Effects of Single Local Injection of Local Anesthetic Agents on Intervertebral Disc Degeneration: Ex Vivo and Long-Term In Vivo Experimental Study

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Introduction: Discogenic low back pain (LBP) is generally caused by progressive intervertebral disc (IVD) degeneration associated with aging or trauma. Analgesic discography (discoblock) can be used to diagnose or treat discogenic LBP by injection of a small amount of local anesthetic. Lidocaine or bupivacaine is one of the most commonly used local anesthetics for discoblock. Several in vitro studies have reported dose- and time-dependent cytotoxic effects of these local anesthetics on IVD cells (1)(2). In addition, a recent prospective, matched cohort clinical study showed that discography was associated with more extensive IVD degeneration at a 10-year follow-up (3). The present study aimed to investigate the deteriorative effects of lidocaine and bupivacaine on rabbit IVDs using an organotypic culture model and an in vivo long-term follow-up model.

Methods: All animal procedures in this study were specifically approved by the Institutional Animal Care and Use Committee of Hokkaido University.

(Organotypic culture study)
The IVDs (T11/T12-L5/6) were surgically harvested from rabbits. A 0.9% saline solution, 1% lidocaine, or 0.5% bupivacaine was intradiscally injected using a micro syringe with a 26-gauge needle (15 µL per disc). IVDs were divided into 5 groups: untreated control, puncture-only group, saline group, lidocaine group and bupivacaine group. 3 or 7 days after the injection NP tissues were collected from IVDs and stained with propidium iodide (PI : dead cell) and Hoechst 33342 (live and dead cells). NP cell death was evaluated using a confocal laser scanning microscopy (n=6). 7 days after the injection histological analysis using hematoxylin and eosin (H&E) staining and TUNEL assays were performed on IVDs. The quantitative data of cell number were presented as the mean of the 3 evaluations (n=6).

(In Vivo study)
Rabbit lumbar IVDs were percutaneously punctured under a fluoroscopic guidance. 15 µL of 0.9% saline solution, 1% lidocaine, or 0.5% bupivacaine was injected into IVDs (L2/L3 and L4/L5). L3/L4 was left intact as a control. A sham procedure was performed by subjecting control animals to IVD needle puncture only. 6 or 12 months after the injection, mid-sagittal images of the treated discs were analyzed qualitatively for evidence of degenerative changes using an ultra-high magnetic field strength, 7.0-Tesla MRI. After MRI analysis, histological analysis was performed on IVDs using Safranin-O fast green and H&E staining. Semiquantitative evaluation of disc degeneration was performed with a grading system ranging from 4 (normal) to 12 (highly degenerative).

(Statistical Analysis)
All data were expressed as the mean ± standard deviation (SD). Statistical analyses were performed using one way analysis of variance followed by a Tukey-Kramer post-hoc test or the Kruskal-Wallis test for multiple group comparisons for nonparametric data. P < 0.05 was considered statistically significant.

**Results:** (Organotypic culture model) NP cell death was measured by PI and Hoechst 33342 staining and visualized by confocal microscopy (Fig. 1-A). Both anesthetic agents induced time-dependent NP cell death; when compared with injected saline solution, significant effects were detected within 7 days. After 7 days, lidocaine and bupivacaine increased dead cells to 72% and 76%, respectively (Fig. 1-B). H&E staining revealed vacuolization in NP cells and a decrease of the normal gelatinous appearance of the extracellular matrix in the saline and local anesthetic agent groups (Fig. 2-A). The percentage of apoptotic NP cells in the lidocaine and bupivacaine groups was significantly higher than that in all other groups (Fig. 2-B).

(In vivo) MRI analysis showed no significant difference among the puncture, saline, and both anesthetic agent groups at 6 or 12 months (Figure 3-A, B).
Histological assessment of IVDs revealed degenerative changes in saline and local anesthetic agent groups. However, there was no significant difference between the saline and anesthetic agent groups (Fig. 4-A). After 12 months, there was a significant difference between anesthetic agent groups and untreated control groups in the number of NP cells (Fig. 4-B).

Discussion: We used an organotypic culture model with a clinically relevant procedure, and local anesthetic groups exhibited time-dependent increases in dead NP cells in confocal laser scanning microscopic analysis. In this organotypic culture model, however, the saline group also exhibited mild
deleterious effects on IVDs in both confocal laser scanning microscopic and histological analyses. The pressure applied by the aqueous fluid increased the percentage of dead cells. As a longitudinal clinical study to assess the long-term effects of discography up to 10 years (3), we followed rabbits up to 12 months, which was comparable with 7 to 10 years of the human lifetime. In this study, MRI analysis did not detect any significant differences, while histological analysis revealed that the saline and the local anesthetics promoted degenerative changes. However, there were no significant differences between the saline-injected group and the local anesthetic groups. Therefore, there was no strong evidence that the local anesthetics caused IVD degeneration in vivo other than the initial mechanical damage of the pressurized injection. Our organotypic culture model clearly revealed that both lidocaine and bupivacaine significantly induced apoptotic NP cell death, which supported our in vitro data (2). Further studies should be performed to investigate the deteriorative effects of local injection of analgesic agents in degenerated IVDs.

**Significance:** There was no strong evidence to suggest that discoblock with local anesthetics has the potential of inducing IVD degeneration in the in vivo model using healthy IVDs.

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