Reactive carbonyls from tobacco smoke increase arterial endothelial layer injury

ADAM E. MULLICK,1 JAMES M. MCDONALD,1 GOAR MELKONIAN,3 PRUDENCE TALBOT,3 KENT E. PINKERTON,2 AND JOHN C. RUTLEDGE1

1Division of Endocrinology, Clinical Nutrition and Vascular Medicine, Department of Internal Medicine, 2Institute of Toxicology and Environmental Health, University of California, Davis, 95616; and 3Department of Cell Biology and Neuroscience, University of California, Riverside, California 92521

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ETS induces pathogenic changes in both the plasma lipids and the vascular wall (2, 21, 36, 48). Previous work (24) performed in our laboratory showed that exposure of native low-density lipoprotein (LDL) to plasma from rats exposed to ETS led to modification of LDL and increased LDL accumulation in normal arteries. In addition, in vitro and in vivo work (12, 36) has demonstrated that ETS modifies LDL by induction of oxidative damage, thus leading to accelerated lipid peroxidation and modified LDL. An oxidative insult, such as one that occurs with ETS exposure, can contribute to glycoxidative damage and/or formation of reactive carbonyls in these lipoproteins, thereby resulting in the accumulation of advanced glycation end products (AGEs) (7, 18, 39). Although these data strongly indicate that ETS exposure can modify LDL, little is known about the direct effects of ETS on the artery wall.

Reactive carbonyls, such as the α-dicarbonyl glyoxal, may play important roles in several disease states (18, 29). These carbonyls are formed during all stages of the glycation process as well as during lipid peroxidation (7, 35). During the Maillard reaction, sugars react with proteins to produce irreversible adducts known collectively as AGEs. Mounting evidence suggests thatα-dicarbonyls are precursors of AGEs (25, 29). The accumulation of these reactive compounds has been termed “carbonyl stress,” which has been most completely described in studies of uremia (18). Previous studies (3, 4, 20) have investigated the role of AGEs and tobacco smoke, but it is unclear which specific constituents of the vapor phase of ETS are responsible for the initiation of smoking-induced vascular injury. Cerami et al. (3) have reported the presence of compounds described as “glycotoxins” that are generated during smoke exposure. These highly reactive compounds were shown to rapidly induce AGE formation on proteins in vitro and in vivo, as well as display mutagenic properties (3). It is unknown which specific effects reactive carbonyls have on vascular function.

Previous studies (11, 31, 47) showed estrogens to be atheroprotective; however, more recent studies (1, 28)
have been skeptical of this concept. Our previous work (8, 43, 44) demonstrated that estrogens reduce arterial endothelial layer permeability and LDL accumulation in the artery wall during an oxidant stress. Furthermore, we showed that estrogen reduces glycoxidative damage in the vascular wall (19, 42). Additional studies showed that estrogens prevent oxidative injury to LDL (23, 27, 40) and may also be protective in smokers (34). Therefore, it was plausible that estrogens could protect against the vasculotoxic effects of ETS, thereby reducing LDL accumulation and preventing atherogenesis.

The studies described above led us to formulate the following hypotheses: 1) ETS exposure generates reactive carbonyls that injure the artery wall, thereby causing increased vascular permeability; and 2) female sex hormones attenuate ETS-mediated LDL accumulation in the artery wall by reducing endothelial layer permeability.

METHODS

Chemicals and Materials

Krebs-Henseleit buffer consisted of (in mM) 116 NaCl, 5 KCl, 2.4 CaCl$_2$·H$_2$O, 1.2 MgCl$_2$, 1.2 NH$_2$PO$_4$, and 11 glucose. Bovine serum albumin (BSA, 1% by weight in perfused, aminoguanidine, and fluorescent-labeled dextran (65,000 molecular weight; estimated Stokes radius = 5.7 nm) were obtained from Sigma (St. Louis, MO). Dextran was labeled with tetramethylrhodamine isothiocyanate (TRITC) (excitation maximum 540 nm, emission maximum 518 nm). Ninety-day hormone pellets were obtained from Innovative Research of America. The fluorophore 1,1'-dioctadecyl-1,3,3',3'-tetramethyl-indocarbocyanine (DiI) was obtained from Molecular Probes.

LDL was isolated and labeled as described by Pitas et al. (22). Briefly, blood from fasting nonsmoking human males was obtained in vacutainers containing EDTA and centrifuged for 10 min at 2,800 rpm at a temperature of 4°C. The plasma was recovered and LDL (density = 1.01 to 1.06) and lipoprotein-deficient plasma were obtained by sequential density gradient ultracentrifugation. LDL was labeled with the fluorescent hydrocarbon probe DiI and diazoyed in phosphate-buffered saline at 4°C for 48 h. The spectral properties of DiI are 540 nm excitation maximum and 556 nm emission maximum.

