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CIGARETTE SMOKE-INDUCED ALTERATIONS IN THE RELEASE OF ARACHIDONATE METABOLITES BY PULMONARY ALVEOLAR MACROPHAGE FROM SELENIUM-FED AND SELENIUM-DEFICIENT RATS

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Abstract—Male weanling F-344 rats were maintained on selenium-supplemented or -deficient diets and were exposed to fresh cigarette smoke daily for 28 weeks. The deficient status of animals was demonstrated by a significant reduction in the pulmonary and hepatic glutathione peroxidase (GSH-Px) activity of rats on selenium-deficient diet. Sham and smoke treatment did not influence the GSH-Px activity in either diet group. Elevated levels of blood carboxyhemoglobin and pulmonary aryl hydrocarbon hydroxylase activity in the smoke-exposed rats of both diet groups indicated effective inhalation of cigarette smoke by animals. Studies of the extracellular release of arachidonate metabolites by pulmonary alveolar macrophages (PAMs) indicated that resting cells released small amounts of prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4). Upon phagocytic challenge by opsonized zymosan particles, the release of the three metabolites was substantially increased in all diet and treatment groups. While the release of cyclooxygenase products, PGE_2 and TXB_2, remained unaffected by cigarette smoke, an inhibition of approximately 50% in the release of lipoxygenase product, LTB_4, was observed in cells from selenium-fed animals. In selenium-deficient animals, cigarette smoke almost completely inhibited (>80%) the zymosan-stimulated release of LTB_4 by PAMs and additionally caused about 50% reduction in TXB_2 release. These results suggest a specific inhibition of lipoxygenase pathway by cigarette smoke in PAMs of selenium-fed rats and suggest that cigarette smoke may additionally impair enzymes of the cyclooxygenase pathway in PAMs of selenium-deficient animals

Cigarette smoking is a major risk factor in the development of various cardiopulmonary diseases in humans. A number of studies have established a relationship between smoking and the incidence of chronic bronchitis, emphysema and lung cancer [1]. Although the mechanisms involved in the development of these diseases remain unclear, it has been observed repeatedly that the number of phagocytic cells is increased markedly in the lungs of smokers, and that the predominant cell type is the macrophage [2, 3]. Various alterations in the properties of macrophages obtained by endobronchial lavage of smokers have been reported [3], thus suggesting a possible role of these cells in the development of smoking-associated diseases.

It is well established that macrophages secrete a variety of biologically active substances [4], many of which are known mediators of inflammatory and immune reactions. Various arachidonic acid metabolites are among such substances, which possess chemotactic and immunoregulatory activities [5–7]. In addition, lipoxygenase products of arachidonic acid metabolism also elicit response in airways and blood vessels [7].

In the present study, we have examined the effect of prolonged cigarette smoke exposure of rats on the ability of their pulmonary alveolar macrophages to secrete representative arachidonic acid metabolites that are derived via cyclooxygenase [prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2)] and lipoxygenase [leukotriene (LTB_4)] pathways under resting and phagocytically-challenged conditions. Since our earlier studies had indicated a selective effect of dietary selenium deficiency on the LTB_4 biosynthesis [8], we have studied the effect of cigarette smoke in both selenium-deficient and selenium-fed rats.

MATERIALS AND METHODS

Animals and diets. Weanling male Fisher 344 rats were obtained commercially (Harlan-Sprague Dawley, Indianapolis, IN) and were kept on Purina rat chow for 1 week in quarantine rooms. The animals showing no abnormal signs during this period were randomly selected for the study and divided into two groups. The first group was maintained on low selenium basal diet and the second group on the same diet supplemented with 1 ppm selenium (as sodium selenite). The diets were received in small instalments from Dyet Inc. PA, and were composed of torula yeast, 30%; sucrose, 59%; tocopherol-stripped lard, 5%; salt mix HMW, 5%, and vitamin mix, 1%, as in Schwartz and Fredga [9]. The selenium content of the low selenium basal diet was 0.03 ppm.

