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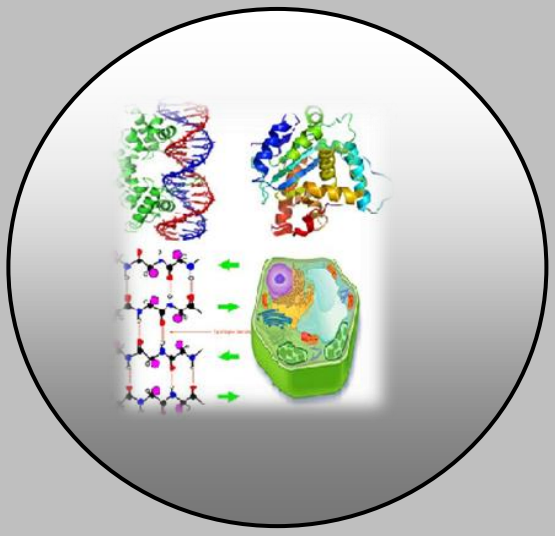

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Cigarette Smoke Induced Programmed Cell Death in Lung Epithelial Cells

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ABSTRACT

Cigarette smoking has been associated with lung epithelial cells damage and considered a major cause of lung cancer. Programmed cell death (PCD) is a highly regulated process and a defect in this process renders the cells to become cancerous. In order to study the role of cigarette smoking and PCD, lung epithelial cells were exposed to a number of chemical constituents of cigarette smoke such as hydrogen peroxide, acetaldehyde, paraldehyde and nicotine. Our results indicate that chemicals such as hydrogen peroxide, acetaldehyde and paraldehyde induce time-dependent PCD in epithelium cells, however, nicotine failed to induce any appreciable PCD as determined by the activation of caspase-3 enzymes, cell survival assay and Flow cytometry analysis.

Key words- Programmed cell death, Caspase-3 enzyme, Flow cytomerty and epithelial cells.

INTRODUCTION

Cigarette smoking is regarded as a major risk factor in developing various lung diseases such as pulmonary fibrosis, emphysema and lung cancer (Elmore, 2007). Programmed cell death (PCD) is a highly conserved and regulated process manifested by several characteristic morphological and biochemical changes in the cells selected for death. These include cell deformability, DNA aggregation and the activation of aspartate-specific cysteine proteinases called caspases (Choi et al. 2000, Nagai and Aoshiba, 2003). Cigarette smoke contains a large number of harmful chemicals and oxidants and can eventually lead to lung cell and tissue dysfunction and destruction (Wyatt et al. 2000). In the present study, we attempted to investigate the effect of some of these chemicals on PCD and found that with the exception of nicotine, all other chemicals used caused significant PCD in lung epithelial cells.

MATERIAL AND METHODS

Reagents- F12 Ham culture media was from Media tech (Herndon, VA) A549 epithelial cells were obtained from ATCC. For Cell survival detection, "CellTitre 96 Aqueous one solution Reagent" was purchased from Promega (Madison, VI). DEVD-AMC was from Sigma (St. Louis).

Cells and culture conditions- A549 lung epithelial cells were grown in F12 Ham media supplemented with 10% fetal bovine serum, and 100 g/ml penicillin, 100 g/ml streptomycin and maintained at 37 ^{O}C in 5% CO_2 atmosphere.

Measurement of Caspase Activation in epithelial Cells- A549 cells were collected and lysed in a buffer containing 20 mM Tris/ Hepes, pH 7.4, 0.1% CHAPS, 5 mM EDTA. Lysates containing 10 g proteins were incubated with 25 M DEVD-7-amino-4-methyl coumarin as substrate for 30 min at 37 ^{O}C. Fluorescence was measured at 380 nm excitation and 460 nm emission wavelengths.

Cell-survival assay- The assay using cell titre 96 Aqueous One reagent from Promega was done as described by manufacturer. Epithelium cells were grown in 1 ml media (in 24-well plates). The cells were treated with various chemicals for various times hours and 20 l cell titre reagents were added to each well and incubated for another 2 hours at CO_2 chamber. For blank, 20 l cell titre reagents were added to 1ml F12 Ham media. The supernatants were transferred in eppendorf tubes and centrifuged on a microcentrifuge for 2 min. The supernatant was read at 490 nm against the media blank.

