Metoprolol Does Not Attenuate Atherosclerosis in Lipid-Fed Rabbits Exposed to Environmental Tobacco Smoke

Yi-Ping Sun, MD; Bo-Qing Zhu, MD; Richard E. Sievers, BS; Stanton A. Glantz, PhD; William W. Parmley, MD

Background We previously demonstrated that exposure to environmental tobacco smoke (ETS) increases the development of atherosclerosis in lipid-fed rabbits. Clinical studies have suggested a protective effect of β-blockers in smokers. Accordingly, we evaluated the effects of metoprolol in this animal model to see whether this β-blocker would block the atherogenic effects of ETS.

Methods and Results Thirty-two New Zealand White male rabbits on a 0.3% cholesterol diet were randomly divided into four groups: ETS-metoprolol (ETS-M), ETS-control (ETS-C), and non-ETS with metoprolol (NETS-M) and without metoprolol (NETS-C). The two metoprolol-treated groups received metoprolol at a dose of 0.4 mg · kg⁻¹ · h⁻¹ administered subcutaneously by an osmotic pump. Rabbits in the ETS groups were exposed to sidestream smoke from four Marlboro cigarettes per 15 minutes, 6 hours a day, for 10 weeks. Average air carbon monoxide (CO), nicotine, and total particulates (TP) in the exposure chambers were 67.2±3.1 (SEM) ppm, 1135.7±78.4 μg/m³, and 37.7±3.0 mg/m³, respectively. Plasma nicotine was significantly higher in ETS-exposed rabbits than in nonexposed rabbits (7.1±1.9 versus 0.5±0.1 ng/mL, P<.01). Blood carbon monoxide hemoglobin (COHb) in the ETS-M group was significantly higher than that in the NETS-M group (4.0±0.2% versus 1.3±0.1%, P<.0001). The lipid lesions in the aorta and pulmonary artery were 57.2±7.6% and 33.1±6.4% (ETS-M), 62.8±8.4% and 58.4±6.1% (ETS-C), 38.7±9.4% and 24.8±7.7% (NETS-M), and 49.8±8.7% and 32.7±7.1% (NETS-C). There were significant differences in lipid deposits of the arteries between the controls and the ETS-exposed rabbits (37±1% versus 53±1%, P=.004) and between the controls and metoprolol-treated rabbits (51±1% versus 38±1%, P=.027). The benefit of metoprolol was independent of ETS exposure (ETS×metoprolol interaction, P=.595).

Conclusions Exposure to ETS significantly accelerated and metoprolol decreased the development of atherosclerosis in lipid-fed rabbits, but there was no interaction between the effects of ETS exposure and metoprolol. Metoprolol did not protect against the effects of ETS on atherosclerosis, suggesting that the β-adrenergic system is not the mechanism of ETS-induced atherosclerosis. (Circulation. 1994;89:2260-2265.)

Key Words • metoprolol • atherosclerosis • smoking

E nvironmental tobacco smoke (ETS), the tobacco combustion products inhaled by nonsmokers, has been linked to heart disease in nonsmokers.1-5 We previously showed that exposure to ETS increases the development of atherosclerosis in lipid-fed rabbits.6 The mechanisms by which cigarette smoke increases the risk of myocardial infarction, sudden death, and death from coronary artery disease are not yet clear, but endothelial damage and platelet activation are prominent proposed mechanisms involved in atherosclerosis and arterial thrombosis.7,8 β-Blockers have been widely used to decrease mortality in patients with cardiovascular disease.9,10 They have been reported to be especially efficacious in smokers.11 The β-blockers propranolol and metoprolol have also produced an antiatherosclerotic effect in rabbits on an atherogenic diet.12-14 We sought to investigate the hypothesis that the increase in atherosclerosis caused by ETS was mediated by an increase in catecholamines secondary to ETS exposure. The present study was designed to evaluate the effects of the β-blocker metoprolol on ETS-induced atherosclerosis in lipid-fed rabbits.

Methods

Study Design Thirty-two New Zealand White male rabbits (2.7 to 3.6 kg) were fed a 0.3% cholesterol diet including 3% soybean oil for 12 weeks. After 2 weeks the rabbits were randomized into four groups, each containing eight rabbits. The four groups were designated as ETS-metoprolol (ETS-M), ETS-control (ETS-C), and non-ETS with metoprolol (NETS-M) and without metoprolol (NETS-C). The rabbits were exposed to ETS (sidestream smoke) in two exposure chambers. A total of 96 cigarettes were smoked per day for 10 weeks by a smoking machine in each chamber as previously described. Four Marlboro filter cigarettes were used every 15 minutes for 6 hours per day, 5 days per week. Three small fans were used to circulate the smoke in each exposure chamber. Another 16 rabbits breathing clean air in another room were unexposed controls. The metoprolol rabbits received the drug subcutaneously by an implanted osmotic pump (ALZA) at a dose of 0.4 mg · kg⁻¹ · h⁻¹ over 10 weeks (three pumps, approximately 3 weeks each). The others had implantation of similar osmotic pumps filled with normal saline. One rabbit in the NETS-C group died at 4 weeks after implantation of the second osmotic pump and was dropped from the study. All rabbits were killed at the end of the study.