All animals were housed in hanging stainless steel wire cages and maintained under a daily light cycle of

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12 hr in environmentally controlled Bioclean rooms that are equipped with HEPA filters and undergo 40 air changes/hr. Animals had free access to food and water *ad lib.*

Cigarette smoke exposures. After 10 weeks on diets, each diet group was divided into three subgroups. One was maintained as room control (RC) and was handled once a week during routine cleaning. The second was maintained as sham control (SH) and received treatment identical to that of the smoke-exposed group except in the absence of cigarette smoke. The third group was exposed to fresh cigarette smoke (SM) from one University of Kentucky Reference cigarette (2R1) daily for 10 min, 7 days a week for 28 consecutive weeks using a peristaltic pump smoke exposure system [10]. The exposure was through the nose only.

Bronchoalveolar lavage. The following day after the last treatment, animals from each group were lavaged to obtain pulmonary alveolar macrophages. Each animal was anesthetized with an intraperitoneal injection of sodium pentobarbital and opened to expose the chest cavity. After severing the abdominal aorta, the trachea was cannulated and the lungs were lavaged with 8-ml aliquots of Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS) eight to nine times per animal. The lavage fluids were pooled and centrifuged at 400 *g* for 15 min to sediment the bronchoalveolar lavage (BAL) cells.

The BAL cell pellets were given a brief hypotonic shock to lyse the erythrocytes, washed once, and resuspended in HBSS. Small aliquots were used for the determination of total, viable and differential cell counts. Viability was determined by trypan blue exclusion, and differential cell counts were made on giemsa-stained cell smears.

Pieces of lung and liver tissue from each animal were immediately frozen in liquid nitrogen and stored at −80°. Homogenates of these tissues were processed for the analysis of glutathione peroxidase (GSH Px) and aryl hydrocarbon hydroxylase (AHH) activities.

Extracellular release of arachidonic acid metabolites. Cell suspensions were adjusted to 10^6 cells/ml in HBSS. All experiments were performed within 2 hr of animal sacrifice, using siliconized glass tubes containing 0.5 ml cell suspension. Release of the metabolites was measured under resting and zymosan-challenged conditions. Zymosan particles were opsonized by incubation at 37° in rat serum for 30 min. The cell suspensions were incubated alone and with opsonized zymosan particles (1:10) for 30 min at 37°, and the tubes were transferred to an ice bath. Supernatant fractions were collected by centrifuging the suspensions at 400 *g* for 10 min at 4° and immediately frozen in liquid nitrogen for determination of arachidonic acid metabolites.

Assay of arachidonic acid metabolites. Levels of PGE_2 and TXB_2 were determined by specific radioimmunoassays described previously [11, 12]. The concentration of LTB_4 was analyzed by a specific radioimmunoassay recently developed in our laboratory. Cross-reactivities of LTB_4 antibodies with other arachidonate metabolites including 6-*trans*-LTB_4 were less than 0.1% with the exception of 12-HETE (0.8%).

In vivo *indicators of cigarette smoke exposure.* The exposure of animals to cigarette smoke was monitored by periodic measurement of the blood carboxyhemoglobin (COHb) levels. The measurements were made on small blood samples obtained from the tail vein of the animals immediately after the completion of sham and smoke treatments using a CO-Oximeter (1L 282, Instrumentation Lab. Inc., MA). At the termination of the experiment, the lung tissue samples were also analyzed for the induction of AHH activity.

Enzyme assays. The lung and liver tissue samples were homogenized in 0.15 M KCl buffer and centrifuged at 9,000 *g* to obtain supernatant fractions. Lung 9,000 *g* fractions were used for AHH assays. Portions of the lung and liver supernatant fractions were further centrifuged at 20,000 *g* for 30 min to obtain supernatant fractions for GSH-Px assays used in assessing the selenium status of animals. Lung tissue AHH activity was assayed using a radiometric procedure [13], and GSH-Px activity was measured as described elsewhere [14].

