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Original research

Chronic chlorinated benzene exposure induces glial cytoskeleton disturbance in the mouse brain and enteric glia

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Abstract. Chlorinated benzenes are widely used solvent for various targets including industrial production of adhesives, drugs, rubber, paints and dry cleaning, and as well as fiber-swelling agent in the textile processing. Similar with other thinners chlorobenzeneis potent to induce detrimental effects in different cell types and could cause the functional disorders of the central nervous system. In spite of the progress in xenobiotic toxicity study, the molecular mechanisms of chlorobenzenecytotoxicity remain undiscovered. The search of molecular markers that are potent to characterize toxic risk chronic thinner exposure is an actual task whole world. Cytoskeleton protein expression is modulated with number of the environmental pollutants. Intermediate filament proteins are histology-specific components of cytoskeleton in the most eukaryotic cells. The glial fibrillar acid protein (GFAP) is a basic component of the astrocyte cytoskeleton and could be used as the reliable biomarker of cytotoxicity. Chlorobenzene effect on the mouse central neural system (CNS) and enteric glial cells was studied with using chronic (30 days) low dose exposure to thinner in vivo model. Obtained results demonstrated that cellular response against thinner toxicity is accompanied by both redox and cytoskeleton disturbance. In the present study, we investigated the GFAP level in the brain and thin intestine to estimate its prognostic value in the model of chlorobenzene chronic intoxication. Prolonged treatment with low doses of chlorobenzene induced in both brain and intestine tissue statistically significant ($P < 0.01$) upregulation of GFAP and redox imbalance. Observed results have shown that low doses of chlorobenzene develop unique gliotoxic features in respect to CNS astrocytes and enteric glial cells.

Keywords: Cytoskeleton; GFAP; Biomarkers; Chlorobenzene; Oxidative stress

Хронічний вплив хлорбензолу спричиняє порушення гліального цитоскелета в мозку та ентеральній глії мишей

Анотація. Хлоровані бензоли є тотально використовуваними розчинниками для різних цілей, включаючи промислове виробництво клеїв, ліків, гуми, фарб і хімчистки, а також як засіб для набухання волокон у обробці текстилю. Подібно до інших розріджувачів та розчинників, хлорбензол спроможний викликати шкідливі ефекти в різних типах клітин і може спричиняти функціональні розлади центральної нервової системи. Незважаючи на прогрес у вивченні токсичності ксенобіотиків, молекулярні механізми цитотоксичності хлорбензолу залишаються нерозкритими. Пошук молекулярних маркерів, здатних характеризувати токсичний ризик хронічного впливу рочинників, є актуальним завданням в усьому світі. Експресія білків цитоскелету модулюється значною частиною сучасних забруднювачів навколишнього середовища. Білки проміжних філаментів є гістологічно специфічними компонентами цитоскелета більшості еукаріотичних клітин. Гліальний фібрилярний кислотний білок (GFAP) є основним компонентом цитоскелета астроцитів і може бути зручним біомаркером ушкоджень. Вплив хлорбензолу на ЦНС і кишкові гліальні клітини мишей досліджували при тривалому (30 днів) впливі низьких доз розчинника *in vivo*. Отримані результати показали, що клітинна реакція на токсичність розчинника супроводжується як окислювально-відновними, так і цитоскелетними порушеннями. У цьому дослідженні ми визначали рівень GFAP у головному мозку та тонкому кишечнику щоб оцінити його прогностичне значення як біомаркера шляхом моделювання хронічної інтоксикації хлорбензолом. Тривале лікування низькими дозами хлорбензолу викликало статистично значуще ($P<0,01$) підвищення регуляції GFAP і окислювально-відновний дисбаланс як у мозку, так і в кишківнику. Результати спостереження показали, що низькі дози хлорбензолу виявляють унікальні гліотоксичні властивості щодо астроцитів ЦНС і кишкових гліальних клітин.

