6
	Collagen only							5	6		

Pred = 2.2 mg/kg prednisolone slow-release subcutaneously; OP-1 = osteogenic protein 1.

without OP-1 with 50 mg collagen carrier. We performed a limited cranial lateral approach to the femur at the diaphysis. A collagen carrier with OP-1 or saline (collagen only) was applied to the fracture site. The incision was closed in a routine fashion. At the end of the specified study interval, each rat was weighed and euthanized by intraperitoneal barbiturate overdose.

We made an effort to feed prednisolone treatment and placebo groups approximately the same amount of food. To control for differences in diet between prednisolone treatment and placebo groups, prednisolone-treated rats were fed *ad libitum*, whereas placebo rats were fed premeasured amounts based on approximate food consumption rates of prednisolone-treated rats.

At the end of specified study intervals, we harvested 72 fractured and contralateral intact hind limbs after euthanasia and performed high-resolution radiographic imaging using a Faxitron[®] x-ray system (Model 43805 N X-Ray System Faxitron[®] Series; Hewlett-Packard, McMinnville, OR) with slow-speed radiographic film. Lateral radiographs were digitized and scanned at a resolution and scale sufficient to ensure accurate analysis (ie, 300 × 300 dots per inch [dpi] at a 300% scale for an equivalent 900 dpi). We imported the scanned images into a personal computer for analysis by a semiautomated image analysis system (Bioquant Image Analysis; R&M Biometrics, Nashville, TN). Using the Bioquant Image Analysis program, lateral radiographic images were evaluated by one blinded investigator (RSG) by tracing the amount of hard (calcified) external callus at each time interval (pixels/area traced).

For mechanical testing, femurs were frozen in normal saline and stored at -20°C until the day of testing, at which time they were thawed at room temperature. The femurs were wrapped in saline-soaked gauze sponges to keep them moist until just before testing. Each femur was placed in a specially designed potting/alignment device and the distal and proximal ends of the femur were potted in aluminum sleeves filled with metal alloy potting medium. We then installed each femur in an axial-torsion test machine (Model AT 3045; Bose EnduraTEC Systems Corp, Eden Prairie, MN) with a low-capacity 50-in-lb torque cell (Transducer Techniques, Temecula, CA). The femurs were loaded in torsion to failure at a rate of 0.5°/sec. The torque versus angular displacement data were recorded (Wintest software; Bose EnduraTEC Systems Corp); maximum angular deformation to failure, maximum torque to failure, and torsional stiffness were recorded and these values were normalized to percentages of contralateral intact femurs.

Femurs with closed fractures were harvested for histomorphometry and placed in 10% buffered formalin for 24 hours. Specimens were decalcified at 25°C using a

commercially available hydrochloric acid solution (Decalcifier II[®]; Surgipath Medical Industries Inc, Richmond, IL) for 24 to 36 hours to completely decalcify the specimens. They were transferred to 70% ethanol until histologic processing. Femurs were prepared for paraffin embedding using standard histologic techniques. Decalcified midsagittal sections 4 µm thick included the entire middiaphyseal fracture callus and adjacent proximal and distal femur. Sections were stained with hematoxylin and eosin.

Histomorphometric analyses were performed blinded by a single investigator (RSG) on a Bioquant workstation (R&M Biometrics) at 100 × magnification. One midsagittal section was examined for each fracture and measurements traced using the Bioquant Image Analysis program. Fracture callus measurements included noncartilaginous soft callus, cartilaginous soft callus, hard callus, and total external callus (the sum of these three histologic parameters). All animals used for histomorphometric analyses were first used for radiography.

We determined differences in fracture healing between prednisolone treatment (Group 1) and placebo (Group 2) groups with no OP-1 or collagen for all parameters using a two-way analysis of variance in which time and prednisolone treatment were the two main effects. A *p* value < 0.05 and < 0.2 for main effects and the interaction term, respectively, was considered significant. Post hoc pairwise comparisons were accomplished using the *t*-test. Significance levels for post hoc comparisons were adjusted for using the Bonferroni correction. Significance levels varied depending on the number of preplanned pairwise comparisons made; for five comparisons *p* < 0.01, for three comparisons *p* < 0.016, and for two comparisons *p* < 0.025. Differences in fracture healing between prednisolone treatment (Group 3) and placebo (Group 4) groups with OP-1 with collagen for all parameters were also determined using a two-way analysis of variance as described previously. Differences in fracture healing between prednisolone treatment (Group 5) and placebo (Group 6) groups with collagen only were evaluated at 28 days using the *t*-test for each measured parameter. We further determined differences in fracture healing between OP-1 and collagen (Groups 3 and 4) and collagen alone (Groups 5 and 6) using a two-way analysis of variance.

