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Ramipril Sensitizes Platelets to Nitric Oxide

Implications for Therapy in High-Risk Patients

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Objectives Using 2 sequential studies in HOPE (Heart Outcomes Prevention Evaluation) study–type patients, the aims of this study were: 1) to test the hypothesis that ramipril improves platelet nitric oxide (NO) responsiveness: and 2) to explore biochemical and physiological effects of ramipril in a cohort selected on the basis of platelet NO resistance.

Background Ramipril prevents cardiovascular events, but the bases for these effects remain uncertain. NO resistance at both the platelet and vascular levels is present in a substantial proportion of patients with diabetes or ischemic heart disease and is an independent risk factor for cardiovascular events.

Methods Study 1 was a double-blind, randomized comparison of ramipril (10 mg) with placebo in a cohort of patients ($n = 119$) with ischemic heart disease or diabetes plus additional coronary risk factor(s), in which effects on platelet responsiveness to NO were compared. Study 2 was a subsequent short-term evaluation of the effects of ramipril in a cohort of subjects ($n = 19$) with impaired platelet NO responsiveness in whom additional mechanistic data were sought.

Results In study 1, ramipril therapy increased platelet responsiveness to NO relative to the extent of aggregation ($p < 0.001$), but this effect occurred primarily in patients with severely impaired baseline NO responsiveness ($n = 41$). In study 2, ramipril also improved platelet NO responsiveness ($p < 0.01$), and this improvement was correlated directly with increased NO-stimulated platelet generation of cyclic guanosine monophosphate ($p < 0.02$) but not with changes in plasma thrombospondin-1 levels.

Conclusions Ramipril ameliorates platelet NO resistance in HOPE study–type patients, with associated increases in soluble guanylate cyclase responsiveness to NO. This effect is likely to contribute to treatment benefit and define patients in whom ramipril therapy is particularly effective. (J Am Coll Cardiol 2012;60:887–94) © 2012 by the American College of Cardiology Foundation

The HOPE (Heart Outcomes Prevention Evaluation) study with ramipril (1) and the EUROPA (European Trial on Reduction of Cardiac Events With Perindopril in Stable Coronary Artery Disease) study with perindopril (2) proved that angiotensin-converting enzyme (ACE) inhibitors reduce the risk for cardiovascular events in aging, high-risk populations. Furthermore, rates of myocardial infarction, stroke, cardiac arrest, heart failure, and complications relating to diabetes were also decreased. However, the mechanism(s) underlying these beneficial effects have never been delineated and have been the subject of considerable speculation (3–8). Although ACE inhibitors reduce the incidence of fatal and nonfatal atherothrombotic-related events in patients with either chronic heart failure (9,10) or evidence of heart failure after acute myocardial infarction (11,12), evaluation of potential interactions between ACE inhibitors and platelets has been limited. Enalapril has been reported not to affect aggregation (13) and lisinopril not to change soluble P-selectin levels (14).

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In our previous investigation of perindopril effects in patients with heart failure (15), there was also no significant change in platelet aggregability. However, platelet responses to nitric oxide (NO) donor (both inhibition of aggregation and increase in intraplatelet cyclic guanosine monophosphate [cGMP] concentration), which were impaired at baseline, were normalized with perindopril therapy, thus manifesting an improvement

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ADMA = asymmetric dimethylarginine
ADP = adenosine 5′-diphosphate
AIx = augmentation index
cGMP = cyclic guanosine monophosphate
MDA = malondialdehyde
NO = nitric oxide
PRP = platelet-rich plasma
sGC = soluble guanylate cyclase
SNP = sodium nitroprusside
TSP = thrombospondin

in NO/cGMP signaling. Indeed, platelet hyporesponsiveness to NO, as a part of a general phenomenon of "tissue NO resistance" (16), occurs commonly in patients with symptomatic ischemic heart disease (17–19) and in those with diabetes (20) and represents an independent marker of increased cardiovascular risk (21). The NO/cGMP signaling pathway is a physiologically important modulator of both vasomotor tone and platelet function. NO resistance is defined as an impaired physiological response to endogenous and exogenous NO and may predispose to increased risk for ischemic or thrombotic events (16).

