Journal of the American College of Cardiology Vol. 49, No. 12, 2007

ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2006.10.074

Once Daily Therapy With Isosorbide-5-Mononitrate Causes Endothelial Dysfunction in Humans

Evidence of a Free-Radical–Mediated Mechanism

George R. Thomas, PHD,* Jonathan M. DiFabio, MSC,* Tommaso Gori, MD, PHD,† John D. Parker, MD, FACC*

Toronto, Canada; and Siena, Italy

Objectives The aim of the study was to determine if isosorbide-5-mononitrate (IS-5-MN) 120 mg, taken once daily for 7 days, is associated with evidence of endothelial dysfunction and whether this effect is determined by increased free radical production.

Background Tolerance to nitroglycerin is associated with increased free radical production and abnormal endothelial function. To date, no data is available concerning the effect of IS-5-MN, administered in clinically employed dosages, on endothelial function in humans.

Methods A total of 19 healthy volunteers were randomized in a double-blind fashion to therapy with IS-5-MN (120 mg once daily) or placebo. After 7 days of treatment, forearm blood flow responses to acetylcholine (Ach; 7.5, 15, and 30 μg/min) and N-monomethyl-L-arginine (L-NMMA; 1, 2, and 4 μmol/min) were measured. In a separate study, after 7 days of therapy with IS-5-MN 120 mg once daily, the responses to Ach were assessed during intra-arterial coinfusion of vitamin C (24 mg/min) or saline.

Results As compared with placebo, IS-5-MN caused significant blunting of the responses to both Ach (peak responses: placebo 127 ± 31%; IS-5-MN 52 ± 24%) and L-NMMA (peak responses: placebo 41 ± 5%; IS-5-MN 22 ± 8%). Vitamin C completely restored the forearm blood flow responses to Ach (peak responses: vitamin C 180 ± 33%; saline 107 ± 17%).

Conclusions We document for the first time that IS-5-MN impairs endothelial function in humans in vivo. Suggesting a role of oxygen free radicals, nitrate-induced abnormalities in endothelium-dependent vasomotor responses were reversed by the antioxidant vitamin C. (J Am Coll Cardiol 2007;49:1289–95) © 2007 by the American College of Cardiology Foundation

Nitroglycerin (GTN) and other organic nitrates have been widely used in the management of cardiovascular disease.

See page 1296

The major clinical limitation of nitrates is the rapid loss of their hemodynamic and anti-ischemic effects during chronic therapy, a phenomenon termed tolerance. The etiology of tolerance remains incompletely understood and is almost certainly multifactorial (1–7). For the past decade, many lines of observation have documented that sustained nitrate therapy, particularly with GTN, is associated with increased vascular production of free radicals (8). Multiple mechanisms of this increased production and/or bioavailability of free radical species have been described. The "free radical" hypothesis of nitrate tolerance suggests that the primary cause of tolerance is an increased bioavailability of vascular superoxide radical anion and related reactive oxygen species (ROS) (8–11). Sources of these ROS might include the mitochondrial respiratory chain, the nitric oxide synthase, and membrane oxidases (2,3). Such an increase might lead to tolerance and vascular dysfunction by: 1) inactivation of nitric oxide (the active metabolite of organic nitrates); 2) inhibition of the mitochondrial aldehyde dehydrogenase, the enzyme responsible for the bioactivation of GTN and pentaerithrityl tetranitrate (PETN); 3) dysfunction of the endothelial nitric oxide synthase; and 4) by causing a series of events (such as increased angiotensin II and endothelin-1 activity as well as autonomic dysfunction) associated with increased vascular tone (2,3).

From the *Division of Cardiology, Department of Medicine, University Health Network and Mount Sinai Hospitals and the Department of Pharmacology, University of Toronto, Toronto, Canada; and the †Department of Internal, Cardiovascular and Geriatric Medicine, University of Siena, Siena, Italy. Supported by a research donation from the Henry White Kinnear Foundation. Dr. Parker holds a Career Investigator Award from the Heart and Stroke Foundation of Ontario, Canada. Dr. Gori is the recipient of a grant from the Italian Ministry of Research.

Manuscript received May 19, 2006; revised manuscript received September 13, 2006, accepted October 12, 2006.

Abbreviations and Acronyms

Ach = acetylcholine
FBF = forearm blood flow
GTN = nitroglycerin
IS-5-MN = isosorbide-5-mononitrate
L-NMMA = N-monomethyl-L-arginine
PETN = pentaerithrityl tetranitrate
ROS = reactive oxygen species

Increased vascular ROS bioavailability has implications beyond the development of nitrate tolerance, and it is now recognized that sustained therapy with organic nitrates causes important abnormalities in vascular function. Of note, it has been repeatedly shown that ROS-dependent endothelial dysfunction is associated with increased cardiovascular mortality and morbidity (12); while it is unknown whether nitrate-induced endothelial dysfunction has the same prognostic implications, the observation that therapy with GTN causes ROS production and impaired responses to endothelium-dependent vasomotor stimuli both in the resistance and conductance circulation in humans in vivo (10) does suggest that these agents could have negative prognostic effects.