Results

Pharmacologic doses of glucocorticoid inhibited fracture healing in prednisolone treatment compared with placebo groups. Radiographic analysis revealed less mineralized bone resulting from prednisolone treatment (*p* < 0.001) and this treatment effect differed over time (*p* = 0.028).

Calcified external fracture callus formation was reduced at 28 ($p < 0.001$) and 42 ($p = 0.002$) days postfracture for prednisolone treatment when compared with placebo (Table 2; Fig. 1). Mechanical analyses at 28 days postfracture showed fracture torque to failure ($p = 0.024$), fracture stiffness ($p = 0.025$), percent of intact femur torque ($p = 0.043$), and percent of intact femur stiffness ($p = 0.01$) were decreased in prednisolone treatment compared with placebo groups (Table 3). Histomorphometric analyses revealed a decrease ($p < 0.001$) in hard callus area with prednisolone treatment compared with placebo. The treatment effect differed over time ($p = 0.016$) with a decrease ($p < 0.001$) in hard callus area at 42 days postfracture for prednisolone treatment compared with placebo (Fig. 2). Total callus area decreased with prednisolone treatment ($p < 0.001$). This treatment effect differed over time ($p = 0.06$) with reductions at 21 ($p = 0.009$) and 42 ($p = 0.001$) days with prednisolone treatment compared with placebo (Fig. 2).

OP-1 and collagen application to the fracture site overcame the inhibition of fracture healing seen in prednisolone-treated rats. All rats with OP-1 and collagen application had exuberant callus at all time points (Fig. 3). Quantitative radiography revealed no difference ($p = 0.97$) in calcified external fracture callus between prednisolone treatment and placebo groups with OP-1 and collagen application (Table 4). The application of OP-1 with collagen carrier prevented the reductions in maximum torque to failure and torsional stiffness (also normalized to percent of contralateral intact femurs) observed with prednisolone treatment (Table 5). Histomorphometric analysis showed a treatment effect that differed over time ($p = 0.06$) with greater ($p = 0.001$) cartilaginous soft callus seen with prednisolone treatment compared with placebo at 10 days. However, we observed no differences for any other histomorphometric parameter between prednisolone treatment and placebo groups with OP-1 and collagen applied to the fracture site (Fig. 4).

Collagen application to the fracture site alone improved fracture healing in prednisolone-treated rats at 28 days. At this time point, there were no differences seen in

Table 2. Radiographic callus area (mm^2) in prednisolone-treated and placebo fractured femurs at 10, 28, and 42 days postfracture

Day	Treatment group	Mean	SD	p Value
10-day	Prednisolone	18.065	2.724	0.548
	Placebo	20.144	3.801	
28-day	Prednisolone	20.573	7.475	< 0.001*
	Placebo	35.914	7.888	
42-day	Prednisolone	20.292	4.119	0.002*
	Placebo	32.371	7.089	

* Significant; SD = standard deviation.

