Effects of M-1, a major metabolite of sarpogrelate, on 5-HT-induced constriction of isolated human internal thoracic artery

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Summary

Sarpogrelate, a selective 5-hydroxytryptamine (5-HT)\textsubscript{2A} receptor antagonist, inhibits 5-HT-induced platelet aggregation and vasoconstriction. It improves ischemic symptoms in patients with arteriosclerosis obliterans. M-1 is a major metabolite of sarpogrelate, and has been reported to show a higher affinity for the 5-HT\textsubscript{2A} receptor on platelets than sarpogrelate. However, the effects of M-1 on 5-HT-induced constrictive response in human blood vessels have not been investigated. The internal thoracic artery (ITA) is the key conduit for coronary artery bypass grafting (CABG). 5-HT has been implicated as playing an important role in the pathogenesis of vasospasm. Thus, in the present study, the effects of M-1 on 5-HT-induced vasoconstriction were examined in isolated human endothelium denuded ITA. M-1 inhibited 5-HT-induced vasoconstriction in a concentration-dependent manner. At the highest concentration, M-1 almost completely inhibited the 5-HT-induced vasoconstriction. Expression of 5-HT\textsubscript{2A} and 5-HT\textsubscript{1B} receptor proteins in the membrane fraction of ITA smooth muscle cells was confirmed by western blot analysis. Individually, supramaximal concentrations of sarpogrelate and SB224289, a selective 5-HT\textsubscript{1B} receptor antagonist, only partially inhibited the 5-HT-induced vasoconstriction. However, simultaneous pretreatment with both these antagonists almost completely inhibited the 5-HT-induced vasoconstriction. The inhibitory effect of M-1 pretreatment mimicked the inhibitory effect of simultaneous pretreatment with sarpogrelate and SB224289. These results suggest that M-1 has antagonistic effects not only on the 5-HT\textsubscript{2A} receptor but also on the 5-HT\textsubscript{1B} receptor in human ITA smooth muscle cells. M-1 may be useful as a lead compound for the development of drugs for the treatment of 5-HT-induced vasospasms in CABG.

Key Words:
5-hydroxytryptamine (5-HT), sarpogrelate, vasoconstriction, internal thoracic artery, 5-HT\textsubscript{2A} receptor, 5-HT\textsubscript{1B} receptor
Introduction

The large family of 5-hydroxytryptamine (5-HT) receptor subtypes can be classified into seven sub-families based on downstream signaling mechanisms.\(^1\) The 5-HT\(_{2A}\) and 5-HT\(_{1B}\) receptor subtypes can be found on numerous vascular smooth muscle cells.\(^2,3\) While the composition of this population of receptor subtypes may be species dependent and vascular bed dependent, activation of these specific receptor subtypes always causes vasoconstriction. 5-HT\(_{1B}\) receptor is also present on vascular endothelial cells and its stimulation releases nitric oxide (NO) which induces relaxation of vascular smooth muscle cells via activation of soluble guanylate cyclase.\(^4\) 5-HT synthesized by enterochromaffin cells in the gastro-intestinal tract is incorporated into platelets, and is released into plasma when platelets are activated.\(^5-7\) Platelets adhere to the surface of the vascular bed where endothelial cells have become detached due to plaque rupture at the site of arteriosclerosis, and are subsequently activated.\(^8\) The activated platelets release 5-HT (amongst other substances), which stimulate 5-HT\(_{2A}\) receptors on the platelets in both an autocrine and a paracrine manner. After 5-HT stimulation, the aggregated platelets additionally release large amounts of 5-HT which subsequently induces vasoconstriction.

