Quinapril, an ACE Inhibitor, Reduces Markers of Oxidative Stress in the Metabolic Syndrome

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OBJECTIVE — Patients with the metabolic syndrome often have abnormal levels of proinflammatory and pro-oxidative mechanisms within their vasculature. We sought to determine whether the ACE inhibitor quinapril regulates markers of oxidative stress in the metabolic syndrome.

RESEARCH DESIGN AND METHODS — Forty patients with the metabolic syndrome were randomized in a double-blind manner to either the ACE inhibitor quinapril (20 mg/day) or matching placebo for 4 weeks. Serum markers of vascular oxidative stress were measured.

RESULTS — After 4 weeks of therapy, serum 8-isoprostane was reduced by 12% in the quinapril group when compared with placebo (quinapril, 46.7 ± 1.0; placebo, 52.7 ± 0.9 pg/ml; $P = 0.001$). Erythrocyte superoxide dismutase activity increased 35% in the quinapril group when compared with placebo (quinapril, 826.3 ± 17.1; placebo, 612.3 ± 6.9 units/g Hb; $P < 0.001$). In addition, lag time to oxidation of LDL, a marker of oxidative stress, was increased by 48% in the quinapril group when compared with placebo (quinapril 89.2 ± 9.2 vs. placebo 60.1 ± 12.3 min; $P < 0.001$). Therapy with quinapril was well tolerated.

CONCLUSIONS — The addition of the ACE inhibitor quinapril reduces markers of vascular oxidative stress and may attenuate the progression of the pathophysiology seen in the metabolic syndrome.

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The metabolic syndrome is a constellation of abnormal glucose and lipid metabolism that has reached epidemic proportions over the last decade (1). Patients with the metabolic syndrome are at considerable risk for developing atherosclerosis-related diseases, including a two- to fourfold increased risk of stroke and a three- to fourfold increased risk of myocardial infarction when compared with those without metabolic syndrome (2).

Recent studies (3,4) suggest that pro-oxidative and proinflammatory processes play a significant role in the progression of atherosclerosis. In fact, inflammatory markers are predictors of cardiovascular events and progression to type 2 diabetes in healthy individuals as well as those with the metabolic syndrome, underscoring the link between inflammation, metabolic disorders, and cardiovascular disease (5,6). Chronic inflammation and an abnormal pro-oxidant state are both found in the metabolic syndrome and may play a role in its pathogenesis (7,8).

The renin-angiotensin system (RAS) plays a central role in the pathogenesis of atherosclerosis-related diseases. Angiotensin II, the central molecule in the RAS, has multiple effects on inflammation, oxidation, atherosclerotic plaque initiation, and progression (9). In the present study, we determine potential mechanisms by which the administration of the ACE inhibitor quinapril regulates mechanisms of oxidative stress in subjects with the metabolic syndrome.

RESEARCH DESIGN AND METHODS

— Men and women aged ≥18 years and with the metabolic syndrome were enrolled in the study. The metabolic syndrome was defined using the National Cholesterol Education Program Adult Treatment Panel III criteria (Table 1), and eligible subjects were required to meet at least three of the five criteria (10). Subjects were excluded if they had any of the following: tobacco use <6 months before enrollment, a clinical history of coronary artery disease or congestive heart failure, use of an ACE inhibitor or angiotensin receptor blocker <12 months before enrollment, ejection fraction <50% by echocardiography or contrast ventriculogram, systolic blood pressure >140 or <100 mmHg, diastolic blood pressure >90 or <60 mmHg, HbA_{1c} >7.0%, serum creatinine >2.0 mg/dl, hepatic impairment, or malignancy. Written informed consent was obtained from all subjects.