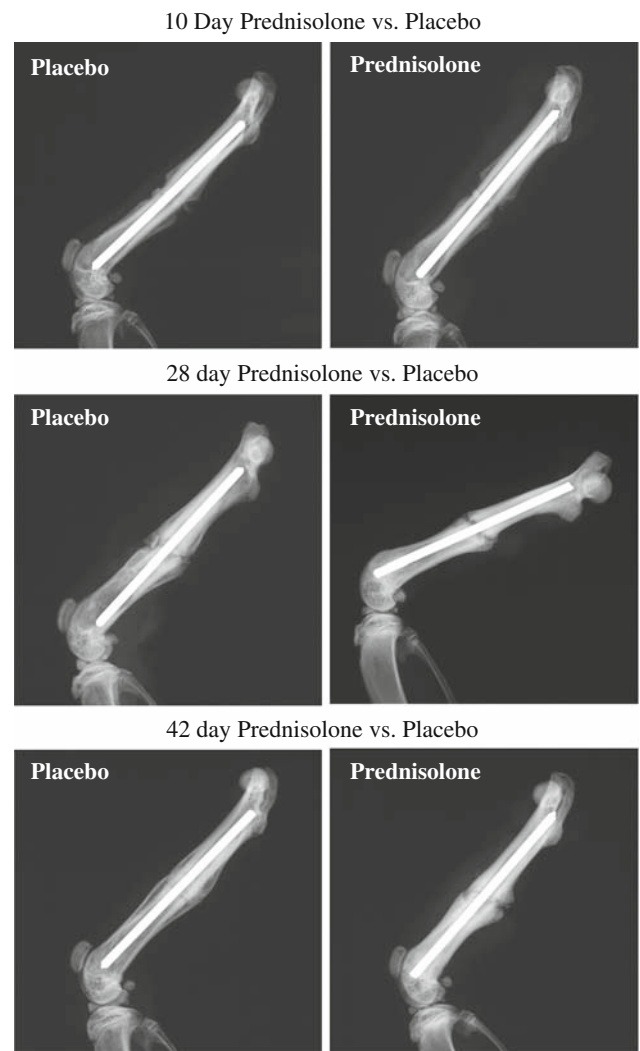


Fig. 1 Lateral radiographs of rat femurs show fracture healing for prednisolone and placebo groups at 10, 28, and 42 days after fracture. There was less calcified external fracture callus formation at 28 ($p < 0.001$) and 42 ($p = 0.002$) days postfracture for prednisolone treatment compared with placebo groups.

Table 3. Fracture and percent of intact torque and stiffness in prednisolone-treated and placebo fractured femurs at 28 days postfracture

Parameter	Treatment group	Mean	SD	p Value
Fracture torque (N-m)	Prednisolone	0.144	0.072	0.024*
	Placebo	0.236	0.025	
Fracture stiffness (Nm/deg)	Prednisolone	0.006	0.003	0.025*
	Placebo	0.018	0.011	
Percent of intact torque	Prednisolone	0.456	0.19	0.043*
	Placebo	0.639	0.089	
Percent of intact stiffness	Prednisolone	0.175	0.088	0.009*
	Placebo	0.479	0.225	

* Significant; SD = standard deviation.

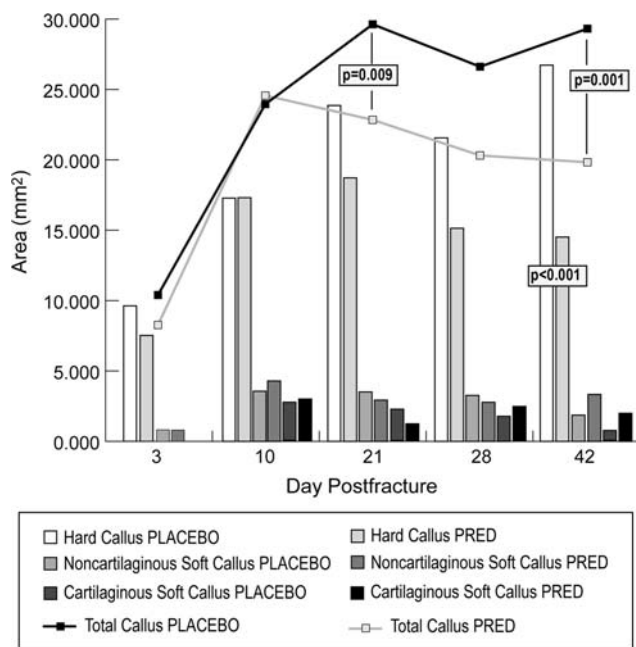


Fig. 2 A graph shows the histomorphometric determination of callus areas for prednisolone treatment and placebo groups at 3, 10, 21, 28, and 42 days. A decrease in total callus area was seen at 21 ($p = 0.009$) and 42 ($p = 0.001$) days and in hard callus area at 42 ($p < 0.001$) days with prednisolone treatment compared with placebo. PRED = prednisolone.

radiographic, mechanical, and histomorphometric analyses for prednisolone treatment compared with placebo groups (Tables 6, 7, 8; Fig. 3).

