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Toxic effects of toluene diisocyanate exposure on neural development

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ABSTRACT. Toluene diisocyanate (TDI) is widely used for the synthesis of polyurethane products and caused serious environmental problems. The accumulation of TDI in human body may cause respiratory disease. However, the toxicity of TDI to neural development has been rarely reported. The current study aimed to investigate the toxic effects of TDI exposure on neural development. TDI was injected to the pregnant ICR mice and the proliferation of neurons in vivo was detected. In addition, the toxicity of TDI on cultured PC12 cells was estimated using cell viability assay. The results showed that the exposure to TDI could significantly inhibit proliferation of neurons, suppress cell proliferation, induce cell injury, and promote cell apoptosis. TDI may be a potential toxicant on nervous system.

Key words: Toluene diisocyanate; Neural development; Toxicity, apoptosis.

INTRODUCTION

Toluene diisocyanate (TDI), the main constituent of polyurethane products, is widely used for the chemical synthesis of foam plastics, coatings, rubber and other related products (Crescentini et al., 2019). TDI causes serious environmental problems and exhibits accumulation and latent in human body, resulting in direct irritation to the skin, eyes and respiratory tract (Aihara et al., 2020; Jiao et al., 2021). TDI has been confirmed as a leading reason for occupational asthma around the world (Lee et al., 2018). The long-time exposure to high concentration of TDI may cause pneumonia, pulmonary edema, and then lead to progressive lung dysfunction, even lung cancer (Pinkerton et al., 2016). Nowadays, TDI is considered to be a chemical substance that poses a threat to human health.

The mechanism of cortical development and brain related diseases is one of the hot topics in the fields of biology and medicine. In the developing cerebral cortex, most of the cells must migrate to the destination, followed by differentiation and connection with other neurons (Hirota and Nakajima, 2017). Dysregulation in brain neuron migration and development may result in nervous system defects and related diseases, such as cerebral fissure, stroke, anencephaly, epilepsy and autism (Lybrand et al., 2021; Yang et al., 2019). Several risk factors have been confirmed for cerebral cortex development, including perinatal exposure to harmful chemical reagents (Li et al., 2021) and metabolic disorders (Lin et al., 2021). To avoid the risk factors for cerebral cortex dysregulation is key for human healthy.

A systematic review based on public databases found that the exposure to diisocyanate might be a risk factor for neurotoxicity (Hughes et al., 2014). However, due to the lack of cell, animal and clinical experiments, there was no sufficient evidence for the causal association between neurotoxic effects and diisocyanate exposure. In the present study, we investigated the toxicity of TDI to neural development.

MATERIALS AND METHODS

Cell line and cell culture

PC12 cells were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cell culture was performed using Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich) with addition of 10% fetal bovine serum (FBS) (Gibco) and 1% penicilin/streptomycin. The cells were incubated at 37 °C with the present of 5% CO_2, then harvested at logarithmic phase for the further analyses.

TDI treatment

TDI was diluted to 100 μM in serum-free medium OPI-MEM, and the cells were washed twice with OPI-MEM before TDI treatment. The cells were treated by 0 nM (negative control), 2 nM, 5 nM, 10 nM, 20 nM and 40 nM TDI, respectively. Then, the cells were incubated at 37 oC with the presence of 5% CO2 for 24 h, and harvested for the further analyses.

Cell viability assay

The viability of PC12 cells exposure to TDI for 24h was estimated by MTT assay. The cells were seeded into 6-well culture plate in the density of 5×10^3 cells/well or 2.5×10^3 cells/well, and

20 μL MTT (5 mg/ml; Sigma, St. Louis, MO, USA) was added to the cell. The plate was incubated in the dark for 4h, and 150 μL dimethyl sulfoxide (Sigma) was added and incubated for additional 15min.

Animal procedures

The animal procedures were performed following the guidance issued by the Laboratory Animal Studies Committee of Huaihe Hospital of Henan University. In the current study, ICR mice were used. The day of vaginal plug detection was defined as gestation day 0.5 (E0.5), and the day of birth was considered as postnatal day 0 (P0). To investigate the toxic effects of TDI on cell proliferation in the VZ (Ventricular zone) and SVZ (subventricular zone) of cerebral cortex, the mice were randomly divided into TDI group and control group. For mice in TDI group, intragastric administration of TDI (1.2 mg/kg) was performed from E10.5 to E16.5. The same volume of corn oil was given to the mice in control group. All the mice received intraperitoneal (i.p) injection of 5-Bromo-2-Deoxyuridine (BrdU, 50 mg/kg) at E15.5. The mice were sacrificed 2h later, and confocal analysis was performed to investigate cell proliferation. Cell cycle assay was performed after 24h of BrdU injection.

