Coronary Microvascular Adaptation to Myocardial Metabolic Demand Can Be Restored by Inhibition of Iron-Catalyzed Formation of Oxygen Free Radicals in Type 2 Diabetic Patients

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Dilation of coronary vessels is impaired in diabetic patients when myocardial metabolic demand is increased. Deferoxamine (DFX) restores a normal dilation of epicardial coronary arteries. To assess the effects of DFX on metabolic coronary microvascular dilation in type 2 diabetic patients, coronary blood flow was measured using intracoronary Doppler and quantitative angiography in 17 type 2 diabetic patients with normal coronary arteries and without any other coronary risk factors. Measurements were made at baseline and during a cold pressor test (CPT), before and after intravenous administration of DFX. With a similar rate-pressure product (RPP) increase during CPT before and after DFX (+21.1 ± 8.7 vs. +20.5 ± 8.9%, respectively), coronary blood flow increase was significantly enhanced after DFX (+31.8 ± 16.7 vs. +6.3 ± 12.9% before DFX, $P < 0.001$). Moreover, coronary resistance increased during CPT before DFX and decreased after DFX (+14.8 ± 21.9 vs. −7.9 ± 10.9%, respectively, $P < 0.001$). Lastly, the negative relationship between coronary blood flow and RPP before DFX ($R = 0.546$, $P < 0.05$) was changed in a positive relationship after DFX ($R = 0.518$, $P < 0.05$). In conclusion, in type 2 diabetic patients, inhibition of iron-catalyzed oxidative reactions by DFX restored dilation of the coronary microcirculation and a normal matching between myocardial metabolic demand and coronary blood flow. *Diabetes* 51:813–818, 2002

Recent studies have shown that in diabetic patients coronary blood flow is unable to match myocardial metabolic demand increase evoked by either atrial pacing or sympathetic stimulation by the cold pressor test (CPT) (1,2). It has been suggested that coronary microvascular functional abnormalities might contribute to the development of left ventricular dysfunction through episodes of silent myocardial ischemia when myocardial oxygen demand is increased and might explain the high frequency of exercise thallium–imaging defects (3–6).

Impairment of dilation of the coronary microcirculation in diabetic patients has been proposed to be related to the degree of sympathetic nerve dysfunction (7). On the other hand, it is well established that oxygen-derived free radical production, which is increased in diabetes (8,9), could impair endothelium-dependent vasodilation since it has been proved that superoxide anions could inactivate nitric oxide (10). Lastly, several studies have shown that nitric oxide production was reduced in diabetes in both peripheral and coronary vascular beds (11,12).

We had previously demonstrated that a low dose of deferoxamine (DFX), an iron chelator that prevents iron-catalyzed generation of hydroxyl radicals, restored normal dilation of epicardial coronary arteries in diabetic patients, suggesting that inactivation of nitric oxide by oxygen species may play a role in impairment of coronary vasomotion (13). Similar results have been obtained in peripheral circulation by antioxidant therapy (14–17).

The purpose of the present study was to determine whether impaired coronary microvascular dilation in response to sympathetic stimulation by the CPT could be improved by administration of DFX.

RESEARCH DESIGN AND METHODS

Patient selection. Seventeen patients with type 2 diabetes undergoing diagnostic coronary angiography were included in this study. Patients were treated by oral hypoglycemic agents (sulfonylurea 4; biguanide 3; α-glucosidase inhibitor 4; sulfonylurea + biguanide 4; biguanide + α-glucosidase inhibitor 2). Mean duration of diabetes was 13.6 ± 9.9 years, and all patients manifested proper glucose homeostasis during the last 3 months and at the time of catheterization, as shown by fasting and postprandial glycemia (5.6 ± 1.1 and 6.1 ± 1.2 mmol/l, respectively) and by HbA_{1c} (5.4 ± 1.0%). Patients who had a history of arterial hypertension (blood pressure >140/90 mmHg), patients (untreated or with lipid-lowering therapy) with total cholesterol serum level >5.70 mmol/l (220 mg/dl) or LDL cholesterol >3.70 mmol/l (143 mg/dl), smokers, patients older than 65 years, and postmenopausal women without substitutive hormonal therapy were excluded. None of the patients had a family history of premature coronary artery disease (defined as a first-degree relative <60 years of age with clinical evidence of coronary atherosclerosis). These patients were selected among 98 diabetic patients referred for coronary arteriography because of abnormal stress test or single photon emission computed tomography (SPECT) stress thallium scintigraphy. All patients had normal left ventricular dimensions, mass, and systolic function assessed by two-dimensional and *M*-mode echocardiography (18,19).