OP-1 and collagen applied to prednisolone treatment and placebo groups resulted in greater indices of healing compared with these groups implanted with collagen alone. Radiographic callus formation ($p < 0.001$), fracture torque ($p < 0.001$), torque as a percent of the contralateral intact femur ($p = 0.009$), fracture stiffness ($p < 0.001$), and stiffness as a percent of the contralateral intact femur ($p < 0.001$) were all greater with OP-1 and collagen compared with collagen alone. Although there was no difference in total external callus formation, histomorphometric analysis revealed greater hard callus ($p < 0.001$) and less cartilaginous ($p < 0.001$) and noncartilaginous soft callus ($p < 0.001$) for the fractures treated with OP-1 and collagen compared with the collagen-only group.

Discussion

Glucocorticoids are administered clinically to treat a variety of diseases and the medical benefits of glucocorticoid use are well-described [5, 28]. Despite this, glucocorticoids promote undesirable complications on many biologic processes [28]. One of the most important of these effects is the influence of glucocorticoids on the healing process of



Fig. 3 Lateral radiographs of rat femurs show fracture healing for prednisolone and placebo groups at 10, 28, and 42 days after fracture with OP-1 in a collagen carrier applied to the fracture site and at 28 days for collagen only. No difference in calcified external fracture callus between prednisolone treatment and placebo groups was seen at any time. OP-1 = osteogenic protein 1.

bone fractures [1, 22–24, 31, 33, 53]. If OP-1 experimentally enhances fracture healing under circumstances of prednisolone-induced inhibition, it may be used clinically to enhance fracture healing in patients receiving

Table 4. Radiographic callus area (mm²) with OP-1 application in prednisolone-treated and placebo fractured femurs at 10, 28, and 42 days postfracture

Days	Treatment group	Mean	SD	p Value
10	Prednisolone/OP-1 and collagen	107.56	11.13	0.07
	Placebo/OP-1 and collagen	123.7	14.68	
28	Prednisolone/OP-1 and collagen	85.0	16.9	0.04
	Placebo/OP-1 and collagen	69.7	12.1	
42	Prednisolone/OP-1 and collagen	70.55	11.22	0.81
	Placebo/OP-1 and collagen	72.32	8.64	

OP-1 = osteogenic protein 1; SD = standard deviation.

glucocorticoid therapy. Recent evidence supporting the use of BMP-2 to create new bone within a critical defect during glucocorticoid treatment [29] raises the following questions: Do pharmacologic doses of glucocorticoid inhibit fracture healing in the rat closed femoral fracture model? Do OP-1 and a collagen carrier overcome the inhibition of fracture healing resulting from glucocorticoid administration in this model? Does collagen alone overcome the inhibition of fracture healing resulting from glucocorticoid administration in this model?

Our dose of glucocorticoid was based on the effects reported to produce osteoporosis in the rat [16, 26, 27, 42]. Although this dose is on the high end of the range administered clinically, we believe these higher doses are reasonable, because the goal was to elicit a bone response; this response parallels that seen clinically. We used a small number of rats at only one time point to test for the effects

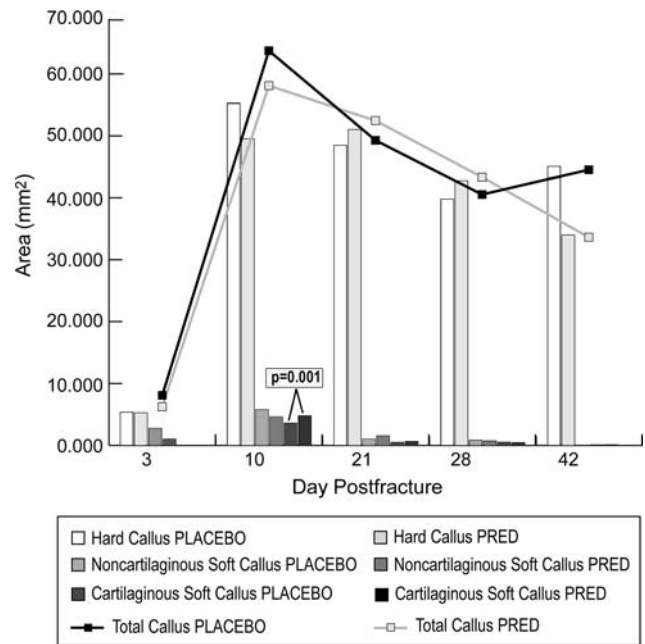


Fig. 4 A graph shows the histomorphometric determination of callus areas for prednisolone treatment and placebo groups at 3, 10, 21, 28, and 42 days after OP-1 with collagen application. We observed an increase in cartilaginous soft callus at 10 days with prednisolone treatment compared with placebo (p = 0.001). No other differences were seen between groups at any time. PRED = prednisolone; OP-1 = osteogenic protein 1.

