Polycyclic aromatic hydrocarbons present in cigarette smoke cause endothelial cell apoptosis by a phospholipase A_2-dependent mechanism[1]

PATRICIA K. TITHOF,*,[2] MONA ELGAYYAR,* YEESOOK CHO,*,† WEI GUAN,* ARON B. FISHER,‡ AND MARC PETERS-GOLDEN§

*Department of Comparative Medicine, The University of Tennessee, Knoxville, Tennessee, USA; †Center for Environmental Biotechnology, ‡Institute for Environmental Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, USA; and §Department of Internal Medicine (Division of Pulmonary and Critical Care Medicine), University of Michigan, Ann Arbor, Michigan, USA

SPECIFIC AIMS

The aim of this study was to examine the role of the phospholipase A_2/arachidonic acid cascade in apoptosis of human coronary artery endothelial cells caused by specific polycyclic aromatic hydrocarbons (PAHs) present in high concentrations in cigarette smoke. Identification of specific components of cigarette smoke that alter pathways known to be important in heart disease will likely provide means for discovering effective preventative and therapeutic strategies in smokers.

PRINCIPAL FINDINGS

1. PAHs induce release of ^{3}H-arachidonic acid or ^{3}H-linoleic acid from human coronary artery endothelial cells

Release of ^{3}H-arachidonic acid from prelabeled cells was used as a measure of PLA_2 activity in response to three different PAHs present in high concentrations in cigarette smoke: 1-methylanthracene (1-MA; 1500 ng/cigarette), benzo(a)pyrene (B(a)P; 25 ng/cigarette), and phenanthrene (PA; 362 ng/cigarette). When endothelial cells were incubated for 1 h with various concentrations of 1-methylanthracene, benzo(a)pyrene, or phenanthrene, all three compounds caused concentration-dependent release of ^{3}H-arachidonic acid. 1-Methylanthracene and phenanthrene also induced release of ^{3}H-linoleic acid, suggesting that these compounds activate an arachidonyl-nonselective PLA_2. In contrast, benzo(a)pyrene did not cause release of ^{3}H-linoleic acid, suggesting that this compound activates an arachidonyl-selective enzyme. Significant release of ^{3}H-arachidonic acid was observed within 5 min from cells exposed to phenanthrene (30 μM) and within 10 min from cells exposed to 1-methylanthracene (30 μM) or benzo(a)pyrene (30 μM).

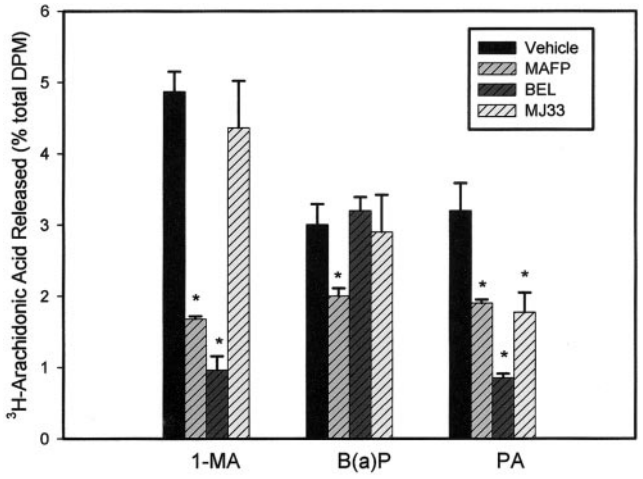

Figure 1. Attenuation of PAH-induced ^{3}H-arachidonic acid release by PLA_2 inhibitors in human coronary artery endothelial cells.

2. Specific PLA_2 inhibitors attenuate PAH-induced release of ^{3}H-arachidonic acid from human coronary artery endothelial cells

PAH-induced release of ^{3}H-arachidonic acid was evaluated in the presence and absence of inhibitors of selective isoforms of PLA_2. Methyl-arachidonyl fluorophosphonate (MAFP), an inhibitor of group IV and group VI enzymes, inhibited ^{3}H-arachidonic acid released in response to all three PAHs. Bromoenol lactone (BEL), a selective inhibitor of group VI calcium-independent PLA_2 ($iPLA_2$), attenuated the response to 1-methylanthracene and phenanthrene, but not benzo(a)pyrene. MJ33, an inhibitor of the acidic, calcium-

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.02–0092fje; to cite this article, use *FASEB J.* (July 1, 2002) 10.1096/fj.02–0092fje

[2] Correspondence: The University of Tennessee, Dept. of Comparative Medicine, A205, College of Veterinary Medicine, 2407 River Dr., Knoxville, TN 37996-4543, USA. E-mail: ptithof@utk.edu

independent PLA_2, aiPLA$_2$ inhibited ^{3}H-arachidonic acid release in response to phenanthrene but not 1-methylanthracene or benzo(a)pyrene (**Fig. 1**). 4-Bromophenacyl bromide, an inhibitor of small molecular weight, calcium-dependent PLA_2s, did not alter the response to any PAH. Reverse transcriptase-polymerase chain reaction analysis of PLA_2 isoforms indicates that human coronary artery endothelial cells contain mRNA for at least five different PLA_2 isozymes: groups IVα, IVβ, IVγ, group VI, and the acidic, calcium-independent PLA_2. Western analysis confirmed the presence of four of these enzymes: group IV β, IVγ, group VI, and the lysosomal, acidic, calcium-independent PLA_2.

