

Risk Factors for Developing Osteonecrosis After Prophylaxis in Steroid-treated Rabbits

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ABSTRACT. *Objective.* Both abnormal lipid metabolisms and coagulopathy have been suggested to be associated with the development of steroid-induced osteonecrosis (ON). We examined plasma risk factors for development of steroid-induced ON in rabbits after prophylaxis with a lipid-lowering agent and/or an anticoagulant.

Methods. Seventy adult male rabbits were injected intramuscularly once with 20 mg/kg methylprednisolone acetate. Fifty-five rabbits received prophylaxis with probucol (a lipid-lowering agent; n = 20) or warfarin (an anticoagulant; n = 14) or both (n = 21). Probuco and warfarin were administered beginning 1 to 2 weeks prior to steroid injection. Two weeks after steroid injection, the bilateral femora and humeri were examined histopathologically for the presence of ON. Based on a logistic regression model, laboratory variables before steroid injection were assessed to determine whether they demonstrated any association with the risk of ON.

Results. Twenty-one rabbits developed ON. In the univariate analyses, significant positive associations were observed between plasma concentrations of triglyceride and low-density lipoprotein and the risk of development of ON. In the multivariate model, only the plasma triglyceride level suggested a positive association. Even after adjusting for probucol and warfarin use, the plasma triglyceride level was still suggested to be a predictor for development of ON. Rabbits with higher baseline triglyceride levels had a more pronounced triglyceride increase in their response to steroids.

Conclusion. Our study suggests that, after prophylaxis with probucol and/or warfarin, plasma triglyceride level is associated with the development of steroid-induced ON in rabbits. (First Release Nov 1 2008; J Rheumatol 2008;35:2391–4; doi:10.3899/jrheum.080416)

Key Indexing Terms:

OSTEONECROSIS

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PROPHYLAXIS

RISK ANALYSIS

Osteonecrosis (ON) of the femoral head is a relatively common disease and accounts for over 10% of the 500,000 total hip arthroplasty procedures performed annually in the United States¹. ON often occurs in young patients who have received steroids for the treatment of underlying diseases, such as systemic lupus erythematosus and renal transplantation². The precise pathomechanisms of steroid-induced ON are unknown; however, several possible mechanisms have been proposed to explain how steroids may lead to the development of ON. These mechanisms involve abnormalities in lipid metabolisms and coagulopathy^{3–7}.

Recently, a lipid-lowering drug and an anticoagulant

have been considered to provide therapeutic benefits for the prevention of development of ON in subjects treated with steroids. In a rabbit ON model, both probucol and warfarin suppressed the incidence of ON⁸. In humans, statin was found to be useful in decreasing the incidence of ON⁹. These findings suggest that both abnormal lipid metabolisms and coagulopathy may play important roles in the development of steroid-induced ON.

The aim of our study was to identify plasma risk factors for the development of steroid-induced ON in rabbits after prophylaxis with a lipid-lowering agent and/or an anticoagulant.

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MATERIALS AND METHODS

We utilized a rabbit model of steroid-induced ON⁶. Details of the animals and methods were as described⁶. Briefly, adult (defined as animals with closed growth plate) male Japanese white rabbits were injected once with 20 mg/kg body weight of methylprednisolone acetate (which corresponds to steroid pulse therapy in the human) intramuscularly into the right gluteus medius muscle.

Rabbits and prophylactic regimen. We examined 70 rabbits in this study. Fifty-five of 70 rabbits received prophylaxis with probucol (a lipid-lowering agent; n = 20) or warfarin (an anticoagulant; n = 14) or both (n = 21). Probuco (300 mg/kg/day; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) was given orally for 4 weeks, beginning 2 weeks before the steroid injection.

tion⁸. Warfarin (1.5 mg/kg/day; Eisai Co., Ltd., Tokyo, Japan) was administered orally for 3 weeks, beginning 1 week before the injection⁸. Body weights of the rabbits were measured prior to each experiment (3.5–4.2 kg) and 2 weeks after the steroid injection. Two weeks after steroid injection, the femora and humeri were histopathologically examined for the presence of ON.

