Oxidative Stress In The Corticosteroid-induced Osteonecrosis Of The Femoral Head Rat Model

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Disclosures:

Introduction: Non-traumatic osteonecrosis of the femoral head (ONFH) is believed to be a multifactorial disease with high-dose corticosteroid therapy and alcohol abuse considered to be major risk factors. However, the pathogenesis of ONFH still remains unclear. It has been reported that oxidative stress induced by Buthionine sulfoximine (BSO) led to ONFH in Wistar rats, and that the concentration of glutathione in the liver, as an index of oxidative stress, decreased within 24 hours after a single BSO administration and that this decrease induced ONFH (1). On the other hand, we previously reported that corticosteroid treatment after a lipopolysaccharide (LPS), a toll-like receptor (TLR) 4 ligand, injection induces ONFH in Wistar rats, and suggested that the TLR4 signaling pathway may play a role in the pathogenesis of ONFH in rats (2). Furthermore, it has been reported that innate immune signaling via TLR contributes to the pathogenesis of autoimmune diseases, and that organ damage observed in patients with systemic lupus erythematosus is induced by proinflammatory responses and oxidative stress, and that the activation of TLR4 signaling contributes to development of oxidative stress (3). Therefore, we hypothesized that corticosteroid treatment after the injection of LPS would lead to oxidative stress, resulting in ONFH. To verify this hypothesis, the incidence of ONFH and oxidative stress in a rat model were examined after the administration of a TLR4 ligand and methylprednisolone.

Methods: All experiments were performed in accordance with the guidelines of the Ministry of Sports, Culture, Science, and Technology, Japan. They also followed protocols were approved by the Animal Care and Use Committee, Sapporo Medical University School of Medicine (Approval Number: 11-042). Male wistar rats (n = 70) were divided into four groups and treated as follows: the Saline+Saline group (n = 10) were given saline (1.0 ml/kg) subcutaneously on Day 1 and saline (1.0 ml/kg) intramuscularly on Day 2; the Saline+MPSL group (n = 20) were given saline (1.0 ml/kg) subcutaneously on Day 1 and 20 mg/kg methylprednisolone intramuscularly on Day 2; the LPS+Saline group (n = 20) were given 1.0 mg/kg LPS, a ligand for TLR4, intravenously on Day 1 and saline (1.0 ml/kg) intramuscularly on Day 2; and the LPS+MPSL group (n = 20) were given 1.0 mg/kg LPS intravenously on Day 1 and 20 mg/kg methylprednisolone intramuscularly on Day 2. All injections were performed at 7:00 p.m.

Animals were sacrificed 1 or 14 days after the last injection. The femurs and liver were harvested and stored at -84°C until analysis. Portions of the femur at 14 days after the last injection were harvested and fixed in a 10% formalin-0.1 M phosphate buffer (pH 7.4). All the injections were performed at 7:00 p.m. Histopathological and glutathione peroxidase assay were performed.

Results: Osteonecrosis of the femoral head was observed in zero of 10 Saline+MPSL, 1 of 10 LPS+Saline, and 5 of 10 LPS+MPSL group rats (Figure 1). The incidence of ONFH in the LPS+MPSL group was significantly greater than that in the Saline+MPSL group (P < 0.05; Fisher’s exact test). Glutathione peroxidase activity in the liver was significantly increased in the LPS+Saline group compared to the other groups (Figure 2). In the LPS+MPSL group, glutathione peroxidase activity was significantly reduced in comparison to the LPS+Saline group (Figure 2). On the other hand, glutathione peroxidase activity in the bone including femoral head and femoral shaft did not change any intergroup (Figure 2).

Discussion: In the liver, GPx activity was significantly higher in the LPS+Saline group than in the Saline+Saline and Saline+MPSL groups, nevertheless ONFH was only observed in one of 10 rats in the LPS+Saline group. In the LPS+MPSL group, in which 50% of rats developed ONFH, GPx activity was significantly lower compared to that in the LPS+Saline group. No significant intergroup differences in GPx activity in the epiphysis and diaphysis. These findings suggested that corticosteroid treatment attenuated the proinflammatory response-induced oxidative stress via TLR.

There have been some reports of a relationship between osteonecrosis and oxidative stress. Mikami et al. and Kuribayashi et al. reported that co-treatment with Vitamin E as an antioxidant prevents corticosteroid-induced osteonecrosis in a rabbit model treated with corticosteroids alone (4, 5). A characteristic of the rabbit model is that osteonecrosis develops in the metaphysis not in the femoral head after the treatment of healthy rabbits with corticosteroids. In contrast, we recently reported that co-treatment with a corticosteroid and N-acetyl-cysteine as an antioxidant exacerbated the incidence of corticosteroid-induced ONFH in rats treated with LPS and a corticosteroid (6). This discrepancy in results may be due to effects of the corticosteroid on the underlying status, either non-inflammation or inflammation in the same way as the following reports; it has been reported...
that corticosteroid suppress oxidative stress through the reduction of proinflammatory response (7). On the other hand, it has been also reported that corticosteroid induced oxidative stress in non-inflammatory conditions (8). The present study shows that GPx activity did not contribute to the development of corticosteroid-induced ONFH in rats treated with corticosteroid and TLR4 ligand.

**Significance:** This study provides that GPx activity did not contribute to the development of corticosteroid-induced ONFH in rats treated with corticosteroid and TLR4 ligand.

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**References:**
Figure 1