Remodeling phenotype of macrophages in steroid-induced osteonecrosis: switching from M1 to M2 polarization

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Osteonecrosis, a common orthopaedic disease, is a worldwide medical problem need to be conquered. Over the past decades, major advances have occurred in the pathophysiological mechanisms of osteonecrosis, however, its etiology is still complex and not clear. A number of different theories have been developed to explain the pathogenic mechanisms of steroid-induced osteonecrosis, including Microcirculation embolism and bone marrow cell hypertrophy induced by thrombus, fat, abnormal red blood cells, nitrogen, etc. Among them, theory of fat metabolism disorder is generally recognized among the kinds of theories of steroid-induced osteonecrosis. However, the pathogenesis of femoral head osteonecrosis very complicated, and it’s difficult to be explained using the single theory. The polarization of Macrophages are involved in many important pathophysiologic processes, such as necrosis and regeneration. Our hypothesis is that M1-polarised Macrophages appear to be disruptive in the early and middle stage of steroid-induced osteonecrosis of femoral head (SANFH), while M2-polarised Macrophages appear to be protective in the late stage, which play an important role in developing SANFH. Our hypothesis could help to explain the pathogenic mechanism of steroid-induced osteonecrosis, and deserves further studies.


Introduction

Osteonecrosis of the femoral, known as "coronary heart disease of the hip", is a worldwide medical problem need to be conquered. The natural history and disease progression of osteonecrosis includes two aspects, namely progressive collapse of femoral head and secondary osteoarthritis of the hip joint. According to the etiology, osteonecrosis of femoral head are divided into two types, traumatic and nontraumatic. Due to the misuse of corticosteroid, the incidence rate of steroid-induced osteonecrosis of femoral head (SANFH) has taken the first place of nontraumatic osteonecrosis. In SANFH, adipogenic differentiation of bone marrow progenitor cell, proliferation and hypertrophy of adipocytes in proximal femur of SANFH, accompanied by intramedullary pressure increase, which oppress the bone marrow vascular structures, resulting in circulatory disturbance, reduced blood flow, lack of oxygen and edema of bone tissues, osteocyte cell death and hypoxia. Because bone is a closed chamber, increase pressure in bone marrow induced by tissue edema in a vicious cycle, which will lead to ischemia and necrosis of osteocytes [1].

Steroid-induced osteonecrosis (SANFH) develops through four stages, which can be identified by symptoms and imageological examination. The relative pathological characteristics of the four stages are: Stage I, the early stage of ischemia are evidenced only in morphological changes in the marrow, such medullary cavity hematopoiesis, plasma leakage, interstitial edema, and so on. Stage II,
necrotic bone absorption (bone nuclei disappeared, empty lacunae appeared) and early repair reaction. A large number of inflammatory cells (like Macrophages) infiltrate into the necrotic area, where the necrotic trabecular bone was gradually absorbed and replaced by new bone. By the time of stage III, it is characterized by bone tissue repair and reconstruction, involving an appositional bone formation phase, followed by a resorptive phase (Macrophages play an important role in the stage). The incorporation process is termed "creeping substitution", within a large number of osteoblasts irregularly distributed in the necrotic trabecular bone surface. Stage IV: At this stage, the articular cartilage is destroyed, the necrotic bone is removed and replaced by new bone, and the bone begins to collapse, involving the vicious cycle “dead - repair - collapse”. Previous studies have confirmed the appositional bone formation on top of dead bone surface. Stage IV: At this stage, the articular cartilage is destroyed, the necrotic bone is removed and replaced by new bone, and the bone begins to collapse, involving the vicious cycle “dead - repair - collapse”. Previous studies have confirmed the appositional bone formation on top of dead trabecular bone during the repair process, consistent with the increased bone mass [2, 3].

