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Cardiovascular Effects of Carbon Monoxide and Cigarette Smoking

Shoshana Zevin, MD,* Sandra Saunders, MD,† Steven G. Gourlay, MBBS, PHD,†‡
Peyton Jacob III, PHD,† Neal L. Benowitz, MD†
Jerusalem, Israel and San Francisco, California

OBJECTIVES This study was designed to compare the effects of inhaled carbon monoxide (CO), administered to achieve concentrations similar to those found in cigarette smoking, with the effects of cigarette smoking and air inhalation on heart rate and blood pressure, catecholamine release, platelet activation and C-reactive protein (CRP), a marker of inflammation.

BACKGROUND Carbon monoxide may contribute to smoking-induced cardiovascular disease. Exposure to environmental CO has been associated with increased cardiovascular morbidity and mortality. Animal and in vitro studies suggest that CO may contribute to atherosclerosis and endothelial injury. There is conflicting evidence about the hemodynamic consequences of exposure to CO and its role in platelet activation.

METHODS In a single-blind, crossover design, 12 healthy smokers inhaled CO at 1,200 ppm to 1,500 ppm to simulate CO intake from cigarette smoking, inhaled air on a similar schedule and smoked 20 cigarettes per day, each for seven days. Mean carboxyhemoglobin was 5 ± 1% on CO treatment, 6 ± 1% while smoking and 0.4 ± 0.2% on air inhalations.

RESULTS There was no difference in blood pressure between the treatments. Mean heart rate was higher during cigarette smoking compared with CO and air inhalations (75 beats/min vs. 66 beats/min; $p < 0.05$). Plasma levels of platelet factor 4 and CRP and urine epinephrine and norepinephrine were higher while smoking, with no effect of CO compared with air.

CONCLUSIONS Carbon monoxide administered under conditions similar to those of cigarette smoking had no significant effect on blood pressure, heart rate, plasma catecholamines, platelet aggregation or CRP. The short-term chronotropic effect, adrenergic-activating, platelet-activating and CRP-increasing effects of smoking in healthy smokers are probably due to components of cigarette smoke other than CO. (J Am Coll Cardiol 2001;38:1633–8) © 2001 by the American College of Cardiology

Cigarette smoking is a well-known risk factor for cardiovascular disease, including ischemic heart disease. Cigarette smoke consists of many chemicals, including nicotine, tar with its many carcinogens, and gaseous compounds including carbon monoxide (CO). Nicotine has well known acute and chronic cardiovascular effects, mainly through sympathetic activation, although the contribution of nicotine per se in cardiovascular morbidity and mortality induced by cigarette smoking is uncertain (1).

Carbon monoxide is suspected to play a major role in cigarette smoke-induced cardiovascular diseases. There is epidemiologic evidence that workers exposed to high CO concentrations have more cardiovascular morbidity and mortality than the expected rate in the population (2–4), and a positive correlation between ambient air CO concentrations and cardiovascular hospital admissions has been reported (5,6).

The main mechanism by which CO causes heart disease is production of hypoxia. The effects of CO are more profound in the myocardium than in peripheral tissues because of very high oxygen extraction by the myocardium at rest (7,8). Carbon monoxide may also have direct myocardial effects. In isolated rat hearts, CO caused a greater decrease in heart rate and pulse pressure compared to the same degree of anoxia produced by the inhalation of nitrogen (9).

Carbon monoxide exposure has been implicated in the process of atherosclerosis. Studies in rabbits and monkeys have reported that exposure to CO produced an accumulation of cholesterol in the aorta and coronary arteries and endothelial damage (10–13). Similar effects have been demonstrated in vitro. Arteries perfused with CO had a higher uptake of cholesterol compared with controls (14). Vascular wall injury has been linked to both abnormal lipid infiltration and increased capillary permeability (15). In humans, CO has been well known to have detrimental effects in the presence of ischemic heart or peripheral vascular disease. Individuals with ischemic heart disease have a decrease in exercise capacity and a lower threshold for ischemia after exposure to low levels of CO that are

From the *Department of Internal Medicine, Shaare Zedek Medical Center, Jerusalem, Israel; †Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, San Francisco General Hospital Medical Center, and the Departments of Medicine, Psychiatry and Biopharmaceutical Sciences, University of California, San Francisco, San Francisco, California; and ‡Pharmacological Sciences, Genentech Inc., South San Francisco, California. Supported by U.S. Public Health Service grants DA02277 and DA12393 from the National Institute on Drug Abuse, National Institutes of Health, and a gift from Sano Corporation. Carried out in part at the General Clinical Research Center at San Francisco General Hospital Medical Center with support of the Division of Research Resources, National Institutes of Health (RR-00083). Dr. Saunders was supported by National Research Service Award T32 GM07546 from the National Institutes of Health. Dr. Gourlay was supported by a National Heart Foundation of Australia Overseas Research Fellowship.

