Immunohistochemical Analysis of Cortical and Cancellous Bone After Radiation and the Effect of Platelet-rich Plasma on Autogenous Bone Grafting

Tara L. Aghaloo, DDS, MD[1]/Anh D. Le, DDS, PhD[2]/Earl G. Freymiller, DMD, MD[3]/
Sean Avera, DDS[4]/Kenneth Shimizu, MD, MPH[5]/Russell D. Nishimura, DDS[6]

Purpose: *The collection of autologous platelet-rich plasma (PRP) has demonstrated favorable affects on wound healing in compromised patients. The purpose of this study was to evaluate the expression of PDGF, bFGF, and TGF-β in irradiated and nonirradiated bone in a rabbit tibia model and the ability of PRP to increase growth factor expression when added to autogenous bone graft in a rabbit cranial defect model.* ***Materials and Methods:*** *Ten New Zealand White rabbit tibiae and calvariae were utilized for this study. Tibiae were irradiated at 60 to 70 cGy and evaluated for expression of PDGF, bFGF, and TGF-β. Rabbit calvariae were also analyzed after grafting with autogenous bone and PRP for determination of growth factor expression.* ***Results:*** *Decreased expression of PDGF, bFGF, and TGF-β was seen in cortical and cancellous bone samples when irradiated bone was compared to nonirradiated rabbit tibiae. An increase in PDGF, bFGF, and TGF-β expression was detected in cortical autogenous bone grafts with PRP at 1 and 2 months compared to autogenous bone alone.* ***Discussion:*** *In this study, growth factors, which were decreased in irradiated cortical and cancellous bone, showed increased expression at 1 and 2 months when PRP was added to autogenous bone grafts. Thus, PRP may have potential therapeutic applications when bone grafting is required in patients with reduced bone vascularity, including patients with previous head and neck irradiation, diabetes, and smoking habits.* ***Conclusions:*** *Decreased expression of PDGF, bFGF, and TGF-β was seen in radiated rabbit tibia as compared to nonirradiated controls, and increased expression of these growth factors was seen in PRP-containing autogenous bone grafts.* (Basic Science) (More than 50 references) Int J Oral Maxillofac Implants 2006;21:535–542

Key words: *animal study, bone grafting, cranial defects, growth factors, immunohistochemistry, platelet-rich plasma, radiation*

The emergence of growth factors and their availability for research and clinical utilization has presented new opportunities for bone healing and regeneration.[1–6] The use of platelet-rich plasma (PRP) has demonstrated favorable results in bone healing in animal and human studies.[7–18] Potential also exists to restore vascularly compromised wound healing to that of normal healing.[19–26] With these promising results, growth factors may be considered for use in patients with compromised vascular states, including diabetics, smokers, and irradiated patients. However, minimal literature exists on the vascular changes that occur in irradiated bone. The literature on the topic consists only of a few animal studies,[19–21,27–31] which demonstrate a decrease in important wound healing growth factors in irradiated bone. These factors include basic fibroblast growth factor (bFGF) as well as factors with potential osteoinductive properties such as transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF). Specific growth factors with angiogenic properties have been reported to improve bone repair in skin and bone wounds after irradiation.[19–26] The purpose of this preliminary study was to evaluate the expression of PDGF, bFGF, and TGF-β in irradiated and nonirradiated bone in a rabbit tibia model. The ability of concentrated growth factors, including PDGF, bFGF, and TGF-β, in the form of PRP to increase growth factor expression when added to autogenous bone graft in a rabbit cranial defect model was also evaluated.

[1]Assistant Professor, Oral and Maxillofacial Surgery, UCLA School of Dentistry, Los Angeles, California.
[2]Visiting Professor, Center for Craniofacial Molecular Biology, Los Angeles, California.
[3]Chairman, Oral and Maxillofacial Surgery, UCLA School of Dentistry.
[4]Private Practice, Periodontics, Auburn, California.
[5]Private Practice, Radiation Oncology, Vista, California.
[6]Professor, Division of Restorative Dentistry, UCLA School of Dentistry, Los Angeles, California.

Correspondence to: Dr Tara Aghaloo, UCLA Den-Oral Surg, Box 951668, 53-009 Den Bldg, Los Angeles, CA 90095-1668. E-mail: taghaloo@ucla.edu

MATERIALS AND METHODS

Rabbit Tibia Model for Irradiation

Tibiae of 10 adult New Zealand white rabbits were exposed to 65 cGy gamma irradiation, with an average energy of 1.25 MeV, delivered using a Theratron 780 Cobalt 60 unit (AECL, Toronto, ON, Canada). A latex balloon filled with water was positioned under the tibiae as a radiation bolus, providing a more uniform dose distribution to treatment volume by compensating for different thicknesses of soft tissue surrounding the bone. Eight fractions were delivered in split-course fashion: 4 fractions at 3-day intervals followed by a 2-week break and then the final 4 treatments at 3-day intervals. Radiation dose was verified using thermoluminescent dosimetry (Harshaw/Bicron, Solon, OH). Animals were sacrificed 4 months after irradiation, and the nonirradiated tibia was utilized as an internal control. The University of California at Los Angeles (UCLA) Animal Research Committee (ARC) in accordance with staff in the UCLA Department of Laboratory and Animal Medicine approved all animal protocols.

Rabbit Calvarial Grafting Model

Isolation and preparation of platelet concentrate were performed as previously described.[32] Bilateral defects were created in the rabbit cranium with an 8-mm trephine drill. Sites were grafted with autogenous bone or autogenous bone + PRP. The animals were sacrificed at 1 and 2 months. The bony specimens were collected and stored in 10% formalin until analyzed by immunohistochemistry. Protocols were approved by the UCLA ARC.