To date, experimental evidence documenting that organic nitrate therapy is associated with increased free radical production and abnormal endothelial function is mostly limited to the use of GTN in doses associated with the development of nitrate tolerance. Recently, a number of investigations have suggested that the organic nitrates may differ in terms of their ability to increase vascular free radical production and cause abnormalities in endothelial function. For example, animal data have suggested that therapy with PETN is associated with a lesser increase in ROS production as compared with GTN. These differences in ROS production might explain why, in humans, as compared with GTN, PETN did not cause hemodynamic tolerance, was not associated with evidence of increased vascular ROS bioavailability, and caused less of an impact on endothelial function (13,14). While these considerations seem to favor PETN, this drug is not commonly prescribed in North America and in several European countries, and GTN and isosorbide-5-mononitrate (IS-5-MN) remain the most commonly employed nitrates. To date, there is no human data available concerning the effect of IS-5-MN on ROS production or endothelial function. Recently, Muller et al. (15) found that long-term, eccentric treatment of rabbits with high dose IS-5-MN did not increase vascular ROS production or induce endothelial dysfunction. Further, IS-5-MN therapy was also found to prevent intimal lesion formation and endothelial dysfunction in rabbits prone to develop atherosclerosis (16). Given this background, the objective of the current study was to determine whether IS-5-MN, taken once daily for 7 days (i.e., using a dosing regimen that is clinically employed), is associated with evidence of endothelial dysfunction in the human forearm circulation. If IS-5-MN was associated with the development of endothelial dysfunction, the second objective of the study would be to determine whether the antioxidant vitamin C would reverse this phenomenon.

Methods

Study population. Twenty-six healthy, nonsmoking male volunteers 18 to 30 years old participated in the study. Before enrollment, subjects completed a questionnaire detailing their medical history and underwent a brief physical examination. None of the subjects were taking any medications at the time of investigation. Baseline heart rate (pulse rate) and blood pressure were also measured. All subjects were requested to abstain from caffeine on each of the study days, and from alcohol and supplemental vitamins for the duration of the study. The study protocol was approved by the Mount Sinai Hospital Research Ethics Board. The objective of the study and study procedures were explained to individual subjects, and written informed consent was obtained from all subjects.

Study protocol. MEASUREMENT OF FOREARM BLOOD FLOW (FBF). Forearm blood flow was measured simultaneously in both arms by venous occlusion strain-gauge plethysmography (D. E. Hokanson Inc., Bellevue, Washington) using techniques previously reported by our laboratory (10,17). Briefly, circulation of the hand was excluded by inflating wrist cuffs to 200 mm Hg during measurement periods. The upper arm cuffs were inflated to 40 mm Hg and deflated at 10 s intervals (Hokanson rapid cuff inflator, D. E. Hokanson Inc.), and FBF was recorded as the average of 5 consecutive measurements. On visit 1, only basal FBF was measured. On visit 2, FBF was measured at baseline and in response to brachial artery infusions of normal saline and vasoactive agents as described in detail in the following text.

Experiment 1. STUDY DAY 1. Nineteen healthy volunteers age between 18 to 30 years participated in this randomized, double-blind placebo-controlled study. After screening (inclusive of anamnesis and brief physical examination) for admission into the study, standing blood pressure and heart rate measurements were obtained using an automatic, calibrated sphygmomanometer (Critikon Company LLC, Tampa, Florida). The mean of 3 measurements was determined. Baseline FBF was measured as described in the preceding text. Subjects were then randomized in a double-blind fashion to receive either a phasic release formulation of IS-5-MN (Imdur, AstraZeneca Canada Inc., Mississauga, Ontario, Canada) 120 mg given once daily (IS-5-MN group; n = 10) or placebo (placebo group; n = 9). After the first dose of study medication, repeat standing blood pressure and heart rate measurements were taken 3 h later. All subjects were instructed to take their study medication daily at 9:00 AM until the end of the study.

STUDY DAY 2. Subjects returned to the laboratory after 7 days of continuous therapy with IS-5-MN or placebo. Standing blood pressure and heart rate measurements were repeated. The brachial artery was cannulated, and FBF in

the nondominant arm was measured at baseline and in response to drug infusions. Forearm blood flow was also measured in the opposite arm using strain gauge plethysmography at each measurement time point. The endothelium-dependent vasodilator acetylcholine (Ach) chloride (Novartis Pharmaceuticals, Ontario, Canada) was infused at 7.5, 15, and 30 μg/min. Subsequently, FBF responses to N-monomethyl-L-arginine (L-NMMA) (1, 2, and 4 μmol/min; Clinalfa AG, Laufelfingen, Switzerland) were measured. The infusion rate was kept constant at 0.4 ml/min with a precision pump (Harvard Apparatus, South Natick, Massachusetts). Each concentration was infused for 6 min, and FBF measurements were performed during the last 3 min. All responses were evaluated as changes from a baseline value (normal saline infusion) immediately before each drug infusion. Intra-arterial blood pressure was recorded after each infusion (Horizon 2000, Mennen Medical Inc., Clarence, New York) using the average of at least 15 cardiac cycles. Electrocardiogram was monitored continuously. Between different drug infusions, normal saline was infused until the flow returned to baseline values. At the end of the study, the arterial line was removed, all study medications discontinued, and subjects were discharged from the laboratory. A schematic flow of the study protocol is represented in Figure 1A.

