INHIBITION OF OSTEOBLAST DIFFERENTIATION AND PROLIFERATION BY THE UP-REGULATION OF HEME-OXYGENASE-1

Tzu-Hung Lin, Rong-Sen Yang, Wen-Mei Fu

Departments of +,*Pharmacology and **Orthopaedics, College of Medicine, National Taiwan University, Taipei, Taiwan

Email: d93443005@ntu.edu.tw

INTRODUCTION:

Heme-Oxygenase-1 (HO-1) is an important enzyme involved in vascular disease, transplantation, and inflammation. Recently, it has been reported that HO-1 plays an important role in inflammatory bone loss in humans and up-regulation of HO-1 negatively regulates osteoclastogenesis. However, the effect of HO-1 on osteoblasts is still unclear. In the present study, we demonstrated that HO-1 inducer, hemin, inhibits the differentiation of rat’s osteoblasts including mineralized bone nodule formation and alkaline phosphatase activity. In addition, we transfected adenoviral vectors expressing HO-1 into osteoblasts and it was found that over-expression of HO-1 inhibited the differentiation of osteoblasts as well as the expression of COX-2, BMP-2, and osteocalcin (OCN).

METHODS:

Primary Osteoblast Cultures

Primary osteoblasts were obtained from the calvaria of fetal rats. The calvaria were divided into small pieces and were treated with 1mg/ml collagenase solution for 20-30 minutes at 37°C. The next two 20 minute sequential collagenase digestions were then pooled and filtered through 70μm nylon filters. The cells were grown on the plastic cell culture dishes in 95% air-5% CO2. L-ascorbic acid (50 μg/ml) and β-glycerophosphate (10 mM) were added to caused the maturation of osteoblasts. Maturation and mineralization of osteoblasts were measured by alkaline phosphatase (ALP) activity and Alizarin-red staining.

RT-PCR for mRNA Analysis

mRNA was analyzed by Reverse Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from primary osteoblasts by using TRIzol kit after 3 days of treatment. RNA was analyzed by using two-step SuperScriptIII and Taq polymerase. Amplification was accomplished with 28-37 cycles. PCR products were then separated electrophoretically in a 2% agarose DNA gel and stained with ethidium bromide.

Adenovirus Infection

Adenovirus containing human HO-1 (Ad-HO-1) and control virus (Ad) were used in this study. Primary rat osteoblasts were infected at a m.o.i. between 0.25 and 25 in α-MEM medium containing 10% FBS 24 hours before further treatment. The medium was changed to osteogenic medium and cells were then incubated for 72 hours.

Cell Proliferation Assay Using BrdU

For the 5-bromo-deoxyuridine (BrdU) assay, cells were seeded onto 96 well plates at a density of 5000 cells/well. Cells were cultured for one day with 10% FBS/α-MEM (90μl). Then medium was changed and cells were then treated with test substance for 24 hours. BrdU (100μM stock solution, 10μl) was added to each well and the cells were incubated for further 4 hours. After removal of medium, cells were fixed and the DNA was denatured using FixDenat reagent. The FixDenat reagent was removed after 30 minutes incubation, anti-BrdU-POD solution was added and the plate was incubated for 90 minutes at room temperature. The cells were then washed three times with washing solution, 100μl of the substrate solution was added to each well, and the relative light units were measured by using a microplate luminescence reader.

Statistics

The values given are means ± S.E.M. The significance of difference between the experimental group and control was assessed by Student’s t test. The difference is significant if the p value is less than 0.05.

RESULTS:

In this study, cultured rat osteoblasts were incubated with HO-1 inducer, hemin for 14 days. It was found that hemin inhibits mineralized bone nodule formation (Fig.1A) and alkaline phosphatase activity (Fig.1B). In addition, the osteoblasts were transfected with adenovirus containing human HO-1. It was found that over-expression of HO-1 decreased alkaline phosphatase activity on Day-3 (Fig.2A). Moreover, overexpression of HO-1 decreased the expression of BMP-2, osteocalcin (OCN), and COX-2 mRNA on Day-3 (Fig.2B). Cell proliferation assay was evaluated using BrdU incorporation, is was found that both hemin and Ad-HO-1 down-regulated proliferation of osteoblasts.

DISCUSSION:

Recently, induction of HO-1 has been demonstrated to negatively regulate osteoclastogenesis in vitro and in vivo and has an anti-inflammatory potential of therapeutic avenues for rheumatoid arthritis (RA). However, our data demonstrate that induction of HO-1 also inhibits osteoclastogenesis. Over-expression of HO-1 decreases BMP-2, OCN, and COX-2 which are important for osteoblastic differentiation and bone repair. Our results suggest that there is a risk to up-regulate HO-1 for the antagonism of inflammatory bone loss.

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