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Intensive treatment of risk factors in patients with type-2 diabetes mellitus is associated with improvement of endothelial function coupled with a reduction in the levels of plasma asymmetric dimethylarginine and endogenous inhibitor of nitric oxide synthase

Satoshi Yasuda*†, Shunichi Miyazaki, Munetake Kanda, Yoichi Goto, Masaaki Suzuki, Yutaka Harano, and Hiroshi Nonogi

Division of Cardiology, Department of Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan

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Aims Vascular endothelium is a major organ involved in hyperglycaemia and is affected by plasma asymmetric dimethylarginine (ADMA). ADMA is an endogenous, competitive inhibitor of nitric oxide synthase and is induced by inflammatory cytokines of tumour necrosis factor (TNF)-α *in vitro*. We hypothesized that a tight glycaemic control may restore endothelial function in patients with type-2 diabetes mellitus (DM), in association with modulation of TNF-α and/or reduction of ADMA level.

Methods and results In 24 patients with type-2 DM, the flow-mediated, endothelium-dependent dilation (FMD: %) of brachial arteries during reactive hyperaemia was determined by a high-resolution ultrasound method. Blood samples for glucose, cholesterol, TNF-α, and ADMA analyses were also collected from these patients after fasting. No significant glycaemic or FMD changes were observed in 10 patients receiving the conventional therapy. In 14 patients who were hospitalized and intensively treated, there was a significant decrease in glucose level after the treatment [from 190 ± 55 to 117 ± 21 (mean ± SD) mg/dL, $P < 0.01$]. After the intensive control of glucose level, FMD increased significantly (from 2.5 ± 0.9 to 7.2 ± 3.0%), accompanied by a significant ($P < 0.01$) decrease in TNF-α (from 29 ± 16 to 11 ± 9 pg/dL) and ADMA (from 4.8 ± 1.5 to 3.5 ± 1.1 μM/L) levels. The changes in FMD after treatment correlated inversely with those in TNF-α ($R = -0.711$, $P < 0.01$) and ADMA ($R = -0.717$, $P < 0.01$) levels.

Conclusion The intensive correction of hyperglycaemia is associated with the improvement of endothelial function, which is coupled with the decrease in the levels of reduction of plasma TNF-α and ADMA in patients with type-2 DM. A strict glycaemic control may exert anti-cytokine and anti-atherogenic effects and may therefore be pathophysiologically important.

Introduction

Cardiovascular disease is the major cause of morbidity and mortality in patients with type-2 diabetes mellitus (DM),[1] in whom hyperglycaemia is one of the main metabolic abnormalities.[2] Blood glucose control occupies the centre stage in DM management.[3] A recent controlled trial, i.e. the United Kingdom Prospective Diabetes Study (UKPDS), suggested that an intensive glucose-lowering treatment decreases the occurrence of macrovascular complications.[4] However, the exact roles of hyperglycaemia and glycaemic control in cardiovascular complications remain to be determined in patients with type-2 DM.

Previous studies demonstrated that acute hyperglycaemia impairs endothelium-dependent vasodilation in healthy subjects[5,6] and further depresses it in patients with type-2 DM.[6] These findings indicate a possible link between glucose level and endothelial function in humans. Endothelial dysfunction is an important phenomenon in the pathogenesis of atherosclerosis[7] and is related to the derangements of nitric oxide (NO) synthase in the vessel wall.[8] Asymmetric dimethylarginine (ADMA) is an endogenous, competitive inhibitor of NO synthase.[9] Its concentration is increased by tumour necrosis

* Corresponding author. Tel: +81 22 717 7153; fax: +81 22 717 7156.
E-mail address: syasuda@cardio.med.tohoku.ac.jp
† Present address: Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai 980-8574, Japan.

factor-α (TNF-α),[10] which is implicated as an important factor in the pathogenesis of type-2 DM.[11]

Thus, the present study was designed to investigate whether an intensive therapy of hyperglycaemia may improve endothelial function in association with the modulation of the cytokines and/or decrease in plasma ADMA level in patients with type-2 DM.

Methods

Study patients

The study protocol was approved by the Institutional Review board, and all these patients gave their written informed consent to participate in the study. Type-2 DM was defined according to the criteria of the Diagnosis and Classification of Diabetes Mellitus.[12] Between May 1999 and June 2000, type-2 DM patients with poor glycaemic control [fasting blood glucose >200 mg/dL and/or haemoglobin A-1C (Hb A-1C) >9%] were recruited for intensive treatment of hyperglycaemia during hospitalization. Twenty-four patients were initially assessed for inclusion in the study. Among them, 14 patients [nine men and five women, mean age 61 ± 12 (SD) years] gave their consent and were admitted to the Hospital of the National Cardiovascular Center (intensive treatment group). The remaining 10 patients [seven men and three women, mean age 63 ± 15 (SD) years], who refused to be hospitalized and were obliged to keep conventional (non-intensive) diabetes treatment, served as the control group in the present study.

All the patients underwent history screening, physical examination, and laboratory analysis, including a complete blood count: the levels of plasma electrolyte, glucose, insulin, Hb A-1C, blood urea nitrogen, creatinine, transaminases and urinary protein levels, and lipid profile. Moreover, the patients were assessed for the presence of diabetic complication, i.e. retinopathy, neuropathy, nephropathy, a history of myocardial infarction, and the presence of angina pectoris and arteriosclerosis obliterans. Patients with nephrotic-range proteinuria, thyroid disease, apparent infections, or haematologic, hepatic, or renal disease were excluded from the study. Before admission, five patients had been receiving angiotensin-converting enzyme inhibitors for hypertension and five patients receiving statin for hyperlipidaemia for over 6 months. These medications were not changed throughout the study period. In addition, no new drugs other than insulin or oral hypoglycaemic agents were administered to any of these patients.

Study design

On admission, following an overnight fasting, a non-invasive assessment of brachial arterial vasoreactivity in response to reactive hyperaemia or nitroglycerin was performed with blood sampling for the determination of the levels of glucose, insulin, Hb A-1C, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, TNF-α, and ADMA in the plasma. We also measured plasma hepatocyte growth factor (HGF) level. The HGF may protect against endothelial dysfunction, and its production is suppressed by high glucose levels.[13] Body mass index (BMI) was calculated using the formula BMI = weight (kg)/height^2 (m^2). All measurements were repeated after ~1 month of intensive treatment for hyperglycaemia.

The intensive therapy was aimed at maintaining normal fasting glucose (80–115 mg/dL) and pre-prandial blood glucose (<130 mg/dL) levels. Throughout the study, the patients followed a 1200–1300 Kcal diet regimen of 60–65 g of protein, 30–35 g of fat, and 160–170 g of carbohydrates. The level of dietary cholesterol was 350 g/day. The dose of oral anti-diabetic drugs was adjusted accordingly and/or insulin therapy was administered to improve glycaemic control. The patients were examined once or twice a week over a 4–5-week period of blood glucose monitoring. None of the patients experienced a hypoglycaemic reaction during the study.

Brachial artery ultrasound

Flow-mediated, endothelium-dependent vasodilation (FMD) following reactive hyperaemia and endothelium-independent nitroglycerin-induced vasodilation of the brachial artery were assessed using a high-resolution ultrasound machine (System Five, General Electronics) equipped with a 7.5 MHz linear array transducer.[6] After a 10 min rest in a temperature-controlled room (22–23 °C), the diameter of the right brachial artery and baseline forearm flow velocity were measured. Increased forearm blood flow was induced by the inflation of a blood pressure cuff placed around the forearm to 200 mmHg or to a pressure of 50 mmHg greater than the systolic blood pressure. This was followed by deflation (RD2 Cuff Deflator, Hokanson Inc., Bellevue, WA, USA) after 5 min. Repeated blood flow scans were obtained to measure the diameter of the brachial artery. After 15 min of vessel recuperation, a repeated measurement of the diameter of the resting brachial artery and repeated blood flow scans were obtained. Sublingual nitroglycerin (0.4 mg) was administered, and then final scans were obtained after 3 min. Throughout the study, a single lead electrocardiogram was obtained, and blood pressure was measured in the left arm every 2 min by an automated blood pressure recorder.

Ultrasound images were recorded on an S-VHS videocassette recorder. Depth and gain settings were used to optimize the images of the lumen–arterial wall interface. Vessel diameter was measured in triplicate at end diastole, from the anterior to the posterior interface between the media and the adventitia. Flow-mediated vasodilation was calculated as the ratio of brachial artery diameter after reactive hyperaemia to baseline diameter and expressed as a per cent increase. Nitroglycerin-mediated vasodilation was calculated in an analogous manner. Volumetric flow rate was calculated by multiplying the time velocity integral of the angle (~70°)-corrected Doppler flow signal by the heart rate and the vessel cross-sectional area. Changes in blood flow were expressed as the percentages of the resting flow measurements. All measurements were performed with the observers blind to patient information and study date. Using this methodology and analysis, the intra- and inter-observer variabilities in brachial artery diameter were 0.03 ± 0.02 (mean ± SD) and 0.06 ± 0.02 mm, respectively, and the variability in FMD performed on two different days was 1.4 ± 0.5%.

Laboratory measurements

Fasting plasma glucose level was measured by the glucose oxidase method and Hb A-1C level was measured by automated high-performance liquid chromatography. Insulin level was measured by the conventional radioimmunoassay. To assess insulin resistance, we used the following homeostasis model assessment (HOMA) parameters: HOMA-R = [fasting blood glucose (mg/dL) × fasting insulin (μU/mL)]/405.[14]

Total cholesterol, triglyceride, and HDL cholesterol levels were determined as described previously.[15] LDL cholesterol level was calculated using the Friedewald equation.[16]

TNF-α and HGF levels were determined by enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co., Tokushima, Japan). The detection limits of these methods are 2 pg/mL for TNF-α and 0.1 ng/mL for HGF. The intra- and inter-assay coefficients of variation were both ~7% for the enzyme-linked immunosorbent assay.

