Reduced Coronary Vasodilation in Patients With Familial Hypercholesterolemia Following Intracoronary Injection of Isosorbide Dinitrate

Keijiro Saku, M.D., Shiro Jimi, Ph.D.*, Nobuo Sasaki, M.D.
Takanobu Nii, M.D., Kazuyuki Shirai, M.D.
Nobuhiko Koga, M.D.** and Kikuo Arakawa, M.D.

To clarify the relationship between the dilatation of angiographically non-stenotic coronary artery segments in response to isosorbide dinitrate (ISDN), and serum lipids, 5 coronary segments in 7 patients with familial hypercholesterolemia (FH) and 43 patients with non-familial hypercholesterolemia (non-FH), who had either 1- or 2-vessel coronary heart disease, were investigated. The serum total cholesterol level was significantly greater in FH than in non-FH. Before and after the direct intracoronary injection of ISDN, coronary diameter was measured by a computer-assisted coronary angiography analysis system. The order of the dilative responses in coronary segments in non-FH patients (segments: #5 > #11 > #6 > #13 > #7) was exactly the same as the order of their original diameters, while the ratio of the increase in diameter (after/before ISDN injection) did not differ among any of the segments (mean: 1.08-fold increase). In the non-FH group, no correlation was found between the serum total cholesterol, triglyceride, low-density lipoprotein-cholesterol, or high-density lipoprotein-cholesterol, and the ratio of the coronary diameter after and before injection of ISDN. Moreover, hypercholesterolemia in non-FH did not affect the coronary dilative response to ISDN injection. In the FH group, although the original diameter of each segment did not differ from that in the non-FH group, the ratio of the diameter after and before injection of ISDN was significantly smaller in FH than in non-FH ($p < 0.01$). These results suggest that a non-stenotic coronary artery in FH has a lower capacity for vasodilation in response to ISDN. Although hypercholesterolemia was excluded as a factor which may suppress the capacity for coronary dilation in non-FH, spontaneous hypercholesterolemia in FH may affect medial smooth muscle functions of the coronary artery.

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HYPERCHOLESTEROLEMIA is a well-recognized risk factor for coronary heart disease (CHD). Familial hypercholesterole-

mia (FH), an autosomal dominant disorder characterized by an abnormal low-density lipoprotein (LDL) receptor function, is associated with a marked elevation of serum total cholesterol, atherosclerosis and tendon xanthomatosis, and frequently with an early onset of CHD! Computer-assisted coronary

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Departments of Internal Medicine and *Pathology, Fukuoka University School of Medicine, Fukuoka 814-01, Japan

**Department of Cardiology, Koga Hospital, Kurume 830-00, Japan

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Mailing address: Keijiro Saku, M.D., Ph.D., Department of Internal Medicine, Fukuoka University School of Medicine, 45-1-7 Nanakuma Jonan-ku, Fukuoka 814-01, Japan

angiography has recently made it possible to derive quantitative estimates in coronary angiography^{2,3}. Based on the analysis of coronary artery diameters by coronary angiography, the effects of several drugs have been estimated quantitatively^{4,5}. However, most previous studies have focused on lesions in coronary artery segments, while few studies have used non-stenotic coronary artery segments, and studies concerned with the lipoprotein metabolism are also few. In this report, we investigated the change in the diameter of angiographically non-stenotic coronary arteries in patients with FH and non-familial hypercholesterolemia (non-FH) before and after intracoronary injection of isosorbide dinitrate (ISDN), an endothelium-independent vasodilator, and devoted particular attention to the effects of serum cholesterol on coronary vasomotion.

METHODS

Patients

Fifty patients (37 men, 13 women), aged 38 to 81 years, with either 1- or 2-vessel CHD were enrolled in this study. These patients underwent coronary angiography for diagnosis of CHD. Patients with spastic angina or microvascular angina, and patients who were suspected to have these conditions, were excluded. Patients who had experienced myocardial infarction within the previous month were also excluded. Seven patients showed familial hypercholesterolemia (FH). They exhibited hypercholesterolemia greater than 250 mg/dl at the first laboratory examination, Achilles tendons more than 11-mm thick, and characteristic familial traits. None of the remaining 43 patients were diagnosed as familial combined hyperlipidemia, familial endogenous hypertriglyceridemia, or type III dyslipoproteinemia. The severity of coronary atherosclerosis, as assessed by the number of affected vessels and Gensini's severity scores, was similar in the FH and non-FH groups (1.3 ± 0.3 vs 1.2 ± 0.2 vessels and 33.0 ± 11.6 vs 36.0 ± 7.3 scores, respectively).

