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Alteration in mitochondrial function and glutamate metabolism affected by 2-chloroethanol in primary cultured astrocytes☆

Qi Sun [a], Yingjun Liao [b], Tong Wang [a], Gaoyang Wang [a], Fenghong Zhao [a], Yaping Jin [a,*]

[a] Department of Occupational and Environmental Health, School of Public Health, China Medical University, Shenyang, Liaoning, People's Republic of China
[b] Department of Physiology, China Medical University, People's Republic of China

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ABSTRACT

The aim of this study was to explore the mechanisms that contribute to 1,2-dichloroethane (1,2-DCE) induced brain edema by focusing on alteration of mitochondrial function and glutamate metabolism in primary cultured astrocytes induced by 2-chloroethanol (2-CE), a metabolite of 1,2-DCE in vivo. The cells were exposed to different levels of 2-CE in the media for 24 h. Mitochondrial function was evaluated by its membrane potential and intracellular contents of ATP, lactic acid and reactive oxygen species (ROS). Glutamate metabolism was indicated by expression of glutamine synthase (GS), glutamate–aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1) at both protein and gene levels. Compared to the control group, exposure to 2-CE could cause a dose dependent damage in astrocytes, indicated by decreased cell viability and morphological changes, and supported by decreased levels of nonprotein sulfhydryl (NPSH) and inhibited activities of Na^+/K^+-ATPase and Ca^{2+}-ATPase in the cells. The present study also revealed both mitochondrial function and glutamate metabolism in astrocytes were significantly disturbed by 2-CE. Of which, mitochondrial function was much vulnerable to the effects of 2-CE. In conclusion, our findings suggested that mitochondrial dysfunction and glutamate metabolism disorder could contribute to 2-CE-induced cytotoxicity in astrocytes, which might be related to 1,2-DCE-induced brain edema.

1. Introduction

1,2-Dichloroethane (1,2-DCE, CAS number: 107-06-2) is one of the highest volume synthetic organic chemicals manufactured in the world. It is mainly used in the manufacture of vinyl chloride. Moreover, this chemical is also used as a general organic solvent, especially as the thinner of adhesives in China. As a volatile solvent, 1,2-DCE may be evaporated quickly into the air in the workplace. At high vapor concentrations, it is known to cause acute and subacute toxic effects on the nervous system, liver, kidney and respiratory system in both humans and laboratory animals (Davidson et al., 1982; Hotchkiss et al., 2010). Accumulated data from the case reports have suggested that subacute poisoning of this chemical has become one of the serious occupational problems in China (Liu et al., 2010). Of which, toxic encephalopathy is the primary clinical manifestation, and brain edema is deemed to be the main pathological change and the cause of death. However, the mechanisms involved 1,2-DCE induced brain edema remain poorly understood.

Abundant experimental studies have suggested that the principal pathway involving 1,2-DCE metabolism is mediated by microsomal cytochrome P450 2E1 (CYP2E1) in animals and, most likely, in humans as well (Guengerich et al., 1980; Reitz et al., 1982; Sweeney et al., 2008). Metabolites generated in CYP2E1 mediated 1,2-DCE metabolism are 2-chloroethanol (2-CE), chloroacetaldehyde and chloroacetic acid. Of which, 2-CE and chloroacetaldehyde are the intermediate metabolites, and assumed to be involved in the mechanisms underlying 1,2-DCE induced toxic effects since they are more reactive than their parent compound. (Guengerich et al., 1980; McCall et al., 1983; Storer and Conolly 1985; Igwe et al., 1986; Raucy et al., 1993). Thus, it is essential to explore the neurotoxic effects induced directly by these chemicals.