Plasma ADMA concentration was measured using high-performance liquid chromatography with pre-column derivatization, as previously described.[17] In brief, equilibrated CBA columns

Table 1 Patient characteristics

	Standard therapy (control), $n = 10$	Intensive therapy, $n = 14$	P-value
Age (years), mean ± SD	63 ± 15	61 ± 12	0.7201
Male, *n* (%)	7 (70%)	9 (64%)	0.7697
Risk factors			
Hypertension, *n* (%)	5 (50%)	7 (50%)	1.000
Hyperlipidaemia, *n* (%)	6 (60%)	7 (50%)	0.9448
Smoking, *n* (%)	4 (40%)	5 (36%)	0.8307
Retinopathy, *n* (%)	2 (20%)	2 (14%)	0.7111
Proteinuria, *n* (%)	2 (20%)	4 (29%)	0.6326
CAD, *n* (%)	2 (20%)	3 (21%)	0.9323
Peripheral artery disease, *n* (%)	0 (0%)	2 (14%)	0.6175
Stroke, *n* (%)	1 (10%)	1 (7%)	0.8028
Medications, baseline[a]			
ACE-inhibitor, *n* (%)	4 (40%)	5 (36%)	0.8307
Calcium blocker, *n* (%)	2 (20%)	1 (7%)	0.7543
Beta-blocker, *n* (%)	2 (20%)	1 (7%)	0.7543
Statin, *n* (%)	6 (60%)	5 (36%)	0.4462
Sulphonylurea, *n* (%)	7 (70%)	10 (71%)	0.9395
Biguanide, *n* (%)	1 (10%)	1 (7%)	0.7543
α-Glucosidase inhibitor, *n* (%)	4 (40%)	2 (14%)	0.3380

ACE, angiotensin-converting enzyme.
[a]Medications immediately before additional therapy for dysglycaemia.

(Bond Elut, Varian Inc., CA, USA) were used for three-fold washing with 1 mL serum samples with methanol and distilled water. Thereafter, the samples were eluted with 10% ammonia and dried. The sediment obtained was dissolved in 1 mL of water, the solution was centrifuged, and the supernatant was subjected to high-performance liquid chromatography using ODS columns (Fisher Scientific, St Louis, MO, USA). ADMA concentration was calculated on the basis of the recovery rate of L-monomethyl-arginine (Sigma, St Louis, MO, USA), used as the internal standard. Intra- and inter-assay variabilities were both ~6%, with a detection limit of 0.1 μM/L.

Statistical analyses

Sample size calculations were performed using a primary endpoint variable of FMD. Power calculations indicated that to detect a mean difference in FMD of 4% (SD, 3%), 13 subjects would be needed to complete the study (α statistics, 0.05; power >0.9). All data are expressed as mean ± SD. Two-tailed *t*-tests or the Mann-Whitney *U* test was used to compare the changes in response to treatment. To compare the proportions of patients, Fisher's exact test was used. Linear regression curves and correlations were calculated according to the least-squares method. *P*-values less than 0.05 were considered significant.

Results

The baseline characteristics of 10 control patients who received standard therapy and 14 intensively treated patients are summarized in *Table 1*. All 24 patients completed 3–4-week follow-up measurements.

The control patients were treated by diet alone (three patients) or diet plus oral hypoglycaemic agents (an increased dose of sulphonylurea, six patients and addition of metformin to sulphonylurea, one patient). *Table 2* shows no significant improvements in clinical and biochemical parameters during the observation period of 28 ± 5 days of standard therapy. Neither the fasting blood glucose (from 181 ± 42 to 186 ± 38 mg/dL) nor the response of FMD to reactive hyperaemia (from 3.0 ± 1.3 to 2.6 ± 1.0%) changed.

Table 2 Changes in biochemical and clinical parameters before and after standard treatment of hyperglycaemia in 10 control patients with type-2 DM

	Before	After	P-value
Hb A-1C (%)	9.4 ± 2.2	9.4 ± 2.0	>0.999
Insulin (μU/mL)	4.2 ± 2.0	4.4 ± 2.2	0.834
HOMA-R	1.9 ± 1.2	1.8 ± 1.0	0.842
Total cholesterol (mg/dL)	212 ± 28	210 ± 25	0.868
TG (mg/dL)	128 ± 40	129 ± 45	0.959
HDL cholesterol (mg/dL)	50 ± 19	51 ± 20	0.910
LDL cholesterol (mg/dL)	128 ± 22	127 ± 25	0.925
Systolic BP (mmHg)	139 ± 18	138 ± 20	0.908
Diastolic BP (mmHg)	76 ± 8	78 ± 10	0.627
BMI (kg/m^2)	23.8 ± 2.7	23.4 ± 3.1	0.763

TG, triglyceride; BP, blood pressure. Values are expressed as mean ± SD.

Biochemical and clinical changes after intensive treatment of hyperglycaemia

In the intensive therapy group, the patients were all treated by diet alone (three patients), diet plus oral hypoglycaemic agents (sulphonylurea newly given, one patient; an increased dose of sulphonylurea, one patient; addition of metformin to sulphonylurea, two patients; and addition of α-glucosidase inhibitor to sulphonylurea, one patient), or diet plus insulin (switched from oral hypoglycaemic agents, six patients). The duration of intensive treatment of hyperglycaemia was 34 ± 13 days. Clinical and biochemical parameters at baseline (before treatment) were similar between the standard therapy group and the intensive therapy group (*Tables 2* and *3*). After the intensive

Table 3 Changes in biochemical and clinical parameters before and after intensive treatment of hyperglycaemia in 14 patients with type-2 DM

	Before	After	*P*-value	*P*-value (vs. control after)
Hb A-1C (%)	9.7 ± 1.6	8.6 ± 1.4	0.032	0.287
Insulin (μU/mL)	4.4 ± 2.6	5.3 ± 2.0	0.314	0.233
HOMA-R	2.0 ± 1.1	1.6 ± 0.5	0.226	0.524
Total cholesterol (mg/dL)	202 ± 33	173 ± 28	0.019	0.003
TG (mg/dL)	121 ± 43	105 ± 51	0.378	0.246
HDL cholesterol (mg/dL)	52 ± 21	52 ± 17	>0.999	0.896
LDL cholesterol (mg/dL)	125 ± 25	101 ± 29	0.027	0.032
Systolic BP (mmHg)	134 ± 18	128 ± 14	0.779	0.1626
Diastolic BP (mmHg)	77 ± 7	74 ± 8	0.301	0.2880
BMI (kg/m^2)	23.6 ± 3.6	21.4 ± 3.2	0.049	0.1405

Values are expressed as mean ± SD.

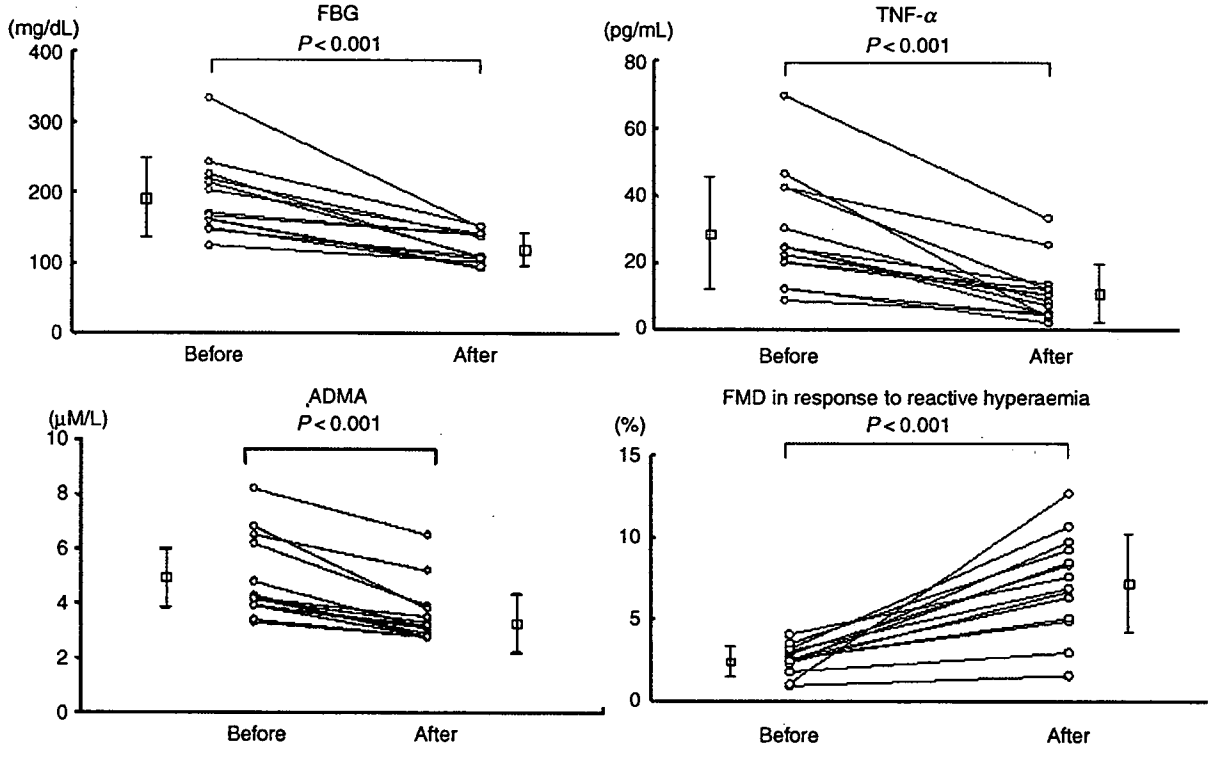

Figure 1 Individual measurements of fasting blood glucose (FBG), TNF-α and ADMA levels, and FMD in response to reactive hyperaemia before and after intensive treatment of hyperglycaemia in 14 patients with type-2 DM.

treatment, the fasting glucose level significantly decreased from 190 ± 55 to 117 ± 21 mg/dL ($P < 0.001$), as shown in *Figure 1*. Significant decreases in Hb A-1C, total cholesterol, and LDL cholesterol levels and BMI were observed, whereas no changes in HOMA-R index; insulin, triglyceride, or HDL cholesterol levels; and systolic and diastolic blood pressures were observed (*Table 3*). Two of three patients with coronary artery disease were taking statins at the time of the study.

The levels of plasma TNF-α (from 29 ± 16 to 11 ± 9 pg/dL, $P < 0.001$) and ADMA (from 4.8 ± 1.5 to 3.5 ± 1.1 μM/L, $P < 0.001$) significantly decreased after the intensive control of glucose level (*Figure 1*). However, HGF level did not significantly change throughout the study (from 0.19 ± 0.05 to 0.20 ± 0.08 ng/mL).