We therefore have conducted 2 sequential investigations in HOPE study–type patients: the first to test the hypothesis that ramipril improves platelet NO responsiveness in such patients across an entire study cohort and the second to explore the cellular mechanism underlying this effect in a preselected subset of patients exhibiting NO resistance. The results of both studies suggest that restoration of NO responsiveness by ramipril therapy contributed to its beneficial clinical effects in the HOPE study.

Methods

Study 1: randomized comparison: SUBJECTS. In study 1, a total of 119 patients who were at high cardiovascular risk were enrolled. Inclusion criteria were similar to those of the HOPE study (1). Eligible patients were men and women age ≥50 years (vs. 55 years in the HOPE study) who had histories of coronary artery disease, stroke, peripheral vascular disease, and/or diabetes plus 1 other risk factor (hypertension, elevated total cholesterol level, low high-density lipoprotein cholesterol level, cigarette smoking, or documented microalbuminuria). Patients were excluded if they had symptomatic heart failure; were taking ACE inhibitors, angiotensin receptor antagonists, or adenosine 5′-diphosphate (ADP) receptor antagonists; had uncontrolled hypertension; had clinically overt renal insufficiency; or had had a myocardial infarction or stroke within 4 weeks of study entry. Platelet hyporesponsiveness to ADP (<4 Ω response) was also an exclusion criterion (see the following discussion).

STUDY DESIGN. The study consisted of a randomized, placebo-controlled, blinded evaluation of the effects of ramipril on platelet responsiveness to sodium nitroprusside (SNP). Additionally, the effects of ramipril on endothelial function were assessed, using plasma levels of asymmetric dimethylarginine (ADMA), a marker of endothelial dysfunction (22), together with the augmentation index (AIx), a marker of apparent arterial stiffness (23,24). Changes in plasma concentrations of malondialdehyde (MDA) were determined as indexes of oxidative stress (25).

Potentially eligible patients (n = 202) attended a screening session at which blood samples were collected for the measurement of platelet aggregation. Patients who had more than 4 Ω of ADP-induced aggregation (n = 119; see "Platelet Aggregation Studies") were randomized to receive ramipril or matched placebo (provided by Aventis Pharmaceuticals, Bridgewater, New Jersey) on the basis of a randomization program. Ramipril was initiated at 5 mg/day. After 1 week of therapy, all patients returned, and ramipril dose was up-titrated to 10 mg/day unless contraindicated (e.g., because of significant cough or symptomatic hypotension). Blood samples were collected from all patients at baseline and after 4 and 12 weeks of therapy; AIx was also measured at each visit.

BLOOD SAMPLING. Blood samples were drawn by venipuncture from an antecubital vein. Blood was collected into plastic tubes containing 1:10 volume of acid citrate (2 parts 0.1 mol/l citric acid to 3 parts 0.1 mol/l trisodium citrate) and used for whole-blood platelet aggregation studies.

PLATELET AGGREGATION STUDIES. All aggregation studies were performed using a dual-channel impedance aggregometer (model 560, Chrono-Log, Havertown, Pennsylvania) as previously described (17). Aggregation was induced with ADP (final concentration 1 or 2.5 μmol/l), and responses were recorded for electrical impedance (Ω) using a computer interface system (Aggrolink, Chrono-Log). Responses to the NO donor SNP rather than nitroglycerin were used as indexes of platelet responsiveness to NO to avert any potential effect of nitrate tolerance on assessment of platelet function (26). SNP (final concentration 10 μmol/l) was added to samples 1 min before ADP. Inhibition of aggregation by SNP was evaluated as a percent of maximal aggregation in the absence of SNP. To minimize inaccuracies in the calculation of the inhibitory effect of SNP, at least 4 Ω of ADP response was required.

APPLANATION TONOMETRY. We used applanation tonometry to evaluate the effects of ramipril on arterial stiffness. Pulsed-wave analysis was used to determine AIx (AtCor Medical, Sydney, Australia) as previously described (23,24). Briefly, a micromanometer probe (SPT-301B, Millar Instruments, Houston, Texas) was used to obtain recordings of the peripheral pressure waveforms by flattening, but not occluding, the radial artery of the dominant arm. Data were collected directly into the SphygmoCor system (AtCor Medical), and after 20 sequential waveforms had been acquired, an average peripheral waveform was generated. The waveform was then scaled from brachial artery blood pressure. The corresponding central (ascending aortic) waveform was derived from the radial artery waveform using a validated transfer function; from this augmentation, a value of AIx was then derived and corrected for heart rate.