Sarpogrelate, a selective 5-HT\(_{2A}\) receptor antagonist, inhibits 5-HT-induced platelet aggregation, vasoconstriction, vascular smooth muscle cell proliferation, and can improve ischemic symptoms in arteriosclerosis obliterans patients.\(^9,10\) A major metabolite of sarpogrelate, M-1, has been reported to show an even higher affinity for 5-HT\(_{2A}\) receptor on platelets than sarpogrelate.\(^9-11\) Thus, M-1 demonstrates a stronger inhibitory effect on platelet aggregation than sarpogrelate. However, the effects of M-1 on 5-HT-induced constrictive response in human blood vessels have not been investigated. The internal thoracic artery (ITA) is a key conduit for coronary artery bypass grafting (CABG) because of its excellent long-term patency.\(^12,13\) Endothelium denuded ITA (to avoid the influence of endothelial cells) is an excellent model system for investigating the effects of vasoactive substances. In the present study, the effects of M-1 on 5-HT-induced constriction of vascular smooth muscle were examined in isolated human endothelium denuded ITA.
MATERIALS AND METHODS

Preparation of blood vessels and contractile studies

Human blood vessels were obtained from patients (n=12) undergoing CABG performed at Miyazaki Prefectural Nobeoka Hospital (Miyazaki, Japan). After portions of each ITA were sectioned for bypass graft, the remaining portions were retained. The ITA samples were then transported to the laboratory for subsequent experimentation. The constrictive responses of endothelium denuded ITA strips were measured using a purposely designed method for ITA samples as described previously.\(^{14}\) In brief, upon obtaining the maximum constriction after the first application of 5-HT (1.0 \(\mu\)M), the preparation was washed twice with Krebs buffer solution (all in mM: NaCl 118.0, KCl 4.7, NaHCO\(_3\) 25.0, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.1, CaCl\(_2\) 2.5, EDTA 0.01 and glucose 11.0: pH 7.4). The preparation was then allowed to gradually recover from 5-HT-induced vasoconstriction. After to confirm that the preparation had completely returned to the steady state level, M-1 (0.03, 0.1, 0.3 or 1.0 \(\mu\)M) was added to the bath. After 30 min pre-incubation, 5-HT (1.0 \(\mu\)M) was administered for a second time. In a separate series of experiments, designed around our previous experiments,\(^{14}\) supramaximal concentrations of sarpogrelate (1.0 \(\mu\)M) and SB224289 (1.0 \(\mu\)M) were also applied. Vasoconstrictive responses to each concentration of 5-HT (0.3–3.0 \(\mu\)M) were obtained in a cumulative fashion before and after treatment with sarpogrelate (1.0 \(\mu\)M), SB224289 (1.0 \(\mu\)M), and sarpogrelate (1.0 \(\mu\)M) plus SB224289 (1.0 \(\mu\)M) or M-1 (1.0 \(\mu\)M).

Western blot analysis of the 5-HT\(_{2A}\) and 5-HT\(_{1B}\) receptors in the membrane fraction

Preparations for obtaining the membrane proteins, and immunoblot analyses were performed as described previously.\(^{15}\) Briefly, membrane proteins were extracted from ITA homogenates using a transmembrane protein extraction kit (Merck Millipore Corp., Darmstadt, Germany). Equivalent amounts of membrane protein (5 \(\mu\)g each lane) were separated by polyacrylamide gel electrophoresis. The separated proteins were then transferred to polyvinylidene difluoride membranes using electrophoretic transfer, and then blocking with 2% skim milk in phosphate buffered saline-0.1% Tween 20 (PBS-T, pH 7.4), the blotting membranes were washed with PBS-T,
and incubated with primary antibody (anti-5HT\textsubscript{1B} and anti-\textbeta-actin, 1:1000 dilution; anti-5HT\textsubscript{2A}, 1:200 dilution) at 4°C for 15 h. The membranes were again washed with PBS-T and then incubated with HRP-coupled anti-rabbit or anti-mouse IgG (1:2000 dilution) at room temperature for 1 h. After a final wash with PBS-T, bound antibodies were visualized by chemiluminescence regent using Luminata Forte Western HRP Substrate (Merck Millipore Corp., Darmstadt, Germany). Antibody binding levels were subsequently detected by quantitative densitometry (ImageQuant LAS 4000, GE Healthcare, Tokyo, Japan).

**Ethics statement**

The Ethics Committee of Kyusyu University of Health and Welfare and Miyazaki Prefectural Nobeoka Hospital approved this project (acceptance number 09-004).