Ключові слова: цитоскелет; ГФКБ; біомаркери; хлорбензол; окислювальний стрес

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Introduction

Industrial solvents are complex chemical mixtures containing toluene, benzene, acetone, methanol, hexane and other substances. They are widely used in the textile industry in the production of solvent-based paints and cleaning fluids. These substances, in particular toluene and benzene, make up approximately 70% of most industrial solvents. They are well known as neurotoxic agents (Hodgkinson, 2006). Chlorobenzene is widely used as a solvent, a component of organic synthesis to construct synthetic polymers (Zhang et al., 2017). Most of thinners enter the body of animals and human mainly through the respiratory tract, but can also be ingested with a food. They are mainly neurotoxic agents and cause functional disorders of the central nervous system (CNS) (Nagyeri, 2012; Nedzvetsky et al., 2021). The progress in industrial organic synthesis accompanied by global thinner application. The pivotal mechanism of chlorobenzene toxicity was confirmed in several reports where endocrine system disruption is recognized as a key component (Molnár et al., 2015). However, neurotoxicity of chlorobenzene remains undiscovered. Several data are evident that chlorobenzene should be considered as an ecotoxic compound which poses a threat to significant environmental areas including industrial urban location.

Chronic solvent abuse leads to structural and functional disorders of various organs. Inhalation of more rarefied vapors is an important cause of encephalopathy and can lead to irreversible brain damage (Lee, 2006; Lacerda et al., 2012). Chronic exposure to low doses of chlorobenzenes lead to initiation of both physiological and behavioral abnormality (Valkusz, 2011). Recent data have shown that low doses of similar environmental contaminants may pose a potential risk of anxiogenic and detrimental effects in exposed subjects, including humans (Sepp et al., 2018).

The designing of animal models with use of molecular markers makes it possible to adequately and reliably assess the functional state of the cell, and indirectly characterize common organism functions. Thus, the search of molecular markers is important part ofthe early impact estimation of detrimental ecotoxic effect on the nervous tissue cells. Tissue-specific proteins are particularly sensitive to environmental stressors, including different thinners and organochlorine-containing solvents. Intermediate filament protein, especially glial fibrillar acidic protein (GFAP), is a most contained component of the astrocyte cytoskeleton. GFAP is recognized as glia-specific marker. Its upregulation reflects adverse CNS disorders across species from fish to mammals (Nedzvetsky et al., 2021).

The mammals are widespread species which has high number in the different world areas and the climate zones. In some ecosystems their number may exceed the number of another animals. Thus, mammal species may be considered as useful bio-indicative object. On the other hand, large amount mammal species suffer from environmental toxicants exposure to high extent as the carnivorous link in a food chain.

Evidence observed in the epidemiological study suggests that household exposure to chlorobenzene in concentrations below 10 μg/m^3 is associated with allergic sensitization as well as dysregulation in T-cell immunity in children (Gascon et al., 2014). Occupational studies have shown that chronic exposure to chlorobenzene can lead to the number of diseases including CNS depression, eye and respiratory irritation (Willhite & Book, 1990), and immune dysfunctions (NIOH/NIOSH, 1994).

The legal limits imposed on the environmental and/or food concentrations of organochlorines are largely based on the results of investigations relating to toxic amounts far higher than those usually found in the environment and much less so on lower in respect with usually subtoxic concentrations (McKinlay et al., 2008). We therefore set out to examine the effects of long-term, oral exposure to a subtoxic dose lower than usually used in toxicological study. There was reported that combined with the lowest observed toxic effects of an arbitrarily chosen mixture contained various thinners and chlorobenzenes, since the contamination of the environment seldom tends to be due to a single compound (Diamanti-Kandarakis et al., 2009). Therefore, neurotoxic effect of chlorinated organic thinners is an actual item of both human and animal toxicology.

The aim of our study was the detection GFAP level in the brain and enteric glia of mouse to estimate glia specific cytoskeleton protein measuring as a prognostic molecular marker of neurotoxicity induced by chronic exposure to low doses of chlorobenzene.