Experiment 2: studies with IS-5-MN and vitamin C. In a separate study, 7 subjects were treated with IS-5-MN 120 mg daily for 7 days. Subsequently, subjects returned to the laboratory at which time the brachial artery was cannulated and FBF in the nondominant arm was measured at baseline and in response to drug infusions. Forearm blood flow was also measured in the opposite arm using strain gauge

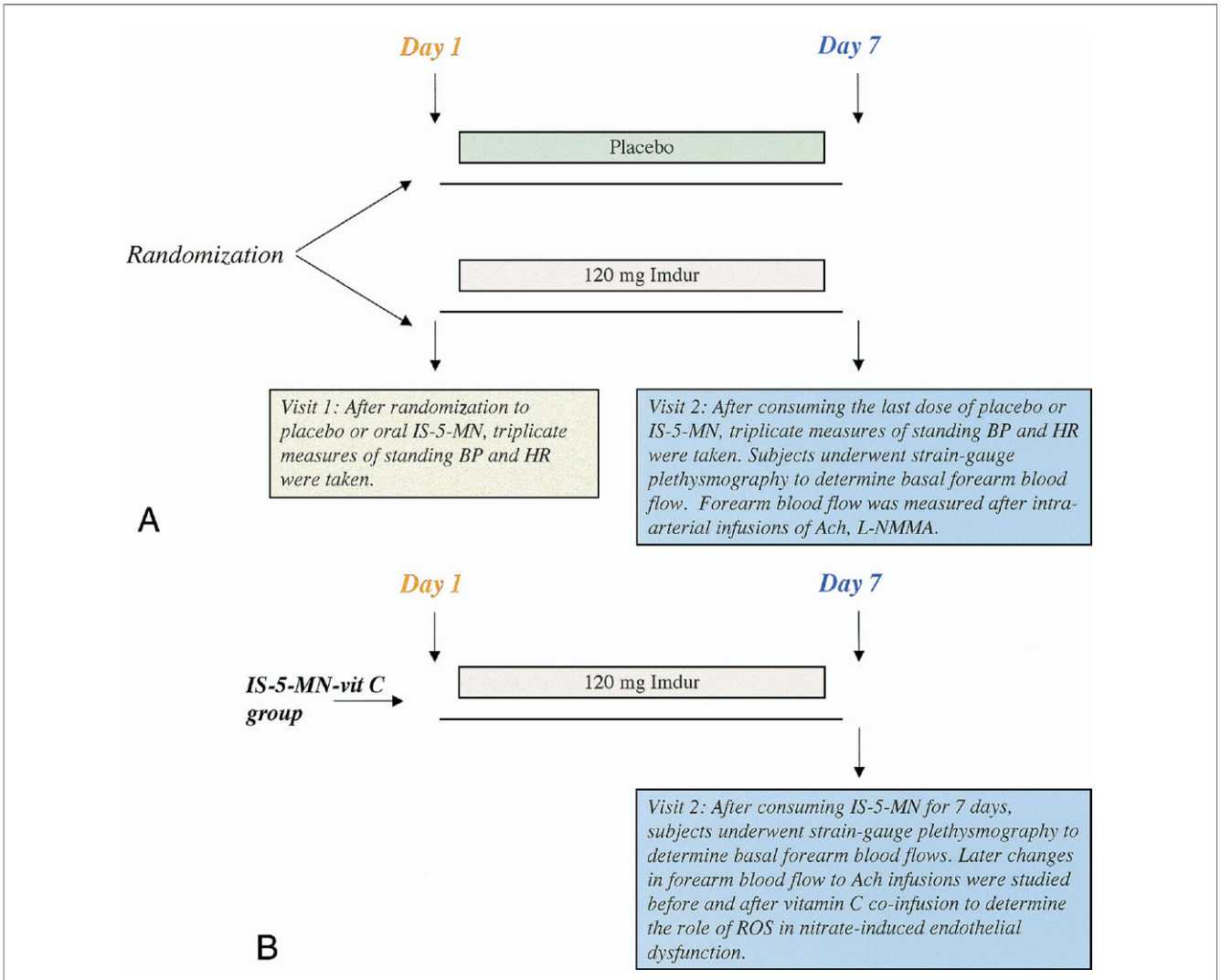

Figure 1 **A Schematic Diagram of the IS-5-MN Study Protocol**

(A) Diagram of the study protocol to determine the effects of a 7-day isosorbide-5-mononitrate (IS-5-MN) therapy on endothelial function. **(B)** Diagram of the study protocol to determine the effect of vitamin C (vit C) on IS-5-MN–induced changes in endothelial function. Ach = acetylcholine; BP = blood pressure; HR = heart rate; IS-5-MN = isosorbide-5-mononitrate; L-NMMA = N-monomethyl-L-arginine; ROS = reactive oxygen species.

