Short Communication

Comparison of Effects of Phosphodiesterase Inhibitors, a Rho-kinase Inhibitor, Ca^{2+}-channel Blockers and a K^{+}-channel Opener on Isolated Human Internal Mammary Arteries

Koki Chiba[1,2] Yuji Nakamura[3] Xin Cao[4]
Shoji Fukuda[5] Koso Egi[6] Mihoko Hagiwara-Nagasawa[3]
Hiroko Izumi-Nakaseko[1,3] Kentaro Ando[1,3] Koichiro Tanaka[2]
Atsuhiko T. Naito[1,3] and Atsushi Sugiyama[1)3)*]

[1)]Department of Pharmacology, Toho University Graduate School of Medicine, Tokyo, Japan
[2)]Department of Traditional Medicine, Faculty of Medicine, Toho University, Tokyo, Japan
[3)]Department of Pharmacology, Faculty of Medicine, Toho University, Tokyo, Japan
[4)]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan
[5)]Department of Cardiovascular Surgery, National Center for Global Health and Medicine, Tokyo, Japan
[6)]Department of Cardiovascular Surgery, Tokyo Yamate Medical Center, Tokyo, Japan

ABSTRACT: Perioperative spasm of the grafts has been one of the most lethal complications during coronary artery bypass grafting. We compared the spasmolytic effects of 4 different pharmacological classes of vasodilators; namely, 3 kinds of phosphodiesterase inhibitors (papaverine, olprinone and milrinone), a Rho-kinase inhibitor (Y-27632), 3 of Ca^{2+}-channel blockers (nicardipine, nifedipine and benidipine) and a K^{+}-channel opener (nicorandil), using the ring preparations made of human skeletonized internal mammary artery grafts. Ring preparations showing physiological dilatation with acetylcholine were used for the assay. The potency of vasodilator action was in the order of benidipine ＞milrinone ＞nifedipine ＞olprinone ＞papaverine ＞Y-27632 ＞nicardipine ＞nicorandil, which is new finding in this study. Thus, benidipine can be most expected to become an efficacious candidate for treating the vasospasm of internal mammary artery in clinical settings.

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1, 2, 3) 5–21–16 Omorinishi, Ota, Tokyo 143–8540, Japan
4) 2–2–1 Miyama, Funabashi-shi, Chiba 274–8510, Japan
5) 1–21–1 Toyama, Shinjuku, Tokyo 162–8655, Japan
6) 3–22–1 Hyakunincho, Shinjuku, Tokyo 169–0073, Japan
*Corresponding Author: tel: ＋81–3–3762–4151 (Ext 2361)
e-mail: atsushi.sugiyama@med.toho-u.ac.jp
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Introduction

Refractory vasospasm has been reported in the arterial bypass graft during coronary artery bypass surgeries.[1] The incidence of arterial bypass-graft spasm ranges from 0.8 to 1.3%, which may result in acute myocardial infarction, occasionally leading to patient's death during the perioperative phase.[1] Several vasodilators have been empirically used for preventing vasospasm of the bypass-graft including Ca^{2+}-channel blockers, phosphodiesterase inhibitors and nitroglycerin. However, there is still no well-accepted protocol against the spasm.[2] In order to develop new pharmacological strategy, we have evaluated vasodilator effects of nitroglycerin and Ca^{2+}-channel blockers (nifedipine, cilnidipine and diltiazem) on the noradrenaline-constricted human internal mammary arterial rings, and found that nitroglycerin was the most potent.[3] Further to explore more efficacious and reliable pharmacotherapy against perioperative bypass-graft spasm, in this study we examined the potency and speed of vasodilator action of 4 different pharmacological classes of drugs; namely, 3 kinds of phosphodiesterase inhibitors (papaverine, olprinone and milrinone), a Rho-kinase inhibitor (Y-27632), 3 of Ca^{2+}-channel blockers (nicardipine, nifedipine and benidipine) and a K^{+}-channel opener (nicorandil), on human skeletonized internal mammary artery.

