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Research Article

Anti-Inflammatory Effects of -3Hydroxy-3-Methylglutaryl-Coenzyme a Reductase Inhibitor on Acute Coronary Arteritis in a Rabbit Model of Kawasaki Disease

Seiichiro Ozawa MD[1], Kenji Hamaoka MD[1*], Kazuyuki Ikeda MD[1]

[1]*Department of Pediatric Cardiology and Nephrology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan*

**Corresponding author:* *Dr. Kenji Hamaoka, Department of Pediatric Cardiology and Nephrology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Kawaramachi-Hirokouji, Kamigyouku 602-8566, Japan. Tel: +81-75-251-5832; Fax: +81-75-251-5833; Email: khamaoka@koto.kpu-m.ac.jp*

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Abstract

Recent observations suggest that some of the clinical benefits associated with statin therapy are pleiotropic, i.e., they are independent of their cholesterol-inhibiting action. In this study, we attempted to evaluate the anti-inflammatory effects of statins on coronary arteritis in a rabbit model of Kawasaki disease (KD).

Allergic vasculitis rabbit models were used in this study and divided into 3 groups as follows: no treatment (A), fluvastatin treatment (B), and pravastatin treatment (C). In group a, histological examinations demonstrated severe panvasculitis with endothelial destruction, marked mononuclear cell infiltration of all layers, and edematous thickening of the medial layer. These inflammatory findings were most prominent on day 3 and were similar to the histopathological features in KD. However, in both groups B and C, the inflammatory findings were significantly suppressed even on day 3 in comparison with those in group A.

Our study showed that statins had significant anti-inflammatory effects in a rabbit model of acute coronary arteritis typical of KD. It is suggested that statins may be effective for preventing the development of coronary aneurysmal changes.

Keywords: 3-Hydroxy-3-Methylglutaryl-Coenzyme a Reductase Inhibitor; Allergic Vasculitis; Endothelial Cell; Animal Model; Kawasaki Disease

Introduction

Kawasaki disease (KD) is an acute inflammatory disease of childhood that most frequently and severely affects the coronary artery [1]. Coronary artery dilatation and aneurysms develop in approximately 15% of the patients, which may eventually induce ischemic heart disease in 3% of the patients [2]. Intravenous administration of immunoglobulin (IVIG) effectively reduces the incidence of coronary artery lesions [3].

To understand the mechanism of panvasculitis and aneurysmal formation in KD, it is essential to establish an animal model that represents typical histopathological features of the disease. We have previously proposed a rabbit model of allergic coronary vasculitis initiated by immunizing weaning rabbits with horse serum. In this animal model, panvasculitis with destruction of internal elastic lamina and edematous thickening of medial smooth muscle cell were detected, similar to the histopathological changes of KD [4].

Recently, 3-hydroxy-3-hethylglutaryl-hoenzyme A (HMG-CoA) reductase inhibitors (statins) were found to inhibit inflammatory cell invasion of the vascular walls, migration of the vascular endothelial cells, and consequent blood vessel destruction [5,6].

To elucidate whether statins inhibit vascular injury, we investigated the serial process of histopathological features of acute coronary arteritis in a rabbit model of KD during the acute stage of allergic coronary vasculitis. After administration of statins, infiltration of macrophages and destruction of arterial wall structures was most suppressed at the intima and adventitia of the coronary arteries. These results suggest that statin treatment was effective in acting upon the intimal endothelial cells of systemic arteries without obvious inflammation from the very early stage of the illness.

Methods

Animal model for Kawasaki disease and statin treatments

Japanese white male rabbits between 5 and 7 weeks of age (weight, 700–800 g) were used in this study. All animals were treated and cared for in accordance with the Rules and Regulations of Animal Research, Kyoto Prefectural University of Medicine. Rabbits were treated with intravenous administration of horse serum (GIBCO-BRL, 10 ml/kg) twice at an interval of 2 weeks. Animals were sacrificed and the hearts were removed on days 1, 3, 5, and 7 after the second administration of horse serum. Rabbits with two injections of the same amount of saline were used as controls (control group). To study the anti-inflammatory effect of statins on coronary arteritis, we investigated the serial process of histopathological features during the acute phase of coronary arteritis in 3 groups of animals: no treatment (A), fluvastatin treatment 20 mg/kg/day (B), and pravastatin treatment 10 mg/kg/day (C). Statins were administered from the day after the second administration of horse serum until day 14.

Histological Examination

Hearts were excised and fixed in cold periodate lysine paraformaldehyde solution for 24 h. Prior to embedding, the fixed specimen was divided serially into segments of 2–3 mm thickness along the long axis of coronary arteries. Sections were stained with hematoxylin–eosin (HE) or Verhoeff elastic stain and examined microscopically.

Immunohistochemistry Method

To evaluate endothelial cell abnormalities, tissue sections were immunostained by the avidin–biotin complex (ABC) method [7]. Two-step immunoperoxidase staining was performed using anti-CD31 antibodies (primary antibodies) against endothelial cells (Dako Cytomation; diluted 1:100). Goat anti-mouse IgG conjugated with peroxidase was used as the secondary antibody. To inhibit endogenous peroxidase activity, the sections were initially incubated with 1% H_2O_2 for 30 min. Then the sections were incubated with 5% skim milk (Nakarai, Japan) to block non-specific background reactions. After overnight incubation with the primary antibody at 4°C, the sections were rinsed twice in phosphate buffered saline for 15 min. Immunoreactivities were detected with an ABC kit (Vector Labs, Funakoshi co. Japan). Peroxidase activity was visualized using 0.1% 3,3′-diaminobenzidine tetrahydrochloride (DAB) as a substrate according to the manufacture's recommendation.

Evaluation of Vasculitis

The extent of vasculitis in each group was rated for each of the intima, media, and adventitia using the following 4-point scale on samples obtained 7 days after initiating vasculitis:

1. No infiltration of inflammatory cells or blood vessel wall thickening was observed.

2. Mild infiltration of inflammatory cells and thickening of the blood vessel wall were observed.

3. Extensive infiltration of inflammatory cells and thickening of the blood vessel wall were observed.

4. Extremely extensive infiltration of inflammatory cells and thickening of the blood vessel wall were observed.

Results

Establishment of coronary panvasculitis in a rabbit model and its histopathological analysis

To understand the mechanism of formation and progression of panvasculitis in KD, we investigated histopathological features of an experimental model of rabbit allergic coronary vasculitis. In group A (no treatment group), severe infiltration of mononuclear cells was noted at the intima (Figure 1b-1) and the adventitia (Figure 1b-2) on day 1 after two dosages of horse serum. Thickening of the media due to intercellular edema of smooth muscle cells was also observed (Figure 1b-3).

Group A: no treatment

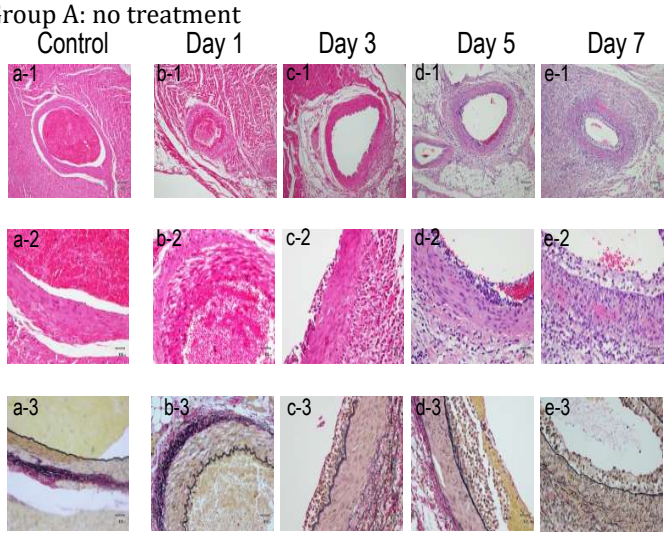

Figure 1. Comparison between control and vasculitis model groups; Panvasculitis with severe mononuclear cell invasion of all coronary artery layers and disruption of the internal elastic lamina is visible 1 day after inducing vasculitis (b). Thickening of the media is most prominent on day 3 (c). Morphological abnormalities of the intima are observed even on day 7 (e).
(a-1–e-1 ×10, a-2–e-2 ×40, Hematoxylin and eosin stain, a-3–e-3 ×40, Verhoeff elastic stain)

Panvasculitis was most prominent on day 3 (Figure 1c). Fibrous deposition (Figure 1c-2, arrow) as well as marked mononuclear cell infiltration was seen in the thickened intima. The most characteristic finding on day 7 was elongation and disruption of the internal elastic lamina (Figure 1e), which is often seen in KD patients and is thought to underlie aneurysm formation. After day 7, panvasculitis gradually regressed. Mononuclear cell infiltration in the intima and the adventitia, and intracellular edema in the media improved after day 7.

Anti-inflammatory effects of statin on acute coronary arteritis in a rabbit model of KD

To evaluate the effect of statin on acute coronary arteritis, we compared group A with groups B and C. In group B (fluvastatin group), thickening of media due to intercellular edema of smooth muscle cells was observed on day 3, similar to group A. (Figure 2, a1-2; HE stain, 3; Verhoeff elastic stain) However, intracellular edema, subsequent thickening of medial smooth muscle cells, and mononuclear cell infiltration of adventitia were remarkably reduced on day 5. Consequently, the thickness of the arterial wall remained significantly decreased on days 5 and 7 in group B compared to group A. (Figure 2, b and c: 1-2; HE stain, 3; Verhoeff elastic stain).

Group B: Patients treated with fluvastatin

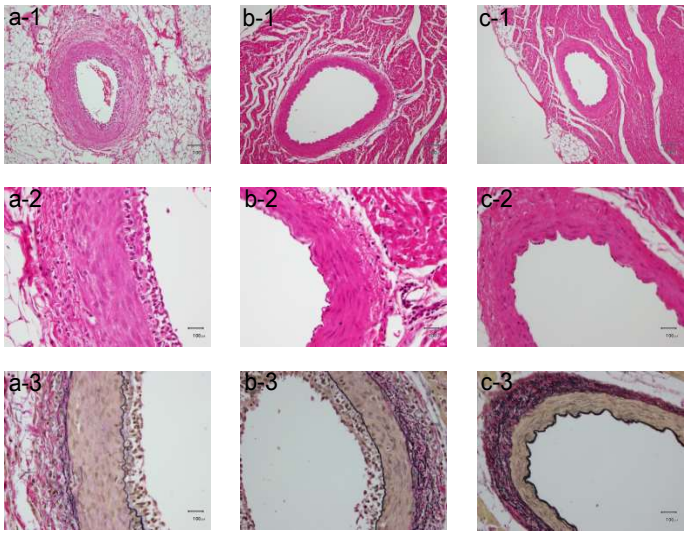

Figure 2. Fluvastatin – 20 mg/kg/day dose group; Panvasculitis findings such as mononuclear cell infiltration are markedly suppressed, and proliferation and dissection of the intima are reduced by day 7 after treatment with fluvastatin compared to those in untreated controls. (a-1-c-2, hematoxylin and eosin stain; a-3–c-3, Verhoeff slastic stain; a-1–c-1 ×10, a-2–c-3 ×20).

Similarly, in group C (pravastatin group), intracellular edema, subsequent thickening of medial smooth muscle cells, and mononuclear cell infiltration of adventitia were remarkably reduced compared to group A. Consequently, the thickness of the arterial wall remained significantly decreased on days 3, 5, and 7. (Figure 3, a–c; HE stain, d–f; Verhoeff elastic stain).

Group C: Patients treated with pravastatin

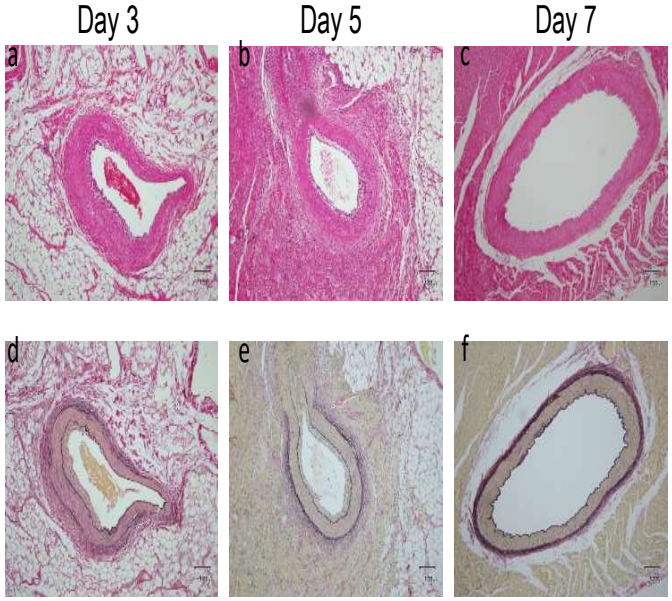

Figure 3. Pravastatin – 10 mg/kg/day dose group; Panvasculitis is markedly suppressed and vessel wall thickness is reduced from day 3 to day 7 after treatment with Pravastatin, comparable to the fluvastatin-treated group, as opposed to the untreated controls.
(a–c, hematoxylin and eosin stain; d–f, Verhoeff elastic stain; a–f ×20).

Evaluation of vasculitis

In group A, histological examinations revealed severe panvasculitis with endothelial destruction, marked mononuclear cell infiltration in all layers, and edematous thickening of the medial layer. These inflammatory findings were prominent and similar to the histopathological features in KD. (Figure 4 ratio of panvasculitis; 2.0 ± 0.33). However, in both groups B and C, the inflammatory findings were significantly suppressed compared to group A. Edematous thickening in the medial layer was insignificant in groups B and C. (Figure 4 ratio of panvasculitis; group B 1.55 ± 0.47 and group C 1.36 ± 0.43 vs. control 0.69 ± 0.66).

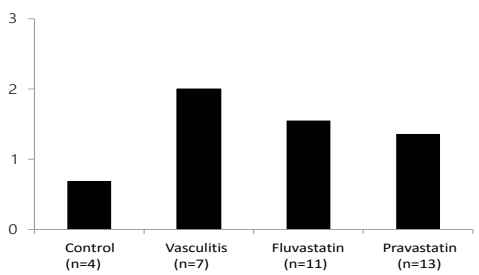

Figure 4. Evaluation of vasculitis; The extent of vasculitis in each group was rated for each of the intima, media, and adventitia using the following 4-point scale from samples obtained 7 days after initiating vasculitis:
0: No infiltration of inflammatory cells or thickening of the blood vessel wall is observed.
1: Mild infiltration of inflammatory cells and thickening of the blood vessel wall are observed.
2: Extensive infiltration of inflammatory cells and thickening of the blood vessel wall are observed.
3: Extremely extensive infiltration of inflammatory cells and thickening of the blood vessel wall are observed.
Vasculitis was ameliorated in the treated groups.

Immunohistochemistry stain for endothelial cells (anti-CD31 antibody)

In normal rabbits injected with saline, the intima was a monolayer of endothelial cells. In group A (no treatment), the intima was destroyed by day 7. Endothelial cell abnormalities were remarkable. However, in both groups B and C, the endothelial cell abnormalities were significantly suppressed compared to group A (Figure 5).

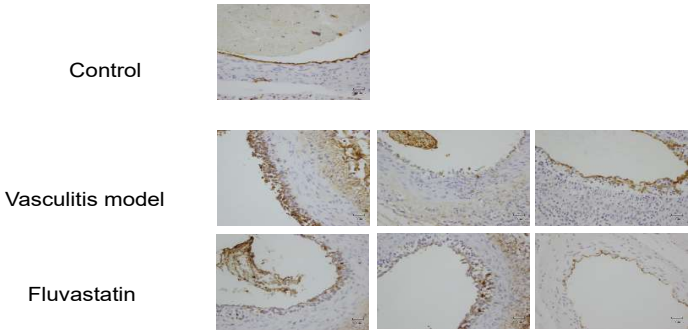

Figure 5. Morphological evaluation of endothelial cells by immunostaining using anti-CD31 antibody;

Morphological abnormalities of vascular endothelial cells, such as dissection and stratification, are reduced since day 3 and markedly improved on day 7 in the treated groups compared with those in the untreated controls.

Discussion

Histopathological studies have revealed that during the first week of KD, inflammatory cells such as macrophages, polymorphonuclear leukocytes, eosinophils, and lymphocytes infiltrate the intima and the adventitia of coronary arteries (stage I). During the second and third weeks, these cells infiltrate all vessel layers and induce panarteritis (stage II). The integrity of coronary arteries is impaired by the panarteritis during stage II, and consequently, aneurysmal formation develops. We have previously proposed a rabbit model of allergic coronary vasculitis induced via immunizing weaning rabbits with horse serum. In this animal model, panvasculitis with destruction of internal elastic lamina and edematous thickening of medial smooth muscle cells were detected, similar to the histopathological features in KD [4, 8-10].

The involvement of the cardiovascular system in rabbits with serum sickness is similar to that in KD, both with respect to the histological changes of the myocardium, valves, coronary arteries, and aorta, and with respect to the chronological sequence with which these changes appear. Although coronary arteritis appears histologically similar to KD, aneurysms did not occur in rabbits, even in cases of necrosis of an arterial segment with deletion of muscle and elastic tissue. However, weaning rabbits with serum sickness tended to develop cellular infiltration and reactive fibro-cellular hyperplasia. They also tended to exhibit markedly increased permeability through degenerated endothelial cells or muscle cells with marked thinning of the media without significant inflammatory cellular reaction. This study provides insight into the long-term prognosis of transient dilatation of coronary arteries in the acute stage of KD.

In KD, an intravenous administration of IVIG effectively reduces the incidence of coronary artery lesions. However, in recent clinical studies, endothelial dysfunction persisted and led to atherosclerosis in adulthood [11-13]. Therefore, it is suggested that protection of endothelial function in the acute stage of KD is important for prevention of cardiac events.

Statin is readily available for the treatment of hypercholesterolemia in adults. In addition, the pleiotropic effects of statin, including anti-thrombosis, anti-inflammation, and protection against low-density lipoprotein oxidation are well known. In this study, it was revealed that statins had significant anti-inflammatory effects on acute coronary arteritis in a model of KD. It has been reported that statin suppresses progression of cerebral aneurysms through inhibition of nuclear factor κB activation in aneurysmal walls [14]. Statins may prevent the development of coronary aneurysmal change. In addition, statin is expected to improve endothelial function. The limitation of the present study is the use of an animal model. In addition, further study needs to be undertaken to determine the optimum dosage and administration periods of statins for

protection again.

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