SANFH involves a series of pathophysiological reactions, such as enhanced adipogenesis, decreased lipid transfer, and accumulated adipocytes, resulting in avascular necrosis of bone tissue. Steroid intervention could down-regulate the gene expression of osteoblast differentiation, while the gene expression of adipocytes differentiation are up-regulated, leading to enhanced adipogenic differentiation and inhibited osteogenic differentiation; therefore, necrotic bone couldn't be repaired effectively, femoral head collapsed, gradually aggravated pathological changes, eventually forming of osteonecrosis [4]. Zhang et al. [5] found that high level of adipogenic-positive clones (index of osteogenic differentiation potential) occurred during the process of glucocorticoid-induced avascular necrosis, suggesting that excessive adipogenic differentiation play an important role in the development of the disease. Yeh, etc. [6] confirmed that hormone could decrease the related gene and protein expression of osteogenesis and increase the gene and protein expression of adipogenesis. Increased adipogenic differentiation generates a large number of adipocytes in the bone marrow; meanwhile, decreased osteoblastic differentiation leads to fewer new bone formation, which will eventually deteriorate, collapse the femoral head [7]. Additionally, disorder of lipid metabolic and impairment of lipid transport associated within the process of steroid-induced osteonecrosis, thus, Lipid Removal Agent (LRA) could exert preventive effects for osteonecrosis [8]. In conclusion, during the progression of osteonecrosis, intensified adipogenic differentiation, weakened lipid transport and abnormal accumulation of adipocytes arouse a series of pathophysiological reactions, leading to Ischemic bone necrosis (avascular necrosis).

Adipocyte hyperplasia and hypertrophy, macrophage infiltration occur in bone marrow of SANFH. During the progress of the osteonecrosis, adipocytes accumulate constantly, inflammatory response becomes worse, thus more macrophages infiltrate into the diseased area. Direct or indirect interactions between macrophages and adipocytes may impair the microcirculation of the femoral head, lead to tissue ischemia, hypoxia, edema, a vicious circle of high intramedullary high pressure, resulting in ischemia and necrosis of femoral head. The pathogenesis of femoral head osteonecrosis very complicated, and it’s difficult to be explained using a single theory. Researchers have developed a number of different theories to explain the pathogenic mechanisms of osteonecrosis, including Microcirculation embolism and bone marrow cell hypertrophy induced by thrombus, fat, abnormal red blood cells, nitrogen, etc. At present, theory of fat metabolism disorder is generally recognized among the kinds of theories of steroid-induced osteonecrosis. However, the theory could not explain why some patients develops to SANFH but some patients don’t with the same usage of steroid, why some of necrotic type are diffused necrosis and some of them arelocalized necrosis? The occurrence of steroid-induced osteonecrosis may be related to other potential systems.

Hypothesis

Our hypothesis is that M1-polarised Macrophages appear to be disruptive in the early and middle stage of steroid-induced osteonecrosis of femoral head (SANFH), while M2-polarised Macrophages appear to be protective in the late stage, which play an important role in developing SANFH.
Macrophages can be activated by a wide variety of stimuli, including IL-4, IL-13, IL-1β, lipopolysaccharide (LPS), interferon-gamma (IFN-gamma), transforming growth factor-β (TGF-β), glucocorticoids, bacterial endotoxins, and so on \[12, 13\]. Activated macrophage can be broadly classified in two main types: classically activated macrophages (M1) and alternatively activated macrophages (M2). According to this classification, M1 macrophages acquire M1 phenotype after activation by IFNγ or LPS, whereas macrophages acquire M2 following stimulation with IL-4 or IL-13. M1 macrophages secrete high levels of proinflammatory cytokines (e.g. tumor necrosis factor (TNF-α), IL-6, IL-1β), and generate reactive nitrogen and oxygen intermediates \[14, 15\]. Conversely, M2 macrophages secrete anti-inflammatory cytokines (e.g. IL-10, TGF-β, IL-4), which play an essential role in the resolution of inflammation response. The balance of M1/M2 macrophage activity is suggested to play a significant role in human physiology and pathology.

In the course of osteonecrosis, it’s characterized by an inflammatory process. Tumor necrosis factor alpha (TNF-α), secreted by both adipocytes and macrophages, which could promote the two types of programmed cell death: apoptosis and death. SANFH is associated with the infiltration of macrophages into the necrotic area of femoral head, which may contribute to an elevated inflammatory status by secreting a variety of pro-inflammatory factors. Appearance of pycnotic nuclei, increase of empty bone lacuna, replacement of adipocytes in bone marrow are the early pathological changes of osteonecrosis; while necrotic bone marrow and bone cells are filled with hypertrophic adipocytes. Along with the deposition of adipocytes and increase in cell size, dysfunctional adipocytes will release some inflammatory factors (such as TNF-α and IL-6). Inflammation factors attract macrophages continuously gathering into the necrotic area; on the other hand, Macrophages themselves will release more inflammatory factors, such as TNF-α, IL-6, IL-12, and so on \[16\]. Then, a paracrine loop between adipocytes and macrophages will be established through the secretion of TNF-α, aggravating inflammatory changes of adipose tissue through the up-regulated expression of MCP-1and TNF-α, and down-regulated expression of adiponectin \[17\]. TNF-α combined to the surface receptors of hypertrophic adipocytes, through dependent NF-kappa B signaling pathway and MAPK signal pathway, promote the expression of pro-inflammatory factors and disintegration of adipocytes \[18\]. Both clinical and animal experiments have confirmed that, during the process of steroid-induced osteonecrosis, serum concentration of TNF alpha elevated, and the expression of TNF-α in bone marrow of femoral head increased significantly \[19, 20\].