RESULTS

Body weights and dietary status. In general, the average body weights of animals in the selenium-supplemented diet group were higher than in the

Table 1. Body weights and lung tissue aryl hydrocarbon hydroxylase (AHH) activity of selenium-fed and -deficient rats

	Body weight (g)		Lung tissue AHH (pmoles [^{3}H]BP metabolized/min/mg protein)	
	Selenium		Selenium	
Treatment	Added 1 ppm	Low basal	Added 1 ppm	Low basal
Room control	352 ± 7*	301 ± 24	0.76 ± 0.06	0.77 ± 0.03
Sham control	304 ± 16	276 ± 20	0.71 ± 0.10	0.83 ± 0.12
Smoke-exposed	277 ± 13	265 ± 5	2.10 ± 0.21†	1.96 ± 0.32†

Values are means ± S.E., N = 4.
* Significantly different from all other groups ($P < 0.05$).
† Significantly different from corresponding sham and room control groups ($P < 0.05$).

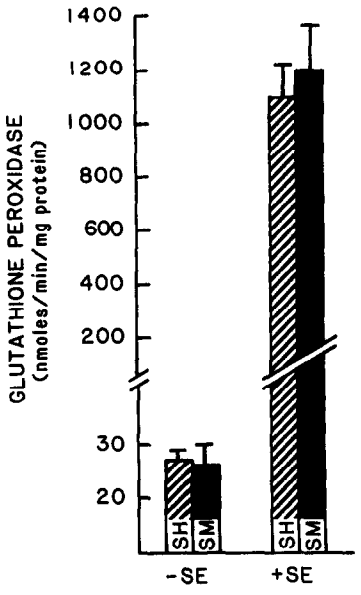

Fig. 1. Glutathione peroxidase activity of liver 20,000 *g* supernatant fractions from sham and smoke-exposed rats fed with basal diets supplemented with selenium or without selenium. Values are means ± S.E., N = 4.

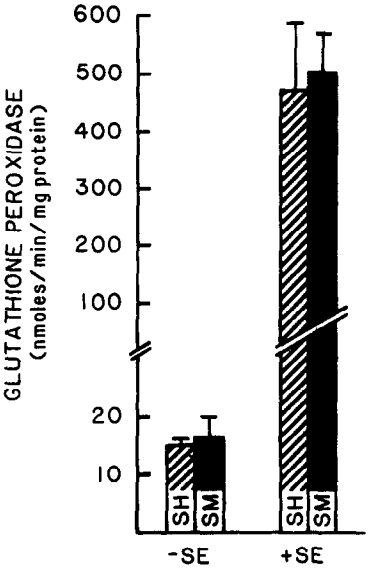

Fig. 2. Glutathione peroxidase activity of lung 20,000 *g* supernatant fractions from sham and smoke-exposed rats fed basal diets supplemented with selenium Se or without selenium.

selenium-deficient group, but such differences between the two diet groups were statistically significant only for the room control animals (Table 1). Reduced food intake may possibly account for the decreased body weight of animals on low selenium diet. Sham and cigarette smoke treatments caused reduced weight gains in both dietary groups. The GSH-Px activity of the lung and liver tissue was reduced significantly in the animals fed low selenium basal diets, thus indicating their selenium-deficient status (Figs. 1 and 2). Sham and smoke treatments did not affect the GSH-Px activity in either of the diet groups.

