Original Article

Inhibition of Nitrate Tolerance without Reducing Vascular Response during Eccentric Dosing of Nitrates

Hideki MORII[1)], Norikatsu NAITO[1)], Kazue NAKANO[1)], and Ken KANAMASA[1)]

It has been reported that the nitrate tolerance related to continuous dosing of nitrates reduces drug efficacy, and therefore eccentric dosing of nitrates is recommended. In this study, we investigated the appearance of nitrate tolerance related to continuous dosing of nitrates and prevention of nitrate tolerance during eccentric dosing by comparing the grade of coronary dilatation after sublingual nitroglycerin. Of 26 patients with ischemic heart disease who underwent elective cardiac catheterization, 8 patients were continuously administered nitrates, 8 patients were eccentrically administered nitrates, and 10 patients were not treated. We compared the coronary response to sublingual nitroglycerin among the 3 groups. In a coronary vessel without significant stenosis, the coronary vessel area, coronary lumen area, and mean coronary blood flow velocity after sublingual nitroglycerin were measured using intravascular ultrasound (IVUS). In the continuous dosing group, the maximal rate of change in the vessel area after sublingual nitroglycerin was 105±1 (mean±SEM) %, significantly lower than those in the untreated group and the eccentric dosing group (114±2%, 114±2%) ($p<0.01$, respectively). In conclusion, eccentric dosing of nitrates inhibited the appearance of nitrate tolerance without reducing vascular response. (*Hypertens Res* 2006; 29: 797–804)

***Key Words*: nitroglycerin, intravascular ultrasound, vasodilation**

Introduction

Nitrates are routinely used to treat ischemic heart disease; however, it is known that continuous dosing of nitrates induces tolerance (*1*, *2*). With respect to systemic circulation and pulmonary circulation, it has been reported that 24-h administration of nitrates induces nitrate tolerance (*3*). With respect to their direct actions on coronary circulation, nitrates dilate the coronary arteries (*4*), increasing coronary blood flow (CBF) (*5*). In many studies examining nitrate tolerance in patients with ischemic heart disease, improvement in exercise tolerance was used as an index (*6*), and thus the actions of nitrates on the coronary circulation and the appearance of nitrate tolerance remain unclear in many respects. It has been proposed that an attenuation of nitrate efficacy may be overcome by allowing a daily nitrate-free period. Eccentric dosing of nitrates with a daily nitrate-free period has been considered to produce encouraging results. De Milliano *et al*. (*7*) showed that a nitrate-free interval of 10 h prevents nitrate tolerance. Waters *et al*. (*8*) observed that the time-to-angina during exercise did not differ among a group given continuous nitroglycerin, a group given nitroglycerin with a "6 h-off" regimen, and a group given placebo, but was significantly longer in a group given nitroglycerin with a 10 h-off regimen. DeMots and Glasser (*9*) reported that eccentric (12 h on/12 h off) transdermal nitroglycerin therapy improved exercise tolerance in both a lower dose group (equivalent to 5 or 10 mg/24 h) and a higher dose group (equivalent to 15 or 20 mg/24 h). Therefore, we used eccentric (12 h on/12 h off) transdermal nitroglycerin therapy in this study.

For the first time, we compared the actions of sublingual nitroglycerin on coronary circulation between continuous dosing of nitrates and eccentric dosing of nitrates using intra-

From the [1)]Department of Vascular and Geriatric Medicine, Kinki University School of Medicine, Osaka-Sayama, Japan.

Address for Reprints: Ken Kanamasa, M.D., Department of Vascular and Geriatric Medicine, Kinki University School of Medicine, Ohno-Higashi 377–2, Osaka-Sayama 589–8511, Japan. E-mail: kanamasa@med.kindai.ac.jp

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Table 1. Clinical Characteristics of the Study Population

	No dose	Eccentric dose	Continuous dose
Gender (M/F)	7/3	7/1	8/0
Age* (years)	62±9	60±11	62±6
OMI/AP	6/4	6/2	6/2
Nitrate administration period* (month)	—	1.7±0.7	2.0±0.6
Coronary risk factors (%)			
Hyperlipidemia	20	25	63
Hypertension	50	25	38
Diabetes mellitus	40	38	25
Smoking	50	88	78
Gout	10	0	0
Obesity	20	13	13
Combined medications (%)			
Antiplatelet agents	90	100	100
β-Blockers	70	88	63
Lipid lowering agents	50	25	63
Nicorandil	40	38	13
Ca blockers	30	25	38
ACE inhibitors	30	63	25
Warfarin	0	0	0

*Mean±SEM. M, male; F, female; OMI, old myocardial infarction; AP, angina pectoris; ACE, angiotensin converting enzyme.

vascular ultrasound (IVUS) and a coronary Doppler flow-wire.

