The Effect Of Platelet-rich-plasma On Adipogenesis And Myogenesis In C2C12 Myoblast Cells

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Disclosures:

Introduction: Fatty degeneration is often seen in rotator cuff muscles with torn tendons. It is known that re-rupture after rotator cuff repair is related to fatty degeneration of them. Platelet-Rich-Plasma (PRP) has been reported to enhance tissue repair processes after tendon ruptures.(1,2) However the effect of PRP on fatty degeneration has not been elucidated yet. We examined the PRP effect on adipogenesis and myogenesis in C2C12 myoblast cells in in vitro model.

Methods: PRP preparations
PRP was prepared following the double-spinning method previously described.(3,4) A fixed volume(100ml) of whole blood was initially centrifuged at 2400 rotations per minutes(rpm) for 10 min to separate plasma from the red cell fraction. A second centrifugation cycle at 3600rpm for 15min was performed to separate PRP from Platelet-Poor-Plasma(PPP). The two cycles yielded in 8ml of PRP preparation. The PRP was activated with autologous thrombin and 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 Dulbecco’s Modification of Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (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, and DMEM without FBS was used for myogenesis. The cells were then seeded on twelve-well plates, and after the cells had reached over confluence, they were cultured in five different medium as below. Regular medium only (group C), adipogenic medium only (group A), adipogenic medium with 10%PRP (group A+P), myogenic medium only (group M), and myogenic medium with 10%PRP (group M+P). All experiments were performed with 1 to 3 passaged cells, and the same passage of the cells was used for each experiment. The medium was changed every 24-48 hours.

Cell Morphology
Cell morphology was observed by phase microscopy to examine the degree of myogenic differentiation. After 7 days of exposure to myogenic medium with or without PRP, medium was removed and cells were fixed in 4% formaldehyde.

Real-time PCR
At day3, 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^ΔΔCt method. We used Peroxisome Proliferator-Activated Receptor (PPAR)γ and CCAAT/enhancer binding protein (C/EBP)α as adipogenic markers and MyoD and Pax7 as myogenic markers.

Results: Cell Morphology
C2C12 showed myotube formation in the group C., There was extensive myotube formation in the group M. In the group M+P, the size and volume of myotube was suppressed.(Fig.1)

Real-time PCR
PPARγ and C/EBPα gene expression in the group A was increased compared to the group C, and the expression was decreased in the group A+P compared to the group A.(Fig.2) MyoD and Pax7 gene expression in the group M was increased compared to the group C, and the expression was decreased in the group M+P compared to the group M.(Fig.3)

Discussion: PRP has received increasing interest across many musculoskeletal research and has been widely applied clinically to stimulate tissue healing.(5) PRP injection has been reported to have beneficial effects on tendon-to-bone healing of rotator cuff and enhance remodeling of tendon-to-bone.(6) However, the effect of PRP on degenerative condition such as fatty degeneration of the muscle has not been elucidated yet. Our results showed that PRP had an inhibitory effect on adipogenic change of myoblast in vitro, indicating the possibility of PRP to inhibit fatty degeneration of rotator cuff muscle. On the other hand, it has been reported that PRP affects both myogenic proliferation and differentiation on C2C12.(7) In present study, administration of
PRP inhibited myogenesis, indicating the possibility of PRP to reduce muscle mass and strength. Therefore, further research will be required for clinical applications.

Significance: Our study indicated that PRP has an inhibitory effect on adipogenic change of C2C12 myoblast cells. The result implied the possibility of use of the PRP to prevent fatty degeneration of torn rotator cuff. On the other hand, PRP has an inhibitory effect on myogenic change of C2C12 myoblast cells. Therefore, further research will be required for clinical applications.

Acknowledgments: None.

References: (1) Kaux JF et al; Acta Orthop Belg(2013)
(5) Brian C. Halpern et al; HSS J(2012)
(6) Hapa O et al; Acta Orthop Traumatol Turc(2012)

Fig. 1

control(C)

myogenic medium(M)

myogenic medium+PRP(M+P)

Fig1: Cell Morphology
Myotube formation was observed in the group C, and more extensive myotube formation in the groupM. However, the size and volume of myotube was suppressed in the group M+P.
Fig. 2

Real-time PCR (PPARγ, C/EBPα)
Expression levels of PPARγ and C/EBPα were quantified by real-time PCR. Columns represent mean values with SD. Values were normalized to GAPDH expression. The asterisks indicate statistically significant differences.

Fig. 3

Real-time PCR (MyoD, Pax7)
Expression levels of MyoD and Pax7 were quantified by real-time PCR. Columns represent mean values with SD. Values were normalized to GAPDH expression. The asterisks indicate statistically significant differences.