BIOCHEMICAL MARKERS. Plasma concentrations of the endogenous NO synthase inhibitor ADMA were measured as previously described (22) using high-performance liquid chromatography. MDA was determined using a modified thiobarbituric acid method (25).

Study 2: mechanistic considerations: Study 2, performed subsequent to the evaluation of study 1 results, was designed to evaluate the relationship between the effects of ramipril treatment on responsiveness of platelet soluble guanylate cyclase (sGC). In view of the heterogeneity of results in study 1, we selected a more NO resistant cohort by excluding patients with baseline SNP response >50%. A total of 19 patients with similar inclusion and exclusion criteria as described previously were enrolled for this study. Ramipril was initiated at 5 mg/day for 1 week, after which the dose was up-titrated to 10 mg/day. Blood samples were collected from patients at baseline and after 2 weeks of therapy. Platelet aggregation studies were performed as described previously.

CGMP STUDIES. For cGMP studies, blood was centrifuged at 250 *g* for 10 min at room temperature to obtain platelet-rich plasma (PRP). Platelet-poor plasma was prepared by further centrifugation of the remaining blood at 2,500 *g* for 20 min. Platelet counts were performed on the STKS Coulter Counter (Coulter Electronics Inc., Hialeah, Florida) and the PRP was adjusted with platelet-poor plasma to a constant count of 250,000/μl.

PRP (0.5 ml) was pre-incubated at 37°C with SNP (10 μmol/l) for 1 min to assess the effect of NO donor on intraplatelet cGMP content. Intraplatelet generation of cGMP in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (0.5 mmol/l, 5 min pre-incubation with PRP before SNP addition) was determined as a measure of NO responsiveness of platelet sGC. Intraplatelet cGMP content was assayed as described previously (27). Briefly, after incubation, PRP was filtered through GF/C Glass Microfibre Filters (Whatman, Maidstone, United Kingdom) for harvesting the platelets. Filters with absorbed platelets were rinsed with physiological saline and placed into 0.5 ml of 4 mmol/l ethylenediaminetetraacetic acid for further extraction of cGMP in a boiling water bath for 5 min. After centrifugation of samples at 3,000 *g* for 10 min, cGMP concentration in supernatant was estimated using cGMP radioimmunoassay kits [^{125}I] (Biomedical Technologies Inc., Stoughton, Massachusetts).

THROMBOSPONDIN (TSP)-1. Plasma concentrations of TSP-1, a platelet-derived endogenous inhibitor of intracellular NO signaling and particularly of sGC (28), were determined before and after ramipril therapy. For determination of TSP-1 levels, blood was centrifuged at 1,800 *g* for 15 min at 4°C, and the supernatant was collected. The supernatant was recentrifuged at 10,000 *g* for 10 min, collected, and stored at −70°C. Determination of TSP-1 levels was then performed per the Quantikine Human TSP-1 enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minnesota).

Both studies were approved by the Ethics of Research Committee of the Queen Elizabeth Hospital, and written informed consent was obtained before study entry.

Statistical analysis: All data were analyzed via intention to treat. Patients were categorized according to entry platelet NO responsiveness. Study 1 was powered to detect >0.75-SD variability in platelet SNP responses between treatment groups. In accordance with previous results (21), we used SNP response of <32% inhibition of aggregation as a criterion of severe platelet NO resistance for evaluation of potential heterogeneity of response to ramipril. We have previously demonstrated (29) that inhibition of aggregation by SNP is dependent on ADP response as a reflection of physiological antagonism. Therefore, changes in SNP response were analyzed relative to changes in ADP response. In study 1, analysis of covariance was used to test both the primary hypothesis of the study (effects of ramipril on the ΔSNP/ΔADP relationship in the entire cohort) and the principal secondary hypothesis (effects of ramipril in the presence of platelet NO resistance). Using this analysis, a potentiating effect of ramipril on SNP responses would be reflected in an alteration of the competitive relationship between SNP and ADP response toward sensitization to SNP. Patient characteristics in various groups were compared using nonpaired *t* tests (all data being normally distributed). Nonpaired *t* tests were also used to compare changes in other parameters (AIx, MDA, ADMA, and cGMP) between treatment groups. Changes in parameters after 4 weeks of treatment were used for examination of the time course of the effects of ramipril. In study 2, all parameters were analyzed using paired *t* tests. All data are expressed as mean ± SD unless otherwise stated. All statistical analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, Illinois).