**Statistical Analyses**

The number of subjects expressed in the figures is the number of patient studied. The data were statistically evaluated by one-way or two-way analysis of variance (ANOVA) with multiple comparison using Dunnett *post hoc* test. Significance was assumed at p<0.05. We used SPSS 21.0 J (IBM, Corp., Armonk, NY, U.S.A.) for Statistical analyses.

**RESULTS**

The 5-HT (1.0 \textmu M)-induced vasoconstriction was significantly inhibited by pre-incubation with M-1 (0.1, 0.3 and 1.0 \textmu M) in a concentration-dependent manner. At the highest concentration of M-1 used (1.0 \textmu M), the 5-HT-induced vasoconstriction was almost completely inhibited (Figure 2).

Vasoconstriction induced by 5-HT was in a concentration-dependent manner (0.3–3.0 \textmu M) (Figure 3). Individually, sarpogrelate (1.0 \textmu M) or SB224289 (1.0 \textmu M) significantly inhibited the 5-HT-induced vasoconstriction; however, about half of the vasoconstriction caused by 5-HT at 3.0 \textmu M still remained. Simultaneous administration of sarpogrelate (1.0 \textmu M) and SB224289 (1.0 \textmu M) almost completely inhibited the 5-HT-induced vasoconstriction. M-1 inhibited 5-HT-induced...
vasoconstriction in a concentration dependent manner. At 1.0 µM concentration, M-1 completely inhibited the 5-HT-induced vasoconstriction. No significant differences between the inhibitory effects of sarpogrelate plus SB224289 and M-1 were observed (Figure 3).

To confirm the presence of 5-HT_2A and 5-HT_1B receptors on ITA cells, we examined the expression of 5-HT_2A and 5-HT_1B receptors in membrane fractions prepared from ITA. Expression of 5-HT_2A and 5-HT_1B receptor proteins was confirmed in ITA cells (Figure 4).

DISCUSSION

M-1 is a main metabolite of sarpogrelate which is a selective 5-HT_2A receptor antagonist. Our results demonstrate that M-1 almost completely inhibited 5-HT-induced constriction of isolated human endothelium denuded ITA. We previously reported that 5-HT induced constriction of endothelium denuded ITA via activation of both 5-HT_2A and 5-HT_1B receptors. Here, we also confirmed the presence of the 5-HT_2A and 5-HT_1B receptor proteins in the membrane fraction of ITA smooth muscle cells by western blot analysis. The inhibitory effect of M-1 pretreatment mimicked the inhibitory effect of simultaneous pretreatment with sarpogrelate and SB224289, a selective antagonist of 5-HT_1B receptor. These results suggest that M-1 has antagonistic effects not only on 5-HT_2A receptor but also on 5-HT_1B receptor in human ITA smooth muscle cells.

CABG still remains the gold standard therapy for severe coronary artery disease, such as a left main coronary disease.\textsuperscript{16} One of the most serious problems in CABG is that the bypass grafts often induce spasms after implantation into coronary arterial circulation.\textsuperscript{17,18} We previously reported that 5-HT-induced platelet aggregation and vasoconstriction in experimental animals is closely related to occlusive thrombus formation.\textsuperscript{19,20} Clinical studies have reported that 5-HT levels in coronary artery circulation are significantly increased in patients with acute coronary artery disease.\textsuperscript{2,21} It has been also revealed that 5-HT is released during coronary angioplasty and angiography, which induces clinically relevant vasoconstriction in patients with coronary artery disease.\textsuperscript{22,23} Thus, among the possible vasoactive substances responsible for spasms, 5-HT is a crucial factor in coronary artery disease and subsequent cardiac events.\textsuperscript{22,24–28} In addition, the release of 5-HT from
activated platelets may play a more crucial role in the constriction of bypass grafts when surgical handling removes the endothelium in CABG, because 5-HT also releases NO from endothelial cells via activation of 5-HT<sub>1B</sub> receptor. Thus, a drug which prevents 5-HT-induced platelet aggregation and vasospasm may possibly improve premature occlusion and postoperative morbidity in CABG.<sup>25,29</sup>