Materials and Methods

All experiments were approved by the ethics committee of Toho University (#23022, approved on September 29, 2011, #26048, approved on July 17, 2014), National Center for Global Health and Medicine (#1046, approved on July 19, 2011) and Tokyo Yamate Medical Center (#177, approved on July 23, 2014). Twenty-three patients who underwent off pump coronary artery bypass graft surgery in National Center for Global Health and Medicine (n=7) and Tokyo Yamate Medical Center (n=16) were enrolled in this study. All patients gave their informed consent prior to their inclusion in this study. Internal mammary artery was skeletonized and harvested with the ultrasonic scalpel.[4] Distal end sections of the bypass graft were used for the current study. Experiments were performed in Toho University. Ring preparations were incubated in organ bath with Tyrode's solution of the following composition (mM): NaCl 137, KCl 5.4, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6, that was oxygenated with a mixture of gases consisting of 95% O_2 and 5% CO_2 and maintained at 31℃ to prevent their rhythmic and phasic contractions. Tension of the preparation was isometrically measured under a resting tension of 1 g (TBM4M, World Precision Instruments, Inc., Sarasota, FL, USA). After the stable contraction was induced by noradrenaline (Noradrenalin Inj™; Daiichi Sankyo Company Ltd., Tokyo, Japan) in a concentration of 0.1 or 0.2 μM reflecting its local concentration around the sympathetic nerve terminals, acetylcholine (Ovisot Inj™; Daiichi Sankyo Company Ltd.) was added to attain a concentration of 10 μM. The preparation exhibiting ≥70% relaxation by acetylcholine was judged to have intact endothelium, which was used for the following pharmacological assessment.

After washout, the ring preparations were constricted with noradrenaline in a concentration of 0.1 or 0.2 μM again, which was defined as 100% contraction. They were treated with olprinone (Coretec Inj™; Eisai Co., Ltd., Tokyo, Japan), papaverine (Papaverine Hydrochloride; Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan), milrinone (Milrila™ Inj; Astellas Pharma Inc., Tokyo, Japan), Y-27632 (Welfide Co., Ltd., Osaka, Japan), benidipine (Coneal™ Tablets; Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), nicardipine (Perdipine™ Powder; Astellas Pharma Inc., Tokyo, Japan), nifedipine (Sigma, St. Louis MO, USA) and nicorandil (Sigmart™ Inj; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). The concentrations were 0.01, 0.1, 1 and 10 μM for olprinone, papaverine, milrinone, benidipine, nicardipine, nifedipine and nicorandil, and 0.01, 0.1 and 1 μM for Y-27632. Olprinone, papaverine, milrinone, Y-27632 and nicorandil were dissolved in saline, whereas benidipine, nicardipine and nifedipine were initially dissolved in dimethyl sulfoxide (Wako Pure Chemical Industries, Osaka, Japan) in 2 mM and then diluted with saline.

The EC_{50} values were calculated by using nonlinear regression analysis with GraphPad PRISM 6.0 software (GraphPad Software, Inc., San Diego, CA, USA). Speed of vasodilator action was evaluated by measuring the time required to reach the 1/2 peak effect after the highest dose administration, which was defined as onset half time. Statistically significant difference among the EC_{50} values and onset half time were analyzed by one-way factorial analysis of variance (ANOVA) followed by Bonferroni test. Data are shown in mean ± SE (n=6 for olprinone, papaverine, milrinone, benidipine, nicardipine, nicorandil and nifedipine; n=5 for Y-27632). A p value <0.05 was considered to be statistically significant.