Methods

Subjects

The subjects were 26 patients (22 males and 4 females; mean age, 61±2 [mean±SEM] years; range, 42–77 years) with chronic ischemic heart disease. Eighteen of the patients had old myocardial infarction and 8 patients had effort angina. Agents prescribed at the outpatient clinic were not discontinued. We excluded patients in whom cardiac catheterization revealed 50% or more stenosis in the left anterior descending coronary artery or abnormalities in anterior wall movement. The 26 patients were randomly divided into 3 groups: 10 patients who were not treated with nitrates (untreated group), 8 patients who were eccentrically treated with nitrates (eccentric dosing group), and 8 patients who were continuously treated with nitrates (continuous dosing group). Randomization of this study was performed by the envelope method. Briefly, a sequential number was ascribed to each envelope. A dosing method (no treatment, eccentric dosing, or continuous dosing) was assigned randomly to each piece of paper, and the envelope was sealed. The person who performed this work was not the same person who opened the envelopes. After registration of the patient, the sequentially assigned envelope was opened.

Study Design

As nitrates, nitroglycerin patches (10 cm^2 per patch, 25 mg) (25 patients) and isosorbide mononitrate (ISMN, 20 mg tablets, po) (1 patient) were used. For eccentric dosing (*1*, *2*, *10*), patches were applied at 8:00 AM, and exfoliated at 8:00 PM in patients with anginal attacks on effort during the daytime, whereas patches were applied after bathing, and exfoliated at 8:00 AM the next morning in patients with attacks at night or early in the morning. For continuous dosing, patches (nitroglycerin) were applied for 24 h, and an oral agent (ISMN) was administered in the morning and evening twice a day.

In the eccentric dosing group, intervals between the start of eccentric application and measurement ranged from 0.2 to 5.0 months, with a mean of 1.7±0.7 months, whereas in the continuous dosing group, intervals between the start of application of the nitrates and measurement ranged from 0.2 to 4.9 months with a mean of 2.0±0.6 months. In the eccentric/continuous dosing groups, the mean interval from application or oral administration of nitrates until sublingual nitroglycerin during cardiac catheterization was 3.8±1.5 h, respectively. Prior to cardiac catheterization, the purpose, methods, and risk of this examination were explained, and written informed consent was obtained from all patients. An institutional review committee approved the study and the subjects gave informed consent.

Cardiac Catheterization

Nitrates and other combined agents were continued on car-

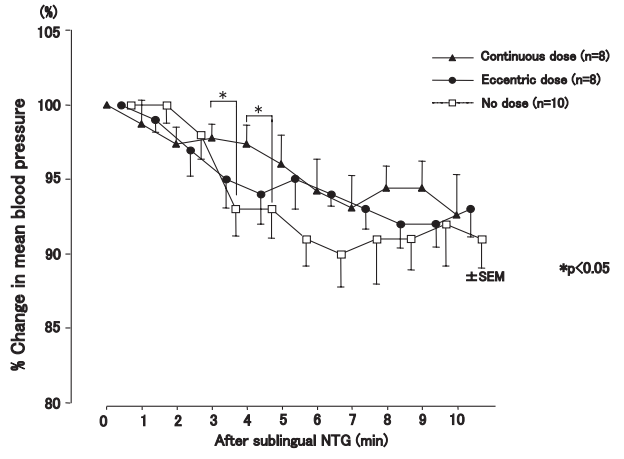

Fig. 1. *Percent change in mean blood pressure after sublingual nitroglycerin (NTG) (mean±SEM).*

