Chronic Carbon Monoxide Exposure Is Associated with the Increases in Carotid Intima-Media Thickness and C-Reactive Protein Level

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Being the most common cause of death from poisoning worldwide, cardiovascular manifestations of acute carbon monoxide (CO) poisoning have been subject of various studies but current evidence about effects of chronic CO exposure on atherosclerosis is limited which is very common. We aimed to investigate association of chronic CO exposure with atherosclerosis by measuring carotid intima-media thickness (CIMT) and high-sensitivity C-reactive protein (hs-CRP). Forty healthy male non-smoker indoor barbecue workers (mean age; 33.0 ± 9.0 years) working in different restaurants for at least three years and 48 age-matched healthy men (mean age; 34.3 ± 6.6 years) enrolled in the study. Clinical characteristics of indoor barbecue workers and control group were comparable in terms of body mass index, blood pressure, and lipid profile. However, carboxyhemoglobin (COHb) (6.4 ± 1.5% vs. 2.0 ± 1.1%), hs-CRP (2.7 ± 2.0 mg/L vs. 1.1 ± 0.8 mg/L) and CIMT (1.1 ± 0.3 mm vs. 0.9 ± 0.1 mm) were higher in indoor barbecue workers (p < 0.001 for each). In Pearson correlation analysis, CIMT was correlated with COHb concentration (r = 0.635, p < 0.001) and hs-CRP level (r = 0.466, p < 0.001). Among indoor barbecue workers, the years worked (years exposed to CO) are correlated with COHb, hs-CRP and CIMT. In multivariate analysis, COHb concentration is the only independent predictor of CIMT (β = 0.571, p < 0.001). The increased CIMT and hs-CRP in indoor barbecue workers suggest that chronic CO exposure may increase the risk of atherosclerotic cardiovascular events. Carbon monoxide; carotid intima-media thickness; hs-CRP; atherosclerosis; indoor barbecue.


Carbon monoxide (CO) is a colorless, odorless, tasteless and non-irritant gas. It is present in the atmosphere both naturally and artificially as a result of oxidation or combustion of carbonaceous substances. CO exposure at high concentrations might be lethal and is the most common cause of death from poisoning worldwide (Gorman et al. 2003). Sources of poisoning include but not limited to accidental exposure of high doses of CO through indoor burning of charcoal for barbecues or heating, automobile exhaust, industrial solvents and petrol powered generators. Cardiovascular manifestations of acute CO poisoning include myocardial dysfunction, ischemia and infarction, arrhythmias and cardiac arrest which have been the subject of various studies (Ernst and Zibrak 1998; Satran et al. 2005; Yildirim et al. 2005; Aslan et al. 2006; Davutoglu et al. 2006; Kalay et al. 2007). However, our knowledge about the effects of chronic CO exposure on cardiovascular system and atherosclerosis is limited and results of both animal and human studies are conflicting (Astrup et al. 1967; Astrup et al. 1970; Davies et al. 1976; Schneiderman and Goldstick 1978; Weir and Fabiano 1982; Kleinman et al. 1989; Penn et al. 1992; Smith and Steichen 1993; Siow et al. 1999; Sorhaug et al. 2006; Sari et al. 2008).

Carotid intima-media thickness (CIMT) measurement is a non-invasive assessment of atherosclerosis. It is known to be not only a valid marker of early atherosclerosis but also reflects its extent and progression (Mack et al. 2000; Bots and Grobbee 2002; Van Bortel 2005; Devine et al. 2006). Increased CIMT has been shown to predict future cardiovascular events and is considered as a surrogate marker of generalized atherosclerosis (Bots et al. 1997; Hodis et al. 1998; O’Leary et al. 1999; Mack et al. 2000; Bots and Grobbee 2002; Van Bortel 2005, Devine et al. 2006). C-reactive protein (CRP), an acute-phase reactant
has been shown to be an independent predictor of atherosclerosis and cardiovascular events (Ridker et al. 2002; Danesh et al. 2004; Libby and Ridken 2004). It is also considered to be related to extent and progression of coronary and extra-coronary atherosclerosis (Memon et al. 2006; Elias-Smale et al. 2007).