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Abbreviations and Acronyms

AUC = area under the concentration or response-time curve
BTG = beta thromboglobulin
CO = carbon monoxide
COHb = carboxyhemoglobin
CRP = C-reactive protein
ELISA = enzyme-linked immunosorbent assay
NPD = nitrogen phosphorus detection
PF4 = platelet factor 4

comparable to those observed in light to moderate smokers (16,17).

The hemodynamic and hormonal effects of CO are less clear (18). Research involving various animals—dogs, goats, rabbits and rats—and different doses of CO administration has been reported. Some of the studies were confounded by the effects of anesthesia on the animals. In some cases, CO increased heart rate, whereas in others CO produced no change or bradycardia (8,19–23). In humans, the results have also been variable (24–26). Some studies have found that CO decreases blood pressure; others found no change (20,22,27,28).

Cigarette smoking is known to potentiate platelet aggregation and thrombosis (29,30). Several studies indicate that nicotine does not cause platelet aggregation (30–32). The role of CO in platelet aggregation is not completely clear, but some studies have indicated increased platelet activation and aggregation (33,34).

Inflammation appears to play a role in the pathophysiology of coronary heart disease (35). C-reactive protein (CRP) is an acute-phase reactant that reflects inflammatory activity in the cardiovascular system and is a predictor of the risk of future cardiovascular events (36,37). Cigarette smoking is associated with increased levels of CRP (36,38). Carbon monoxide exposure might also influence an inflammatory response to smoking (39).

Thus, although there is convincing evidence from human studies that CO can exacerbate ischemic heart disease, the acute hemodynamic and hormonal effects of CO are less clear. An understanding of how CO affects the cardiovascular system is important for an understanding of effects of cigarette smoking as well as possible effects of air pollution. The aim of our study was to compare the effects of sustained exposure to CO (seven days), in concentrations similar to those of cigarette smokers, with the effects of cigarette smoking and air inhalation on heart rate, blood pressure, catecholamine release, platelet activation and CRP, a marker of inflammation.

METHODS

Subjects. The subjects were 12 healthy male smokers aged 27 to 47 years. The average usual number of cigarettes smoked per day was 28 (range 18 to 40). The mean baseline plasma cotinine level was 316 ng/ml (range 184 ng/ml to 603 ng/ml). The average Fagerström dependence questionnaire score was 7.6 (range 6 to 10), consistent with a high level of dependence.

Written informed consent was obtained from all subjects and the study protocol was approved by the Committee on Human Research at the University of California, San Francisco.

Experimental protocol. The study was conducted in the General Clinical Research Center at San Francisco General Hospital Medical Center over 21 days. The study was of a crossover design with three treatments: inhalation of CO, cigarette smoking and inhalation of air. Each treatment was administered for seven days without additional washout days. Because of the relatively short half-lives of nicotine (averaging 2 h) and CO (averaging 3 h to 6 h), the first three days of each treatment were judged to be an adequate time interval to allow washout of previous treatment effects. The smoking treatment could not be blinded, but the inhalation treatments were single-blinded for the subjects. In inhalation treatments, subjects inhaled CO or air from 1-l bags, once every minute for 10 min, to simulate smoke inhalation from cigarette smoking. The inhalations were repeated 20 times a day, once every 45 min from 8 AM to midnight. The concentration of CO (in air) was 1,200 ppm for the first three subjects, and 1,500 ppm for the others.

In the smoking treatment, the subjects smoked 20 cigarettes per day, one every 45 min. Expired CO levels were monitored daily at 8 AM and 12 PM, and blood carboxyhemoglobin (COHb) concentration measured on day 5 every 4 h between 8 AM and midnight. On day 3 of each treatment, blood pressure and heart rate were recorded for 24 h using an ambulatory blood pressure monitor (Accutracker, SunTech Industries, Raleigh, North Carolina). On day 4 of each treatment, blood was collected at 8 AM and 12 PM for determination of white blood cell count, CRP, plasma platelet factor 4 (PF4), plasma beta thromboglobulin (BTG) and plasma catecholamines. Urine was collected for 24 h for measurement of catecholamines. On days 5 and 6 of each treatment, a 30-min intravenous infusion of 3′, 3′-dideuteronicotine and 2,4,5,6-tetradeuterocotinine and oral doses of caffeine and clorzoxazone were administered to determine the effect of CO on nicotine and drug metabolism. The results of the metabolism study are reported elsewhere (40).