plethysmography at each measurement time point. Acetylcholine was infused at 7.5, 15, and 30 μg/min at a rate of 0.4 ml/min. Normal saline was coinfused at a rate of 0.4 ml during the Ach infusions. Ten minutes after the completion of the Ach + saline infusions, vitamin C was infused at a rate of 24 mg/min for 15 min. This infusion rate, which is expected to achieve a local plasma concentration of 1 mg/ml or 5 mM (i.e., approximately 50 times the normal concentration), has been used in previous studies to demonstrate a role of ROS in the pathogenesis of endothelial dysfunction in patients with cardiovascular disease (12). Subsequently, while the vitamin C infusion was continued, Ach was again administered at 7.5, 15, and 30 μg/min. Each concentration was infused for 6 min, and FBF measurements were performed during the last 3 min. A schematic flow of the study protocol is represented in Figure 1B. All responses were evaluated as changes from a baseline value (normal saline infusion) immediately before each drug infusion. Data were digitally acquired, and each series of infusions was given a coded identifier. Analysis was performed off-line, in random order, by an investigator blinded to this code.

Statistical analysis. All results are expressed as mean ± SE. Comparisons between groups for baseline values were performed with unpaired *t* tests. The effect of IS-5-MN on blood pressure and heart rate was tested using a 2-way analysis of variance (ANOVA) with Bonferroni correction. All FBF values were compared as the ratio of the infused versus the noninfused arm. Differences between groups in blood flow responses were tested using 2-way repeated measures ANOVA on the % and absolute changes in FBF as compared with the corresponding baseline. A value of $p < 0.05$ was set as the threshold for significance. Statistical analyses were performed using Sigmastat (Jandel Scientific, San Rafael, California).

Results

Study 1. BLOOD PRESSURE AND HEART RATE RESPONSES. On visit 1, baseline standing heart rate and systolic blood pressure did not differ significantly between groups (Table 1). Compared with baseline values, in the IS-5-MN group, standing blood pressure was significantly lower ($p < 0.05$, ANOVA with Bonferroni correction) and heart rate was significantly higher ($p < 0.05$) at 3 h after the administration of the first dose. The blood pressure and heart rate responses observed in the IS-5-MN group were significantly different from those observed in the placebo group (significant group interaction by 2-way ANOVA). On visit 2, these hemodynamic effects of IS-5-MN were still evident but were no longer statistically significant. Blood pressure and heart rate in the placebo group were similar at all time points. Intra-arterial drug infusions had no significant effect on blood pressure and heart rate.

Table 1 Standing Blood Pressure and Heart Rate Responses to IS-5-MN

	Visit 1		
	Baseline	3 h After IS-5-MN	Visit 2 Day 7
Systolic blood pressure, mm Hg			
IS-5-MN group	117 ± 2	105 ± 2*	111 ± 2
Placebo group	123 ± 4	120 ± 3	119 ± 4
Heart rate, beats/min			
IS-5-MN group	72 ± 3	83 ± 3*	78 ± 3
Placebo group	78 ± 4	79 ± 4	80 ± 3

*$p < 0.05$ as compared with baseline and with all data points in the placebo group, analysis of variance $p < 0.05$ for the effect of group and of time, p = NS for the interaction.
IS-5-MN = isosorbide-5-mononitrate.

Table 2 FBF Responses: Ratio of Infused to Noninfused Arm

Infusions	Placebo	IS-5-MN	
Saline	1.56 ± 0.17	1.57 ± 0.24	
Ach 7.5 μg/min	2.29 ± 0.31	1.96 ± 0.35	*
Ach 15 μg/min	2.87 ± 0.59	1.91 ± 0.35	
Ach 30 μg/min	3.58 ± 0.70	2.49 ± 0.69	
Saline	1.81 ± 0.20	1.53 ± 0.34	
L-NMMA 1 μmol/min	1.74 ± 0.26	1.41 ± 0.31	*
L-NMMA 2 μmol/min	1.26 ± 0.16	1.46 ± 0.29	
L-NMMA 4 μmol/min	1.09 ± 0.15	1.06 ± 0.13	

Values are mean ± SE and are expressed as the ratio of the infused to the noninfused arms. *$p < 0.05$ between groups, 2-way analysis of variance, % change, and delta change from saline infusion.
Ach = acetylcholine; FBF = forearm blood flow; IS-5-MN = isosorbide-5-mononitrate; L-NMMA = N-monomethyl-L-arginine.