Materials and methods

Animals, experimental design

Male Balb/c mice (n=10; 4 monthes aged) weighing 30–40 g were obtained from the Animal Experimental Unit, Dnipro State Agrarian and Economic University. Mouse were kept under standard condition of 22 ± 3°C temperature; relative humidity 55±5 % and maintained on a 12 h light: 12 h dark cycle. The animals were maintained on a standard rodent pellet diet with tap water available ad libitum. The experimental protocols were reviewed and approved by the Regional Committee for the Ethical Use of Animals (Dnipro, Ukraine).

The exposure to solvent of the mouse group (n=5) was carried out by chronical schem following 30 days. The animals were treated with a mixture (1:1) of 1 mg/kg each of hexachlorobenzene and 1,2,4-trichlorobenzene dissolved in olive oil (1:100) via a gastric tube every day in a morning 10 AM. The animals of control group (n=5) were treated with the same volume of olive oil.

Protein sample processing

The animals of both groups were sacrificed by cervical dislocations with using mild diethyl ether narcosis and the brain and thin intestine were removed. Duodenum, jejunum, ileum, hippocampus, cortex and cerebellum were separated and washed with phosphate buffer saline (PBS). The tissue samples were used fresh or kept at -800 C. Fresh or frozen tissue samples were homogenized 1:10 (w/v) in buffer 10 mM Tris–HCl, pH7.4, 0.1 mM NaCl, 0.1 mM phenylmethylsulfonylfluoride (PMSF), 5 μM soybean trypsin inhibitor and 0.1 mM aprotinin (Sigma, St. Louis, MO, USA). The homogenates were centrifuged at 60,000 g for 60 min. After that the fraction of water-soluble proteins was took away with a supernatant. The residue was washed with PBS, resuspended in homogenizing buffer and recentrifuged at 60,000 g for 60 min. The resulting pellets were washed and resuspended in buffer (25 mM Tris–HCl, pH 7.4, 0.1 mM PMSF and 4 M urea). The fraction of water-insoluble proteins was taken after following centrifugationat 60,000 g for 60 min.

Immunoblot

The determination of both the content and the polypeptide fragment composition of the glial intermediate filaments in both brain and intestine tissue were carried out with immunoblot technique. Obtained protein extracts were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with using reducing sample buffer containing 2% β-mercaptoethanol. PAAG electrophoresis performed according to Laemmly (Laemmly, 1970). Equal amount of total protein 50 μg/track were applied on each lane as described previously (Nedzvetsky et al., 2012). Separated proteins were transferred to nitrocellulose filters (Schleicher and Schuell Inc., Keene, NH, USA) with an electro blotter 170 mA during 90 min. After saturation of the nonspecific sites of binding with 1% bovine serum albumin in 100 mM NaCl, 20 mM Na_2HPO_4, and 20 mM NaH_2PO_4 at pH 7,2, the membranes were incubated overnight at 40 C with anti-GFAP antibodies (Santa Cruz, sc-9065, 1:2000). The membranes

were washed and incubated for 1 h with a secondary antibody, a goat anti rabbit antibodies HRP conjugate (Sigma-Aldrich, 12-348, 1:20000). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. The relative amount of GFAP from mouse brain on the immunoblot membrane was quantified as the arbitrary units by scanning the blots with an adapted software program. Densitometric analysis of the pictures was performed with using of densitometry software TotalLab TL120 (USA) and normalized to the intensity of the respective bands obtained for according β-actin detection. Each trace was corrected for background by subtracting a tracing of nonreactive area on the immunoblot membrane. Protein bands of various molecular weights were identified by extrapolation of plots for relative mobilities of prestained proteins with marked molecular weight (PageRulerPrestained Protein Ladder, Fermentas, Germany, cat. no. 26616).

The detection of oxidative stress generation

The measuring a level of oxidative stress was performed via measuring final products of lipid peroxidation in homogenates of tissue. Total protein levels were measured according to Bradford method with modification (Bradford, 1976). Tissue lipid peroxidation (LPO) (malondialdehyde + 4-hydroxyalkenals: MDA + 4-HDA) was determined using an LPO-586 kit (Oxis International, Inc., Corvallis, OR, USA). Used method is based on a reaction of N-methyl-2-phenylindole with MDA + 4-HDA at 450 C.

