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SHORT COMMUNICATION

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Programmed Cell Death in Cigarette Smoke-exposed Lung Cells requires Calcium and Mitochondrial Component of Apoptotic Cascade

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ABSTRACT

Programmed cell death (PCD) plays a special part in the development and aging of a cell and aberration in PCD can have significant consequences from cell dysfunction to cancer. Cigarette smoking is also known to produce PCD in lung epithelial cells. We undertook this study to further elucidate the mechanism of PCD in lung epithelial cells. A special protease called caspase-3 becomes activated during the course of PCD. The activation of caspase-3 in A549 epithelial cells was found to be insensitive to effect to the calcineurin inhibitor FK506 but was completely blocked by cyclosporine-A pretreatment. Our data indicate the involvement of calcium and some mitochondrial component of the apoptotic cascade in H_2O_2 and acetaldehyde -induced epithelial cell death.

Key words: Programmed cell death, Cell dysfunction, Calcineurin inhibitor and Caspase-3

INTRODUCTION

Apoptosis or programmed cell death (PCD) is a highly conserved and regulated process manifested by several characteristic morphological and biochemical changes in the dying cells (Elmore, 2007). These include activation of aspartate-specific cysteine proteinases called caspases. Certain constituents of cigarette smoke such as hydrogen peroxide, acetaldehyde and paraldehyde are known to cause PCD lung epithelial cell.

The role of calcium in cell death is source of great interest (Mc Farlaine et al, 2000; Zhang et al 2008; and Tithof et al, 2002). In this study, we investigated and provided the evidence for the involvement of calcium in lung epithelial cells as a result of cigarette smoke -induced PCD.

MATERIAL AND METHODS

Reagents- Cyclosporine, FK506 and CHAPS were purchased from Sigma. DEVD-7-aminomethyl coumarin was purchased from Alexis. For Cell survival detection, "Cell Titre 96 Aqueous one solution Reagent" was purchased from Promega.

Cells and culture conditions- A549 epithelial cells were grown in F12 Ham media supplemented with 10% fetal bovine serum and antibiotics at 37 ^{o}C in 5% CO_2 atmosphere.

Measurement of Caspase Activation in A549 Cells- Epithelial 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 with some modifications. Briefly, 0.5 million cells were grown in 1 ml media (in 24-well plates). The cells were treated with the chemicals for 8 hours and 20 □l CellTitre reagents was added to each well and incubated for another 2 hours at CO_2 chamber. For blank, 20 □l CellTitre reagents were added to 1ml F12 Ham media. The supernants were transferred in eppendorf tubes and centrifuged on a microcentrifuge for 2 min. The supernatant was read at 490 nm against the media blank.

RESULTS AND DISCUSSION

Cigarette smoke ingredients –induced cell death requires calcium- Cell survival was measured using a MTS assay. Control untreated epithelial cells showed significantly lower amount of cell death as compared to various cigarette-smoke constituents -treated cells from earlier time points to even up to 24 hours (Table 1).

Calcium- dependence for Cigarette smoke ingredient- induced caspase 3-activation- A549 cells were incubated with H_2O_2 and acetaldehyde in absence and presence of EGTA (an extra cellular Ca^{2+} chelator) and BAPTA-AM (an intracellular Ca^{2+}-chelator). Caspase 3-activity was measured after 8 hours. Presence of Ca^{2+} is essential for the cigarette-smoke -induced caspase 3 –activation in epithelial cells (Table 2). A role of calcium has been shown in lung cancers and thymocytes (Haile et al 2004; and Shen and Dong, 2001).

Effect of various calcineurin –inhibiting agents on H_2O_2 and acetaldehyde –induced caspase 3 –activation- Calcium could be involved in apoptosis in several ways. The Ca^{2+} -activated protein phosphatase calcineurin dephosphorylates BAD (a pro-apoptotic member of the Bcl-2 family), and leads to BAD translocation to the mitochondria.

To analyze the role of calcineurin in caspase 3-activation in epithelial cells, they were exposed to calcineurin inhibitors -FK506 and cyclosporine. The lack of effect of FK506 indicates that calcineurin /and BAD are not involved in caspase 3 –activation in epithelial cells. Cyclosporine is also known to prevent the mitochondrial permeability which would inhibit the release of cytochrome c [*Kroemer et al, 2007*].

The marked inhibition of caspase 3 activation by cyclosporine may suggest the possible role of some mitochondrial components in H_2O_2 and acetaldehyde -induced epithelial cell death (Table 3). In summary, our data indicate that lung epithelial cells undergo programmed cell death as a result of exposure to certain cigarette-smoke ingredients and this process is dependent on calcium since it can be blocked with intracellular and extracellular chelators of calcium. Further studies are required to identify and investigate the role of specific mitochondrial components in this process.

Table 1. H_2O_2 and acetaldehyde -induced cell death in A549 epithelial cells.
Cells were pretreated with either 1mM EGTA or 100 □M BAPTA for 30 minutes.
The cells were further treated with H_2O_2 (10 □M) and acetaldehyde (10 □M) for 8 hours.

Treatment	**Cell viability (% of Control)**		
	None	+ EGTA	+ BAPTA
- H_2O_2	100	100	100
+ H_2O_2	23 ± 4.0	88 ± 4.9	96 ± 5.4
- Acetaldehyde	100	100	100
+ Acetaldehyde	39 ± 3.3	75 ± 4.2	68 ± 2.3
+ EGTA alone	80 ± 6.4		
+ BAPTA alone	73 ± 6.0		

Table 2. Caspase-3 activation by H_2O_2 and acetaldehyde in A549 epithelial cells.
Cells were pretreated with either 1mM EGTA or 100 □M BAPTA for 30 minutes.
The cells were further treated with H_2O_2 (10 □M) and acetaldehyde (10 □M) for 8 hours.

Treatment	**Caspase 3 activity (% of Control)**		
	None	+ EGTA	+ BAPTA
- H_2O_2	100	100	100
+ H_2O_2	264 ± 17.4	87 ± 7.9	78 ± 7.9
- Acetaldehyde	100	100	100
+ Acetaldehyde	235 ± 12.7	79 ± 6.8	78 ± 6.5
+ EGTA alone	88 ± 5.0		
+ BAPTA alone	93 ± 4.0		

Table 3. Caspase-3 activation by H_2O_2 and acetaldehyde in A549 epithelial cells.
Cells were pretreated with either 1mM EGTA or 100 □M BAPTA for 30 minutes.
The cells were further treated with H_2O_2 (10 □M) and acetaldehyde (10 □M) for 8 hours.

Treatment	**Caspase 3 activity (% of Control)**		
None	+ FK 506	+ Cyclosporine A	.
- H_2O_2	100	100	100
+ H_2O_2	279 ± 13.5	258 ± 16.0	43 ± 5.0
- Acetaldehyde	100	100	100
+ Acetaldehyde	243 ± 23.0	265 ± 7.0	45 ± 6.5
+ FK506	30 ± 4.0		
+ Cyclosporine	40 ± 6.0		

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