ISCHEMIC INJURY OF THE FEMORAL HEAD INDUCES APOPTOSIS

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Introduction: Ischemic necrosis of the femoral head is one of the most serious complications that can arise following injury or treatment of the pediatric hip. The hallmark of ischemic necrosis is cell death that occurs in the bone marrow and trabecular bone of the femoral head. Even though it is assumed that cells in the femoral head die by necrosis, studies performed in cardiac and neural tissues show that ischemic injury can induce cell death through the pathway of apoptosis, a programmed cell death. The mechanism of cell death following ischemic injury of femoral head has not been investigated. The purpose of this investigation was to determine whether apoptosis is one of the pathways of cell death that is induced in the femoral head following ischemic injury.

Methods: The study was approved by the Local Animal Care and Use Committee. The piglet model of ischemic necrosis of the femoral head, as described by Salter, was used in the study because it consistently produces ischemic necrosis and it is believed to be a clinically relevant animal model. Ischemic necrosis was induced in 14 piglets by placing a non-absorbable ligature tightly around the right femoral neck to disrupt the blood supply to the femoral head. The left side was not operated upon and was used as an internal control. Animals were sacrificed 2, 6, 7, 10, 14, 21, and 28 days after disrupting the blood supply to the femoral head. The femoral heads were obtained and bisected. One half of the femoral head was used to isolate genomic DNA from the ossification center of the femoral head using a Genomic DNA Purification Kit. The isolated genomic DNA was then assayed for the presence of nucleosomal ladders (generated during apoptosis) using a Ligation Mediated (LM)-PCR Ladder Assay Kit. Since LM-PCR produces semi-quantitative results, the femoral head samples were first subjected to 15 to 25 cycles of LM-PCR to determine which number of PCR cycles would be optimal for analysis. The LM-PCR products were then visualized by electrophoresis on agarose/ethidium bromide gels. The other half of the femoral head was fixed in formalin, decalcified in EDTA, embedded in paraffin, and sectioned for routine histologic staining (hematoxylin and eosin) and caspase-3 immunostaining.

Results: The operated femoral head samples clearly contained more nucleosomal ladders than the non-operated femoral head samples and the difference was most evident at 19 cycles. When all DNA samples were analyzed after 19 cycles of LM-PCR, a presence of nucleosomal ladders in the operated femoral head samples was clearly evident in the piglets sacrificed 6, 7, 10, and 14 days following the induction of ischemic injury (Fig. 1). In contrast, the non-operated femoral head samples either failed to amplify nucleosomal ladders at all or they were significantly underrepresented as compared to the operated samples. The femoral head samples from 2, 21, and 28 days following the induction of ischemic injury failed to amplify nucleosomal ladders in both the operated and the non-operated femoral head samples.

Discussion: In contrast to previous belief, our results clearly demonstrate that cell necrosis is not the sole mechanism of cell death following ischemic injury of the femoral head. Both apoptosis and necrosis seem to be involved. Our results open up the possibility of therapeutically targeting the apoptotic pathway to improve the outcome following ischemic injury of the femoral head. Further studies are required to define the specific cell types in the femoral head that die by apoptotic pathway.

Histologic assessment of H&E stained sections revealed morphologic evidence of cell death involving most hematopoietic cells in the bone marrow space on the operated side. The morphologic changes included shrunken cells, pyknosis, karyolysis, presence of ghost cells, and cell debris. Increased caspase-3 staining was observed in sections from the operated side in comparison to the non-operated side. Positive staining for caspase-3 was observed in bone marrow cells undergoing cell death but it was difficult to clearly identify their cell types.

Figure 1. A digital image of an agarose gel showing the products of LM-PCR analysis of DNA extracted from the ossification center of femoral heads 2, 6, 7, 10, 14, 21, and 28 days after the induction of ischemic injury. Days 2, 21, and 28 showed no nucleosomal ladders present on the right operated femoral head samples. The non-operated side lacked the presence of nucleosomal ladders at all time periods.

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