Treatment of Rat Bone Marrow Cells with PGE2 or KMN-159: Effect on Osteoclastogenesis and Adipogenesis

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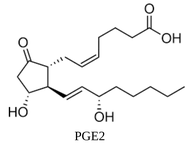
1. Introduction

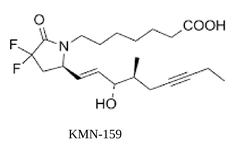
In bone marrow, osteoblastic precursors are of the mesenchymal stem cell lineage (MSCs) whereas osteoclastic precursors are of the hematopoietic lineage. MSCs can be induced to differentiate into osteoblasts by exposure to natural prostaglandins such as PGE2. The osteoblasts then produce RANK ligand (RANKL) which activates the fusion of monocytes into functional osteoclasts in order to maintain bone density. Our study is beginning to examine whether prostanoid compounds have a direct effect on osteoclastogenesis, an effect which could perturb the balance of bone mass. Similarly, MSCs can also be driven to differentiate into adipocytes if exposed to insulin and as with the osteoclasts, we are exploring whether exposure to prostanoid compounds has an effect on this differentiation scheme. In the first part of our studies, we are inducing osteoclast formation in rat bone marrow cells with 25 µg/ml G-CSF and RANKL (0-25 µg/ml) and testing whether simultaneous exposure to a novel prostanoid (KMN-159) has an effect on the number of osteoclasts formed as determined by TRAP staining. We are also inducing adipocyte formation in the bone marrow cells by treatment with insulin (5 µM) and dexamethasone (10 nM) and testing the number of adipocytes formed by Oil Red O staining in the presence of PGE2 and KMN-159 as compared to vehicle treated cells. These data could provide relevant information for studies in which osteoblastic bone fracture repair is stimulated by prostanoid compound treatment.

2. Prostaglandins

Prostaglandins are derivatives of arachidonic acid– an omega 6 fatty acid. Arachidonate is converted into prostaglandins through enzymes like cyclooxygenases (COX) and various PG synthases. Prostaglandins are known for their hormone-like effects on different cell and tissue types . PGE2 and KMN-159 are both EP4 receptor agonists, known to promote MSC differentiation into osteoclasts.



PGE2



KMN-159

3. Effects of PGE2 and KMN-159 on Adipocyte Formation in BMC Cultures after Treatment with Insulin

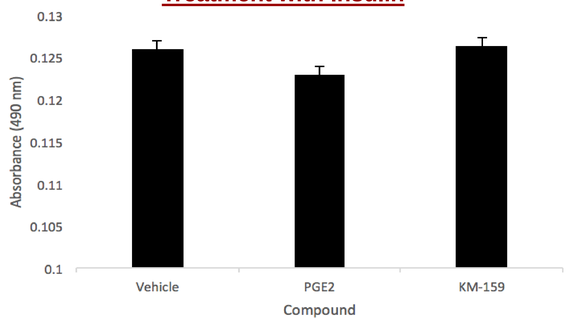
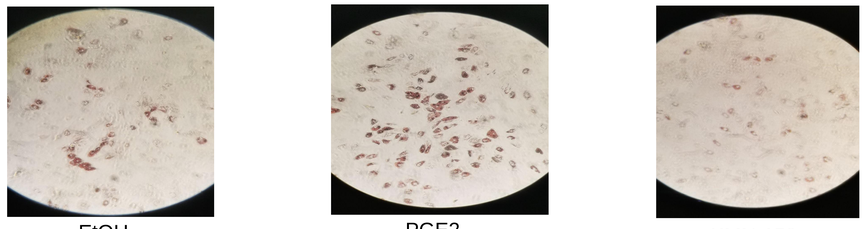


Figure 1. Absorbance of Isopropanol-Oil Red O Dye Mixture

Adipocyte formation after 12-day culture and treatment at plating with either vehicle (0.1% ethanol, PGE2 (1 uM), or KMN-159 (1 uM). Insulin (10 ug/ml) was added beginning on day 3. Cells were stained with Oil Red O dye (staining fat droplets in the cells) and eluted with 100% isopropanol. Isopropanol and dye were then transferred to a 96-well dish and quantitated by absorbance at 490 nm using the plate reader. Values are the means of n=8 wells and error bars are +/- 1 SD.