Astrocytes, the most numerous glial cells in the mammalian brain, are now thought to be indispensable for neuronal survival and function (Chen and Swanson 2003; Benarroch 2005; De Keyser et al., 2008). For example, they may contribute to prevent the excitotoxicity by removing excess glutamate from the extracellular space through glutamate transporters (Ekblad-Sekund and Walum 1991; Finsterwald et al., 2015). Glutamate, the major excitatory neurotransmitter in the brain, is amidated by glutamine synthetase (GS) to form glutamine following taken up by astrocytes (Anderson and Swanson 2000). GS is localized almost exclusively in astrocytes (Bak et al., 2006). Since no extracellular

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* Corresponding author at: Department of Occupational and Environmental Health, School of Public Health, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang 110122, People's Republic of China.
E-mail address: ypjin@cmu.edu.cn (Y. Jin).

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enzymes exist for glutamate breakdown, its normal levels in the extracellular space are maintained exclusively by a family of Na^+-dependent glutamate transporters (Nicholls and Attwell 1990). Of which, glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST) are localized predominantly in astrocytes (Danbolt et al., 1992; Rothstein et al., 1994; Lehre et al., 1995; Danbolt 2001). It has been disclosed that up to 80% glutamate released into the synaptic cleft is taken-up by astrocytes through GLAST and GLT-1 (Phillis et al., 1994; Levy et al., 1998; Anderson and Swanson 2000).

Studies have indicated that glutamate in the extracellular space may activate three types of ionotropic glutamate receptors, i.e. NMDA, AMPA and kainate receptors. AMPA and kainate receptors are permeable to Na^+ and K^+, and also to low levels of Ca^{2+} (Hollmann et al., 1991; Guerguerian et al., 2002), however NMDA receptors are more permeable to Ca^{2+} than other receptors. Thus, excessive stimulation to these receptors may cause consistently influx of Ca^{2+} and Na^+, leading to calcium overload and water excess in the neurons, as a consequence, cytotoxic edema occurs (Loftis and Janowsky 2003; Han et al., 2004; Osteen et al., 2004). On the other hand, since a high outwardly-directed gradient of glutamate across the cell membrane is maintained in astrocytes, the activities of glutamate transporters are highly dependent on cellular energy supply to ensure efficient uptake of glutamate. Substantial evidence indicated that astrocytic dysfunction has been implicated in a variety of cerebral injuries and neurodegenerative diseases (Maragakis and Rothstein 2006).

Taken together, it is necessary to investigate the alteration of mitochondrial function and glutamate metabolism induced by 2-CE in astrocytes. However, to the best of our knowledge, no studies concerning this subject have been reported.

2. Materials and methods

2.1. Reagents

2-Chloroethanol (2-CE) with purity >99.0% was obtained from the Sinopharm Chemical Reagent Co., Ltd., China. Dulbecco's modified Eagle's medium (DMEM), trypsin, penicillin and streptomycin were purchased from Invitrogen, USA. Heat inactivated fetal bovine serum (FBS) was the product of Hyclone, USA. Poly-L-lysine, dichlorofluorescein diacetate (DCF-DA), 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1) and alamarBlue were obtained from Sigma, USA. Goat and donkey serum, diamidino-phenylindole (DAPI), and secondary antibodies conjugated fluorescein isothiocyanate (FITC), tetraethyl rhodamine isothiocyanate (TRITC) or horseradish peroxidase were purchased from Beyotime Institute of Biotechnology, China. The primary antibody against glial fibrillary acidic protein (GFAP, mouse anti-rat) was the product of Millipore, USA; the primary antibodies against GS (goat anti-rat) and β-actin (rabbit anti-rat) were obtained from Santa Cruz Biotechnology, Inc. USA; GLAST (rabbit anti-rat) and GLT-1 (rabbit anti-rat) were purchased from Abcam, USA. Trizol Reagent, SYBR Premix Ex Taq II, and PrimeScript RT reagent kit were purchased from Takara, Japan. Enhanced chemiluminescence (ECL) plus kit was obtained from Amersham Life Science, UK. The commercial assay kits for analysis of intracellular activities of Na^+/K^+-ATPase and Ca^{2+}-ATPase, and levels of lactic acid, ATP and nonprotein sulfhydryl (NPSH) were purchased from the Nanjing Jiancheng Bioengineering Institute, China. BCA protein assay kit was the product of Thermo Fisher Scientific, USA.