Serum Samples

Blood samples were taken in the morning after an overnight fast. Serum total cholesterol (TC) and triglyceride (TG) were mea-

sured by enzymatic methods^{6,7}. High-density lipoprotein-cholesterol (HDL-C) was measured by the heparin- Ca^{2+} precipitation method⁸, and low-density lipoprotein-cholesterol (LDL-C) was determined by the method of Friedewald et al⁹.

Coronary Angiography

Coronary arteries were cannulated by the Judkins technique with 6F catheters, and were recorded on Kodak 35 mm cinefilm at a rate of 25 frames/sec. Coronary arteries were divided into 15 segments, according to the classification of the American Heart Association Grading Committee. All measurements were performed primarily in the right anterior oblique projection. In this study, reliable and reproducible measurement was obtained in coronary segments #5, #6, #7, #11, and #13, but some images were excluded due to indistinct figures. A non-stenotic coronary artery segment was defined as stenosis of less than 10%, compared with the segment's adjusted luminal borders. One hundred two non-stenotic segments were carefully selected by two expert cardiologists on the basis of smooth luminal borders and the absence of stenotic changes, and examined using a computer-assisted coronary angiography analysis system (Mipron 1; Kontron Co). Triplicate measurements were performed in each segment, and the mean diameter, which represented the average of the entire segment, was used as the diameter of the segment. The diameters of the coronary arteries were measured before and after the direct intracoronary injection of isosorbide dinitrate (ISDN) (2.5 mg/5 ml solution) using the Mipron 1 system. One minute after injection of ISDN through the Judkins catheter over 20 sec, coronary angiography was performed from several projections over 5 min.

Other Methods

Most of the patients were receiving Ca^{2+} -blocking agents (nifedipine, diltiazem, etc) or nitrates, but not beta-blocking agents. All medications were discontinued at least 24 h before the study. Informed consent was obtained from each patient before entering the study, and the project was assessed and approved by the Ethics Committee of Fukuoka University.

TABLE I PATIENTS, SERUM LIPIDS AND LIPOPROTEINS

	age	sex		vessel		TC	TG	HDL-C	LDL-C
		M	F	1V	2V				
non-FH (n=43)	61±2	32	11	31	12	208±6	120±10	40±1	146±6
FH (n=7)	58±6	5	2	3	4	291±12*	129±18	36±2	223±16*

value: mean ± SEM

1V: one-vessel disease, 2V: two-vessel disease.

*: $p < 0.001$ vs non-FH

Fig. 1. The diameter of non-stenotic coronary arteries was measured angiographically before and after the intracoronary injection of ISDN in non-FH patients. The original coronary luminal diameter (a), the change in diameter after ISDN injection (b) and the ratio of the diameter after and before ISDN injection (c) in each of the coronary artery segments (#5, #6, #7, #11, #13) are shown. No differences in the ratio of the diameter after and before ISDN were observed between the segments.

Statistics

Data were statistically analyzed with the paired- and unpaired-Student's t-test, and by an analysis of covariance (ANOVA) followed by Fisher's least significance test. P values of less than 0.05 were considered to indicate significant differences. All data are expressed as the mean ± the standard error of the mean (SEM).

RESULTS

Table I shows the patient characteristics and their lipid and lipoprotein data. No differences in age or the sex ratio were found between the non-FH and FH groups. Levels of serum TC and LDL-C were significantly greater in FH than in non-FH ($p < 0.001$). However, no differences in TG or HDL-C were found between the groups.