In this experiment, we used 1.0 µM supramaximal concentrations of sarpogrelate and SB224289, as previously reported.<sup>14</sup> Inhibition of 5-HT-induced vasoconstriction by each antagonist separately was incomplete at supramaximal concentrations, and approximately half of the vasoconstriction induced by 5-HT at 3.0 µM still remained. However, simultaneous treatment of sarpogrelate and SB224289 at supramaximal concentrations almost completely inhibited the 5-HT-induced vasoconstriction. As noted above, 5-HT<sub>2A</sub> and 5-HT<sub>1B</sub> receptors are both expressed on the membranes of ITA smooth muscle cells. Therefore, to prevent 5-HT-induced constriction of blood vessels which express both 5-HT<sub>2A</sub> and 5-HT<sub>1B</sub> receptors (such as ITA and coronary artery), it is necessary to block both 5-HT<sub>2A</sub> and 5-HT<sub>1B</sub> receptors. Kim et al. recently reported that in a prospective randomized trial that evaluated sarpogrelate efficacy for patients with vasospastic angina (VSA) in addition to standard treatment including calcium channel blocker and/or vasodilators, sarpogrelate did not significantly improve the angiographic remission rate in patients with VSA.<sup>30</sup> The results of this trial are consistent with our proposal that it is necessary to block both 5-HT<sub>2A</sub> and 5-HT<sub>1B</sub> receptors to prevent 5-HT-induced vasospasms.

M-1 is a major metabolite of sarpogrelate (Figure 1). In this experiment, M-1 inhibited 5-HT-induced constriction of human endothelium denuded ITA in a concentration dependent manner. At the highest concentration, M-1 almost completely inhibited the 5-HT-induced vasoconstriction. The inhibitory effect of M-1 pretreatment mimicked the inhibitory effect of simultaneous pretreatment with a selective 5-HT<sub>2A</sub> receptor antagonist and a selective 5-HT<sub>1B</sub> receptor antagonist. These results suggest that M-1 has antagonistic effects both on 5-HT<sub>2A</sub> receptor and on 5-HT<sub>1B</sub> receptor in human ITA smooth muscle cells.

Because M-1 is immediately metabolized to other compounds, M-1 does not have long-lasting
effects in vivo. Therefore, it would be difficult to use M-1 as a medicine in clinical practice. However, we suggest that M-1, which has agonistic effects on both 5-HT$_{2A}$ and 5-HT$_{1B}$ receptors, can be used as a lead compound for further optimization and drug development, and that this process may yield promising compounds for the treatment of 5-HT-induced vasospasms in CABG.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interests.
REFERENCES

1) Kroeze WK, Kristiansen K, Roth BL. Molecular biology of serotonin receptors structure and function at the molecular level. *Curr. Top. Med. Chem.*, 2, 507–528 (2002).

2) Nagatomo T, Rashid M, Abul Muntasir H, Komiyama T. Functions of 5-HT$_{2A}$ receptor and its antagonists in the cardiovascular system. *Pharmacol. Ther.*, 104, 59-81 (2004).

3) Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.*, 46, 157-203 (1994).

4) Gamoh S, Hisa H, Yamamoto R. 5-hydroxytryptamine receptors as targets for drug therapies of vascular-related diseases. *Biol. Pharm. Bull.*, 36, 1410-1415 (2013).

5) Vanhoutte P.M. Endothelial dysfunction and vascular disease. *Verh. K. Acad. Geneeskd. Belg.*, 60, 251-266 (1998).

6) Hara K, Hirowatari Y, Yoshika M, Komiyama Y, Tsuka Y, Takahashi H. The ratio of plasma to whole-blood serotonin may be a novel marker of athero-sclerotic cardiovascular disease. *J. Lab. Clin. Med.*, 144, 31-37 (2004).

7) Figueras J, Domingo E, Cortadellas J, Padilla F, Dorado DG, Segura R, Segura R, Galard R, Soler JS. Comparison of plasma serotonin levels in patients with variant anginapectoris versus healed myocardial infarction. *Am. J. Cardiol.*, 96, 204-207 (2005).

