Molecular Mechanisms of Sulfur Mustard Vesicant-Induced Cell Death: Early and late cell responses

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# Molecular Mechanisms of Sulfur Mustard Vesicant-Induced Cell Death: Early and late cell responses

## Abstract

Introduction
Sulphur mustard reacts with a wide range of biological molecules, including proteins and nucleic acids. It possesses mutagenic, carcinogenic, cytotoxic, vesicating effects, and results in cell death. However, the biomedical mechanism of cell death induced by Sulphur mustard is not completely understood. To reveal this mechanism, we examined the specific genes involved in the regulation of cell survival and death pathway.
Experimental Results
CEES Induced-Apoptosis

Early Stage

0 30 90 (min)

Late Stage

0 6 12 24 (h)

Perinuclear margination Of chromatin
Cell Response to CEES Damage in Early Stage
Cells are attacked by CEES, leading to apoptosis. The early stage involves the activation of MEKK, which activates MKK, leading to the activation of JNK, which in turn activates p-JNK and p-jun. The late stage involves the activation of Akt, which leads to the activation of caspases through PDK1 and PDK2. Mitochondria play a role in both stages, with 14-3-3 and Bcl-2 family proteins involved in the regulation of caspases and p-JNK, respectively.
CEES (200 µM) Induced c-Jun and phosphorylation of Jun

(Western Blotting)
CEES (200 µM) Induced JNK and Phosphorylation of JNK

(Western Blotting)
**Cytokines Induced in Early Stage of CEES (200 µM) Damaged in Jurkat cells**

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*IL-8 increased from 0 hour to 0.5 hour.*
Cells Response to CEES Damage in Late Stage
cDNA array
50 µM CEES/24 hrs/Jurkat cells
Microarray reveals most caspases expression was induced by CEES

![Graph showing relative gene expression (% of control) against CEES concentrations ranging from 0µM to 50µM. The graph displays different caspase isotypes (CASPASE-1 to CASPASE-9) with varying trends and degrees of induction.]
Array data was validated by RPA, RT-PCR, WB and activity assay.

(A) CEES [µM] 0 0.05 0.5 5 50

- Caspase 8
- Caspase 3
- Caspase 6
- Caspase 7
- Caspase 1
- L32
- GAPDH
- Unprotected (nt)

(B) β-actin

- Caspase 4

(C) CEES [µM] 0 0.5 5 50

- Caspase-3
  - 32 kDa
- Caspase-4
  - 45 kDa
  - 40 kDa
  - 35 kDa

(D) Relative AMC Fluorescence (380 nm)

Caspase-3 enzyme Activity

- 0 0.5 5 50
CEES INHIBITED BCL FAMILY EXPRESSION

S 0 0.5 5 50 µM

Bcl-XL
Blk
Bax
Bcl-2
Mcl-1
L32

RNAse Protection

Relative Gene Expression

CEES [µM]
0 0.5 5 50

0% 20% 40% 60% 80% 100% 120%
CEES Inhibited Akt/PKB expression

(A) RT-PCR

(B) Western Blotting
Phosphorylation and activities of Akt were affected by CEES

(A) Phosphorylation of Akt (Western blotting)

(B) Akt kinase activity assay

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<td>Relative Akt activity</td>
<td>120%</td>
<td>100%</td>
<td>80%</td>
<td>60%</td>
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CEES [µM] 0 0.5 5 50

α-Thr

α-Ser

60 kDa
PDKs expression was affected by CEES

(A) RT-PCR

(B) Western Blotting
Cytokines Were Induced in Late Stage of CEES Treatment (200 µM) in Jurkat cells

0 hour

24 hours

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(Protein array)
CEES Attacks Cells

Early Stage

MEKK
MKK
JNK
p-JNK
p-jun
Mitochondria

Late Stage

Akt
PDK1
PDK2
BAD
BclS
14-3-3
BclS
caspases

Apoptosis
Conclusion
Sulphur mustard causes cell death via apoptosis:
- In early stage, it induces JNK activity and then triggers apoptosis pathway.
- In late stage, sulphur mustard attacks the Akt pathway, by inhibiting Akt transcription, translation, and post-translation modification. Concomitantly, the anti-apoptotic genes, Bcl family, were down-regulated, in sharp contrast to the striking up-regulation of some death executioner genes, caspases 3, 8, 6 and 5.
- Sulphur mustard also induces some cytokines expression.
- Take together, sulphur mustard induces apoptosis by inhibiting the cellular survival factors which suppresses the expression of caspases.