Coronary Artery Diameter Increase Induced by a Phosphodiesterase 5 Inhibitor, E4021, in Conscious Pigs

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ABSTRACT—The effects of intravenous infusions of 1, 3 and 10 $\mu\text{g/kg/min}$ of the phosphodiesterase 5 inhibitor, E4021, at 30-min intervals on coronary artery diameter were studied in 8 conscious pigs monitored with a pair of piezoelectric crystals. The highest dose increased the diameter by $2.9 \pm 0.5\%$ ($P < 0.01$ vs vehicle) of the baseline diameter, with a significant decrease in mean pulmonary arterial pressure. However, there were no changes in mean aortic pressure and heart rate. Additionally, E4021 significantly prolonged the duration of the diameter increase induced by nitroglycerin. Thus, phosphodiesterase 5 inhibition causes coronary artery diameter increase and produces an amplifying effect with nitroglycerin.

Keywords: Phosphodiesterase 5 inhibitor, Coronary diameter, Conscious pig

Cyclic 3',5'-guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE5) is one of at least seven distinct but related gene families of cyclic nucleotide PDEs. PDE5 hydrolyzes cGMP synthesized from guanosine triphosphate (1). We found that E4021 (sodium 1-[6-chloro-4-(3,4-methylenedioxybenzyl) aminoquinazolin-2-yl]piperidine-4-carboxylate) selectively and potently inhibits PDE5 (2). E4021 relaxes porcine coronary artery preparations precontracted with prostaglandin $F_{2\alpha}$ and has a synergistic effect with nitroglycerin in both the relaxant effect and the elevation of cGMP levels (2). In addition, E4021 administered intravenously (i.v.) ameliorates myocardial ischemia evoked by a vasoconstricting substance similarly to nitrovasodilators in guinea pigs (3). Although these findings suggest the clinical effectiveness of E4021 as a remedy for coronary artery disease including angina pectoris, it is not clear whether the compound may directly dilate the epicardial large coronary artery, a target tissue of coronary vasodilators, in whole animals.

In the present study, we evaluated the effects of PDE5 inhibition by E4021 on the coronary artery diameter, and we examined the interaction of E4021 and a representative nitrovasodilator, nitroglycerin, in coronary artery diameter increase in conscious pigs chronically monitored with a pair of piezoelectric crystals.

Experiments were performed according to our company's guidelines for animal experimentation (Eisai Research Laboratories, Tsukuba). Eight male farm pigs (18.5 ± 1.3 kg; Nihon Nosan Kogyo, Yokohama) were

used after an overnight fast. They were anesthetized with 20 mg/kg of sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA) injected into the dorsal ear vein. After each animal was ventilated by the inhalation of a mixture of O_2 and N_2O (1:2) to which 0.6–1.0% enflurane (Abbott) was added, the right external jugular vein and the right common carotid artery were exposed by a midline incision in the neck and cannulated with polyethylene catheters (6Fr; Atom, Tokyo) for the infusion of E4021 and measurement of aortic pressure, respectively.

A left thoracotomy was carried out through the fifth intercostal space. A miniature pressure gauge (P6.5; Konigsberg Instruments Inc., Pasadena, CA, USA) was inserted into the left ventricular cavity through a small incision in the apex for the measurement of left ventricular pressure (LVP). Two polyethylene catheters (6Fr, Atom) were inserted, one into the left atrium for the measurement of left atrial pressure and intra-atrial injection of nitroglycerin and one into the base of the pulmonary artery for the measurement of pulmonary arterial pressure. The LVP gauge was calibrated in vivo with measurements of pressure from two catheters (one in the aortic arch and one in the left atrium) connected to external transducers (TP-2001; Nihon Kohden, Tokyo). The left circumflex coronary artery was dissected minimally 2–4 cm from the left main coronary artery. A pair of 7 MHz piezoelectric crystals (Murata, Kyoto) was glued to the opposite adventitial surfaces of the coronary artery using a medical adhesive agent (Aron Alpha; Sankyo,

Tokyo) as described previously (4, 5). The epicardial coronary artery diameter was measured with an ultrasonic dimension system (UDM-5C; MECC, Fukuoka). A Doppler flow probe (HDP 2520S; Crystal Biotech, Holliston, MA, USA) was placed around the coronary artery distal to the piezoelectric crystals to measure the coronary flow velocity.