Immunohistochemical Staining

Specimens were treated with hydrochloric acid decalcifying solution (Fisher Scientific, Tustin, CA). Specimens were then dehydrated with graded alcohols and embedded in paraffin. The specimens were subsequently sectioned at 6 μm with a steel knife. Following 3 washes of phosphate buffered saline (PBS), slides were blocked with 5% normal goat serum (Gibco, Gaithersburg, MD) for 30 minutes. Murine monoclonal PDGF, bFGF, or TGF-β antibody in 2.5% normal goat serum were added at 1:100 concentration to slides and incubated overnight at 4°C. After being washed with PBS, slides were incubated with a secondary biotinylated anti-mouse IgG (1:200 Dako, Carpinteria, CA) for 1 hour at room temperature. Controls for each antibody consisted of incubation with secondary antibody in the absence of primary antibody. Slides were then washed 3 times with PBS. Substrate chromogen (3,3'-diaminobenzidine (DAB); Sigma Chemical, St Louis, MO) 1% in distilled water was used to develop the slides. Slides were mounted with Permount (Biomeda, Foster City, CA) and imaging was performed on an Optronics Imaging System with K.S 300 software (Zeiss, Oberkochen, Germany).

RESULTS

PDGF

In irradiated rabbit tibial bone, the level of PDGF was notably depressed in endothelial cells lining vascular channels of cortical bone as compared with those of nonirradiated controls (Figs 1a and 1b). Similarly, irradiated cancellous bone failed to show PDGF expression, whereas nonirradiated controls stained positively throughout trabecular bone and vessels (Figs 2a and 2b). To determine whether PRP had an effect on PDGF expression in a bone graft model, immunohistochemistry was performed. At 1 and 2 months, rabbit cranial samples grafted with autogenous bone + PRP showed increased expression of PDGF throughout the cortical bone sample when compared to autogenous bone grafting alone (Figs 3a, 3b, 4a, and 4b).

bFGF

Immunohistochemical staining of normal or nonirradiated bone shows abundant expression of bFGF in endothelial cells of the cortical vasculature, but no expression in irradiated bone (Figs 1c and 1d). Similar suppression of bFGF expression was observed in vasculature of irradiated cancellous bone (Figs 2c and 2d). Again at 1 and 2 months, rabbit cranial samples grafted with PRP and autogenous bone demonstrated an increased expression of bFGF in the vascular channels of the cortical bone as compared to autogenous bone alone (Figs 3c, 3d, 4c, and 4d).

TGF-β

Radiation treatment at 60 to 70 cGy of rabbit tibiae revealed a marked reduction in TGF-β expression in both cortical and cancellous bones. In nonirradiated cortical bone, endothelial cells lining the vascular channels stained positively for TGF-β. However, in the irradiated cortical bone, no TGF-β expression was detected on immunohistochemistry (Figs 1e and 1f). In nonirradiated cancellous bone, trabeculations and blood vessels stained positively for TGF-β, whereas irradiated bone showed no TGF-β expression (Figs 2e and 2f). When rabbit cranial bone grafts were evaluated for TGF-β expression, samples of autogenous bone + PRP showed increased expression compared to bone graft alone at both time points (Figs 3e, 3f, 4e, and 4f).

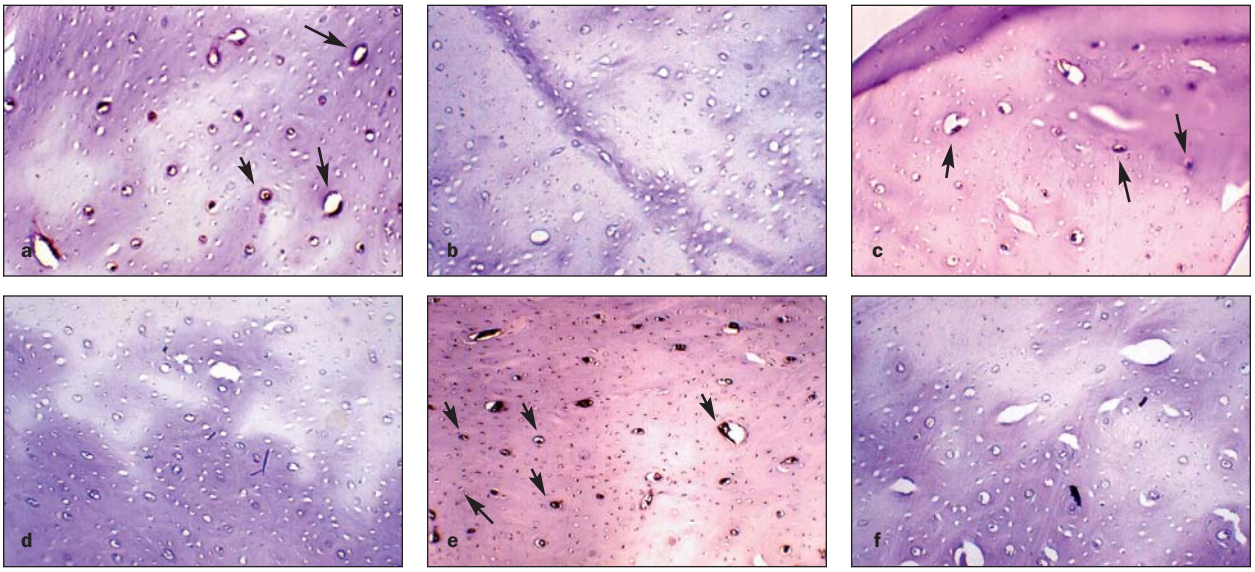

Fig 1 Immunohistochemistry comparison of normal and irradiated cortical rabbit tibial bone. Immunohistochemistry was performed to determine expression of growth factors in cortical nonirradiated and irradiated rabbit tibiae. *(a)* Expression of PDGF in nonirradiated cortical bone. Arrows point to representative areas of staining in the cortical bone and in the vascular channels. *(b)* Expression of PDGF in irradiated cortical bone. No staining can be seen in the irradiated cortical bone. *(c)* Expression of bFGF in nonirradiated cortical bone. A minimal increase in staining can be seen in the *vascular* channels *(arrows). (d)* Expression of bFGF in irradiated cortical bone. No staining can be seen in this irradiated cortical bone. *(e)* Expression of TGF-β in nonirradiated cortical bone. Arrows point to many areas of increased staining within the cortical bone and vascular channels. *(f)* Expression of TGF-β in irradiated cortical bone. No expression of TGF-β can be seen in irradiated samples.