8) Asada Y, Yamashita A, Sato Y, Hatakeyama K. Pathophysiology of atherothrombosis: Mechanisms of thrombus formation on disrupted atherosclerotic plaques. *Pathol. Int.*, 70, 309-322 (2020).

9) Maruyama K, Kinami J, Sugita Y, Takada Y, Sugiyama E, Tsuchihashi H, Nagatomo T. MCI-9042: high affinity for serotonergic receptors as assessed by radioligand binding assay. *J. Pharmacobiodyn.*, 14, 177-181 (1991).

10) Hara H, Osakabe M, Kitajima A, Tamao Y, Kikumoto R. MCI-9042, a new antiplatelet agent is a selective S$_2$-serotonergic receptor antagonist. *Thromb. Haemost.*, 65, 415-420 (1991).

11) Qi R, Ozaki Y, Satoh K, Kurota K, Asazuma N, Yatomi Y, Kume S. Quantitative measurement...
of various 5-HT receptor antagonists on platelet activation induced by serotonin. *Thromb. Res.*, 81, 43-54 (1996).

12) Del Campo C. Pedicled or skeletonized? A review of the internal thoracic artery graft. *Tex. Heart Inst. J.*, 30, 170–175 (2003).

13) Tatoulis J. Total arterial coronary revascularization-patient selection, stenosis, conduits, targets. *Ann. Cardiothorac. Surg.*, 2, 499–506 (2013).

14) Tanaka N, Nakamura E, Ohkura M, Kuwabara M, Yamashita A, Onitsuka T, Asada Y, Hisa H, Yamamoto R. Both 5-hydroxytryptamine 5-HT_{2A} and 5-HT_{1B} receptors are involved in the vasoconstrictor response to 5-HT in the human isolated internal thoracic artery. *Clin. Exp. Pharmacol. Physiol.*, 35, 836-840 (2008).

15) Yokota A, Gamoh S, Tanaka-Totoribe N, Shiba T, Kuwabara M, Nakamura E, Hayase T, Hisa H, Nakamura K, Yamamoto R. Angiotensin II, as well as 5-hydroxytryptamine, is a potent vasospasm inducer of saphenous vein graft for coronary artery bypass grafting in patients with diabetes mellitus. *Biochem. Biophys. Rep.*, 6, 82–87 (2016).

16) Mohr FW, Morice MC, Kappetein AP, Feldman TE, Ståhle E, Colombo A, Mack MJ, Holmes Jr. DR, Morel MA, VanDyck N, Houle VM, Dawkins KD, Serruys PW. Coronary artery bypass graft surgery versus percutaneous coronary intervention in patients with three-vessel disease and left main coronary disease: 5-year follow-up of the randomized, clinical SYNTAX trial. *Lancet*, 381, 629–638 (2013).

17) Chanda J, Canver CC. Reversal of preexisting vasospasm in coronary artery conduits. *Ann. Thorac. Surg.*, 72, 476–480 (2001).

18) Fabricius AM, Gerber W, Hanke M, Garbade J, Autschbach R, Mohr FW. Early angiographic control of perioperative ischemia after coronary artery bypass grafting. *Eur. J. Cardiothorac. Surg.*, 19, 853–858 (2001).

19) Nishihira K, Yamashita A, Tanaka N, Kawamoto R, Imamura T, Yamamoto R, Eto T, Asada Y. Inhibition of 5-hydroxytryptamine receptor prevents occlusive thrombus formation on neointima of the rabbit femoral artery. *J. Thromb. Haemost.*, 4, 247-255 (2006).
20) Nishihira K, Yamashita A, Tanaka N, Moriguchi-Goto S, Imamura T, Ishida T, Kawashima S, Yamamoto R, Kitamura K, Asada Y. Serotonin Induces vasoconstriction of smooth muscle cell-rich neointima through 5-hydroxytryptamine2A receptor in rabbit femoral arteries. *J. Thromb. Haemost.*, 6, 1207-1214 (2008).

21) van den Berg EK, Schmitz JM, Benedict CR, Malloy CR, Willerson JT, Dehmer GJ. Transcardiac serotonin concentration is increased in selected patients with limiting angina and complex coronary lesion morphology. *Circulation*, 79, 116-124 (1989).