of collagen alone on fracture healing with glucocorticoid administration. The use of only one time point at 28 days could have limited the ability to detect major differences

Table 5. Fracture and percent of intact torque and stiffness with OP-1 application in prednisolone-treated and placebo fractured femurs at 28 and 42 days postfracture

Parameter (days)	Treatment group	Mean	SD	p Value
Fracture torque (Nm) (28 days)	Prednisolone/OP-1 and collagen	0.40	0.067	0.71
	Placebo/OP-1 and collagen	0.37	0.067	
Fracture stiffness (Nm/deg) (28 days)	Prednisolone/OP-1 and collagen	0.03	0.010	0.70
	Placebo/OP-1 and collagen	0.03	0.010	
Percent of intact torque (28 days)	Prednisolone/OP-1 and collagen	120.6	38.7	0.11
	Placebo/OP-1 and collagen	81.7	20.6	
Percent of intact stiffness (28 days)	Prednisolone/OP-1 and collagen	117.8	81.4	0.35
	Placebo/OP-1 and collagen	89.9	24.5	
Fracture torque (Nm) (42 days)	Prednisolone/OP-1 and collagen	0.52	0.20	0.28
	Placebo/OP-1 and collagen	0.61	0.12	
Fracture stiffness (Nm/deg) (42 days)	Prednisolone/OP-1 and collagen	0.04	0.02	0.07
	Placebo/OP-1 and collagen	0.05	0.02	
Percent of intact torque (42 days)	Prednisolone/OP-1 and collagen	120.6	37.5	0.97
	Placebo/OP-1 and collagen	119.9	50.6	
Percent of intact stiffness (42 days)	Prednisolone/OP-1 and collagen	89.4	19.5	0.27
	Placebo/OP-1 and collagen	120.9	50.6	

OP-1 = osteogenic protein 1; SD = standard deviation.

Table 6. Radiographic callus area (mm²) with collagen application in prednisolone-treated and placebo fractured femurs at 28 days postfracture

Treatment group	Mean	SD	p Value
Prednisolone/collagen	33.88	4.32	0.60
Placebo/collagen	37.23	4.83	

SD = standard deviation.

Table 7. Fracture and percent of intact torque and stiffness after collagen application in prednisolone-treated and placebo fractured femurs at 28 days postfracture

Parameter	Treatment group	Mean	SD	p Value
Fracture torque (Nm)	Prednisolone/collagen	0.24	0.08	0.26
	Placebo/collagen	0.29	0.04	
Fracture stiffness (Nm/deg)	Prednisolone/collagen	0.01	0.005	0.88
	Placebo/collagen	0.01	0.002	
Percent of intact torque	Prednisolone/collagen	69.51	23.69	0.89
	Placebo/collagen	67.91	10.92	
Percent of intact stiffness	Prednisolone/collagen	30.30	11.17	0.88
	Placebo/collagen	29.29	9.89	

SD = standard deviation.

Table 8. Histomorphometric analysis (mm²) with collagen application in prednisolone-treated and placebo fractured femurs at 28 days postfracture

Parameter	Treatment group	Mean	SD	p Value
Noncartilaginous soft callus	Prednisolone/collagen	3.64	2.41	0.12
	Placebo/collagen	5.88	1.90	
Cartilaginous soft callus	Prednisolone/collagen	3.16	1.08	0.95
	Placebo/collagen	3.20	0.69	
Hard callus	Prednisolone/collagen	27.07	4.71	0.64
	Placebo/collagen	28.16	2.80	
Total external callus	Prednisolone/collagen	33.88	4.32	0.26
	Placebo/collagen	37.23	4.83	

SD = standard deviation.

between prednisolone treatment and placebo with collagen alone. This possibility is suggested by the histomorphometric evaluation for Groups 1 and 2. At the 28-day time point, we observed no difference for all parameters of fracture callus measurement with prednisolone treatment compared with the placebo group (Fig. 2). This is similar to the results of collagen used alone with prednisolone treatment (Table 8). It is unknown whether the inclusion of other time points may have revealed a difference, because we observed a decrease at 21 and 42 days with prednisolone treatment (Fig. 2). Thus, further study is needed to

evaluate collagen at different time points to confirm that collagen alone reverses the inhibition of fracture healing during glucocorticoid therapy, as shown at multiple time points with OP-1 and collagen.

