Continuous Presence of both Insufficient Neovascularization and Elevated Vascular Permeability during Inadequate Repair of Steroid-associated Osteonecrosis Lesion in A Rabbit Model

Ge Zhang1, Hui Sheng1, Ling Qin1, Yixiang Wang2, David Yeung2, Simon Lee3, James Griffith3, Kwoksui Leung1
1Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong, China; 2Department of Organ & Imaging, The Chinese University of Hong Kong, Hong Kong, China; 3Lee Hysan Clinical Laboratory, The Chinese University of Hong Kong, Hong Kong, China
Lingqin@cuhk.edu.hk

Introduction: Subchondral collapse in steroid-associated osteonecrosis (ON) is induced by inadequate repair, that is, bone resorption that exceeds bone formation, which remains unclear in vascular pathophysiology. We have accordingly formulated our hypothesis that local intraosseous vasculature might be abnormal during inadequate repair of steroid-associated osteonecrosis lesion. The present study was to examine both bio-imaging-based function-architecture-morphology characteristics of local vasculature and underlying cell-molecule events involved in angiogenesis during inadequate repair of steroid-associated ON lesion.

Materials and Methods: Fifty-six 28-week old male New-Zealand white rabbits received induction protocol for establishment of steroid-associated ON. At 0, 1, 2, 4 and 6 weeks after induction, in vivo Dynamic MRI was performed on bilateral proximal femurs for vascularization index and permeability index, and bone marrow aspiration from iliac crest was also conducted for determining each compartment activity of marrow stem-cell-pool by in vitro culture of granulocyte-macrophage progenitor cells (haematopoietic compartment) and fibroblast colony-forming units (mesenchymal compartment). After finishing Dynamic MRI scan and bone marrow aspiration, 8, 8, 16 and 16 rabbits were euthanized at 0, 1, 2, 4 and 6 weeks post induction, respectively. Bilateral proximal femurs were dissected for the following evaluation on intraosseous vasculature: three-dimensional architecture by Micro-CT-based micro-angiography, two-dimensional micro-morphometry by Optical Microscopy and two-dimensional ultra-morphology of micro-vessel endothelium by Transmission Electron Microscopy. Local vascular endothelial growth factor A (VEGF-A) expression at mRNA and protein level was examined by Reverse Transcription-Polymerase Chain Reaction and immunohistochemistry, respectively. Type of osteonecrotic lesion repair was classified by two blinded pathologists for consensus.

Results: Intravascular thrombosis, foci edema and marrow-cell loss were found at 1 week after induction (8/8). ON lesion formation was found at 2 weeks after induction (7/8). Rabbits with dominant destructive repair (DR Positive) were identified at 4 weeks (5/16) and 6 weeks (6/16) after induction, whereas rabbits with dominant reparative bone apposition (DR Negative) were also identified at 4 weeks (6/16) and 6 weeks (8/16) after induction.

For MRI-derived vascular function indexes, either a significant decrease from baseline in vascularization index or a significant increase from baseline in permeability index was found with same extent in both the DR Positive rabbits and DR Negative rabbits at 1 week after induction. Then, a significant increase over baseline for either vascularization index or permeability index was found with different extent in the DR Positive rabbits and DR Negative rabbits at 2 weeks after induction, respectively. Thereafter, a gradual change toward baseline was found for both the two indexes in the DR Negative rabbits, whereas an almost constant level for both the two indexes was still preserved in the DR Positive rabbits. The vascularization index was significantly higher in the DR Negative rabbits than that in the DR Positive rabbits from 2 weeks to 6 weeks after induction, whereas the permeability index was significantly lower in the DR Negative rabbits than that in the DR Positive rabbits from 2 weeks to 6 weeks after induction.

For Micro-CT-based angiography, disseminated angiographic substance by leaking was found more in the DR Positive rabbits than that in the DR Negative rabbits, whereas newly formed vasculature was found less in the DR Positive rabbits than that in the DR Negative rabbits (Figure 1).

For Optical Microscopy based micro-morphometry and Transmission Electron Microscopy based ultra-morphology, Micro-vessel Density was found significantly higher in the DR Negative rabbits than that in the DR Positive rabbits, whereas extensive edema, many leakage radiopaque particles by perfusion for angiography and broadened inter-endothelial junction were found dominant in the DR Positive rabbits compared to the DR Negative rabbits throughout the experimental period.

For compartment activity indexes of marrow stem-cell-pool, a significant decrease from baseline in either the two indexes was found with different extent in the DR Positive rabbits and DR Negative rabbits at 1 week after induction, respectively. Then, a significant increase from 1-week-level in either the two indexes was found with different extent in the DR Positive rabbits and DR Negative rabbits at 2 weeks after induction, respectively. Thereafter, the two activity indexes were both recovered toward baseline in the DR Negative rabbits, whereas an almost unchanged level was still maintained for both the two activity indexes in the DR Positive rabbits. From 1 week to 6 weeks after induction, the two compartment activity indexes were significantly higher in the DR Negative rabbits than those in the DR Positive rabbits.

For local VEGF-A expression, continuously increased expression from baseline at both mRNA and protein level were found from 1 week to 2 weeks after induction. Thereafter, local VEGF-A expression at the two levels were both unchanged toward baseline in the DR Negative rabbits, whereas continuously maintained high expression (i.e. uncontrolled expression) were still found at both the two levels in the DR Positive rabbits.

Discussion: Local vasculature during inadequate repair of steroid-associated ON lesion may be characterized as both insufficient neovascularization and elevated permeability. The underlying mechanistic events might be involved in decreased marrow stem-cell-pool activity and subsequent uncontrolled VEGF-A expression.