Measurements

All rabbits were observed daily for general appearance. Body weight and food intake were recorded every 3 weeks. We
measured average air carbon monoxide (CO), nicotine and total suspended particulates, plasma nicotine, cotinine, blood carbon monoxide hemoglobin (COHb), total serum cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), plasma metoprolol, epinephrine and norepinephrine levels, bleeding time, and platelet aggregation. After the rabbits were killed, the percentages of aorta and pulmonary artery covered by lipid lesions were determined.

Monitoring ETS Exposure
A model L15 CO Personal Exposure System (Langan Products, Inc) with a resolution of 0.5 ppm in CO (range, 0 to 128 ppm) was used to measure average air CO, and a Miniram PDM-3 Optical Scattering Particle Monitor (MIE, Inc; precision of measurement is ±10 μg/m³) was used for average total particulates. We also monitored air nicotine levels by a passive diffusion monitor in the middle of the exposure chambers. Air CO, nicotine, and total particulates were measured during the exposure time every other week. The concentrations of plasma nicotine and cotinine were determined by gas chromatography with nitrogen-phosphorus detection modified for simultaneous extraction of nicotine and cotinine.17 Radiometer OSM3 Hemoximeter (Radiometer) was applied for measurement of COHb in blood. The samples of plasma nicotine, cotinine (at week 8), and blood COHb (at week 10) were collected at the end of a day of ETS exposure.

Biochemical and Hematologic Analyses
Total serum cholesterol and triglycerides were measured by automated enzymatic methods with DART cholesteral reagent (AMSCO) with the DACOS XL analyzer (Coulter Electronics). HDL-C was determined after precipitation of other lipoprotein classes with dextran and magnesium ions (HDL-cholesterol precipitant, catalogue No. 236141, Ciba Corning Diagnostics Corp). As a measure of cumulative exposure of arterial walls to cholesterol, the area under the cholesterol-time curve was calculated as cholesterol-weeks.

Plasma epinephrine and norepinephrine levels were determined by high-performance liquid chromatography with electrochemical detection.16,18 These blood samples were withdrawn immediately after 1 day of ETS exposure at the end of week 8. Plasma metoprolol levels were measured by gas chromatography with electron capture detection.19

The bleeding time was determined by the time from the initial bleeding to cessation of bleeding after a small standard prick in the rabbit’s ear.20 A platelet-count–ratio method22 was modified and used for quantitative determination of circulating platelet aggregates. ADP (1 mmol/L) was added to a citrated venous blood sample before stirring. The sample was divided into two tubes, one containing EDTA/formalin and the other EDTA only. Platelet-rich plasma was collected after centrifugation. The platelet aggregate ratio was calculated from the platelet count in the two solutions by standard techniques (Operator Reference Manual, Cell-Dyn 900 hematology analyzer, Unipath Co). The higher the ratio, the fewer the platelet aggregates.

Morphological Studies
After intravenous pentobarbital 130 mg/kg, we removed the aorta from its origin (2 cm distal to the aorta valve) down to the bifurcation of the internal iliac arteries and the pulmonary artery from its beginning at the pulmonary valve to just above the bifurcation. The vessels were opened by a linear vertical incision, stained with Sudan 4, and photographed. The lipid lesions were measured quantitatively by planimetry of stained regions in photographs.

Statistical Analysis
Pollution levels associated with ETS in the metoprolol and saline groups were compared by one-way ANOVA. Two-way ANOVA was used for most comparisons in the study; the two factors are presence or absence of ETS and metoprolol. For analysis of the lipid deposits, we used a three-factor ANOVA, with site (aorta or pulmonary artery) as the third grouping factor. Measures done before and after ETS exposure were analyzed separately. Statistical analysis was done with a general linear model implementation of ANOVA using the Minitab (Minitab Statistical Software) GLM procedure, version 7.2. Results are reported as mean±SEM obtained from the least-squares means estimated with the general linear model used to compute the ANOVA. Significance was judged at P<.05.