Study design

Subjects were randomized in a double-blinded fashion to either quinapril 20 mg/day or matching placebo for 4 weeks. Allocation concealment was maintained until the end of the study. The dose of the study drug was chosen based on the results of prior studies in which we found

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Abbreviations: E-SOD, erythrocyte superoxide dismutase; RAS, renin-angiotensin system;

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Diagnostic criteria for the metabolic syndrome*

Factor	Criteria
1	Abdominal girth >40 in (102 cm) in men or >35 in (88 cm) in women
2	HDL cholesterol <40 mg/dl in men or <50 mg/dl in women
3	Fasting triglycerides >150 mg/dl (1.69 mmol/l)
4	Blood pressure >130/85 mmHg
5	Fasting glucose ≥110 mg/dl (≥ 6.1 mmol/l)

Three out of five criteria are required for diagnosis of the metabolic syndrome.

that the addition of quinapril 20 mg daily to standard therapy in subjects with coronary artery disease reduced markers of inflammation and oxidative stress; these changes were independent of blood pressure reduction (11). Subjects were advised to self-administer one-half of the full dose during the initial 2 days of therapy, after which they were to take the full study dose. After 2 weeks, blood pressure was rechecked and blood was drawn to measure serum creatinine and potassium. Fasting blood samples were drawn before and at the end of therapy at a similar time of day. The study protocol complies with the Declaration of Helsinki and was approved by the institutional review board at the participating institution before its implementation.

Measurement of total serum 8-isoprostane and erythrocyte superoxide dismutase

Plasma samples were centrifuged and stored at −80°C. An aliquot was drawn, and enzyme immunoassay (EIA; Cayman Chemical, Ann Arbor, MI) for serum 8-isoprostane was performed on each sample in triplicate. Serum (60 μl) was used for analysis, and enzyme immunoassay was performed as previously described (12). The levels of total serum 8-isoprostane were determined on a plate reader at an optical density of 420 nm. Erythrocyte superoxide dismutase (E-SOD) activity was determined using hemolysates and commercially available kits (cat. no. SDI 25; Randox Laboratories, Crumlin, Ireland). Briefly, superoxide radicals produced by xanthine and xanthine oxidase reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye (13). The E-SOD activity is then measured by the degree of inhibition of this reaction. E-SOD activity was expressed as units per gram of Hb (14). We found no evidence of interference of quinapril or its metabolite in the isoprostane or E-SOD assays.

LDL oxidation and lag time

We have previously observed that the administration of an RAS inhibitor to subjects with coronary artery disease increases the lag time to oxidation of LDL cholesterol, suggesting increased resistance to the modification of LDL cholesterol (15). LDL cholesterol was isolated from blood samples. Using $CuSO_4$, oxidation of LDL was performed. Spectrophotometric analysis was used to determine the lag time to oxidation of LDL (16). We found no evidence of interference of quinapril or its metabolite in the LDL oxidation assays.

Blood glucose was measured using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. LDL and HDL fractions were separated from fresh serum by combined ultracentrifugation and precipitation. Lipoprotein fraction cholesterol and triglycerides were measured enzymatically.

Statistical analysis

All data are presented as the mean ± SE. Comparisons were determined within the ACE inhibitor and placebo groups using paired-sample *t* tests. A *P* value of <0.05 was considered statistically significant, and all *P* values were two sided. Calculations were performed with SPSS software (version 10.0; Statistical Package for the Social Sciences, Chicago, IL).

RESULTS — Forty subjects (23 men and 17 women) were enrolled in the study and followed for 4 weeks. Follow-up was 100% complete. The two experimental groups (quinapril and placebo) had similar demographic characteristics (Table 2). The mean age was 36.2 years; mean BMI 29.3 kg/m^2; mean LDL cholesterol, HDL cholesterol, and triglyceride levels were 125.9 ± 20.3 mg/dl (3.3 ± 0.5 mmol/l), 39.7 ± 5.9 (1.04 ± 0.13), and 229 ± 33 (6 ± 0.8), respectively; mean systolic and diastolic blood pressures were 125.9 ± 8.2 and 79.5 ± 10.6 mmHg, respectively; and the mean fasting glucose was 112 mg/dl. Nine subjects (23%) were on lipid-lowering therapy. No statistically significant differences were noted in baseline characteristics between the two treatment groups.

