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Simvastatin Stimulates Avascular Meniscus Healing - In vivo and In vitro Study

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Summary:
Simvastatin Stimulates Avascular Meniscus Healing--In Vivo, ex vivo, and In vitro Study

Abstract:
PURPOSE
To assess whether simvastatin can stimulate avascular meniscus healing in vivo and in vitro

METHODS
In vivo
In 24 Japanese White rabbits, a reproducible 1.5-mm-diameter full thickness cylindrical defect was created in the avascular inner two-thirds of the anterior portion of the medial meniscus bilaterally. Simvastatin-conjugated gelatin hydrogel was implanted into the defect of left knee (Simvastatin Group), while gelatin hydrogel was placed in the right knee (Control Group). Meniscal regeneration was evaluated histologically at 4 (n=6), 8 (n=8), and 12 weeks (n=10) for (1) quality[4] with use of an established three-component scoring system, (2) quantity with use of the measurement tool in Photoshop CS3[5, 6], and (3) immunochemistry assay of BMP-2, type-I collagen and type-II collagen.

In vitro
Human lateral menisci were harvested from 7 patients who underwent Total Knee Arthroplasty for knee osteoarthritis in the medial compartment. The lateral meniscus was divided into out-side and inner-side, and the cells from the inner-side were isolated separately and cultured in alginate beads in the presence or absence of 0.5uM simvastatin for 7 days. Real-time polymerase chain reaction (PCR) was used to quantify gene expression of BMP-2, BMP-7, SOX-9, COL2A1, aggrecan and MMP-13.

Ex vivo
An organ culture model was used to evaluate the potential effect of simvastatin on meniscal healing. In the organ culture model, a 1-cm vertical tear was created in the inner avascular zone of the meniscus, and the meniscus was cultured in the presence or absence of 0.5uM simvastatin for 2 weeks. Hematoxylin and eosin staining, and safranin O staining were preformed to evaluate the healing of the meniscal tears.

RESULTS
In vivo

Regenerative tissue quality scores were superior in the simvastatin group compared with controls at all end points (4 weeks, p=0.035; 8 weeks, p=0.014; 12 weeks, p=0.001). Additionally, the quantity of regenerated tissue in the group treated by simvastatin was greater at all end points, reaching significance at 8 ($p<0.05$) and 12 weeks ($p<0.05$). Moreover, the immunohistochemistry assays demonstrated a strongly positive staining for BMP-2, type-I collagen and type-II collagen in the meniscal reparative tissue at 12-week time point in the simvastatin group.

In vitro
The real-time PCR analysis showed that in the inner side of meniscus, simvastatin significantly up-regulated BMP-2 (inner-side: ratio=2.77, p=0.031), BMP-7 (Inner-side: ratio=3.06, p=0.040), and SOX-9 (Inner-side: ratio=2.45, p=0.041) gene expression, and down-regulated MMP-13 (Inner-side: ratio=0.60, p=0.025). However, COL2A1 (inner-side: ratio=0.942, p=0.306) and aggrecan (inner-side: ratio=0.605, p=0.187) were not significantly changed by the simvastatin treatment after 7 days.

Ex vivo
The histological analysis showed that more tissue ingrowth in the tear site of the simvastatin-treated explants compared with control explants

CONCLUSION
In conclusion, our study suggests that simvastatin could be a new therapeutic drug to enhance healing in meniscal repair, however further investigations are required.