Available online at www.sciencedirect.com
ScienceDirect
Free Radical Biology & Medicine 42 (2007) 798–810
www.elsevier.com/locate/freeradbiomed

Original Contribution

Activation of the death receptor pathway of apoptosis by the aldehyde acrolein

André Tanel, Diana A. Averill-Bates *,[1]

Département des Sciences Biologiques, TOXEN, Université du Québec à Montréal, CP 8888, Succursale Centre Ville, Montréal, QC, Canada H3C 3P8

Received 5 July 2006; revised 26 November 2006; accepted 5 December 2006
Available online 14 December 2006

Abstract

Reactive α,β-unsaturated aldehydes such as acrolein are major components of common environmental pollutants. As a toxic by-product of lipid peroxidation, acrolein has been implicated as a possible mediator of oxidative damage to cells and tissues in a wide variety of disease states, including atherosclerosis and neurodegenerative and pulmonary diseases. Although acrolein can induce apoptotic cell death in various cell types, the biochemical mechanisms are not understood. This study investigates the implication of the death receptor pathway in acrolein-induced apoptosis. Exposure of Chinese hamster ovary cells to acrolein caused translocation of adaptor protein Fas associated with death domain to the cytoplasmic membrane and caspase-8 activation. Kp7-6, an antagonist of Fas receptor activation, blocked apoptotic events downstream of caspase-8, such as caspase-7 activation and nuclear chromatin condensation. Acrolein activated the cross-talk pathway between the death receptor and mitochondrial pathways. Bid was cleaved to truncated-Bid, which was translocated to mitochondria. Activation of the mitochondrial pathway by acrolein was confirmed by caspase-9 activation. Inhibition of activation of either the Fas receptor or caspase-8 partially decreased acrolein-induced caspase-9 activation. These findings indicate that acrolein activates the Fas receptor pathway, which occurs upstream of the mitochondrial pathway. Caspase-9 activation still occurred despite inhibition of the Fas receptor pathway, suggesting that acrolein could also trigger the mitochondrial pathway independent of the receptor pathway. These findings improve our understanding of mechanisms of toxicity of the reactive aldehyde acrolein, which has widespread implications in multiple disease states which seem to be mediated by oxidative stress and lipid peroxidation.

Keywords: Acrolein; Death receptor; Apoptosis; Caspase; Mitochondria; Free radicals

Introduction

Acrolein is a highly reactive, α,β-unsaturated aldehyde and humans are exposed to this compound in multiple contexts [1]. It is a ubiquitous environmental pollutant which is present in food. Acrolein is found in the vapors of overheated cooking oil and severe human toxic exposures have been reported [2]. It is used industrially as a starting material for acrylate polymers and in the production of acrylic acid and as a herbicide [3]. Acrolein is also a metabolic product of the widely used anticancer drug cyclophosphamide and has been implicated in its toxic side effects [4].

As well as being a ubiquitous environmental pollutant, acrolein is one of the toxic aldehyde by-products of endogenous lipid peroxidation, together with 4-hydroxy-2-nonenal [5,6]. Lipid peroxidation is a deleterious chain reaction occurring mainly in biological membranes, resulting from oxidative stress. Acrolein also reacts with glutathione [7,8]. In fact, acrolein causes more rapid and severe depletion of this important cellular antioxidant compared to the well-known oxidant H_2O_2 [9]. Furthermore, acrolein and its glutathione adduct, glutathionylpropionaldehyde, were shown to cause

Abbreviations: AFC, amino trifluorocoumarin; CHO, Chinese hamster ovary; FADD, Fas-associating protein with death domain; Fas, fibroblast-associated; FasL, Fas ligand; FasR, Fas receptor; FBS, fetal bovine serum; MEM, minimum essential medium; Mops, 3-(*N*-morpholino)-propane sulfonic acid; PARP, poly(ADP-ribose) polymerase; PBS, phosphate-buffered saline; PS, phosphatidylserine; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEM, standard error of mean.

* Corresponding author. Fax: +1 514 987 4647.
E-mail address: averill.diana@uqam.ca (D.A. Averill-Bates).
[1] Formerly Diana A. Bates.

0891-5849/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2006.12.009

formation of oxygen radicals [10], which may be responsible for induction of lipid peroxidation by acrolein. Together, these studies show that acrolein causes an oxidative redox imbalance.

