Neurotoxicity of Bupivacaine was Associated with Mitochondrial Membrane Depolarization and Intracellular Production of Reactive Oxygen Species, which was not Modulated by Diazoxide in SH-SY5Y Cells .

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Background and Aims:

- Local anesthetics including bupivacaine are neurotoxic even at normal clinical dose. The depolarization of mitochondrial membrane potential (MMP) results in the increase in intracellular reactive oxygen species (ROS) and subsequent apoptosis. The first objective of this study was to investigate the cytotoxicity and the effects of bupivacaine on MMP and intracellular ROS production.
- Mitochondrial ATP-sensitive potassium (K) channel plays a key role in the maintenance of MMP. Bupivacaine has been reported to block K channel. Thus, our hypothesis was diazoxide, K channel opener could modulate the cytotoxic effect of bupivacaine.

Methods:

- The SH-SY5Y human neuroblastoma cells were grown in MEM and Ham's F12 nutrient mixture.
- After SHSY5Y cells were treated with cumulative dose of bupivacaine, the effects of bupivacaine on cell viability and caspase-3 expression were evaluated.
- Then the effects of bupivacaine on MMP and production of intracellular ROS were determined by JC-1 staining and flow cytometry, respectively.
- Furthermore, SH-SY5Y cells were pretreated with diazoxide (50-200μM) for 2hours before exposure to bupivacaine and then the modulatory effect of diazoxide was evaluated.

Results:

- Bupivacaine reduced cell viability in dose (Fig.1) and time (Fig.2) dependent manner.
- Bupivacaine increased intracellular ROS production (Fig.3) while depolarizing MMP (Fig.4).
- Caspase-3 expression was induced by bupivacaine (Fig.5). However, pretreatment of SH-SY5Y cells with diazoxide did not affect the cell viability reduced by bupivacaine (Fig.6).

Fig.1

Fig.2

Fig.3

Fig.4

Fig. 5

Fig.6

Conclusions:

Neurotoxicity of bupivacaine was associated with intracellular ROS production and depolarization of MMP. Diazoxide, K_{ATP} channel opener, did not affect that neurotoxicity of bupivacaine in human neuroblastoma SH-SY5Y cells.