Concise report

Gender difference in the development of steroid-induced osteonecrosis in rabbits

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Abstract

Objective. To investigate the incidence of steroid-induced osteonecrosis (ON) among male and female rabbits.

Methods. Forty-seven adult rabbits (male, n = 24; female, n = 23) were injected once intramuscularly into the right gluteus medius muscle with 20 mg/kg of methylprednisolone acetate. Haematological examinations were performed just before and at 1 and 2 weeks after the steroid injection. Two weeks after the injection, both femora and humeri were histopathologically examined for the presence of ON, and the bone marrow fat cells were examined morphologically.

Results. Sixteen of 24 male rabbits (66.7%) and 5 of 23 female rabbits (21.7%) developed ON. There was a significant difference in the rate of incidence of ON between male and female rabbits (P = 0.0032). Haematologically, at 1 week after the steroid injection, both the mean low-density lipoprotein (LDL) and the ratio of LDL cholesterol to high-density lipoprotein cholesterol in the male rabbits were significantly higher than those in the female rabbits (P = 0.0001 for both comparisons). The bone marrow fat cells of the male rabbits [61.5 (5.6) μm] were significantly larger than those of the female rabbits [58.9 (3.7) μm; P = 0.0102].

Conclusion. This study indicates that gender may be an important factor in considering the pathogenesis of steroid-induced ON.

Key words: Osteonecrosis, Gender, Corticosteroid, Animal model.

Introduction

Osteonecrosis (ON) is known to occur in patients who have received steroids for the treatment of underlying diseases such as SLE, nephrotic syndrome and renal transplantation [1]. The natural history of ON generally involves a progressive collapse that often results in secondary degenerative OA [1]. To treat this condition, prosthetic replacement is one of the surgical options. However, a higher rate of early failure has been reported in younger ON patients [1, 2]. Thus, there is a need for the development of preventative methods, which requires that the pathogenesis of ON be clarified.

The precise aetiology of ON remains unclear. Several possible factors in the pathogenesis of ON have been suggested based on human and animal studies, including coagulation abnormalities, hyperlipidaemia and oxidative stress [3–6]. For the prevention of ON, a recent study reported that treatment with both anti-coagulants and lipid-lowering agents prevented the development of ON in rabbits more effectively than either treatment alone [7]. To our knowledge, there have been no studies assessing the effect of gender on the development of steroid-induced ON in rabbits. In this experimental study, we investigated the development of steroid-induced ON in male and female rabbits.

Materials and methods

We utilized a rabbit model of steroid-induced ON for this study [4]. All experiments were conducted in accordance...
with the Guidelines for Animal Experiments of Kyushu University, Japanese Law (No. 105) and notification No. 6 of the Government of Japan.

Animals
We studied 47 adult (defined as animals with closed growth plate; male, \( n = 24 \); female, \( n = 23 \)) Japanese white rabbits (Kyudo, Tosu, Japan), ranging in age from 28 to 32 weeks. Animals were housed at the Animal Center of Kyushu University and maintained on a standard diet and water.

Treatment
The rabbits were injected once with 20 mg/kg body weight of methylprednisolone acetate (MPSL; Upjohn, Tokyo, Japan) intramuscularly into the right gluteus medius muscle before the start of the investigation (Week 0) [4]. Two weeks after the MPSL injection, the rabbits were sacrificed and tissue specimens were prepared as described [4].

Evaluation of ON
The diagnosis of ON was determined at 2 weeks after steroid administration, a time point that has been reported to be critical in the development of ON [4, 5, 7]. The complete areas of the proximal one-third and distal condyle of both the femora and humeri (eight regions) were examined histologically for the presence of ON. Diagnosis of ON was made blindly by four authors (S.I., T.Y., K.N. and G.M.) [4, 5, 7] on the basis of the presence of diffuse empty lacunae or pyknotic nuclei of osteocytes within the bone trabeculae, accompanied by surrounding bone marrow cell necrosis (Fig. 1). If the diagnoses differed between the four investigators, a consensus was reached by discussion of the histological findings without knowledge of the group from which the sample was obtained. Rabbits with at least one ON lesion among the eight areas examined were considered to have ON.