Patients were included in the present study by consensus of two experienced investigators upon immediate review of the angiograms and only if coronary arteries were angiographically normal and completely smooth, without luminal irregularities. The study protocol was approved by the

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CPT, cold pressor test; CSA, cross-sectional area; DFX, deferoxamine; LAD, left anterior descending artery; RPP, rate-pressure product.

TABLE 1
Characteristics of the study population

Age (years)	50.1 ± 5.3
BMI (g/m^2)	29.3 ± 6.8
Sex (M/F)	11/6
Heart rate (bpm)	73 ± 17
Systolic blood pressure (mmHg)	138 ± 14
Diastolic blood pressure (mmHg)	81 ± 12
Total Cholesterol (mmol/l)	4.97 ± 0.66
Triglycerides (mmol/l)	1.46 ± 0.24
HDL cholesterol (mmol/l)	1.68 ± 0.39
LDL cholesterol (mmol/l)	3.00 ± 0.64
LV end-diastolic diameter (mm)	51 ± 6
Fractional shortening (%)	38.6 ± 7.1
LV mass (g/m^2)	91 ± 13

Data are means ± SD or *n*. LV, left ventricular.

institutional review committee of the University of Kremlin-Bicêtre. All patients gave written informed consent before cardiac catheterization.

Catheterization protocol. Patients were studied in the fasting state. No premedication was administered; 1% lidocaine was used for local anesthesia and 5,000 units i.v. heparin was administered. After documentation of normal coronary arteries, an additional 5,000 units i.v. heparin was given, and a 8F guiding catheter was positioned in the left coronary artery. Each patient then underwent the following study protocol. A 3F 20-MHz coronary Doppler catheter (Monorail Doppler 3; Schneider Europe AG, Zuerich, Switzerland) connected to a single-channel 20-MHz pulsed Doppler velocimeter (model MDV-20 Single Channel Velocimeter; Millar Instruments, Houston, TX) was placed in the left anterior descending artery (LAD). The Doppler catheter was placed in the midportion of the LAD, and catheter position was adjusted to obtain an optimal audio signal and phasic tracing of coronary blood flow velocity. The use of this device to assess intracoronary blood flow velocity has been previously discussed in detail (20).

Coronary angiograms were performed using an injection of 8 ml low osmolarity contrast medium (meglumine ioxaglate) in the left coronary artery. Serial injections of the left coronary artery were performed at intervals of at least 5 min to exclude contrast-induced coronary dilation. Measurements of the diameters of LADs were made on each angiogram.

Intracoronary blood flow velocity was measured near the tip of the Doppler catheter, just before each angiogram, for avoiding the hyperemic effect of the contrast material. Heart rate, aortic pressure (through the guiding catheter), mean and phasic blood flow velocity (kilohertz shift), and electrocardiogram were continuously monitored throughout the protocol.

Protocol design. Thirty minutes after the coronary arteriography, baseline hemodynamic measurements and left coronary arteriography were carried out. Five minutes later, a CPT was performed. The patients' hands were immersed in ice water for 120 s. Hemodynamic measurements and coronary angiography were repeated at the peak of the CPT, immediately before removal of the hands from ice water. After completion of the first series of measures, DFX was administered intravenously. Patients were given DFX (Desferal; Ciba-Geigy Laboratories, Rueil-Malmaison, France) at a rate of 50 mg/min (10 ml/min) during 10 min (500 mg DFX were dissolved in 5 ml of distilled water and were then diluted in 95 ml saline 0.9%). The dose of deferoxamine was chosen according to experimental data in dogs that have shown that low doses of DFX exhibited a cardioprotective action in the stunned myocardium (21–23). The above described procedure was then repeated. Hemodynamic recordings, measurements of blood flow velocity in distal LAD, and left coronary angiograms were thus performed at baseline and at the peak of the CPT. The final hemodynamic measurements and coronary arteriography were repeated after intracoronary infusion of a bolus of 2 mg isosorbide dinitrate through the guiding catheter in order to dilate maximally epicardial coronary arteries, followed 4 min later by an injection of 12 mg papaverine in the left coronary artery in order to assess maximal coronary blood flow (24).

