

## Effects of Propranolol on Osteoclasts Cultured in Vitro

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**Abstract—Objective:** To study the effect of propranolol on osteoclasts. **Methods:** (1) propranolol at the doses of  $10^{-2}$   $\mu\text{mol/ml}$ ,  $10^{-3}$   $\mu\text{mol/ml}$ ,  $10^{-4}$   $\mu\text{mol/ml}$ , were added into the osteoclasts for coculture for 72 h, then tartrate-resistant acid phosphatase (TRAP) and toluidine blue staining was used to detect the effects of propranolol on osteoclasts. **Results:** compared with the control group,  $10^{-2}$   $\mu\text{mol/ml}$ ,  $10^{-3}$   $\mu\text{mol/ml}$ ,  $10^{-4}$   $\mu\text{mol/ml}$  group of propranolol were able to make the number of osteoclasts and bone resorption area decreased ( $P < 0.05$ ) **Conclusion** different concentrations of propranolol can inhibit osteoclast bone resorption ( $P < 0.05$ ).

**Keywords-** *propranolol, osteoclasts, TRAP*

### I. INTRODUCTION

The normal growth and metabolism of bone includes osteogenetic effect of osteoblast and bone resorption effect of osteoclast, both of which interact with each other to achieve a basic balance [1]. The absorption and bone formation depends on osteoblasts and osteoclasts in the basic multicellular unit recruitment, proliferation and differentiation, and it is also effected by the local micro environment and the external mechanical force and systemic neurohumoral factors [2]. The metabolism of bone is affected by the innervation, which has been valued by many researchers [3]. At present, a number of studies have made it clear the sympathetic fibers play an important role in normal bone metabolism and fracture healing [4]. Eleferiou reported in the Journal of Nature that sympathetic nerve exciting can promote bone resorption and decreased bone formation, and the mechanism of which is by stimulating osteoclast activity

and promoting osteoclast formation [5]. The increase in sympathetic activity can increase the number and activity of osteoclasts, and increase bone resorption and decreased bone formation, bone loss, and the effect of which is closely related to the activity of beta 2- adrenergic receptor on osteoblasts. The activation of the receptor can increase the expression of RANKL, stimulate osteoclast formation, and then lead to the decrease of bone mass [6]. But there is not yet any report about a beta 2- adrenergic receptor related drugs directly effecting on osteoclasts, so this experiment choose non selective beta adrenergic receptor antagonist - propranolol effecting on osteoclasts, to observe the effects of different concentrations of propranolol on osteoclasts, providing a basis for further exploring the mechanism of effects of propranolol on bone metabolism.

### II. MATERIALS AND METHODS

#### A. Materials

1) *laboratory animal:* 6 newborn rabbits in 24h, either size or gender is not limited;

2) *Major laboratory products and chemical reagents:* Propranolol (Sigma), DMEM medium and fetal bovine serum (Hyclone), penicillin streptomycin (Gibco), glutamine (Sigma). Tartrate resistant acid phosphatase (TRAP) staining kit, toluidine blue (Nanjing Jiancheng Biological Engineering Institute);

### B. Experimental Methods

1) *Extraction and culture of rabbit osteoclasts:* The newborn rabbits were sacrificed by broken neck, soaking in 75% alcohol for 15min, taking limbs long bone in aseptic conditions, then putting in cold PBS medium after wiping out soft tissue and epiphyses. Then longitudinally split the backbone in the cold low glucose DMEM medium (containing 15% fetal bovine serum, penicillin 100IU/ml, chain enzyme 100mg/ml), lightly scraping the inner surface with a sharp knife, and rinsed repeatedly the surface of the medullary cavity with pointed straw until white color, standing 1min, taking the upper cell suspension seeded on 24 well plates in which preset bone slices or coverslip. Add 0.5ml cell suspension into each hole, cultured in carbon dioxide incubator. Replaced the culture solution after 30min, rinsing off the cells not attached. Later replace the culture fluid after 24h.

#### 2) Identification of osteoclast

a) *Morphological observation:* The growth and morphological changes of osteoclasts were observed under the inverted microscope. The visual field was randomly selected and recorded.

b) *Tartrate-resistant acid phosphatase (TRAP) staining:* Operation according to TRAP staining kit manual.

3) *Experimental group:* Cells were placed in 37 C and 5% CO<sub>2</sub> for cultured 30min, replaced the culture solution, rinsing off the cells not attached. Then randomly divided into 4 groups (10<sup>-2</sup>μmol/ml, 10<sup>-3</sup>μmol/ml, 10<sup>-4</sup>μmol/ml group of propranolol), each group of 6 holes, the experimental group and the control group were changed for conditioned medium after 24h.

4) *Count the positive cell of TRAP:* Taking out the coverslip after cultured 72h. According to the TRAP staining kit manual, take photos and record the number of positive cells.

5) *Area of bone resorption:* Taking out the coverslip after cultured 72h. Soak in 2.5% glutaraldehyde solution for 7~10min, and then ultrasonic cleaning by double distilled for 3-5min×3 times, dehydration by the series of ethanol, naturally dried, then placed in 1% toluidine blue for 4-5min at room temperature. Microscope, and photography after cleaning. And the absorption area of the system is calculated by the image analyzer system.

