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N. Ogawa
I. Koyama
T. Shibata
T. Watanabe
N. Akimoto
Y. Taguchi
N. Shinozuka
R. Omoto

Pravastatin prevents the progression of accelerated coronary artery disease after heart transplantation in a rabbit model

N. Ogawa · I. Koyama (✉) · T. Shibata · T. Watanabe · N. Akimoto · Y. Taguchi · N. Shinozuka · R. Omoto
Department of Surgery,
Saitama Medical School, 38,
Morohongo, Moroyama-machi, Iruma-gun,
Saitama, Japan, 350-04

Abstract This study was designed to investigate the effects of pravastatin (Pr) on accelerated coronary arteriosclerosis in transplanted hearts. The rabbit hearts were transplanted to the recipients' neck heterotopically, and received FK506. The rabbits in group 1 were fed a normal diet (ND), and cholesterol-rich diet (CD) in group 2 and 3. Pr (10 mg/kg) was given to group 3. They were sacrificed at 4 weeks and the severity of myocardial rejection and arteriosclerosis was assessed and scored histologically. The serum lipid levels were significantly elevated by a CD. However, addition of Pr had no effect on the levels of LDL and total cholesterol (TC). There was no significant difference in myocardial rejection in each group. Transplanted hearts in group 2 showed more severe arteriosclerotic lesions than those in group 1. Pr treatment in group 3 diminished the severity of coronary arteriosclerosis. Pr may prevent the accelerated coronary arteriosclerosis after heart transplantation without significant changes in TC and LDL.

Key words Heart transplantation · Coronary arteriosclerosis · Pravastatin · FK506

Introduction

The accelerated coronary arteriosclerosis after heart transplantation [7] has been one of the great problems determining long term prognosis [2]. It has been suggested that coronary arteriosclerosis in transplanted hearts may be caused by chronic rejection of the vascular type, but cytomegalovirus infection [8], hypercholesterolemia [6], and toxicity of immunosuppressants [7] have been also considered as other causes. We reported that hypercholesterolemia produced by a cholesterol-rich diet enhanced the progression of coronary arteriosclerosis after heart transplantation [13]. Moreover, it has been reported that coronary arteriosclerosis after heart transplantation was inhibited by the administration of anti-hyperlipidemics such as fish oil [12] and HMG-CoA reductase inhibitor [10]. In this experiment, we studied the effect of the HMG-CoA reductase inhibitor, pravastatin (Pr), on coronary arteriosclerosis after heart transplantation under hyperlipidemia.

Materials and methods

Japanese white male rabbits weighing 3–4 kg were used. After intramuscular injection of ketamine hydrochloride (10 mg/kg) and intravenous injection of sodium pentobarbital (10 mg/kg), thoracotomy of the donors was carried out under anesthesia maintained by 1.5 % halothane. The hearts were excised after cardiac arrest by infusion of cold cardioplegia having added 1000 units of heparin, and were immersed in cold cardioplegic solution. The left carotid artery and vein of the recipients wee exposed and anastomosed to the ascending aorta and pulmonary artery of the grafts, respectively [4]. The total ischemic time of the graft was approximately 60 min. After heart transplantation, the immunosuppressant, FK506 (0.3 mg/kg per day, i.m.), was administered to all rabbits. The antihyperlipidemic, Pr (10 mg/kg per day), was administered orally. Rabbits were fed with a 1 % cholesterol diet (CD) or a normal diet (ND). Both transplanted and native hearts were excised after sacrifice at 4 weeks and were fixed in 10 % buffered formalin solution for histological evaluation. Total cholesterol levels (TC) and LDL-cholesterol levels (LDL) in samples were determined.

Experimental groups

Recipients were classified into the following three groups: group 1, ND only ($n = 4$), group 2, CD only ($n = 6$), and group 3, CD + Pr ($n = 7$).

