**1,2-Dichloroethane induced hepatotoxicity and apoptosis by
inhibition of ERK 1/2 pathways**

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Abstract

1,2-Dichloroethane (DCE) is a ubiquitous occupational environmental contaminant. Subacute exposure of DCE could cause severe toxic encephalopathy and obvious toxic effects on the liver. However, the toxicity of DCE on liver and the molecular mechanism still remain elusive. In the present study, we established a DCE-exposed animal model by inhalation in SD rats and used HepG2 cells in in vitro tests. Hepatic dysfunction was found in DCE-exposed groups compared with the control group. Moreover, apoptotic cells and the decreased phosphorylation of ERK1/2 were found in liver tissue of rats in three DCE-exposed groups. In in vitro tests, we also found that short-term exposure to DCE induced apoptosis in HepG2 cells. Furthermore, the incubation of cells with DCE significantly decreased the phosphorylation of ERK1/2 in a concentration-dependent manner. Additionally, the incubation of HepG2 cells with the ERK1/2 activator EGF significantly increased apoptosis in HepG2 cells. In conclusion, our results suggested that DCE induced apoptosis in HepG2 cells by inhibition of ERK1/2 pathways.

Key words: 1,2-Dichloroethane, liver lesions, ERK1/2, apoptosis, HepG2 cell

41 **Introduction**

42 1,2-Dichloroethane (DCE) is a short-chain, chlorinated aliphatic hydrocarbon
43 which used mainly in the manufacture of vinyl chloride in dry cleaning, and as
44 metal-degreasing agents in many industries. In China, it is also commonly utilized as
45 the thinner of adhesives and because DCE is highly volatile, therefore, the main route
46 of occupational exposure is inhalation through the respiratory route. After absorbed
47 through the lungs, DCE could rapidly distribute and accumulate in the blood, lung,
48 liver, brain, kidney, and abdominal fat(Sweeney et al. 2008; Take et al. 2013). It could
49 cross the blood-brain barrier easily and a line of evidence has proved occupational
50 exposure to DCE may lead to acute DCE toxic encephalopathy in both exposed
51 workers(Liu et al. 2010; Wang et al. 2013)and had the same effects in mice (Wang
52 et al. 2013; Wang et al. 2014). Meanwhile, Experimental toxicology studies showed
53 DCE exposure have obvious toxic effects on the liver, and kidney. Based on the data
54 reported, DCE also causes toxicity of liver, and several animal tests have showed that
55 DCE resulted in an increase in the absolute and relative liver weights,
56 histopathological changes and abnormal serological indexes (Brondeau et al. 1983;
57 Cottalasso et al. 1994; Daniel et al. 1994; Hotchkiss et al. 2010). However, the
58 mechanisms responsible for these adverse effects remain to be elucidated. Apoptosis
59 is long recognized as an important process in biological systems under both
60 physiological and pathological conditions. Caspases are a family of cysteine proteases
61 that play an essential role in apoptosis. In vitro, DCE could obviously induced kidney
62 cells apoptosis in both animal tests and human embryonic kidney 293 cells(Wenxue
63 Li 2015). However, the cellular mechanisms responsible for DCE-induced
64 hepatotoxicity remain to be determined. In this study, we determined three major mitogen-activated protein kinases
65 (MAPKs) that include the extracellular signal-regulated kinase (ERK), C-JUN
66 N-terminal kinase(JNK) and p38 kinase, which are a family of serine/threonine
67 kinases. MAPK pathways are central components of the intracellular signaling
68 networks that control many aspects of mammalian cellular physiology, including cell
69 proliferation, differentiation, and apoptosis (Matsuoka and Igisu 2002; Wada and
70 Penninger 2004). MAPKs are activated by upstream protein kinases, and then regulate

gene expression through phosphorylation of downstream transcriptional factors (Li et al. 2014; Sui et al. 2014). In general, the activation of p38 MAPK and JNK by certain toxins and cytokines activate the stress response and produce growth arrest and apoptosis. Conversely, the ERK signaling cascade is activated by growth factors and play an important role in cell survival and proliferation (Chao and Yang 2001; McCubrey et al. 2007). However, the role of ERK signaling in liver cellular apoptosis following exposure to DCE has not been clearly delineated.