EFFECT OF IS-5-MN TREATMENT ON FBF. On visit 1, FBF was similar between the 2 groups (data not shown). On visit 2, baseline FBF remained similar between groups, and within each group it did not significantly differ from the values obtained on visit 1.

RESPONSES TO ACH INFUSIONS. The intra-arterial infusion of Ach caused a dose-dependent increase in FBF in both groups ($p = 0.05$, effect of the three levels of infusion rate,

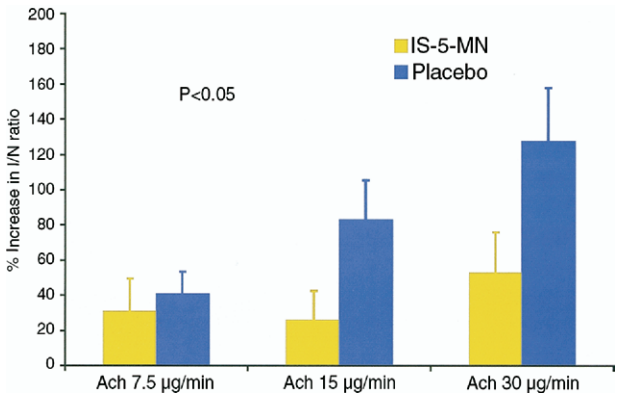

Figure 2 FBF Responses to Ach Infusion

$p < 0.05$ for the effect of treatment group, 2-way analysis of variance. Ach = acetylcholine; FBF = forearm blood flow; IS-5-MN = isosorbide-5-mononitrate; I/N = ratio of forearm blood flow, infused to noninfused arms.

2-way ANOVA) (Table 2, Fig. 2). When compared between groups, the responses to each Ach step were significantly blunted in the IS-5-MN group as compared with placebo (group effect, $p < 0.05$, 2-way ANOVA). Showing that this difference was consistent through the 3 infusion rates, there was no statistically significant interaction between group and infusion level ($p = 0.3$, 2-way ANOVA). In the placebo group, during infusion of the highest concentration of Ach, FBF increased by 127 ± 31% as compared with baseline, whereas in the IS-5-MN group, it increased by only 52 ± 24% ($p < 0.05$).

RESPONSES TO L-NMMA INFUSIONS. N-monomethyl-L-arginine caused a dose-dependent vasoconstriction in both groups ($p < 0.05$, 2-way ANOVA) (Table 2, Fig. 3). In the IS-5-MN–treated group, the decreases in FBF in response to the 2 μmol and 4 μmol/min infusions were significantly blunted as compared with the placebo group ($p < 0.05$ for group effect, $p < 0.05$ for the interaction of group and infusion rate). At the highest infused concentration of L-NMMA, the decrease in FBF was 41 ± 5% in the placebo-treated group and 22 ± 8% in the IS-5-MN group.

Study 2. THE EFFECT OF VITAMIN C COADMINISTRATION ON IS-5-MN–INDUCED CHANGES IN FBF. In the subjects who had been treated with IS-5-MN 120 mg once daily for 7 days, intra-arterial infusion of vitamin C (in a dosage previously shown to reverse endothelial dysfunction in patients with cardiovascular disease [12]) significantly increased FBF responses to Ach as compared with Ach alone ($p < 0.05$ for vitamin C vs. saline coinfusion). This difference did not depend on the infusion rate of Ach ($p < 0.05$ for the effect of the infusion rate, p = NS for the interaction of coinfused drug and Ach infusion rate) (Table 3, Fig. 4). During coinfusion of vitamin C, FBF increased by 180 ± 33% in response to the highest infused concentration of Ach. In contrast, during coinfusion of saline, FBF increased by only 107 ± 17% in response to the highest infused concentration of Ach.

Table 3 FBF Responses: Ratio of Infused to Noninfused Arms

Infusions	IS-5-MN	IS-5-MN + Vitamin C	
Saline	1.1 ± 0.06	1.0 ± 0.10	
Ach 7.5 μg/min	1.4 ± 0.17	1.8 ± 0.22	*
Ach 15 μg/min	1.8 ± 0.20	2.1 ± 0.31	
Ach 30 μg/min	2.2 ± 0.23	2.8 ± 0.31	

Values are mean ± SE and are expressed as the ratio of the infused to the non-infused arms. *$p <$ 0.05 between groups, 2-way analysis of variance, % change, and delta change from saline infusion. Ach = acetylcholine; other abbreviations as in Table 2.

Discussion

Over the past decade there have been several reports documenting that GTN therapy has adverse effects on endothelial function. Our laboratory and other groups have shown that long-term treatment with GTN causes abnormalities in nitric oxide synthase and endothelial function (10,17,18). Nitric oxide synthase dysfunction associated with GTN therapy has been attributed to uncoupling of the enzyme in the setting of increased vascular production of free radical species.