Flow cytometry- Detection of caspase-3 was carried out according to manufacturer's instructions using a caspase-3 reagent (FLICA, Invitrogen-Molecular Probes; and Reference (Lin et al. 2010). After apoptosis induction, the epithelial cells were analyzed on a Flow cytometer with 488 nm excitation using 530 nm band pass and 670 nm long pass emission filters.

RESULTS AND DISCUSSION

Cigarette smoking causes direct oxidant –induced injury and cell loss in lung. Cigarette smoking also induces DNA damage by producing single-strand breaks. PCD brings about a number of changes in the cell shape including, cytoplasmic shrinkage membrane deformability, nuclear condensation and translocation of phosphatidylserine (Elmore, 2007).

In accordance with other studies (Ramage et al. 2006), nicotine did not induce any significant PCD measured by all the three detection methods such as caspase-3 activation, cell survival and flow cytometry analysis even after 24 hours. On the contrary marked increase in the activation was detected when cells were incubated with hydrogen peroxide, acetaldehyde or paraldehyde when compared to vehicle DMSO alone. The increase was found to be time dependent and increases with time. Most of the treatments also show the maximum PCD at about 24 hours. Among all the above chemicals, hydrogen peroxide potentiated the maximum apoptosis.

Although, we can not be certain to what extent these in vitro studies represent the true physiological damage incurred by a smoker's lungs as a result of cigarette smoking, nevertheless, our data shed some light on the potential harmful effects of the cigarette smoke constituents. A role of mitochondrial damage and increased inflammation in epithelial cells was observed as a result of cigarette smoke exposure (Ramage et al. 2006, Yokohori et al. 2004).

The characterization of the various mechanisms of cigarette-induced PCD and necrosis may greatly help in understanding lung diseases and the advancement of the treatments of cigarette-smoking related lung injuries.

Table 1. Activation of caspase 3 in A549 lung epithelial cells.

Time	DMSO (%)	H_2O_2 (%)	Acetaldehyde (%)	Paraldehyde (%)	Nocotine (%)
0h	100	100	100	100	100
2h	100 ± 5.3	133 ± 4.4	110 ± 5.5	115 ± 3.2	98 ± 6.9
6h	98 ± 4.1	198 ± 8.7	138 ± 5.4	146 ± 7.6	95 ± 6.0
8h	93 ± 3.2	220 ± 6.9	190 ± 6.9	186 ± 5.4	88 ± 4.2
24h	90 ± 5.8	239 ± 3.4	215 ± 3.2	223 ± 4.0	88 ± 5.7

Note-Cells were incubated with various chemicals such as DMSO (1%) alone, hydrogen peroxide (10 M), acetaldehyde (10 M), paraldehyde (10 M) and nicotine (1mM) for following time intervals.

Table 2. Cell survival assay.

Time	DMSO (%)	H_2O_2 (%)	Acetaldehyde (%)	Paraldehyde (%)	Nocotine (%)
0h	100	100	100	100	100
2h	111 ± 4.9	50 ± 7.8	55 ± 8.9	62 ± 12.9	105 ± 11.0
6h	108 ± 7.8	37 ± 5.6	43 ± 7.7	47 ± 7.9	108 ± 9.7
8h	97 ± 6.8	25 ± 8.9	30 ± 0.8	34 ± 9.0	96 ± 4.7
24h	89 ± 7.4	9 ± 2.0	22 ± 6.5	23 ± 8.6	89 ± 4.5

Note-Cell survival assay was done using an MTS assay. The supernatant was read at 490 nm against the suitable media blank.

Table 3. FACS analysis of PCD in A549 cells.

Time	DMSO (%)	H_2O_2 (%)	Acetaldehyde (%)	Paraldehyde (%)	Nocotine (%)
0h	100	100	100	100	100
8h	93 ± 3.2	185 ± 5.4	148 ± 5.6	145 ± 2.7	129 ± 5.9
24h	123 ± 8.9	315 ± 6.1	270 ± 6.7	223 ± 2.3	134 ± 6.5

Note-Epithelial cells were exposed to the chemicals as described in the Legends to Table 1 for Zero, 8 and 24 hours.

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