Developing a Small Animal Trauma Model for Osteonecrosis of the Femoral Head

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INTRODUCTION: Osteonecrosis (necrosis of the bone) is a condition in which blood supply is inadequate for the needs of bone metabolism and femoral head is one of the regions mostly affected. Decreased blood flow to femoral head causes the disruption of the bone remodeling by disabling the osteogenic - angiogenic coupling. This uncoupling results in diminishment of femoral perfusion and concomitantly ends up with osteonecrosis of the femoral head (ONFH).

METHODS: 8 week old male C57BL/6J mice were gone under surgery by dislocating the left femoral head from hip joint and by doing a slight circular incision around the femoral neck to disturb the blood supply to femoral head. The remnant of ligamentum teres on the femoral head was cut to impair the possible regeneration. Right hip joint was left untreated as control. Animals (n=5 per time point) were sacrificed on certain time points as 2-3-4-6-8-10-12 weeks and safranin orange (SO) and hematoxylin eosin (HE) stainings were performed. Ex vivo μCT was taken at 2-3-4-6-8 and 12 weeks for defining the bone structural parameters. The immunohistochemical staining (IHCS) for CD31 (endothelial cell marker) and EMCN (with CD31 labels bone specific regenerative H type -CD31EMCN- vessels) were done to observe the changes in vascular structure. All animal procedures were conducted following the guidelines of the Regulations on Animal Experimentation at our institution.

RESULTS SECTION: Loss of trabecular bone is reported to be an indicator of osteonecrosis. Between 2nd and 8th weeks, a decrease in trabecular bone in the operated (left) side (ONFH) was observed with μCT (Figure 1A). Bone structural parameter analysis results quantitatively confirmed the BV/TV ratio was decreased in the ONFH side compared to control side starting from the 2nd week until 12th week. This difference between the ONFH side and Control side was significant by week 6 (Figure 1B). Bone marrow necrosis, which is defined by the eosinophilic staining, and presence of empty lacunae are the signs of osteonecrosis. HE staining showed the presence of empty lacunae in the ONFH side as early as 24 hours after the operation (Figure 2A, black arrows). By 6th week eosinophilic staining of the bone marrow and accumulation of bone marrow fat (BMF), which is a sign of oxidative stress and associated with weak bone mass, was observed (Figure 2B, red arrows). IHCS of the frozen sections by CD31 and EMCN showed the vascular invasion of epiphysis through the growth plate by H type vessels by week 4 in the ONFH side (white arrow heads) while the growth plate was observed to be intact in the control side by week 4 (Figure 3, white dots define the articular cartilage surface).

DISCUSSION: In the literature loss of trabecular bone, increased number of empty lacunae and bone marrow necrosis are reported as the signs of osteonecrosis. The bone fat imbalance is reported to be related with the presence of proinflammatory cytokines in increasing oxidative stress. The bone marrow fat is also stated to be related with the weak bone mass. In this study from 2 weeks to 8 weeks the loss of trabecular bone in ONFH was observed by μCT. By 24th hour empty lacunae were able to be observed in the ONFH side. By 4th week IHCS showed the invasion of the growth plate by the bone specific H type vessels which might be related by the increased oxidative stress which is related with HIF1a (a factor regulating the cellular response to hypoxia and an upstream regulator of VEGF). The increase of HIF1a might have increased the VEGF release, an angiogenic factor, that might have caused the invasion through the growth plate. Also, by 6th week the HE staining showed the presence of bone marrow necrosis and bone fat accumulation. Hence the results until now are supporting the development of necrosis in the ONFH side and it seems that we have succeeded to generate a trauma model for ONFH in mice. However there are some limitations to this study. First, we need to use higher number of mice to confirm the results and to observe the significance rate in bone parameters. Additionally, the HIF1a staining and hypoxia-related inflammatory factors should be observed. The vessel density difference between ONFH and Control sides should also be shown quantitatively.

SIGNIFICANCE/CLINICAL RELEVANCE: The action mechanisms and the molecular targets in ONFH are not well known worldwide and the present animal models for traumatic ONFH are not small animal models which makes researching the onsets of ONFH difficult. Developing a small animal surgery model specific for ONFH would help scientific world to study the mechanisms leading to ONFH and to find less invasive treatment strategies that are applicable in clinic before the disease progresses.

IMAGES AND TABLES:

Figure 1A

ONFH

2 weeks 3 weeks 4 weeks 6 weeks 8 weeks

Decrease in Trabecular bone

Figure 1B

BV/TV

Figure 2A

No Ope (control)

Figure 2B

ONFH

Control

Figure 3

Intact Growth Plate

Control

ONFH