Increased vascular oxidative stress secondary to prolonged GTN exposure is well documented and with a number of sources described, including xanthine oxidase (8,19), nicotinamide adenine dinucleotide oxidase (20), the mitochondrial electron transport chain, cytochrome P450 oxidase (21), as well as nitric oxide synthase itself (9–11). Of importance, this increased vascular free radical bioavailability has implications beyond the development of nitrate tolerance as ROS oxidizes carbohydrates, lipids, proteins, and DNA (22–25) and are thus capable of disrupting

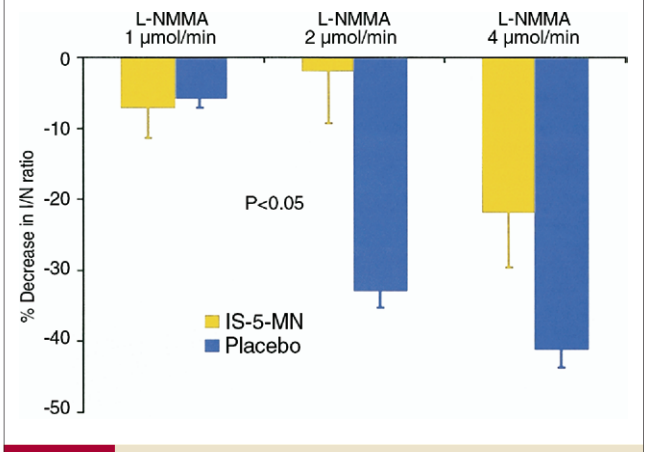

Figure 3 FBF Responses to L-NMMA Infusion

$p < 0.05$ for the effect of treatment group, 2-way analysis of variance. L-NMMA = N-monomethyl-L-arginine, other abbreviations as in Figure 2.

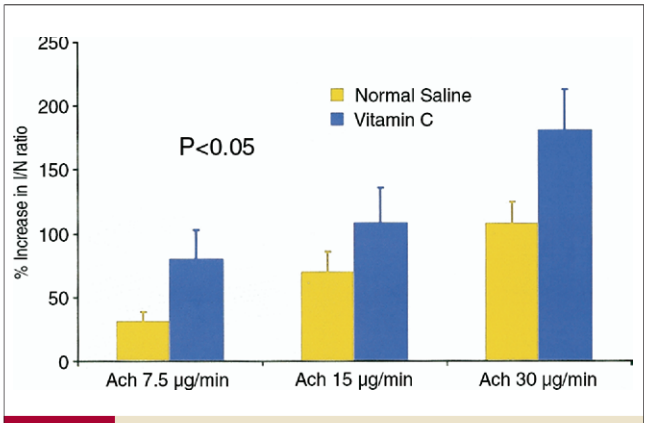

Figure 4 FBF Responses to Ach and Vitamin C Infusion

$p < 0.05$ for the effect of vitamin C versus saline coinfusion, 2-way analysis of variance. Abbreviations as in Figure 2.

biochemical homeostasis and inducing biological injury. Accordingly, studies in both humans and animals have shown that prolonged exposure to GTN induces abnormalities in the function of several enzyme systems as well as neurohormonal activation and impaired nitrate biotransformation. Although the relative contribution of these abnormalities to the induction of nitrate tolerance is unknown, they appear to be caused and result in increased bioavailability of ROS.

It is now known that prolonged exposure to GTN causes endothelial dysfunction in healthy subjects (17) and in subjects with risk factors for vascular disease (18,26). Importantly, there is also experimental evidence to suggest there may be within-class differences in terms of the impact of organic nitrates on endothelial function and nitrate tolerance development. For example, we have previously shown that therapy with pentaerythrityl tetranitrate, which has antioxidant properties, is associated with less severe abnormalities in human endothelial function as compared with GTN, and it does not cause tolerance (13,14). As well, there are within-class differences in terms of the site of biotransformation: while GTN and PETN are activated within mitochondria, the site of IS-5-MN biotransformation is yet unknown (27). Finally, there are also reports that certain organic nitrates, in experimental conditions, can have protective vascular effects. For example, one group has hypothesized that nitrate-derived nitric oxide might have protective effects that are similar to those of endogenous nitric oxide. In particular, using a rabbit model of accelerated atherogenesis, Muller et al. (15,16) recently demonstrated that long-term treatment with IS-5-MN retards intima-media thickening, prevents the development of endothelial dysfunction, and is associated with a decrease in vascular ROS production. In sum, while evidence exists that GTN can cause ROS production and endothelial dysfunction, PETN seems to be devoid of these effects, and animal studies—which need human confirmation—show that IS-5-MN might protect from hypercholesterolemia. To date, there have been no studies examining the impact of the most commonly employed nitrate (i.e., IS-5-MN) on endothelial function in humans in vivo.