Brachial artery reactivity after intensive treatment of hyperglycaemia

Before treatment under hyperglycaemic condition, the baseline brachial arterial diameter was 4.5 ± 0.3 mm, and FMD in response to reactive hyperaemia was 2.4 ± 0.9%. After the intensive control of glucose level, FMD significantly ($P < 0.001$) increased to 7.2 ± 3.1% (*Figure 1*), whereas the baseline diameter (4.5 ± 0.2 mm) did not change. There was a similar increase in blood flow during reactive

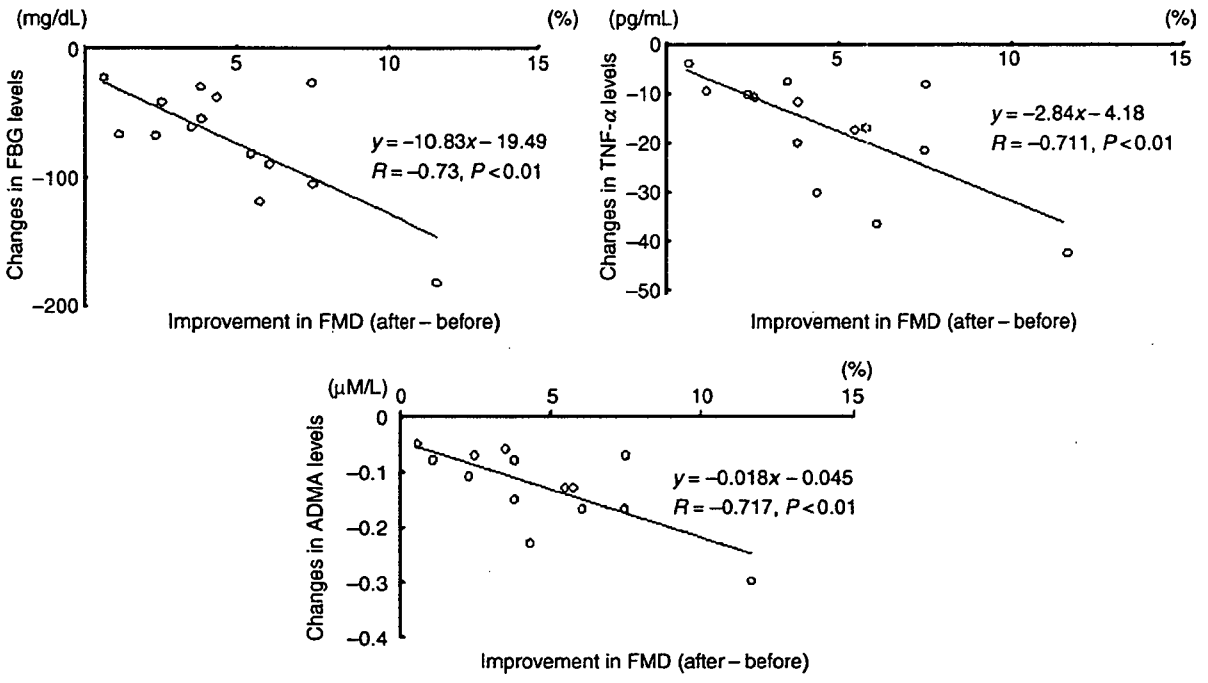

Figure 2 Correlation of improvement of FMD after treatment with decreases in levels of FBG, TNF-α, and ADMA.

hyperaemia (293 ± 16 vs. 296 ± 20%) and a similar baseline heart rate (67±7 vs. 65 ± 8 bpm) before and after the treatment.

Nitroglycerin-mediated vasodilation was 9.8 ± 1.0% before treatment; however, in contrast to FMD, it did not change after treatment (10.0 ± 1.6%).

Correlation with FMD improvement

As shown in *Figure 2*, the improvement of FMD after treatment correlated inversely with the changes in fasting glucose ($R = -0.730$, $P < 0.01$), TNF-α ($R = -0.711$, $P < 0.01$), and ADMA ($R = -0.717$, $P < 0.01$) levels. However, the improvement of FMD did not correlate significantly with the changes in Hb A-1C level ($R = 0.408$, $P = 0.148$), total cholesterol level ($R = 0.325$, $P = 0.256$), or BMI ($R = 0.270$, $P = 0.351$).

Six-to-12-month follow-up

A follow-up study was performed 6–12 months after the discharge. In eight of 14 patients with an Hb A-1C level of <8.0% at this follow-up period, fasting blood glucose level and FMD remained at 127 ± 26 mg/dL and 8.4 ± 1.0%, respectively. In contrast, in the remaining six patients with an Hb A-1C level of ≥8.0%, fasting blood glucose level and FMD worsened to be 178 ± 30 mg/dL and 3.1 ± 1.1%, respectively. There were inverse correlations of FMD with fasting blood glucose ($R = -0.577$) and Hb A-1C levels ($R = -0.860$).

Discussion

The major finding in the present study is that the intensive treatment of hyperglycaemia is associated with the improvement of endothelial function, coupled with the decrease in plasma TNF-α and ADMA (an endogenous inhibitor of NO synthase) levels in patients with type-2 DM.

Previous studies revealed that an acute increase in blood glucose level impairs endothelium-dependent vasodilation in healthy subjects[5,6] and further inhibits it in patients with type-2 DM.[6] DM is a state of chronic hyperglycaemia, and glycaemic control is one of the major goals of diabetes management.[18] As shown in *Figure 1*, endothelial dysfunction improves after a 5-week intervention targeting hyperglycaemia in type-2 diabetes patients, accompanied by a relatively small but significant decrease in Hb A-1C level. In contrast, either hyperglycaemia or endothelial function did not change in control outpatients who received routine treatment. These findings suggest that hyperglycaemia may be a fundamental abnormality underlying the mechanism that causes endothelial dysfunction in DM. However, we must acknowledge a potential limitation that an appropriate control group should have included patients who were admitted to the hospital, but did not receive intensive treatment. In addition, the number of statistical tests performed and relatively small sample size of the study population may potentially infiltrate type-I error.

In patients with type-2 DM, TNF-α levels were elevated in both blood and tissue.[19-21] Taken together with results from knockout mice deficient in TNF-α or its receptors,[11] it is suggested that TNF-α is a factor contributing to the pathogenesis of type-2 DM. Hyperglycaemia is an important stimulus for TNF-α synthesis in human peripheral monocytes *in vitro*.[22] A previous *in vivo* study demonstrated that the administration of TNF-α impairs endothelial-dependent vasodilation in rats.[23] In the present study, as shown in *Figure 1*, plasma TNF-α level decreased after the intensive treatment of hyperglycaemia. This finding indicates the therapeutic potential of a strict glycaemic control against inflammatory cytokines that play a prominent role in atherogenesis.[7]

TNF-α and hyperglycaemia could impair dimethylarginine dimethylaminohydrolase and cause the accumulation of ADMA, an endogenous, competitive inhibitor of NO synthase, contributing to the derangements of NO pathways in the vessel.[10,24] The intra-arterial infusions of ADMA significantly impair endothelium-dependent flow responses in the human forearm.[25] In the present study, we found that the ADMA level increased in patients with type-2 DM (*Figure 1*), and its decrease after the strict glycaemic control correlated significantly with the improvement of FMD (*Figure 2*). Not only ADMA, but also TNF-α itself downregulates NO synthase by decreasing mRNA's half-life.[26] Moreover, both inflammatory cytokines and high glucose levels enhance the production of oxygen-derived free radicals,[27,28] which rapidly inactivate NO.[29] In patients with type-2 DM, the extent of urinary excretion of the isoprostanes (8-iso-prostaglandin $F_{2\alpha}$) significantly decreased ~4 weeks after an intensive therapy for hyperglycaemia, an intervention similar to that used in the present study.[30] Taking together a recent report that lowering serum TNF-α level alone (without glycaemic control) does not improve endothelial function,[31] these findings suggest that the hyperglycaemia-induced oxidative stress could be a key factor in the pathophysiology of diabetes.

HGF is characterized to be one of the most potent mitogens among the growth factors for vascular endothelial cells and contributes to vascular protection or repair.[13] Because its production is suppressed by glucose in a dose-dependent manner *in vitro*,[13] we hypothesized that endothelial dysfunction might be associated with the decreased production of HGF in diabetic patients. However, this was not the case. The level of HGF did not change throughout this study. Moreover, as shown in *Table 3*, insulin sensitivity, as assessed using HOMA-R index,[14] did not change significantly. Insulin resistance contributes, in part, to the pathogenesis of type-2 DM and may be potentially linked with endothelial dysfunction and ADMA.[32] To address this important issue, we need to further assess insulin sensitivity with a more specific method such as steady-state plasma glucose measurement.

Impaired endothelium is a key factor for diabetic macroangiopathy.[7] Thus, restoring endothelial function has important clinical implications for reducing the risk of cardiovascular diseases in diabetic patients. The present results, although obtained in a short period, suggest that a long-term maintenance of strict glycaemic control is important. If hyperglycaemia continues, then the expression level of NO synthase and the generation of NO may be chronically reduced, leading to a persistent dysfunction of the vascular endothelium and the consequent atherogenesis. In the UKPDS conducted for more than 15 years,[4] the difference in Hb A-1C level between the conventionally and intensively treated groups was significant throughout the study. However, Hb A-1C level progressively increased in both groups. The median Hb A-1C level was 6.6% in the first 5 years, but increased to 8.1% in the last 5 years, even in the intensively treated group. A difficulty in maintaining a good glycaemic control may explain, in part, the borderline decrease in the extent of myocardial infarction ($P = 0.05$) induced by the intensive treatment. Taking the multifactorial aetiology of macrovascular disease into account, the results of the UKPDS also suggest that the optimum treatment of patients with type-2 DM would include the control of blood pressure and correction of lipid abnormalities in addition to the control of glucose level. For the assessment of the effectiveness of therapeutic/dietary interventions and for the early detection of vascular dysfunction, plasma ADMA may be useful as a potential biochemical marker.[9,33] Metformin,[34] angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker,[35] and statins[36] could decrease ADMA level. Although these drugs were not newly given in the present patients, it is possible that an increased utilization of and compliance with medications and an improved diet during hospitalization may contribute, at least in part, to endothelial function improvement. Insulin-sensitizing rosiglitazone also decreases ADMA level.[37] A recent study has suggested that obese and insulin resistance are not strongly associated with the development of type-2 DM in Japanese patients with a BMI of ~23 kg/m^2 (from the Japan Diabetes Complications Study), unlike in European patients with a BMI of ~29 kg/m^2 (from the UKPDS).[38]

In conclusion, in patients with type-2 DM, the intensive treatment of hyperglycaemia is associated with the improvement of endothelial dysfunction, coupled with decreases in TNF-α and ADMA levels. A strict glycaemic control may exert anti-cytokine and anti-atherogenic effects and may therefore be pathophysiologically important.

