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# Acrolein-induced cell death in PC12 cells: Role of mitochondria-mediated oxidative stress

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**Abstract**

Oxidative stress has been implicated in acrolein cytotoxicity in various cell types, including mammalian spinal cord tissue. In this study we report that acrolein also decreases PC12 cell viability in a reactive oxygen species (ROS)-dependent manner. Specifically, acrolein-induced cell death, mainly necrosis, is accompanied by the accumulation of cellular ROS. Elevating ROS scavengers can alleviate acrolein-induced cell death. Furthermore, we show that exposure to acrolein leads to mitochondrial dysfunction, denoted by the loss of mitochondrial transmembrane potential, reduction of cellular oxygen consumption, and decrease of ATP level. This raises the possibility that the cellular accumulation of ROS could result from the increased production of ROS in the mitochondria of PC12 cells as a result of exposure to acrolein. The acrolein-induced significant decrease of ATP production in mitochondria may also explain why necrosis, not apoptosis, is the dominant type of cell death. In conclusion, our data suggest that one possible mechanism of acrolein-induced cell death could be through mitochondria as its initial target. The subsequent increase of ROS then inflicts cell death and further worsens mitochondria function. Such mechanism may play an important role in CNS trauma and neurodegenerative diseases.


*Keywords:* PC12 cells; Acrolein; Oxidative stress; Reactive oxygen species; Mitochondrial membrane potential; Oxygen consumption

## 1. Introductory statement

Oxidative stress has been implicated in various neurodegenerative conditions and disorders including ischemia-reperfusion, traumatic injury, Parkinson's disease (PD) and Alzheimer's disease (AD) (Coyle and Puttfarcken, 1993). Recent evidence suggests the products of lipid peroxidation, such as 4-hydroxynonenal (HNE) and 2-propenal (acrolein), may mediate oxidative stress related tissue damage (Esterbauer et al., 1991; Luo and Shi, 2004). Consistent with such notion, tissue levels of acrolein and HNE are elevated in neurodegenerative diseases, such as Alzheimer's disease (Markesbery and Lovell, 1998; Calingasan et al., 1999; Lovell et al., 2001), as well as after CNS injury (Springer et al., 1997; Baldwin et al., 1998; Luo et al., 2003).

Acrolein is the strongest electrophile among the unsaturated aldehydes (Dennis and Shibamoto, 1990; Esterbauer et al., 1991; Lovell et al., 2000). Acrolein formed in vivo exhibits facile reactivity with various biomolecules including proteins, DNA and phospholipids, and thus has the potential to disrupt the function of these molecules (Esterbauer et al., 1991; Kehrer and Biswal, 2000). Acrolein can react with sulfhydryl groups of cysteine, histidine, and lysine residues of proteins (Kehrer and Biswal, 2000). Acrolein has been shown to rapidly incorporate into proteins and generate carbonyl derivatives (Uchida, 1999). Acrolein can also react with

*Abbreviations:* ROS, reactive oxygen species; PD, Parkinson's disease; AD, Alzheimer's disease; HNE, 4-hydroxynonenal; NAC, *N*-acetylcysteine; CsA, cyclosporin A; $DCFH_2$-DA, dichlorofluorescein-diacetate; Rh123, Rhdamine 123; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GSH, glutathione; PHPA, *p*-hydroxyphenylacetate; PEG, polyethylene glycol; MPTP, mitochondrial permeability transition pore

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nucleophilic sites in DNA and modify DNA bases with the formation of exocyclic adducts. Available data indicates that, compared to other alkenals such as HNE, acrolein may play a particularly important role in inflicting damage to cells, especially in CNS trauma and neurodegeneration. Although it is clear that acrolein is capable of damaging neuronal tissues (Shi et al., 2002; Luo and Shi, 2004), the mechanisms of how acrolein inflicts tissue damage are not fully established. Existing data suggests that acrolein may inflict neuronal tissue damage via oxidative stress (Luo and Shi, 2004). There are two lines of evidence that support such hypothesis. First, acrolein causes significant depletion of glutathione (Kehrer and Biswal, 2000) and increased generation of reactive oxygen species (ROS) (Luo and Shi, 2004), both of which lead to oxidative injury. Second, acrolein-induced neuronal membrane damage in spinal cord tissue can be lessened by antioxidants (Luo and Shi, 2004). Since mitochondria are the main cellular organelles that produce ROS in physiological conditions (Balaban et al., 2005), we hypothesize that mitochondria may also be the main source of ROS over-production in pathological conditions in the presence of acrolein.