Laboratory data collection. Before and 2 weeks after the steroid injection, fasting blood samples were collected from the auricular arteries in all rabbits. Laboratory data on plasma concentrations of cholesterol, triglyceride, free fatty acid, low density lipoprotein (LDL), very low density lipoprotein, and platelets were examined.

Statistical analysis. Univariate and multivariate analyses were performed to assess the relationship between the development of ON and laboratory variables before steroid injection using a logistic regression model. The laboratory variables were categorized into 3 levels (tertiles) based on the continuous distribution of the ON-negative rabbits' data. For example, plasma triglyceride levels were divided into the following 3 categories — levels < 19 mg/dl, levels between 19 and 25.9 mg/dl, and levels > 26 mg/dl. The odds ratios (OR) associated with the tertiles of laboratory variables were estimated using the lowest level as the reference category. The corresponding 95% confidence intervals (95% CI) were derived, and the linear trends were tested for significance.

To construct a multivariate model, laboratory variables were selected as follows: (1) variables found to be statistically significant according to the crude OR; and (2) variables that were biologically considered to possibly be associated with the development of ON whether the crude OR was statistically significant or not. Finally, analyses were conducted for selected variables and 2 prophylactic drugs (probucole and warfarin). Statistical analyses were conducted using SAS, Version 8.2 (SAS, Cary, NC, USA).

RESULTS

In this study, 6 of the 70 rabbits died. Five of the rabbits with warfarin died of bleeding from the site of blood sampling. One rabbit with probucole died of pneumonia. After exclusion of these 6, 64 rabbits were examined. Twenty-one of the 64 rabbits developed ON (Figure 1), including 6 (32%) receiving probucole, 3 (33%) with warfarin, one (5%) with both prophylactic drugs, and 11 rabbits (73%) with no prophylactic treatment. Before the steroid injection, the mean

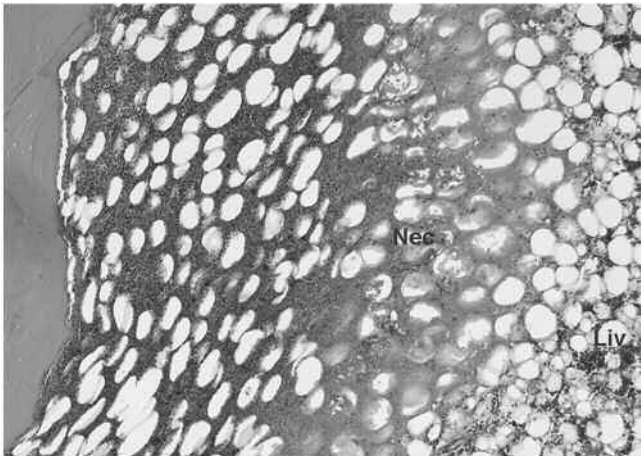


Figure 1. Histological features of osteonecrosis in a steroid-treated rabbit. Bone trabeculae show empty lacunae with surrounding necrotic bone marrow cell debris (H&E stain, original magnification $\times 40$). Nec: osteonecrotic bone marrow; Liv: living bone marrow.

body weight was 3778 g and 3748 g in rabbits with ON and those without ON, respectively.

In the univariate analyses (Table 1), significant positive associations were seen between plasma levels of triglyceride and the LDL and the risk of ON development (p for trend = 0.012 and 0.041, respectively). There were no associations between the other variables and the risk of development of ON.

When plasma levels of triglyceride, LDL, platelets, and free fatty acid were entered into a multivariate model, only plasma level of triglyceride suggested a positive association with the risk of development of ON (p for trend = 0.064), while the initial associations with the other variables were attenuated (Table 2). The highest category of plasma triglyceride level (> 26.0 mg/dl) suggested an association with a 6.4-fold increase in the risk of development of ON (95% CI 0.83, 49.19; Table 2).