The chest cavity was closed after all wires and catheters

were passed subcutaneously to the back of the neck and secured between the scapulae. Ampicillin 0.5 g (Viccillin; Meiji Seika, Tokyo) was administered orally once daily for 6 days to prevent infection. The catheters were maintained patent during the experiment by flushing with fresh heparin solution (100 U/ml; Green Cross, Osaka).

The cardiohemodynamic variables of coronary artery diameter, coronary flow velocity, pulmonary arterial

Fig. 1. Time courses of the effects of i.v. infused E4021 (solid circles, $n=6-8$) at doses 1, 3 and 10 μ g/kg/min and its vehicle (0.01 N NaOH solution, open circles, $n=3-5$) on coronary diameter (CoD, A), coronary flow velocity (CFV, B), mean pulmonary arterial pressure (MPAP, C), mean aortic pressure (MAoP, D), maximal rate of increase in left ventricular pressure (LV dP/dt max, E) and heart rate (HR, F) in conscious pigs. The baseline values of coronary diameter before the infusions of E4021 and its vehicle were 2.74 ± 0.19 and 2.49 ± 0.24 mm, respectively. All values are the mean $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ vs vehicle.

pressure, aortic pressure, LVP, maximal rate of increase in the LVP (LV dP/dt max) and heart rate were recorded simultaneously on a multichannel pen-recorder (Polygraph System, Nihon Kohden).

The experiments were started about 7 days after surgery. The pigs were placed in a sling cage during the experiments. The increase in the coronary artery diameter with nitroglycerin (10 µg/kg, i.v.), a potent vasodilator of large coronary arteries (4, 6), was evaluated to ensure that the vasomotion of the coronary artery was not impeded by the surgical procedure or instrumentation. The effects of E4021 and its vehicle were examined on separate days.

Protocol 1: To examine the cardiohemodynamic effects of E4021, three sequential 30-min i.v. infusions of E4021 (1, 3 and 10 µg/kg/min) or one infusion of the vehicle (0.01 N NaOH solution) for 90 min was carried out in eight conscious pigs.

Protocol 2: To examine the amplifying effects of E4021 on the coronary artery diameter increase with nitroglycerin, nitroglycerin (1 and 10 µg/kg, n=7) was injected into the left atrium before and after i.v. infusion of E4021, carried out in Protocol 1.

E4021 (Eisai Tsukuba Research Laboratories) was dissolved in 5 ml of 0.01 N NaOH solution and used for the 30-min infusion. The volume of infusion was fixed at 5 ml/30 min for each dose of E4021. Nitroglycerin (Nihonkayaku, Tokyo) was dissolved in 0.9% saline. The solutions of the drugs were freshly prepared before each experiment.

All data are expressed as the mean ± S.E.M. The value for each cardiohemodynamic parameter after the E4021 infusion was compared with the value after the vehicle infusion by Student's unpaired *t*-test. The comparison of the coronary artery increase with nitroglycerin was performed by the paired *t*-test. *P* values less than 0.05 were considered significant.

The time courses of the effects of the three sequential 30-min i.v. infusions of E4021 at 1, 3 and 10 µg/kg/min on cardiohemodynamic parameters are shown in Fig. 1. The vehicle did not affect any parameters measured in the present study. E4021 increased the coronary artery diameter from the baseline diameter (2.74 ± 0.19 mm) in a dose-dependent manner. The maximum dilation, produced by the highest dose was 2.9 ± 0.5% (n=8, *P* < 0.01 vs vehicle). At this time, the mean pulmonary arterial pressure decreased by -16.7 ± 3.6% (n=7, *P* < 0.05) from the baseline value of 20.5 ± 1.1 mmHg, whereas the coronary flow velocity, mean aortic pressure, LV dP/dt max and heart rate were not influenced.

The maximum increase and the duration of the increase in coronary diameter (time from an intra-left atrial injection to the point of 50% of its maximum increase) induced by nitroglycerin at 1 and 10 µg/kg before and after

Table 1. Effects of E4021 on coronary artery diameter increase with nitroglycerin in conscious pigs

E4021			
	before	after	
Nitroglycerin (1 $\mu\text{g/kg}$)			
CoD (basal value, μm)	2779 $\pm$ 199	2798 $\pm$ 202	NS
ΔCoD (%)	5.6 $\pm$ 0.7	5.5 $\pm$ 0.9	NS
T 1/2 (min)	2.0 $\pm$ 0.4	3.6 $\pm$ 0.9	NS
Nitroglycerin (10 $\mu\text{g/kg}$)			
CoD (basal value, μm)	2797 $\pm$ 195	2799 $\pm$ 198	NS
ΔCoD (%)	8.2 $\pm$ 1.3	7.1 $\pm$ 0.8	NS
T 1/2 (min)	4.7 $\pm$ 0.9	6.6 $\pm$ 1.1	P<0.01