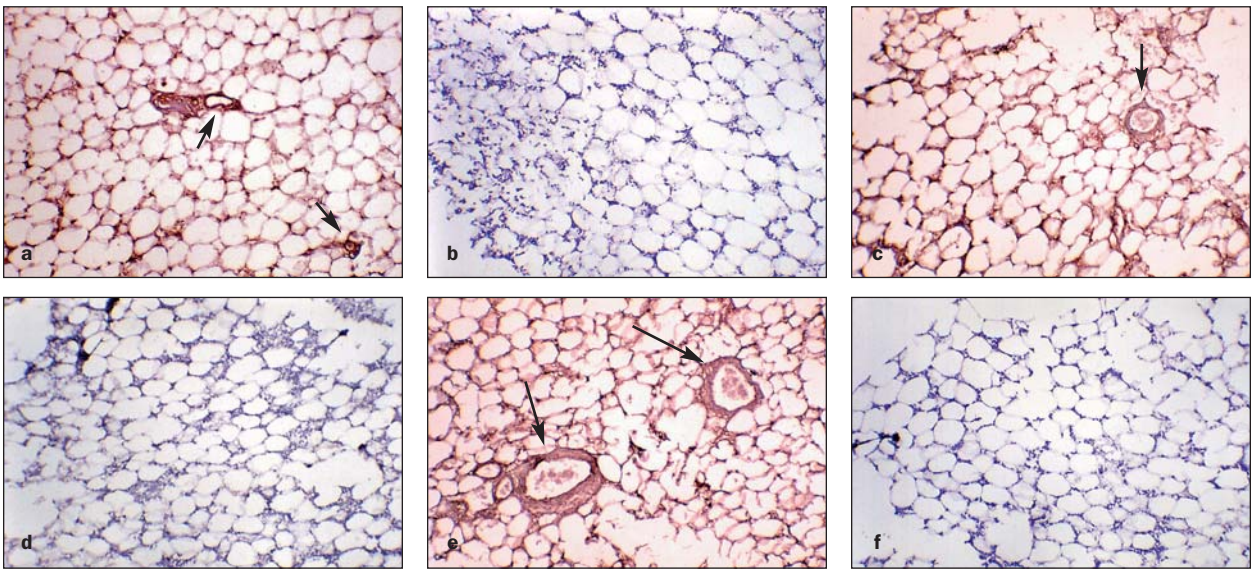

Fig 2 Immunohistochemistry comparison of normal and irradiated cancellous rabbit tibial bone. Immunohistochemistry was performed to determine expression of growth factors in cancellous nonirradiated and irradiated rabbit tibiae. *(a)* Expression of PDGF in nonirradiated cancellous bone. An increase in staining can be seen throughout the cancellous bone and blood vessels *(arrows). (b)* Expression of PDGF in irradiated cancellous bone. No staining and no vessels can be seen. *(c)* Expression of bFGF in nonirradiated cancellous bone. An increase in staining is seen throughout the trabecular bone, and more blood vessels are seen *(arrow). (d)* Expression of bFGF in irradiated cancellous bone. No staining is seen, and no vessels are seen. *(e)* Expression of TGF-β in nonirradiated cancellous bone. A greater amount of staining is detected, with large blood vessels visible *(arrows). (f)* Expression of TGF-β in irradiated cancellous bone. No staining and no blood vessels can be seen.

