Effect Of Platelet Rich Plasma On Fatty Degeneration Of Rotator Cuff Muscles In Vitro And In Vivo.

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Introduction: Rotator cuff tear is a common disease and cause a dysfunction of the shoulder. It has been reported that massive tears of the rotator cuff cause fatty degeneration of muscle. (1,2) Regarding the pathogenesis of fatty degeneration, satellite cells in the muscular tissue served as a source of adipocytes.(3) Therefore, to control the fate of muscle satellite cell differentiation is a key factor for treatment of rotator cuff tear. Recently, platelet rich plasma (PRP) has been reported to inhibit the adipogenesis of pre-adipocyte cell line 3T3-L1(4), and to enhance tissue repair processes after tendon ruptures.(5) We hypothesize that PRP can prevent fatty degeneration of rotator cuff muscle via suppressing adipogenesis of muscle sattelite cell. To verify the hypothesis, we examined the PRP effect on adipogenesis in C2C12, a satellite cell-derived cell line, in vitro and massive rotator cuff tear in vivo rat model.

Methods: PRP preparation
PRP was prepared following the double-spinning method. The PRP was activated with freeze-thaw method to release growth factors before each examination.

Cell Culture
A murine myogenic cell line, C2C12 was obtained from RIKEN cell bank (Tsukuba, Japan). The cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin (PS)(regular medium) in 5%CO2 chamber at 37°C. For adipogenesis, StemMACS AdipoDiff Media (Miltenel Biothech, Auburn CA, USA) was used. The cells were then seeded on twelve-well plates at a concentration of 50000 cells per well. After the cells had reached over confluence, they were cultured in three different medium; regular medium only (control group), adipogenic medium only (adipogenic group), and adipogenic medium with 10%PRP (adipogenic + PRP group). The medium was changed every 24-48 hours. Oil red staining was performed at day 7 and quantitative PCR was performed at day 3.

Rat Rotator Cuff Tear Model
Six Sprague-Dawley rats were used in this study. One of six rats was used to prepare PRP. Full thickness supraspinatus and infraspinatus tear was created in bilateral shoulders under general anesthesia in order to create fatty degeneration rotator cuff muscles. Then, intraarticular injections of 50 µL of saline solution in the right shoulder (saline group) and PRP in the left shoulder (PRP group) were given. 30 days after operation, rats were anesthetized and the supraspinatus and infraspinatus muscles were harvested.Oil red-O staining
The cells were fixed with Paraformaldehyde (PFA). The muscles were also fixed with PFA, then frozen section were used for histological analysis. The samples were stained with Oil Red-O solution to identify lipid droplets. For quantitative analysis, the extent of Oil red-O staining were quantified with the number of lipid droplets in five nonoverlapping microscopic fields per well.

**Real-time PCR**

Total RNA was extracted from the cell using an RNeasy mini kit (Qiagen, Valencia, CA). Total RNA was reverse transcribed into single-strand cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed in triplicate on the cDNA with an Applied Biosystems 7900HT Fast Real-Time PCR System and SYBR Green regents (Applied Biosystems). Results were normalized to housekeeping gene expression levels and expressed relative to the control (untreated) culture levels using the $2^{-\Delta\Delta Ct}$ method. We used Peroxisome Proliferator-Activated Receptor (PPAR)y and CCAAT/enhancer binding protein (C/EBP)α as adipogenic markers.

**Results:** In Vitro Experiments

**Oil red-O staining**

C2C12 myoblast cells in the control group showed no staining with Oil red-O (Fig1a). Cells cultured in the adipogenic medium showed positive staining of Oil red-O (Fig1b). The number of lipid droplets was decreased by PRP administration (Fig1c) and the number from five nonoverlapping microscopic fields was 56.6±9.8 in an adipogenic group and 24.0±3.4 in an adipogenic + PRP group. (Fig.1d)

**Real-time PCR**

PPARy and C/EBPα gene expression was significantly suppressed by PRP administration compared to an adipogenic group. (Fig.2)

In Vivo Experiments **Oil red-O staining**

Lipid droplets were observed around muscle fibers as well as around intramuscular tendons in saline solution administration group. In PRP administration group, lipid droplets were observed around intramuscular tendons, but not around muscle fibers. (Fig.3a)

**Real-time PCR**

PPARy and C/EBPα gene expression was significantly suppressed by PRP administration compared to a saline solution administration. (Fig.3b)

**Discussion:** PRP has received increasing interest across many musculoskeletal researches and has been widely applied clinically to stimulate tissue healing. The application of PRP for rotator cuff repair significantly improved the shoulder function (6) and decreased retear rate (7). Our results showed that PRP had an inhibitory effect on adipogenic change of myoblast in vitro, and on fatty degeneration of rotator cuff muscles in vivo. These results indicates that administration of PRP for patients after rotator cuff repair could prevent the fatty degeneration change of the rotator cuff muscles that is one of the important prognostic factor after surgical repair.

**Significance:** Our study showed that PRP had an inhibitory effect on adipogenic change of C2C12 myoblast cells in vitro, and fatty degeneration change of rotator cuff muscles in vivo. These results indicate that PRP could be useful treatment tool for prevention of fatty degeneration of rotator cuff muscles.
Fig. 2

Graphs showing the fold change of PPARγ and CEBPα under different conditions:

- **PPARγ**
  - Control
  - Adipogenic medium
  - Adipogenic medium + 10%PRP

- **CEBPα**
  - Control
  - Adipogenic medium
  - Adipogenic medium + 10%PRP

* indicates statistical significance.
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