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Conflict of interest: none declared.

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Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction

Satoshi YASUDA, Shunichi MIYAZAKI, Hideyuki KINOSHITA, Noritoshi NAGAYA, Munetake KANDA, Yoichi GOTO and Hiroshi NONOGI

Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan

ABSTRACT

Previous experimental studies have demonstrated that MMPs (matrix metalloproteinases) contribute to LV (left ventricular) remodelling. We hypothesized that cardiac MMPs are activated in patients with AMI (acute myocardial infarction) and, if so, MMP production may be attenuated by statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) through their cardiovascular protective actions. We studied 30 patients, ten control patients with stable angina pectoris and 20 patients with AMI, in whom LV catheterization at the chronic stage was performed 22 ± 12 days (value is mean ± S.D.) after the onset of AMI. Blood samples were collected from the CS (coronary sinus) and a peripheral artery. In patients with AMI, the levels of MMP-2 and MMP-9 were significantly ($P < 0.05$) higher in the CS than the peripheral artery (MMP-2, 853 ± 199 compared with 716 ± 127 ng/ml; MMP-9, 165 ± 129 compared with 98 ± 82 ng/ml), whereas no significant differences were observed in the patients with angina pectoris. The CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with BNP (brain natriuretic peptide) levels (MMP-2, $R = 0.68$, $P < 0.01$; MMP-9, $R = 0.59$, $P < 0.05$) and LV end-diastolic volume index (MMP-2, $R = 0.70$, $P < 0.01$; MMP-9, $R = 0.70$, $P < 0.01$). When patients with AMI treated with 10 mg of pravastatin or without ($n = 10$ in each group) were compared, this statin therapy significantly ($P < 0.05$) decreased the CS–arterial concentration gradients of MMP-2 (69 ± 43 compared with 213 ± 185 ng/ml) and MMP-9 (14 ± 27 compared with 119 ± 84 ng/ml). In conclusion, the enhanced production of cardiac MMP-2 and MMP-9 is associated with LV enlargement and elevated BNP levels in patients with AMI. A pleiotropic effect of statins appears to be associated with the modulation of cardiac MMP activation, which may be potentially beneficial in the attenuation of post-infarction LV remodelling.

Key words: acute myocardial infarction, angina pectoris, brain natriuretic peptide (BNP), metalloproteinase (MMP), remodelling, statin, tissue inhibitor of metalloproteinases (TIMP).

Abbreviations: ACE-I, angiotension-converting enzyme inhibitor; AMI, acute myocardial infarction; Ang II, angiotensin II; AP, angina pectoris; BNP, brain natriuretic peptide; CK, creatine kinase; CRP, C-reactive protein; CS, coronary sinus; LDL, low-density lipoprotein; LV, left ventricular; LVEDVI, LV end-diastolic volume index; LVEF, LV ejection fraction; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; WBC, white blood cell.

Correspondence: Dr Satoshi Yasuda, at the present address: Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai, 980-8574, Japan (email syasuda@cardio.med.tohoku.ac.jp).

INTRODUCTION

The loss of myocytes as a consequence of AMI (acute myocardial infarction) results in progressive changes in ventricular architecture [1,2]. This process, defined as post-infarction ventricular remodelling, is associated with a higher mortality and a higher incidence of complications, such as the development of heart failure, aneurysm formation and ventricular rupture [3,4]. During the remodelling process, as well as intrinsic changes in cardiac myocytes, it has been recognized that important alterations also occur within the extracellular matrix of the myocardium [5,6].

MMPs (matrix metalloproteinases) belong to a family of zinc-containing endoproteinases responsible for extracellular protein degradation, and are inhibited by specific tissue inhibitors [TIMP (tissue inhibitor of metalloproteinases)] [5,6]. In experimental myocardial infarction, MMPs are up-regulated in myocardial tissues, and are the driving force in extracellular matrix remodelling and infarct expansion [7,8]. Among the MMPs, the importance of MMP-9 during the processes of infarct healing and LV (left ventricular) remodelling has been demonstrated in previous studies using genetically modified mice [9,10]. Infarcted mice with the targeted deletion of MMP-9 had a decreased incidence of early myocardial rupture [9] and progressive LV dilation [10]. However, in the clinical setting, there has been little evidence regarding the production of MMPs in the infarcted human heart.

Statins have various cardiovascular protective actions, including anti-inflammatory and anti-apoptotic actions, independent of their effects on cholesterol levels. A study using a mouse AMI model demonstrated that statin treatment attenuated LV remodelling [11], which was associated with decreased MMP activity [12].

In the present study, we hypothesized that cardiac MMP activation may be associated with the degree of LV enlargement and the level of BNP (brain natriuretic peptide), a biochemical marker of post-infarction remodelling [13,14]. If so, MMP production may be attenuated by statin treatment in patients with AMI.

MATERIALS AND METHODS

Patients

This study included 30 male patients. All of the patients gave their written informed consent prior to participation in the study. The Institutional Ethical Committee on Human Research approved the study protocol. Patients with the following disorders were excluded from the study: prior myocardial infarction, and liver (elevated activities of aminotransferases), kidney (elevated level of creatinine or urea) or lung dysfunction (restrictive or obstructive pattern in spirometry).

The control group consisted of ten patients with stable AP (angina pectoris), who complained of symptoms consistent with Canadian Cardiovascular Society Classification of angina level I, II or III, with evidence of myocardial ischaemia. All of the control patients had no evidence of a previous AMI, and had severe coronary artery stenosis and therefore underwent coronary angioplasty (with adjunctive stenting in five patients). The treated sites were the left anterior descending artery in four patients (40 %), the right or left circumflex artery in four patients (40 %), and both the left anterior descending and right coronary arteries in two patients (20 %).

We also studied 20 patients with AMI who fulfilled the following criteria: typical chest pain >30 min of duration, ST segment elevation > 0.1 mV in two or more ECG leads with the subsequent evolution of a typical infarct pattern, and increased serum CK (creatine kinase) level. A total of 14 patients underwent PTCA (percutaneous transluminal coronary angioplasty) of the infarct-related artery (with adjunctive stenting in nine patients), and the remaining six patients received an intravenous administration of a tissue-type plasminogen activator and/or heparin in the acute phase. In all the patients, coronary angiography immediately after treatment showed a TIMI 3 grade flow in the infarct-related artery. The elapsed time to reperfusion was 4.6 h on average. The infarct sites were in the anterior wall in ten patients (50 %), the inferior wall in seven patients (35 %) and the postero-lateral wall in three patients (15 %). In this study, all of the patients with AMI were treated with the ACE-I (angiotension-converting enzyme inhibitor) enalapril (5 mg) after their hospital admission. Among them, ten patients with hyperlipidaemia (total cholesterol level > 220 mg/dl) were treated with 10 mg of pravastatin; the remaining ten patients did not have hyperlipidaemia and thus did not receive pravastatin. A recent Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) trial [14a] has shown a similar decrease in coronary artery disease incidence following treatment with 10–20 mg of pravastatin used in Asia to that observed for 20–40 mg doses used in Europe and the United States.

Cardiac catheterization and analysis of LV function

In patients with AMI, chronic-stage cardiac catheterization was repeated approx. 3–4 weeks after the onset of AMI. A 5 French multipurpose catheter (Cathex) was introduced into the CS (coronary sinus) through the left subclavian vein under fluoroscopic guidance [14]. The position of the catheter tip was confirmed by the injection of contrast medium. Blood samples were collected from the CS before the intravenous administration of heparin. Following the collection of blood samples from the right brachial artery (as peripheral blood samples) through a 6 French sheath, heparin was administered and coronary

angiography and left ventriculography were performed, according to the conventional Judkins' technique. LV pressure was measured using a 2 French high-fidelity micromanometer catheter (Miller Instruments) advanced into the left ventricle via the lumen of a 6 French pig-tail catheter. The restenosis of a treated artery was defined as an arterial narrowing of > 75 %, as determined by coronary angiography.

LV volume was evaluated angiographically by a cardiologist who was blinded to the results of the biochemical assays. Ventricular silhouettes in a 30° right anterior oblique projection were digitized using an ANCHOR ventriculography analysis system (Siemens-Elema). Using the area-length method, LV end-systolic volume index, LVEDVI (LV end-diastolic volume index) and LVEF (LV ejection fraction) were calculated.

Biochemical assessment

Blood samples were centrifuged and serum was stored at − 80 °C until assay. A sandwich enzyme immunoassay was performed to determine MMP-2 level (Fuji Chemical Industries) [15]. In addition, the level of MMP-9, another gelatinase-like MMP-2, and that of MMP-13, an interstitial collagenase, were analysed using MMP Biotrak enzyme-linked immunoadsorbent assay kits (Amersham Biosciences). The levels were back-calculated from the standard curve determined with the enzyme-linked immunoadsorbent assay kits using a 96-well microplate reader (Emax; Molecular Devices). These kits detect the pro-enzyme and the pro-enzyme complexed with TIMP. The detection limits were 0.5 ng/ml for MMP-2, 0.6 ng/ml for MMP-9 and 0.03 ng/ml for MMP-13.

We also measured levels of TIMP-1 (Fuji Chemical Industries) and TIMP-2 (Amersham Biosciences) using sandwich enzyme immunoassays [15]. The detection limits for TIMP-1 and TIMP-2 were 1.2 and 8.0 ng/ml respectively.

BNP was measured using specific immunoradiometric assay kits (Shionogi). The sensitivity of these kits was 2 pg/ml. Ang II (angiotensin II) and TGF-β (transforming growth factor-β) levels were also measured, as reported previously [16].