22) Golino P, Piscione F, Willerson JT, Cappelli-Bigazzi M, Focaccio A, Villari B, Indolfi C, Russolillo E, Condorelli M, Chiariello M. Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. *N. Engl. J. Med.*, 324, 641-648 (1991).

23) Golino P, Piscione F, Benedict CR, Anderson HV, Cappelli-Bigazzi M, Indolfi C, Condorelli M, Chiariello M, Willerson JT. Local effect of serotonin released during coronary angioplasty. *N. Engl. J. Med.*, 330, 523-528 (1994).

24) Vanhoutte PM, Shimokawa H. Endothelium-derived relaxing factor and coronary vasospasm. *Circulation*, 80, 1–9 (1989).

25) Miyata K, Shimokawa H, Higo T, Yamawaki T, Katsumata N, Kandabashi T, Tanaka E, Takamura Y, Yogo K, Egashira K, Takeshita A. Sarpogrelate, a selective 5-HT2A serotonergic receptor antagonist, inhibits serotonin-induced coronaryartery spasm in a porcine model. *J. Cardiovasc. Pharmacol.*, 35, 294-301 (2000).

26) Shimokawa H, Tomoike H, Nabeyama S, Yamamoto H, Araki H, Nakamura M, Ishii Y, Tanaka K. Coronary artery spasminduced in atherosclerotic miniature swine. *Science*, 221, 560–562 (1983).

27) Hesistad DD, Armstrong ML, Marcus ML, Piegors DJ, Mark AL. Augmented responseto vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. *Circ. Res.* 54, 711–718 (1984).

28) McFadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW, Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. *N. Engl.*
29) Thatte HS, Khuri SF. The coronary artery bypass conduit: I. Intraoperative endothelial injury and its implication on graft patency. *Ann. Thorac. Surg.*, **72**, S2245–S2252 (2001).

30) Kim SR, Choi KH, Song YB, Lee JM, Park TK, Yang JH, Hahn JY, Choi JH, Choi SH, Gwon HC. Effect of sarpogrelate and high-dose statin on the reduction of coronary spasm in vasospastic angina: a two by two factorial, pilot randomized study. *Clin. Cardiol.*, **42**, 899-907 (2019).
Fig. 1. Chemical structures of sarpogrelate and M-1. M-1 is metabolized in humans mainly from sarpogrelate by deesterification of succinate.
Fig. 2. Effects of M-1 (0.03, 0.1, 0.3 and 1.0 µM) on 5-HT (1.0 µM)-induced vasoconstriction. M-1 was added to the bath for 30 min prior to the administration of 5-HT. Data are expressed as a percentage of the response to 60 mM KCl in each vessel are shown as mean ± SEM (n=5). The absolute force of ITA rings induced by the second 60 mM KCl in control was 1.63 ± 0.30 g (n=5). *P<0.05 compared with control (C).
Fig. 3. The effects of antagonists on 5-HT (0.3–3.0 μM)-induced vasoconstriction. ¶, 1.0 μM sarpogrelate; §, 1.0 μM SB224289; ¶§, 1.0 μM sarpogrelate + 1.0 μM SB224289; ¶, 1.0 μM M-1; □, control. Sarpogrelate, SB224289, Sarpogrelate + SB224289, and M-1 were added to the bath for 30 min prior to the administration of 5-HT. Data are expressed as a percentage of the response to 60 mM KCl in each vessel and reported as mean ± SEM (n=4). The absolute force of ITA rings induced by the second 60 mM KCl in control (0.3 μM 5-HT) was 1.68 ± 0.28 g (n=4). *P<0.05 compared with each control.
Fig. 4. Western blot analysis of 5-HT$_{2A}$ and 5-HT$_{1B}$ receptors. Expression of 5-HT$_{2A}$ and 5-HT$_{1B}$ receptor proteins was confirmed in the membrane fraction of internal thoracic artery (ITA) smooth muscle cells harvested from patients undergoing coronary artery bypass grafting (CABG) (n=3).