Quantitative coronary arteriography. Left coronary arteriograms were obtained by electrocardiogram-triggered digital subtraction at a rate of 6 frames/s on a 512-pixel matrix (General Electric CGR DG 300). The angiographic system was set up in a right anterior oblique position with adequate cranial or caudal angulation, allowing optimal view of the LAD segment on end-diastolic frames without overlap by side branches. Relations between focal spot, patient, and height of image tube were kept constant throughout the procedure. Analysis of coronary angiograms was performed by a previously validated technique (20). A segment of the guiding catheter filled with saline was placed close to the center of the image and used as a scaling device for calibration before the procedure was begun.

Measurements. Cross-sectional area (CSA) of the LAD was calculated from diameters (d), assuming a circumferential model: CSA = $\pi d^2/4$. Each angiogram was analyzed at random without knowledge of the sequence of the procedure. Estimates of blood flow in the LAD (F) were calculated using mean coronary blood flow velocity (v) and CSA (F [ml/min] = v [cm/s] × CSA [cm^2]). Coronary resistance (mmHg · ml^{-1} · min^{-1}) was calculated as the ratio of F to mean aortic pressure (mmHg) measured through the guiding catheter in each condition. Coronary flow reserve was calculated as peak-to-resting coronary flow velocity ratio after intracoronary papaverine (24).

Statistical analysis. All data are expressed as means ± SD. Statistical comparisons of hemodynamic parameters, coronary vessel dimensions, and coronary hemodynamics throughout the procedure were made by two-way ANOVA with repeated measures for experimental condition factor, followed by the Fisher protected least-significant difference test. Statistical significance was assumed if the null hypothesis could be rejected at the 0.05 probability level.

RESULTS

Clinical data. Characteristics of patients are summarized in Table 1. Echocardiographic data show that left ventricular end-diastolic diameter, fractional shortening, and left ventricular mass index were within the normal range (14). Among the 17 patients, 10 had lipid-lowering therapy, and the lipid profile showed normal values. Four women had postmenopausal hormonal substitutive therapy and two women were nonmenopaused.

Hemodynamic parameters. At baseline, there was no significant differences for heart rate, systolic pressure, mean aortic pressure, and rate-pressure product before and after DFX administration (Table 2). Heart rate was not changed by CPT, which produced similar increase in aortic pressures and rate-pressure product before and after DFX administration. So in this study, the increase in rate-pressure product during CPT was only due to the elevation of arterial pressure.

Coronary artery dimensions and coronary blood flow velocity. CPT induced a significant reduction from baseline value in a cross-sectional area of the LAD and a

TABLE 2
Hemodynamic data

	Before DFX		After DFX	
	Baseline	Cold-pressor test	Baseline	Cold-pressor test
Heart rate (bpm)	72 ± 9	73 ± 9	72 ± 8	73 ± 10
Systolic pressure (mmHg)	135 ± 11	163 ± 11*	134 ± 10	161 ± 11*
Mean aortic pressure (mmHg)	97 ± 8	116 ± 11*	97 ± 7	117 ± 11*
Rate-pressure product (mmHg · bpm)	9,756 ± 1,235	11,798 ± 1,553*	9,722 ± 1,193	11,697 ± 1,502*
Δ Rate-pressure product (mmHg · bpm, %)	+ 2,042 ± 845 + 21.1 ± 8.7		+ 1,975 ± 843 + 20.5 ± 8.9	

Data are means ± SD. *$P < 0.001$ vs. baseline values.