Statistical analyses

Data are presented as mean ± standard error (M±SE). Between-group differences in biochemical data were analyzed by one-way analysis of variance (ANOVA) with the post hoc Duncan's multiple range tests. Between-group differences in GFAP content were assessed by variance analysis for repeated measurements (ANOVA) followed by Fisher's post hoc test for all groups.

Results

The detection LPO level in the tissue samples was carried out to assess possible cell membrane damages caused by thinner exposure. The measuring LPO products content in all selected brain areas shown significantly increased LPO level in exposed to solvent group in compare to control mouse group (P<0.01). This result is evidence of oxidative stress generation into neural tissue treated with solvent mouse group (Fig. 1).

The detection GFAP level in the brain and thin intestine samples was carried out to assess gliotoxic effect caused by thinner exposure in GFAP-positive glial cells. Quantitative analysis of the GFAP level in the mouse brain showed considerable increase of the GFAP under chlorobenzene treatment. The insoluble cytoskeletal GFAP fraction in the mouse brain exposed to chlorobenzene increased in all investigated areas hippocampus, cortex and cerebellum in comparison with a control group (Fig. 2).

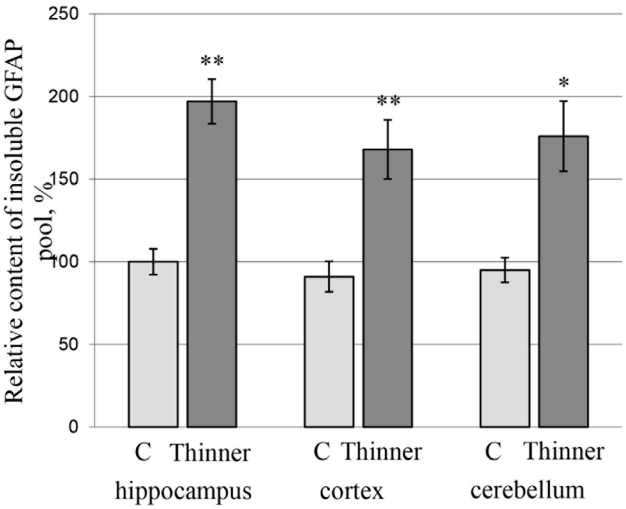

Fig. 2. The content of insoluble GFAP pool in the hippocampus, cortex and cerebellum of control (C) and exposed to chlorobenzene group (Thinner). Significance of the differences in compare to control group: * - P < 0.05, ** - P < 0.01.

The increasing of soluble fraction of GFAP in the animal brain was less pronounced and observed in all of these areas (Fig. 3).

Immunoblot analysis revealed a significant increase in GFAP content of the group treated with thinner inhalation (Fig. 3). In addition to the intact polypeptide 49-kDa GFAP, degraded GFAP (lower molecular weight GFAP protein) content was also significantly higher in thinner-exposed animals compared to those the non-exposed animals (Fig. 4 - 5). The level of GFAP degradation products was higher in a brain of animals of thinner-exposed. Considerable increase of the GFAP degraded polypeptide fragments of 40-49 kDa was found in the brain of experimental animal group.

The content of GFAP in the intestine system was measured to characterize the detrimental effect of chlorobenzene on enteric

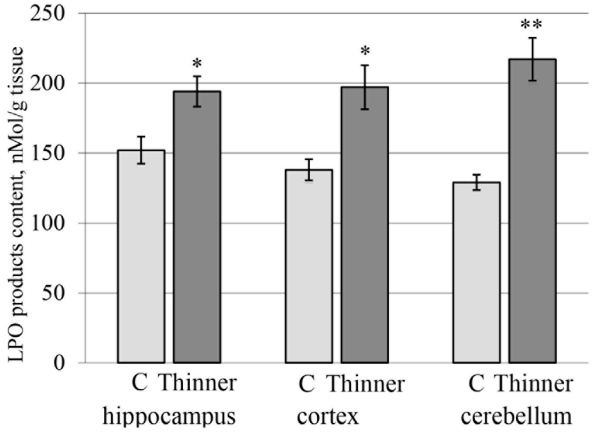

Fig. 1. The content of LPO products in a brain of control and treated with chlorobenzene mouse groups. Significance of the differences in compare to control group: * - P < 0.05, ** - P < 0.01.