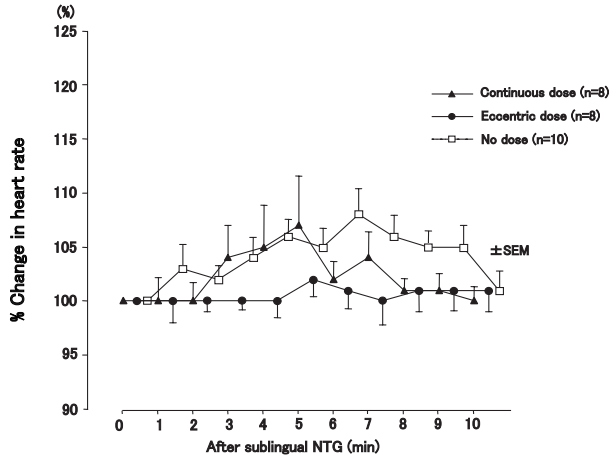

Fig. 2. *Percent change in heart rate after sublingual nitroglycerin (NTG) (mean±SEM).*

diac catheterization, and cardiac catheterization was performed between 10:00 AM and 3:00 PM. Bilateral coronary angiography was performed without coronary infusion of isosorbide dinitrate (ISDN). A coronary Doppler flow-wire was inserted to the proximal left anterior descending coronary artery, and an IVUS catheter was inserted in a position where good blood flow waveforms and IVUS findings could be obtained. After stable blood flow waveforms and IVUS findings were obtained for 5 min or more, 0.3 mg of nitroglycerin was administered *via* the sublingual route, and the aortic pressure, heart rate, CBF velocity, coronary vessel area, and coronary lumen area were recorded over 10 min at 1-min intervals. The vessel area obtained by tracing the border between the media and adventitia at the end-diastole of the R wave peak on electrocardiograms was regarded as the external elastic membrane cross-sectional vessel area, which was defined as the coronary vessel area. The vessel area obtained by tracing the border between the coronary lumen and the intima was regarded as the lumen cross-sectional area, which was defined as the coronary lumen area. Aortic pressure was measured using a guiding catheter, and heart rate was measured on electrocardiograms. Ten min later, the Doppler flow-wire was removed. As an anticoagulant, 10,000 U/ml of heparin was intravenously administered.

Doppler Flow-Wire

We used a Flomap-5500 (Cardiometrics Inc., Rancho Cordova, USA) and a Doppler flow-wire containing a transducer (transmission frequency: 12 MHz) at the end of a percutaneous transluminal coronary angioplasty guidewire measuring 0.018 inches (0.46 mm) in outer diameter and 175 cm in length, respectively, which facilitated blood flow recording at an area 5 mm from the flow-wire end. The frequency was 40 kHz, and the sample volume measured 0.65 mm in thickness and 1.7 mm in diameter. To evaluate Doppler blood flow signals, we used an instrument (*11*) in which spectrograms were indicated after fast Fourier transform (FFT) analysis. Diastolic peak CBF velocity and mean CBF velocity were calculated in comparison to the pretreatment values. CBF and coronary vascular resistance (CVR) were calculated using the formulae described by Doucette *et al*. (*11*):

$$\text{CBF} = \{\pi(\text{CD})^2/4\} \times \{\text{APV} \times 60/2\}$$
$$= \text{coronary lumen area} \times \text{APV} \times 7.5,$$

where CD is the coronary artery diameter and APV is the average peak velocity; and

$$\text{CVR} = \text{mBP}/\text{CBF},$$

where mBP is the mean blood pressure.

Intravascular Ultrasound

We used an Ultra Cross 3.2 (30 MHz intravascular ultrasound; Boston Scientific, Fremont, USA) and a Clear View Ultra (Boston Scientific) for analysis. A coronary guiding catheter was inserted into the left coronary main trunk, and a catheter for IVUS was inserted into the guiding catheter using a guide-wire, then guided to the right coronary artery or proximal left anterior descending coronary artery. On coronary tomograms recorded in videotape, the following areas were measured using a planimeter: the area inside a high-brightness inner echogenic ring (*12*) (*A*), and the area occupied by the outer circumference of the media (*B*). The area calculated by subtracting *A* from *B* was regarded as the plaque area, and the %plaque area was calculated using the following formula:

$$\%\text{plaque area} = (B - A)/B \times 100.$$

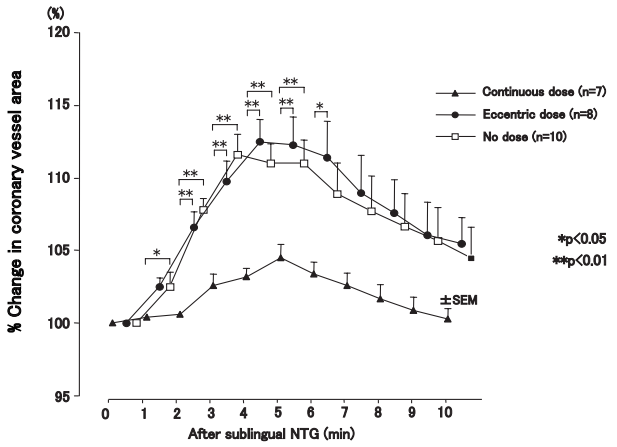

Fig. 3. *Percent change in coronary vessel area after sublingual nitroglycerin (NTG) (mean±SEM).*

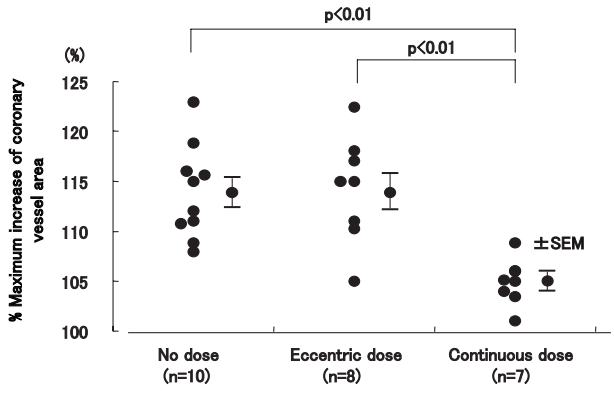

Fig. 4. *Percent maximum increase of the coronary vessel area (mean±SEM).*

Statistical Analysis

All values are expressed as the mean±SEM. For statistical analysis, one-way ANOVA was used, and values of $p<0.05$ were regarded as statistically significant.

Results

Clinical Background

The subjects were 18 patients with old myocardial infarction and 8 patients with effort angina; all 26 patients had significant stenosis in 1 of 3 coronary vessels. The mean intervals from the start of administration of nitrates until coronary angiography were 1.7±0.7 months in the eccentric dosing group and 2.0±0.6 months in the continuous dosing group (Table 1).

Mean Aortic Blood Pressure

There were no significant differences in mean aortic blood pressure before sublingual nitroglycerin among the 3 groups (continuous dosing group, 105.9±6.6 mmHg; eccentric dosing group, 90.5±2.6 mmHg; untreated group, 107.4±8.5 mmHg). There was a significant difference in the rate of change in mean aortic blood pressure between the continuous dosing group and the untreated group 3 and 4 min after sublingual nitroglycerin (Fig. 1).

Heart Rate

There were no significant differences in heart rate before sublingual nitroglycerin among the 3 groups (continuous dosing group, 67.4±4.8 bpm; eccentric dosing group, 67.0±3.6 bpm; untreated group, 73.5±3.9 bpm). Furthermore, there were no significant differences in the rate of change in heart rate among the 3 groups (Fig. 2).

Coronary Vessel Area

There were no significant differences in the absolute values of this parameter before sublingual nitroglycerin among the 3 groups (continuous dosing group, 16.8±2.4 mm^2; eccentric dosing group, 13.8±1.6 mm^2; untreated group, 14.9±1.1 mm^2). In the continuous dosing group, the rate of vascular dilatation was significantly smaller than that in the eccentric dosing group between 2 min and 6 min after sublingual administration of nitroglycerin, and it was also significantly smaller than that in the untreated group between 1 min and 5 min after sublingual administration of nitroglycerin (Fig. 3). In the continuous dosing group, the maximal rate of change in the vessel area was 105±1%, which was significantly smaller than those in the eccentric dosing group and the untreated group (114±2%, 114±2%, $p<0.01$, respectively) (Fig. 4).We excluded 1 patient in the continuous dosing group in whom it was difficult to measure the external elastic membrane cross-sectional area on IVUS.