Chronic ETS Exposure

Animals. Ovariectomized and intact female Sprague-Dawley rats were obtained from Zivic Miller (Zelienople, PA). Ovariectomy was performed at 8 wk of age. To test the hypothesis that the estrogen status of an animal could affect the injury response of ETS exposure, rats were separated into three groups 2 wk after ovariectomy: 1) intact control, 2) ovariectomized supplemented with subcutaneous estradiol pellets (2.5 mg), and 3) ovariectomized supplemented with placebo pellets. Our (19, 42) previous experiments showed that 2.5-mg estradiol pellets produced plasma estradiol concentrations in the physiological range (69 ± 21 pg/ml) in rats. The Animal Use Committee at the University of California (UC) Davis approved all procedures.

Exposure protocol. The rats were housed at the Institute of Toxicology and Environmental Health on the UC Davis campus. The temperature was maintained at 23°C with a 12:12-h light-dark cycle. Rat chow and water were available ad libitum. One week after hormone pellet implantation, rats were placed in ETS exposure chambers at UC Davis that previously have been described in detail (33). Chamber conditions during ETS exposure were as follows: relative humidity (34 ± 3.3%), temperature (23°C), carbon monoxide (90 ± 5.7 ppm), nicotine (5.3 ± 2.0 mg/m³), and total suspended particulates (31 ± 1.8 mg/m³). Animals were exposed to ETS for 6 h/day and 5 days/wk for 6 wk.

Measurement of arterial LDL accumulation. Arterial perfusion experiments were performed 6 wk after ETS exposure. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (0.1 ml of 50 mg/ml per 100 g body wt). The carotid arteries were isolated and adjacent tissues were carefully dissected away. The isolated arteries were placed in the perfusion apparatus and the rate of LDL accumulation and efflux from the artery wall was determined by quantitative fluorescence microscopy. A baseline level of fluorescence intensity was established during perfusion of a nonfluorescent solution [1% BSA-Krebs-Henseleit buffer]. DiI-LDL (70 µg protein/ml), in a separate reservoir, was then perfused into the carotid artery. At the end of a 10-min perfusion with the solution containing DiI-LDL, the vessel lumen was cleared of DiI-LDL. Once the lumen was cleared of the fluorescent-labeled solution, any remaining fluorescence is a measure of the number of molecules of DiI-LDL that remain bound to the endothelial surface or in the vessel wall.

It is during the washout phase that the analysis of LDL accumulation is performed. Measurement of accumulation involves analysis of washout data as two distinct processes: a rapid wash out of the lumen fluorescence, followed by a slower vessel wall fluorescence washout. Calculation of fluorescence intensity (I) accumulation (the amount of fluorescent-labeled molecules in the artery wall) involves finding the intersection of tangents drawn to approximate these two processes. The time required for I accumulation to reach half of its original value was referred to as the half-life (T½) of DiI-LDL in the artery wall. To determine accumulation rate, I accumulation is divided by the length of dye perfusion (10 min). Finally, an appropriate conversion factor is used to convert millivolts per minute to ng·min$^{-1}$·cm$^{-2}$. This conversion factor comes from four measurements: 1) the surface area and 2) lumen volume of the vessel in the photometric window; 3) the maximum I at time 0 (I$_{max}$), which occurs at the beginning of dye perfusion; and 4) the concentration of fluorophore. Throughout the perfusion experiment the vessel was perfused at a rate of 7 ml/min at 37°C and pH 7.4. Distal resistance was adjusted to maintain 100 mmHg of hydrostatic pressure within the vessel at all times.