Our data suggest OP-1 overcomes the inhibition of fracture healing resulting from glucocorticoid administration in this model. Other investigations also suggest BMPs enhance bone formation in a compromised clinical environment. OP-1 overcomes the inhibitory effect of nicotine in a rabbit spinal fusion model [34] and induces bone formation in an infected segmental defect in the rat femur [7]. BMP-2 enhances osteotomy healing in rabbits under the inhibitory effects of glucocorticoid treatment [29]. Thus, the current work in conjunction with these previously reported studies suggest the clinical application of exogenous BMP may be of particular value when there is inhibition to the normal bone healing process.

The data suggest, at Day 28, collagen alone overcomes the inhibition of fracture healing resulting from prednisolone administration. This was unexpected because the collagen carrier lacks the necessary osteoinductive peptides necessary for bone healing [9, 10]. The purified bovine Type I collagen used as a carrier for OP-1 in this study was osteoconductive only [38]. Thus, some contribution to bone healing may result from the collagen matrix alone. However, these findings for collagen alone could be by chance because of multiple uncorrected comparisons. We chose the 28-day time point because healing was expected to be near complete and it was a time when comparisons could be made for all measured parameters in this study. Despite the improvement matrix alone appeared to provide, adding OP-1 to the same collagen carrier produced greater radiographic and histologic hard callus, and the callus formed was stronger in torsion than if collagen was used alone. Thus, OP-1 was more effective at healing fractures than collagen alone in this model.

The reason collagen contributed to fracture healing in this study may be related to events occurring during the inflammation stage of fracture healing, which is important in the fracture repair process [2, 14]. Application of a foreign collagen may cause inflammation at the fracture site and migration of inflammatory cells to the fracture. Cells involved with inflammation during fracture healing release inflammatory mediators and cytokines [12, 14]. Many of these cytokines are important to the maturation and proliferation of osteoprogenitor cells [14]. Thus, collagen may induce the release of cytokines important to fracture healing from cells participating in this process.

The role of BMP in fracture healing has been well-described [2, 12, 49]. However, the details regarding the underlying molecular mechanisms involved in exogenous BMP enhancement of fracture healing continue to be elucidated. The isolation of osteoblast-specific BMP receptors

during bone formation and fracture healing reinforces the theory that BMPs are important in the differentiation of osteoprogenitor cells to mature osteoblasts [36]. For example, BMP upregulates the expression of the transcriptional factor *Cbfa1* [11, 55] which is essential to osteoblast and chondrocyte differentiation and proliferation [11, 21, 55], whereas glucocorticoids downregulate the expression of this protein [6]. Considering this, OP-1 may be offsetting the inhibitory action of glucocorticoid in part through counteracting the downregulation of *Cbfa1*. This theory has been proposed previously [29]; however, the findings in the current investigation provide further support for the ability of BMP to overcome the inhibitory effects of glucocorticoid administration in a fracture healing model.

Our data suggest the local application of OP-1 to the fracture site overcomes the inhibitory effects of glucocorticoids on fracture healing. These results support the hypotheses that radiographic callus, mechanical strength of the fracture, and histomorphometric measurements of callus formation are the same in prednisolone-treated and placebo rats when OP-1 is applied to the fracture site. The fundamental questions of how OP-1 is working to reverse the inhibitory effects of glucocorticoids must be examined hand in hand with how the glucocorticoid is affecting cellular metabolic pathways. Thus far, these effects are incompletely understood. Investigations using molecular biologic and nuclear magnetic resonance techniques will provide insight into these questions. Despite the present gap in mechanistic information, our data suggest OP-1 has potential for clinical use in patients treated with glucocorticoids.

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