Chronic exposure to moderate doses of CO without acute toxicity is not rare. It is common especially in some professions such as in indoor barbecue workers, toll operators, firemen and miners who are working in environments deprived of adequate ventilation. In this study we therefore aimed to investigate whether CIMT and CRP levels were increased in indoor barbecue workers who are chronically exposed to CO.

Methods

Study population and protocol

40 healthy male indoor barbecue workers (mean age; 33.0 ± 9.0 years) who were working in various restaurants of the city of Gaziantep for at least three years were enrolled into the study and 48 age matched healthy men (mean age; 34.3 ± 6.6 years) were assigned as the control group. Control group was formed by the administrative staff of our hospital. They were carefully evaluated in terms of below mentioned sources of uncontrolled exposure of CO. The study was conducted in June 2007. Study protocol conforms to the principles of Helsinki Declaration and Gaziantep University Ethics Committee approved it. All participants have given written informed consent.

All enrolled subjects were free of acute or chronic medical disorders and were of normal body habitus. All subjects underwent a detailed medical history and physical examination by the investigator physicians. Electrocardiograms of all participants were obtained. Transthoracic echocardiographic examinations of all participants were performed. Subjects with the possibility of coronary artery disease after medical history, physical examination, electrocardiographic and echocardiographic examinations were excluded from the study (such as anginal symptoms, ischemic findings in the electrocardiogram, low ejection fraction and/or segmental wall motion abnormalities in the echocardiography etc.). Exclusion criteria were smoking any amount, history of any known cardiovascular disease including coronary artery disease, hypertension, valvular heart disease, myocardial or pericardial disease, arrhythmia, metabolic syndrome and diabetes mellitus. Patients with concomitant inflammatory diseases such as infections and autoimmune disorders, neoplastic diseases, major depression, liver and kidney diseases and recent major surgical procedure, those with dyslipidemia and/or history of cholesterol-lowering therapy such as statins were also excluded from the study. Toll operators, firemen, miners, taxi, ambulance and truck drivers, repairmen and those working in car services, painters, those working in petrol stations, air conditioning and heating departments and persons using portable heaters, wood or coal for heating and those diving for occupational or recreational purposes were not included into the study in order to exclude uncontrolled environmental exposure of CO.

CIMT measurement of the participants was performed. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), carboxyhemoglobin (COHb) and high-sensitivity CRP (hs-CRP) levels were measured.

CIMT measurements

CIMT measurement was performed as previously described (Lueckemann et al. 2002). Measurements were performed by the same physician who had no information about the participants’ clinical data, using a commercially available device (Vivid 7, GE Vingmed Ultrasound AS, Horten, Norway) with a 12-MHz linear array transducer. Measurements were performed in a quiet, semi-darkened room with the subject lying in the supine position. Intima-media thickness was measured at distal straight portion (1 cm in length) of the common carotid artery at sites. The CIMT was defined as the distance between the leading edge of the lumen–intima and the leading edge of the media-adventitia interfaces. The average value of the 3 points was calculated for each side, and the largest value was used for the analysis. The intra-observation coefficient of variation for repeated measures of CIMT was 2%.

Definitions and laboratory measurements

Body mass index was calculated by dividing weight in kilograms by height in meters squared (kg/m²). Blood pressure of the participants was measured 3 times, 5 minutes apart after 10 minute rest and average of 3 measurements was recorded. All blood pressure measurements were performed in the participants’ work place. Venous blood samples of the participants were collected from the antecubital vein resting in the supine position after an overnight fast. TC, HDL-C and TG levels were determined by enzymatic-colorimetric methods (Roche-Hitachi, Mannheim, Germany) on a Beckman DU-7 spectrophotometer (Beckman, Fullerton, CA, USA). LDL-C was calculated by the Friedewald’s formula. Hs-CRP was measured by using a commercially available assay kit with CardioPhase hs-CRP (Dade Behring Inc. Newark, DE, USA). COHb was measured from arterial blood by using Cobas b 221 Blood Gas System (Roche Diagnostics Inc, Indianapolis, IN, USA). COHb measurement of the indoor barbecue workers was performed in their workplace. COHb measurement of the control group was performed in the hospital. All COHb measurements were done between 11 and 12 AM. All laboratory analyses were done in duplicate and average of the two values were recorded.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation and categorical data were expressed as percentages. The Student t test was used for comparing continuous variables and chi-square test was used to analyse categorical data. The correlation between CIMT and demographic and laboratory parameters was assessed by the Pearson correlation test. Multiple linear regression analysis was performed to identify the independent predictor(s) of CIMT by including the parameters, which were correlated with CIMT in bivariate analysis. A two-tailed p < 0.05 was considered statistically significant. All statistical studies were carried out with SPSS program (version 11.5, SPSS Inc., Chicago, IL, USA).

