INTRODUCTION:
Single-dose intra-articular injections of local anesthetics such as lidocaine, bupivacaine, and ropivacaine are frequently used to relieve pain after injury or surgery, to provide anesthesia for diagnostic tests, or to decrease chronic joint pain due to inflammation in combination with corticosteroids. The chondrotoxicity of local anesthetics has been documented in vitro and is a cause for clinical concern. Continuous administration of local anesthetics into human and animal joints has also been linked to subsequent chondrolysis. The effect of single-doses of local anesthetics has not been widely investigated, and the evaluation of chondrocyte viability has not been performed with respect to the average clinical duration of action of the medications. This study was performed to evaluate the in vitro chondrotoxicity of single-doses of 1% lidocaine, 0.25% bupivacaine and 0.5% ropivacaine on human chondrocytes over the clinical duration of action of each drug.

METHODS:
Human chondrocytes were seeded at a density of 0.5 X 10^6 cells/well in 6 well plates. A bioreactor was used to simulate normal joint fluid metabolism. The clinically acceptable dose of 10 cc was adjusted to account for decreased cartilage surface area of experimental conditions versus human knee, and three anesthetics were tested: 1% lidocaine, 0.25% bupivacaine and 0.5% ropivacaine. Each medication was delivered to the chondrocytes over the average duration of chemical action. This information is summarized below in Table 1. Cell viability was assessed with a two-color fluorescence assay.

Data were analyzed by chi-square test and z-test. A priori power analysis was performed. The power of chi-square tests for the analysis of n=1300 chondrocytes per culture condition (5000 chondrocytes per condition were actually analyzed) is 95% assuming an alpha value 0.05 and a small effect size (w=0.1).

RESULTS:

<table>
<thead>
<tr>
<th>Anesthetic Name</th>
<th>Flow rate (ml/h)</th>
<th>Average Duration of Action (hrs)</th>
<th>Half-Life (hrs)</th>
<th>Dose per 10cc (mg)</th>
<th>Surface area adjusted dose (mg/6ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Lidocaine</td>
<td>2.0</td>
<td>3</td>
<td>1.5</td>
<td>100</td>
<td>12.5</td>
</tr>
<tr>
<td>0.25% Bupivacaine</td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>25</td>
<td>3.13</td>
</tr>
<tr>
<td>0.5% Ropivacaine</td>
<td>0.5</td>
<td>12</td>
<td>6</td>
<td>50</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Table 1. Flow rate, average duration of action, half-lives, and doses of all medications used in experimental conditions.

Live/dead counts from 24 high power fields were summed for each culture condition. The results are summarized in Figure 1. Chondrocytes treated for three hours with 1% lidocaine exhibited significantly higher cell death than those in control media by chi-square test (7.9 vs. 2.9% cell death, p<0.001). Chondrocytes treated for six hours with 0.25% bupivacaine exhibited no difference in cell death compared to those in control media (2.7 vs. 2.8% cell death, p=0.856). Similarly, cells treated for 12 hours in 0.50% ropivacaine showed no difference in cell death compared with the control group (2.9 vs. 2.4% cell death, p=0.084). The results did not change with the use of z-tests. The pH of 1% lidocaine/DMEM was maintained at 7.7, bupivacaine/DMEM at 7.8, and ropivacaine/DMEM at 7.3.

DISCUSSION:
Our data suggest that when normal human chondrocytes are exposed to single injection doses of 0.25% bupivacaine and 0.5% ropivacaine over the medication’s average duration of action (six and twelve hours, respectively), there is no significant decrease in cell viability. Conversely, when chondrocytes are exposed to a single injection dose of 1% lidocaine for the average duration of action of three hours, there is a significant decrease in chondrocyte viability when cell cultures are compared with controls.

The molecular mechanism of anesthetic chondrotoxicity has not been fully elucidated, but several studies have demonstrated that lidocaine causes mitochondrial injury, which ultimately leads to cell death. Grishko et al. showed that 1% lidocaine caused detectable mitochondrial dysfunction in chondrocytes, ultimately resulting in apoptosis in cells cultured for 24 hours. Johnson et al. found that lidocaine induced both necrosis and apoptosis in neuronal cells, with the mechanism of cell death dependent on anesthetic dose. Cells exposed to 37mM lidocaine for 30 minutes underwent cell death via apoptosis, while those exposed to 185mM lidocaine for 10 minutes underwent necrosis. In our study, significant cell death was observed when chondrocytes were exposed to a total dose of 12.5 mg 1% lidocaine for three hours. It appears likely that the toxicity of lidocaine is related to mitochondrial dysfunction, with the mechanism of cell death dependent on the dose of anesthetic.

Chemical incompatibility may also contribute to the observed chondrotoxicity of 1% lidocaine. Bogatch et al. found minimal cell death after one hour exposure to 1% lidocaine and 0.25% bupivacaine, but found significant cell death when chondrocytes were exposed to 1% lidocaine mixed with DMEM or human synovial fluid. Our results corroborate this finding, as significant cell death was observed in cultures with 1% lidocaine/DMEM. This may suggest an inherent chemical incompatibility between culture media and injectable anesthetics.

SIGNIFICANCE:
Although there have been many studies examining the chondrototoxic effect of this class of medications, this investigation has been the only one to focus on identifying whether these medications are chondrotoxic in a model of single-dose administration based on the clinical duration of action. The observed chondrotoxicity in this study suggests that intra-articular administration of lidocaine should be used with caution, even in a single dose.