Biochemical analysis. Carboxyhemoglobin was measured using a Ciba-Corning-2500 co-oximeter. Plasma concentrations of nicotine and cotinine were measured by gas chromatography with nitrogen phosphorus detection (NPD) using 5-methyl-nicotine and 1-methyl-5-(3-pyridyl)-pyrrolidin-2-one (ortho-cotinine) as internal standards. The method is essentially identical with a published gas chromatography-mass spectrometry method (41), with the exception that NPD is used instead of mass spectrometry for detection and that different internal standards are used. Urinary catecholamines were analyzed by high-performance liquid chromatography using an electrochem-

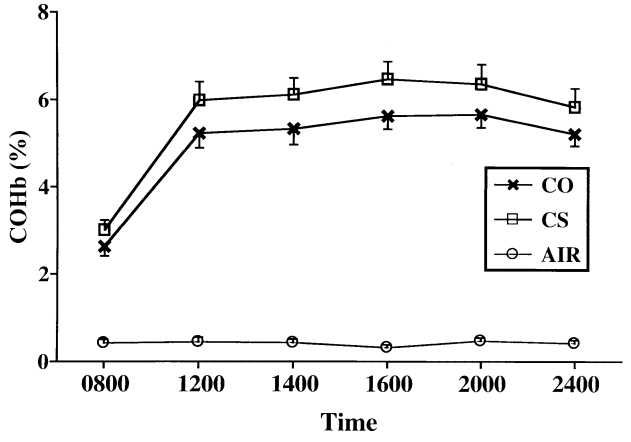

Figure 1. Mean (± SEM) carboxyhemoglobin (COHb) time course on day 5 of carbon monoxide inhalations (CO), cigarette smoking (CS) and air inhalations (AIR). Smoking and inhalation treatments started at 8 AM and were administered every 40 min till midnight.

ical detector (42). Plasma catecholamines were assayed by a modification of the method of Anton and Sayre, as described previously (43). Plasma CRP was measured in platelet-poor plasma with enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Hemagen Diagnostics, Inc., Waltham, Massachusetts). Platelet factor 4 and BTG were assayed in platelet-poor plasma with ELISA using commercial kits (Asserachrom PF4 and Asserachrom BTG from Diagnostica Stago, Asnières-sur-Seine, France).

Data analysis. Areas under the response-time curves (AUC) for blood pressure and heart rate, and areas under concentration-time curves for nicotine were computed using the trapezoidal method. The mean values for 24 h were calculated as $AUC_{7AM–7AM}$/24 h. For blood pressure and heart rate, mean daytime (7 AM to 10 PM inclusive) and nighttime (11 PM to 6 AM inclusive) values were calculated as: $AUC_{7AM–10PM}$/15 h and $AUC_{11PM–7AM}$/9 h, respectively. Treatment effects were compared by repeated-measures analysis of variance. Data were log-transformed when variance across treatments was not homogeneous. Post-hoc comparisons were made using the Tukey test. The power of the study to detect a difference of about 25%, which is typically taken as clinically meaningful, varied from 65% to 95% depending on the measure.

RESULTS

Six subjects underwent the sequence CO/smoking/air and six subjects the reverse. The levels of COHb throughout the day were similar in the CO inhalation and cigarette smoking conditions. Mean (± SD) COHb levels were 5 ± 1% on CO inhalation treatment, 6 ± 1% on smoking treatment and 0.4 ± 0.2% on air inhalation treatment (Fig. 1). Plasma nicotine concentrations averaged 5 ng/ml to 24 ng/ml throughout the day during cigarette smoking, and <1 ng/ml in the no-smoking conditions. Plasma cotinine concentration averaged 270 ng/ml in the smoking condition.