Coronary Lumen Area

There were no significant differences in the absolute values of this parameter before sublingual nitroglycerin among the 3 groups (continuous dosing group, 13.4±1.9 mm^2; eccentric dosing group, 9.5±1.0 mm^2; untreated group, 11.5±1.0 mm^2). In the continuous dosing group, the rate of lumen cross-sectional area increase was significantly smaller than that in the eccentric dosing group between 2 min and 5 min after sublingual nitroglycerin (Fig. 5). Furthermore, the maximal rate of change in the coronary lumen area in the continuous dosing group was 108±2%, whereas the rates were 119±3% and 114±2% in the eccentric dosing group and the untreated group, respectively, with a significant difference between the continuous dosing group and the eccentric dosing group ($p<0.05$, Fig. 6). However, we excluded 1 patient in

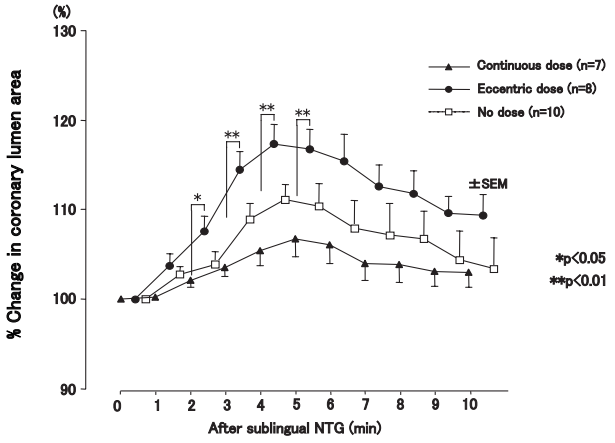

Fig. 5. *Percent change in coronary lumen area (mean±SEM).*

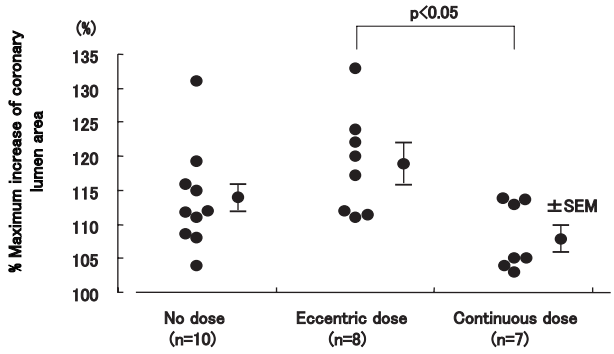

Fig. 6. *Percent maximum increase of the lumen area (mean±SEM).*

the continuous dosing group in whom it was difficult to measure the coronary lumen area due to marked non-uniform rotational distortion (NURD) on IVUS.

Rate of Change in the Average Peak Velocity

There were no significant differences in absolute APV values before sublingual nitroglycerin among the 3 groups (continuous dosing group, 17.3±3.3 cm/s; eccentric dosing group, 19.6±3.2 cm/s; untreated group, 26.6±7.5 cm/s). In the continuous dosing group, the rate of change in APV was 93±7% at 9 min after sublingual nitroglycerin, whereas the rate in the untreated group was a significantly smaller 66±9% (Fig. 7). However, we excluded 1 patient in the untreated group in whom no good Doppler flow waveforms could be obtained.

Average Systolic Peak Velocity

There were no significant differences in absolute average systolic peak velocity (ASPV) values before sublingual nitroglycerin among the 3 groups (continuous dosing group, $n=8$: 13.7±4.1 cm/s; eccentric dosing group, $n=8$: 13.1±3.5 cm/s; untreated group, $n=9$: 17.4±5.0 cm/s). In the 3 groups, the rate of change in ASPV was serially decreased after sublingual nitroglycerin; there were no significant differences among the 3 groups, although the rate of change in the continuous dosing group was smaller.