Markers of smoke exposure. The blood COHb levels, determined immediately after the daily treatments at different exposure times, showed a range of 0.6 to 0.8% and 4.1 to 5.3% for the sham and smoke-exposed groups respectively. The lung tissue AHH activity was also elevated significantly in smoke-exposed animals of both diet groups (Table 1). Since in these experiments the lungs were lavaged prior to their use for AHH assays, the observed cigarette smoke-induced increase in enzyme activity was slightly over 2-fold instead of the 3- to 4-fold that we normally observe in unlavaged lungs from smoke-exposed rats frozen immediately at sacrifice. The lung lavage procedure takes approximately 3–5 min per animal during which the lungs are washed repeatedly with warm HBSS. This may result in a decrease of the induced lung tissue AHH activity. Although BAL cells do metabolize benzo[*a*]pyrene, their contribution to overall lung tissue AHH is believed to be minimal.

Bronchoalveolar lavage cell recovery. The number of free lung cells recovered by bronchoalveolar lavage (BAL) of animals was similar for all diet and treatment groups (Table 2). Practically all (>97%) of the BAL cells in each group were macrophages, as judged by morphology and latex particle phagocytosis. In the past, we have observed that a few rats (<1%), regardless of their treatment status, yield higher number of BAL cells with larger percentages of lymphocytes and polymorphonuclear neutrophils (PMN). Such animals are generally infected, and the data from these animals were excluded from analysis. Exposure to cigarette smoke failed to induce infiltration of PMNs into the lungs of rats at any exposure point.

Extracellular release of arachidonic acid metabolites by bronchoalveolar lavage cells. The incu-

Table 2. Bronchoalveolar lavage cell recovery

Dietary selenium	Bronchoalveolar lavage cells ($\times 10^6$/rat)		
	Room control	Sham control	Smoke-exposed
Added 1 ppm	3.0 ± 0.1	2.92 ± 0.57	3.09 ± 0.22
Low basal	2.8 ± 0.3	2.70 ± 0.31	3.16 ± 0.26

Values are means ± S.E., N = 4.

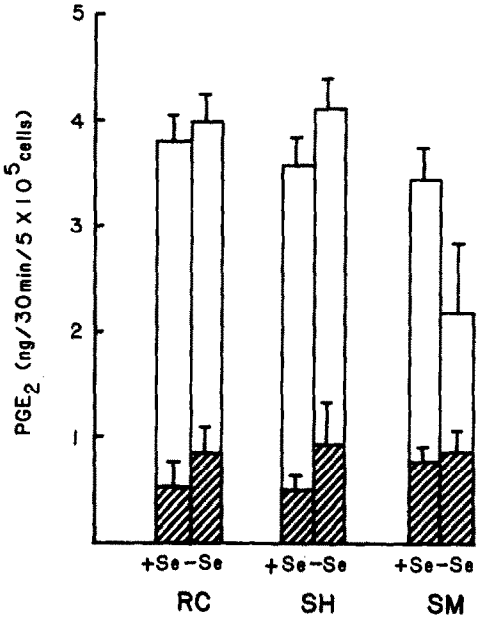

Fig. 3. Effect of dietary selenium and cigarette smoke exposure on resting and zymosan-induced release of PGE_2 from BAL cells of room control (RC), sham control (SH) and smoke-exposed (SM) rats. The hatched area indicates basal release under resting conditions.

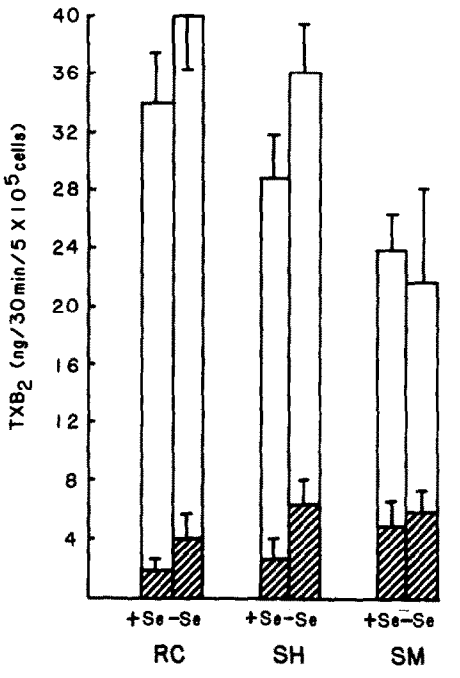

Fig. 4. Effect of dietary selenium and cigarette smoke exposure on resting and zymosan-induced release of TXB_2 from BAL cells of room control (RC), sham control (SH) and smoke-exposed (SM) rats. The hatched area indicates basal release under resting conditions.