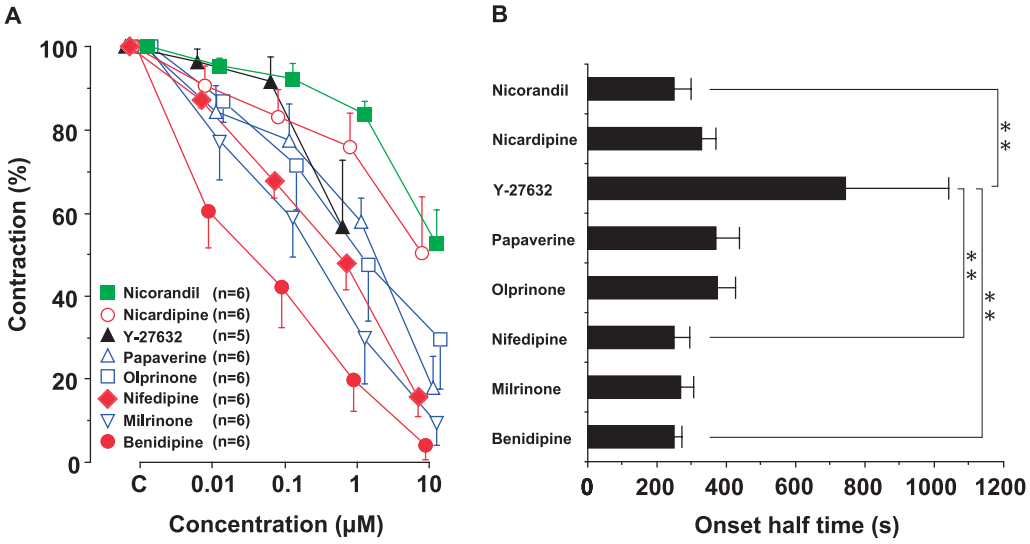

Fig. 1 Summary of the potency (A) and speed (B) of vasodilating action of a K^+-channel opener (nicorandil), 3 kinds of Ca^{2+}-channel blockers (nicardipine, nifedipine and benidipine), a Rho-kinase inhibitor (Y-27632) and 3 of phosphodiesterase inhibitors (papaverine, olprinone and milrinone) on the noradrenaline-induced contraction model of human internal mammary artery. Onset half time: speed of vasodilating action evaluated by measuring the time required to reach the 1/2 peak effect after the highest dose administration. Data are presented in mean ± SE (n = 6 for phosphodiesterase inhibitors, Ca^{2+}-channel blockers and K^+-channel opener; n = 5 for Rho-kinase inhibitor). $**P < 0.01$.

Results

Forty-seven out of 68 preparations showed dilatation after the treatment with acetylcholine, reflecting the presence of intact endothelial cells. All the drugs showed vasodilator effect on the preparations in a concentration-related manner as shown in Fig. 1A, whereas the results of the onset half time were depicted in Fig. 1B. Typical traces showing the vasodilator effects of nicorandil, Y-27632, milrinone and benidipine are depicted in Fig. 2. Benidipine showed the most potent effect on the dilatation against the noradrenaline-induced contraction, whereas nicorandil exerted the least relaxation among the eight drugs. The potency of relaxation was in the order of benidipine, milrinone, nifedipine, olprinone, papaverine, Y-27632, nicardipine and nicorandil with pEC_{50} (= $-\log EC_{50}$) (M) of 7.32±0.34, 6.78±0.34, 6.22±0.20, 6.05±0.47, 5.99±0.19, 5.98±0.25, 5.14±0.38 and 4.99±0.18, respectively. There were significant differences in these pEC_{50} values between benidipine and nicorandil ($p<0.05$); benidipine and nicardipine ($p<0.05$); milrinone and nicorandil ($p<0.05$); and milrinone and nicardipine ($p<0.05$). On the other hand, the speed of vasodilator action was in the order of benidipine, nifedipine, nicorandil, milrinone, nicardipine, papaverine, olprinone and Y-27632. There were significant differences in the onset half time between Y-27632 and nicorandil ($p<0.01$); Y-27632 and nifedipine ($p<0.01$); and Y-27632 and benidipine ($p<0.01$).

Discussion

We compared the potency and speed of vasodilator action of 4 different pharmacological classes of drugs on human internal mammary arteries in vitro by using 3 kinds of phosphodiesterase inhibitors, a Rho-kinase inhibitor, 3 of Ca^{2+}-channel blockers and a K^+-channel opener, each of which dilated the arteries in a concentration-related manner, whereas their potencies may not be necessarily related to the pharmacological classification. Meanwhile, Y-27632 showed the slowest onset speed for vasodilator action, whereas no significant difference was observed in the onset half time among the 7 vasodilators other than Y-27632.