Results

Study 1: randomized comparison: SUBJECT CHARACTERISTICS AND CLINICAL COURSE. One hundred nineteen patients were randomized to receive the study medication (Table 1). Placebo (n = 59) and ramipril (n = 60) groups were well matched with regard to all measured parameters, except statin therapy, which was more frequent in the placebo group. During the 12-week study period, 11 patients of the total 119 withdrew (9 from the active treatment group). Of these, 3 patients receiving ramipril were withdrawn because of cough or dizziness and 1 from each group as a result of an acute coronary syndrome.

PLATELET AGGREGATION AND INHIBITION WITH SNP. Platelet responsiveness to ADP in blood samples from subjects was similar between groups at baseline (7.6 ± 2.6 Ω vs. 7.4 ± 2.4 Ω, placebo vs. ramipril). There was no differential change in the extent of ADP-induced aggregation over the 3-month period in both groups (Table 2). At study entry, platelet responsiveness to the antiaggregatory

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Table 1 Patient Demographics at Study Entry

Variable	Placebo Group (n = 59)	Ramipril Group (n = 60)	p Value
Men	38 (64%)	35 (58%)	0.57
Age (yrs)	66.4 ± 9.7	67.2 ± 10.6	0.65
Height (cm)	166.8 ± 12.0	165.1 ± 18.1	0.43
Weight (kg)	81.5 ± 18.7	81.3 ± 19.5	0.97
BMI (kg/m^2)	29.6 ± 9.0	30.2 ± 9.4	0.73
SBP (mm Hg)	144.7 ± 23.2	145.3 ± 24.5	0.88
DBP (mm Hg)	82.6 ± 8.9	80.6 ± 12.7	0.33
HR (beats/min)	68 ± 8.9	66 ± 11.0	0.25
Past MI	28 (48%)	20 (33%)	0.13
CABG	14 (24%)	23 (38%)	0.11
PCI	31 (53%)	22 (37%)	0.09
Diabetes	9 (15%)	15 (25%)	0.25
Smokers	19 (32%)	12 (20%)	0.15
Hypertension	26 (44%)	21 (35%)	0.35
Cholesterol level (mmol/l)	4.2 ± 0.9	4.5 ± 0.9	0.05
Creatinine level (μmol/l)	0.092 ± 0.015	0.094 ± 0.019	0.53
Therapy			
Aspirin	51 (86%)	54 (90%)	0.58
Statins	57 (97%)	49 (82%)	0.02*
Beta-blockers	9 (15%)	16 (27%)	0.18
Calcium antagonists	41 (69%)	31 (52%)	0.06
Nitrates	22 (37%)	21 (35%)	0.85
Warfarin	5 (8%)	2 (3%)	0.27
Perhexiline	1 (2%)	4 (7%)	0.36

Values are n (%) or mean ± SD. *Statistically significant ($p < 0.05$).

BMI = body mass index; CABG = coronary artery bypass grafting; DBP = diastolic blood pressure; HR = heart rate; MI = myocardial infarction; PCI = percutaneous coronary intervention; SBP = systolic blood pressure.

effect of SNP was similar between the study groups (47.3 ± 28.5% vs. 42.4 ± 28.9% inhibition of aggregation, placebo vs. ramipril group, p = 0.35). After 3 months of ramipril therapy, average platelet SNP response increased, but not significantly (Table 2). On correction of changes in SNP response for those in ADP response (29), the ΔSNP/ΔADP relationship demonstrated an increase in NO responsiveness in the ramipril treatment group (analysis of covariance, $p < 0.001$) (Fig. 1).

HETEROGENEITY OF RAMIPRIL EFFECT ON NO RESPONSIVENESS. Severe platelet NO resistance (SNP response ≤32%) was present in 41 patients at baseline (23 of whom were randomized to ramipril therapy). There was no difference in the extent of ADP-induced aggregation between the normal NO responder and impaired NO responder groups. ADP-induced aggregation was not significantly affected by 3 months of therapy within each subgroup.

