The Expression of Adiponectin Receptor 1 and 2 of Rat Intervertebral Disc Cells

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Introduction: Adipose tissue is well known as the biggest endocrine organ that secretes various adipokines such as tumor necrosis factor (TNF)-α, Interleukin (IL)-6, leptin, resistin and adiponectin(reference 1). Among these adipokines, adiponectin is known to have anti-diabetic, anti-atherogenic and anti-inflammatory effects by mediating its biological effects(reference 2). We hypothesized that adiponectin in adipose tissue of spinal canal involved in the pathomechanism of low back pain. The aim of this study was to assess the expression of adiponectin receptor 1 and 2 (Adipo R1 and Adipo R2) in rat intervertebral disc (IVD) cells and, as well, to investigate whether adiponectin has its anti-inflammatory effect on rat IVD cells.

Methods: All animal procedures were performed under approval and guidance of Animal Care and Use Committee at the author’s institution.

The expression of adiponectin receptor in the rat IVD cells
Immunohistochemical staining: Rat intervertebral disc tissues were fixed in 4% paraformaldehyde for 2 days and decalcified with EDTA. After dehydrated in graded series of alcohol, they were embedded in paraffin, and then all samples were cut at a thickness of 6µm. Deparaffinized in xylene, they were rehydrated in graded series of alcohol. All sections were divided in two and each were stained with goat antibodies against Adipo R1 (Santa Cruz, CA, USA) or rabbit antibodies against Adipo R2 (Santa Cruz, CA, USA). These sections were incubated with the antibodies at the dilution of 1:100 for 60 minitues at room temperature. The samples were stained using diaminobezidine (DAB) detection kit and counterstained with hematoxylin. The percentage of positive cell (% positive immunostained cells): Specimens were evaluated and scored based on a semiquantitative approach of percentage of Adipo R1 and Adipo R2 positive cells. The number of immunostained cells were counted in at least three randomly selected high power fields (150 cells or more) for each area of samples.

Cell culture and treatments
Coccygeal IVD were aseptically dissected from eight skeletally matured (12-week-old) male SD rats. The nucleus pulposus (NP) and anulus fibrosus (AF) were separately isolated. Each IVD cells were pre-cultured for 7 days at 37°C in 5% CO2 and 95% air in complete tissue culture media, Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% FBS, 25µg/ml ascorbic acid, 100 U/ml penicillin, and 100 mg/ml streptomycin. After pre-culture, NP cells and AF cells were seeded in 6 multiwell plate up to complete adhesion (70% confluence). NP cells and AF cells were then treated with recombinant adiponectin (1.0µg/ml) and/or IL-1β (0.2µg/ml) for 24 hours. It was divided into 3 groups as following; control group; without IL-1β and adiponectin, IL-1β group; treated with IL-1β (0.2µg/ml) only, IL-1β+adiponectin group; treated with both IL-1β (0.2µg/ml) and adiponectin(1.0µg/ml). The mRNA expression of TNF-α and IL-6 in the rat IVD cells
mRNA quantification of TNF-α and IL-6: Total RNA was isolated from each NP cells and AF cells, and real time reverse transcription polymerase chain reaction (RT-PCR) performed for TNF-α and IL-6. GAPDH was used as endogenous control.

Statistical analysis
Datas were expressed as mean ± standard error (SE) and analyzed using ANOVA with Fisher’s PLSD post hoc test. P values of <0.05 were regarded as statistically significant.

Results: The expression of adiponectin receptors in the rat IVD cells
Immunohistochemical staining: the expression of AdipoR1 and AdipoR2 was observed in both NP and AF cells. In addition, it was also observed a strong staining in adipose tissue of bone marrow and chondrocyte like cells in the endplate (Figure 1).
% positive immunostained cells: mean % positive cells of Adipo R1 and Adipo R2 was 43% and 46.7% of NP cells, and 33.3% and 43.3% of AF cells, respectively. When the expression levels of Adipo R1 and Adipo R2 were compared, there were no significant difference on either NP or AF cells. There were also no significant difference of the expression of Adipo R1 and R2 compared between NP and AF (Figure 2).

The mRNA expression of TNF-α and IL-6 in the rat IVD cells
The mRNA expression of pro-inflammatory cytokines, TNF-α and IL-6, was significantly stimulated by IL-1β treatment in both NP and AF cells. In the NP cells, the mRNA expression of TNF-α of IL-1β+adiponectin group was lower than IL-1β group, however the difference did not reach to the statistical significance. Whereas in AF cells, it of IL-1β+adiponectin group was significantly lower than IL-1β group (p<0.05). The mRNA expression of IL-6 was not affected by the treatment with adiponectin in NP and AF cells (Figure 3).

Discussion: The results of present study, for the first time, demonstrated the expression of adiponectin receptor, Adipo R1 and R2, in the NP and AF cells. Previously anti-inflammatory effect of adipocetin was reported in the other organs. In the present study, the TNF-α expression induced by IL-1β stimulation was significantly down-regulated by the treatment with adiponectin in AF cells. The same trend was also observed in NP cells. Adiponectin may play the role in homeostasis of rat IVD cells. Further study are now undergoing to investigate the effects of adiponectin on IVD cells.

Significance: Adiponectin may have an anti-inflammatory effect on rat IVD cells.
ORS 2015 Annual Meeting
Poster No: 1603