Histological staining methods

Four-micron-thick paraffin sections were stained with hematoxylin and eosin (H&E) and Elastica van Gieson (EVG). Also, the ABC method with monoclonal mouse anti-rabbit macrophage (DAKO) or anti-mouse smooth muscle actin antibody (ZYMED) was used for immunohistochemical staining.

Pathological evaluation method

The severity of myocardial rejection after heart transplantation was evaluated by 7 grades of 0, 1A, 1B, 2, 3A, 3B, and 4, and scored to 0.0, 1.0, 1.5, 2, 3.0, 3.5, and 4, respectively, according to the standardized cardiac biopsy grading [3].

All coronary arteries more than 50 μm in diameter were light microscopically studied. According to the references of the standard of Stanford University [12], the severity of arteriosclerosis was judged by the degree of intimal thickening and scored from 0 to 6 points. The mean value of the intimal thickening per blood vessel was used for comparison.

Statistical procedure

The assay of the statistically significant differences was performed by Student's *t*-test, and the probability rate of the significant difference was defined as < 0.05.

Results

Measurement of serum lipid

The serum lipid level in group 2 was significantly elevated by a 1 % cholesterol diet (TC 1275 ± 51), compared with that in group 1 with a normal diet (TC 50 ± 2, LDL 3.3 ± 6). However, the decrease in serum lipid level was not found in group 3 (TC 1327 ± 354, LDL 1297 ± 9) which was administered Pr as an antihyperlipidemic (Table 1).

Severity of myocardial rejection in transplanted hearts

The pulsation in all transplanted hearts was observed until sacrifice. The severity of the myocardial rejection was 1.0 ± 0.0 in group 1, 2.5 ± 1.56 in group 2, and 1.64 ± 0.37 in group 3. There was no significant difference in the myocardial rejection in each group.

Table 1 Serum cholesterol levels after 4 weeks. Data are expressed as mean ± SD (*ND* normal diet, *CD* cholesterol-rich diet, *Pr* pravastatin, *TC* total cholesterol, *LDL* LDL-cholesterol)

Group	n	TC (mg/dl)	LDL (mg/dl)	
1	ND	4	50 ± 32	3.3 ± 2.6
2	CD	6	1275 ± 960*	1260 ± 951*
3	CD + Pr	7	1327 ± 354*	1297 ± 49*

* $P < 0.05$ vs group 1

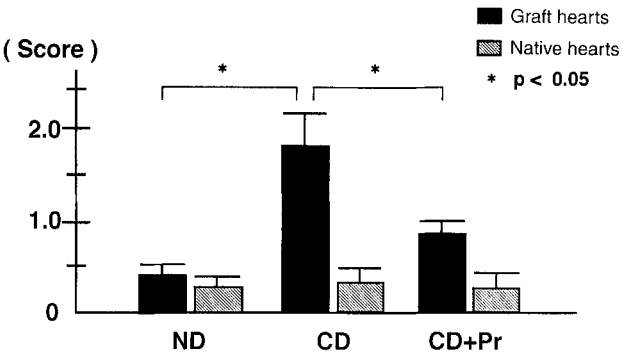


Fig. 1 Coronary arteriosclerosis in grafted and native hearts (*ND* normal diet, *CD* 1 % cholesterol diet, *CD + Pr* 1 % cholesterol diet and added pravastatin)

Severity of coronary arteriosclerosis

The score of the coronary intimal thickening in transplanted hearts in group 1 fed a normal diet was 0.43 ± 0.15. A cholesterol-rich diet enhanced the intimal thickening, which was found in group 2 (1.78 ± 0.45). The severity of coronary intimal thickening was significantly decreased in group 3 (0.80 ± 0.32), suggesting that pravastatin would inhibit the progression of the arteriosclerosis in transplanted hearts (Fig. 1). Moreover, coronary intimal thickening in native hearts in each group was minimum (0.34 ± 0.13 in group 1, 0.36 ± 0.16 in group 2, and 0.25 ± 0.18 in group), indicating that the influence of the hyperlipdemia or pravastatin was not found in native hearts without immunological injury.