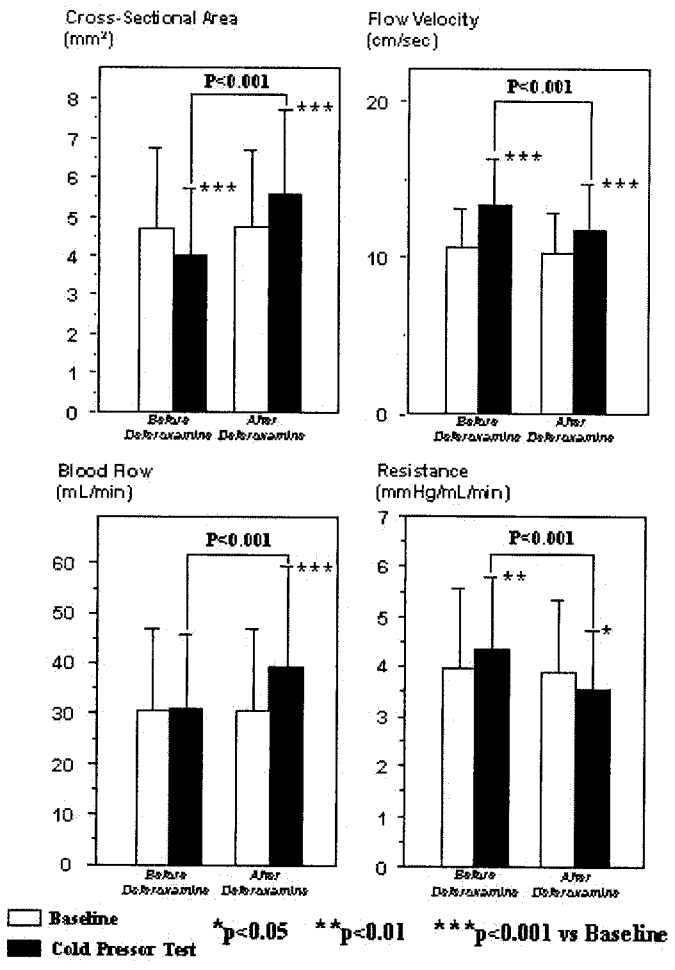

FIG. 1. Bar graphs of cross-sectional area, flow velocity, blood flow, and resistance in the LAD at baseline (□) and during the CPT (■), before and after DFX infusion.

significant increase in blood flow velocity before DFX (Fig. 1). After DFX administration, coronary cross-sectional area was not modified at baseline but increased significantly during the CPT, and flow velocity increase was significantly lower than before DFX.

Coronary blood flow and coronary resistance. Values of coronary blood flow and coronary resistance at baseline were not significantly different before and after DFX (Fig. 1). Conversely, during CPT after DFX, coronary blood flow was significantly higher and coronary resistance was significantly lower compared with CPT before DFX (Fig. 1). Thus before DFX, during the CPT, there was a weak and insignificant increase in coronary blood flow (+0.9 ± 4.5 ml/min, +6.3 ± 12.9%, NS), but a marked and significant rise in coronary resistance (+0.40 ± 0.72 mmHg · ml^{-1} · min^{-1}, +14.8 ± 21.9%, $P < 0.01$). The response to CPT was strikingly changed after DFX with a significant increase in coronary blood flow (+8.8 ± 6.1 ml/min, +31.8 ± 16.7%, $P < 0.001$) and a significant decrease in coronary resistance (−0.38 ± 0.58 mmHg · ml^{-1} · min^{-1}, −7.9 ± 10.9%, $P < 0.05$). Moreover, the negative correlation between coronary blood flow and rate-pressure product changes before DFX was turned in a positive correlation between these two parameters (Fig. 2). Last, a positive correlation was observed between coronary resistance and rate-pressure changes before DFX, and no correlation was found between these two parameters after DFX (Fig. 3). As the rate-pressure product increase was mainly due to the arterial pressure increase, there was also a negative correlation between coronary blood flow (CBF) and mean aortic pressure (MAP) changes ($\Delta CBF = -0.98\ \Delta MAP + 25.3$, $R = 0.633$, $P < 0.001$) and a positive correlation between coronary resistance (CR) and mean aortic pressure changes ($\Delta CR = +2.02\ \Delta MAP - 24.4$, $R = 0.766$, $P < 0.001$) before DFX.

Coronary flow reserve estimated through the peak-to-resting coronary flow velocity ratio was measured at the end of the procedure. Data showed that the ability of coronary blood flow to increase was preserved in our patients with a ratio of 4.05 ± 0.88.

DISCUSSION

The main results of the present study in type 2 diabetic patients with angiographically normal coronary arteries is that a low dose of DFX restores the normal matching between coronary blood flow and myocardial oxygen demand during sympathetic stimulation by the CPT. Because DFX is an iron chelator that prevents hydroxyl radical formation OH$^{\cdot}$ (25), this study shows that coronary microvascular dysfunction may be partly due to inactivation of nitric oxide in diabetic patients.