Actually, TNF-α is mainly secreted by macrophages; during the process of osteonecrosis, macrophages differentiate into different functional types of cells. M1-polarized macrophages secrete higher levels of pro-inflammatory genes and possibly contribute to steroid-induced inflammation and osteonecrosis. Classically activated M1 macrophages, driven by TNF-α secreted from enlarged adipocytes is thought to be the primary trigger for the recruitment \[11\]. Besides, MCP-1, IL-6 and adiponectin play important roles through other pathways than the paracrine loop. After stimulation with proinflammatory cytokine, Monocyte chemotactic protein-1 (MCP-1) was produced by preadipocytes and endothelial cells, which would recruit monocytes into the adipose tissue and stimulate the differentiation of M1 macrophages \[21, 22\]. The increase in the expression of MCP-1 will lead an influx of monocytes and macrophages \[23\]. In addition, both adipocytes and osteocytes are derived from bone marrow stromal cells, which are capable of transdifferentiating into each other under certain conditions. Therefore, it makes good sense that macrophages play a very important role in the development of osteonecrosis through the secretion of TNF-α. We hypothesized that steroid intervention would result in increased macrophages infiltration and a shift to an M1 macrophage polarization as TNF-α expression in the early and middle stage of SANFH.

Changes in cell polarity of macrophages will influence the pathological development of osteonecrosis. If the polarization of M1/M2 macrophages is switched to M1 type (classically activation), inflammatory response will be strengthened, resulting the promoted development of osteonecrosis. In addition, macrophages could also suppress the adipogenic differentiation of stromal cells, which will have the effect of limiting fat tissue expansion \[24, 25\]. If the polarization is switched to M2 type (Alternative activation), macrophages will play an extremely important role in inhibiting bone necrosis and promoting tissue repair. Therefore, macrophages are closely associated with the development of steroid-induced osteonecrosis. The increase in adipose tissue mass and adipocyte hypertrophy in the femoral head of SANFH. Increased adiposity and proinflammatory signals promotes the recruitment of M1-polarized monocytes, which could eventually overwhelm the protective effect of M2 macrophages, leading to a dominant role for M1-polarized, with high secretion of TNF-α, IL-6, and NO \[26\]. Tissue repair and cell proliferation can be linked to alternatively activated macrophages (M2 type). If M2 macrophages (alternatively activation) possess mainly anti-inflammatory properties, the switch in macrophage phenotype from M1 to M2 may be partially responsible for the suppression of inflammatory gene expression, leading to impaired bone repair and regeneration.
Therefore, it can be used to explain why the regenerative repair reaction in the necrotic region of the femoral head appeared in the late stages of osteonecrosis.

In addition, the peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors, among them, PPAR-\(\gamma\) plays an important role in adipogenic differentiation of adipocytes. Previous study indicated PPAR-\(\gamma\) activation resulted in a reduction of MCP-1 [27]. In the early-stage of SANFH, the gene expression of PPAR-\(\gamma\) increases significantly, highly related to the proliferation of adipogenic-positive clones [28]. Besides, the role in adipocyte differentiation during early adipogenesis, PPAR-\(\gamma\) also exhibits potent anti-inflammatory activity. The activation of PPAR-\(\gamma\) in local environment might induce a switch from M1- toward M2-activated macrophages, which will play a crucial role in initiating bone repair and regeneration in the necrotic area in the late stage of SANFH.

Therefore, it makes good sense of the phenotypic switch of macrophage polarization from M1 to M2 during the development of SANFH. Classically activation (M1) and regulation of macrophages will be the new regulatory target for the treatment of osteonecrosis. Through the further study the molecular mechanism for activation and regulation of macrophages, the pathogenic mechanism of steroid-induced osteonecrosis will be explored, and new pathways for clinical treatment and drug screening will be opened.

Conflicts of interest statement

None declared.

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References


