**Introduction:** Raloxifene, a drug for osteoporosis, belongs to the class of selective estrogen receptor modulators (SERMs). Raloxifene acts like estrogen as preventing bone loss in postmenopausal women. Recently, the presence of estrogen receptor in osteoblast is well known, therefore it suggests that estrogen agonists has a direct role in the regulation of osteoblast function. Qu et al. demonstrated that raloxifene have direct effect on osteoblasts and bone formation in bone marrow derived female mouse. However, the point at which raloxifene has effect on bone marrow-derived mesenchymal stem cells (MSCs) regardless of sex difference. The purpose of this study was to examine the osteogenic effect of raloxifene on MSCs derived from male and female rat and to assess the sex difference of raloxifene with dexamethasone in the regulation of bone formation.

**Materials and Methods:** Preparation and culture of MSCs

Rat bone marrow cells were prepared according to the method previously reported. The femora were excised aseptically from 7-week-old female and male Fisher 344 rats, and both ends of the femora were cut at the epiphyses. The bone marrow was flushed out using 10 ml of culture medium expelled by a syringe through a 20-gauge needle. The released cells were collected in two T-75 flasks and cultured in Dalbecco's Modified Eagle Medium containing 10% fetal bovine serum (removed sex steroids by charcoal treatment) and antibiotics. Cultures were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C. After 14 days of primary culture, marrow stromal cells were detached from the flask using 0.25% trypsin and seeded on a 12-well plate at 104 cells/cm2 for subculture. The cells were subcultured in 1 ml of the standard medium supplemented with 80 μg / ml vitamin C phosphate, 10 mM Na-β-glycerophosphate and with or without dexamethasone (Dex). A 10-6M Raloxifene added to the MSCs culture medium. As a control group, the cells were cultured in standard medium with or without Dex. The medium was replaced 3 times a week. The cultures at 2 weeks were used for biochemical analysis.

Alkaline phosphatase (ALP) activity

The cultured cells were washed twice in PBS and scraped in 0.2% Nonidet-P40 containing 1 mM MgCl2. The suspension was sonicated and centrifuged at 13,000 rpm for 10 minutes. The supernatants were incubated in 1 ml of buffer containing 50 mM p-nitrophenylphosphate and 1 mM MgCl2 at 37°C for 30 minutes. To stop the reaction, 2 ml of 0.2N NaOH was added to the solution. Absorbance was measured at 410 nm to detect the p-nitrophenol that was released after incubation. The ALP activity of each well was expressed as μmol of p-nitrophenol/30 min.

Osteocalcin assay

Cells were scraped, sonicated, and centrifuged as described for ALP activity. The supernatant was removed, and the sediment was soaked in 20% formic acid for 2 weeks at 4°C to extract the osteocalcin. The extract was applied to NAP-5 columns and eluted with 10% formic acid to remove calcium phosphate salt. The purified extract was evaporated to remove calcium phosphate salt. The extract was evaporated to dryness and redissolved in 100 μl 0.2N NaOH was added to the solution. Absorbance was measured at 410 nm to detect the p-nitrophenol that was released after incubation. The ALP activity of each well was expressed as μmol of p-nitrophenol/30 min.

**Results:** ALP activities was not significant in all groups without Dex. In the groups with Dex, ALP activity in the 10-6M raloxifene female group was significantly higher than the control group (P<0.05). But ALP activities in the 10-6M raloxifene male group was not significantly different from the control group.

**Discussion:** The results of this study indicate that 10-6M raloxifene had ossification-promoting effect on MSCs derived from female rat. Thus, under the presence of dexamethasone, raloxifene have osteogenic effect on female MSCs. On the contrary, raloxifene has no osteogenic effect on male MSCs. Therefore, raloxifene has sex difference with regard to osteogenic effect on MSCs. These results provide a useful insight into the possible influence of raloxifene after MSCs transplantation in clinical practice.

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**References:**

**Fig. 1** Alkaline phosphatase (ALP) activity of female and male cultured mesenchymal stem cells after 2 weeks of subculture. Dex + indicates cells cultured with dexamethasone and 10-6 R indicated cells culture added to10-6M raloxifene. Values represent the mean ± SD. P < 0.05 using the Mann-whitey U test.

**Fig. 2** Osteocalcin content of female and male cultured mesenchymal stem cells after 2 weeks of subculture. Dex + indicates cells cultured with dexamethasone and 10-6 R indicated cells culture added to10-6M raloxifene. Values represent the mean ± SD. P < 0.05 using the Mann-whitey U test.