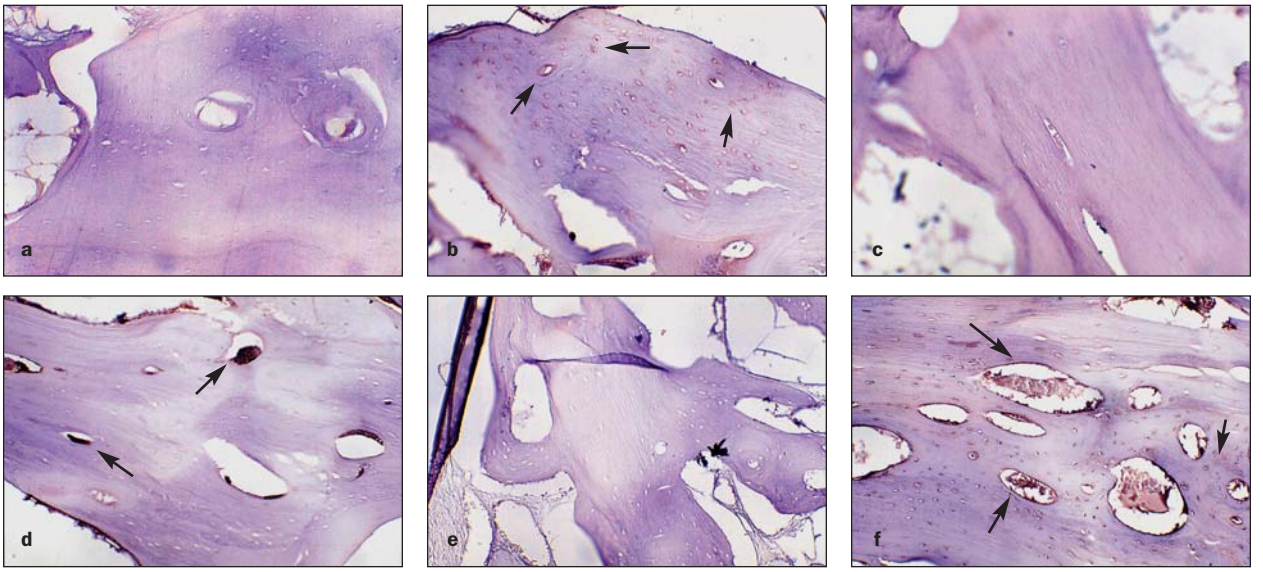

Fig 3 Immunohistochemistry comparison of autogenous bone and autogenous bone + PRP after 1 month. Autogenous cortical bone was grafted into rabbit cranial defects alone and in combination with PRP. Animals were sacrificed after 1 month, and immunohistochemistry was performed to determine the expression of growth factors, including PDGF, bFGF, and TGF-β. *(a)* Autogenous bone graft alone, stained for PDGF. No staining was detected in the cortical bone or vascular channels. *(b)* Autogenous bone + PRP, stained for PDGF. PDGF expression was detected throughout the cortical bone graft and in the vascular channels *(arrows)*. *(c)* Autogenous bone graft alone, stained for bFGF. No staining was detected in the cortical bone or vascular channels. *(d)* Autogenous bone + PRP, stained for bFGF. An increase in bFGF staining was detected in the vascular channels of the bone graft *(arrows)*. *(e)* Autogenous bone graft alone, stained for TGF-β. Autogenous bone graft alone, stained for PDGF. No staining was detected in the cortical bone or vascular channels. *(f)* Autogenous bone + PRP, stained for TGF-β. Arrows point to the increased staining throughout the cortical bone and in the vascular channels of the bone graft.

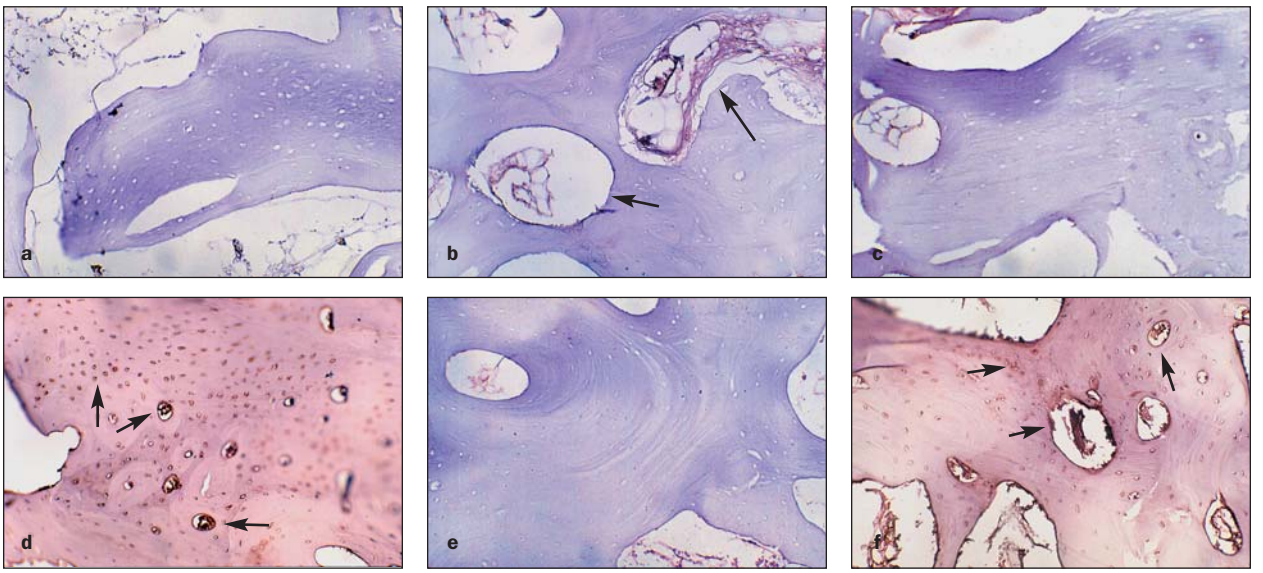

Fig 4 Immunohistochemistry comparison of autogenous bone and autogenous bone + PRP after 2 months. Autogenous cortical bone was grafted into rabbit cranial defects alone and in combination with PRP. Animals were sacrificed after 2 months, and immunohistochemistry was performed to determine the expression of growth factors, including PDGF, bFGF, and TGF-β. *(a)* Autogenous bone graft alone, stained for PDGF. No staining is detected in the cortical bone or vascular channels. *(b)* Autogenous bone + PRP, stained for PDGF. PDGF expression was detected in the vascular channels *(arrows)*. *(c)* Autogenous bone graft alone, stained for bFGF. No staining was detected in the cortical bone or vascular channels. *(d)* Autogenous bone + PRP, stained for bFGF. Increased expression of bFGF was detected in the vascular channels and throughout the entire autogenous bone graft (arrows). *(e)* Autogenous bone graft alone, stained for TGF-β. Minimal staining was detected in the vascular channels of the cortical bone graft. *(f)* Autogenous bone + PRP, stained for TGF-β. Arrows point to the increased staining throughout the cortical bone and in the vascular channels of the bone graft.