Summary of our findings. The results of the current human study suggest that the administration of a phasic-release formulation of IS-5-MN, given once daily, is associated with the development of pronounced endothelial dysfunction. Of note, this is the first demonstration of an adverse effect observed by using a clinically employed dosing regimen of IS-5-MN. Consistent with our prior studies of transdermal GTN, the blunted flow responses to Ach, an accepted marker of endothelial dysfunction, were associated with very abnormal responses to the administration of L-NMMA. The latter finding suggests that the abnormal responses to Ach are caused, at least in part, by abnormalities in nitric oxide synthase function as previously demonstrated for GTN (4,17). In a second set of experiments, we prove that oxidative stress plays a major role in the IS-5-MN–induced development of endothelial dysfunction. Collectively, these data provide evidence suggesting that IS-5-MN, in a clinically employed dosage, stimulates vascular production of ROS and causes endothelial dysfunction.

These data are in agreement with those of Sekiya et al. (28), who showed that isosorbide dinitrate, of which IS-5-MN is a metabolite, impairs endothelium-dependent dilation and worsens intima-media thickness in patients with cardiovascular disease. Furthermore, we provide the first human evidence suggesting that this vascular dysfunction is secondary to increased ROS production as it is completely reversed by the coadministration of intra-arterial vitamin C. The explanation for the differences between our findings and those of the animal reports from Muller et al. (16) is not entirely clear but might reside in the differences in species, experimental model (healthy volunteers vs. hypercholesterolemic animals), nitrate dosing, as well as in the vascular beds studied. Whatever these differences, we show that, in humans, a dosage of IS-5-MN that is commonly employed, and that is believed to be free of nitrate tolerance, causes (ROS-mediated) endothelial dysfunction.

Of note, our study involved normal volunteers rather than patients with cardiovascular disease, and we recognize that our results may not necessarily be extrapolated to the clinical setting. However, since endothelial vasomotor responses are already blunted in most patients with coronary artery disease, the effect of interventions that lead to further endothelial dysfunction (such as nitrate therapy) would be very difficult to study with the model of endothelium-dependent vasomotor responses that we employed. While this limitation is recognized, the authors would like to emphasize that, in this study, nitrates induced severe, ROS-mediated, endothelial dysfunction in individuals with intact ROS scavenging capacities.

Clinical relevance of our findings. Our findings have important clinical implications. Isosorbide-5-mononitrate is the most commonly used long-acting nitric oxide donor in clinical practice (29). The fact that it can cause profound endothelial dysfunction in healthy subjects is both surprising and a cause for concern given the traditional concept that nitric oxide donors might have beneficial effects in the setting of decreased nitric oxide bioavailability (30). Our findings with IS-5-MN are similar to our previous observations with GTN; therapy with GTN causes important abnormalities in endothelial function in forearm resistance vessels, epicardial coronary arteries, as well as in peripheral conduit arteries (14,18). Therefore, it seems that commonly used organic nitrates, despite a (blunted) residual hemodynamic and symptomatic effect, cause abnormalities in endothelial function, which might have clinical relevance during sustained therapy. Furthermore, our data suggest that nitrate-induced production of ROS, and subsequent endothelial dysfunction, associated with nitrate administration can be prevented by concurrent administration of an antioxidant, thereby restoring the beneficial effects of these

drugs. These data agree with observations from A-HEFT (African-American Heart Failure Trial) (31), where the combination of IS-5-MN and hydralazine (which is more reliably absorbed after oral administration as compared with vitamin C and has antioxidant properties [32]) improved the clinical outcome of patients with chronic congestive heart failure. Taken together, these considerations emphasize the need for more clinical data concerning the impact of organic nitrates (± antioxidant therapy) on long-term clinical outcome and the process of atherogenesis.

Acknowledgments

The authors would like to thank the members of the Clinical Cardiovascular Research Laboratory at Mount Sinai Hospital for their assistance in completing this study.

Reprint requests and correspondence: Dr. John D. Parker, Mount Sinai Hospital, 600 University Avenue, Room 1609, Toronto, Ontario, Canada, M5G 1X5. E-mail: jdp@ca.inter.net.