3. PAHs induce apoptosis of human coronary artery endothelial cells

Apoptosis was evaluated by three techniques: Western analysis for the cleavage product of poly(ADP) ribose polymerase (PARP), ELISA for histone fragmentation, and terminal deoxyribonucleotide nick-end labeling (TUNEL). There was no evidence of PARP cleavage in vehicle-treated cells; however, significant amounts of the cleavage fragments were observed in cells after treatment for 6 h with 30 μM 1-methylanthracene, benzo(a)pyrene, phenanthrene, or 10 μM arachidonic acid or linoleic acid. To evaluate the time course of apoptosis, TUNEL assays were performed. No apoptotic cells were observed at 60 min, but a significant number of TUNEL-positive cells were seen at 2 h after treatment with the PAHs or fatty acids. For a more quantitative measure of PAH-induced apoptosis, histone fragmentation was evaluated by ELISA. These studies were performed in the presence and absence of MAFP, BEL, and MJ33. As can be seen in **Fig. 2**, the same inhibitor profile observed for ^{3}H-arachidonic acid release was seen with the apoptotic response. The response to 1-methylanthracene was attenuated by MAFP and BEL, but not MJ33. In contrast, the response to phenanthrene was inhibited by all three inhibitors, but benzo(a)pyrene-induced apoptosis was inhibited only by MAFP.

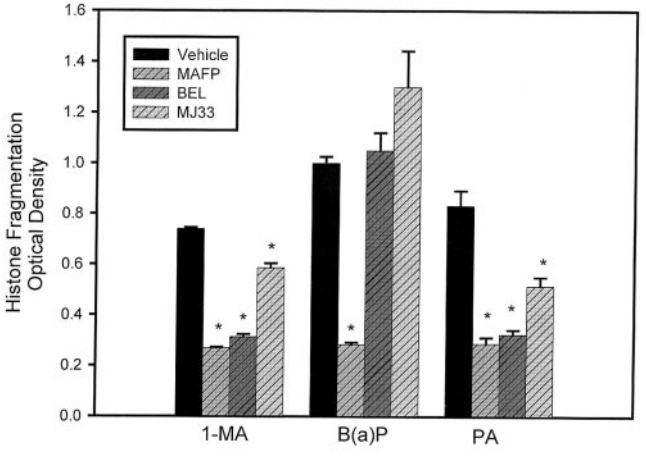

Figure 2. Inhibition of PAH-induced apoptosis of human coronary artery endothelial cells by PLA_2 inhibitors.

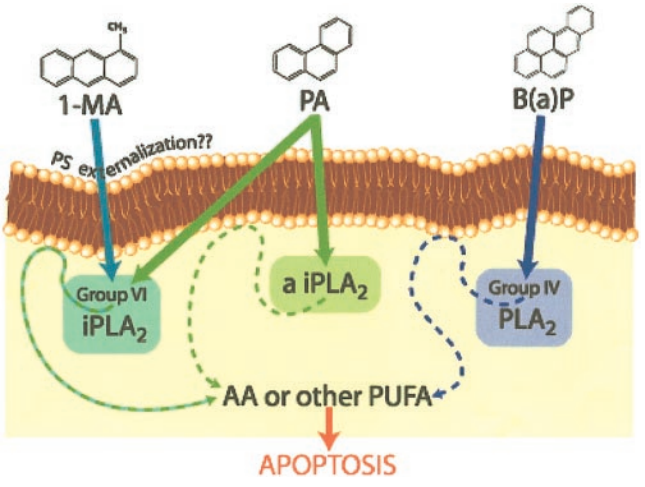

Figure 3. Schematic diagram.

CONCLUSIONS

It is well known from epidemiologic studies that the PLA_2/arachidonic acid cascade is important in the mechanism whereby cigarette smoking causes heart disease; however, little work has focused on identifying specific components of cigarette smoke responsible for this effect. This study is the first to identify specific PAHs in high concentrations in cigarette smoke that stimulate PLA_2-mediated release of membrane fatty acids. These results suggest that three distinct isoforms of PLA_2 are activated by three different PAHs. The data indicate that 1-methylanthracene, a three-ring compound, activates the group VI iPLA$_2$ whereas benzo(a)pyrene, a five-ring compound, activates a group IV enzyme. In contrast, phenanthrene, with a four-membered ring, activates both group VI and the acidic iPLA$_2$. Moreover, this study is the first to link exposure of endothelial cells to cigarette smoke components with PLA_2 activation, fatty acid release, and apoptosis, events known to be important in the etiology of atherosclerosis. FJ