Oxidative stress and lipid peroxidation have been implicated in a variety of human disease states. Because acrolein is an exogenous product of lipid peroxidation and can itself induce lipid peroxidation, this reactive aldehyde may have a possible role as a mediator of oxidative damage to cells and tissues. Indeed, acrolein has been implicated in the development of multiple disease states involving oxidative stress, such as atherosclerosis

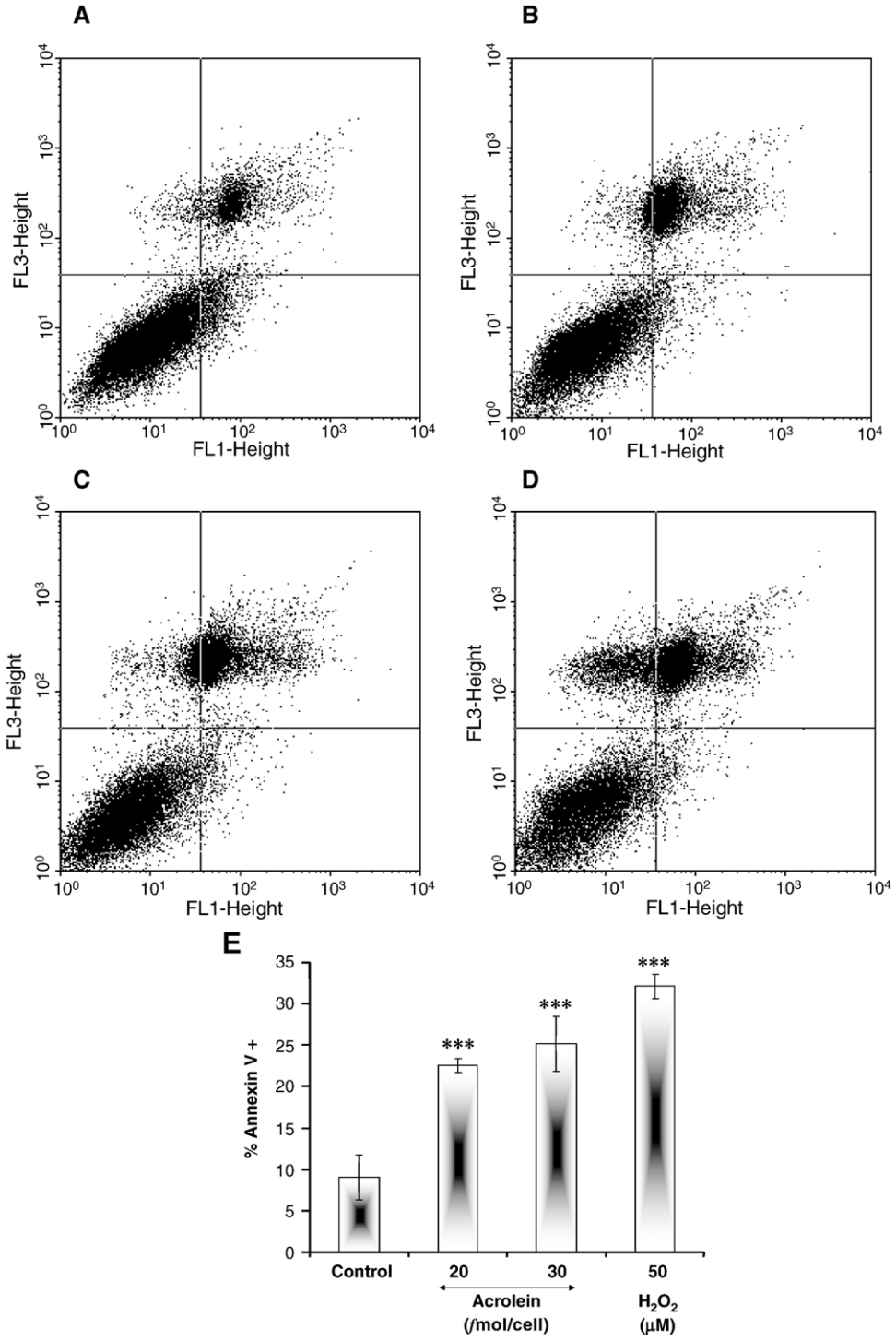

Fig. 1. Acrolein induces externalization of phosphatidylserine. Apoptosis was analyzed by flow cytometry using annexin V–FITC in CHO cells after treatment with acrolein. Cells (10^6/ml) were either (A) untreated (controls) or treated with (B) 20 or (C) 30 fmol/cell acrolein or (D) 50 μM hydrogen peroxide for 2 h. Cells were stained with annexin V–FITC (*x* axis) and PI (*y* axis). Twenty thousand cells were analyzed using a FACS scan to determine the percentage of annexin V^+-labeled cells. One representative experiment is shown from six independent experiments. (E) ***$p < 0.001$ indicates a statistically significant difference between treatment with acrolein or H_2O_2 and the control.