Table 1. The Effect of CO, CS and Air Inhalation on HR and Blood Pressure

	CO	CS	Air
HR (beats/min) 24-h mean	66 ± 6	75 ± 7*	67 ± 6
Day	69 ± 6	79 ± 7*	70 ± 6
Night	61 ± 7	67 ± 7*	61 ± 6
SBP (mm Hg) 24-h mean	120 ± 8	124 ± 9	120 ± 9
Day	124 ± 19	129 ± 9	123 ± 11
Night	113 ± 6	115 ± 13	112 ± 9
DBP (mmHg) 24-h mean	66 ± 6	68 ± 6	67 ± 7
Day	69 ± 7	72 ± 7	69 ± 7
Night	62 ± 5	63 ± 6	62 ± 7

Mean ± SD. *$p < 0.05$ compared to carbon monoxide (CO) or air.
CS = cigarette smoking; DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure.

There was no effect of CO inhalations on mean heart rate. Cigarette smoking caused a significant increase in heart rate compared to other treatments including overnight (Table 1). On all treatments, daytime heart rate was significantly higher than nighttime (Table 1). There were no differences in either systolic or diastolic blood pressure across the treatments. For each treatment, daytime values for blood pressure were significantly higher than nighttime values (Table 1).

Plasma epinephrine (mean ± SD) levels were higher during cigarette smoking compared to the other treatments (25 ± 12 pg/ml; 19 ± 10 pg/ml and 19 ± 8 pg/ml on cigarette smoking, CO and air inhalations, respectively), but the difference was not statistically significant. Plasma norepinephrine levels (mean ± SD) also trended higher on the smoking treatment (205 ± 92 pg/ml; 166 ± 61 pg/ml and 182 ± 120 pg/ml, on cigarette smoking, CO and air inhalations, respectively). Urine 24 h excretion of norepinephrine and epinephrine were significantly higher on cigarette smoking, and there was no effect of CO (Fig. 2). Plasma CRP and PF4 were significantly higher on cigarette smoking ($p < 0.05$), and there was no significant difference between CO and air treatments. The CRP data are shown in Figure 3. Platelet factor 4 values averaged 3.6 ± 1.6

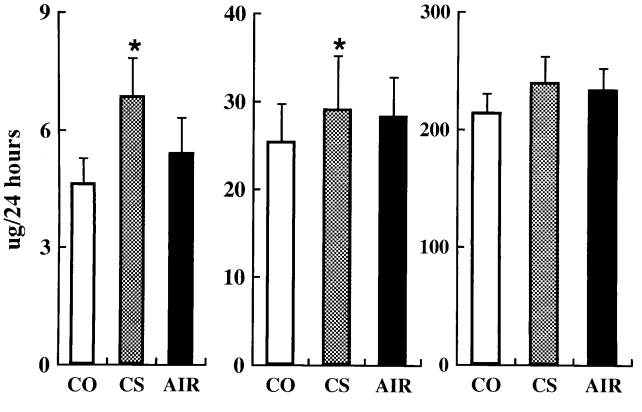

Figure 2. Mean (± SEM) urinary levels of epinephrine, norepinephrine, and dopamine on carbon monoxide inhalations (CO), cigarette smoking (CS) and air inhalations (AIR), n = 10. *$p < 0.05$. Urine was collected for 24 h. The levels while smoking cigarettes were significantly ($p < 0.05$) higher compared to other treatments.

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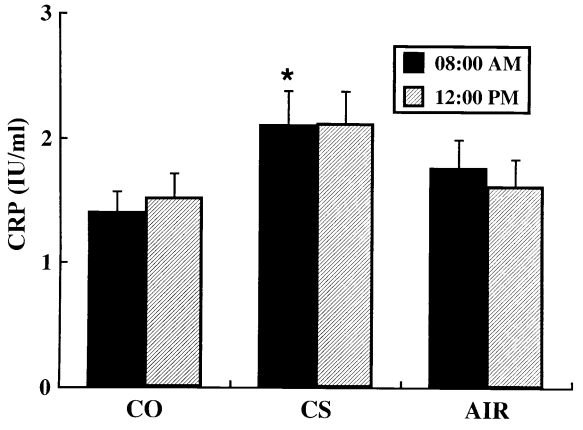

Figure 3. Mean (± SEM) plasma levels of C-reactive protein (CRP) on carbon monoxide inhalations (CO), cigarette smoking (CS) and air inhalations (AIR). Analysis of variance of log CRP revealed a significant effect of cigarette smoking (*$p < 0.005$). Differences were significant both at 8 AM and 12 PM.