All values are the mean ± S.E.M. of seven animals. Nitroglycerin (1 and 10 µg/kg) was injected into the left atrium before and after the i.v. infusion of E4021 (1, 3 and 10 µg/kg/min). T 1/2 indicates the time from the nitroglycerin injection to the point of 50% of its maximum increase. CoD: coronary diameter, NS: not significant.

the E4021 infusion are summarized in Table 1. The injection of nitroglycerin after E4021 treatment was carried out after recovery from the coronary artery diameter increase with E4021. The maximum increases produced by the two doses of nitroglycerin were not influenced by the E4021 treatment, but the duration of the coronary artery diameter increase with the high dose was significantly prolonged; from 4.7 ± 0.9 min before the E4021 infusion to 6.6 ± 1.1 min after the E4021 infusion (n=7, *P* < 0.01).

Nitrovasodilators activate soluble guanylate cyclase with a resulting increase in the biosynthesis of cGMP and activation of cGMP-dependent protein kinase. The protein kinase activation is thought to decrease the cytosolic calcium concentration in vascular smooth muscle cells and lead to smooth muscle relaxation (7). If cGMP has a key role in the modulation of physiological vascular function and in the relaxant responses elicited by nitrovasodilators and endogenous nitric oxide, agents that inhibit PDE5 and then increase the cGMP level should produce vasodilation. The elucidation of the role of PDE5 in regulating coronary vascular tone and the clinical effectiveness of PDE5 inhibitors in treating coronary vascular disease has awaited the development of more potent and selective inhibitors for several years.

Although some selective PDE5 inhibitors including WIN 58237 (8), 1,3-dimethyl-6-(2-propoxy-5-methane sulphonylamidophenyl)-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (9, 10) and sildenafil (11, 12) have been recently developed, the characteristics of the vasodilating effects of these compounds on large coronary arteries have not been clarified in whole animals. We have also found a novel, selective and potent PDE5 inhibitor, E4021 (2). Although E4021 relaxed porcine coronary artery with endothelium,

its vasorelaxant action was weaker after pretreatment with a nitric oxide synthetic inhibitor or in preparations without endothelium (2). On the other hand, the coronary artery diameter, which is influenced by changes in systemic hemodynamics (13), was not affected by E4021, as evidenced by aortic pressure, heart rate and coronary flow velocity in the present experiment. These results suggest that E4021 potentiates coronary artery diameter increase due to an increase in cGMP level induced by nitric oxide. Previously, E4021 was shown to have a synergetic effect with nitroglycerin in the relaxant effect in isolated porcine coronary artery. In addition, the combination of nitroglycerin and E4021 produced a greater and more sustained increase in cGMP level than that with nitroglycerin alone and the sustained effect was marked (2). These *in vitro* findings could be extrapolated to the results that we have shown for the first time: that E4021 prolonged the coronary artery diameter increase with nitroglycerin without influencing its maximum response.

In the present study, E4021 showed a significant reduction in pulmonary arterial pressure unlike its effect on aortic pressure. Pulmonary vascular tone is relieved through the enhanced cGMP level produced by endogenous pulmonary vasodilators such as atrial natriuretic peptide (14) and nitric oxide (15). Accordingly, the selective pulmonary vasodilation of E4021 may be associated with the high level of guanylate cyclase activity activated by atrial natriuretic peptide and nitric oxide in the pulmonary vascular smooth muscle.

Nitrovasodilators that have been clinically used for the treatment of coronary vascular disease are characterized by undesirable side effects such as nitrate tolerance, headaches, hypotension and reflex tachycardia. In addition to the evidence that PDE5 inhibition with E4021 produces coronary artery diameter increase, the absence of side effects including overdepression of blood pressure, reflex tachycardia and marked small coronary artery dilation with E4021 treatment are positive aspects of its cardiohemodynamic profile.

In conclusion, PDE5 inhibition by E4021 caused coronary artery diameter increase without any changes in aortic pressure, heart rate and coronary flow velocity, and E4021 had an amplifying effect with nitroglycerin on coronary artery diameter increase. Accordingly, the development of a selective PDE5 inhibitor could represent a novel therapeutic approach to the management of cardiovascular disorders such as angina pectoris.

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