We selected non-stenotic coronary artery segments by examining angiograms. Fig. 1 shows the diameter and the change in the diameter of the non-stenotic portions of segments #5, #6, #7, #11, and #13, from 43 non-FH patients. The original diameters of the coronary artery before the intracoronary injection of ISDN were 2.97 ± 0.09 mm, 2.32 ± 0.10 mm, 1.73 ± 0.08 mm, 2.58 ± 0.10 mm, and 2.25 ± 0.08 mm, respectively, and, after the injection of ISDN, the diameters increased 0.26 ± 0.05 mm, 0.13 ± 0.05 mm, 0.06 ± 0.05 mm, 0.20 ± 0.04 mm, and 0.12 ± 0.06 mm, respectively. Therefore, the order of the original luminal diameter (#5 > #11 > #6 > #13 > #7, $p < 0.05$; Fig. 1-a) was exactly the same as the order of the dilative response to ISDN (#5 > #11 > #6 > #13 > #7; Fig. 1-b). However, the ratio of the diameter after and before injection of ISDN was fairly similar among all of the segments; 1.09, 1.06, 1.03,

Fig.2. The relationship between the ratio of the diameter after and before ISDN injection in coronary segments and the levels of serum total cholesterol (a), triglyceride (b), and HDL-cholesterol (c) in non-FH patients. No significant correlation was found.

1.08, and 1.05, respectively (Fig. 1-c). Using these results, we were able to directly compare the changes in the diameters of coronary segments with different diameters. Thus, the mean ratios of the increase in diameter in different coronary segments in each patient were calculated and used for various comparisons.

The effects of serum TC, TG, LDL-C and HDL-C on changes in the diameters of coronary arteries in response to ISDN were examined using 102 segments from 43 non-FH patients (Fig. 2). However, no significant correlation was found between the ratio of the diameter after and before injection of

Fig.3. The ratio of the diameter after and before ISDN injection in coronary segments was compared between FH patients ($n=7$), non-FH patients ($n=43$), non-FH patients with $TC \geq 220$ mg/dl ($n=11$) and non-FH patients with $TC \geq 50$ mg/dl ($n=5$). FH patients showed significantly lower coronary dilative responses. *: $p < 0.05$, **: $p < 0.01$.

ISDN and either TC (Fig. 2-a), TG (Fig. 2-b), LDL-C (data not shown), or HDL-C (Fig. 2-c). Furthermore, hypercholesterolemic ($TC \geq 220$ mg/dl) non-FH patients (11 patients) showed a similar ratio of the diameter after and before ISDN as non-hypercholesterolemic patients ($TC < 220$ mg/dl) (Table II).

Non-stenotic segments were examined in 7 FH patients. The mean diameters of segments #5, #6, #7, #11, and #13 before ISDN injection were 2.94 mm, 1.68 mm, 1.92 mm, 2.74 mm, and 2.03 mm, respectively, which were nearly identical to those in non-FH. The ratio of the diameter after and before ISDN was compared between the non-FH and FH groups (Fig. 3). The dilative response in non-stenotic segments was significantly lower in FH than in non-FH ($p < 0.01$; 1.01 ± 0.01 vs 1.08 ± 0.01). Moreover, when FH patients were compared with hypercholesterolemic non-FH patients ($TC \geq 220$ mg/dl; $n=11$, mean $TC=258 \pm 12$ mg/dl), a significant difference in the ratio of the diameter after and before ISDN was found ($p < 0.05$) (Fig. 3). A similar result was obtained ($p < 0.05$), when FH patients (mean $TC=291 \pm 12$) were compared to non-FH patients with $TC \geq 250$ mg/dl ($n=5$, mean $TC=287 \pm 17$ mg/dl).

TABLE II THE RATIO OF THE DIAMETER AFTER AND BEFORE ISDN INJECTION IN NON-STENOTIC CORONARY SEGMENTS IN NON-FH PATIENTS WITH/WITHOUT HYPERCHOLESTEROLEMIA

Total cholesterol	Segment					
	#5	#6	#7	#11	#13	(mean)
<220 mg/dl	1.09±0.02	1.07±0.03	1.03±0.05	1.10±0.02	1.08±0.04	(1.08±0.01)
≥220 mg/dl	1.12±0.03	1.06±0.04	1.09±0.07	1.10±0.04	1.11±0.01	(1.09±0.02)

value: mean ± SEM

DISCUSSION

In this study, the non-stenotic portion of coronary arteries in FH patients showed a lower capacity for vasodilation in response to ISDN than that in non-FH. FH is characterized by spontaneous hypercholesterolemia, and is believed to be frequently accompanied by CHD.¹

Several factors have been shown to influence non-stenotic coronary artery diameters, ie, sex,¹⁰ left ventricular hypertrophy,¹⁰ dilated cardiomyopathy,^{10,11} and aortic valve stenosis.¹¹ However, none of these conditions, excluding sex, were noted in the patients examined here. In the present study, coronary artery diameters of non-stenotic segments in a total 50 patients with either 1- or 2-vessel CHD were examined angiographically. It has been demonstrated that even non-stenotic portions, as estimated by angiography, contain intimal lesions that can be detected by intravascular ultrasound imaging.¹² Therefore, our non-stenotic segments may possess some intimal lesions. On the other hand, it has also been reported that coronary arteries which possess stenosis to different degrees and at different sites in chronic stable angina pectoris, show identical responses to ISDN.¹³ Thus, such concealed lesions, which may not be detected by angiography, may not significantly affect the dilative response of the coronary artery.