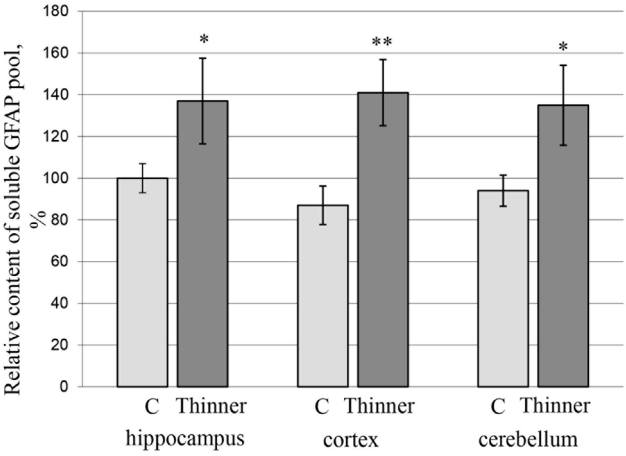

Fig. 3. The content of soluble GFAP pool in the hippocampus, cortex and cerebellum of control (C) and exposed to chlorobenzene group (Thinner). Significance of the differences in compare to control group: * - P < 0.05, ** - P < 0.01.

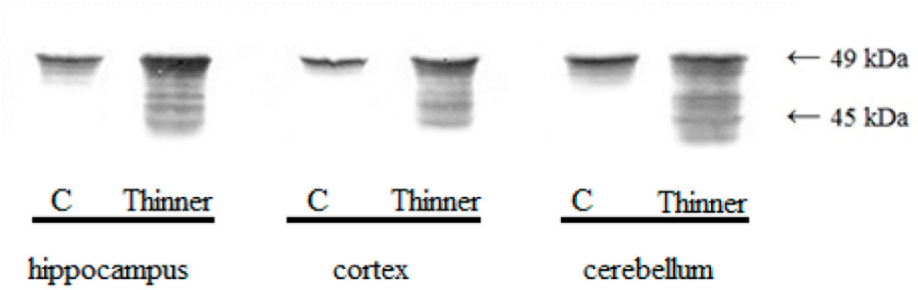

Fig. 4. Immunoblot results of GFAP insoluble pool in hippocampus, cortex and cerebellum of control (C) and exposed to chlorobenzene group (Thinner).

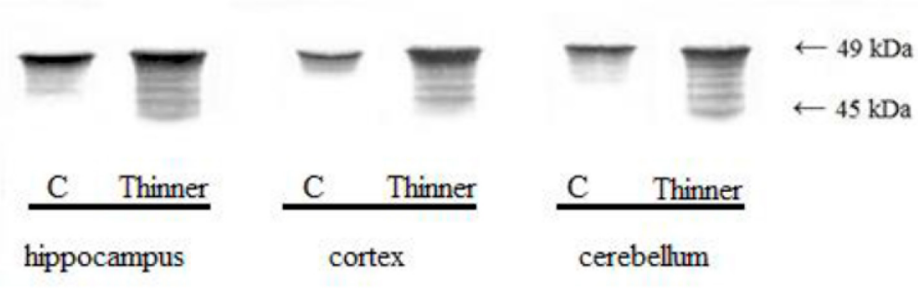

Fig. 5. Immunoblot results of GFAP soluble poolin hippocampus, cortex and cerebellum of control (C) and exposed to chlorobenzene group (Thinner).

neural system. The detection of total GFAP pool in thin gut areas has showed the increase in the content of this cytoskeleton protein in thinner exposed group compared with control group (Fig. 6-7).

The total enteric GFAP pool content in exposed group was statistically increased in duodenum ($P < 0.05$), jejunum ($P < 0.01$) and ileum ($P < 0.05$). The relativeincrease in GFAP content in the intestinal system was comparable with GFAP upregulation in the brain of thinner exposed animals.

