Effect Of Direct Intra-articular N-acetyl-D-glucosamine Injection For Osteochondral Repair In Rabbit Model

Wen-Hui Cheng1, You-Ching Chih1, Hsin-Yi Liu1, Yi-Ting Lin1, Nai-Jen Chang1, Horng-chaung Hsu, Phd, MD.2, Ming-Long Yeh1.
1Department of Biomedical Engineering, NCKU, Tainan, Taiwan, 2Department of Orthopaedic Surgery, China Medical University Hospital, Taiwan, R.O.C., Taichung, Taiwan.

Disclosures:

Introduction: Numerous approaches have been proposed in the recent decades to improve healing of articular cartilage surfaces, including micro-fracture of subchondral bone, mosaicplasty, autologous cell implantation (ACI), oral administration, and intravenous injection (IV) [1]. In which, oral delivery and intravenous injection are the two most convenient ways; however, the diffusion of the drug from peripheral vascular circulation into the identified joint is very inefficient. Therefore, in this study, we evaluated the outcome of direct intra-articular injection of N-acetyl-D-glucosamine (GlcNac) for repairing osteochondral defects.

Methods: And male New Zealand big white rabbits were used in all experiments as our animal models. A 3 mm in diameter and 3 mm in depth of full-thickness osteochondral defect was created on medial femoral condyle. The G group was injected twice a week for a period of 3 weeks for group at 4 weeks, initiated after a week postoperatively. As for a control group, the rabbits were treated with intra-articular normal saline injection twice a week for 3 weeks. For groups 12 weeks, the injection last 5 weeks. Six rabbits in each group were sacrificed at 4-week and 12-week after defect-model establishment, respectively.

Results: All gross appearance of articular cartilage defects was assessed and the representative morphology in each group at 4 weeks and 12 weeks (Fig 1). The microscopic observation of the treatment joints revealed that entire injection treatments were performed with no reported severe infections. Macrosopic appearance at 4 weeks, the defect areas in both control and G groups were concave and the defects were readily visible. New tissue developed inwards from the outer area of the defect edges in both groups. For macrosopic appearance at 12 weeks, the defects of both groups were fully covered with neo-tissue where were still reddish. In empty group, some defect surface or cavity had yellow convex tissue. Gross appearance grading according to the modified Wayne's grading scale scoring system [2] of two groups (Fig 2). Quantitative scores at 4 weeks, the total scores in the control group (6 ± 0.40) were higher than that in the G group (5.36 ± 0.37). Conversely, the total score in the G group (8.33 ± 0.47) was higher than that in the control group (7.33 ± 0.76) at 12 weeks. Although G group showed beneficial trends for macroscopic observation, they did not have statistical significance in difference. In addition, the difference between 4 weeks and 12 weeks was statistically significant (p < 0.01) in the G group but not in the control group (Fig 2).

Horizontal micro-CT images of medial condyles in each group at 4 weeks and 12 weeks postoperatively reveal the healing condition at defect area (Fig 3). The quantities of reconstituted tissue of control and G group are similar with each other. More newly formed mineralized tissue was observed in both groups at 12 weeks than at 4 weeks. The defect was still not completely filled and the newly tissue was grew inwards from the outer area of the defect edges in both groups. Bone Volume/Tissue Volume (BV/TV) and Trabecular Thickness (Tb.Th) were used to quantitative and qualitative measurement of subchondral bone regeneration. With respect to BV/TV results, differences between both groups were non-significant statistically, although the G group showed beneficial trends for normal BV/TV at 12 weeks. In addition, the difference in BV/TV between 4 weeks and 12 weeks was significant only in the G group (p = 0.002) but not in the control group (Fig 4). The Tb.Th value of G group (p < 0.001) was significantly higher than control group at 12 weeks. In addition, significant difference in Tb.Th between 4 weeks and 12 weeks was found only in the G group (p < 0.001) but not in the control group (Fig 5).

