The Controlled Intradiscal Release of Simvastatin Retards the Progression of Degeneration and Facilitates Autogeneous Repair at Rat Caudal Intervertebral Disc Injured by Needle Stab

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Introduction

Our earlier work indicated that cholesterol-lowering drug simvastatin was anabolic to chondrogenic expression of rat intervertebral disc (IVD) cells and therefore elicited the potential of simvastatin in IVD regeneration [1]. To test the effectiveness of the drug in vivo, we developed a modified stab incision at the rat IVD to induce degeneration, followed by the injection of a simvastatin-loaded hydrogel. As this model has become widely used for the IVD degeneration due to the cost-efficiency and convenient procedure, the needle puncture model using small rodents has never been utilized. It has been recognized that needle puncture can induce mild and progressive disc degeneration that is suitable for testing the potential treatments for the degenerative disc disease[2]. The aims of this study are: (1) to develop an easier and less invasive disc degeneration model by needle puncture at rat tails (2) to test the effectiveness of the controlled intradiscal release of simvastatin to retard or repair the perturbed disc using the proposed model.

Materials and Methods

Animals Male Sprague-Dawley rats (3 months old) obtained from Harlan were used in this study.

Surgical Technique With the aid of fluoroscopy,18G or 21G needle was inserted in the middle of the nucleus pulposus (NP) of Co5/Co6 or Co7/Co8, rotated 180°, and held for 5s. Depth of penetration was controlled at 5 mm from the needle. Co6/7 remained intact.

Simvastatin Treatment 6 weeks after stabbed injury by 21G needle, 2 μl of simvastatin at the concentration of 5 mg/ml loaded in a thermo-sensitive PEG-PLGA-PEG hydrogel or hydrogel alone that degrades persistently in vivo was slowly injected into the injured NP using microsyringe attached with 31G needle.

MRI Procedures and Processing In vivo MRI was serially scanned post-surgery using a 7.0T Varian MR scanner. T2-weighted midsagittal images of the intact and stabbed discs were processed and analyzed qualitatively using Analyze 7.0 and an IDL code.

Histologic Analysis After animals were sacrificed, the control and stabbed discs with or without treatment were processed and sectioned. Sections were stained with H&E and safranin O.

Statistical analysis: Data were expressed as mean ± SEM. One-way ANOVA and Dunnett post-hoc test were used to determine the significant difference.

Results

The progressive loss in both T2 densities and MRI indices (MRI index = number of pixels × corresponding image densities) were noticed in discs stabbed by 18G and 21G needles, especially in the discs intervened by 18G needles (Fig 1). H&E staining showed that the stab perturbation induced decreases of the NP area and the border between the NP and AF became less well-defined. The NP area in the intact disc stained strongly for safranin O, whereas the red stain in 21G-stabbed discs were non-homogenous and the positively stained areas in 18G-stabbed discs were scarcely noticed (Fig 2). 2 weeks after simvastatin injection, the T2 signals and MRI indices in the perturbed discs were all significantly increased compared to those of the stabbed discs with the hydrogel injection only (Fig 3). H&E staining revealed that in the stabbed disc injected with hydrogel alone, the NP became irregular and the border between AF and NP was less apparent. On the other hand, after the treatment with simvastatin, the NP size of the treated discs returned normal although the shape of NP was still irregular. Safranin O staining also illustrated that the treated discs presented recovered density level and stained region compared to those with hydrogel injection only (Fig 4).

Discussion

The present study demonstrates a creation of a rat model that is well representative for developing IVD degeneration. Needle puncture induced a decrease in the T2-weighted density and histological changes at the injured disc, which are degenerative signs similar to those of human discs, indicating that disc degeneration is able to be produced with such a perturbation in rat tail. Furthermore, our data also supported that the localized, controlled release of simvastatin significantly reversed the decayed T2 signals and the diminished constitutional substances of the extracellular matrix in the stabbed disc, suggesting the potential of using simvastatin to retard /regenerate of degenerative disc.