Cell fluorescence immunostaining

The cells were fixed with 4% formaldehyde solution for half an hour, and then washed by 0.1 M PBS buffer for three times. After blocked for 1h, the cells were incubated with the primary antibodies at 4 °C for overnight. Subsequently, the cells were incubated with the secondary antibody for 1 h at room temperature. 1μg/ml DAPI was added for nuclear staining.

Statistical analysis

All the data analysis was performed using SPSS 18.0 software, and the figures were plotted by GraphPad Prism version 5.0. The continuous variables were shown as mean ± Standard deviation (SD). The differences of continuous data between two groups were estimated via student's t test, and their comparison among three or more groups was performed using ANOVA.

RESULTS

To investigate the toxic effects of TDI on cell proliferation in the VZ (Ventricular Zone) and SVZ (Subventricular Zone) of cerebral cortex, the mice were randomly divided into TDI group and control group from E10.5 to E16.5. Cell cycle assay was performed after 24 hours of BrdU injection. MTT analysis was performed to show the influences of TDI treatment on proliferation of PC12 cells. Compared to negative control, the exposure to TDI could significantly impair viability of PC12 cells. Moreover, the inhibitory effects of TDI on cell viability exhibited dose-dependent manner. Moreover, the cell injury caused by TDI exhibited dose-dependent manner. In addition, the exposure to TDI could also cause toxicity to Hela cells and PC9 cells (the data were not shown). The toxicity of TDI exposure to neurons proliferation in VZ and SVZ of cerebral cortex was estimated via confocal analysis. The newly proliferative neurons were labeled by BrdU staining, and all the neurons were stained by PI. Ki67 is a hallmark for cell proliferation which is increased in S phase of cell cycle. The neural stem cells and progenitor cells could be labeled by Pax6. As shown in figure 1, the number of Brdu-positive cells was significantly decreased in TDI group, compared to control group. TDI exposure could significantly reduce the numbers of proliferative neurons in IZ/VZ and

DCP. Based on Ki67 staining results, we found that the number of Ki67-positive cells was decreased after TDI exposure, revealing the inhibition of TDI on proliferation of neurons. In addition, Pax6 staining results showed that TDI exposure resulted in inhibition on proliferation of neural stem cells and progenitor cells in IZ/VZ. Quantitative analysis showed that the percentages of BrdU-positive cells, Ki67-positive cells and Pax6-positive cells were significant differences in TDI and control groups, respectively.

