J Musculoskel Neuron Interact 2004; 4(4):388-389

Original Article

Hylonome

Anabolic agents and gene expression around the bone implant interface

A.S. Virdi[1], A. De Ranieri[1], S. Kuroda[1,2], Y. Dai[3], D.R. Sumner[1]

[1]Department of Anatomy and Cell Biology, Rush Medical College, Rush University Medical Center,Chicago, IL, USA, [2]Oral Implantology and Regenerative Dental Medicine, Department of Masticatory Function Rehabilitation, Tokyo Medical and Dental University, Tokyo, Japan, [3]Department of Bioengineering, University of Illinois at Chicago, Chicago, IL, USA

Keywords: Implant Interface, Bone Regeneration, Marrow Ablation, Gene Expression

Joint replacement using implants is a common surgical procedure in orthopaedics. Approximately 200,000 total hip replacement procedures alone were performed in 2001 in the US (http://www3.aaos.org/research/imca/OAhipContents/OAHip_replace.pdf). Cementless implant fixation is currently the preferred option for the acetabular component and is commonly used on the femoral side as well. While the vast majority of orthopaedic implants succeed in restoring function, a significant proportion fail due primarily to aseptic loosening brought about by wear particle-induced osteolysis – this topic will be discussed in detail elsewhere in the Workshop. One of the main reasons for late stage loosening is thought to be the consequence of early loosening resulting from failure to achieve biological fixation in the initial phases of repair. It is now well known that implant fixation can be enhanced by various means which includes the use of exogenous applied anabolic agents such as growth factors[1].

Over the past number of years our laboratory has shown the positive effects of TGF-β and BMP-2 on bone ingrowth and ongrowth around an implant in a canine gap model[2-5]. While these studies have demonstrated the action of these growth factors in terms of bone volume over tissue volume and the strength of fixation, the findings are limited to a phenomenon rather than mechanistic interpretation. In addition to the fact that canine models are inherently costly, they are also less amenable to molecular analysis due mainly to the lack of genetic information available for this species. In order to circumvent these limitations and better understand the underlying molecular mechanisms in growth factor enhanced bone tissue regeneration around an implant we have sought to utilize a rat bone marrow ablation model. This model was originally described for studying hematopoesis[6,7] and was later characterized for osteogenesis and bone remodeling[8-10]. We have modified the model by placing an implant in the ablated space in the medullary cavity to emulate the biological environment surrounding the implant in our canine model. Due to the significant reduction in cost for the rat model, it provides greater flexibility in experiment design to accommodate time course studies. We have used the rat bone marrow ablation model to characterize the molecular events occurring during the reparative process in the absence of an implant (ablation alone), in the presence of an implant and with implants carrying TGF-β_2.

We examined the time-dependent gene profiles in the rat model at 1, 3, 5, 7, 10 and 14 days post-ablation. Expression levels of a total of 39 genes related to osteogenesis and GAPDH were measured by real-time PCR[11]. One group was used as an intact control (time point 0 day). During the inflammatory phase between days 1 and 5 there was down-regulation of several cytokine genes, including COX-1 and -2, although TNF-α and IL-1 showed upregulation. The repair phase of days 3 to10 was characterized by upregulation of a number of growth factor, receptor and inhibitor genes related to all three TGF$\beta_{1,2}$ and $_3$, bFGF, BMP-2, -4 and -7, VEGF and IGF-1. During this time, Cbfa-1, alkaline phosphatase, collagen type I, osteonectin, osteopontin and osteocalcin exhibited peak expression at days 5 or 7. At later time points of days 10 to 14 when remodeling begins, there were peak levels of RANK, RANKL, cathepsin K, IL-6 and COX-2. Principal component analysis used to identity 8 underlying components that together explained over 80% of the variance in the data. When components are arranged chronologically, there is a clear picture of the molecular cascade of sets of genes which may be co-regulated *in vivo* during bone regeneration.

In another study using the rat model, we examined the ability of TGF-β_2 to enhance implant fixation by comparing

The authors have no conflict of interest.