The potency of 3 of Ca^{2+}-channel blockers was in the order of benidipine >nifedipine >nicardipine, which could be partly explained by the following previous knowledge. Intracoronary infusion of benidipine and nifedipine for 5 min in dogs has been reported to increase coronary blood flow via the NO-dependent mechanism in addition to the blockade of Ca^{2+} channels.[5,6] Benidipine has been shown to have a triple Ca^{2+} channels (L, N and T) blocking action with a

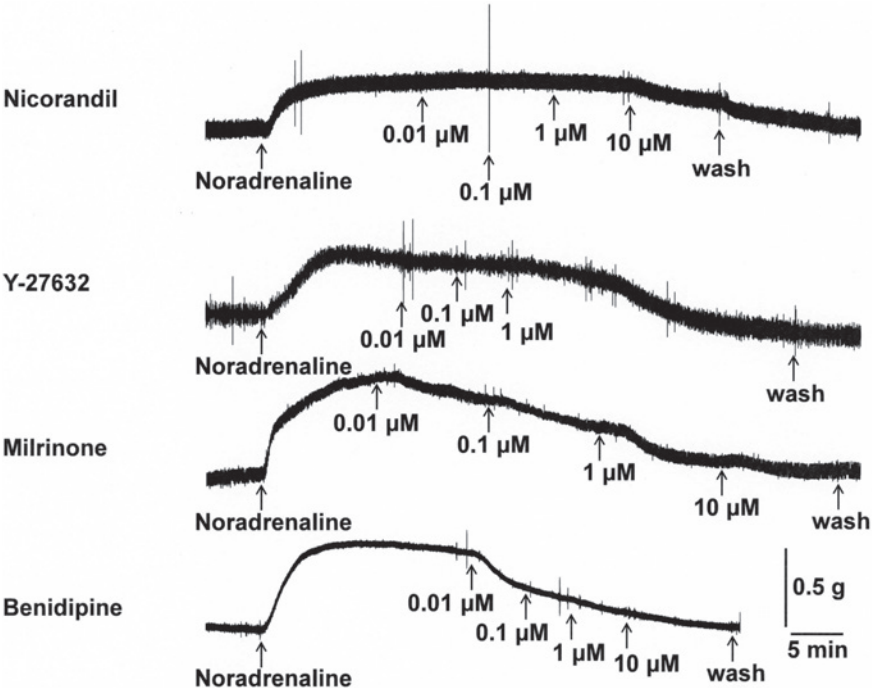

Fig. 2 Typical traces showing the vasodilator effects of nicorandil, Y-27632, milrinone and benidipine on the noradrenaline-induced contraction model of human internal mammary artery.

membrane approach, whereas nifedipine and nicardipine selectively inhibit L-type Ca^{2+} channel.[7] Moreover, benidipine can modestly suppress receptor-operated Ca^{2+} channels in the internal mammary artery graft.[8] The EC_{50} value of benidipine (pEC_{50} (M) = 7.32) is almost equal to that of nitroglycerin (pEC_{50} (M) = 7.42) in our previous study,[3] suggesting that benidipine could be also effective strategy against perioperative bypass-graft spasm like nitroglycerin.

Milrinone and olprinone belong to phosphodiesterase-3 inhibitor, whereas papaverine is a nonspecific phosphodiesterase inhibitor.[9, 10] These three phosphodiesterase inhibitors induced concentration-dependent relaxations against noradrenaline-constricted human internal mammary arterial rings with similar potency, suggesting that the difference of subtype selectivity for phosphodiesterases may not affect their vasodilator action.

Nicorandil is a sarcolemmal ATP-sensitive K^+-channel opener having nitrate component, which can potently dilate coronary and peripheral vessels and has been used for patients with angina pectoris.[11, 12] The potency of relaxation by nicorandil was the weakest among 8 drugs, suggesting that effects of nicorandil on the sarcolemmal ATP-sensitive, K^+-channels may be least associated with vasospasm of internal mammary artery in this study. On the other hand, Y-27632, a specific inhibitor of Rho-kinase,[13, 14] was also assessed; however, its vasodilator effect was not so great, suggesting that Rho-kinase may be modestly related to the pathophysiology of vasospasm in this study.

There are some limitations in this study. First, we do not have information on the activity of Rho or phosphodiesterase in the currently used internal mammary arteries, which needs to be analyzed using much more samples from patients who will undergo bypass graft surgery. Second, there were 2 reports describing the increase of NO production by intracoronary infusion of nifedipine and benidipine for 5 min in dogs;[5, 6] however, there was no such report for the internal mammary artery grafts. Third, although there was one report describing the expression of mRNA levels as well as pharmacological activity of L- and T-type Ca^{2+} channels in human internal mammary artery grafts,[15] there was no report demonstrating the presence of N-type Ca^{2+} channel in them.