DISCUSSION

Much of the initial research and many of the applications of the growth factors evaluated in this study come from orthopedic surgery literature, specifically from the literature on fracture healing, where it has been shown that PDGF, bFGF, and TGF-β have positive roles in promoting long-bone fracture healing.[1–6] PDGF is a glycoprotein produced by platelets, macrophages, and endothelial cells. It has important functions in mitogenesis, angiogenesis, and recruitment of fibroblasts and osteoblasts to a wound site to assist in the initiation of vascularization, collagen synthesis and deposition, and bone regeneration.[7,33,34] Its crucial role in fracture healing is demonstrated by decreased intramembranous bone formation when PDGF inhibitors are utilized.[35] PDGF has been shown to increase new bone formation in calvarial defects; complete bony union was seen in 2 weeks when it was released from a poly(L-lactide) membrane.[36] PDGF has also shown an improvement in alveolar bone and periodontal ligament repair, with less inflammation than control in Class III molar furcation defects in beagle dogs.[37] In a human clinical trial with a split-mouth design, high-dose PDGF + insulin-like growth factor-1 (IGF-1) (150 mg/mL) showed a significant increase in vertical bone height and osseous fill when compared to controls; however, no difference was observed with lower doses of 50 mg/mL.[38]

FGFs can enhance cell proliferation, motility, differentiation, mitogenesis, wound healing and tissue repair, and angiogenesis.[39,40] Basic FGF is released by neutrophils, lymphocytes, blood monocytes, and tissue macrophages.[40] Both animal and human studies have shown the importance of bFGF in fracture repair.[41,42] One study demonstrated the benefit of bFGF specifically in stimulating cell proliferation during mandibular fracture healing.[43] In a study of alveolar bone defects created in beagle dogs and primates, improvement in bone formation and cementum was observed after treatment with bFGF as compared to control sites.[44] Also, bFGF can improve bone defect filling in mongrel dogs with Class III molar furcation defects.[45]

TGF-β has also been studied for its potential role in bone regeneration and repair. It is involved in bone and connective tissue regeneration and has chemotactic and mitogenic properties influencing osteoblastic progenitor cells and inhibition of osteoclasts and bone resorption.[33,46,47] TGF-β has been shown to trigger rapid maturation of collagen in early wounds.[48] TGF-β1 has been studied in periodontal defects. It can increase gingival healing by inducing fibroblast proliferation, formation of vascularity, and extracellular matrix remodeling.[49] It can also enhance bone regeneration in molar furcation defects of sheep when combined with barrier membranes.[50] Also, in alveolar bone defects in the canine model, the use of recombinant human TGF-β1 (rhTGF-β1) enhanced bone regeneration significantly, with further improvement when barrier membranes were used in combination with TGF-β1.[51] Taken together, this study, in combination with previous literature, shows the importance and potential roles of PDGF, bFGF, and TGF-β in bony wound healing.

Irradiation and Growth Factors

Radiation therapy is a common treatment modality for head and neck malignancy. Commonly used radiation protocols in the United States and around the world utilize doses from 65 to 70 cGy and 52 to 70 cGy, respectively.[52–55] Radiation at these doses has many deleterious effects; the most relevant to bony and soft tissue healing include hypocellularity, hypovascularity, and hypoxemia.[27,28,56] There is a documented decrease in bone healing after irradiation.[29] These changes in irradiated tissues, specifically bone, may be due in part to a decrease in growth factors that are involved in wound healing. Decreased expression of PDGF, bFGF, and TGF-β was seen immunohistochemically in both cortical and cancellous bone samples when irradiated rabbit tibia at 60 to 70 cGy was compared to nonirradiated bone (Figs 1 and 2). To the authors' knowledge, this is the first report of a decrease in vascular growth factor expression in autogenous bone grafts after irradiation.

Since the 1990s, TGF-β's effect on radiated skin wounds, surgical flaps, and bone healing has been studied.[19–21,30] The findings of this study are consistent with previous reports showing decreased TGF-β in irradiated vasculature and irradiated osteoblasts in vitro.[30,31] Local TGF-β administration has been shown to overcome irradiation-induced impaired wound healing by increasing wound breaking strength, possibly via an increase in the synthesis of type I collagen by fibroblasts.[20,21] It may also contribute to improved random flap survival in irradiated skin.[19]

PDGF has also been used to improve wound healing after radiation damage,[22,23] resulting in a treated irradiated rat surface skin wound 50% stronger than paired controls.[23] In this irradiated rabbit model, the expression of PDGF was significantly depressed in endothelial cells lining vascular channels of cortical bone as compared with those of nonirradiated controls (Figs 1a and 1b).[30]

The angiogenic growth factor bFGF has been demonstrated to improve wound healing in chronic and irradiated skin wounds when delivered in a topical form.[24,25] Immunohistochemical staining demonstrated expression of bFGF (Fig 1c) in endothelial cells of the cortical vasculature of nonirradiated bone

but none in irradiated bone (Fig 1d). Similar suppression of bFGF expression was observed in vasculature of irradiated cancellous bone (Figs 2c and 2d). Since bFGF expression is dramatically reduced in cortical bone after high doses of irradiation (60 to 70 cGy), evaluation of bFGF as an adjunct therapy for bone healing in patients undergoing irradiation therapy is important. One study evaluated the induction of angiogenesis and osseous healing of irradiated mandibular resection sites when the grafting material was pretreated with bFGF. Active bone formation and reestablishment of mandibular contours occurred in the bFGF-treated rabbits, but sequestration, necrosis, and failure to heal occurred in the control animals despite treatment.[26] These preliminary animal studies are promising, but more research is required to make definitive conclusions about the role of growth factors in the improvement of wound healing in irradiated tissues.

