N-acetyl Cysteine Protect Cell from Chondrocyte Death Induced by Local Anesthetics

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Introduction: Local anesthetics (LA), such as Ropivacaine, Bupivacaine, and Lidocaine, are among the drugs most frequently used for musculoskeletal problems in procedures ranging from diagnosis to postoperative pain control1-4. However, intra-articular local anesthetic injection has recently been reported as one of the possible causes of chondrolysis after arthroscopic surgery5-8. It is known that commonly used aminoamide local anesthetics have a cytotoxic effect on chondrocytes9-13. However, there is no study that has offered suggestions regarding methods of protecting chondrocyte from those adverse effects of aminoamide local anesthetics. The purpose of the current study was to determine whether a well-known antioxidant, N-acetyl cysteine (NAC), has cytoprotective effects on aminoamide local anesthetics induced chondrocyte cell death and to evaluate the cytoprotective mechanism of the NAC.

Methods: This study used a control group, an LA, and a NAC-LA group. Cytotoxicity was induced in the LA group through exposure to Ropivacaine (0.075%), Bupivacaine (0.05%) and Lidocaine (0.2%) for 24 hr. The NAC-LA group was exposed to 10 mM NAC for 1 hr, before LA exposure. These study groups were evaluated for cell viability, apoptosis rate, intracellular ROS production, caspase-3/7 activity, and expression levels of caspase-3, phospho-ERK, phospho-JNK, phospho-p38, and cleaved PARP-1. All statistical analyses were performed via one-way ANOVA.

Results: Cell viability in all LA subgroups was significantly lower than in the control group (p<0.001) (Fig. 1). Cell viability in the NAC-LA subgroups was significantly higher than that of their paired LA subgroups (p<0.002). Apoptosis and necrosis were higher than those of the control group. All of the NAC-LA subgroups were lower than the LA subgroups (Fig. 2). According to the apoptotic cells showed higher numbers of cells with DNA fragmentation in the LA group than in the control group. The number of cells with DNA fragmentation was considerably lower in the cyanidin-LA subgroups than in their paired LA subgroups (Fig. 3). According to the FACS analysis and confocal microscope (Fig. 4), the level of intracellular ROS production in the LA subgroups was significantly higher than that of the control group (Ropivacaine: p=0.029, Bupivacaine: p=0.002, Lidocaine: p=0.001). All of the NAC-LA subgroups showed significantly lower intracellular ROS production than the LA subgroups (Ropivacaine: p=0.023, Bupivacaine: p=0.002, Lidocaine: p=0.008). Caspase-3/7 activity of LA subgroups was significantly higher than that of the control group, except that of the Lidocaine subgroup (Ropivacaine: p=0.029, Bupivacaine: p=0.002, Lidocaine: p=0.001). All of the NAC-LA subgroups showed significantly lower caspase-3/7 activity than that of the control group, except that of the Lidocaine subgroup (Ropivacaine: p=0.004, Bupivacaine: p=0.001, Lidocaine: p<0.001). Caspase-3/7 activity of NAC-LA subgroups was significantly lower than that of their paired LA subgroups (Ropivacaine: p=0.004, Bupivacaine: p=0.001, Lidocaine: p<0.001). According to the results of the western blot analysis, the expression levels of phosphorylated ERK, p38, and JNK, caspase-3, and cleaved PARP-1 in all the LA subgroups were higher than those of the control...
group. Their expression levels in the NAC-LA subgroups were significantly lower than those of the LA subgroups.

**Discussion:** The current study demonstrated that NAC had cytoprotective effects against local anesthetic-induced cytotoxicity on chondrocyte; these effects were achieved by reducing intracellular ROS production and down-regulating ERK, p38, JNK, caspase-3, and cleaved PARP-1.

**Significance:** The current study suggests that antioxidants can be candidates for preventing local anesthetic-induced chondrocyte death.

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**Figure 1.** Cytoprotective effects of NAC against chondrocyte death induced by local anesthetic. (A) The CCK-8 analysis demonstrated that cell viability in all LA subgroups was significantly lower than in the control group (p<0.001). Cell viability in the NAC-LA subgroups was significantly higher than in their paired LA subgroups (p<0.002). (B) Confocal microscopics.

**Figure 2.** According to the annexin V/PI double staining, Apoptosis and necrosis were higher than control group. All of the NAC-LA subgroups were lower than the LA subgroups.

**Figure 3.** TUNEL staining study demonstrated that LA induced DNA fragmentation, a marker of apoptosis, and the rate of DNA fragmentation was markedly decreased in the NAC-LA subgroups.

**Figure 4.** Intracellular ROS levels were significantly increased in the LA subgroups, as compared with the control. Intracellular ROS levels in the NAC-LA subgroups were all lower than in the LA subgroups. (A) According to the morphological analysis using a confocal microscope, intracellular ROS levels were higher in the LA subgroups than in the control; however, ROS levels were markedly lower in the NAC-LA subgroups than in the LA subgroups.

**Figure 5.** Caspase-3 activity of LA subgroups were significantly higher than in the control group, except the Lidocaine subgroup (Repacaine: p<0.001, Bupivacaine: p=0.001) (Fig. 4). Caspase-3 activity of NAC-LA subgroups were significantly lower than in their paired LA subgroups (Repacaine: p=0.001, Bupivacaine: p<0.001, Lidocaine: p=0.001).

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