In conclusion, the potency of vasodilator action was in the order of benidipine >milrinone >nifedipine >olprinone >papaverine >Y-27632 >nicardipine >nicorandil, which is new finding in this study. Benidipine can be most expected to become an efficacious candidate for ameliorat-

ing vasospasm of internal mammary artery in clinical settings.

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Conflicts of interest: None declared.

Author Contributions: Koki Chiba and Yuji Nakamura contributed equally to this manuscript.

References

1) Kleszczewski T, Buzun L, Lisowska A, Modzelewska B. Potassium induced contraction of the internal thoracic artery in vitro is time related: the potential consequences in the analysis of the mechanism of the spasm after coronary artery bypass grafting and in the analysis of the results of in vitro studies. Heart Vessels. 2016; 31: 616-21.
2) Harskamp RE, McNeil JD, van Ginkel MW, Bastos RB, Baisden CE, Calhoon JH. Postoperative internal thoracic artery spasm after coronary artery bypass grafting. Ann Thorac Surg. 2008; 85: 647-9.
3) Fukuda S, Nakamura Y, Egi K, Fujioka S, Nagasaka S, Minh PN, et al. Comparison of direct effects of clinically available vasodilators; nitroglycerin, nifedipine, cilnidipine and diltiazem, on human skeletonized internal mammary harvested with ultrasonic scalpel. Heart Vessels. 2016; 31: 1681-4.
4) Higami T, Yamashita T, Nohara H, Iwahashi K, Shida T, Ogawa K. Early results of coronary grafting using ultrasonically skeletonized internal thoracic arteries. Ann Thorac Surg. 2001; 71: 1224-8.
5) Kitakaze M, Node K, Minamino T, Asanuma H, Kuzuya T, Hori M. A Ca channel blocker, benidipine, increases coronary blood flow and attenuates the severity of myocardial ischemia via NO-dependent mechanism in dogs. J Am Coll Cardiol. 1999; 33: 242-9.
6) Kitakaze M, Asanuma H, Takashima S, Minamino T, Ueda Y, Sakata Y, et al. Nifedipine-induced coronary vasodilation in ischemic hearts is attributable to bradykinin- and NO-dependent mechanism in dogs. Circulation. 2000; 101: 311-7.
7) Yao K, Nagashima K, Miki H. Pharmacological, pharmacokinetic, and clinical properties of benidipine hydrochloride, a novel, long-acting calcium channel blocker. J Pharmacol Sci. 2006; 10: 243-61.
8) Hou HT, Wang J, Wang ZQ, Liu XC, Marinko M, Novakovic A, et al. Effect of benidipine in human internal mammary artery and clinical implications. Ann Thorac Surg. 2016; 101: 1789-95.
9) Onomoto M, Tsuneyoshi I, Yonetani A, Suehiro S, Matsumoto K, Sakata R, et al. Differential pharmacologic sensitivities of phosphodiesterase-3 inhibitors among human isolated gastroepiploic, internal mammary, and radial arteries. Anesth Analg. 2005; 101: 950-6.
10) Abusnina A, Lugnier C. Therapeutic potentials of natural compounds acting on cyclic nucleotide phosphodiesterase families. Cell Signal. 2017; 39: 55-65.
11) Luo B, Wu P, Bu T, Zeng Z, Lu D. All-cause mortality and cardiovascular events with nicorandil in patients with IHD: systematic review and meta-analysis of the literature. Int J Cardiol. 2014; 176: 661-9.
12) Yanagisawa T, Teshigawara T, Taira N. Cytoplasmic calcium and the relaxation of canine coronary arterial smooth muscle produced by cromakalim, pinacidil and nicorandil. Br J Pharmacol. 1990; 101: 157-65.
13) Liao JK, Seto M, Noma K. Rho kinase (ROCK) inhibitors. J Cardiovasc Pharmacol. 2007; 50: 17-24.
14) Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, et al. Calcium sensitization of smooth muscle mediated by a rho-associated protein kinase in hypertension. Nature. 1997; 389: 990-4.
15) Aley PK, Wilkinson JA, Bauer CC, Boyle JP, Porter KE, Peers C. Hypoxic remodelling of Ca^{2+} signalling in proliferating human arterial smooth muscle. Mol Cell Biochem. 2008; 318: 101-8.