4. Adipogenesis in EP4 Agonist-Treated and Non-Treated Cells After Treatment with Insulin



EtOH PGE2 KMN-159

Figure 2. Adipocytes Stained with Oil Red O

Adipocyte formation (corresponding to Figure 1) after 12-day culture and treatment with either 0.1% ethanol, PGE2 (1 uM), or KMN-159 (1 uM); insulin treatment on day 3. All cells were stained with Oil Red O and viewed at 300x.

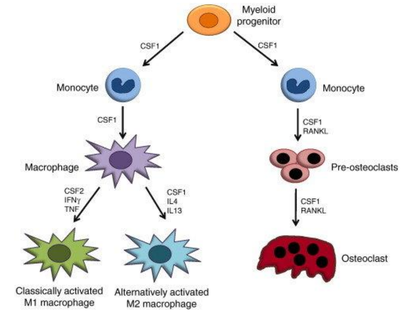
5. Procedure

Tibiae were removed from a 75-day-old male rat, the bone marrow was extracted and suspended in MEMα supplemented with 10% FCS. Cells were quantified to obtain an initial plating count of 1.1 x 10^6 cells/well and 2.2 x 10^6 cells/well for 24-well and 12-well cell culture plates, respectively. Cells in 12-well plates were treated with Vehicle (0.1% EtOH); PGE2 1000nm; or KM-159 100, 300, and 1000 nm final concentrations. Cells in 24-well plates were treated with Vehicle (0.1% EtOH), PGE2 1000 nm, or KM-159 1000 nm final concentrations.12- well plates were fed with adipogenic mediu (MEMα + 10% FCS + 10 ug/ml insulin + 1 uM dexamethasone + corresponding drug). 24-well plates were initially fed with MEMα + 10% FCS and their corresponding drug. On the third day of the twelve day treatment period, one of the 24-well plates was supplemented with 10 ug/ml insulin to assess whether day 0 addition of insulin was required to stimulate adipogenesis. Both 24-well plates were also supplemented with 1uM dexamethasone on day 3. All other plates were fed every 3 days with the same initial media. On Day 12, all plates were fixed with 2% paraformaldehyde and stained with **[CONC?]** Oil Red O dye to identify the adipocytes. Following staining, 24-well-plates 200 uL of 100% isopropanol was added to each well to elute the Oil Red O dye which was quantitated by absorbance at 490 nm.

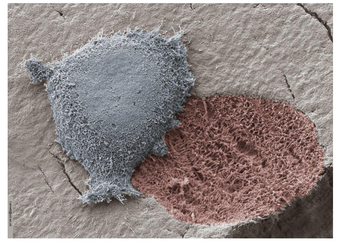
6. Monocyte Differentiation into Osteoclasts

This diagram displays the differentiation scheme of the fusion of monocytes to form osteoclasts (right side) as performed in this experiment, or monocytes to macrophages (left side). Osteoclasts are defined as having 3 or more nuclei.

Our goal was to 1) generate osteoclasts from rat bone marrow and 2) determine if KMN-159 influenced their formation.



Source: BBA- Reviews on Cancer



Pictured is a scanning electron micrograph of an activated osteoclast and the resorption pit left behind on the bone surface. On the underside of the osteoclast is a ruffled border that secretes H^+ and enzymes that destroy the mineralized bone matrix.

Source: Bone Research Society

7. Osteoclast Formation

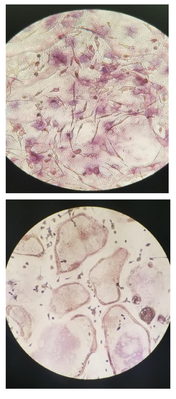


Figure 3. Mature vs. Developing Osteoclasts

Rat bone marrow cells were incubated with 25 ug/mL G-CSF and RANK ligand (RANKL) to induce osteoclast formation. The cells in both pictures were stained for tartrate-resistant acid phosphatase (TRAP), a marker for active osteoclasts. The smaller cells (top photo) are early stage osteoclasts while the large cells (bottom photo) are mature osteoclasts with >20 nuclei each. Initial data suggest that KMN-159 might induce osteoclast formation but the experiment needs to be repeated.

8. References and Acknowledgements

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