Metabolic coronary vasodilation in diabetes. Several animal and human studies have shown that coronary metabolic vasodilation is impaired in diabetes. In diabetic rats, coronary blood flow increase in response to norepinephrine infusion and to pacing was significantly lower than that in control rats (26). In diabetic dogs, coronary arterioles did not dilate when coronary perfusion pressure was decreased (27). In humans, it has been shown that atrial pacing was not followed by appropriate coronary blood flow increase in diabetic patients (1), and we have shown that CPT did not induce any change in coronary blood flow in type 2 diabetic patients despite the increase in rate-pressure product (2). These microvascular functional abnormalities might contribute to the development of left ventricular dysfunction through episodes of silent myocardial ischemia when myocardial oxygen demand is increased and might explain the high frequency of exercise thallium–imaging defects (3–6).

Potential mechanisms for coronary microcirculation dysfunction in type 2 diabetic patients. Although it is well established that coronary reserve is depressed in diabetic patients (1,28), the impairment of coronary blood flow adaptation to metabolic demand cannot be linked to structural abnormalities of coronary microcirculation, because the depression of coronary reserve cannot explain the absence of flow increase at baseline when, in our patients, coronary flow reserve measured after intracoronary papaverine was 4.05 ± 0.88. Moreover, even though heart rate was not increased by the CPT, myocardial metabolic demand was increased by >20% either before or after DFX. This increase should have normally induced a significant increase in coronary blood flow at baseline. Lastly, in our patients before DFX, the more the rate-pressure product was increased, the less the coronary blood flow was increased, and the more the coronary resistance was increased (Figs. 2 and 3). These correlations suggest that the greater the sympathetic stimulation

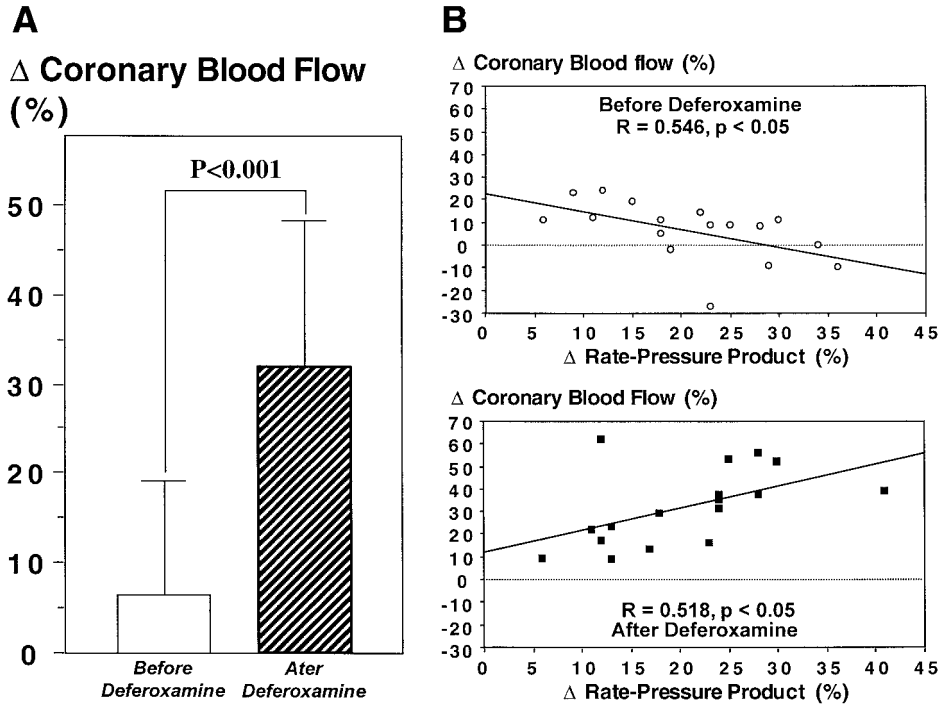

FIG. 2. *A*: **Relative change in coronary blood flow showing a highly significant increase after DFX administration.** *B*: **Correlations between changes in coronary blood flow and changes in rate-pressure product. The significant negative correlation at baseline was turned in a significant positive correlation by DFX infusion.**

(and arterial pressure increase), the greater the coronary microvascular constriction.

However, it might be postulated that improvement in coronary blood flow after DFX could be due to the second CPT alone. It must be pointed out that in a previous study (13), diameter of epicardial coronary arteries was not modified during a second CPT made after L-arginine administration and increased after DFX. Thus, it seems very improbable that CPT alone is responsable for the improvement of coronary blood flow after DFX. On the other hand, we have previously shown in a control group of comparable age that coronary blood flow was increased during CPT (2).