Discussion

The harmful effect of the thinners exposure well known in respect to both human and animals. Chlorobenzene is a volatile organic compound that evaporates into the atmosphere at room temperature. It is mainly used as a solvent in pesticide production, as a degreasing agent, and as an intermediate in the synthesis of other halogenated organic chemicals. The total accumulation of pesticides into soil and water recourses requires detailed elucidation of the mechanisms of detoxication these chemicals (Wang, 2023). Chlorinated benzenes are widely used as a solvent for adhesives, rubber and paints and included as a fiber swelling agent in textile processing (Diez et al., 2006; Merck, 1989; Willhite & Book, 1990). Chlorobenzene has been found in sewage, surface water, groundwater and even drinking water. Based on various national studies, the US Environmental Protection Agency (EPA) has estimated concentrations of chlorobenzene in groundwater to be less than 1–5 µg/L and in surface water to be less than 1 µg/L (Ehrlich, 1988). Because of its high volatility, chlorobenzene is expected to

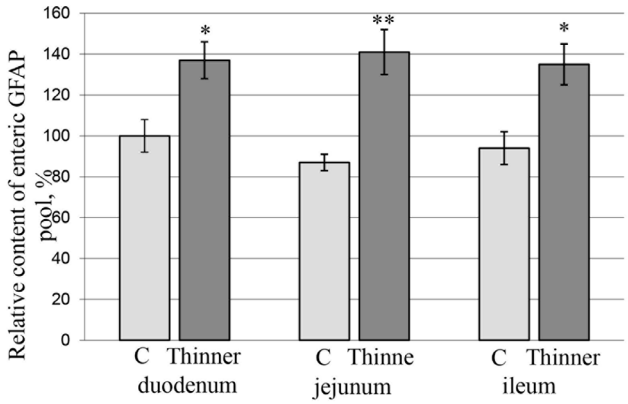

Fig. 6. The content of GFAP in duodenum, jejunum and ileum of control (C) and exposed to chlorobenzene (Thinner) mouse groups. Significance of the differences in compare to control group: * - $P < 0.05$, ** - $P < 0.01$.

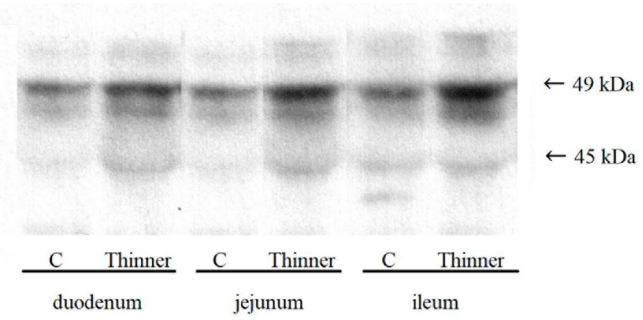

Fig. 7. The immunoblot results of GFAP in duodenum, jejunum and ileum of control (C) and exposed to chlorobenzene (Thinner) mouse groups.

rapidly evaporate into the air when it enters surface water, but when it enters the ground, it is expected to first bind to soil and then slowly migrate to groundwater. Scientific literature data indicate average concentrations of chlorobenzene in contaminated soils as high as 37 ppm (Ehrlich, 1988). Given the fact that chlorobenzene evaporates quickly, its levels in the air can exceed those in water and soil. Compared to general outdoor levels of chlorobenzene, much higher levels of chlorobenzene in the air are found in work environments. Reported levels in the workplace range from 18.7 mg/m^3 to 488 mg/m^3 in different countries (Kusters & Lauwerys, 1990). Indoor air concentrations of chlorobenzene have been reported to be relatively low (Diez et al., 2000; Rolle-Kampczyk et al., 2006). However, long-term exposure to low concentrations of thinners, particularly while either oral consumption or inhalation occurred the potential of harmful effect for human health should be assessed.