We included the plasma level of triglyceride and the 2 prophylactic drugs (probucole and warfarin) in the same model, a model that was also controlled for other laboratory variables, including the plasma levels of LDL, platelets, and free fatty acid. As a result, plasma level of triglyceride was suggested to be a predictor for the development of ON (Table 2). Regarding the plasma triglyceride level, the odds ratio for the highest category (> 26.0 mg/dl) was 8.20 (95% CI 0.87, 77.63), and the trend test also suggested a positive

Table 1. Plasma risk factors associated with development of steroid-induced osteonecrosis in rabbits based on univariate logistic regression analyses.

Variables	ON Development, %	Crude OR (95% CI)	p trend
Cholesterol, mg/dl			
< 16	40	1	
16.0–19.9	19	0.35 (0.07–1.62)	0.850
20.0+	36	0.83 (0.26–2.72)	
Triglyceride, mg/dl			
< 19	12	1	
19.0–25.9	24	2.31 (0.36–14.72)	0.012
26.0+	48	7.00 (1.35–36.28)	
Free fatty acid, mEq/l			
< 0.2	50	1	
0.20–0.29	28	0.39 (0.11–1.44)	0.215
0.30+	28	0.39 (0.09–1.67)	
Low-density lipoprotein, mg/dl			
< 5	12	1	
5.0–22.9	38	4.62 (0.83–25.73)	0.041
23.0+	44	5.89 (1.11–31.41)	
Very low-density lipoprotein, mg/dl			
< 4	50	1	
4.0–4.9	25	0.33 (0.09–1.22)	0.392
5.0+	35	0.55 (0.14–2.20)	
Platelets, $10^4/\mu\text{l}$			
< 27.1	46	1	
27.10–32.9	17	0.24 (0.05–1.04)	0.234
33.0+	30	0.51 (0.15–1.77)	

Table 1. Plasma risk factors associated with development of steroid-induced osteonecrosis in rabbits based on univariate logistic regression analyses.

Variables	Model Adjusting for Other Laboratory Variables		Model Also Adjusting for Prophylactic Drugs	
	OR (95% CI)	p trend	OR (95% CI)	p trend
Triglyceride, mg/dl				
< 19	1		1	
19.0–25.9	2.93 (0.33–26.11)	0.064	3.42 (0.30–38.64)	0.063
26.0+	6.40 (0.83–49.19)		8.20 (0.87–77.63)	
Free fatty acid, mEq/l				
< 0.2	1		1	
0.20–0.29	0.46 (0.09–2.36)	0.354	1.26 (0.17–9.22)	0.734
0.30+	0.41 (0.06–3.01)		1.56 (0.12–20.40)	
Low-density lipoprotein, mg/dl				
< 5	1		1	
5.0–22.9	3.43 (0.36–32.58)	0.813	2.33 (0.15–35.33)	0.507
23.0+	1.89 (0.19–18.75)		0.67 (0.05–9.08)	
Platelets, 10 ⁴ /μl				
< 27.1	1		1	
27.10–32.9	0.14 (0.02–0.86)	0.407	0.14 (0.02–1.26)	0.653
33.0+	0.81 (0.14–4.64)		4.94 (0.33–73.49)	
Probucol			0.09 (0.01–0.55)	
Warfarin			0.07 (0.01–0.92)	

association with the risk of ON development ($p = 0.063$). The odds ratios for probucol use and warfarin use were 0.07 (95% CI 0.01, 0.92) and 0.09 (95% CI 0.01, 0.55), respectively (Table 2), thus indicating that both probucol and warfarin significantly decreased the risk of development of ON.

We also investigated whether the sequential change in the plasma triglyceride level after steroid treatment differed between rabbits with plasma triglyceride level > 26.0 mg/dl before steroid treatment ($n = 29$) and those with a triglyceride level < 26.0 mg/dl ($n = 35$), and found a significant difference between the groups, as assessed by a repeated measure analysis of variance ($p < 0.01$; Figure 2).