The serum CRP (C-reactive protein) level was measured by N Latex CRP II monoassay using a nephelometric analyser (BN II; Dade Behring). The lower detection limit of this test was 0.06 mg/dl. Total cholesterol, triacylglycerol (triglyceride) and HDL (high-density lipoprotein) cholesterol concentrations were determined by enzymatic methods using a Toshiba TBA 80M analyser. LDL (low-density lipoprotein) was calculated using Fredewald's formula. We also measured WBC (white blood cell) number.

Statistical analysis

The two groups were compared by Student's t test. Measurements from the CS and the peripheral artery were compared within a group by ANOVA. When a significant difference among groups was indicated by the initial analysis, individual paired comparisons were determined using the Student–Newman–Keuls method. A linear regression line was calculated by the least-square method to assess the correlation between two parameters. To investigate independent predictors, we used multivariate logistic regression analysis. In all cases, differences were considered significant at $P < 0.05$. Results are presented as means ± S.D., or medians.

Table 1 Clinical characteristics

*$P = 0.05$ and **$P < 0.01$ compared with control (patients with stable AP).

Characteristic	Patients with AMI ($n = 20$)	Patients with stable AP ($n = 10$)
Age (years)	66 ± 9	67 ± 6
Peak CK (units/l)	1986 (801–8574)	–
Cardiac function		
LVEF (%)	48 ± 7**	58 ± 7
LVEDVI (ml/m^2)	95 ± 18**	55 ± 21
Vessels > 75 % stenosed (n)	1.5 ± 0.7	1.6 ± 0.7
Risk factors (n)		
Hypertension	11 (55 %)	7 (70 %)
Diabetes mellitus	15 (75 %)	6 (60 %)
Hyperlipidaemia	10 (50 %)	6 (60 %)
Smoking	12 (60 %)	6 (60 %)
Biochemical parameters†		
Total cholesterol (mg/dl)	193 ± 27	198 ± 20
LDL (mg/dl)	120 ± 30	122 ± 31
WBC count (cells/μl)	6615 ± 1571	5600 ± 1063
CRP (mg/dl)	0.34 ± 0.33*	0.13 ± 0.06
Medication used (n)		
ACE-I	20 (100 %)	4 (40 %)
β-Blockers	11 (55 %)	6 (60 %)
Statins	10 (50 %)	6 (60 %)
Calcium antagonists	7 (35 %)	5 (50 %)
Nitrates	4 (20 %)	2 (20 %)
Aspirin	20 (100 %)	10 (100 %)

† Data obtained on the day when cardiac catheterization was performed.

RESULTS

The baseline clinical characteristics of the patients with AMI and the control patients with AP (without evidence of AMI) are summarized in Table 1. In the patients with AMI, cardiac function data were obtained at chronic-stage cardiac catheterization performed 22 ± 12 days after the onset of AMI. Coronary angiography revealed 90 % stenosis of the infarct-related artery in two patients and 100 % stenosis in three patients. These five patients with restenosis had received intravenous thrombolysis alone in the acute stage. In the remaining 15 patients, the treated

Table 2 Comparisons of BNP, MMP and TIMP levels in the CS and peripheral artery

*$P < 0.05$ compared with levels in artery; †$P < 0.05$ compared with control (patients with stable AP).

	Patients with AMI ($n = 20$)		Patients with stable AP ($n = 10$)	
Peptide	CS	Artery	CS	Artery
BNP (pg/ml)	400 ± 376*†	126 ± 176	54 ± 25	52 ± 25
MMP-2 (ng/ml)	853 ± 199*†	716 ± 127	631 ± 44	630 ± 46
MMP-9 (ng/ml)	165 ± 129*†	98 ± 82	68 ± 25	71 ± 24
MMP-13 (ng/ml)	0.05 ± 0.04	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
TIMP-1 (ng/ml)	155 ± 59	150 ± 53	130 ± 33	134 ± 32
TIMP-2 (ng/ml)	112 ± 18	108 ± 14	94 ± 11	97 ± 16

sites remained patent. With the exception of cardiac function (LVEF and LVEDVI) and the prevalence of ACE-I use, clinical characteristics were similar between patients with AMI and AP.

Enhancement of cardiac MMP production in patients with AMI

Table 2 shows the comparison of BNP, MMP and TIMP levels between blood samples from the CS and peripheral artery. In patients with AMI, levels of BNP, MMP-2 and MMP-9 were significantly ($P < 0.05$) higher in the CS than in the peripheral artery, whereas the levels of MMP-13, TIMP-1 and TIMP-2 were similar. In control patients with AP, no significant differences in the levels of BNP, MMPs and TIMPs were observed between the CS and peripheral artery. These findings indicate that the production of MMP-2 and MMP-9, as well as that of BNP, is enhanced in an infarcted heart.

Correlation of cardiac MMP production with post-infarction LV remodelling

In patients with AMI, the CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with those of BNP and LVEDVI respectively (Figure 1), but not with LVEF, peak CK level and circulating WBC counts. These myocardial gradients were not different between patients with and without progression to restenosis (MMP-2, 87 ± 32 compared with 152 ± 173 ng/ml; MMP-9, 83 ± 86 compared with 61 ± 82 ng/ml).

Comparisons between pravastatin-treated patients with AMI and non-pravastatin-treated patients with AMI

We then compared levels of MMPs between ten patients treated with 10 mg of pravastatin and ten patients not treated with pravastatin (Table 3). Although the total cholesterol level before treatment was higher ($P < 0.05$) in the pravastatin-treated patients with AMI (223 ± 7 mg/dl in treated patients compared with 195 ± 17 mg/dl in

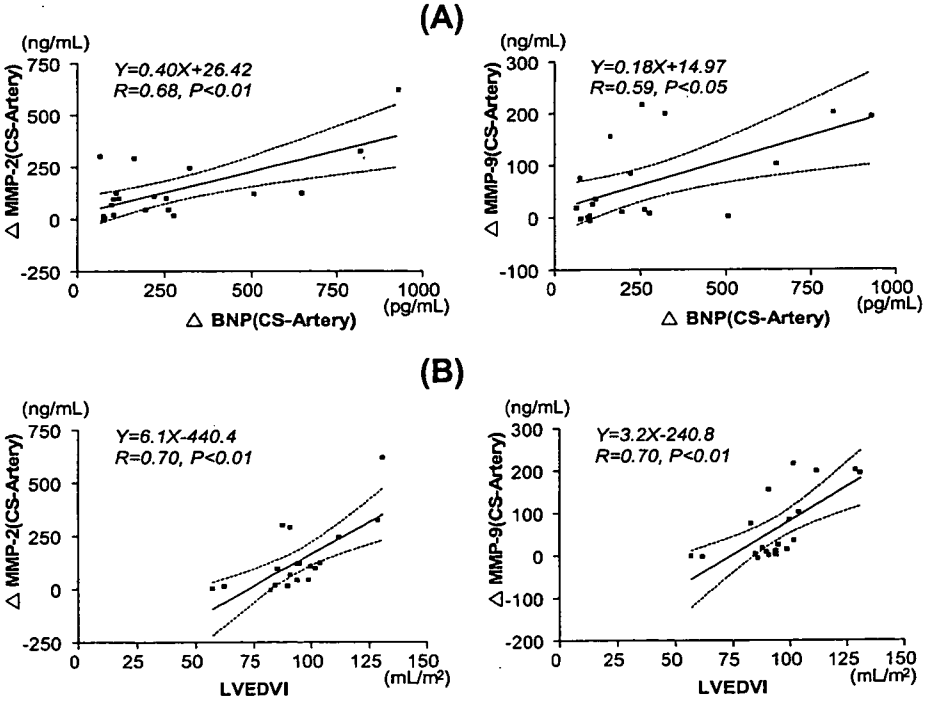

Figure 1 Correlations between CS–arterial concentration gradients of MMP-2 and -9 and BNP (A) and LVEDVI (B) in 20 patients with AMI

non-treated patients), no significant differences were observed after treatment between the two groups (183 ± 31 mg/dl in treated patients compared with 201 ± 20 mg/dl in non-treated patients). Levels of CRP (0.18 ± 0.13 mg/dl in treated patients compared with 0.50 ± 0.40 mg/dl in non-treated patients; $P = 0.03$) and the CS–arterial concentration gradients of MMP-2 and MMP-9 (Table 3) were significantly different between the two groups. However, the concentration gradients of TGF-β and Ang-II were similar between patients treated with pravastatin and those not treated (Ang-II, 19.5 ± 20.2 compared with 36.9 ± 32.4 pg/ml respectively; TGF-β, 1.2 ± 3.3 compared with 2.1 ± 4.7 pg/ml respectively).

We then performed multivariate analysis for the predictors of CS–arterial concentration gradients of MMP levels, including age, sex, coronary risk factors, peak CK, infarct site (anterior wall), CRP, TIMP, pravastatin treatment, LVEF and LVEDVI. The association between pravastatin treatment and cardiac MMP-2 production was modest, with an odds ratio of 0.074 (95 % confidence interval, 0.005–1.109; $P = 0.06$), and did not reach statistical significance.

DISCUSSION

The major findings of the present clinical study are that after AMI, the cardiac production of MMP-2 and MMP-9 is enhanced and associated with LV enlargement and BNP secretion, and that the pleiotropic effect of statins appears to be associated with the modulation of cardiac MMP activation.

Among the MMP species, MMP-2 and MMP-9 play an important role in LV remodelling, as these MMPs are activated in the myocardium and it has been reported that the targeted deletion of these MMPs prevents post-infarction cardiac dysfunction and rupture [9,10]. In the clinical setting, circulating MMP-2 and MMP-9 levels have been measured in previous studies of patients with AMI [17–19]; however, these results were conflicting. Squire et al. [17] reported that circulating MMP levels were inversely correlated with LV dilatation, whereas Matsunage et al. [18] and Nakaya et al. [19] found that serum MMP levels and activity were positively correlated with LV dilatation. In addition, circulating MMP levels could be affected at the acute stage following reperfusion therapy and by the clinically vulnerable state [20–23]. In the present study, we focused on cardiac production of MMP [14], and the measurement was performed at the clinically stable stage following AMI. As shown in Table 2, despite similar levels of TIMPs, significant differences in levels of BNP, MMP-2 and MMP-9 were observed between the CS and the peripheral artery in patients with AMI. To our knowledge, this is the first study demonstrating the enhanced production of MMP-2 and MMP-9 in a human infarcted heart. Moreover, as shown

Table 3 Comparisons of MMPs between pravastatin-treated and non-pravastatin-treated patients

*$P < 0.05$ compared with levels in artery; †$P < 0.05$ compared with levels in non-pravastatin-treated patients. CS–artery, CS–arterial concentration gradient.