Corresponding author: Amarjit S. Virdi, Department of Anatomy and Cell Biology, 600 S. Paulina Street AcFac 507, Chicago, Illinois 60126, USA
E-mail: amarjit_virdi@rush.edu

Accepted 4 August 2004

three doses (0.1μg, 1μg and 10μg) to a control group (0μg) by placing HA/TCP treated rod coated with TGF-β_2 in the ablated femoral marrow space[12]. Bone volume per tissue volume (BV/TV) for the region between the endocortical envelope and surface of the implant was calculated μCT scans which showed that the 10μg group had noticeably more bone formed in the vicinity of the implant. The mechanical pull-out testing performed to measure implant strength of fixation also showed the same picture. In order to determine the molecular events responsible for the TGF-β_2 mediated enhancement of bone formation and implant fixation, we examined the temporal gene expression profiles of 21 genes at 1, 3, 5, 7, 10, 14 and 28 days post-operatively using real-time PCR[13]. When compared to time point matched controls (no TGF-β_2) the locally applied TGF-β_2 accelerated, delayed and in some cases augmented gene expression. For example, the expression of IGF-1, VEGF, BMP-4, IGF-1R, TβRI, TβRII, osteocalcin and osteonectin occurred at earlier times in the TGF-β_2 treated animals than in controls. TGF-β_1, TGF-β_3, alkaline phosphatase and osteopontin expression was delayed while TGF-β_2, BMP-2, BMP-7 and Flt-1 had up-regulation without a change in timing of peak expression. Principal components analysed identified four factors which explained 93% of the variance in the data in the control group and four factors explaining 94% of the variance in the TGF-β_2 treatment group. Closer examination of the genes grouped in each component revealed that sets of genes that were co-expressed in the control group were generally also co-expressed in the treatment group. In all, 14 of the 21 genes examined exhibited this pattern. This observation strongly suggests that the molecular events during bone regeneration are governed by strict expression of certain sets of genes whether or not an anabolic agent is used. However, the presence of an anabolic agent may enhance the regenerative process by altering their expression profile.

References

1. Sumner DR. Fixation of implants. In: Callaghan JJ, Rosenberg AG, Rubash HE, Simonian PT, Wickiewicz TA (eds) The Adult Knee. Lippincott Williams & Wilkins, Philadelphia; 2003:289-296.
2. Sumner DR, Turner TM, Purchio AF, Gombotz WR, Urban RM, Galante JO. Enhancement of bone ingrowth by transforming growth factor beta. J Bone Joint Surg Am 1995; 77-A:1135-1147.
3 Sumner DR, Turner TM, Urban RM, Leven RM, Hawkins M, Nichols EH, McPherson JM, Galante JO. Locally delivered rhTGF-β_2 enhances bone ingrowth and bone regeneration at local and remote sites of skeletal injury. J Orthop Res 2001; 19:85-94.
4. Sumner DR, Turner TM, Cohen M, Losavio P, Urban RM, Nichols EH, McPherson JM. Aging does not lessen the effectiveness of TGF-β_2 enhanced bone regeneration. J Bone Miner Res 2003; 18:730-736.
5. Sumner DR, Turner TM, Urban RM, Turek T, Seeherman H, Wozney JM. Locally delivered rhBMP-2 enhances bone ingrowth and gap healing in a canine model. J Orthop Res 2004; 22:58-65.
6. Amsel S, Maniatis A, Tavassoli M, Crosby WH. The significance of intramedullary cancellous bone formation in the repair of bone marrow tissue. Anat Rec 1969; 164:101-112.
7. Patt HM, Maloney MA. Bone marrow regeneration after local injury: a review. Exp Hemat 1975; 3:135-148.
8. Suva LJ, Seedor JG, Endo N, Quartuccio HA, Thompson DD, Bab I, Rodan GA. Pattern of gene expression following rat tibial marrow ablation. J Bone Miner Res 1993; 8:379-388.
9. Liang CT, Barnes J, Seedor JG, Quartuccio HA, Bolander M, Jeffrey JJ, Rodan GA. Impaired bone activity in aged rats: alterations at the cellular and molecular levels. Bone 1992; 13:435-441.
10. Tanaka H, Wakisaka A, Ogasa H, Kawai S, Liang CT. Local and systemic expression of insulin-like growth factor-I (IGF-I) mRNAs in rat after bone marrow ablation. Biochem Biophys Res Commun 2001; 287:1157-1162.
11. Kuroda S, Virdi AS, Sumner DR. Expression profiling of regenerate after bone marrow ablation. J Bone Miner Res 2003; 18:S305.
12. De Ranieri A, Kuroda S, Virdi AS, Sumner DR. Locally delivered TGF-β enhances implant fixation and bone regeneration in rat model. J Bone Miner Res 2003; 18:S331.
13. De Ranieri A, Virdi AS, Kuroda S, Sumner DR. Exogenously applied rhTGF-β_2 enhances bone regeneration and implant fixation by accelerating and up-regulating the innate gene expression profile in a rat model. Trans ORS 2004; 29:1418.