There was a higher incidence of cough in the quinapril group when compared with the placebo group (4 vs. 1; *P* =

Table 2—*Patient demographics and baseline characteristics*

	Quinapril	Placebo
n	20	20
Age (years)	35.2	39.6
Sex (M/F)	11/9	12/8
BMI (kg/m^2)	29.8	29.1
Systolic blood pressure (mmHg)	126 ± 8	130 ± 9
Diastolic blood pressure (mmHg)	80 ± 10.4	77.8 ± 11.1
LDL cholesterol (mg/dl)	124.7 ± 21.4	130 ± 16.8
HDL cholesterol (mg/dl)	40.3 ± 7.0	38.9 ± 6.1
Triglycerides (mg/dl)	238 ± 36	219 ± 30
Fasting glucose (mg/dl)	113 ± 15	111 ± 19
HbA_{1c} (%)	5.8 ± 0.7	5.6 ± 0.8
Hypertension	5 (25)	6 (30)
Smoking history	7 (35)	6 (30)
Concomitant medications		
Lipid lowering therapy*	5 (25)	6 (30)
β-Blocker	2 (10)	4 (20)
Diuretic	3 (15)	4 (20)
Calcium channel blocker	3 (15)	4 (20)

Data are means ± SE or *n* (%). *Includes statin, niacin, resin, or fibrates.

Table 3—*Effects of therapy on markers of oxidative stress and metabolic indexes*

	Baseline			After 4 weeks of therapy		
	Quinapril	Placebo	*P*	Quinapril	Placebo	*P*
n	20	20	—	20	20	—
8-isoprostane (pg/ml)	54.4 ± 1.4	53.2 ± 1.2	NS	46.7 ± 2	52.7 ± 0.9	0.001
E-SOD activity (units/g Hb)	609.3 ± 9.8	612.7 ± 9	NS	826.3 ± 17.1	612.3 ± 6.9	0.001
Lag time to LDL oxidation (min)	60.5 ± 10.1	61.0 ± 8.7	NS	89.2 ± 9.2	60.1 ± 12.3	0.001
LDL cholesterol (mg/dl)	124.7 ± 21.4	130 ± 16.8	NS	122.9 ± 19.2	133.1 ± 17.8	NS
Triglycerides (mg/dl)	238 ± 36	219 ± 30	NS	234 ± 31	227 ± 24	NS
Fasting glucose (mg/dl)	113 ± 15	111 ± 19	NS	116 ± 17	115 ± 13	NS
HbA_{1c} (%)	5.8 ± 0.7	5.6 ± 0.8	NS	5.6 ± 0.6	5.7 ± 0.7	NS

Data are means ± SE.

0.05). Furthermore, there was a rise in serum potassium or creatinine of >20% in 2 of 20 subjects in the quinapril group (P = NS). There was an average reduction of systolic blood pressure by 4 ± 2 mmHg and diastolic blood pressure by 3 ± 2 mmHg in the group of subjects treated with quinapril during the study period (P = NS). No subject in either group experienced hypotension (i.e., systolic blood pressure <100 mmHg) during the study. No changes in glycemic control were observed in either of the two study groups.

Treatment with quinapril reduces serum 8-isoprostane in the metabolic syndrome

Activity of 8-isoprostane was significantly reduced after 4 weeks of therapy with quinapril compared with the placebo (46.7 ± 2 vs. 52.7 ± 0.9 pg/ml; P = 0.001). Furthermore, the decrease in the expression of 8-isoprostane was observed in every study subject treated with quinapril (data not shown).

Treatment with quinapril increases E-SOD activity in the metabolic syndrome

Activity of E-SOD was significantly increased after 4 weeks of therapy with quinapril compared with placebo (826.3 ± 17.1 vs. 612.3 ± 6.9 units/g Hb; $P < 0.001$). Furthermore, the increase in E-SOD activity was observed in every study subject treated with quinapril (data not shown).

Treatment with quinapril increases lag time to LDL oxidation in metabolic syndrome

Lag time to oxidation of LDL was significantly increased after 4 weeks of therapy with quinapril compared with placebo (quinapril 89.2 ± 9.2 vs. placebo 60.1 ± 12.3 min; $P < 0.001$). Furthermore, the increase in lag time to LDL oxidation was observed in every subject treated with quinapril (data not shown). We determined mean values for 8-isoprostane, E-SOD, and lag time to LDL oxidation in a healthy control population (n = 19) to be 44.4 ± 5.3 pg/ml, 773 ± 86 units/g Hb, and 86.0 ± 10.0 min, respectively. No changes in levels of LDL cholesterol, triglycerides, serum glucose, or HbA_{1c} were observed in either the placebo or quinapril study groups (Table 3).