	Patients with AMI						Patients with stable AP					
	Pravastatin-treated ($n = 10$)			Non-pravastatin-treated ($n = 10$)			Pravastatin-treated ($n = 6$)			Non-pravastatin-treated ($n = 4$)		
MMP (ng/ml)	CS	Artery	CS–artery	CS	Artery	CS–artery	CS	Artery	CS–artery	CS	Artery	CS–artery
MMP-2	808 ± 182	739 ± 158	69 ± 43†	897 ± 216*	684 ± 84	213 ± 185	631 ± 53	624 ± 51	7 ± 23	629 ± 32	639 ± 43	−9 ± 53
MMP-9	94 ± 61†	80 ± 59	14 ± 27†	236 ± 142*	117 ± 100	119 ± 84	68 ± 20	72 ± 16	−4 ± 4	68 ± 20	69 ± 29	0 ± 5
MMP-13	0.06 ± 0.06	0.03 ± 0.03	0.03 ± 0.06	0.03 ± 0.02	0.05 ± 0.03	−0.01 ± 0.03	0.03 ± 0.04	0.04 ± 0.02	−0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.03

in Figure 1, the CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with those of BNP and LVEDVI. Taking into account the delicate balance between MMPs and TIMPs in tissue remodelling, the present findings indicate that excessive cardiac production of MMPs may play an important pathological role in the progression of post-infarction LV dysfunction.

A previous experimental study of an AMI model using BNP-transgenic mice demonstrated a potential interaction of BNP with inflammation [24]. The overexpression of BNP leads to neutrophil infiltration and MMP-9 expression in the infarct region and increases the incidence of cardiac rupture. These findings suggest the significance of inflammatory reaction in the heart accompanied by changes in LV function. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors, such as statins, exert various cardiovascular protective effects beyond their lipid-level lowering actions [12,25]. These pleiotropic effects include the inhibition of inflammatory responses. In the present study, we have shown that the CS–arterial concentration gradients of MMP-2 and MMP-9 were smaller in the pravastatin-treated group than in the non-pravastatin-treated group, which was accompanied by a decrease in CRP level. These findings indicate that pravastatin may modulate cardiac MMP production in patients with AMI, probably via its anti-inflammatory effects. Similar observations of decreased circulating MMP-2 levels in patients with AMI treated with 10 mg of paravastain have been reported previously [19].

There are several potential limitations of the present study. First, this study was not randomized. Pravastatin was administered to a small number of patients with AMI with hyperlipidaemia. In such a pro-inflammatory state, tissue MMPs might have been activated before treatment [26], which could affect the results. Therefore prospective studies will be required to determine if pravastatin has a causal role in reducing cardiac MMP production in patients with AMI. Secondly, the present study was carried out over the short term, whereas ventricular remodelling is known to progress over months or years. Thirdly, previous studies have shown that the renin–angiotensin system is also involved in the induction of post-infarction ventricular remodelling [27] and can be inhibited by statins [28,29]. However, we have shown that the CS–arterial concentration gradients of Ang II were similar between pravastatin-treated patients and non-pravastatin-treated patients. This may be related, in part, to the fact that all our patients with AMI had been treated with 5 mg of enalapril.

In conclusion, the present study demonstrates the enhancement of MMP production in an infarcted heart. Pleiotropic effects of statins may be associated with the modulation of cardiac MMP activation, which is potentially beneficial in the attenuation of post-infarction LV remodelling.

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Prevention of Life-Threatening Ventricular Tachyarrhythmia by a Novel and Pure Class-III Agent, Nifekalant Hydrochloride

Junko Ohashi, MD, Satoshi Yasuda, MD, Shunichi Miyazaki, MD, Wataru Shimizu, MD, Isao Morii, MD, Takashi Kurita, MD, Atsushi Kawamura, MD, Shiro Kamakura, MD, and Hiroshi Nonogi, MD

Abstract: Nifekalant hydrochloride (NIF) is a novel intravenous class-III antiarrhythmic agent with a pirimidinedione structure that purely blocks the K^+channel without inhibiting β-adrenergic receptors. The authors investigated the efficacy of NIF for refractory ventricular tachycardia/fibrillation (VT/VF). They studied 30 patients treated with an intravenous infusion of NIF [26 men, 4 women; age: 63 ± 17 (mean ± SD) years] at a dose of 0.19 ± 0.14 mg/kg body weight per hour. Sixteen were patients with acute coronary syndrome (ACS), and 14 were patients with chronic structural heart disease (Chr-HD). Amiodarone and sotalol had already been administered to 9 patients with Chr-HD before the administration of NIF. The QT and T peak-end (Tp-e) intervals were measured and corrected by Bazett's method (QTc, cTp-e). The left ventricular ejection fraction was depressed (28 ± 9%). NIF was effective for preventing VT/VF without proarrhythmia and hemodynamic deterioration in 21 patients (70%; 12 with ACS; 9 with Chr-HD), but ineffective in 4 patients (all with Chr-HD). The QTc prolongation in the responders was more pronounced than in the nonresponders (25% ± 15% versus 5% ± 7% increase; $P < 0.05$). Proarrhythmic torsade de pointes (TdP) developed transiently in the remaining 5 patients in whom the cTp-e was markedly increased compared with that in the responders (93% ± 49% versus 37% ± 41% increase; $P < 0.05$). In conclusion, these findings indicate that the intravenous administration of NIF is useful in the emergent treatment of inhibiting drug-refractory VT/VF, although proarrhythmic TdP owing to an enhancement of transmural dispersion of repolarization needs to be taken into account.

Key Words: antiarrhythmia agents, electrocardiography, potassium, tachyarrhythmia, torsade de pointes

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INTRODUCTION

Nifekalant hydrochloride (NIF) is a class-III antiarrhythmic agent having a pirimidinedione structure.[1] NIF inhibits HERG channels, suggesting the selective inhibition of the rapid component of the delayed rectifier K^+current (IKr).[2] The major adverse effect of NIF is proarrhythmic torsade de pointes (TdP).[3,4] As class-III antiarrhythmic agents, NIF and amiodarone are similar, but they do have some differences. NIF is characterized as a pure K^+ channel blocker with a minimal negative inotropic effect,[5,6] which amiodarone has via a β-blocking action.[7] Negative inotropic effect of amiodarone is disadvantageous, particularly when amiodarone is administered rapidly to a failing heart. Moreover, NIF decreases the defibrillation threshold;[8] whereas, amiodarone does not.[9] On the basis of these unique features of NIF, we hypothesized that NIF may effectively inhibit refractory ventricular tachycardia/fibrillation (VT/VF) in patients with severe left ventricular dysfunction. We also investigated the electrocardiographic characteristics related to antiarrhythmic and proarrhythmic actions of NIF in electrocardiography. Therapy using NIF did not include the general advice of the Cardiopulmonary Resuscitation and Emergency Cardiovascular Care to administer amiodarone,[10] but instead was used as a replacement for this guideline.

MATERIALS AND METHODS

Study Patients

We studied 30 patients who were hospitalized between May 1999 and May 2004 in the National Cardiovascular Center (Suita, Japan) and were treated with intravenous administrations of NIF for refractory VT/VF [26 men, 4 women; age, 63 ± 17 (mean ± SD) years]. When VT/VF appeared in patients pretreated with oral amioradone or sotalol or when VT/VF did not disappear after intravenous administration of class Ia and Ib drugs, we considered it refractory to conventional treatments. Patients in whom VT/VF was

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From the Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Osaka, Japan.
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Reprints: Satoshi Yasuda, MD, PhD, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai, 980-8574, Japan (e-mail: syasuda@cardio.med.tohoku.ac.jp).

refractory to direct counter shock were also included. All the patients gave their written informed consent, and the institutional ethical committee on human research approved the study protocol.

The left ventricular ejection fraction was depressed [28% ± 9% (mean ± SD)]. Sixteen patients had acute coronary syndrome (ACS). Ten had anterior acute myocardial infarction, and 6 had unstable angina pectoris. The remaining 14 had chronic structural heart diseases (chr-HD). Eight had dilated cardiomyopathy, 4 had old myocardial infarction (OMI), and 2 had valvular heart disease. All the patients with ACS underwent revascularization by percutaneous coronary intervention or coronary bypass surgery, and 7 underwent hemodialysis because of renal failure. Among the patients with chronic heart disease, 9 had been administered oral amiodarone or sotalol, and implantable cardioverter defibrillators (ICDs) had been implanted in 7 patients.

Study Design

Before and during the administration of NIF, we measured QT and the interval between the peak and the end of the T wave (Tp-e) in lead V4 or V5 reflecting the transmural dispersion of repolarization in the free wall of the left ventricle.[11] The QT and Tp-e intervals were corrected by Bazett's method to obtain corrected QT (QTc) and corrected Tp-e (cTp-e).

When original VT/VF remained even though the dose of NIF increased to at least 0.2 mg/kg/hr and were refractory to direct counter shock, we considered that the response to NIF was ineffective. When TdP developed even if original VT/VF disappeared, we considered that it was proarrhythmic effect of NIF.

Comparisons between 2 groups were made using 2-tailed Student's *t*-test. Differences were considered significant at $P < 0.05$. Data are presented as mean ± SD.

RESULTS

Efficacy of NIF Inhibiting VT/VF

When NIF was administered intravenously at a maintenance dose of 0.05 to 0.6 (0.19 ± 0.14) mg/kg/ hr, none of the patients had hemodynamic deterioration during administration. NIF was effective in preventing VT/VF without proarrhythmia and hemodynamic deterioration in 21 of the 30 patients (Figure 1). Thus, the overall efficacy rate was 70% in this study group. NIF was ineffective in 4 patients. In 5 patients, proarrhythmic TdP developed transiently, but it disappeared soon after NIF administration was discontinued without additional treatment. The group was limited to the 9 patients pretreated with oral amiodarone or sotalol, NIF was effective in 6 patients (67% of efficacy) (Figure 1).