These chemical reagents were prepared as stock solutions with sterile water, and then diluted to the final concentrations before application. Water used in this study was doubly distilled.

2.2. Primary culture for astrocyte

Briefly, the cerebral cortices of newborn Wistar rats, obtained from the animal laboratory of China Medical University were cautiously removed, and washed three times by ice-cold Hanks balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}. They were chopped into pieces <1 mm on each side, and then dissociated with 0.125% (w/v) trypsin solution at 37 °C for 20 min. The cell suspension was filtered through a 200 meshes stainless steel screen. Following centrifugation, dissociated cells were suspended in DMEM containing 1% penicillin-streptomycin and 10% FBS. They were plated in the culture dishes precoated with poly-L-lysine (BD Biosciences, USA) at a density of 1×10^6/ml, and maintained at 37 °C, in 5% CO_2 and 100% humidified atmosphere. The cells were incubated continually, and the media was changed every three days. When a confluent layer was formed, the culture dishes were shaken for 15 h at 250 rpm in the orbital shaker for removing oligodendrocytes. Following vibration, the media was changed immediately, and a nearly pure layer of astrocyte was remained in the culture dishes. The target cells were grown for five days, and then resuspended by trypsin and reseeded in another culture dishes at a density of 1×10^5/ml. A glass coverslip was placed beforehand in a culture dish and a part of cells were plated on it for GFAP staining by immunofluorescence method to identify the astrocyte. After five days in culture, >95% cells were positive for GFAP as illustrated in Fig. 1.

All experiments were carried out in accordance with the National Institutes of Health guidelines in a manner that minimized animal suffering and animal numbers, and were approved by Institutional Animal Care and Use Committee (IACUC) of China Medical University.

2.3. Treatments

Astrocytes were exposed to different levels of 2-CE for 24 h in the media with 5% FBS when a confluent layer was formed. The stock solution of 1 M (mol/l) 2-CE was prepared with redistilled water, and then diluted to the target concentrations by the media containing 5% FBS before application. The cells in the control group were exposed to the media containing 5% FBS without 2-CE for 24 h. After exposure to 2-CE, the cells were collected for the following analysis.

2.4. Analysis

2.4.1. Immunofluorescence staining

Briefly, after washing three times in PBS, the cells were fixed with 4% paraformaldehyde, permeabilized in 0.2% Triton X-100, and incubated with 10% normal goat or donkey serum at room temperature to block nonspecific binding of antiserum. They were then incubated with primary antibodies against GFAP (1:400), GS (1:50), GLAST (1:100,) or GLT-1 (1:100) at 4 °C overnight. Labeled cells were visualized by the

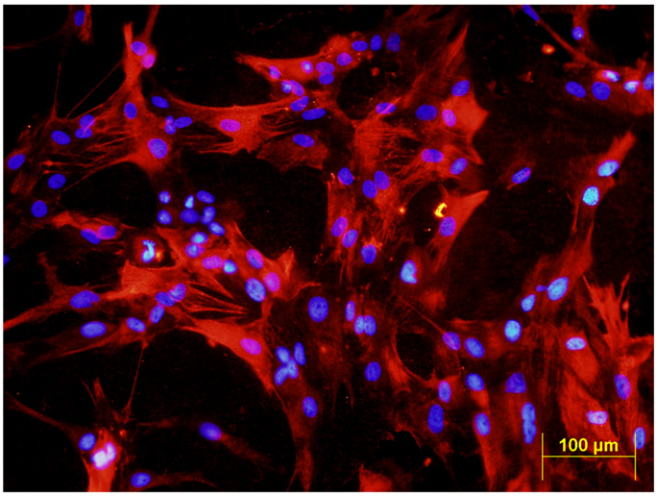

Fig. 1. Immunofluorescence staining for GFAP. Notes: Astrocytes grown on the glass coverslips were stained with antibody of glial fibrillary acidic protein (GFAP) conjugated tetraethyl rhodamine isothiocyanate. The photomicrographs were captured with an Olympus fluorescence microscope (200×).