IU/ml, 5.0 ± 2.6 IU/ml and 3.9 ± 1.2 IU/ml in the CO, smoking and air conditions, respectively. Beta thromboglobulin also tended to be higher on smoking treatment, without any effect of CO inhalation.

DISCUSSION

We have developed an experimental CO intervention to simulate the exposure of CO from cigarette smoking. Average CO concentrations were similar during CO inhalation and cigarette smoking in our subjects compared with breathing air alone. Carbon monoxide delivered in this way had no effect on heart rate, catecholamine release, platelet activation or CRP. Cigarette smoking had the expected effects on these parameters.

Heart rate and blood pressure. Heart rate was significantly higher with cigarette smoking compared to the other treatments. Cardiac acceleration while smoking is well known, and is mediated by nicotine via sympathetic neural activation (44–46). In previous studies in humans, the reported effects are variable: most reports concerned CO poisoning and tachycardia was present (26,47), whereas in moderate exposures with COHb up to 15%, no change in heart rate was seen (24,28,48). Our study supports the latter results.

Systolic and diastolic blood pressure did not differ across the treatments. Acute exposure to CO in humans produces a decrease in systemic vascular resistance (49). However, prolonged exposure for eight days with COHb levels of 12% did not result in any changes in blood pressure (50), and acute exposures resulting in COHb levels of up to 20% did not cause any change in blood pressure, at rest or during exercise (25,28). Hausberg and Somers found that acute exposure to CO inhalation did not have any effect on muscle sympathetic nerve activity, forearm blood flow or blood pressure or heart rate (48). Our results are in agreement with those demonstrating no change at levels of COHb observed in smokers.

Catecholamine release. We found significantly increased levels of urinary epinephrine and norepinephrine levels during cigarette smoking, but catecholamine excretion was not affected by CO compared with air. Pankow and Ponsold found a transient increase in urinary catecholamines in rats after exposure to CO with COHb levels of 50%; however, after continuous CO exposure this effect disappeared (51). Our data indicate that CO does not contribute to the elevated catecholamine levels found in cigarette smokers.

Platelet activation. Cigarette smoking is associated with increased coagulation; both the platelet function and the coagulation factors are affected (29,52–54). Cigarette smoking, but not nicotine, has been shown to increase plasma concentrations of PF4 and BTG (29,30). One possible candidate for inducing platelet activation is CO. In dogs with stenosed coronary arteries, non-tobacco burning cigarettes caused the same rise in platelet activation and thrombus formation as tobacco-burning cigarettes (33). In human in vitro studies, increasing COHb levels, but not nicotine, were associated with increase in platelet aggregation (34).

We used the concentrations of the constituents of platelet alpha-granules, PF4 and BTG, as markers for in vivo platelet activation. We found increased levels of PF4 with cigarette smoking but not with CO inhalations, compared with air. Beta thromboglobulin was also higher with smoking, but the differences did not reach statistical significance. Thus, our data indicate that CO in the concentrations found in cigarette smoke does not cause platelet activation.

Cigarette smoking is associated with an inflammatory state, indicated by higher levels of CRP compared to nonsmokers (36,38). The level of CRP is reported to increase in relation to the number of pack-years of cigarette smoking (38). The latter study suggested that CRP remains elevated after smoking cessation, and is no different in current smokers compared with former smokers. Our study provides novel data indicating that within a few days of not smoking, CRP levels decline significantly, indicating that cigarette smoking has an acute effect to stimulate inflammation.

We are unaware of data on the effects of inhalation of CO on CRP or other inflammatory markers. However, CO is released in inflammatory conditions, and the expression of heme oxygenase, the enzyme that generates endogenous CO, is associated with the suppression of inflammation (39). Thus, one might expect that CO would have an inhibitory effect on the inflammatory response. In the present study, CO inhalation was associated with a CRP level that was no different than that in the air inhalation condition.

Conclusions. The results of our study indicate that inhaled CO, delivered to healthy smokers in concentrations similar to those in cigarette smoke, has little or no effect on heart rate, blood pressure, catecholamine secretion or platelet activation, at least with exposure for several days, compared to inhalation of air. The effects of smoking on these

parameters in healthy smokers are most likely due to other constituents of cigarette smoke.

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Reprint requests and correspondence: Dr. Neal L. Benowitz, Chief, Division of Clinical Pharmacology and Experimental Therapeutics, University of California, San Francisco, Box 1220, San Francisco, California 94143-1220. E-mail: nbeno@itsa.ucsf.edu.

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