Results

Average working time of the indoor barbecue workers in their jobs was 15.6 ± 7.1 years. They were working approximately 8 hours a day and 6 days a week. Clinical characteristics of indoor barbecue workers and the control group was comparable in terms of age, sex, body mass index, blood pressure, TC, HDL-C, LDL-C and TG levels.
However, COHb concentration (6.4 ± 1.5% vs. 2.0 ± 1.1%), hs-CRP level (2.7 ± 2.0 mg/L vs. 1.1 ± 0.8 mg/L) and CIMT (1.1 ± 0.3 mm vs. 0.9 ± 0.1 mm) were higher in the indoor barbecue workers than the control group ($p < 0.001$ for all) (Table 1).

Relationship of CIMT with demographic and laboratory parameters is presented in Table 2. CIMT was positively correlated with COHb concentration ($r = 0.635$, $p < 0.001$) and hs-CRP level ($r = 0.466$, $p < 0.001$) (Table 2) (Fig. 1). Among indoor barbecue workers, the years worked (years exposed to CO) was related with COHb ($r = 0.575$, $p < 0.001$), hs-CRP ($r = 0.687$, $p < 0.001$) and CIMT ($r = 0.335$, $p = 0.035$). In multivariate analysis COHb concentration was the only independent predictor of CIMT ($\beta = 0.571$, $p < 0.001$).

### Discussion

The present study demonstrates that CIMT and hs-CRP levels are increased in subjects with chronic CO exposure. This investigation is the first to assess and find an association between chronic CO exposure and CIMT and also is the second in the literature to assess the association between chronic CO exposure and hs-CRP levels and the first demonstrating an association (Zevin et al. 2001).

### Previous data about association of CO with atherosclerosis

Being the most common cause of death from poisoning worldwide, cardiovascular manifestations of acute CO poisoning have been the subject of various studies but current...
Evidence about the effects of chronic CO exposure on the cardiovascular system and atherosclerosis is limited. In our recent study (Sari et al. 2008), we demonstrated that chronic carbon monoxide exposure prolonged electrocardiographic P-wave and QT dispersion in the same population. However, studies investigating the association of chronic CO exposure with atherosclerosis revealed inconsistent results. Astrup et al. (1967) reported that after several months of CO exposure at COHb levels of 20-33%, cholesterol and lipid deposits were formed in the aortic wall of rabbits. Davies et al. (1976) investigated the effect of intermittent carbon monoxide exposure on experimental atherosclerosis in 12 rabbits for 10 weeks. They reported that extent of coronary artery atherosclerosis was significantly higher in the CO exposed group than in the control group. Weir and Fabiano (1982) reported that they have found no convincing evidence to support the conclusion that chronic CO exposure increases the risk of developing clinically significant atherosclerotic disease. Penn et al. (1992) reported that in an animal model of cockerels, daily exposures to moderately high CO levels were without discernable effect upon arteriosclerotic plaque development, although high COHb levels were attained. Sørhaug et al. (2006) exposed 51 female Wistar rats to CO at doses comparable to tobacco smoking for 72 weeks and reported that no signs of atherosclerosis have been observed in the aortas and femoral arteries of CO exposed rats.