NO responsiveness increased markedly after ramipril therapy in this NO-resistant subgroup of patients, compared with the placebo group (ΔSNP response 36.5 ± 31.9% vs. 15.9 ± 22.7% inhibition of aggregation, ramipril vs. placebo, p = 0.03) (Fig. 2B). In contrast, platelet NO responsiveness was unaltered by ramipril in the subgroup with normal platelet responses at baseline (ΔSNP response −4.6 ± 22.2% vs. −5.8 ± 29.1% inhibition of aggregation, ramipril vs. placebo, p = NS) (Fig. 2A). Thus, effects of ramipril varied markedly according to the presence or absence of severe platelet NO resistance at baseline.

TIME COURSE OF RAMIPRIL EFFECT. Interestingly, the week 4 data suggested that some effect of ramipril was already present at this stage, with a significant sensitization of platelets (on the basis of the ΔSNP/ΔADP response relationship) in the entire cohort ($p < 0.001$).

VASOMOTOR EFFECTS. Three months of ramipril therapy marginally reduced both systolic blood pressure (−11.7 ± 20.9 mm Hg vs. −5.2 ± 17.5 mm Hg, ramipril vs. placebo, p = 0.07) and diastolic blood pressure (−6.2 ± 10.3 mm Hg vs. −1.8 ± 10.0 mm Hg, ramipril vs. placebo, p = 0.03). There was no difference in heart rate between the placebo and ramipril groups.

We also examined the effect of ramipril therapy on AIx, a measure of arterial stiffness and wave reflection (24,25). At study entry, there was no difference in AIx between the placebo and ramipril groups (23.7 ± 8.9% vs. 24.9 ± 9.3%, respectively). Three months of ramipril therapy significantly reduced AIx (−1.3 ± 8.1 vs. −4.8 ± 10.9, placebo vs. ramipril, p = 0.02) (Table 2).

OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION. Plasma concentrations of MDA and ADMA, used as markers of oxidative stress and endothelial dysfunction, respectively, are summarized in Table 2. MDA concentrations were not significantly affected by ramipril therapy. ADMA levels were reduced by ramipril therapy (p = 0.05) (Table 2).

Study 2: mechanistic considerations: Study 2 was designed to determine whether sensitization of platelets to NO by ramipril was associated with potentiation of NO responsiveness of sGC. In view of the apparent heterogeneity of response to ramipril therapy in study 1, we selected patients with relatively impaired NO responsiveness. Although the 19 patients recruited for this study were selected on a similar basis to those in study 1, they tended to be older (mean age 70 ± 9 years), and 11 of the 19 participants had diabetes.

Table 2 Effect of 3 Months of Ramipril on Measured Parameters

Parameter	Change From Baseline: Placebo Group (n = 57)	Change From Baseline: Ramipril Group (n = 51)	p Value
HR (beats/min)	1.1 ± 15.5	2.2 ± 8.7	0.63
ADP-induced aggregation (Ω)	−1.5 ± 2.4	−0.9 ± 2.6	0.22
Inhibition of aggregation by SNP (%)	1.7 ± 28.2	12.3 ± 36.8	0.10
AIx (%)	−1.3 ± 8.1	−4.8 ± 10.9	0.02
MDA (μmol/l)	0.006 ± 0.15	−0.042 ± 0.26	0.25
ADMA (nmol/l)	6.9 ± 6.1	−15.9 ± 5.9	0.05
SNP-induced intraplatelet cGMP response (%)	−3.1 ± 42.0	−3.0 ± 41.7	0.99

Values are mean ± SD.

ADMA = asymmetric dimethylarginine; ADP = adenosine diphosphate; AIx = augmentation index; cGMP = cyclic guanosine monophosphate; HR = heart rate; MDA = malondialdehyde; SNP = sodium nitroprusside.