PRP

A combination of growth factors would seem to have an added benefit to graft healing, since the literature shows great promise in bone and soft tissue healing with these factors.[8–12,33,57–61] PRP, which created from blood collected from the patient and centrifuged into a concentrated platelet plug consisting of multiple growth factors released from the platelet alpha granules, has shown some success in enhancing bone graft density and maturation when added to autogenous, bovine, and/or freeze-dried bone.[7–18,32,60] Platelet counts from PRP are significantly higher than peripheral platelet counts,[7,59] and thus should contain an increased concentration of growth factors. TGF-β1, PDGF-AB, and IGF-1 concentrations have been measured and are increased in PRP, but 1 study could make no correlations between platelet counts and growth factor levels.[59] In the present study, concentrations of individual growth factors were not measured, since the aim of this study was to determine whether the presence of PRP (confirmed by an adequate platelet count) was sufficient to increase specific vascular growth factors in an autogenous bone graft. It has been reported that 1,000,000 platelets contain 0.06 ng PDGF.[62] However, studies on PDGF's effects on wound healing used much higher concentrations (0.5 to 100 μg) to enhance wound healing.[63–65] To address this discrepancy, it has been speculated that if multiple growth factors are utilized, as in PRP, lower concentrations of individual growth factors can have a synergistic effect to promote wound healing.[62] Though the present study demonstrated an increase in PDGF, bFGF, and TGF-β expression in autogenous bone grafts where PRP was added, the authors do not infer that expression of any of these individual factors was responsible for an improved wound healing response or an increase in bone regeneration. Other studies have shown various growth factor concentrations in PRP, but it is unknown whether the presence of these growth factors in PRP actually leads to increased expression in the bone graft.[62,66–68] Future studies to establish a direct correlation between the doses of each specific growth factor in the PRP with an increased or decreased expression of that specific factor will be necessary to establish a direct dose-response effect. In addition, studies to determine the duration of bioactivity of the specific growth factors contained in PRP will be needed to determine their temporal effect on wound healing. In this study, expression of PDGF, bFGF, and TGF-β was seen at 1 and 2 months when PRP was added to the autogenous bone graft. This does not signify, however, that these factors were active in the wound healing process at that time. Since little is known regarding growth factor temporal expression, evaluating these factors by immunohistochemistry allows a preliminary evaluation of the time sequence in bone regeneration.[69]

In this study, an increase in PDGF, bFGF, and TGF-β expression was detected in cortical autogenous bone grafts at 1 and 2 months with PRP when compared to autogenous bone alone (Figs 3 and 4). Based on this and previous studies, the combined effects of growth factors contained in PRP may present a therapeutic benefit to bone grafting and should be investigated further in cases of vascular compromise where wound healing is often impaired. PDGF, bFGF, and TGF-β are the most commonly studied and have angiogenic, mitogenic, and osteoinductive properties. Applications of growth factors in implant dentistry, particularly the use of recombinant growth factors in the form of PRP with autogenous bone grafts, are currently being investigated. Since this was a pilot immunohistochemical study, no quantitative histomorphometric analysis was performed to determine statistical differences in expression of these growth factors. However, based on these preliminary findings, growth factors hold promise for wound healing applications, especially for potential use in patients with vascular compromise and impaired wound healing.

CONCLUSIONS

This preliminary study demonstrated a decrease in the expression of vascular growth factors PDGF, bFGF, and TGF-β in irradiated rabbit tibial bone. In addition, an increase in expression of these same growth factors was seen when PRP was added to an autogenous bone graft in a rabbit calvarial defect model.

ACKNOWLEDGMENTS

The authors want to thank Mr Hai Duong for excellent technical assistance and Dr Diana Messadi for help in assimilating the data.