Acute ETS Exposure

Exposure protocol. To investigate the effect of acute ETS exposure on the artery wall, arteries from 3- to 4-mo-old male Sprague-Dawley rats not exposed to ETS were used. Carotid arteries were harvested and placed in the perfusion apparatus as described above. Quantitative fluorescence microscopy was used to measure the rate of TRITC-dextran accumulation in these vessels before and after administration of a LDL perfusate solution (1% BSA-Krebs-Henseleit buffer + 50 µg LDL protein/ml) exposed to ETS. Exposure of the LDL perfusate solution to ETS was performed by “puffing” three-fourths of a cigarette into a 500-ml sidearm flask containing our LDL perfusate, as described by Eiserich et al. (5). Briefly, the sidearm of a 500-ml flask was connected to both a vacuum pump and cigarette via a Y connector, stopcock, and tubing.
In preparation for each puff, the flask was evacuated by opening the stopcock between the vacuum pump and flask, whereas the connection between the cigarette and flask was closed. With the cigarette lit, the connection between the pump and flask was closed, whereas the connection between the cigarette and flask was opened, allowing for gas-phase filtered cigarette smoke to slowly enter the flask, burning about one-fifth of the cigarette. Once the pressure within the flask equilibrated with ambient pressure, the connection between the cigarette and flask was closed and the flask was once again evacuated with the vacuum pump. This cycle was repeated until three-fourths of the cigarette was consumed. The resultant mixture of tobacco smoke and LDL perfusate solution was placed into a 37°C water bath for 2 h for preparation for the perfusion experiments.

Measurement of arterial permeability. Vascular permeability was evaluated by measuring TRITC-dextran accumulation in the absence and presence of this ETS-exposed LDL perfusate solution. Triplicate trials established baseline levels of dextran (65K molecular wt) accumulation with three additional trials performed in the presence of ETS-exposed LDL perfusate in each carotid. As described above, each trial consisted of 10 min of perfusion with the fluorophore solution, followed by 10 min of washout with the nonfluorescent solution. Both carotid arteries were examined in each animal.

Glyoxal measurement. To determine the content of glyoxal following ETS exposure, the LDL perfusate solution was exposed to tobacco smoke as described above in the absence or presence of aminoguanidine. Aliquots of the solution were removed at various time points for glyoxal quantification. Glyoxal levels were measured by ultraviolet absorbance at 294 nm after sample reaction with 0.5 M Girard T in 0.50 M sodium formate at pH 2.9 (46).

Statistical Analysis

In each perfusion experiment, a carotid artery was alternately perfused with a nonfluorescent buffer solution and a buffer solution containing either Dil-LDL or TRITC-dextran. A mean value for the rate of LDL or dextran accumulation in each artery was determined from 3 to 5 repeated trials. Differences between treatment effects were analyzed using two-factor ANOVA for both the chronic studies of LDL accumulation and the acute studies of dextran accumulation in the presence of ETS-exposed LDL perfusate and aminoguanidine. The analysis of factor level effects was done with the use of Tukey’s multiple-comparison test and a Bonferroni t-test was used to determine confidence intervals among the groups. Because of a nonnormal distribution in the rate of LDL accumulation, we used Tukey’s nonparametric test. Student’s t-test was used to find significance in glyoxal levels after ETS exposure and to find significance with dextran accumulation after glyoxal administration. A P value < 0.05 was considered significant.

RESULTS

Chronic ETS Exposure

Across all groups (intact, ovariectomized, and ovariectomized + estradiol treated), ETS exposure (n = 19 arteries) increased the rate of LDL accumulation more than fourfold compared with LDL accumulation in arteries (n = 18 arteries) from filtered air-exposed rats (4.0 ± 0.7 vs. 0.8 ± 0.7 ng protein·min⁻¹·cm⁻²; P < 0.05). With 95% confidence, arteries from animals exposed to ETS, regardless of estrogen status, accumulated between 1 and 5 ng·min⁻¹·cm⁻² more LDL than arteries from animals exposed to filtered air. Because the LDL perfused into these arteries had never been exposed to ETS, the increased LDL accumulation was mediated by an artery wall effect induced by chronic exposure to ETS. There was no attenuation of the ETS-induced increase in the rate of LDL accumulation in the intact group or the ovariectomized group treated with estradiol (Fig. 1).

Analysis of LDL efflux from the artery wall revealed that LDL had a significantly shorter residence $T_{1/2}$ in the artery walls of ETS-exposed arteries compared with arteries from filtered air-exposed rats (6.0 ± 0.3 vs. 8.1 ± 0.3 min; $P < 0.05$). With 95% confidence, animals exposed to ETS had a LDL residence $T_{1/2}$ that was 1 to 3 min less than animals exposed to filtered air. As with LDL accumulation rates, there were no differences in the $T_{1/2}$ within ETS subgroups (intact vs. estradiol treated vs. placebo treated) and filtered air subgroups. Thus ETS-exposed arteries had greater LDL accumulation and efflux than arteries from filtered air-exposed animals.

Artery Morphometry

Carotid artery samples from intact females exposed to either 6 wk of filtered air or ETS exposure were compared using transmission electron microscopy.