Observed in present study both increase in LPO and GFAP upregulation is evident link between oxidative damage and glial cell response against thinner-induced injury. Thinner initiated oxidative injury can provoke multiple disturbances in various regulatory pathways. It is generally assumed that the toxic effects of chlorobenzene are indirectly associated with reactive metabolites and its toxicity in respect with cellular structures in target organs. Similar upregulation GFAP in CNS and in enteric nervous system caused with chronic chlorobenzene exposure is reported first time. However, the small number of potential molecular sensors that cause various toxic effects of chlorobenzene are still unknown. Currently, there was discovered several critic molecular mechanisms of proinflammatory processes in response to chlorobenzene. Symptom relief NF-κB is a central intracellular signaling pathway involved in inflammatory responses to external stressors (Thoma & Lightfoot, 2018). This pathway is known to be the central target for the toxic effect of various substances, including polycyclic aromatic hydrocarbons (Qin et al., 2012). Since activation, genes responsible for inflammatory processes in the body, including MCP-1, are expressed through NF-κB pathway. The mitogen-activated protein kinase (MAPK) pathway is another signaling pathway involved in the development of inflammatory processes in response to environmental pollutants (Prabhakaran et al., 2011). The effect of chlorobenzene exposure on the NF-κB activation and MAPK signaling pathways as well as the significance of this pathway for chlorobenzene-induced release of MCP-1 was reported in lung epithelial cells (Röder-Stolinski et al., 2008). Chlorobenzene exposure accompanied by oxidative stress initiation in human lung epithelial cell culture (Feltens et al., 2010). Furthermore, there was demonstrated that chlorophenols exposure induces endoplasmic reticulum (ER) stress which linked to the programmed cell death initiation in mammalian cells (Zhang et al., 2016).

Other detrimental effect of this solvent is a disturbance in endocrine system. This effect was confirmed in respect to adrenocorticotrophic hormone release (Molnár et al., 2015), prolactin secretion (Sepp et al., 2018) and behavioral disturbance (Nagyeri et al., 2012).

Neurotoxicity of halogenated hydrocarbons is the undoubtable fact, but the mechanisms of its detrimental effects remain unknown. This fact can be explained as result multifactorial effects used halogenated aromatic hydrocarbons on neural and glial cells. There was demonstrated that intraperitoneal injection of industrial thinner causes a significant elevation in the rate of ROS generation and a reduction in GSH levels in the rat brain (Mattia et al., 1993). Quite possible, biology effects of halogenated aromatic hydrocarbons are different chlorobenzene mechanisms of effects on neural tissue cells. On the other hand, this probably relates to the fact that much larger quantities of thinner translocation into the brain tissue after its intraperitoneal administration.

In the CNS, astrocytes contain the basic pool of antioxidant (Zhu et al., 2022). Glial cells have been shown to play a key role in the maintenance of neuronal GSH and to protect neurons from oxidation-caused neurotoxicity (Wei et al., 2020). Thus, astrocytes are believed to maintain neuronal oxidant-antioxidant homeostasis. The sufficiency of intracellular concentration of antioxidants, especially of GSH, is essential to protect neurons from neurotoxic insults (Jia et al., 2009). The astrocytes protect the neurons against various toxic agents. Glia-dependent protection is realized through the growth of metabolic activity of the glial cells firstly. It is accompanied with upregulation GFAP expression.

Astrocytes exhibit the earliest cellular responses following the CNS insult. This response is characterized by an overexpression of GFAP (Kuryata et al., 2021). In the present study, we demonstrated that thinner exposure promotes GFAP expression and its fragmentation in the brain tissue. Enhanced GFAP expression, reflecting reactive gliosis, has described in rat brain affected with toluene fume inhalation (Baydas et al., 2003; Nedzvetskii et al., 2012). In that study, toluene exposure augmented the GFAP content, particularly in the hippocampus. Toluene induced reactive gliosis is believed to be due to toluene-induced generation of ROS. Thus, our results show that chlorobenzene and toluene possess similar neurotoxic effects.

Glial cells appear to be more vulnerable than neurons to neurotoxic insults and also more sensitive to neuroprotective treatment (Lohren et al., 2015; Nedzvetsky et al., 2021). They play a vital role in regulating both the environmental milieu of neurons and the interactions between neurons and capillaries. The collective data indicates that antioxidants may protect various tissues against neurotoxic chemicals such as thinner by enhancing cell surviving (Baydas et al., 2003; Faraj et al., 2022).