The overall objective of the present study is to further explore the role of oxidative stress in acrolein toxicity. Particularly, we plan to examine the role of mitochondrial dysfunction in acrolein-induced oxidative stress and subsequent cell death. We chose to carry out this study in a well-established neuronal PC12 cell tissue culture where the oxidative stress, mitochondria function, and cell viability can be assessed at the same time and therefore can be correlated in the same sample. We have found that acrolein-induced PC12 cell death was accompanied by severe oxidative stress and mitochondrial dysfunction. Our data is consistent with the hypothesis that acrolein induces cell death through stimulating the production of mitochondrial ROS and inflicting mitochondrial dysfunction.

## 2. Experimental procedures

### 2.1. Chemicals

Acrolein (product number: 48501, solvent: neat), *N*-acetylcysteine (NAC) and cyclosporin A (CsA), polyethylene glycol (PEG, MW ~2000) and Adenosine 5′-triphosphate (ATP) Bioluminescent Assay Kit were obtained from Sigma-Aldrich (St. Louis, MO). Cell culture media and reagents were purchased from Invitrogen (Carlsbad, CA). Dichlorofluorescein-diacetate ($DCFH_2$-DA) and Rhodamine 123 (Rh123) were purchased from Molecular Probes (Eugene, OR). Other routine laboratory reagents were obtained from Sigma-Aldrich (St. Louis, MO).

### 2.2. Cell culture

PC12 cells were obtained from the American Type Culture Collection (Rockville, MD). PC12 cells were grown in DMEM with 12.5% horse serum, 5% fetal bovine serum and 1% Pen–Strep (penicillin–streptomycin). PC12 cells were placed in 12-well cell culture dishes at 37 °C under a humidified atmospheric condition of 5% $CO_2$ and 95% air, and 3–6-day-old cells were used for the experiments. When used, cells were either suspended in Krebs-Ringer solution (125 mM NaCl, 5 mM KCl, 25 mM HEPES, 6 mM glucose, 5 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, and 2.4 mM $CaCl_2$, pH 7.4) at different concentrations or maintained in 12-well culture dishes depending on the treatment paradigms.

### 2.3. Treatment paradigms

PC12 cells were exposed to acrolein (final concentrations: 1, 10 and 100 μM) freshly prepared in distilled water everyday before use. These concentrations equaled to 0.35, 3.5 and 35 nmol/mg protein, respectively, calculated based on the average protein concentration of the cells. The in vivo acrolein concentrations in Alzheimer's disease brain are 2.5 nmol/mg in amygdale and 5.0 nmol/mg in parahippocampal gyrus (Lovell et al., 2001). Therefore, the concentrations used in this study were pathologically relevant. Two treatment paradigms were used. For cell viability and ATP content assays, the cells were maintained in the cell culture dishes and exposed to acrolein. Pretreatment with *N*-acetylcysteine, cyclosporin A and polyethylene glycol (PEG 2000) were performed 30 min before acrolein exposure. Twenty-four hours after exposure, the cells were collected and assays were performed according to the following protocols. For all other experiments, the cells were suspended in Krebs-Ringer solution at the $2 \times 10^6$ cells/ml and acrolein was added before the beginning of each assay.

### 2.4. MTT assay of cell viability

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously (Monner, 1988; Varming et al., 1996). The assay was initiated by removing the culture medium and adding MTT (0.3 mg/ml). Following 1 h incubation at 37 °C, the medium was aspirated, and 0.3 ml of isopropanol was added to lyse the cells and dissolve the formazan crystals. Aliquots (100 μl) of this solution were pipetted into 96-well microplates and the absorbance was recorded at 570 nm (with background subtraction at 620 nm) in a microplate reader. Cell viability was expressed as a percentage of the absorption in control cultures (100%).

### 2.5. Determination of necrosis and apoptosis

Necrotic and apoptotic cell death was determined as previously described (Delgado-Esteban et al., 2000; Delgado-Esteban et al., 2002). Necrosis was assessed by