There was no obvious correlation between the ratio of the diameter after and before ISDN and TC, TG, HDL-C, or LDL-C in non-FH patients. Moreover, the mean ratio of the diameter after and before ISDN in hypercholesterolemic non-FH patients with TC ≥ 220 mg/dl (11 patients) or TC ≥ 250 mg/dl (5 patients) was almost the same as that in patients with TC below those levels.

Therefore, these results suggest that serum cholesterol does not regulate coronary vasodilation in non-FH patients. Egashira et al.¹⁴ reached a similar conclusion. They reported that vasodilation of the large epicardial coronary artery in CHD patients evoked by nitrate did not differ between high- and low-cholesterol groups, although the details of the origin of hypercholesterolemia in these patients were not fully described.

In FH patients, the ratio of the diameter of the coronary artery after and before ISDN was significantly decreased, compared with that in non-FH patients. In FH patients, the TC level was moderately high (mean: 291 mg/dl, range: 261–324 mg/dl), while in non-FH patients the TC level covered a much wider range. Therefore, we examined the dilative capability of the coronary artery in non-FH patients with hypercholesterolemia (TC ≥ 220 mg/dl), and compared the results to those in FH patients. A significant difference was also observed between the two groups ($p < 0.02$).

It is unclear why cholesterol had no appreciable effects on coronary dilation in non-FH patients, even though FH patients with hypercholesterolemia showed a lower vasodilative reaction. There are several possible explanations for this lower sensitivity of the coronary artery in FH patients in response to ISDN, an endothelium-independent vasodilator. First, this difference may be due to the serum TC level, which was higher in FH patients (291 ± 12 mg/dl) than in non-FH patients with hypercholesterolemia (258 ± 12 mg/dl). However, when we selected non-FH patients with TC ≥ 250, the mean TC level in non-FH (289 ± 17 mg/dl) was nearly identical to that in FH (291 ± 12 mg/dl). The ratio of the diameter after and before ISDN in non-FH patients with

TC $\geq$ 250 was the same as that in non-FH patients with TC $\geq$ 220 mg/dl, and was constantly greater than that in FH patients. Therefore, the serum TC level can be excluded as a potential influencing factor. Second, a difference in coronary flow!⁵ which we did not examine, may exist between the two groups. Third, arterial tonus in FH may be suppressed compared to that in non-FH. Regarding cholesterol, it has been shown that when membrane cholesterol in smooth muscle cells is increased by high cholesterol-liposomes, the intracellular calcium level rises!⁶ Another report has indicated that elevated HDL levels ameliorate abnormal vasoconstriction in early atherosclerosis!⁷ A decreased HDL-C level, while not significant in the present FH patients, is a known characteristic of FH patients!⁸ and may lead to vasodilative abnormality of the coronary artery. Furthermore, if arterial cells in FH were impaired by oxidized lipids!⁹ other tonus regulatory factors derived from endothelial cells!^{14,20} may also be involved. Finally, the media may be affected in the coronary artery of FH, and this may in turn affect arterial dilative functions. We recently reported that the medial thickness of the aorta in homozygous WHHL rabbits, which are a model animal for FH, decreased due to atrophy and loss of aortic medial smooth muscle cells!²¹ If such loss and/or atrophy of medial smooth muscle cells occurs in the coronary artery in man, it may lead to vasomotive disorders in the coronary artery. Therefore, although the exact mechanisms of these differences are still unknown, spontaneous and/or prolonged hypercholesterolemia in FH may affect the medial layer's functions and/or tonus.

In summary, in this angiography-based study, we showed that FH, which is associated with a risk for the development of coronary atherosclerosis, induces a lower dilative response to ISDN, which may accelerate the occurrence of CHD in FH. An overall understanding of coronary circulation, including small vessels, is required for the proper management of coronary artery disease.

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