CONCLUSIONS — This study demonstrates possible mechanisms by which the ACE inhibitor quinapril may affect vascular oxidative processes in subjects with the metabolic syndrome. Therapy with quinapril resulted in increased E-SOD activity, suggesting that quinapril has antioxidative effects in subjects with the metabolic syndrome. In addition, levels of serum 8-isoprostane were decreased, whereas lag time to LDL oxidation was increased, findings that are consistent with decreased oxidative stress within the vasculature. Furthermore, our findings suggest that these effects may be at least partly independent of blood pressure reduction. In comparison with placebo, no significant changes in systolic blood pressure, LDL cholesterol, or metabolic control were noted with quinapril therapy in these patients.

Pro-oxidative mechanisms are thought to be a hallmark of the atherogenic process. We examined several markers of oxidation within the vasculature in this study. The isoprostanes are a family of free-radical–dependent metabolites of arachidonic acid that are used as clinical biomarkers of lipid peroxidation (i.e., oxidative stress) (17). Oxidized LDL is believed to be the most atherogenic form of LDL; the time required for LDL to undergo oxidation, or lag time, is another indirect measure of oxidative stress (18,19). Finally, superoxide dismutase catalyzes the reaction of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2), making it a central element in the maintenance of the vascular redox balance. As such, superoxide dismutase is indirectly involved in regulating levels of nitric oxide (NO) bioavailability. Inhibition of bradykinin degradation by ACE inhibitors may increase the activity of superoxide dismutase and modulate the production of NO, leading to the inactivation of reactive oxygen species, while also inhibiting various pro-oxidative mechanisms within the vasculature (20).

The RAS plays a central role in the pathogenesis of atherosclerosis-related diseases. Angiotensin II, the central molecule in the RAS, has multiple effects on atherosclerotic plaque initiation and progression. On a molecular and cellular level, blockade of the RAS reduces the extent of vascular lesions in atherosclerosis (21), and it appears that these effects may be independent of blood pressure reduction (22). Although not fully established, these mechanisms include improved endothelial function, plaque stabilization, and regulation of hemodynamic stress; NO bioactivity; pulse pressure; and the ability of macrophages to oxidize LDL (23). Our study highlights mechanisms by which these agents may be of benefit in subjects with the metabolic syndrome.

Limitations of the study

Our investigation is a short-term study (4 weeks) to determine potential mecha-

nisms by which ACE inhibitors may be effective in the metabolic syndrome. Due to its small size ($n = 40$), we were not able to evaluate differences among various subsets within our study population. In addition, it is possible that the response to therapy with quinapril may have been more pronounced if a higher dose of quinapril had been used (e.g., 40 mg/day). The subjects in our study are at significant risk for the development of atherosclerosis and its related complications; therefore, these results may not be applicable to lower-risk populations. Nevertheless, in comparison with placebo, therapy with quinapril for 4 weeks reduced serum levels of 8-isoprostane, increased E-SOD activity, and prolonged lag time to LDL oxidation in all subjects.

Summary

The incidence of the metabolic syndrome is increasing throughout the world, with a concomitant increase in the risk of atherosclerosis-related diseases. Pro-oxidative and proinflammatory mechanisms are important in the pathogenesis of atherosclerosis, and the present study suggests in part the mechanisms by which the ACE inhibitor quinapril may be beneficial in the prevention of atherosclerosis-related diseases. These findings also reinforce the growing evidence of oxidative mechanisms in the pathogenesis of atherosclerosis. Inhibitors of the RAS, such as ACE inhibitors, have powerful anti-inflammatory and antioxidant effects within the vasculature, and clinical outcome studies should be considered to determine the utility of these agents in the primary prevention of atherosclerosis-related diseases in subjects with the metabolic syndrome.

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