Cell Toxicity in Fibroblasts, Tenocytes, and Human Mesenchymal Stem Cells—A Comparison of Necrosis and Apoptosis-Inducing Ability in Ropivacaine, Bupivacaine, and Triamcinolone

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Purpose: To analyze the ability of ropivacaine, bupivacaine, and triamcinolone to induce apoptosis and necrosis in fibroblasts, tenocytes, and human mesenchymal stem cells. **Methods:** Human dermal fibroblasts, adipose-derived human mesenchymal stem cells (hMSCs), and tenocytes gained from the rotator cuff tendon were seeded with a cell density of $0.5 \times 10^4/cm^2$. One specimen of ropivacaine, bupivacaine, and triamcinolone was tested separately on the cells with separate concentrations of 0.5%, 0.25%, and 0.125% for each specimen. The negative control received no agent, only a change of medium. The incubation period for each agent was 30 minutes. After a change of medium and 1 hour, 24 hours, and 7 days of incubation, 10^4 cells were harvested and analyzed via fluorescence-activated cell sorting with double-staining with annexin V and propidium iodide. Statistical analysis to determine significant difference ($P < .05$) between the groups with SPSS statistics 23 through one-way analysis of variance with a univariate general linear model was performed. **Results:** Bupivacaine showed necrosis-inducing effects on fibroblasts and tenocytes, with the necrotic effect peaking at 0.5% and 0.25%. Ropivacaine and triamcinolone caused no significant necrosis. Compared with fibroblasts and tenocytes, hMSCs did not show significant necrotic or apoptotic effects after exposure to bupivacaine. Overall, no significant differences in apoptosis were detected between different cell lines, varying concentrations, or time measurements. **Conclusions:** Bupivacaine 0.5% and 0.25% have the most necrosis-inducing effects on fibroblasts and tenocytes. Ropivacaine caused less necrosis than bupivaine. Compared with fibroblasts and tenocytes, hMSCs were not affected by necrosis using any of the tested agents. A significant apoptosis-inducing effect could not be detected for the different cell lines. **Clinical Relevance:** Possible cell toxicity raises questions of concern for intra-articular injections using local anesthetics and corticosteroids. The present study demonstrates the necrotic and apoptotic effects of ropivacaine, bupivacaine, and triamcinolone and may give recommendations for intra-articular use of local anesthetics and corticosteroids.

Local anesthetics and corticosteroids frequently are preferred for intra-articular pain management and in arthroscopic surgery.[1] Bupivacaine and its cytotoxic effect on chondrocytes has been the most investigated local anesthetic to date.[2,3] The cytotoxic effects on cartilage chondrocytes by comparing ropivacaine with bupivacaine via the use of cell-viability assays have shown better viability of the cells after treatment with ropivacaine.[4] Previous studies have investigated the necrotic and apoptotic effects of fentanyl on fibroblasts and mesenchymal stem cells (MSCs) with intention of using a different morphine and its derivatives instead of ropivacaine or bupivacaine as an analgesic agent.[5-7] Fentanyl, however, is not suggested as an alternative to ropivacaine regarding fibroblasts because of its greater necrosis-inducing effects, even though it shows less necrosis in MSCs than ropivacaine.[5,6] Another study found morphine to be less toxic to progenitor stem cells than ropivacaine and bupivacaine.[7]