Results
All rabbits exhibited a similar weight gain throughout the 10-week ETS exposure period (average, 3.06±0.04 kg before ETS exposure and 3.63±0.06 kg at the end of the study; P=.513 and P=.431 before and after ETS exposure among the groups). The ETS-exposed rabbits did eat slightly less than unexposed rabbits (108±5 versus 130±9 g/d; P=.04); metoprolol had no effect on food consumption (P=.83).

Monitoring ETS Exposure
There were no significant differences in average air CO, nicotine, and total particulates between ETS-M and ETS-C groups (Table 1). Rabbits in the ETS-M group showed a significant increase in blood COHb compared with those in the NETS-M group (4.0±0.2% versus 1.3±0.1%, P<.0001). There were significant elevations of plasma nicotine and cotinine in the ETS groups compared with the nonexposed rabbits, but

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ETS indicates environmental tobacco smoke; M, metoprolol; CO, carbon monoxide; and COHb, carbon monoxide hemoglobin.
there were no significant differences between the metoprolol and control groups.

**Biochemical and Hematologic Analyses**

Before ETS exposure, serum cholesterol levels were similar among the groups (P=.842). There were no significant differences in total serum cholesterol, triglyceride, HDL-C, and cholesterol-weeks among the groups at the end of the study (Table 2). Plasma metoprolol levels in the metoprolol-treated groups were 412.6±25.8 nmol/L, while drug concentrations in the control groups were undetectable (minimum detectable level, 10 nmol/L; Table 3). The average plasma epinephrine and norepinephrine levels were not significantly different among the groups (Table 3). ETS and metoprolol had no effects on plasma catecholamines in the study. ETS shortened the bleeding time (P<.05, Table 4).

**Morphological Studies**

The lipid lesions in the aorta and pulmonary artery were 57.2±7.6% and 33.1±6.4% (ETS-M), 62.8±8.4% and 58.4±6.1% (ETS-C), 38.7±9.4% and 24.8±7.7% (NETS-M), and 49.8±8.7% and 32.7±7.1% (NETS-C). The rabbits exposed to ETS had significantly higher levels of lipid deposits in their arteries (average lipid deposits of the aorta and pulmonary artery, 37±1% for controls versus 53±1% for ETS-exposed rabbits, P=.004), with higher levels in the aorta than the pulmonary artery (P=.009, Fig 1). Metoprolol also significantly reduced the amount of lipid deposits (51±1% for controls versus 38±1% for metoprolol-treated rabbits, P=.027), but this benefit was independent of whether the rabbits were exposed to ETS (P=.595 for ETS×metoprolol interaction). In addition, neither the increase in lipid deposits associated with ETS exposure nor the effects of metoprolol depended on whether one considered the aorta or pulmonary artery (P=.908 for ETS×site; P=.457 for metoprolol×site).

The negative conclusion about the ETS×metoprolol interaction was based on the fact that our analysis had a 55% power to detect the observed effect. Although this power falls below the desired value of 80%, it is still reasonably high. In addition, the fact that the P value for the interaction term does not approach statistical significance adds to our confidence in this negative conclusion. Nevertheless, the findings displayed in Fig 1 show a slight tendency for metoprolol to have a greater effect with ETS in the aorta but a lesser effect on the pulmonary artery. These tendencies may be due to chance, and the three-factor interaction is not significant, but the power of our experiment may be too low to detect this difference. Over both sites, any effect that might be present might cancel out so that the metoprolol and placebo trends appear nearly parallel. These tendencies require some caution in interpreting our conclusion that metoprolol has no specific effect in the presence of ETS.

There was a positive relation between cholesterol-weeks and lipid lesions in the aorta and pulmonary artery (r=.59, P<.001 and r=.55, P=.001, Fig 2). These results, combined with the lack of differences in levels of epinephrine and norepinephrine in the different experimental groups, suggest that the increased lipid deposits that occurred with ETS exposure are not mediated by an increase in circulating catecholamines.