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olated, the cells generally were flush with the underlying internal elastic membrane, and intercellular junctions were intact and normal in appearance. In treated samples, some cells appeared normal. However, cells were also seen that showed characteristics rarely seen in controls. These included inclusion of vacuoles in the endothelial cytoplasm (Fig. 2B). These vacuoles were sometimes filled with flocculent material or sometimes appeared empty. In addition, the basal surface of the endothelial cells in treated samples often appeared elevated off of the internal elastic membrane (Fig. 2C). In some cells, the junctional complexes between adjacent endothelial cells were disrupted (Fig. 2C) or the intercellular space appeared swollen. In several treated samples, the endothelial cytoplasm contained bundles comprised of microtubules (Fig. 2D).

**Acute ETS Exposure**

The highly reactive carbonyl glyoxal was measured to explore the possibility that ETS exposure results in the generation of reactive carbonyls. The LDL perfusate solution was incubated at 37°C in the presence or absence of aminoguanidine (10 mM) and exposed to ETS. Aminoguanidine prevents glycoxidative damage by scavenging reactive carbonyls (46). Three separate trials were performed to investigate the effect ETS has on glyoxal formation. A large significant increase in glyoxal was observed within 0.5 h of ETS treatment (Fig. 3, P < 0.05). Glyoxal levels were maintained at this high level for up to 18 h (Fig. 3). The solutions containing aminoguanidine had significantly less glyoxal formation at the first time point, with levels thereafter reduced significantly relative to the solution that did not contain aminoguanidine (Fig. 3, P < 0.05).

The role that LDL or BSA plays in glyoxal generation during ETS exposure was determined by incubation of a LDL-free or a LDL and BSA-free solution in the presence of ETS. In the absence of LDL, 13% less glyoxal was generated at the first time point (0.5 h), with levels reduced thereafter. In the absence of BSA and LDL, 52% less glyoxal was generated at the first time point with levels reduced thereafter. Thus although glyoxal generation during ETS exposure does not require LDL or BSA as a substrate, as much as ~50% of the generation of glyoxal is dependent on LDL and protein.

To address whether acute exposure of LDL to ETS increases arterial permeability, experiments were performed by perfusing the LDL perfusate solution, exposed to ETS for 2 h, through rat carotid arteries. Simultaneous with the LDL perfusion, endothelial layer permeability was determined by measuring TRITC-dextran (65K molecular wt) accumulation in the artery wall. Triplicate trials established baseline levels of dextran accumulation with three additional trials performed in the presence of ETS-exposed LDL in each carotid artery. ETS-exposed LDL increased dextran accumulation and thus vascular permeability.

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**Fig. 2.** Transmission electron micrographs of cross sections of carotid arteries after 6 wk of filtered air (A) or ETS exposure (B–D) in intact female rats. A: filtered air control. The nucleus and cytoplasm appear normal. Although the junctional complex between adjacent cells is not well resolved in this plane of section, the cell boundaries are closely apposed, and the intercellular space is not swollen. B: ETS exposed. The cell contains a large vacuole (V) filled with a flocculent material. C: elevation of an endothelial cell off the basal lamina (BL) and disruption of cell junctions between adjacent endothelial cells (*) was associated with ETS exposure. D: high-magnification transmission electron micrograph of endothelial cell cytoplasm from ETS-exposed female reveals bundles of fibers and individual microtubules.

**Fig. 3.** Effect of ETS on in vitro glyoxal formation in a LDL perfusate solution. Glyoxal levels (measured using ultraviolet spectroscopy) were assayed over an 18-h time period after an initial exposure to tobacco smoke. A greater than sixfold increase in glyoxal levels was observed immediately after ETS exposure, with levels thereafter remaining significantly elevated (*P < 0.05). Administration of aminoguanidine (AG) resulted in a significant attenuation of glyoxal formation during ETS exposure.
by 105% (ETS = 4.4 ± 0.9 vs. control = 2.1 ± 0.6 ng dextran·min⁻¹·cm⁻²; P < 0.05; Figs. 4 and 5). Seven additional vessels were evaluated to determine whether aminoguanidine (10 mM) could protect against the increased vascular permeability seen with ETS exposure. Aminoguanidine did not provide a statistically significant protective effect against ETS-induced increased permeability, although a trend of decreased permeability with aminoguanidine appears to be present (Fig. 5).

