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Molecular Mechanisms of Cadmium Toxicity

by

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'This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree'.

Signed: Date:

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ABSTRACT

Cadmium is a heavy metal which has been associated with a number of pathological diseases. However, despite the known toxicity of this metal, there is inconclusive evidence about its mechanism(s) of action in the cells. The present study was therefore undertaken with the aim of defining the role of oxidative stress, intracellular Ca^{2+} alteration via phospholipase C- Inositol-1,4,5-triphosphate (PLC-IP_3) and mitochondrial-cytochrome c dependent pathways in the etiology of cadmium-induced toxicity in three human cell lines: HepG2 (human hepatoma), 1321N1 (human astrocytoma) and HEK 293 (human embryonic kidney) cell lines after 24hrs exposure to 5, 10 and 50 μM cadmium chloride ($CdCl_2$). The role of the Nrf2-Keap1-ARE pathway in the adaptive response of these cell lines to Cd exposure was examined, and the possible mechanism(s) involved in the protective response of garlic extracts to $CdCl_2$ were also investigated. Finally, this work examined changes in the proteomic profile of the three human cell lines after Cd exposure in order to develop suitable biomarkers for Cd toxicity. In summary, this study shows that the oxidative stress induced by Cd occurs by different mechanisms depending on cell type and that Ca^{2+} alteration may play an important role in Cd-induced toxicity in HEK 293 cells, while the mitochondrial-cytochrome c dependent pathway is important in Cd toxicity in all three cell lines. Also, the study shows that Nrf2-Keap1-ARE mediated adaptive response to Cd may be activated by PKCδ and that Cd generally alters the metabolism of exposed cells.

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ABBREVIATIONS

AGE	Aged garlic extract
ARE	Antioxidant response element
Apaf-1	Apoptotic peptidase activating factor1
Bax	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2
BMA	Bisindolymaleimide VIII acetate
Cadherin	Calcium dependent adhesion molecule
CBP	cAMP-Response Element-Binding Protein
Cd	Cadmium
$CdCl_2$	Cadmium chloride
CDNB	1-chloro-2,4-dinitrobenzene
CNC	Cap'n'collar
C_T	Cycle threshold
Cys	Cysteine
DADS	Diallyldisulfide
DAG	Diacylglycerol
DAS	Diallylsulfide
DAT	Diallyltrisulfide
dATP	Deoxyadenosine triphosphate
DEVD	Asp-Glu-Val-Asp
DMEM	Dulbecco's Modified Eagle Medium
EDTA	Ethylenediaminetetraacetic acid
Ellman	5,5'-dithio(2-nitrobenzoic acid)

FADD	Fas-associating protein with a novel death domain
Fe^{2+}	Iron(II) ion
Fe^{3+}	Iron(III) ion
g	Gram
g	Centrifugal force equivalent to gravitational acceleration
GA	Aqueous garlic extract
γ-GCSc	Gamma glutamyl cysteine synthase
GCLM	Glutamate cysteine ligase modifier subunit
GCLC	Glutamate cysteine ligase catalytic subunit
GST	Glutathione S-transferase
GSK	Glycogen synthase kinase
HEK 293	Human embryonic kidney cells
HepG2	Human hepatoma cells
HO1	Heme oxygenase 1
H_2FDA	Dihydrofluorescein diacetate
JNK	c-Jun N-terminal Kinases
Keap1	Kelch-like ECH associating protein 1
LDH	Lactate dehydrogenase
MafK	Macrophage activating factor K
MALDI	Matrix assisted Laser Desorption and Ionization
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

Nrf2	Nuclear factor erythroid 2-related factor 2
NR3C1	Nuclear receptor subfamily 3 Group C member of glucocorticoid receptor
NQO1	NADP(H):quinone oxidoreductase
PCR	Polymerase chain reaction
OSCs	Organosulfur compounds
PKC	Protein kinase C
PLC-IP$_3$	Phospholipase c-inositol-1,4,5-triphosphate
PIDD	p53-Induced Protein with Death Domain
qRT	Quantitative reverse transcriptase
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SAC	S-allylcysteine
SAMC	S-allylmercaptocysteine
TAE	Tris-acetic acid-EDTA buffer
TBA	Thiobarbituric acid
TOF	Time of flight
TRITC	Tetramethyl Rhodamine Isothiocyanate
FITC	Fluorescence Isothiocyanate
TMP	Tetramethoxypropane