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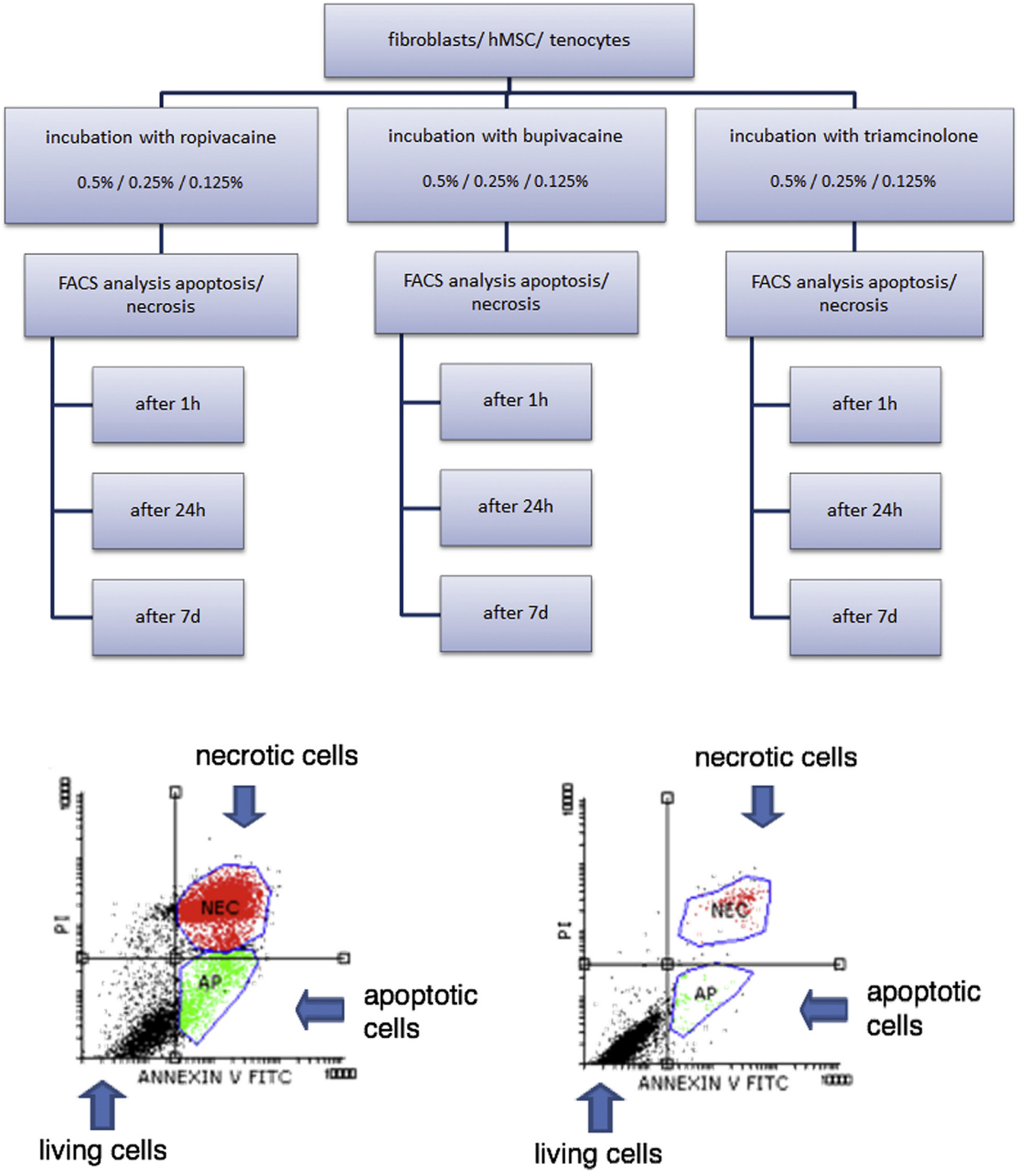

Fig 1. Top: Overview of the study setup. Cells were harvested at a confluency of 70%. Every cell count using FACS measured 10^4 cells. Bottom: FACS analysis example showing dot plots. Necrotic cells are shown in red; apoptotic cells are green. Living cells are counted in the left bottom quadrant of each plot (see arrows). To the left is an example of high-significance necrosis whereas the right example shows a minimum of the necrosis rate. (AP, apoptotic cells; FACS, fluorescence-activated cell sorting; hMSC, human mesenchymal stem cells; NEC, necrotic cells.)

In addition to local anesthetics or as a replacement, corticosteroids commonly are used as pain and inflammation-reducing medication intra-articularly.[8-11] Lidocaine was found to potentiate toxic effects of methylprednisolone when used on chondrocytes.[10] A comparison of dexamethasone, triamcinolone, methylprednisolone, and betamethasone on MSCs showed that dexamethasone had the least cytotoxic