Comparisons of Usage Dose and ECG Parameters Between Responders and Nonresponders to NIF

There was no significant difference in the dose of NIF used between responders and nonresponders to NIF (0.19 ± 0.15 vs. 0.22 ± 0.05 mg/kg/hr). In 4 nonresponders, 2 patients had been treated with oral amiodarone.

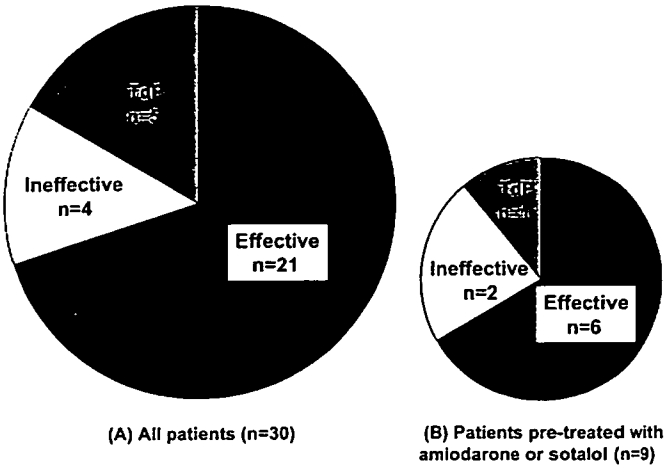


FIGURE 1. Efficacy of nifekalant hydrochloride (NIF) for preventing refractory ventricular tachycardia/fibrillation (VT/VF) in all patients studied (n = 30) and in patients pretreated with oral amiodarone or sotalol (n = 9).

Despite similar baseline QTc values (responders, 451 ± 64 msec; nonresponders, 478 ± 37 msec), the QTc after the administration of NIF differed between the 2 groups (responders, 559 ± 60 msec; nonresponders, 503 ± 68 msec; $P = 0.06$). The percent increase in QTc was 25% ± 15% in responders, whereas it was 5% ± 7% in nonresponders; these values were significantly different ($P = 0.018$), as shown in Figure 2. However, the cTp-e and the percent changes were similar between responders and nonresponders to NIF (Figure 3). Figure 4 shows representative cases.

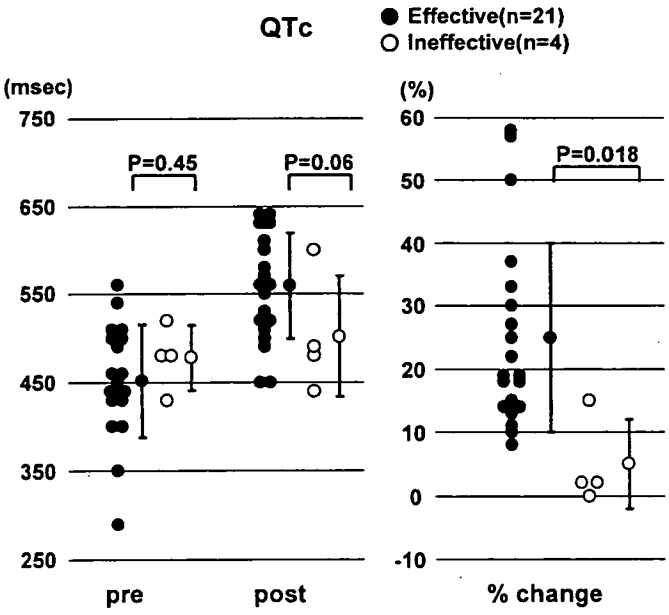


FIGURE 2. Comparisons of corrected QT between effective group (closed circles) and ineffective group (open circles). The QT interval was corrected by Bazett's method (QTc). % change = (post QTc − pre QTc) × 100/pre QTc.

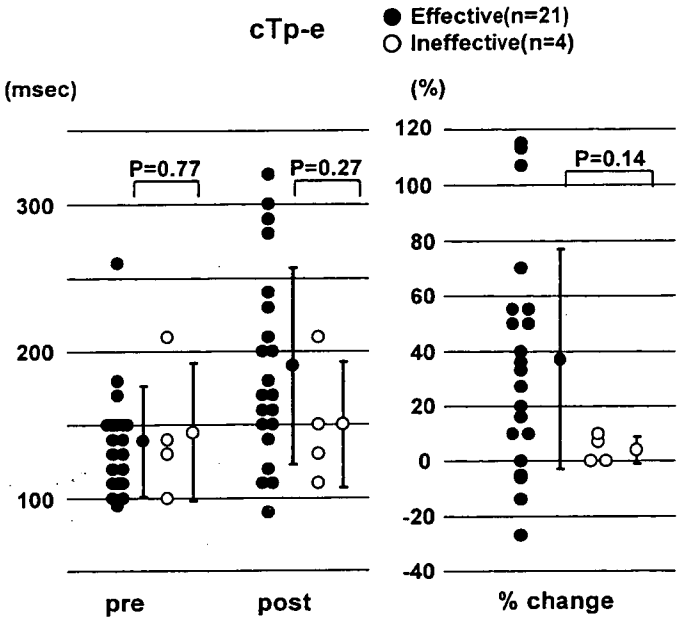


FIGURE 3. Comparisons of corrected T peak-end interval (cTp-e) between effective group (closed circles) and ineffective group (open circles). The Tp-e is the interval between the peak and the end of the T wave.

Comparisons of the Usage Dose and ECG Parameters Between Patients Effective to NIF and Those Developing Proarrhythmic TdP

The dose used in the TdP group was significantly lower than that used in the effective group (TdP, 0.09 ± 0.04 mg/kg/hr; effective, 0.19 ± 0.15 mg/kg/hr; $P = 0.01$). One of five patients in the TdP group was pretreated with oral amiodarone. ECG measurement before NIF administration was not performed in 1 patient with dilated cardiomyopathy who progressively failed in decompensate state due to incessant form of VT and VF.

Before NIF administration, the baseline QTc values of the 2 groups were similar (TdP, 485 ± 39 msec; effective, 452 ± 64 msec) (Figure 5). However, NIF increased QTc to be significantly longer in the TdP group than in the effective group (TdP, 636 ± 70 msec; effective, 559 ± 60 msec; $P = 0.02$). Also, percent increase in the QTc following NIF administration differed between the 2 groups (TdP, 33% ± 6%, effective, 25% ± 15%; $P = 0.09$).

Despite similar baseline cTp-e values (TdP, 133 ± 49 msec; effective, 139 ± 38 msec), the cTp-e after the administration of NIF differed between the 2 groups (TdP, 246 ± 50 msec; effective, 190 ± 67 msec; $P = 0.09$). The percent increase was 93% ± 49% in the TdP group, whereas it was 37% ± 41% in the effective group; these values were significantly different ($P = 0.02$) (Figure 6). Figure 7 shows representative cases.

Figure 8 shows serial changes in QTc and cTp-e in individual patient of TdP group. QTc prolongation was seen immediately after NIF administration. When TdP was induced, QTc interval increased more, accompanied by cTp-e prolongation. However, both QTc and cTp-e returned to the control (pre-) level after discontinuing NIF (-off).

Subclassification by Underlying Heart Diseases

Twenty-one responders consisted of 12 patients in the ACS group and 9 patients in the Chr-HD group. Four nonresponders belonged to the Chr-HD group; 2 patients had

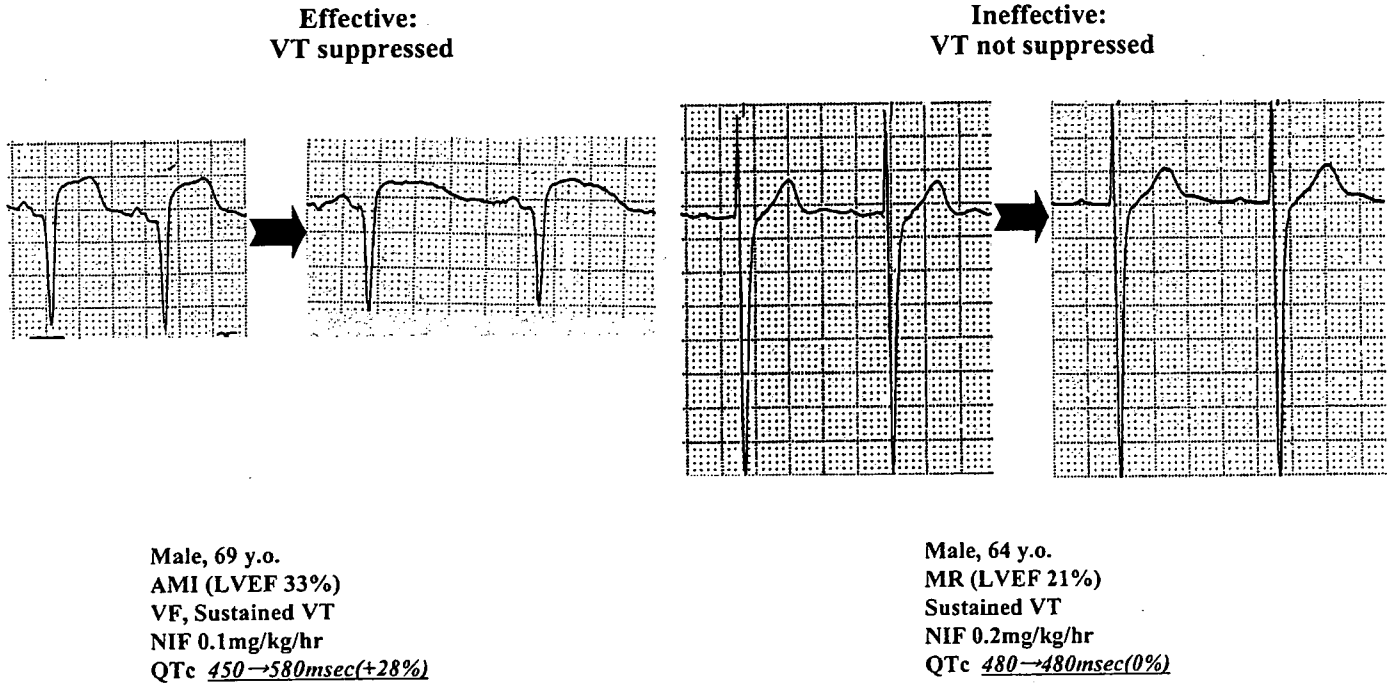


FIGURE 4. Representative patient in whom NIF was effective (left panel) and that in whom NIF was not effective (right panel). AMI indicates acute myocardial infarction; LVEF, left ventricular ejection fraction; MR, mitral regurgitation.

