Activation of HIF-1 Pathway and Angiogenesis in the Cartilage Following the Induction of Ischemic Osteonecrosis

INTRODUCTION:
Osteonecrosis of the femoral head remains one of the most challenging conditions to treat in pediatric orthopaedics due to the lack of understanding of the biology of the disease and our inability to modulate the healing process. Understanding the repair response, including the angiogenic response or lack of it, may potentially yield new insight into the disease process and lead to the development of new treatment strategies. Although the role of HIF-1α as the central mediator of cellular response to hypoxia is well established, the role it plays following ischemic osteonecrosis of the femoral head has not been investigated. In addition to severe hypoxia or anoxia, an abrupt disruption of blood supply to the femoral head would produce rapid deficiencies of nutrients for cell function and survival, leading to extensive cell death in the ischemic region. Under these circumstances, it is unclear where and how rapidly the activation of HIF-1 pathway and the angiogenic response occur in the femoral head. In this study we propose a novel hypothesis that the induction of ischemic osteonecrosis will lead to activation of HIF-1 pathway in the cartilage surrounding the necrotic bone since the cartilage remains viable whereas the bone becomes necrotic, and that the cartilage plays an important role in initiating the revascularization process. A large animal model (piglet) of juvenile ischemic osteonecrosis was used to test the hypothesis.

METHODS:
This study was approved by our IACUC. A total of 52 immature pigs were used for the following studies. Right femoral neck was surgically ligated to induce femoral head ischemia. For controls, unoperated contralateral femoral heads were used. At 24 hrs following the induction of ischemia, hypoxprobe immunohistochemical (n=6) method was used to detect the cells subjected to severe hypoxia (PO2 <10mmHg). In addition, a chemical staining for lactate dehydrogenase (LDH) activity was performed on fresh tissue sections to determine the extent of cartilage and bone necrosis (n=6). LDH is an enzyme that is ubiquitously expressed by cells. A loss of its activity denotes a loss of cell viability. RNA (n=16) and protein (n=9) were isolated from bone and cartilage of the femoral heads at 24 hrs, 2 and 4 wks after the surgery to determine HIF-1α, VEGF, VEGFR2, and PECAM expression and HIF-1 protein levels using quantitative real-time PCR and western blot analysis, respectively. Angiogenesis was assessed at 4 wks using Scanco Micro-CT 35 scanner at the resolution of 30 microns/voxel following microfil infusion (n=8) and using quantitative histology (n=8) of transverse sections of the femoral heads. A paired t-test was used to compare the difference between the ischemic and the contralateral normal sides.

RESULTS:
Hypoxprobe staining revealed a presence of severe hypoxia in the entire bony epiphysis at 24 hrs. In the immature articular cartilage (epiphyseal cartilage), the hypoxprobe staining was present only in the deep layer of the cartilage. This layer consisted of proliferative and hypertrophic chondrocytes. Cell viability staining at 2 weeks revealed a loss of LDH activity in the bone and marrow cells of the entire bony epiphysis. In the immature cartilage, LDH activity was also absent in the deep layer, indicating cell death in the region. The pattern of staining was similar between the hypoxprobe staining and the loss of LDH staining.

DISCUSSION:
These results provide new insights into the ischemic damage and repair response following ischemic osteonecrosis of the immature femoral head. The study reveals that a complete disruption of the blood supply to the femoral head produces severe hypoxia and diffuse cell death in the bony epiphysis and the deep layer of the cartilage. The chondrocytes in the superficial layer of the cartilage remain viable and show a rapid increase in the HIF-1α protein level and VEGF mRNA expression. Subsequent rise in the PECAM expression and vessel number in the cartilage provides further support for the hypothesis that cartilage plays an active role in responding to the ischemic injury and initiating the revascularization of the immature femoral head.

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