When coronary arteries in transplanted hearts were observed light microscopically, foam cells or fibrous thickening was found in the arterial intima (Fig. 2). On the other hand, macrophages and smooth muscle cells in the thickened intima were observed by immunohistochemical stainings with the anti-smooth muscle cell actin and anti-macrophage antibodies (Fig. 3).

Discussion

Various factors are proposed to accelerate coronary arteriosclerosis in transplanted hearts. Hyperlipidemia is considered to be one of these factors. Alonso et al. [1]

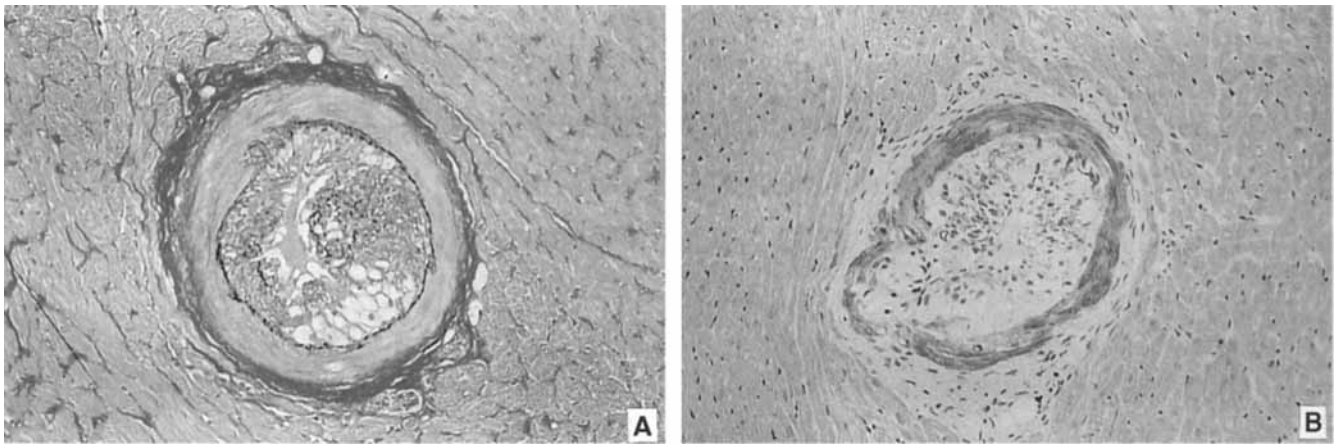


Fig. 2 **A** An intramyocardial branch of the transplanted heart, group 2. The intima is remarkably thickened with numerous foam cells (scored as 6). Elastica van Gieson (EVG) original magnification × 100. **B** Similar artery as in **A** with immunohistochemical staining. It shows the smooth muscle cells stained with anti-actin antibody. There are numerous positive cells in the thickened intima. Anti-actin antibody stain, original magnification × 100