bations to determine the ability of the BAL cells to release PGE_2, TXB_2 and LTB_4 under resting and phagocytically-stimulated conditions were carried out in siliconized glass tubes to minimize the adherence of cells and metabolites to container surfaces. Under resting conditions, the BAL cells released minimal amounts of the three metabolites, and the exposure of animals to cigarette smoke did not alter appreciably any of these basal levels (Figs. 3–5). Phagocytic stimulation with opsonized zymosan particles significantly increased the extracellular release of these metabolites by the cells from all diet and treatment groups (Figs. 3–5). There were no significant differences in the zymosan-induced release of PGE_2 (Fig. 3) and TXB_2 (Fig. 4) between the control or sham and smoke-exposed rats of the selenium-fed group. However, cigarette smoke induced a marked decrease in the release of TXB_2 by cells from selenium-deficient rats.

In contrast to the cyclooxygenase products, the exposure to cigarette smoke markedly inhibited the release of LTB_4 by BAL cells in both diet groups (Fig. 5). Such inhibition amounted to about 50% in the selenium-fed group and over 80% in the selenium-deficient group.

DISCUSSION

The main aim of the present study was to determine if daily exposure to fresh mainstream cigarette smoke for prolonged periods can alter the arachidonate metabolism in rat PAMs. In addition, we were interested in determining how exposure to cigarette smoke influences the arachidonate metabolism of PAMs in selenium-deficient rats which were found earlier to exhibit impaired leukotriene synthesis [8].

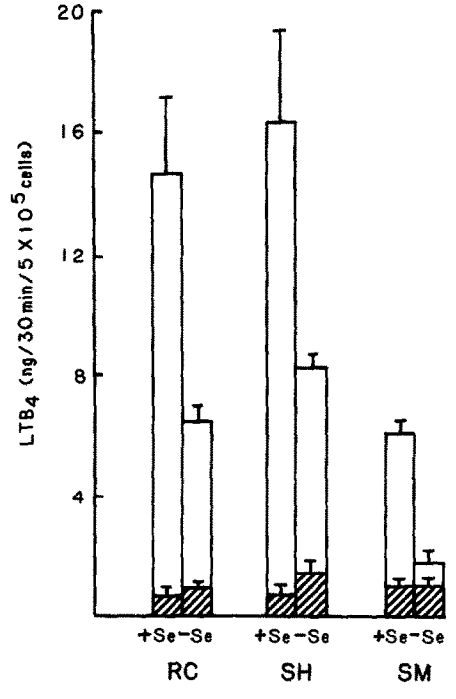

Fig. 5. Effect of dietary selenium and cigarette smoke exposure on resting and zymosan-induced release of LTB_4 from BAL cells of room control (RC), sham control (SH) and smoke-exposed (SM) rats. The hatched area indicates basal release under resting conditions.

Our daily estimates of total particulate matter (TPM) removed by animals from the exposure chambers [10] indicated that each animal had received approximately 200 mg of smoke TPM during the entire exposure period of 28 weeks. Elevated levels of blood COHb and lung tissue AHH in smoke-exposed animals confirmed effective inhalation of cigarette smoke by the animals. That animals on the low selenium basal diet did develop selenium-deficiency was also demonstrated by a significant reduction in the GSH-Px activity of the lung and liver tissue (Figs. 1 and 2).

