Gender Difference in the Cytochrome P4503A Activity and its Correlation with the Development of Osteonecrosis in Steroid-treated Rabbits

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INTRODUCTION
Cytochrome P4503A is the major drug metabolizing enzyme in the gastrointestinal tract and liver. The activity of cytochrome P4503A is highly variable, and may influence responses to half of all oxidatively metabolized drugs, as well as endogenous substances such as corticosteroids. The low cytochrome P4503A activity leads to a remarkable increase of corticosteroid levels and its effects. Therefore, cytochrome P4503A activity, which metabolizes corticosteroids, was suggested to be associated with the development of osteonecrosis (ON) (1). In both human and animal studies, there have been reports that cytochrome P4503A activity in female was higher than that in male, which relates to the effects of gender-specific factors, such as testosterone or estradiol (2). However, there have been no studies assessing the gender difference in the cytochrome P4503A activity in steroid-induced ON models.

We therefore evaluated i) cytochrome P4503A activity and ii) the levels of estradiol in male and female rabbits, and iii) whether these factors related to the gender difference in the development of ON.

METHODS
Animals. We studied 40 (male, n = 20; female, n = 20) Japanese white rabbits (Kyudo, Tous, Japan), ranging in age from 30 to 32 weeks. Growth plate closure was confirmed histologically when they were sacrificed. 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) (3). Two weeks after MPSL injection, rabbits were sacrificed and tissue specimens were prepared as described (4).

Evaluation of ON. The diagnosis of ON was determined at 2 weeks after the steroid injection. Rabbits were sacrificed, and the 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.

Laboratory data examination. We collected blood samples from the auricular arteries while the animals were in a fasting state. Regarding the cytochrome P4503A activity, Midazolam (MDZ) and 1′-hydroxymidazolam (1′-OH-MDZ) concentrations were determined at two points: just before (week 0) and 48 hours after the MPSL injection. After an interval of 60 minutes from the administration of estradiol at 1 week significantly decreased in comparison to those before the steroid injection (*P = 0.044).

RESULTS
Prevalence of ON. The incidence of ON in the male rabbits was 75% (15 of 20), while that in the female rabbits was 30% (6 of 20). There was a significant difference in the rate of incidence of ON between male and female rabbits (*P = 0.010).

Cytochrome P4503A activity. (Fig. 1). The mean 1′-OH-MDZ:MDZ ratio in female rabbits at just before the steroid injection (0 week), as well as that at 48 hours after the steroid injection was significantly higher than those in male rabbits (*P = 0.039 and *P = 0.001, respectively). In addition, the mean 1′-OH-MDZ:MDZ ratio in female rabbits significantly increased at 48 hours after the steroid injection (*P = 0.044), while that in male rabbits did not so (*P = 0.978). The 1′-OH-MDZ:MDZ ratio in ON-negative rabbits at 0 week (0.073 ± 0.054), as well as that at 48 hours (0.082 ± 0.063) were higher than those in ON-positive rabbits (0 week; 0.047 ± 0.027, 48 hours; 0.054 ± 0.022).

Laboratory data examination. Estradiol (Fig. 2A). The levels of serum estradiol between male and female rabbits had a significant interaction with gender (*P = 0.008). In male rabbits, the levels of estradiol at 1 week significantly decreased in comparison to those before the steroid injection (*P = 0.009).

LDL:HDL cholesterol ratio (Fig. 2B). The ratio of LDL cholesterol to HDL cholesterol in male and female rabbits had no significant interaction with gender (*P = 0.333). Serum LDL:HDL cholesterol ratio in male rabbits at 1 week was significantly higher than that in female rabbits (*P = 0.025).

DISCUSSION
Masada et al. reported that the rate of incidence of ON in rabbits treated with cytochrome P4503A inducer (phenobarbital (33%)) was significantly lower than that in rabbits treated with cytochrome P4503A inhibitor (itraconazole) (100%) or that in control rabbits (83%) (1). We speculate that the gender differences in the development of ON might be partly explained by a gender difference in the cytochrome P4503A activity.

Chou CP et al. compared the cytochrome P450 in intact female rats and that in ovariolectomized (OVX) female rats (5). Cytochrome P450 activity as well as serum estradiol levels in OVX female rats were lower than those in intact female rats. In addition, cytochrome P450 activity can be regulated by the effects of estradiol, both before and after the steroid injection.

SIGNIFICANCE
Gender difference in the cytochrome P450 activity may be one of the important factors in considering the gender difference in the development of steroid-induced ON in rabbits.

REFERENCES