6) *Statistical analysis:* The data were analyzed with SPSS version 19.0 software package. The differences in the values between the two groups were assessed by the Student's t-test. The statistics results described by means and standard deviations (x+s). The level of statistical significance was set P<0.05.

### III. RESULTS AND ANALYSIS

#### A. Cell Morphology Observation

The number of osteoclast directly extracted is small, the volume is big, and growth is fast. Osteoclast has multiple nuclei, and the number and sizes of the nuclei is different. Cytoplasm is fullness, and Pseudopodia and swelled can be seen between cells. (Fig. 1)

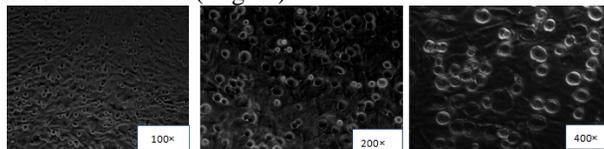


Figure 1. Morphology of osteoclasts

#### B. TRAP Staining Results

After TRAP staining, positive cells can be seen with multiple nuclei, usually 3-5, irregularly in shape, and its nucleolus is obvious, cytoplasm is purple (Fig 2)

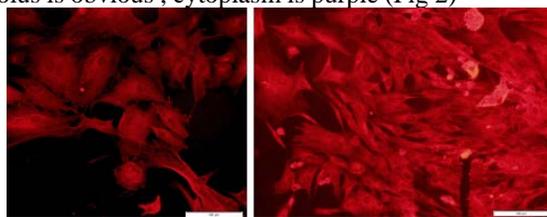


Figure 2. TRAP staining

#### C. Effects of Propranolol on Osteoclast Number of TRAP Positive Cells

The results showed that after 72h, the number of osteoclasts of 10<sup>-2</sup>μmol/ml, 10<sup>-3</sup>μmol/ml, 10<sup>-4</sup>μmol/ml group of propranolol were lower than the control group, the difference was statistically significant (P<0.05), indicating 10<sup>-2</sup>μmol/ml, 10<sup>-3</sup>μmol/ml, 10<sup>-4</sup>μmol/ml group of propranolol can reduce the number of osteoclasts; and the difference between the three groups had statistically significant (P < 0.05), indicating 10<sup>-3</sup> group of propranolol on osteoclast is most obvious. (Table I)

TABLE I. EFFECT OF PROPRANOLOL ON POSITIVE CELLS OF TRAP AND AREA OF BONE RESORPTION

| Group                    | positive cells of TRAP | the area of bone resorption            |
|--------------------------|------------------------|--|
|                          | $\bar{X} \pm s$        | $\bar{X} \pm s$<br>( $\mu\text{m}^2$ ) |
| Control group            | 122.67±9.87            | 2315.56±412.66                         |
| 10 <sup>-2</sup> μmol/ml | 53.00±5.00             | 1345.24±249.02                         |
| 10 <sup>-3</sup> μmol/ml | 22.67±5.13             | 344.43±143.63                          |
| 10 <sup>-4</sup> μmol/ml | 33.33±1.53             | 674.83±105.45                          |

#### D. Analysis of Bone Resorption Lacunae Area

Osteoclast formed bone resorption lacunae on bone slices, presenting blueviolet after toluidine blue staining, which was

round, oval or botuliform (Fig. 3). The results showed that bone resorption area of  $10^{-2}$   $\mu\text{mol/ml}$ ,  $10^{-3}$   $\mu\text{mol/ml}$ ,  $10^{-4}$   $\mu\text{mol/ml}$  group of propranolol, were lower than the control group, the difference was statistically significant ( $P < 0.05$ ); and the difference between the three groups had statistically significant ( $P < 0.05$ ), indicating  $10^{-3}$  group of propranolol on osteoclast is most obvious.

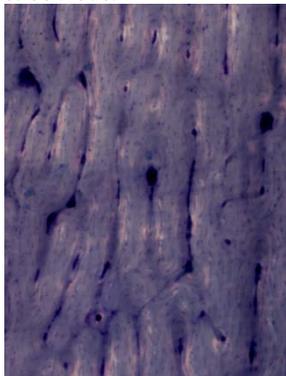


Figure 3. Toluidine blue staining

#### IV. DISCUSSION

Osteoclast cells contain abundant acid phosphatase, which was not specific to osteoclast cells, so do osteoblast. [7]. TRAP is one of the six kinds of acid phosphatase isozyme, is a specific marker of osteoclasts, its expression and the secretion are closely related with the function of osteoclasts, which can be used as important identification markers of osteoclasts [8]. This experiment found that the cells were red after TRAP staining, containing multiple nuclei, and pseudopodia could be seen between cells, indicating that the cells was osteoclasts.

Bone resorption lacunae directly reflects the ability of osteoclastic bone resorption, therefore bone resorption lacunae area is an important indicator of bone resorption of osteoclasts [9-10]. The experimental results show that  $10^{-2}$   $\mu\text{mol/ml}$ ,  $10^{-3}$   $\mu\text{mol/ml}$ ,  $10^{-4}$   $\mu\text{mol/ml}$  group of propranolol can reduce the area of bone resorption on bone slices, and this conclusion is consistent with the result after TRAP staining.

#### V. CONCLUSIONS

The effects of different concentrations of propranolol after 72 hours can inhibit osteoclastic bone resorption.

#### VI. ACKNOWLEDGEMENTS

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