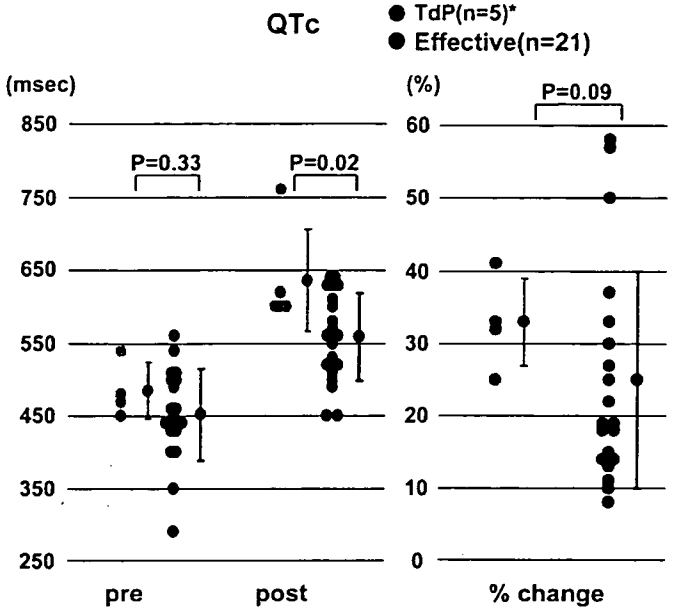


FIGURE 5. Comparisons of QTc between effective group (closed circles) and torsade de pointes (TdP) group (gray circles). *In one patient in the TdP group, ECG measurement before NIF administration was *not* performed.

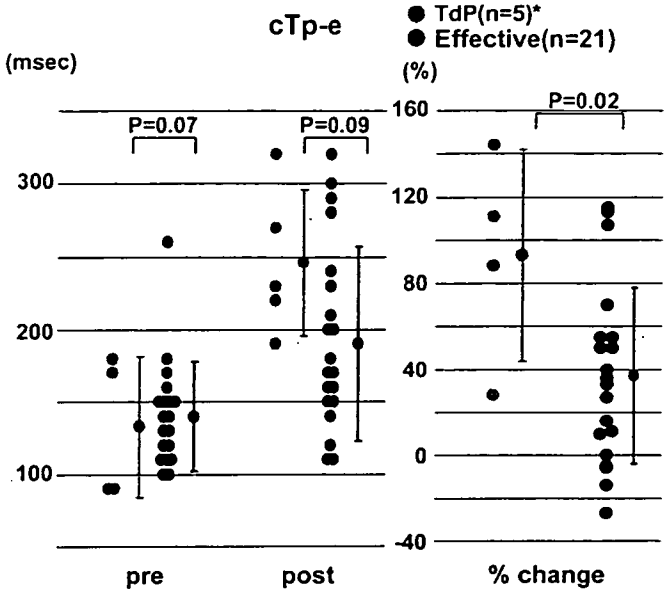


FIGURE 6. Comparisons of cTp-e between effective group (closed circles) and TdP group (gray circles). *In one patient in the TdP group, ECG measurement before NIF administration was *not* performed.

undergone valve replacement, 1 patient had dilated cardiomyopathy, and 1 patient had OMI.

DISCUSSION

The major findings of the study are as follows. The intravenous administration of NIF at low doses effectively suppresses VT/VF refractory to conventional therapy, including oral amiodarone or sotalol by prolonging repolarization (QT). However, these class III antiarrhythmic agents also have a proarrhythmic potential to induce TdP probably owing to an increase in transmural dispersion of repolarization (Tp-e).

Efficacy of NIF for Preventing VT/VF

The patients were considered to be high risk, including those with acute anterior myocardial infarction and those who had been already treated with oral amiodarone, oral sotalol, and/or ICD. Moreover, left ventricular function was depressed; the averaged ejection fraction was less than 30%. In these particular patients, intravenous administration of amiodarone may induce hypotension through its negative inotropic actions, as demonstrated in the previous studies.[7,12,13]

As shown in Figure 1, without hemodynamic deterioration, NIF was sufficiently effective inhibiting refractory VT/VF. Note that NIF does not significantly alter cardiac function. In the open-chest anesthetized canine model, left ventricular pressure was significantly decreased by sotalol and amiodarone, but not by NIF.[6,7] Also, in the canine myocardial infarction model, NIF at neither a low nor a high dose significantly changed the maximum rate of increase in left ventricular pressure (LV dp/dt).[5] Moreover, organ toxicity, which can be induced in the lung, liver, or thyroid by amiodarone, is low in NIF treatment.

Factors for Antiarrhythmic Effect of NIF

Figure 2 shows that QT prolongation is associated with the antiarrhythmic effects of NIF. Also, in the present study, all the patients with ACS responded well to NIF. Action potential duration and effective refractory period were shortened in an ischemic region compared with those in a nonischemic region, which may induce heterogeneous ventricular repolarization in the heart.[14] It is speculated that NIF can prolong action potential duration sufficiently to improve electrical heterogeneity and therefore exert an antiarrhythmic effect.

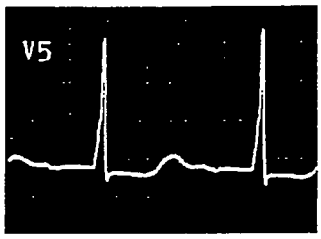
On the other hand, all 4 patients in whom NIF was ineffective in preventing VT/VF may have fixed and chronic substrates. The response of QT prolongation to NIF in these patients was not as sensitive as that in effective patients, even at almost comparable doses. Because the numbers of nonresponders are small and the pre-treatment of amiodarone is a confounding variable, further studies are needed to clarify why NIF failed to prolong the QT interval in some patients.

Factors for Proarrhythmic Effect of NIF

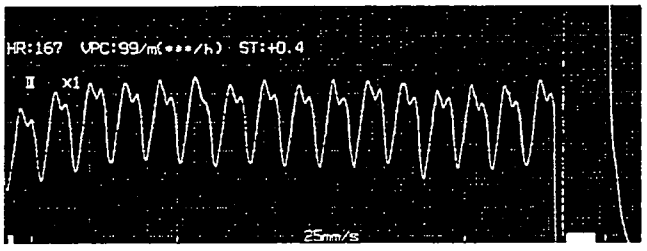
While proarrhytmic properties of amiodarone are relatively minor as demonstrated in the previous studies,[15] proarrhythmic TdP is the major adverse effect of NIF.[16] In this study, 4 (17%) of 30 patients developed TdP (Figure 7). However, TdP was transiently induced and disappeared after discontinuing the administration of NIF because the T1/2 β of NIF is relatively short, 1.53 ± 0.23 hr.[2]

Figure 6 shows that NIF increased cTp-e more significantly in the TdP group than in the effective group, suggesting that Tpeak-end interval reflecting transmural dispersion of repolarization (TDR) plays a significant role in the development of TdP.[11] The increase in cTp-e was induced at low dose of NIF, indicating that the response of I_{Kr} channels to NIF

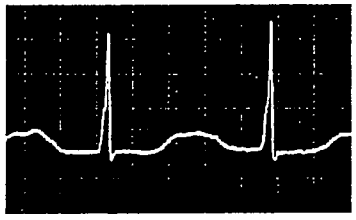
(A) Before administration (QTc 520msec)



(B) Original VT



(C) NIF 0.2mg/kg/hr (QTc 760msec)



(D) Torsade de pointes (TdP)

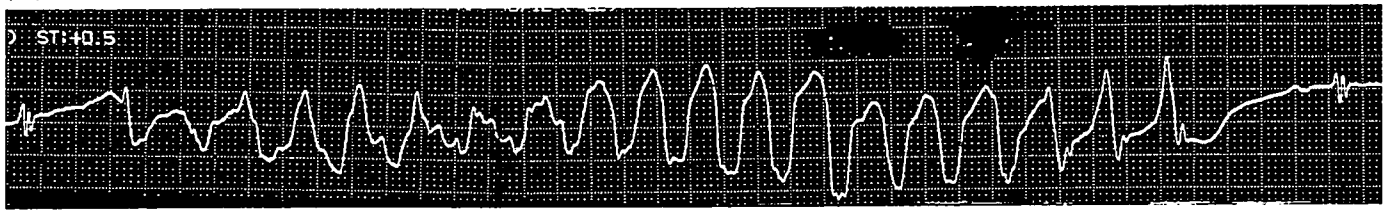


FIGURE 7. Representative patient with NIF-induced TdP. An 83-year-old male with acute myocardial infarction in whom LVEF was depressed to 21%. ECG recording. (A) Before NIF administration (QTc 520msec), (B) sustained VT (rate 167/min) with hemodynamic collapse, (C) during NIF administration at maintenance dose of 0.2 mg/kg/hr (QTc 760msec), and (D) proarrhythmic TdP.

appears to be sensitive, particularly in patients developing TdP. The previous study demonstrated that the sensitivity of I_{Kr} channels could be modified by its genetic polymorphism or surroundings such as catecholamine, potassium, and pH.[17]

The present findings indicate the importance of cTp-e measurement in predicting the risk for TdP. However, in the clinical setting, QTc measurement seems to be more practical. Also, as shown in Figure 5, QTc prolonged for more than 600 msec in all 5 patients developing TdP. Therefore, caution is suggested when QTc is prolonged for this length of time during NIF administration.

Clinical Implications of NIF

According to the Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care,[10] intravenous amiodarone should be considered for VF or pulseless VT patients after 3 unsuccessful direct countershocks.

Compared with a class III agent of amiodarone, NIF has several advantageous characteristics particularly for emergency care. First, NIF is easily soluble and applicable to secure golden time for resuscitation. Second, its half life is relatively short, achieving rapid action and clearance. Third, NIF has only a small cardiac depressant effect and may improve the defibrillation threshold. Fourth, an extracardiac adverse event is not usual. Accumulating evidence in future studies may prove the therapeutic potential of this pure K^+channel blocker.[18]

In conclusion, the present findings indicate that the intravenous administration of NIF is useful in the emergent treatment of inhibiting drug-refractory VT/VF, although