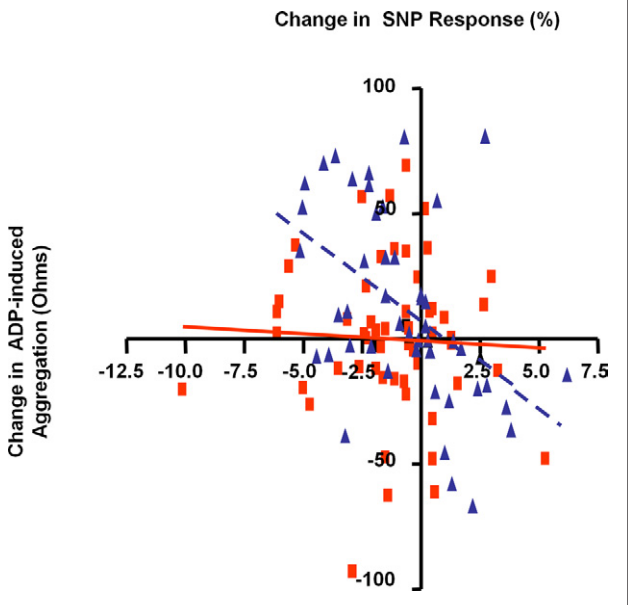

Figure 1 **Effect of Ramipril on the Change in ADP/SNP Response Relationship**

This graph illustrates the effect of 3 months of ramipril **(blue triangles)** or placebo **(red squares)** treatment on the change in adenosine diphosphate (ADP)/sodium nitroprusside (SNP) response relationship in patients at high risk for cardiovascular events (HOPE [Heart Outcomes Prevention Evaluation]–type patients). Ramipril **(blue dashed line)** significantly ($p < 0.001$, analysis of covariance) altered the change in ADP/SNP response relationship compared to placebo **(red solid line)**.

PLATELET AGGREGATION IN RESPONSE TO ADP AND SNP. There was no significant change in the extent of ADP-induced platelet aggregation over the 2-week period of ramipril treatment (data not shown). As intended, baseline SNP responsiveness was substantially less than for study 1, with mean baseline value of 24 ± 13% inhibition. With ramipril treatment, SNP response increased to 37 ± 25% ($p < 0.01$) (Fig. 3A).

INTRAPLATELET CGMP GENERATION IN RESPONSE TO SNP. Platelet cGMP-generating capacity in response to SNP significantly increased after 2 weeks of treatment with ramipril (Fig. 3B), implying improvement in responsiveness of sGC to NO. Specifically, at baseline, SNP-induced stimulation of cGMP generation was 249 ± 85% of control, increasing to 317 ± 114% ($p < 0.02$) after ramipril therapy. There was a significant correlation ($R = 0.63$, $p < 0.01$) between changes in the responsiveness of the platelet-cGMP system and modulation of platelet aggregation by SNP (Fig. 3C).

TSP CONCENTRATIONS. Ramipril treatment induced no significant changes in plasma TSP-1 concentrations (baseline 109.5 ± 80.5 ng/ml, post ramipril therapy 93.9 ± 61.2 ng/ml, $p = 0.50$).

Discussion

The HOPE study with ramipril (1) and the EUROPA study with perindopril (2) demonstrated that these ACE inhibitors reduce the risk for cardiovascular events in aging, high-risk populations. However, the mechanism(s) underlying these beneficial effects have never been delineated and have been the subject of considerable speculation (3–8). In the present study, we provide evidence for an effect of ramipril in HOPE-type patients, which is distinct from previously documented beneficial changes to vasomotor function (14). Specifically, we demonstrate that ramipril, while reducing arterial stiffness and plasma ADMA concentrations (consistent with previous findings [8]), potentiates platelet responsiveness to the antiaggregatory effects of NO by selectively ameliorating NO resistance. Furthermore, we demonstrate that this effect is correlated with the sensitization of platelet sGC to NO.

Previous studies (13,14) have made it clear that ACE inhibition does not substantially affect responsiveness to proaggregatory stimuli in vitro. However, homeostasis of

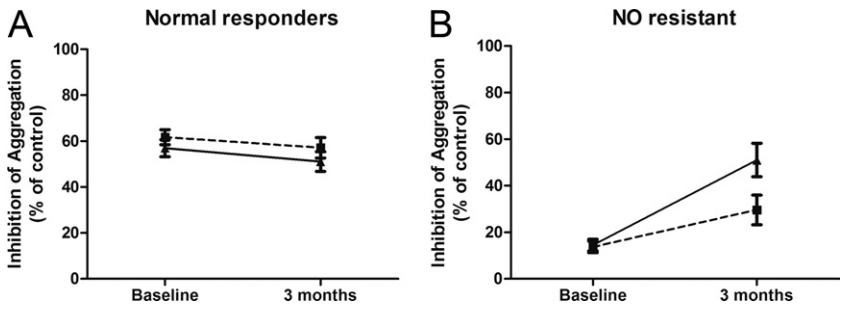

Figure 2 **Effect of Ramipril on Inhibition of Aggregation According to Baseline SNP Responsiveness**