Various studies of the BAL cells obtained from human smokers and smoke-exposed animals have reported a number of morphological, functional and metabolic alterations in the PAMs [3, 15–20]. In the present study, we demonstrated that the phagocytically-stimulated BAL cells of rats, which were predominantly (>97%) macrophages, were altered significantly in their ability to synthesize and secrete lipoxygenase product of arachidonic acid (LTB_4) as a result of prolonged exposure to cigarette smoke. These effects were evident in both selenium-fed and selenium-deficient rats.

All incubations were carried out under conditions that discourage cell attachment to surfaces so as to minimize the effect of adherence-induced metabolic activation of macrophages [21]. Since our studies were performed in the absence of added arachidonic acid, the measured metabolites were derived solely from the endogenous precursor in the macrophage membranes, known to contain as much as 25% of their total fatty acids as arachidonate [22]. Under these conditions, an inhibition of the release of LTB_4 but not that of PGE_2 and TXB_2 by cigarette smoke in selenium-fed rats indicated the specificity of the smoke-induced inhibitory effect on the lipoxygenase pathway of arachidonate metabolism. The greater susceptibility of the lipoxygenase pathway to cigarette smoke was further indicated by markedly higher inhibition of LTB_4, than of TXB_2, release by PAMs in selenium-deficient rats. An inhibitory effect of selenium-deficiency alone on the release of LTB_4 by zymosan-stimulated PAMs was consistent with our earlier findings [8].

Cigarette smoke or selenium-deficiency alone did not affect the excretion of TXB_2, a cyclooxygenase product. However, selenium-deficiency combined with cigarette smoke markedly reduced the ability of the PAMs to release TXB_2, thus indicating an interaction between these two factors in causing an inhibition of TXB_2 synthesis and release. The mechanism(s) involved in causing such an impairment of TXB_2 synthesis remains to be elucidated.

In contrast to TXB_2, selenium-deficiency or cigarette smoke alone caused approximately 50% inhibition of LTB_4 release by PAMs. The combination of selenium-deficiency and cigarette smoke resulted in >80% inhibition of zymosan-stimulated LTB_4 synthesis by PAMs, thus indicating an additive effect of these two factors and suggesting that the sites of inhibitory action of selenium-deficiency and cigarette smoke are different. In this context, we have shown in a separate study that cigarette smoke exposure of rats significantly reduced the 5-lipoxygenase activity in PAMs, as assayed by using [1-^{14}C]arachidonate as a substrate.* Therefore, an impairment of LTA_4 synthase and/or LTA_4 hydrase by selenium-deficiency, as suggested earlier [8], and an inhibition of 5-lipoxygenase by cigarette smoke may exert a composite inhibitory effect on the synthesis of LTB_4 in PAMs.

The significance of these findings is difficult to assess at the present time. It may, however, be pointed out that the secretory activity of PAMs from smoke-exposed rats did not resemble that reported for human smoker PAMs. It has been found that the PAMs obtained from human smokers release significantly smaller quantities of PGE_2 and TXB_2 than nonsmokers [23]. In addition, recent studies indicate a role of LTB_4 in mobilizing phagocytes from the peripheral blood, suggesting its role in maintaining an inflammatory cell response within human lungs [24]. The same study also indicated a slightly greater release of LTB_4 by smoker PAMs than those from nonsmokers [24]. Our studies have consistently failed to observe an inflammatory cell response in lungs of smoke-exposed rats. In fact, we found that mice exhibited a remarkable inflammatory cell response in the lungs upon exposure to cigarette smoke [25], much like that reported for human smokers [2, 3]. It is possible that an inhibition of LTB_4 release by PAMs in smoke-exposed rat lungs may be one of the factors responsible for the absence of an inflammatory cell response in rats. Studies are planned to study arachidonate metabolism in PAMs of mice which exhibit a pronounced pulmonary recruitment of macrophages and PMNs upon exposure to cigarette smoke.

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