Calculation of the size of bone marrow fat cells
We calculated the size of bone marrow fat cells as the average of the maximal diameters of 100 fat cells in randomly selected fields (1 field = \( 4 \times 10^{-8} \text{ m}^2 \)) from viable areas, using NIH Image software (Bethesda, MD, USA), as previously described [5, 7]. The repeatability of this method was confirmed in a previous study [5, 7].

Results

Prevalence of ON
The incidence of ON in the male rabbits was 66.7% (16 of 24), whereas that in the female rabbits was 21.7% (5 of 23). There was a significant difference in the rate of incidence of ON between male and female rabbits (\( P = 0.0032 \); Fig. 2A). Histological appearance of ON was similar in male and female rabbits. In the metaphysis and diaphysis of both genders, yellowish areas were observed in which an accumulation of bone marrow cell debris was seen and the bone trabeculae showed empty lacunae (Fig. 1).

Sizes of bone marrow fat cells
The bone marrow fat cells of the male rabbits [61.5 (5.6) \( \mu \text{m} \)] were significantly larger than those of the female rabbits [58.9 (3.7) \( \mu \text{m} \); \( P = 0.0102 \)]. Among male rabbits, the average size of bone marrow fat cells was significantly larger in rabbits with ON [62.9 (5.0) \( \mu \text{m} \)] than in those without [58.8 (5.9) \( \mu \text{m} \); \( P = 0.0058 \)]. Similarly, among female rabbits, the average size of bone marrow fat cells was significantly larger in rabbits with ON [62.2 (4.0) \( \mu \text{m} \)] than in those without [57.9 (3.0) \( \mu \text{m} \); \( P = 0.0218 \)]. There was no significant difference in the size of bone marrow fat cells between ON-positive male and female rabbits (\( P = 0.9772 \)).

Laboratory data examination

\textit{LDL}. The levels of plasma LDL between male and female rabbits had a significant interaction with gender (\( P = 0.0069 \); Fig. 2B). The levels of LDL in male rabbits at 1 week were significantly higher than those in female rabbits (\( P = 0.0001 \)). Among male rabbits, plasma LDL levels were significantly increased at 1 (\( P = 0.0001 \)) and 2 weeks (\( P = 0.0001 \)), relative to Week 0. In female rabbits, no significant difference in LDL plasma level was observed at 1 week (\( P = 0.4370 \)), but levels were significantly increased at 2 weeks (\( P = 0.0200 \)) relative to Week 0.

\textit{LDL: HDL cholesterol ratio}. The ratio of LDL cholesterol to HDL cholesterol in male and female rabbits had no

Statistical analysis
Data were expressed as the mean (s.d.). The numbers of ON-positive male and female rabbits were compared using Fisher’s exact probability test. The sizes of bone marrow fat cells of male and female rabbits were compared using the unpaired t-test and one-way analysis of variance (ANOVA) with Scheffe’s post hoc test. Haematological data from male and female rabbits was analysed for interaction with gender by repeated-measures ANOVA. Data from each group at 0, 1 and 2 weeks were analysed by Dunnett-type multiple comparison. Data obtained at each point were compared using the unpaired t-test. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, Tokyo, Japan). \( P < 0.05 \) was considered statistically significant.
significant interaction with gender ($P = 0.3330$; Fig. 2C). Plasma LDL: HDL cholesterol ratio in male rabbits at 1 week was significantly higher than that in female rabbits ($P = 0.0001$). There were no significant differences in plasma triglyceride or VLDL levels between male and female rabbits.

### Discussion

The association of SLE with ON is well known. The prevalence of ON in SLE patients has been reported to range from 4 to 40% [1]. In addition, some studies regarding the incidence of ON in male SLE patients have reported a much higher incidence of ON (75–100%) relative to female patients (30–43%) [9, 10]. This finding may indicate that male patients are more vulnerable to steroid-induced ON.

