PGE2 INDUCES DIFFERENTIATION AND SELF-RENEWAL OF OSTEOBLAST PRECURSOR CELLS IN VITRO AND STIMULATES BONE HEALING IN VIVO

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Introduction:

Anabolic action of PGE2 has been demonstrated in animals as well as in humans. PGE2 administrated to rats in vivo increases cortical bone as well as trabecular bone formation. PGE2 was shown to stimulate bone formation and cause hyperostosis in infants. Recent data further illustrated that lack of COX-2, which is the major rate-limiting enzyme in the prostaglandin biosynthesis pathway, delays fracture healing in mice. Despite the extensive documentation of anabolic effects in vivo and in vitro, the cellular and molecular mechanisms that mediate PGE2 action remain unclear. Our current studies aim to elucidate the potential mechanisms of action of PGE2 on mesenchymal stem cell proliferation and differentiation into osteoblasts, and its subsequent impact on bone healing in vivo. A previously characterized femoral segmental allograft model was used in the study (1). Due to the lack of live cells on the processed allografts, the healing at the host-graft junction was very limited. By local infusion of PGE2 at the junction site, we demonstrated that exogenous PGE2 increased host-derived callus formation and improved the junction healing of allografts.

Materials and Methods:

Colony assays for bone marrow stromal cells: Bone marrow stromal cells were flushed out of the bone marrow cavity of mouse long bones and seeded at different density to examine the CFU-F, CFU-ALP and CFU-O. Numbers of the colonies were counted manually and colony area were traced by computer-assisted programs.

Fluorescence activated cell-sorting (FACS) analysis: Cells were pulse labeled with BrdU at day 7 of the culture for 1.5 hours and then proceeded to immunofluorescence staining according to manufacturer’s instruction (BD Pharmingen). ALP staining of bone marrow stem cells was conducted using endogenous phosphatase detection kit from Molecular Probes. Data was acquired using FACScalibur and analyzed by Cellquest software.

Local PGE2 Infusion in bone allograft model: A processed cortical femoral allograft 4mm in length was inserted in a segmental defect created in donor femur. An osmotic minipump was inserted subcutaneously on the back of the mouse and an 18G catheter was used to direct PGE2 to the femoral graft site. PGE2 in ethanol/propylene glycol (40:60, vol/vol) were infused at a rate of 0.25 µl/hr for 4 weeks subcutaneously on the back of the mouse and an 18G catheter was used to deliver PGE2 at the site of grafting and demonstrated marked improvement of callus formation radiographically, host healing completely relies upon limited callus formation derived from the host-bed. In this study we locally infused PGE2 at the site of grafting and demonstrated marked improvement of callus formation radiographically. This study again indicating that PGE2 is involved in bone healing and likely increases the recruitment and differentiation of mesenchymal stem cells. Histological analysis is now underway to further characterize the healing upon PGE2 treatment.

Results:

In vitro analyses of bone marrow stromal cells in response to PGE2: The addition of exogenous PGE2 (10^{-6}M starting from day 1) increased the number of CFU-F by about 1.3 fold, CFU-ALP 2 folds and CFU-O 4 folds in bone marrow stromal cell cultures. PGE2 also increased the area of CFU-ALP colonies. Consistent with the colony assays, FACS analysis demonstrated that PGE2 increased the percentage of ALP+ cells by about 2-3 folds in the culture. BrdU pulse labeling of the culture for 1.5 hour on day 7 demonstrated that PGE2 effects on cell proliferation was variable depending upon the density of the cultures. At low density, PGE2 increased BrdU+ cells while at higher density, PGE2 inhibited it. However, PGE2 consistently increased the net number of ALP+ and BrdU+ (double positive) cells by 2–3 folds, yet the ratio of double positive cells over total ALP+ cells remained unchanged. When added at day 10, PGE2 failed to show any increment in CFU-O formation.

Effects of local infusion of PGE2 in allografting model: Radiographic assessment at weekly intervals denoted an increase in host derived callus formation at the proximal osteotomy site by day 14 in comparison to the vehicle group that showed no or very little creeping callus. By day 28, there was a marked increase in callus formation from the host in PGE2 treated group but very little creeping callus from the vehicle group (Figure1).

Discussions:

Osteoblasts derived from mesenchymal stem cells proceed through various substages of differentiation prior to obtaining bone-forming capacity. The detailed analyses of bone marrow stromal cell differentiation demonstrated that 1) PGE2 increased CFU-F colony formation indicating that PGE2 involved in stem cell recruitment and survival. 2) PGE2 promoted the differentiation of bone marrow stromal cells to become ALP+ without affecting ALP+ cell proliferation (percentage of cycling ALP+ cells in the culture remain unchanged upon PGE2 treatment. 3) PGE2 added at the later stage of differentiation (starting on day 10) has no inducible effects supporting that PGE2 acts on stromal stem cells or its proximate progenies rather than osteoblasts at their late stage differentiation. 4) PGE2 increased the net cycling ALP+ cells in the culture suggesting that PGE2 is involved in maintaining the limited self-renewal of the osteoblast precursor cells. Taken together, our studies show that anabolic action of PGE2 is differentiation stage specific and works at multiple steps in the early differentiation of mesenchymal stem cells towards osteoblast lineage.

Structural allografts are notorious for their poor performance in healing due to the lack of osteoinductive and osteogenetic activity. Allograft healing completely relies upon limited callus formation derived from the host-bed. In this study we locally infused PGE2 at the site of grafting and demonstrated marked improvement of callus formation radiographically. This study again indicating that PGE2 is involved in bone healing and likely increases the recruitment and differentiation of mesenchymal stem cells. Histological analysis is now underway to further characterize the healing upon PGE2 treatment.

Figure 1. PGE2 infusion in vivo increased host derived bone callus formation in mouse femoral allograft model. Callus formation were monitored by X-ray at day 0, 14 and 28. A,C,E were mice received vehicle while B,D,F were mice received infusion of PGE2

Reference:


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