REFERENCES

1. Rundle CH, Miyakoshi N, Ramirez E, Wergedal JE, Lau KH, Baylink DJ. Expression of the FGF receptor genes in fracture repair. Clin Orthop 2002;403:253–263.
2. Schmidmaier G, Wildemann B, Heeger J, et al. Improvement of fracture healing by systemic administration of growth hormone and local application of IGF-1 and transforming growth factor-beta1. Bone 2002;31:165–172.
3. Fujii H, Kitazawa R, Maeda S, Mizuno K, Kitazawa S. Expression of platelet-derived growth factor proteins and their receptor alpha and beta mRNAs during fracture healing in the normal mouse. Histochem Cell Biol 1999;112(2):131–138.
4. Rosier RN, O'Keefe RJ, Hicks DG. The potential role of TGF-beta in fracture healing. Clin Orthop 1998;355(suppl):S294–S300.
5. Radomsky ML, Thompson AY, Spiro RC, Poser JW. Potential role of FGF in enhancement of fracture healing. Clin Orthop 1998;355(suppl):S283–S293.
6. Bostrom MP, Asnis P. TGF-beta in fracture repair. Clin Orthop 1998;355(suppl):S124–S131.
7. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638–646.
8. Kim SG, Kim WK, Park JC, Kim HJ. A comparative study of osseointegration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. J Oral Maxillofac Surg 2002;60:1018–1025.
9. Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstruction: A clinical and radiographic animal study on the use of autogenous scaffolds and PRP. Int J Oral Maxillofac Surg 2002;31:281–286.
10. Rodriguez A, Anastassov GE, Lee H, Buchbinder D, Wettan H. Maxillary sinus augmentation with deproteinated bovine bone and platelet-rich plasma with simultaneous insertion of endosseous implants. J Oral Maxillofac Surg 2003;61:157–163.
11. Aghaloo TL, Moy PK, Freymiller EG. Evaluation of PRP in combination with anorganic bovine bone in the rabbit cranium: A pilot study. Int J Oral Maxillofac Implants 2004;19:59–65.
12. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Kenney EB. Comparison of PRP, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: A reentry study. J Periodontol 2002;73:198–205.
13. Kassolis JD, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing PRP in combination with freeze dried allograft: Case series. J Periodontol 2000;71:1654–1661.
14. Shanaman R, Filstein MR, Danesh-Meyer MJ. Localized ridge augmentation using GBR and PRP: Case reports. Int J Periodontics Restorative Dent 2001;21:345–355.
15. Froum S, Wallace S, Tarnow D, Cho S. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: Three bilateral case reports. Int J Periodontics Restorative Dent 2002;22:45–53.
16. Kim E, Park E, Chuong P. Platelet concentration and its effect on bone formation in calvarial defects: An experimental study in rabbits. J Prosthet Dent 2001;86:428–433.
17. Witlfang J, Schlegel K, Schultze-Mosgau S, Nkenke E, Zimmerman R, Kessler P. Sinus floor augmentation with beta tricalcium phosphate: Does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Implants Res 2003;14:213–218.
18. Philippart P, Brasseur M, Hoyaux D, Pochet R. Human rTF, PRP, and tetracycline induce a high quality human bone graft: A 5 year survey. Int J Oral Maxillofac Implants 2003;18:411–416.
19. Nall AV, Brownlee RE, Colvin CP, et al. Transforming growth factor beta 1 improves wound healing and random flap survival in normal and irradiated rats. Arch Otolaryngol Head Neck Surg 1996;122:171–177.
20. Cromack DT, Porras-Reyes B, Purdy JA, Pierce GF, Mustoe TA. Acceleration of tissue repair by transforming growth factor beta 1: Identification of in vivo mechanism of action with radiotherapy-induced specific healing deficits. Surgery 1993;113:36–42.
21. Bernstein EF, Harisiadis L, Salomon G, et al. Transforming growth factor-beta improves healing of radiation-impaired wounds. J Invest Dermatol 1991;97:430–434.
22. Wollina U, Liebold K, Konrad H. Treatment of chronic radiation ulcers with recombinant platelet-derived growth factor and a hydrophilic copolymer membrane. J Eur Acad Dermatol Venereol 2001;15:455–457.
23. Mustoe TA, Purdy J, Gramates P, Deuel TF, Thomason A, Pierce GF. Reversal of impaired wound healing in irradiated rats by platelet-derived growth factor-BB. Am J Surg 1989;158:345–350.
24. Fu X, Shen Z, Guo Z, Zhang M, Sheng Z. Healing of chronic cutaneous wounds by topical treatment with basic fibroblast growth factor. Chin Med J 2002;115:331–335.
25. Zheng J, Wang S, Guo L. Promotion of wound healing with fibroblast growth factor in combined burn radiation injury. Zhong Zheng Xing Shao Shang Wai Ke Za Zhi 1994;10(2):146–149.
26. Eppley BL, Connolly DT, Winkelmann T, Sadove AM, Heuvelman D, Feder J. Free bone graft reconstruction of irradiated facial tissue: Experimental effects of basic fibroblast growth factor stimulation. Plast Reconstr Surg 1991;88(1):1–11.
27. Marx RE. A new concept in the treatment of osteoradionecrosis. J Oral Maxillofac Surg 1983;41:351–357.
28. Marx RE. Osteoradionecrosis: A new concept in its pathophysiology. J Oral Maxillofac Surg 1983;41:283–288.
29. Wurzler KK, DeWeese TL, Sebald W, Reddi AH. Radiation-induced impairment of bone healing can be overcome by recombinant human bone morphogenetic protein-2. J Craniofac Surg 1998;9:131–137.
30. Ducasse E, Cosset JM, Mazurier J, Eschwege F, Puppinck P, Lartigau E. High-dose external ionizing radiation prevents intimal hyperplasia and limits secretion of growth factors PDGF-BB, bFGF, and TGFb1: Experiments and results [in French]. J Mal Vasc 2003;28(2):68–72.
31. Dudziak ME, Saadeh PB, Mehrara BJ, et al. The effects of ionizing radiation on osteoblast-like cells in vitro. Plast Reconstr Surg 2000;106:1049–1061.
32. Aghaloo TL, Moy PK, Freymiller EG. Investigation of platelet-rich plasma in rabbit cranial defects: A pilot study. J Oral Maxillofac Surg 2002;60:1176–1181.
33. Marx RE. Platelet-rich plasma: A source of multiple autologous growth factors for bone grafts. In: Lynch, SE, Genco RJ, Marx RE (eds). Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics. Chicago: Quintessence, 1999:71–82.
34. Antonaides HN. Human PDGF: Purification of PDGF-I and II and separation of their reduced subunits. Proc Nat Acad Sci USA 1981;78:314–317.
35. Joyce ME, Jingushi S, Scully SP, Bolander ME. Role of growth factors in fracture healing. Prog Clin Biol Res 1991;365:291–416.