Eventually, we use histological and immunohistochemical analysis to confirm the repairing situation. Qualitative evaluation of sulfated GAGs and glycoproteins by Alcian blue, collagen expression observation by Masson's trichrome and general pathological observation by H&E (Fig 6 and 7). Histological findings at 4 weeks after operation (Fig 6) in the control group, shows that the defects were covered by the hyperplasia of capillary vessel, proliferation of fibroblast and connective tissue. In the Alcian blue staining, the defect showed poor positive GAG expression. The control group has more appearance of neutrophils in the defect space by Masson's stain (Fig 7). In the G group, the proliferations of undifferentiated blast cells (fibroblast cartilage cells) were found and GAG expression in the injured defect were presented (Fig 6). In addition, most specimens presented small newly formed bone trabecular at outer area of the defect edges (Fig 7, yellow arrow). At 12 weeks, control and G groups were completely filled with repaired tissue. In the control group, the restoration of subchondral bone and the regeneration of cartilage were observed, but the bone restoration was not complete and the irregular surface was apparently covered with fibrous tissue. In the G group, the defect treated with GlcNac showed the generation of hyaline-like cartilage, and the cells were appeared in better orientation and more normal GAGs expression was observed, although the thickness of regeneration cartilage was uneven and the subchondral bone was still remodeling. Immunohistochemical staining is to further confirm the
related protein and growth factor expression for the osteochondral defect regeneration. At 4 weeks after operation, defect areas of both the control and G groups showed clear positive Collagen type I (Col I) and Collagen type II (Col II) expression. The expression at 12 weeks, the defect area in control group was replaced with fibrous tissues in the surface and bottom of the defect that containing mostly type I collagen. In contrast, the defect in the G group were repaired with hyaline-like cartilaginous tissue containing mostly type II collagen and only expressed type I collagen in bone remodeling area (Fig 8).

**Discussion:** To sum up, GlcNAc injection showed beneficial trends for gross appearance, bone regeneration, hyaline-like cartilage and well cartilage alignment at 12 weeks. In line with our results, Shikhman et al [3] reported that intra-articular administration of GlcNAc twice a week has chondroprotective and anti-inflammatory in Osteoarthritis (OA) model but once a week injections did not had therapeutic efficacy. Severe inflammatory response was observed in the control group at the repairing defect surface, but the severe inflammatory response was not seen in the G group and beneficial effect in cartilage and bone regeneration at 12 weeks were also appeared in GlcNAc group. This study demonstrated that intra-articular injection of GlcNAc promotes positive healing for osteochondral regeneration in the rabbit model. More hyaline-like cartilage and bone regeneration and inhibit inflammation were observed by directly intra-articular GlcNAc injection.

**Significance:** With the injection of supplementation GlcNAc solution, the cartilage shows great gross appearance, hyaline-like cartilage, well collagen alignment and abundant amount of glucosaminoclycan (GAGs) expression.

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**Fig 2.** Quantitative scores of gross appearance of two groups at 4 weeks and 12 weeks. #: between two time point, p<0.05

**Fig 3.** The micro-CT images of bone assessment in two groups at 4 weeks and 12 weeks after operation. Circles enclose the repaired osteochondral defect area.

**Fig 4.** Quantification scores of BV/TV; #: between two time point, p<0.05
Fig 5. Quantification scores of the Tb.Th. #: between two time point, p<0.05

Fig 6. Histological examinations of staining images of the repaired area using H&E, Masson’s trichrome and Alcian blue stain at 4 weeks and 12 weeks. Scale bar: 200μm.
Fig 7. Higher magnifications of the images at defect areas for the control and the G groups. (Masson’s stain; 100X magnification). Yellow arrow: newly formed trabeculae bone. Scale bar: 50μm

Fig 8. Immunohistochemistry of the defect areas for the control and the G groups for collagen type I and type II at 4 weeks and 12 weeks. Scale bar: 200μm

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