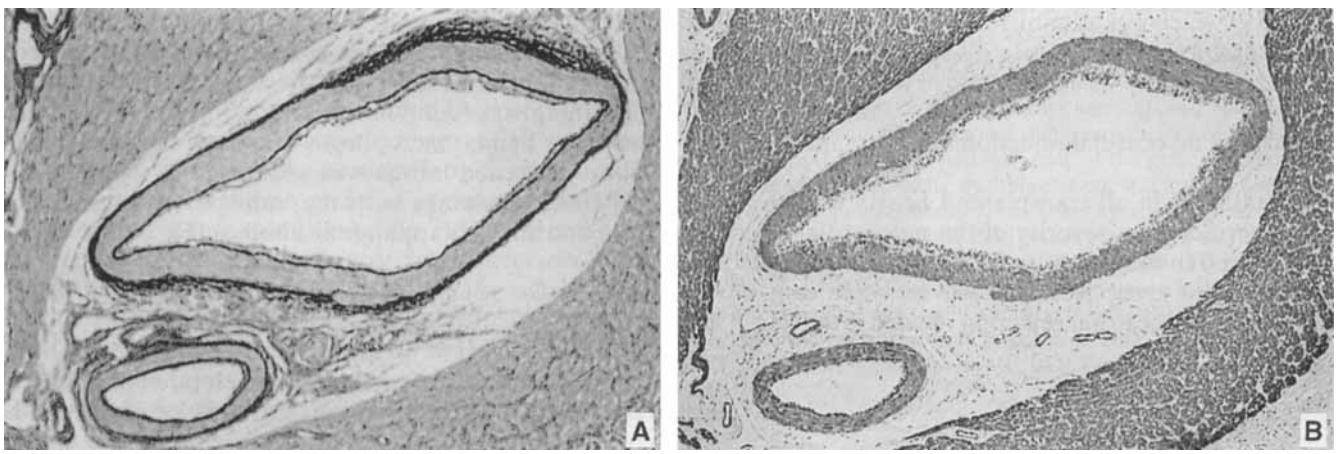
reported that coronary arteriosclerosis in transplanted hearts was rapidly accelerated by the combination of hyperlipidemia and immunological injury. We also reported previously [13] that hyperlipidemia in a heart-transplanted model is an increasing factor in intimal thickening in the coronary artery.

This study was undertaken on the assumption that accelerated coronary arteriosclerosis in transplanted hearts could be inhibited by antihyperlipidemic drugs.

Fig. 3 **A** Main branch of a transplanted heart, group 3. The intima shows slight and patchy thickening (scored as 1). EVG, original magnification × 40. **B** Similar artery as in **A** with immunohistochemical staining with anti-actin antibody. There are positive cells in the thickened intima. Anti-actin antibody stain, original magnification × 40

Sarris et al. [12] reported that fish oil inhibited the coronary arteriosclerosis in transplanted hearts. Meiser et al. [10] demonstrated the beneficial effect of HMG-CoA reductase inhibitor on FK506-induced coronary artery disease using a rat model. But both results showed no changes in serum cholesterol levels despite the use of antihyperlipidemic drugs.

In in vitro experiments, Hidaka et al. [9] reported that HMG-CoA reductase inhibitor inhibited the migration of poricine aortic smooth muscle cells which were stimulated by platelet-derived growth factor. Corisini et al. [5] also demonstrated that the proliferation of smooth muscle cells in rats and humans was inhibited by simvastatin. However, no clinical meaning was indicated because the concentration of simvastatin in those experiments was 100 times higher than the clinical dose. These reports suggested that simvastatin was much more potent than pravastatin in inhibiting the activation of smooth muscle cells. Moreover, Soma et al. [14] showed that HMG-CoA reductase inhibitors inhibited the proliferation of the smooth muscle cells in the intima without decreasing the serum cholesterol level. They reported that the effect of pravastatin was less than other HMG-CoA reductase inhibitors, and the dif-



ference among HMG-CoA reductase inhibitors could be caused by the differing permeability of cellular membranes or the effect of enzyme inhibition. However, this experiment demonstrated that pravastatin was effective for coronary arteriosclerosis in transplanted hearts using a rabbit model and a hypercholesterol diet.

Concerning the development of the arteriosclerosis, the "response to injury theory" which was proposed by Ross et al. [11] has been widely cited. In transplanted vessels, arteriosclerosis would be initiated by immunological injury of the endothelium and followed by proliferation and migration of smooth muscle cells. These processes are induced by many growth factors which are secreted from platelets, endothelial cells, or macrophages. It would be presumed that the progression of the arteriosclerosis can be inhibited by suppressing any step from endothelial injury to smooth muscle cell migration. Further investigations are necessary to elucidate the mechanism of the beneficial effect of the HMG-CoA reductase inhibitor, pravastatin, on the inhibition of arterisclerosis.

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