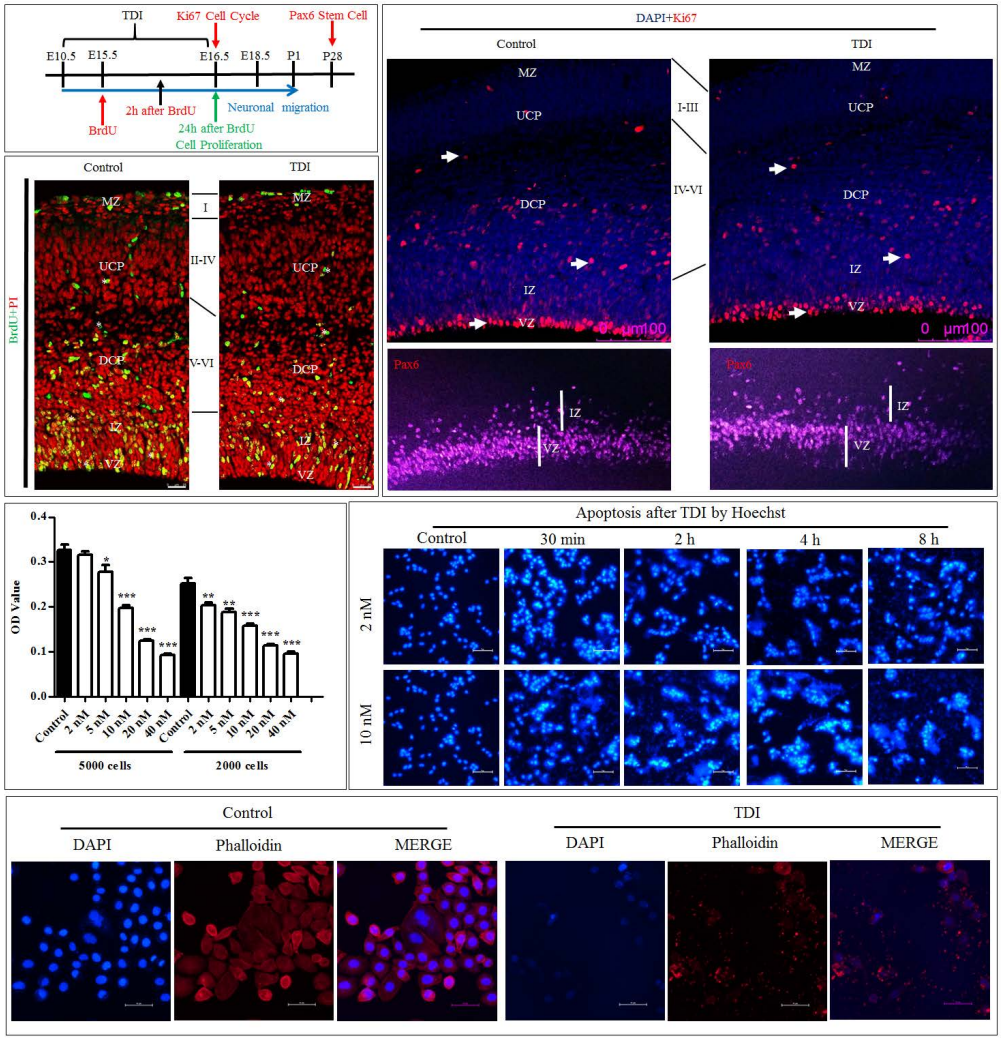

Figure 1. The effect of TDI on nerve cell.

The effect of TDI on proliferation of neural stem cells and progenitor cells staining by BrdU (novel proliferative neurons), Ki67 (cell cycle) and Pax6 (progenitor cells) staining. Compared to the control, the exposure to TDI could significantly impair the proliferation of PC12 cells (Hoechst), exhibiting dose-dependent manner (*p<0.05; **p<0.01; ***p<0.001). TDI exposure could significantly reduce the number of neural progenitor cells in VZ (Ventricular Zone) and SVZ (Sub-Ventricular Zone), and the proliferative neurons in DCP (Down Cortical Plate). The percentage of cells stained by BrdU, Ki67 and Pax6 was significantly lower in TDI group than in control group, revealing the toxicity of TDI on neuron proliferation.

DISCUSSION AND CONCLUSION

TDI is a type of chemical intermediate containing bifunctional cyanante ligands, which is widely utilized for chemically synthesis of high polymer materials. According to the carcinogen list published by World health organization in 2017, TDI is defined as a Class 2B carcinogen. TDI could cause direct harm to human body, and the frequently observed symptoms include eye pain, tears, conjunctival congestion, cough, chest tightness, breath shortness, asthma, red papules, macular papules (Prueitt et al., 2017). The international limit of free TDI is below 5%, and the maximum allowable concentration of TDI in space air is 0.1 mg/m^3, and the limit concentration is 0.02 ppm (0.14 mg /m^3) according to the national occupational health standard (GBZ2.1-2007). The exposure to high concentration of TDP may influences the percentages of innate and adaptive immune cells, as well as the production of cytokines, thus causing toxicity (Pollaris et al., 2021; Tajima et al., 2020). Recently, more and more studies have been devoted to explore the acute TDI poisoning and how to protect, but the chronic and cumulative damages are rarely referred. Until now, the effects of TDI on mammalian embryonic brain development toxicity and nerve damage have been rarely reported. To address the issue, the present study was designed.

The accumulation of TDI in human body may induce systemic T cell-dependent sensitization, and excessive production of inflammatory factors, thus leading to asthma (Deng et al., 2021). TDI exposure is recognized as a major cause of occupational asthma. In addition to respiratory disease, TDI exposure may also cause intestinal obstruction (Shadnia et al., 2013). However, few studies have been designed to explore the toxic effects of chronic and accumulation of TDI on neural development. Only some case reports and cross-sectional studies had reported the neurotoxicity of long-term TDI exposure (Hughes et al., 2014). The exposure to high concentration of TDI might result in headache, inattention, poor memory, irritability, depression. All the signs revealed the possible toxicity of TDI to neural development. However, the causal relationship between TDI exposure and neural diseases requires further verification based on cell experiments, animal models and clinical investigations. In our study, we found that TDI exposure could significantly suppress the proliferation of neural stem cells and progenitor cells in mice model, revealing the neurotoxicity of TDI.

In our study, PC12 cells, a commonly used nerve cell line, were cultured and exposed to different concentrations of TDI. Analysis results showed that the exposure to TDI could obviously impair viability of PC12 cells through suppressing cell proliferation and promoting apoptosis. Moreover, the toxicity of TDI to PC12 cells was in a dose-dependent manner. Several limitations in current study should be stated. First, only one cell line was used in our study. In consideration of the heterogeneous across different types of neural cell lines, further cell lines are required to verity of our results. Second, the animal models are needed to explore the influences of TDI exposure to neural development in vivo. And, the big data analysis based on clinical investigations are also necessary. In addition, the molecular mechanisms underlying the function of TDI in neural development remains poorly known. Therefore, further studies are required to verity and improve our analysis.

In conclusion, the exposure to TDI may cause dysregulation of neural development via suppress proliferation of neural stem cells and progenitor cells.

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Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

CREDIT AUTHOR STATEMENT

Yao Xu: Investigation; Data curation. **Lei An:** Conceptualization; Writing-original draft. **Xuhong Lin:** Conceptualization; Resources; Supervision.

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