CPT has been shown to increase coronary blood flow through the rise in myocardial oxygen demand resulting from sympathetic stimulation (29,30) and to dilate epicardial coronary arteries of normal subjects through a flow-dependent mechanism (31–34). In addition, cardiac efferent sympathetic signals play a major role in adaptative vasodilation of coronary circulation to myocardial metabolic demand (32). Two mechanisms are involved in sympathetic-mediated coronary vasodilation: *1*) direct re-

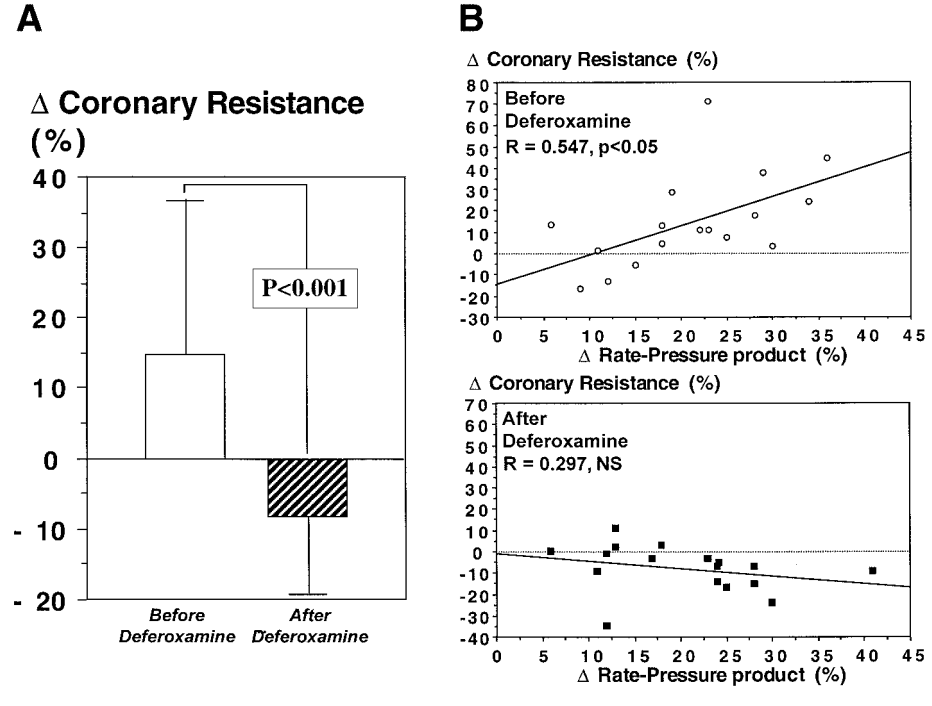

FIG. 3. *A*: **Relative change in coronary resistance showing a coronary resistance increase before DFX infusion that was turned in a decrease after DFX.** *B*: **Correlations between changes in coronary resistance and changes in rate-pressure product. The significant positive correlation at baseline was abolished after DFX infusion.**

laxation of microvessel smooth muscle cells where β-adrenoreceptors are predominating (33) and *2*) nitric oxide synthesis and release by endothelial cells, which contain α2-adrenoreceptors (34). Diabetic autonomic neuropathy with sympathetic denervation is a common complication of diabetes (35), and it has been recently shown by Di Carli et al. (7) that diabetic autonomic neuropathy was associated with impaired vasodilator response of coronary microcirculation during CPT. However in our study, patients without diabetic neuropathy (10 of 17 patients) also had depressed vasodilation, suggesting that sympathetic dysinnervation was not the only responsible for the coronary microvascular dysfunction. Moreover, the abnormal response to increased myocardial oxygen demand evoked by atrial pacing in diabetic patients (1) also suggests that sympathetically mediated dilation of coronary microcirculation might not be involved alone. Moreover, in this latter study, it must be pointed out that β-adrenergic antagonist therapy did not influence the results.