Average Diastolic Peak Velocity

There were no significant differences in absolute average diastolic peak velocity (ADPV) values before sublingual nitroglycerin among the 3 groups (continuous dosing group, 21.7±5.3 cm/s; eccentric dosing group, 22.7±3.9 cm/s; untreated group, 31.7±8.9 cm/s). In the 3 groups, the rate of change in ADPV was serially decreased after sublingual nitroglycerin; there were no significant differences among the 3 groups.

Diastolic/Systolic Velocity Ratio

There were no significant differences in absolute diastolic/systolic velocity ratio (DSVR) values before sublingual nitroglycerin among the 3 groups (continuous dosing group, 1.8±0.2; eccentric dosing group, 2.2±0.4; untreated group, 1.8±0.1). In the 3 groups, the rate of change in DSVR was serially increased after sublingual nitroglycerin; there were no significant differences among the 3 groups.

Coronary Vascular Resistance

There were no significant differences in absolute CVR before sublingual nitroglycerin among the 3 groups (continuous dosing group, 0.13±0.03 mmHg/ml/min; eccentric dosing group, 0.15±0.05 mmHg/ml/min; untreated group, 0.10±0.02 mmHg/ml/min). After sublingual nitroglycerin, there were no marked changes in any groups.

Influence of Angiotensin Converting Enzyme Inhibitors

In this study, we compared various parameters between patients receiving an angiotensin converting enzyme (ACE) inhibitor and those without an ACE inhibitor in the continuous dosing group, the eccentric dosing group, and the untreated group. The continuous dosing group consisted of 2 patients receiving an ACE inhibitor (ACE group) and 6 patients without this therapy (non-ACE group). There were no significant differences in the various parameters between the ACE group and the non-ACE group. The eccentric dosing group consisted of 5 patients receiving an ACE inhibitor (ACE group) and 3 patients without this therapy (non-ACE group). There were no significant differences in the various parameters between the ACE group and the non-ACE group. The untreated group consisted of 3 patients receiving ACE

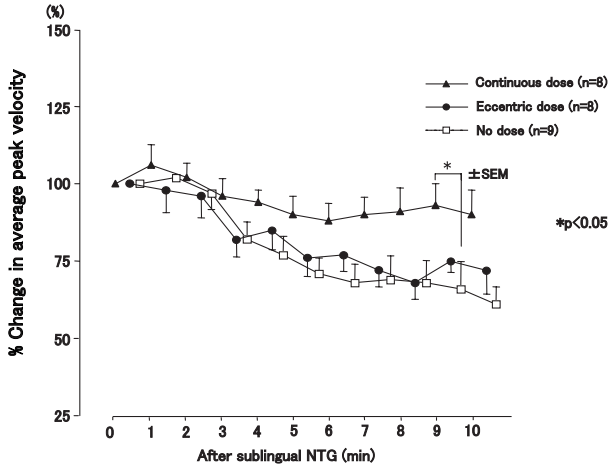

Fig. 7. *Percent change in average peak velocity (mean±SEM).*

inhibitors (ACE group) and 7 patients without this therapy (non-ACE group). There were no significant differences in the various parameters between the ACE group and the non-ACE group.

Discussion

Tolerance to Nitrates

Nitrates have a long history in the treatment of heart disease (*13*). Since continuous dosing of nitrates facilitates the maintenance of a constant high blood concentration, this procedure is routinely used to treat unstable angina or heart failure; however, it is known that nitrate tolerance appears within 24 h with respect to improvement in pulmonary wedge pressure or exercise tolerance (*3*, *5*). As a strategy to overcome such tolerance, eccentric dosing has been recommended (*9*, *14*).