Subsequent experiments were performed to address whether the presence of glyoxal alone could increase vascular permeability as seen with perfusion of ETS-exposed LDL. The addition of glyoxal to perfusate, at a concentration measured in our in vitro assay (150 mM), increased dextran accumulation in the artery wall by 53% (6.8 ± 0.4 vs. 4.4 ± 0.3 ng·min⁻¹·cm⁻²; P < 0.05). These data demonstrate that exposure to tobacco smoke in a chronic or acute setting injures the vascular endothelium, resulting in increased LDL accumulation and increased arterial permeability. This study implicates reactive carbonyls as playing a large role in the vascular injury caused by tobacco smoke. Morphometric examination of the arterial endothelium provides strong evidence that ETS exposure increases vascular permeability by damaging the endothelium. In addition, the estrogen status of the animals did not attenuate the damage seen with ETS.

Previous studies (29) have suggested that reactive carbonyls play an integral part in the transduction of glycoxidative stress in disease states such as diabetes and uremia. The question of whether smoking-induced vascular injury is related to the reactive carbonyls produced in tobacco smoke has not previously been addressed. ETS contains highly reactive carbonyls that can induce AGE formation in vitro and in vivo in a matter of hours (3). Reactive carbonyls, such as α-dicarbonyls, are formed from all stages of the glycation process as well as during lipid peroxidation (7, 35). The accumulation of these compounds, such as glyoxal, has been termed carbonyl stress (18). The downstream products of carbonyl stress, AGEs, have been observed in the coronary arteries of smokers (20). Studies (26) have demonstrated that AGE-modified proteins can activate endothelial receptor for AGE and induce cytotoxic damage.

This study is the first to demonstrate that the highly reactive carbonyl glyoxal is generated by tobacco smoke and is directly injurious in vascular tissue. Previous studies (13, 15) have suggested injurious vascular effects of other specific components of tobacco smoke including nicotine, although it has been demonstrated that nicotine does not effect mortality in rats (41) nor does the presence of nicotine in smoke alter the progression of atheroma in rabbits (32). With the elimination of the systemic inflammatory immune response, our studies suggest that about one-half of the vascular injury mediated by ETS can be attributed to glyoxal. Other compounds present at high concentrations in tobacco smoke, such as other reactive carbonyls and compounds downstream or distinct (16) from carbonyls, can potentially injure the artery wall. These include various aldehyde-containing compounds, such as formaldehyde, acrolein, and acetic acid, in addition to nitric oxide (5, 6, 30). In addition, downstream products of glyoxal generated by the Maillard reaction, such as nonenzymatically modified plasma proteins like apolipoprotein B100-AGE, may mediate some of the damage observed with long-term ETS exposure (3, 25, 26). Finally, our acute model of ETS exposure does not take into account the systemic or alveolar inflammatory immune response toward inhaled ETS. However,
these studies indicate that glyoxal may be one important mediator in ETS-induced arterial injury. Furthermore, these data have implications for other disease states such as diabetes and renal failure, which are also marked by high concentrations of reactive aldehydes (18).

We tested the hypothesis that estrogens could attenuate increases in LDL arterial accumulation caused by ETS exposure. Our laboratory (19, 44) found that estradiol decreased arterial LDL accumulation and vascular permeability. In addition, estradiol was found to be protective during an oxidative challenge (8, 43, 44) and reduced glycoxidative damage in the vascular wall (19, 42). However, despite these previous findings, in this study there was no evidence that would suggest such a protective effect of estrogen. This is in accordance with epidemiological data showing increased cardiovascular disease in premenopausal woman who smoke (37, 38). It is possible that the glycoxidative stress associated with ETS exposure overwhelmed the protective capacity of estradiol, neutralizing any protective effect. Additionally, it is possible that the duration of this study (6 wk) was not long enough to detect a beneficial effect of estrogen. Finally, previous reports (14, 17) have suggested that cigarette smoke is antigenic, although the exact mechanism is unknown.

In conclusion, increased LDL accumulation is well known to be an early, and possibly initiating, event in atherosclerosis. These data illustrate that the carbonyl stress generated during exposure to tobacco smoke can directly initiate changes in the arterial wall leading to vascular injury associated with atherosclerosis. Thus these data suggest a pathophysiological link between reactive carbonyls generated from tobacco smoke and vascular disease. Given the continued popularity of smoking worldwide and the potential health consequences for nonsmokers, it is increasingly important to determine specifically what effects ETS has on the cardiovascular system and how this contributes to the morbidity and mortality associated with tobacco smoke. Future efforts should concentrate on the cellular and molecular consequences of repeated ETS exposure to the artery wall and the role that reactive carbonyls have in this transduction of ETS-induced injury.

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REFERENCES