On the other hand, several studies have stressed the marked role of oxygen free radicals in diabetic vascular dysfunction because cellular oxidant stress due to advanced glycation end products increases oxygen free radical production that may *1*) inactivate nitric oxide to NO_2^- (10,36), *2*) stimulate oxidized LDL cholesterol formation that may also degrade nitric oxide (37,38), and *3*) interfere with receptor-mediated stimulation of nitric oxide or with signal transduction in the release of nitric oxide (39,40). Moreover, production of bioactive vasoconstrictive prostaglandin $F_{2\alpha}$ from arachidonic acid through oxygen free radical catalyzed lipid peroxidation (41) is increased in diabetic patients (14). Thus, it has been shown that diabetes impairs endothelium-dependent nitric oxide–mediated vasodilation of peripheral microvessels (42,43) and coronary circulation, where nitric oxide production is reduced (12).

Beneficial effects of DFX on diabetic coronary microcirculation. The beneficial effects of antioxidant therapies that can restore endothelium-dependent relaxation in diabetes have been demonstrated in rat aortic rings (16,44), in human forearm microcirculation (15), and in epicardial coronary arteries (13), underlining the major role of the nitric oxide system impairment by oxygen free radicals. Indeed, we have previously shown (13) that antioxidant DFX restored flow-dependent dilation and normal dilation during CPT in epicardial coronary arteries of diabetic patients. The present study extends the beneficial effects of DFX to the coronary microvasculature that demonstrates for the first time that the whole coronary vascular bed could be functionaly improved by preventing oxygen-derived free radical production in diabetic patients. Among reactive oxygen-derived free radicals, hydroxyl radical ($OH^\cdot$) is one of the more toxic generated through the Haber-Weiss and Fenton reactions ($Fe^{3+} + O_2^{-\cdot} \rightarrow Fe^{2+} + O_2$, and $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\cdot$), which both require a reduced metal ion, the most abundant of which is iron (37).

DFX has been used to prevent myocardial stunning (21–23) and has been demonstrated to be protective for endothelial cells (45). It has been shown that low doses of DFX prevents iron ions from catalyzing redox reactions leading to generation of $OH^\cdot$ by binding Fe^{3+} ions (21–23). In our study, small doses of DFX were administered to the patients (500 mg infused for 10 min) because free iron ions available to stimulate radical reactions are small (rarely >5 μmol/l [46]) and radical reactions promoted by low levels of iron can be inhibited by DFX at concentrations equimolar to iron (47). Previous studies have shown that intravenous injection of 10 mg/kg body wt of DFX in humans gave plasma concentrations of 80–130 μmol/l that fell rapidly (half time 5–10 min) (48), which is highly sufficient to bind iron ions. Thus, our results demonstrate a rapid restoration of coronary microcirculation vasodilation during CPT that cannot be accounted for by a scavenging effect due to oxydation of DFX by $OH^\cdot$ and $O_2^{-\cdot}$, because this reaction is slow and requires high plasma concentrations (millimolar) (25).

Clinical implications. Dilation of epicardial coronary arteries and microcirculation is a physiological response that allows coronary blood flow to match myocardial oxygen demand. In diabetic patients, the physiological dilation of whole coronary circulation is impaired, which may cause silent episodes of myocardial hypoxia during current life episodes that activate the sympathetic nervous system, such as exercise, mental stress, or cold exposure. Episodes of myocardial ischemia, which are more frequently observed in diabetic patients than in the other patients because of diabetic neuropathy (49,50), might progressively deteriorate the myocardium and lead to diabetic myocardiopathy, even in patients without coronary artery stenosis (3–5). As DFX has been demonstarted to restore coronary epicardial (13) and microcirculation vasomotion in diabetic patients without any other coronary risk factors, antioxidant therapy might be beneficial for long-term preservation of myocardial perfusion and heart function.

Conclusions. This study provides arguments for an alteration of the endothelium-derived nitric oxide system in the coronary microcirculation of diabetic patients that might be due to hydroxyl radicals. This alteration can be reversed by the iron-chelator DFX. Although our results do not provide direct evidence for endothelial-derived nitric oxide being altered by hydroxyl radicals, they confirm that compounds preventing oxygen free radical generation might be useful for preservation of vascular function in diabetic patients. Therefore, further studies are required to establish whether antioxidant therapy has long-term beneficial effect and whether it can be used as a possible prophylactic treatment against coronary large vessels and microvascular dysfunction, development of coronary artery disease, and myocardiopathy in diabetic patients. However, toxicity and short plasma half life of DFX do not make this compound useful in preventing deleterious effects of diabetes on coronary microcirculation.

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