In this study, we examined the potential liver toxicity of DCE in vitro and in vivo, and showed that DCE could obviously induce liver cell apoptosis in both animal tests and HepG2 cells. The mechanism might be related to the inhibition of ERK 1/2 pathways. Moreover, EGF could activate the ERK1/2 pathway and attenuate the effect of apoptosis induced by DCE.

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471 Student's t-test (*P<0.05, **P<0.01 versus control; #P<0.05, ##P<0.01 versus cells
472 treated with 150µM DCE alone).
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Figure 1

Fig 1 The adverse effects in SD rat liver induced by DCE. Male Sprague-Dawley rats were exposed to DCE 6 h per day for 5 days successively in inhalation chambers. (A) the liver/weight ratios of the rats were calculated; (B) alanine aminotransferase (ALT), (C) aspartate aminotransferase (AST), (D) total cholesterol(CHOL) and (E) triglycerides(TG) of the rats were determined by auto-biochemical analyzer. * $p < 0.05$, ** $p < 0.01$, compared to negative control group.

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Figure 2

Fig 2: The pathological changes and apoptosis of rats' liver in DCE dose groups. After the treatment with DCE for 5 days successively, liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. (A) Tissue sections were prepared and stained with hematoxylin and eosin. The pathology (HE, PAS AND Oil Red O) of liver was examined by optical microscopy ($\times 100$, $\times 200$ magnification). (B) Apoptotic cells in liver after DCE exposure were detected using an in situ apoptosis detection kit as described in the Materials and methods. Photographs were taken by optical microscopy ($\times 100$ magnification). Data are representative of at least three independent experiments. (C) Western blotting was used to determine the levels of phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)).

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Figure 3

Fig 3. DCE inhibits proliferation of HepG2 cells and induces cells apoptosis. (A) HepG2 cells were exposed to 0, 5, 25, 50, 75, 100, 125 and 150 μM DCE for 24 h, 48 h, respectively. Cell viability determined by the WST-1 assay. (B) HepG2 cells incubated with 100, 125 and 150 μM DCE for 48 h. Flow cytometry analysis was performed to assess cell apoptosis. Data are shown as the percent for positive cells of necrosis, apoptosis, and live for three independent experiments. Each point represents the mean $\pm$ SD of three independent experiments. The statistical significance was determined by Student's t-test (* $P < 0.05$, ** $P < 0.01$ versus buffer control).

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Figure 4

Fig 4. DCE increases the levels of cleaved caspase-9, cleaved caspase-3 and decreases ERK1/2 phosphorylation and EGF-stimulated ERK phosphorylations could inhibit DCE-induced apoptosis. (A) HepG2 cells incubated with 100, 125 and 150 μ M DCE for 48 h. Western blotting was used to determine the levels of phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) and cleaved caspase-9, cleaved caspase-3, and β -actin. HepG2 cells were starved for 24 h, and then incubated with EGF (100, 200 and 300 nM) for 5 minutes prior to the addition of 150 μ M DCE followed by an additional 48 h incubation period. After 48 hours of incubation, the cells were collected for apoptosis analysis. Western blotting was used to determine the levels of phospho- Erk1/2 and the levels of cleaved caspase-9, cleaved caspase-3. The results were representative of at least three independent experiments. β -actin was used as an internal reference. The intensity of bands was quantified by densitometric analysis. (B) The apoptotic rate was analyzed by flow cytometry. The results are from one representative experiment of three performed that showed similar patterns. Each point represents the mean $\pm$ SD of three independent experiments. The statistical significance was determined by Student's t-test (* P <0.05, ** P <0.01 versus control; # P <0.05, ## P <0.01

versus cells treated with 150 μ M DCE alone).

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