REFERENCES

1. Parker JD, Gori T. Tolerance to the organic nitrates: new ideas, new mechanisms, continued mystery. Circulation 2001;104:2263–5.
2. Gori T, Parker JD. The puzzle of nitrate tolerance: pieces smaller than we thought? Circulation 2002;106:2404–8.
3. Gori T, Parker JD. Nitrate tolerance: a unifying hypothesis. Circulation 2002;106:2510–3.
4. Munzel T, Li H, Mollnau H, et al. Effects of long-term nitroglycerin treatment on endothelial nitric oxide synthase (NOS III) gene expression, NOS III-mediated superoxide production, and vascular NO bioavailability. Circ Res 2000;86:E7–12.
5. Kim D, Rybalkin SD, Pi X, et al. Upregulation of phosphodiesterase 1A1 expression is associated with the development of nitrate tolerance. Circulation 2001;104:2338–43.
6. Chen Z, Zhang J, Stamler JS. Identification of the enzymatic mechanism of nitroglycerin bioactivation. Proc Natl Acad Sci U S A 2002;99:8306–11.
7. Ignarro LJ. After 130 years, the molecular mechanism of action of nitroglycerin is revealed. Proc Natl Acad Sci U S A 2002;99:7816–7.
8. Munzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A novel mechanism underlying tolerance and cross-tolerance. J Clin Invest 1995;95:187–94.
9. Landmesser U, Dikalov S, Price SR, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003;111:1201–8.
10. Gori T, Mak SS, Kelly S, Parker JD. Evidence supporting abnormalities in nitric oxide synthase function induced by nitroglycerin in humans. J Am Coll Cardiol 2001;38:1096–101.
11. Kaesemeyer WH, Ogonowski AA, Jin L, Caldwell RB, Caldwell RW. Endothelial nitric oxide synthase is a site of superoxide synthesis in endothelial cells treated with glyceryl trinitrate. Br J Pharmacol 2000;131:1019–23.
12. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–8.
13. Jurt U, Gori T, Ravandi A, Babaei S, Zeman P, Parker JD. Differential effects of pentaerythritol tetranitrate and nitroglycerin on the development of tolerance and evidence of lipid peroxidation: a human in vivo study. J Am Coll Cardiol 2001;38:854–9.
14. Gori T, Al Hesayen A, Jolliffe C, Parker JD. Comparison of the effects of pentaerythritol tetranitrate and nitroglycerin on endothelium-dependent vasorelaxation in male volunteers. Am J Cardiol 2003;91:1392–4.
15. Muller S, Laber U, Mullenheim J, Meyer W, Kojda G. Preserved endothelial function after long-term eccentric isosorbide mononitrate despite moderate nitrate tolerance. J Am Coll Cardiol 2003;41:1994–2000.
16. Muller S, Konig I, Meyer W, Kojda G. Inhibition of vascular oxidative stress in hypercholesterolemia by eccentric isosorbide mononitrate. J Am Coll Cardiol 2004;44:624–31.
17. Gori T, Burstein JM, Ahmed S, et al. Folic acid prevents nitroglycerin-induced nitric oxide synthase dysfunction and nitrate tolerance: a human in vivo study. Circulation 2001;104:1119–23.
18. Caramori PR, Adelman AG, Azevedo ER, Newton GE, Parker AB, Parker JD. Therapy with nitroglycerin increases coronary vasoconstriction in response to acetylcholine. J Am Coll Cardiol 1998;32:1969–74.
19. Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium-derived contracting factor. Am J Physiol 1989;257:H33–7.
20. Munzel T, Kurz S, Rajagopalan S, et al. Hydralazine prevents nitroglycerin tolerance by inhibiting activation of a membrane-bound NADH oxidase. A new action for an old drug. J Clin Invest 1996;98:1465–70.
21. Janssen YM, Van Houten B, Borm PJ, Mossman BT. Cell and tissue responses to oxidative damage. Lab Invest 1993;69:261–74.
22. Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986;320:454–6.
23. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–9.
24. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys 1991;288:481–7.
25. Huie RE, Padmaja S. The reaction of NO with superoxide. Free Radic Res Commun 1993;18:195–9.
26. Heitzer T, Just H, Brockhoff C, Meinertz T, Olschewski M, Munzel T. Long-term nitroglycerin treatment is associated with supersensitivity to vasoconstrictors in men with stable coronary artery disease: prevention by concomitant treatment with captopril. J Am Coll Cardiol 1998;31:83–8.
27. Daiber A, Oelze M, Coldewey M, et al. Oxidative stress and mitochondrial aldehyde dehydrogenase activity: a comparison of pentaerythritol tetranitrate with other organic nitrates. Mol Pharmacol 2004;66:1372–82.
28. Sekiya M, Sato M, Funada J, Ohtani T, Akutsu H, Watanabe K. Effects of the long-term administration of nicorandil on vascular endothelial function and the progression of arteriosclerosis. J Cardiovasc Pharmacol 2005;46:63–7.
29. Gunasekara NS, Noble S. Isosorbide 5-mononitrate. A review of a sustained release formulation (Imdur) in stable angina pectoris. Drugs 1999;57:261–77.
30. Harrison DG, Bates JN. The nitrovasodilators. New ideas about old drugs. Circulation 1993;87:1461–7.
31. Taylor AL, Ziesche S, Yancy C, et al. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. N Engl J Med 2004;351:2049–57.
32. Daiber A, Oelze M, Coldewey M, et al. Hydralazine is a powerful inhibitor of peroxynitrite formation as a possible explanation for its beneficial effects on prognosis in patients with congestive heart failure. Biochem Biophys Res Commun 2005;338:1865–74.