These graphs illustrate the effect of 3 months of ramipril **(triangles)** or placebo **(squares)** treatment on the inhibition of adenosine diphosphate–induced whole-blood aggregation in blood samples from patients with "normal" (normal responders) **(A)** and "impaired" (sodium nitroprusside [SNP] response ≤32%, nitric oxide [NO] resistant) **(B)** SNP responsiveness at study entry. Ramipril had no effect on SNP responsiveness in the normal responder patient cohort (p = NS). In contrast, ramipril significantly improved SNP responsiveness in patients who were NO resistant at study entry ($p < 0.002$). Data are expressed as mean ± SEM.

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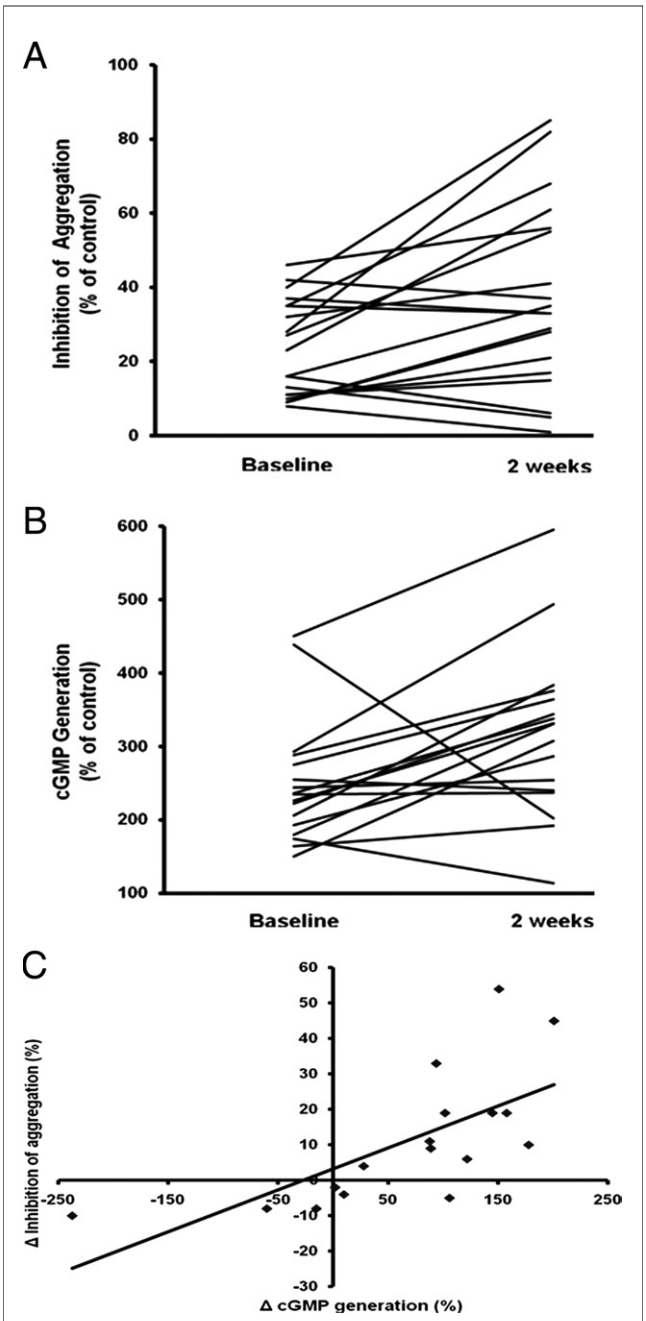

Figure 3 **Effect of 2-Week Ramipril Treatment on SNP Responsiveness and cGMP Generation**