In the current study expanding state of oxidative stress induced by chronic consumption of chlorobenzene in mouse brain and thin intestine was demonstrated. Furthermore, now we found that accumulation of ROS caused by chlorobenzene initiates progression of glial reactivity to be a result of astrogliosis in the brain. For this reason, a major mechanism that is a focus of considerable research is the role of lipid and protein peroxidation as well as astrocyte differentiation and proliferation in brain tissue in thinner toxicity.

Neurodegeneration occurs in both central and peripheral neural system and role of glial cells in various disorders is well-documented fact (Kuryata et al., 2021; Quincozes-Santos et al., 2023). Observed in our study upregulation GFAP content in the enteric tissue supports the hypothesis on similar features of CNS astrocytes and enteric GFAP-positive glial cells. On the other side, the role of enteric glia remains poor studied (Seguella & Gulbransen, 2021). In our studies we used GFAP as additional biochemical indicator for the evaluation of damaging action of chlorobenzene into both neural and intestinal tissue. Observed in our study results have shown similar upregulation in GFAP expression in CNS areas and intestine enteric system. These data could be related with tight cognation between astrocytes and GFAP-positive enteric glial cells. Both of them potent to express GFAP unlike other glial cell populations (Progatzky & Pachnis, 2022). Therefore, glial response against cytotoxic factors is induced by similar to astrogliosis mechanisms and accompanied by upregulation of GFAP expression.

Recently there was reported that chlorinated benzenes potent to induce neurologic deficits (Maruthur et al., 2021). Similar disturbance could be associated with chronic glial cell injury that observed in present study. The major findings of our study are that thinner stimulates GFAP upregulation in both CNS and PNS. These data suggest that chlorobenzene induces glial reactivity against glial cell damage. During reactive gliosis, glial cells secrete numbered active substances including excitatory amino acids, pro-inflammatory cytokines or/and free radicals, which lead to hyper-activation of glial cells and neurons. Reciprocal activation astrocytes and microglial cells have been proposed to be the major causes of neurodegenerative diseases (Franklin et al., 2021). Extremely activated glial cells produce cytokines and other factors that upregulate abnormal response in a brain cells including bot glia

and neurons (Singh, 2022). On the other hand, the overproduction of GFAP indicates possible changes of astrocyte morphology (Moeton et al., 2016). Therefore, regulation of GFAP-positive glial cell reactivity plays the key role in balanced neuronal functions.

The mechanisms by which chlorobenzene induces glial reactivity is not clear. However, chlorobenzene induced oxidative reaction accompanied by the generation of reactive oxygen species, such as hydrogen peroxide or superoxide anion, and reduction in the activity or expression level of GSH-Px might induce reactive gliosis. There is the data that chlorobenzene can lead to the activation of the NF-κB pathway in the human alveolar epithelial cell line A549 (Röder-Stolinski et al., 2008). The exposure the cells within 60 min were exposed to chlorobenzene for 1 h induced phosphorylation NF-κB. Moreover, treatment this epithelial cell line with chlorobenzene activated p38MAP-kinase and the chemokine MCP-1 release. Thus, chlorobenzene may induce the changes that involved in inflammatory reactions in both brain and enteric glial cells. Observed in our study GFAP upregulation could be related with aforementioned NF-κB dependent pathway to modulate glial response against thinner toxicity. Furthermore, increase in GFAP content reflects the harmful effect of chlorinated benzenes on both CNS and peripheric glial cells.

Conclusions

Chronic chlorobenzene exposure can sensitize glial cells response and multiplemechanisms which might contribute to the pathogenesis of neurodegenerative disorders.

Findings allow using the level of the glial cells GFAP expression in the mouse brain and intestinal tissue as a reliable biomarker. Reported upregulation in GFAP expression could be similar to other mammalian species and future study are required.

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Declaration of Competing Interest

The authors declare that they have no any competing financial or personal interests in respect to this paper.

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