36. Park YJ, Ku Y, Chung CP, Lee SJ. Controlled release of platelet-derived growth factor from porous poly (L-lactide) membranes for guided tissue regeneration. J Control Rel 1998;51 (2-3):201–211.
37. Park JB, Matsuura M, Han KY, et al. Periodontal regeneration in class III furcation defects of beagle dogs using guided tissue regenerative therapy with PDGF. J Perio 1995;66:462–477.
38. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-1 in patients with periodontal disease. J Periodontol 1997;68:1186–1193.
39. Basilico C, Moscatelli D. The FGF family of growth factors and oncogenes. Adv Cancer Res 1992;59:115–165.
40. Hurley MM, Marie PJ, Florkiewicz RZ. FGF and FGF receptor families in bone. In: Bilezikian JP, Raisz LG, Rodan GA (eds). Principles of Bone Biology, ed 2. San Diego: Academic Press, 2002:825.
41. Kawaguchi H, Kurokawa T, Hanada K, ed 2. Stimulation of fracture repair by rhbFGF in normal and streptozotocin-diabetic rats. Endocrinology 1994;135:774–781.
42. Wildburger R, Zarkovic N, Egger G, Petek W, Zarkovic K, Hofer HP. Basic fibroblast growth factor immunoreactivity as a possible link between head injury and impaired bone fracture healing. Bone Miner 1994;27:183–193.
43. Gong Z, Zhou S, Cao J, Gu X. Effects of recombinant human basic fibroblast growth factor on cell proliferation during mandibular fracture healing in rabbits. Chin J Traumatol 2001;4(2):110–112.
44. Murakami S, Takayama S, Ikezawa K, et al. Regeneration of periodontal tissues by bFGF. J Perio Res 1993;34:425–430.
45. Rossa C, Marcantonio E, Cirelli JA, Mercantonio RA, Spolidorio LC, Fogo JC. Regeneration of class III furcation defects with basic fibroblast growth factor (bFGF) associated with GTR. A descriptive and histometric study in dogs. J Periodontol 2000;71:775–784.
46. Mohan S, Baylink DJ. Bone growth factors. Clin Orthop Relat Res 1991;263:30–43.
47. Pierce GF, Tarpley J, Yanagihain D. PDGF-BB, TGF-β1, and bFGF in dermal wound healing: Neo-vessel and matrix formation and cessation of repair. Am J Pathol 1992;140:1375–1388.
48. Bonewald LF, Mundy GR. Role of TGF-beta in bone remodeling. Clin Orthop 1990;250:261–276.
49. Okuda K, Murata M, Sugimoto M, et al. TGF-beta1 influences early gingival wound healing in rats: An immunohistochemical evaluation of stromal remodeling by extracellular matrix molecules and PCNA. J Oral Pathol Med 1998;27:463–469.
50. Mohammed S, Pack AR, Kardos TB. The effect of TGF-beta 1 on wound healing, with or without barrier membranes, in a class II furcation defect in sheep. J Periodontal Res 1998;33: 335–344.
51. Ruskin JD, Hardwick R, Buser D, Dahlin C, Schenk RK. Alveolar ridge repair in a canine model using rhTGF-β1 with barrier membranes. Clin Oral Implants Res 2000;11:107–115.
52. Grotz KA, Krummenauer F, al-Nawas B, et al. Retrospective analysis of the prognostic significance of the manner of mandibular resection and adjuvant radiotherapy in para-mandibular carcinomas [in German]. Mund Kiefer Gesichtschir 2000;4:301–308.
53. Celik N, Wei F, Chen H, et al. Osteoradionecrosis of the mandible after oromandibular cancer surgery. Plast Reconstr Surg 2002; 109:1875–1881.
54. Malde R, Agarwal JP, Laskar SG, et al. High dose rate brachytherapy boost for primary nasopharyngeal carcinoma: Preliminary results of an ongoing prospective study. Bull Cancer 2005;92: E45–E50.
55. Butler EB, Teh BS, Grant WH, et al. SMART (Simultaneous modulated accelerated radiation therapy) boost: A new accelerated fractionation schedule for the treatment of head and neck cancer with intensity modulated radiotherapy. Int J Radiat Oncol Biol Phys 1999;45(1):21–32.
56. Marx RE, Johnson RP. Studies in the radiobiology of osteoradionecrosis and their clinical significance. Oral Surg Oral Med Oral Pathol 1987;64:379–390.
57. Zechner W, Tangl S, Tepper G, et al. Influence of PRP on osseous healing of dental implants: A histologic and histomorphometric study in minipigs. Int J Oral Maxillofac Implants 2003;18:15–22.
58. Kim SG, Chung CH, Kim YK, Park JC, Lim SC. Use of particulate dentin-plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. Int J Oral Maxillofac Implants 2002;17:86–94.
59. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in PRP and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg 2002;30:97–102.
60. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. PRP and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. J Periodontal Res 2002;37:300–306.
61. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants 2003;18:93–103.
62. Yazawa M, Ogata H, Kimura A, et al. Basic studies on the bone formation ability by platelet rich plasma in rabbits. J Craniofac Surg 2004;15:439–446.
63. Pierce GF, Tarpley JE, Yanagihara D, et al. Platelet-derived growth factor (bb homodimer), transforming growth factor-β1, and basic fibroblast growth factor in dermal wound healing. Am J Pathol 1992;140:1375–1388.
64. Robson MC, Phillips LG, Thomason A, et al. Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. Lancet 1992;339:23–25.
65. Robson MC, Phillips LG, Thomason A, et al. Recombinant human platelet-derived growth factor-BB for the treatment of chronic pressure ulcers. Ann Plast Surg 1992;29:193–201.
66. Schlegel KA, Donath K, Rupprecht S, et al. De novo bone formation using bovine collagen and platelet-rich plasma. Biomaterials 2004;25:5387–5393.
67. Raghoebar GM, Schortinghuis J, Liem RSB, et al. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res 2005;16:349–356.
68. Weibrich G, Kleis WK, Buch R, et al. The Harvest Smart PReP system versus the Friadent-Schutze platelet-rich plasma kit. Clin Oral Implants Res 2003;14:233–239.
69. Thorwarth M, Rupprecht S, Falk S, Felszeghy E, Wiltfang J, Schlegel KA. Expression of bone matrix proteins during de novo bone formation using a bovine collagen and platelet-rich plasma (prp)—An immunohistochemical analysis. Biomaterials 2005;26:2575–2584.