DISCUSSION

In our analyses, the plasma triglyceride level before steroid injection was strongly suggested to be a predictor for the development of ON after prophylaxis with probucol and/or warfarin in rabbits. In addition, we found that rabbits with higher baseline triglyceride levels had a more pronounced triglyceride increase in their response to steroids.

Our results suggest that, in normolipidemic rabbits, the plasma triglyceride level before steroid injection may be associated with the risk of development of ON. However, it remains unknown whether or not hypertriglyceridemia before steroid injection is a risk factor for ON. Steroids are known to induce transient hypertriglyceridemia in normolipidemic subjects^{6,10}. On the other hand, to our knowledge, whether steroids increase the level of plasma triglyceride in subjects with hypertriglyceridemia remains unclear.

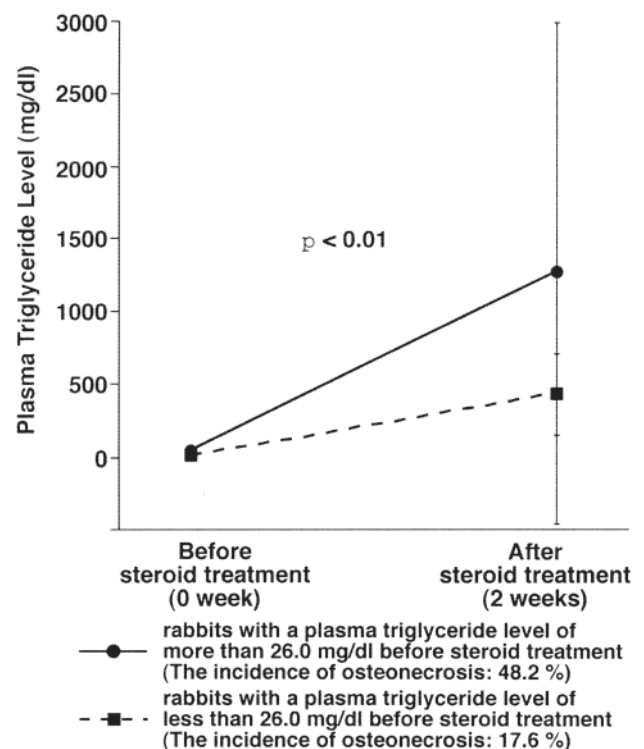


Figure 2. Plasma triglyceride levels after steroid treatment in rabbits with a plasma triglyceride level > 26.0 mg/dl before steroid treatment remained at significantly higher levels than those observed in rabbits with a plasma triglyceride level < 26.0 mg/dl throughout the 2-week period after steroid treatment.

Regarding the association between acute alcohol consumption and hypertriglyceridemia, Pownall, *et al* reported that triglyceride concentration increased only 3% in patients with hypertriglyceridemia, but 53% in normolipidemic subjects¹¹, suggesting that the triglyceride response in patients with hypertriglyceridemia may be different from that in normolipidemic subjects. We consider that our findings cannot be applied directly to subjects with hypertriglyceridemia before steroid therapy.

Hypertriglyceridemia itself has been considered to cause several conditions that are closely related to the postulated risk factors for the development of ON, such as thrombosis¹², alterations in fibrinolytic activity¹³, and endothelial dysfunction¹⁴. However, based on analyses of laboratory data, some studies have shown hypertriglyceridemia was associated with ON^{15,16}, while others did not^{17,18}. These facts suggest that only post-steroid hypertriglyceridemia may not be a decisive factor for the development of ON. We suppose that the plasma triglyceride level before steroid injection may be a determinant of the post-steroid plasma triglyceride level, in which the triglyceride response to steroid administration may play an important role in the development of ON after prophylaxis.