These graphs illustrate the changes in the inhibitory effect of sodium nitroprusside (SNP) on adenosine diphosphate–induced platelet aggregation **(A)** and stimulatory effect of SNP on intraplatelet cyclic guanosine monophosphate (cGMP) generation **(B)** after 2 weeks of ramipril therapy. There was a significant correlation ($R = 0.63$, $p < 0.01$) between these 2 parameters **(C)**.

platelet function is equally dependent on the integrity of responsiveness to antiaggregatory autacoids. Several potential biochemical mechanisms underlie the presence of NO resistance (see Chirkov and Horowitz [16] for a review). "Scavenging" of NO by O_2^- and inactivation of sGC are likely to be the major relevant biochemical disturbances. NO resistance has been studied extensively at both the platelet (17) and vascular (30,31) levels. It appears most likely to occur in the presence of acute coronary syndromes (18), diabetes mellitus (20), stable angina pectoris (17), and acute heart failure (15) and has also been documented in aortic stenosis (32), hypertension (33), and polycystic ovarian syndrome (29). Furthermore, we have demonstrated that therapies that reduce O_2^- generation, such as perhexiline (34) and correction of hyperglycemia (35), may ameliorate NO resistance. More recently, we have also shown that platelet NO resistance represents an independent risk factor for cardiovascular events and mortality ($p < 0.01$ for cardiac events) (21). This is analogous to the previously demonstrated prognostic significance of coronary endothelial vasomotor dysfunction (36).

In study 1, we demonstrated that platelet NO responsiveness was very heterogeneous within a HOPE-type population, ranging from normal to severe NO resistance. Although overall, ramipril therapy potentiated NO responsiveness significantly (with the onset of an effect within 4 weeks), this effect was dependent in magnitude on baseline SNP response, as demonstrated by the data in Figure 2: the increase in responsiveness occurred predominantly within the subset of patients with severe NO resistance.

We therefore performed study 2 to evaluate this phenomenon prospectively by choosing a less NO responsive patient cohort (mean baseline SNP response of 24%). Even after 2 weeks, ramipril potentiated NO responsiveness. Parallel mechanistic investigation revealed that ramipril: 1) increased cGMP generation in response to SNP, suggesting sensitization of sGC to NO as a fundamental mechanism of effect, but 2) did not change plasma levels of TSP-1, a reported modulator of sGC activity (28).

These results therefore suggest that ramipril ameliorates NO resistance by sensitization of sGC in a TSP-1–independent manner. Given the likelihood that ramipril exerts similar biochemical effects in platelets and in vascular smooth muscle, these data in combination suggest that endothelial function is improved both by increased generation of NO and by facilitation of cGMP generation. These findings, together with the reduction in plasma ADMA concentration (Table 2), also have mechanistic implications regarding the previously reported beneficial effects of ACE inhibitors on endothelial function (8–14).

Study limitations. The study was subject to several limitations. A randomized controlled design for study 2 would have been more rigorous but would have imposed considerable recruitment difficulties.

The most important limitation is that NO responsiveness was examined only in platelets, not in vascular muscle, because of the difficulty of performing parallel physiological and biochemical studies in human vasculature. However, the observed changes in platelet NO-related physiology are of independent prognostic importance (21). Similarly, it is impossible to ascertain to what extent these changes in platelet responsiveness to NO might have contributed to improved cardiovascular

outcomes and prognosis in HOPE. Interestingly, a recent evaluation of the genetic determinants of effects of perindopril therapy in EUROPA (37) identified 3 polymorphisms affecting the bradykinin type 1 receptor and angiotensin II type 1 receptor genes that modulated both the risk for cardiovascular events in EUROPA and the extent of the beneficial effect of perindopril. There was an inverse relationship between background event risk and extent of benefit. Because angiotensin II type 1 receptor stimulation increases superoxide production (38), while bradykinin type 1 stimulation induces secondary NO release (39), these observations may reflect additional factors predictive of NO resistance.

Conclusions

The data from the present study demonstrate that ramipril sensitizes platelets to NO in a HOPE-type patient population and that this effect results from sGC-dependent amelioration of platelet NO resistance. These findings provide an additional potential basis for the effects of ramipril in reducing risk for cardiac events.

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Key Words: asymmetric dimethylarginine (ADMA) ■ cGMP ■ nitric oxide resistance ■ platelet aggregation ■ ramipril.