INTRODUCTION:
Steroid-induced osteonecrosis (ON) is known to occur in patients who received corticosteroids for the treatment of such underlying diseases as systemic lupus erythematosus, nephrotic syndrome and after renal transplantation. Since patients with steroid-induced ON are generally young-aged, the ultimate goal is the prevention of the development of ON.

Several possible factors in the pathogenesis of ON have been suggested based on both human and animal studies, including coagulation abnormalities and hyperlipidemia. Human studies demonstrate that vascular occlusion may occur as a result of mechanical interruption by thrombi or lipid emboli in nutrient vessels. In a rabbit model of ON, hyperlipidemia associated with abnormal thrombophilic coagulopathy and bone marrow fat-cell packing was linked to the development of ON.

Recently, the effect of either anticoagulants or lipid-lowering agents alone on the prevention of ON has been assessed. In this study, we tested the hypothesis that combined treatment with both an anticoagulant and a lipid-lowering agent should prevent ON development in rabbits more effectively than either treatment alone.

MATERIALS AND METHODS:
We utilized a rabbit model of steroid-induced ON in this study. This experiment was reviewed by the Committee of the Ethics on Animal Experiment in Faculty of Medicine, Kyushu University.

Ninety-one adult male Japanese rabbits, weighing 3.5-4.2 kg, were intramuscularly injected once with 20 mg/kg of methylprednisolone acetate into the right glutus medius muscle. These rabbits were divided into four groups, warfarin plus probucol treatment group (WP group, n=21), probucol alone (PR group, n=29), warfarin alone (WA group, n=21), and non-prophylactic treatment (NP group, n=20).

Warfarin potassium, 1.5 mg/kg body weight per day, was orally administered once daily for 3 weeks from 2 weeks before the corticosteroid injection till 2 weeks after the injection. Probucol, 300 mg/kg body weight per day, was orally administered once daily for 4 weeks from 2 weeks before the corticosteroid injection till 2 weeks after the injection. Two weeks after the corticosteroid injection, both femora and humeri were histopathologically examined for the presence of ON; the sizes of the bone marrow fat cells were examined morphologically, as previously described.

Blood samples were collected from all rabbits equally through the auricular arteries in a fasting state in the early morning immediately before the corticosteroid injection (0 weeks), and 1 and 2 weeks before and after the injection.

RESULTS:
Incidence of ON was 1/21 (5%), 11/29 (37%), 7/21 (33%), and 14/20 (70%) in the WP, PR, WA, and NP groups, respectively (Figure 1). The incidences of ON in the WP, PR, and WA groups were significantly lower than that observed in the NP group (p<0.0001, p<0.05, and p<0.05, respectively). The incidences of ON in the PR and WA groups were significantly higher than that observed in the WP group (p<0.01 and p<0.05, respectively) (Figure 1).

The average sizes of bone marrow fat cells were significantly smaller in the WP group (53.5 ± 4.1 μm) than those in the NP group (60.0 ± 4.0 μm) (p<0.01). The size of bone marrow fat cells in the PR group (52.0 ± 5.0 μm) was also significantly smaller than that in either the NP (p<0.01) or WA groups (56.9 ± 4.5 μm, p<0.05). There were no significant differences in the sizes of bone marrow fat cells between the WP and PR groups.

The levels of PT-INR in both the WP and WA groups were significantly higher (p<0.01, p<0.05, respectively) than those observed in either the NP or PR group throughout the experimental period. The LDL levels in the WP group remained at significantly lower levels during the study than the NP or WA groups (p<0.05, Figure 2B).

DISCUSSION:
Our current study showed that, in a rabbit model of steroid-induced ON, a combination treatment with warfarin plus probucol significantly prevented the development of ON. This experimental study suggests that both a hypercoagulable state and abnormal lipid metabolisms in the early period after the corticosteroid injection seem to play important roles for developing steroid-induced ON.

Figure 1. A combination treatment with warfarin plus probucol significantly prevented the development of ON.

Figure 2A. The levels of PT-INR in both the WP and WA groups were significantly higher than those in both NP and PR groups.

Figure 2B. The levels of LDL in both the WP and PR group were significantly lower than those in both NP and WA groups.

REFERENCES: