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Effect of sarpogrelate hydrochloride, a 5-hydroxytryptamine$_2$ receptor antagonist, on allograft arteriosclerosis after aortic transplantation in rats☆,☆☆

Susumu Watada [a], Hirohisa Harada [b,*], Kentaro Matsubara [a], Hideaki Obara [a], Kenji Matsumoto [a], Nobutoshi Ando [b], Yuko Kitagawa [a]

[a] Department of Surgery, Keio University School of Medicine, 35, Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[b] Department of Surgery, Tokyo Dental College, Ichikawa General Hospital, 5-11-13 Sugano, Ichikawa-shi, Chiba 272-8513, Japan

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Background: Sarpogrelate hydrochloride, a 5-hydroxytryptamine$_2$ receptor antagonist, is known to prevent serotonin-induced neointimal hyperplasia. We examined the effect of this agent on allograft arteriosclerosis in a rat model of aortic transplantation.

Methods: Rats were given an aortic isograft or allograft and oral administration of either saline vehicle alone or 20 mg/kg daily of sarpogrelate for 8 weeks. The grafts were then harvested, and the lumen diameter and the thickness of the intima and media were measured. Comparisons were made between measurement results in isografts and allografts from rats treated and not treated with sarpogrelate. Immunohistochemistry assessments were used to detect expression of serotonin in graft specimens.

Results: For both allografts and isografts, significantly less intimal thickening was observed in specimens from rats given sarpogrelate compared with rats given saline. Sarpogrelate had no effect on medial thickening in either graft type. Serotonin was detected in allografts from rats given saline alone but not in allografts from rats given sarpogrelate or in isografts.

Conclusions: Sarpogrelate hydrochloride may mitigate arteriosclerosis in allografts. Platelet aggregation and serotonin may be correlated with intimal thickening associated with chronic rejection.

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1. Introduction

The use of immunosuppressive agents has reduced the rate of acute rejection in patients who have undergone transplantation. Chronic rejection remains a considerable problem, partly because its precise mechanisms are unknown. It is well appreciated that chronic rejection may develop arteriosclerosis in vascular allografts, which eventually shortens graft survival. Pathological findings in allograft arteriosclerosis include diffuse concentric intimal thickening, migration and proliferation of vascular smooth muscle cells (VSMC), and intense infiltration by mononuclear cells, including T cells and macrophages. Moreover, it has been reported that platelets largely contribute to allograft arteriosclerosis [1,2]. Early pathological descriptions of transplants recognized the presence of platelets in kidney transplants [3] and some studies indicated that platelets accumulated in renal allografts using indium [4,5]. Recent studies have also identified the presence of prominent intravascular platelet aggregates in experimental and clinical transplants that undergo chronic rejection [6–9]. Thus, control of arteriosclerosis may depend partly on limiting VSMC proliferation and platelet activation.

Serotonin (5-hydroxytryptamine, 5-HT), a hormone released from activated platelets, is a key factor in influencing the magnitude of VSMC proliferation and inducing neointimal hyperplasia [10]. Controlling the function of serotonin may inhibit intimal thickening in the allograft. Therefore, our study was focused on sarpogrelate hydrochloride (Anplag or MCI-9042; Mitsubishi Tanabe Pharma, Tokyo, Japan), a selective 5-HT receptor antagonist that has been found to be effective in reducing serotonin-induced neointimal hyperplasia [11]. Sarpogrelate is also an anti-platelet agent and has been widely used to treat patients with peripheral arterial disease [12] (Fig. 1).

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT$_2$R, 5-hydroxytryptamine$_2$ receptor; Qint, relative thickness of intima, calculated as: intima / (lumen + intima + media) × 100; Qmed, relative thickness of media, calculated as: media / (lumen + intima + media) × 100; TBS, Tris buffered saline; VSMC, vascular smooth muscle cells.

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* Corresponding author. Tel.: +81 47 322 0151; fax: +81 47 325 445.
E-mail address: hharada@tdc.ac.jp (H. Harada).

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2. Objective

We therefore investigated the effect of sarpogrelate administration on allograft arteriosclerosis in a rat model of aortic transplantation. The aortic transplant model has striking morphological similarities to vascular lesions of solid organ grafts in humans and allows standardized quantitative measurement of structural changes in the vessel wall, as well as a convenient evaluation of the results for a particular intervention [13,14].

3. Material and methods

3.1. Experimental animals

Male DA (major histocompatibility class haplotype $RT1^a$) and Lewis ($RT1^l$) rats (initial body weight, 180–250 g) were used in the study in accordance with international guidelines on animal care and experimentation. Allogeneic transplantation involved DA donors and Lewis recipients; syngeneic transplantation was performed in DA rats. Transplant recipients received either an isograft followed by oral administration of saline vehicle alone (1 ml/kg/day) for 8 weeks (group 1); an isograft followed by 20 mg/kg/day of sarpogrelate hydrochloride (dissolved in 10 mg/ml of saline) for 8 weeks (group 2); an allograft followed by saline vehicle for 8 weeks (group 3); or an allograft followed by 20 mg/kg/day of sarpogrelate for 8 weeks (group 4). Each group included four rats. Based on previous studies indicating that 10–30 mg/kg/day of sarpogrelate suppressed peripheral obstruction in experimental rat models, 20 mg/kg/day of sarpogrelate was the chosen dose for this study [15].

3.2. Aorta transplantation

Donor rats were anesthetized by using isoflurane inhalation (Dainippon Sumitomo Pharma, Osaka, Japan). The thoracic aorta was served between the diaphragm and the subclavian artery. The arterial stump of the intercostal artery was electrocoagulated to stop bleeding. The lumen of the blood vessels was washed with heparinized normal saline (0.1 mg/mL). The thoracic aortic graft was cut into a 3-cm section. The recipient animals were anesthetized using isoflurane. A mid-line incision was made and the retroperitoneum was opened. Microclips were placed below the renal arteries and above the aortic bifurcation, and a segment of this section was dissected. The transplanted vessel extended from below the renal arteries to the bifurcation of the abdominal aorta. Interrupted sutures (9-0 monofilament nylon) were used for anastomoses. After transplantation, all recipients received cyclosporin A (5 mg/kg/day given intramuscularly for 5 days; Novartis Pharma, Tokyo, Japan) to prevent acute rejection.

Eight weeks after transplantation, the rats were anesthetized and the grafts were harvested. One segment of each graft was frozen in a mixture of cold isopentane and dry ice and stored at −70 °C until processed for immunohistochemistry; the other was fixed in 4% buffered formaldehyde at room temperature and embedded in paraffin.

3.3. Morphometric analysis measurement

Cross-sections (5 μm) from different levels of the paraffin-embedded grafts were stained with hematoxylin and eosin and studied morphometrically by measuring the lumen diameter and thickness of intima and media. The relative thickness (%) of the intima (Qint) was calculated as: intima / (lumen + intima + media) × 100). The relative thickness (%) of the media (Qmed) was calculated as: media / (lumen + intima + media) × 100). Three measurements were performed on each layer in the vessel wall to obtain mean (±SEM) values for Qint and Qmed. Differences between groups were assessed by using Student's t-test (StatView SE + Graphics; Abacus Concepts, Cary, NC). A P value of less than 0.05 was considered to represent a significant difference.

3.4. Immunohistochemistry

Immunohistochemistry studies were conducted to assess the expression of serotonin in graft specimens (4-μm thick) and a positive control (intestine from a DA rat). The sections were dewaxed in Clear Plus (FALMA, Tokyo, JAPAN), dehydrated in methanol, and washed in 0.1 M Tris buffered saline (TBS). Endogenous peroxidase activity was blocked by incubation for 20 min in methanol containing 3% hydrogen peroxide. After washing in 0.1 M TBS, the sections were incubated in 0.1% trypsin for 30 min at 37 °C. The sections were again washed in 0.1 M TBS and then incubated with 1:50 mouse monoclonal anti-serotonin (clone HT-H209; Abcam, Cambridge, United Kingdom) for 60 min at room temperature. After washing in 0.1 M TBS, the sections were treated with Histofine Simple Stain Max Peroxidase Multi (Nichirei Biosciences, Tokyo, Japan) for 30 min at room temperature. The sections were washed in 0.1 M TBS and developed for 10 min by using diaminobenzidine. After a final washing in 0.1 M TBS, the sections were counterstained with hematoxylin.

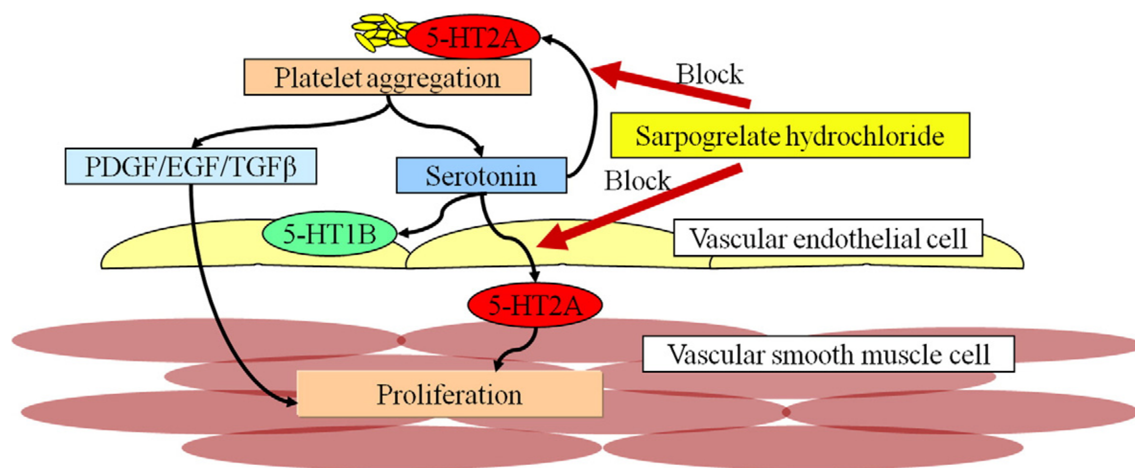

Fig. 1. Action mechanisms of serotonin and sarpogrelate hydrochloride on platelet and blood wall. Serotonin is held in dense platelet granules and released during platelet activation. Moreover, serotonin enhances platelet aggregation in damaged vessel segments and serotonin secretion (positive feedback) through the 5-HT_2 receptor in platelet membranes. In addition, serotonin, through the 5-HT_{2A} receptor in VSMC, induces a vasoconstrictive response and increases thrombotic potential by means of proliferation of VSMC in blood vessels. Sarpogrelate hydrochloride inhibits the function of serotonin as a 5-HT_{2A} receptor antagonist.

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