Concerning the coronary vessel area, there was no significant difference in the pretreatment values between the continuous dosing group and the eccentric dosing group; however, in the eccentric dosing group and the untreated group, the coronary vessel area was significantly dilated compared to that in the continuous dosing group, suggesting that tolerance appeared in the continuous dosing group. In this study, the duration during which nitrate patches were applied ranged from 0.2 to 4.9 months in the continuous dosing group and from 0.2 to 5.0 months in the eccentric dosing group. It has been reported that tolerance related to oral administration or application of nitrates appears within a few days to a few weeks (*2*, *15*). The mean intervals from oral administration or application of nitrates until sublingual nitroglycerin on invasive examination were 3.8±0.5 h in the continuous dosing group and 4.2±0.6 h in the eccentric dosing group. Therefore, in both groups, the results may indicate the coronary response to sublingual nitroglycerin when the blood concentration of NO or ISDN was increased. With regard to the vascular response to sublingual nitroglycerin when the blood concentration of ISDN is extremely low, a study has reported that sublingual nitroglycerin given 14 h after administration of ISDN did not attenuate the antianginal effects (*16*). Another study has indicated that the additional effect of vasodilation of the coronary artery can be achieved by increasing the blood concentration despite the appearance of tolerance to nitroglycerin (*17*). When we compared the rate of change in coronary blood velocity after sublingual nitroglycerin among the 3 groups, APV in the continuous dosing group decreased by 13±6% of the pretreatment value, whereas the rates of decrease were 32±5% in the eccentric dosing group and 39±6% in the untreated group, suggesting the appearance of tolerance in the continuous dosing group. The limitations of eccentric dosing of nitrates include a rebound phenomenon related to a decrease in the blood concentration of nitrates, and this phenomenon has been experimentally and clinically reported as deterioration of anginal symptoms or coronary stenosis related to discontinuation of nitrates (*18–21*). Eccentric dosing does not prevent cardiac events (*22–24*). However, the appearance of tolerance, rebound phenomena, neurohumoral regulation, and enhancement of vascular sensitivity have all been associated with the dosing of nitrates (*1*, *25–28*).

High blood pressure plays a critical role in serious cardiac events in hypertensive patients with moderate coronary artery lesions. A previous study reported that 70 male patients with myocardial infarction still had endothelial dysfunction (*29*). Increased arterial stiffness is a possible simultaneous risk for atherosclerotic cardiovascular disease and diastolic heart failure in patients with hypertension (*30*). Kanamasa *et al.* (*14*) investigated the safety of eccentric and continuous dosing of nitrate to protect against cardiac events in patients with healed myocardial infarction. Among the patients receiving eccentric and continuous dosing of nitrates, cardiac events occurred in 12.7 per 1,000 person-years, and 67.4 per 1,000 person-years, respectively, whereas they occurred in only 19.7 per 1,000 person-years in the patients treated without nitrates. The incidence of cardiac events was significantly greater in patients receiving continuous dosing of nitrates compared with patients not receiving nitrates. Thus continuous dosing of nitrates increases cardiac events, and eccentric dosing of nitrates does not. The prevention of nitrate tolerance by the ACE inhibitors carvedilol and fluvastatin has also been investigated (*31–33*). In the present study, however, we could not analyze the prevention of nitrate tolerance by concomitant administration of ACE inhibitors, because only a few of our patients were taking these drugs.

Antianginal Effects after Sublingual Nitroglycerin

In patients receiving nitrates for a long period, a clinically important issue is whether sublingual nitroglycerin improves anginal symptoms. Bernstein and Ivy (*34*) reported that sub-

lingual nitroglycerin attenuated antianginal effects during long-term administration of nitrates in 3 patients. Zimrin *et al*. (*6*) also indicated that sublingual nitroglycerin after 24-h continuous intravenous injection of nitrates significantly attenuated antianginal effects. In addition, Danahy and Aronow (*16*) investigated the antianginal effects of nitroglycerin administered *via* the sublingual route when 14-h discontinuation was established between the final dose of ISDN and sublingual nitroglycerin, and reported that the antianginal effects of nitroglycerin were not attenuated by this regimen. The occurrence of nitrate tolerance depends on the long-term administration method of nitrates, that is, continuous dosing or eccentric dosing; however, in either case, a rapid increase in the blood concentration of nitroglycerin due to sublingual nitroglycerin administration may have conquered the nitrate tolerance, thereby leading to the antianginal effects. These results suggest that during long-term continuous dosing of nitrates, a reduction of the coronary vascular response to sublingual nitroglycerin attenuates the antianginal effects, whereas during eccentric dosing of nitrates, the antianginal effects may be maintained without marked reduction of the coronary vascular response to sublingual nitroglycerin.

In conclusion, a reduction of coronary vascular response related to continuous dosing of nitrates can be avoided by the eccentric dosing method, suggesting that eccentric dosing prevents nitrate tolerance.

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