Several mechanisms have been implicated in the pathogenesis of ON. Hyperlipidaemia has been identified as a possible contributor to ON [3–5, 7, 8]. Jaffe et al. [11] first suggested that steroid-induced hyperlipidaemia could increase the amount of fat within the femoral head, elevate intracortical pressure and lead to sinusoidal collapse. Wang et al. [12] have undertaken studies to show how altered lipid metabolism might lead to ON. In one such report, the adipocytes within the femoral heads of steroid-treated rabbits had a 25% greater increase in fat content relative to untreated rabbits [12]. Dexamethasone reportedly stimulates the differentiation of bone marrow stromal cells into adipocytes as well as the accumulation of fat in the marrow at the expense of type-1 collagen and OC mRNA expression [13]. On the other hand, a higher LDL: HDL cholesterol ratio apparently reflects increased lipid transport to the peripheral tissue, a potential risk factor for corticosteroid-induced ON in rabbits [8]. Our recent studies in the prevention of steroid-induced ON demonstrated the correlation between LDL: HDL ratio and the prevalence of ON [7, 14]. In the present study, 1 week after the MPSL injection, the plasma LDL: HDL cholesterol ratios of the female rabbits were significantly lower than those of the male rabbits. In addition, the bone marrow fat cells of the female rabbits were significantly smaller on average than those of the male rabbits. Therefore, we speculate that the gender differences on the development of ON might be partly explained by a gender difference in lipid deposition in bone marrow fat cells.

In human studies, although females tend to have more favourable lipid profiles than males from 20 to 50 years of age, cardiovascular disease (CVD) risk increases in females after the onset of menopause, as well as with age in both females and males [15]. Among females, CVD death rates after menopause are two to three times higher than females of the same age before menopause [15]. A previous study showed that basal release of nitric oxide is greater from endothelium-intact aortic rings of female rabbits than from those of males; ovariectomy diminished circulating oestriadiol concentration and basal release of nitric oxide to the levels seen in male rabbits [16]. These data suggest that basal nitric oxide release from endothelium-intact aortic rings depends on circulating oestriadiol concentration, which could explain the protective effect of oestriadiol against the development of atherosclerosis. A recent study in an animal model suggested a possible relationship between the development of ON and oxidative stress caused by steroid administration that ultimately results in damage to the vascular endothelial cells [6].

Cytochrome P450 3A is the major drug-metabolizing enzyme in the gastrointestinal tract and liver [17–19]. The activity of cytochrome P450 3A is highly variable, and may influence responses to half of all oxidatively metabolized drugs, as well as endogenous substances such as corticosteroids. Recently, the activity of hepatic cytochrome P450 3A4, which metabolizes corticosteroids, was suggested to be associated with the development of ON [19]. Masada et al. [19] revealed an inverse relationship between hepatic cytochrome P450 3A activity and the prevalence of ON in rabbits, suggesting the possibility that development of ON may be prevented by reducing corticosteroid doses in patients with low hepatic
Several studies have reported gender differences in cytochrome P450 3A activity [17, 18]. Zhu et al. [18] investigated cytochrome P450 3A activity in 202 healthy subjects (104 men and 98 women) by measuring the plasma 1-hydroxymidazolam:midazolam ratio 1 h after oral administration of 7.5 mg midazolam. Their results suggest that cytochrome P450 3A activity in women was significantly higher than in men. In addition, our preliminary evaluation of the activity of cytochrome P450 3A in rabbits also showed similar findings to those in a previous report [18]. These findings could account for the gender difference in the development of ON in our study. However, other reports have not found gender differences in cytochrome P450 3A4-mediated drug metabolism [17]. Additional studies are required to clarify the mechanisms underlying the gender differences in cytochrome P450 3A activity in rabbits with ON induced by corticosteroid administration.

The major limitation of this study is that only a few serum markers were investigated. In both male and female rabbits, other risk factor markers such as coagulation and apolipoprotein as well as the activity of cytochrome P450 3A should be monitored in future studies [7, 19, 20]. We believe that gender differences are critical to the understanding of the pathogenesis of steroid-induced ON.

Rheumatology key messages

- The incidence of ON in male rabbits was significantly higher than that in female rabbits.
- Gender differences may be one of the important factors in considering the pathogenesis of steroid-induced ON.

Acknowledgements

Funding: This work was supported in part by a Grant-in-Aid from the Japan Society for the Promotion of Science (No. 21591948, 211160), Research Grant for Intractable Diseases from the Ministry of Health and Welfare of Japan and a grant from Takeda Science Foundation.

Disclosure statement: The authors have declared no conflicts of interest.

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