APPLICATION OF CULTURED CELLS IN DISTRACTION EPIPHYSIOLYSIS

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Introduction
Physeal distraction is a relatively simple alternative for lengthening in which the epiphysis is pulled away from the metaphysis at a slow, controlled rate. However the gain in length achieved during the distraction is reduced because of increased retardation of growth with premature epiphyseal closure after the apparatus was removed. The purpose of this study was to evaluate the ability of percutaneously injected cultured chondrocytes to elicit repair at the site of physeal separation and to prevent premature physeal closure. We also wanted to determine if Cox-2 inhibitors could be used for the prevention of premature physeal closure after distraction epiphysioysis.

Materials and Methods
The immature rabbits (6 weeks old) were divided into three study groups: group A, distraction epiphysiolysis alone; group B, distraction epiphysiolysis with injection of cultured chondrocytes; and group C, distraction epiphysiolysis with Cox-2 inhibitors. With the use of general anesthesia, chondrocytes were harvested from the iliac crest and a circular external fixator (Smith-Nephew, Memphis, TN) was applied across the physis by drilling two pairs of 1 mm Kirschner wires transversely through the epiphysis and diaphysis and attaching these to the distraction fixator bars. Distraction was begun immediately and was continued at the rate of 1 mm/day. Rabbits in group C were given a Cox-2 inhibitor (Rofecoxib 9 mg/kg) daily for three weeks.

After radiographic confirmation of physeal separation at 5 days, the distraction gap was increased 5 mm to allow easy introduction of the spinal needle. The correct position of the needle was confirmed radiographically and 7-8 million cultured cells were injected into the distracted physis (Fig 1). The distractor was then compressed to its position before injection. Epiphyseal distraction was further continued daily for the duration of 5 days.

Results
Radiologically, separation of the epiphysis from the metaphysis began as a widening of the line of metaphyseal transparency after 4 or 5 days. Separation always occurred in the zone of the growing cartilage. The differences among mean values of lengthening (compared to the contralateral extremity) in the three groups were statistically different at 4 weeks after operation, while at 10 weeks only the cell-injected group retained any lengthening effect (Table 1) and this difference was statistically (p < 0.001) significant.

<table>
<thead>
<tr>
<th>Time after op.</th>
<th>Distract only</th>
<th>Distract+Cox 2</th>
<th>Distract+cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td>+9.4</td>
<td>+8.9</td>
<td>+8.2</td>
</tr>
<tr>
<td>2 wks</td>
<td>+6.4</td>
<td>+9.1</td>
<td>+8.0</td>
</tr>
<tr>
<td>4 wks</td>
<td>+5.1</td>
<td>+4.2</td>
<td>+7.2</td>
</tr>
<tr>
<td>10 wks</td>
<td>-3.0</td>
<td>-3.0</td>
<td>+4.4</td>
</tr>
</tbody>
</table>

Table 1 *difference in length (mm) compared to contralateral side

Histologic sections showed that the failure plane passed through the lower hypertrophic zone or provisional calcification zone in most specimens (80%), but in 20% specimens, separation was noted at the lower proliferating zone with the presence of vertical septa proximally. In groups A and C, bony bridge formation was present at 3 weeks and full bony bridge formation was present at 10 weeks without significant difference.

In group B specimens, a huge mass of chondrocytes was visible in the lower portion of the separated physis (Fig 3). At 10 weeks after initiation of distraction, the thickness of the physis was restored to normal width, but chondrocyte growth was disordered (Fig 4). In these cell implanted experimental groups, injected cells seemed to attach to the bottom of separated growth plate and questionable grow or behave hypertrophic chondrocytes and delay the premature closure of the epiphyseal plate.

Discussion
Distracted epiphysiolysis seems quite sensitive to experimental variables and it is difficult to achieve reproducible results. Because of the risk of growth disturbance following distraction epiphysiolysis, most investigators have recommended that it be used only toward the end of the growth period and only to achieve fairly small amounts of overall lengthening.

In the present study, after 5 days of distraction, separation was noted primarily at the lower hypertrophic and provisional calcification zones, but in some areas, fracture separation was propagated toward the proliferating zone, possibly causing premature physeal closure. A study in rabbits (1) suggested that reformation of a bar after excision can be inhibited by the use of oral indomethacin without the use of an interpositional material. However, in our current study, Cox-2 inhibitor did not prevent premature bony bridge formation; the frequency of bony bridge formation when a Cox-2 inhibitor was used was the same as in the control group.

In our animal model, the implantation of cultured cells allowed lengthening without bony bridge formation after distraction epiphysiolysis of the proximal tibia. Radiographically, a significant lengthening effect was present after distraction in the cell-implanted group compared to control cell-free groups. Histologically, a slight widening of the distracted proximal tibial physis was visible in the cell-implanted group compared to the cell-free groups. At 10 weeks after distraction epiphysiolysis, both the cell-free distraction group and the Cox-2 inhibitor group showed shortening, while the cell-treated group retained the length gained. The fate of transplanted chondrocytes used to elicit the repair of physeal separation site is unknown. Our hypothesis is that the injected cells attach to the bottom of the separated physis and grew or behaved as hypertrophic chondrocytes to delay premature physeal closure. The huge enlargement of the hypertrophic chondrocytes was like that after slow distraction (chondrodiasis), and 10 weeks after distraction epiphysiolysis, the thickness of the physis was restored to normal size. We believe that this proliferation of hypertrophic chondrocytes was derived partly from the implanted cells and partly from the tension effect on the physis.

Our results in this pilot study indicate that this technique has promise in allowing limb lengthening without premature physeal closure.

References