**Discussion**

We previously showed that exposure to ETS significantly accelerates the development of atherosclerosis in the aorta and pulmonary artery in cholesterol-fed rabbits with a dose-response relation. The effects of ETS on cardiovascular disease may be mediated by multiple mechanisms. Among these, endothelial damage, lipid deposition, and clot formation are major processes in atherogenesis. ETS may increase heart rate, blood pressure, and coronary vasoconstriction, damage the lining of the coronary arteries, and heart muscle, change platelet function, and work via substances such as 3-methylcholanthrene, benzo(a)pyrene, and 7,12-dimethylbenz(a)anthracene. The antiatherogenic effects of the β-blockers may be related to their hemodynamic effects, reduced heart rate and blood pressure, the renin-angiotensin system, platelet function, and ACAT (acyl coenzyme A:...
TABLE 4. Bleeding Times and Platelet Aggregation

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<th>ETS</th>
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<tr>
<td></td>
<td>Metoprolol</td>
<td>Saline</td>
<td>Metoprolol</td>
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<tr>
<td>Bleeding time, s</td>
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<tr>
<td>Before ETS</td>
<td>67±3</td>
<td>83±8</td>
<td>82±10</td>
</tr>
<tr>
<td>After ETS</td>
<td>64±5</td>
<td>62±4</td>
<td>78±4</td>
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<tr>
<td>Platelet aggregate, %</td>
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<tr>
<td>Before ETS</td>
<td>92±4</td>
<td>84±4</td>
<td>93±4</td>
</tr>
<tr>
<td>After ETS</td>
<td>78±4</td>
<td>82±4</td>
<td>87±6</td>
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ETS indicates environmental tobacco smoke; M, metoprolol.

cholesterol acyltransferase; E.C.2.3.1.26),37 or direct cell membrane stabilization in cells of the arterial wall.13 Clinical evidence38 in hypertensive patients has suggested that β-blockers reduce mortality without a similar reduction in myocardial infarction. There may be a special protection of β-blockers in smokers with hypertension and after myocardial infarction.11,39,40 The antiatherogenic effects of sympathetic activation can be assumed to result from a complex interaction of hemodynamic factors and an array of biochemical processes.41 These effects include β-blockade in the central nervous system, in the heart, and in other systems. We tested the hypothesis that ETS induces atherosclerosis by stimulating the β-adrenergic system by giving metoprolol (0.4 mg·kg⁻¹·h⁻¹) to lipid-fed rabbits exposed to ETS for 10 weeks. Our results showed that exposure to ETS significantly increased the development of atherosclerosis. These results are consistent with our previous study4 and with epidemiological studies demonstrating that ETS increases the risk of death from coronary heart disease.2,4,3 Although metoprolol inhibited atherosclerosis in all groups, it did not significantly decrease the additional atherosclerosis caused by ETS in these rabbits. Thus, the β-adrenergic system does not seem to be the key in ETS-induced atherosclerosis.

Another study42 used metoprolol at a dose of 0.35 mg·kg⁻¹·h⁻¹ for 14 weeks. The rabbits were fed a 0.25% cholesterol diet for 21 weeks. Results showed that the treated group had significantly less atherosclerosis in the aorta than the controls (P < .015). Metoprolol had no effects on plasma cholesterol, triglycerides, or apolipoprotein. Similarly, there were no significant differences in serum lipids in our study. An antiatherogenic effect of β-blockade on stress- and diet-induced atherosclerosis in monkeys has been reported.43 Metoprolol was also found to reduce atherosclerotic lesions in the aorta of rabbits with a reduced response to an atherogenic diet.14

Treatment with β-blockers affects fasting serum lipids. These changes (increased triglycerides and reduced HDL-C) might be harmful.44,45 Nevertheless, data suggest that metoprolol might prevent clinical complications of atherosclerosis. A clinical study showed a significant reduction of cardiac death in patients with a large infarct and a significant decrease in sudden death rates and the incidence of nonfatal reinfarction (32.1%, 14.7%, and 21.1% in the placebo versus 12.5%, 5.8%, and 11.7% in the metoprolol group, respectively, P < .05).10 The International Prospective Primary Prevention Study in Hypertension compared a β-blocker treatment in smokers and nonsmokers.46 The results showed that smokers required higher doses to achieve the diastolic target pressure and had a higher heart rate and hematocrit and a higher cardiac event rate than nonsmokers. It seems that higher drug dose and longer study period may be needed to observe the effects of
metoprolol on experimental atherosclerosis in ETS-exposed rabbits.

Although there were no significant differences in plasma catecholamines in the study, it is clear that ETS can stimulate sympathetic activation. Short periods of sympathetic activation may cause disturbances of endothelial integrity and platelet function, both of which are key atherogenic processes. Repeated sympathetic activation may therefore contribute to acceleration of atherosclerosis.

We conclude that the $\beta_1$-adrenergic receptor antagonist metoprolol significantly reduced the development of atherosclerosis in lipid-fed rabbits. However, no additional protective effect on atherosclerosis induced by ETS was demonstrated. Thus, the $\beta_1$-adrenergic receptor system is probably not the mechanism by which ETS induces atherosclerosis in rabbits.

Acknowledgments

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References


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