The development of effective prophylactic regimens against ON represents a significant advance in patients who have received steroid. We found that the plasma triglyceride level before steroid treatment may be associated with the risk for developing ON after prophylaxis. Further investigations into the role of triglycerides in the pathomechanisms of ON may help us to develop a better prophylaxis regimen against ON.

REFERENCES

1. Mankin HJ. Nontraumatic necrosis of bone (osteonecrosis). *N Engl J Med* 1992;326:1473-9.
2. Assouline-Dayana Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and natural history of osteonecrosis. *Semin Arthritis Rheum* 2002;32:94-124.
3. Fisher DE. The role of fat embolism in the etiology of corticosteroid-induced avascular necrosis: clinical and experimental results. *Clin Orthop* 1978;130:68-80.
4. Boskey AL, Raggio CL, Bullough PG, Kinnett JG. Changes in the bone tissue lipids in persons with steroid- and alcohol-induced osteonecrosis. *Clin Orthop* 1983;172:289-95.
5. Jones JP Jr. Intravascular coagulation and osteonecrosis. *Clin Orthop Relat Res* 1992;277:41-53.
6. Yamamoto T, Irisa T, Sugioka Y, Sueishi K. Effects of pulse methylprednisolone on bone and marrow tissues: corticosteroid-induced osteonecrosis in rabbits. *Arthritis Rheum* 1997;40:2055-64.
7. Miyanishi K, Yamamoto T, Irisa T, Noguchi Y, Sugioka Y, Iwamoto Y. Increased level of apolipoprotein B/apolipoprotein A1 ratio as a potential risk for osteonecrosis. *Ann Rheum Dis* 1999;58:514-6.
8. Motomura G, Yamamoto T, Miyanishi K, Jingushi S, Iwamoto Y. Combined effects of an anticoagulant and a lipid-lowering agent on the prevention of steroid-induced osteonecrosis in rabbits. *Arthritis Rheum* 2004;50:3387-91.
9. Pritchett JW. Statin therapy decreases the risk of osteonecrosis in patients receiving steroids. *Clin Orthop Relat Res* 2001;386:173-8.
10. Bagdade JD, Yee E, Albers J, Pykalisto OJ. Glucocorticoids and triglyceride transport: effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat. *Metabolism* 1976;25:533-42.
11. Pownall HJ, Ballantyne CM, Kimball KT, Simpson SL, Yeshurun D, Gotto AM Jr. Effect of moderate alcohol consumption on hypertriglyceridemia: a study in the fasting state. *Arch Intern Med* 1999;159:981-7.
12. De Curtis A, D'Adamo MC, Amore C, et al. Experimental arterial thrombosis in genetically or diet induced hyperlipidemia in rats — role of vitamin K-dependent clotting factors and prevention by low-intensity oral anticoagulation. *Thromb Haemost* 2001;86:1440-8.
13. Hiraga T, Shimada M, Tsukada T, Murase T. Hypertriglyceridemia, but not hypercholesterolemia, is associated with the alterations of fibrinolytic system. *Horm Metab Res* 1996;28:603-6.
14. Bae JH, Bassenge E, Kim KB, et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 2001;155:517-23.
15. Mielants H, Veys EM, DeBussere A, van der Jeught J. Avascular necrosis and its relation to lipid and purine metabolism. *J Rheumatol* 1975;2:430-6.
16. Scribner AN, Troia-Cancio PV, Cox BA, et al. Osteonecrosis in HIV: a case-control study. *J Acquir Immune Defic Syndr* 2000;25:19-25.
17. Gladman DD, Urowitz MB, Chaudhry-Ahluwalia V, Hallet DC, Cook RJ. Predictive factors for symptomatic osteonecrosis in patients with systemic lupus erythematosus. *J Rheumatol* 2001;28:761-5.
18. Nagasawa K, Ishii Y, Mayumi T, et al. Avascular necrosis of bone in systemic lupus erythematosus: possible role of haemostatic abnormalities. *Ann Rheum Dis* 1989;48:672-6.