**Present findings supporting association of CO exposure with atherosclerosis and mechanism**

CIMT is accepted to be a surrogate marker of atherosclerosis, its extent, progression and has been shown to predict future cardiovascular events. Therefore our study is the first and is important by demonstrating that CIMT is increased in subjects exposed to moderate doses of CO on average of 15.6 ± 7.1 years. Moreover, CIMT showed significant correlation with COHb and hs-CRP (Table 2). CIMT increases with age but there was no correlation between CIMT and age in the present study. This may be because we excluded subjects with the diagnosis or possibility of coronary artery disease from the study and cholesterol levels of the study population were not only within normal limits but also similar with the control group. Because daily exposure was more or less same in the indoor barbecue workers, we were not able to calculate possible relation between aforementioned parameters. However, years worked (years exposed to CO) was related with COHb, hs-CRP and CIMT among indoor barbecue workers.

CRP, is also known to be related to extent and progression of coronary and extra-coronary atherosclerosis. So far there is only one study investigating the effect of CO on CRP level in 12 healthy smokers, with the result that CO administered under conditions similar to those of cigarette smoking had no significant effect on CRP (Zevin et al. 2001). The present study is the first to demonstrate that hs-CRP is increased in subjects with chronic CO exposure.
In addition, hs-CRP showed significant correlation with CIMT. Proportion of people living in the crowded cities (where CO emission is high) increases because of industrialization, education, workforce shift etc. Additionally, vast majority of people work in closed places rather than open areas. As chronic exposure to CO without acute toxicity is common in the environment and in some professions, unveiling the possible interaction with atherosclerosis is very important. Finally, epidemiological studies have shown that high levels of environmental CO correlate with higher incidence of cardiovascular diseases and mortality (Stern et al. 1988; Kleinman et al. 1989).

The mechanism by which chronic CO exposure causes increase in CIMT and hs-CRP level is unclear. CO is known to have direct toxic effects on the myocardium. The affinity of hemoglobin for CO is 200 to 250 times greater than its affinity for oxygen causing competitive inhibition of oxygen release due to shift in the oxygen-hemoglobin dissociation curve, reduced oxygen delivery, and tissue hypoxia. Apart from direct toxic effects of CO to the myocardium, it might stimulate chronic inflammatory process known as key element of atherosclerosis which was shown by increase in hs-CRP level in the present study. However, the correlation between CIMP and COHb was stronger than the one between CIMT and hs-CRP and the fact that COHb but not hs-CRP was independent predictor of CIMT suggests that some other pathophysiological events also contribute to increased CIMT in the chronically CO exposed group which needs to be evaluated with future studies.

Study limitations

Several limitations of the present study deserve consideration. First, whether our results which demonstrate an association between chronic CO exposure and atherosclerosis in terms of CIMT and hs-CRP measurements has a causal relationship needs to be clarified with prospective follow-up studies. Second, whether results of the present study is confined to indoor barbecue workers or can be extended to all subjects with chronic CO exposure needs to be evaluated. Third, higher COHb levels in the indoor barbecue workers suggest that CO was responsible for the findings, but measurement of CO levels to which barbecue workers were exposed and other pollutants such as particulate materials (< 2.5 µm in diameter), black carbon, ozone, sulfur dioxide and nitrogen dioxide would strengthen our results. Forth, we were not able to control the environment of the study subjects and control group because they might be exposed to CO in different ways, such as via air pollution by means of living nearby highways and via passive smoking. Finally, whether the findings can be applicable to females needs to be clarified as association of hs-CRP with CIMT and atherosclerosis was stronger in men than in women according to a population based study (Makita et al. 2005).

Conclusion

In conclusion, our results demonstrate that CIMT and hs-CRP levels are increased in subjects with chronic CO exposure. The present study is the first demonstrating the association of chronic CO exposure with atherosclerosis in terms of increased CIMT and hs-CRP in subjects exposed to CO on average of 15.6 ± 7.1 years. This implication deserves further studies for clarifying the possible linkage between chronic CO exposure and atherosclerosis. Long-term follow-up and large-scale studies are mandatory to assess the risk of atherosclerotic cardiovascular events in this commonly encountered but relatively understudied situation.

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References


Hodis, H.N., Mack, W.J., LaBree, L., Selzer, R.H., Liu, C.R., Liu,