NeuroToxicology 51 (2015) 10–19

Contents lists available at ScienceDirect

NeuroToxicology

Acute and subchronic toxicity of inhaled toluene in male Long–Evans rats: Oxidative stress markers in brain☆,☆☆

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ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 31 August 2015
Accepted 1 September 2015
Available online 3 September 2015

Keywords:
Toluene
Oxidative stress
Solvents
Antioxidants
Protein carbonyls
Aconitase
Neurotoxicity

ABSTRACT

The effects of exposure to volatile organic compounds (VOCs), which are of concern to the EPA, are poorly understood, in part because of insufficient characterization of how human exposure duration impacts VOC effects. Two inhalation studies with multiple endpoints, one acute and one subchronic, were conducted to seek effects of the VOC, toluene, in rats and to compare the effects between acute and subchronic exposures. Adult male Long–Evans rats were exposed to toluene vapor ($n = 6$ per group) at a concentration of 0 or 1019 ± 14 ppm for 6 h in the acute study and at 0 ± 0, 10 ± 1.4, 97 ± 7, or 995 ± 43 ppm for 6 h/d, 5 d/week for 13 weeks in the subchronic study. For the acute study, brains were dissected on ice within 30 min of the end of exposure, while for the subchronic study, brains were dissected 18 h after the last exposure. Frontal cortex, hippocampus, cerebellum, and striatum were assayed for a variety of oxidative stress (OS) parameters including total aconitase (TA), protein carbonyls, glutathione peroxidase (GPX), glutathione reductase (GRD), glutathione transferase (GST), γ-glutamylcysteine synthetase (GCS), superoxide dismutase (SOD), total antioxidants (TAS), NADPH quinone oxidoreductase-1 (NQO1), and NADH ubiquinone reductase (UBIQ-RD) activities using commercially available kits. Following acute exposure, UBIQ-RD, GCS and GRD were increased significantly only in the cerebellum, while TAS was increased in frontal cortex. On the other hand, subchronic exposure affected several OS markers including increases in NQO1 and UBIQ-RD. The effect of subchronic toluene exposure on SOD and TAS was greater in the striatum than in the other brain regions. TA activity (involved in maintaining iron homeostasis and an indicator of DNA damage) was inhibited in striatum and cerebellum, increased in hippocampus, and unchanged in frontal cortex. Protein carbonyls increased significantly in both the frontal cortex and cerebellum. In general, the results showed that acute exposure to toluene affected OS parameters to a lesser extent than did subchronic exposure. These results suggest that toluene exposure induces OS in the brain and this may be a component of an adverse outcome pathway for some of the neurotoxic effects reported following toluene exposure.

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☆ Preliminary findings were presented at the 47th Annual Meeting of Society of Toxicology in Seattle, WA (March 16–20, 2008). Abstract # 1957.

☆☆ The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Abbreviations: TAS, total antioxidant substances; GSH, glutathione; GCS, γ-glutamylcysteine synthetase; GST, glutathione-S-transferase; GRD, glutathione reductase; GPX, glutathione peroxidase; NQO1, NAD(P)H:quinone oxidoreductase; UBIQ-RD, NADH ubiquinone reductase; SOD, superoxide dismutase; TA, total aconitase.

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http://dx.doi.org/10.1016/j.neuro.2015.09.001
0161-813X/Published by Elsevier Inc.

1. Introduction

Volatile organic compounds (VOCs) are ubiquitous in our environment and continue to pose a significant health problem in industrial nations. Toluene is a representative of the large class of solvents present in many commercial products, including adhesives, paint thinners, and cleaning agents (Low et al., 1988). Toluene also is used to produce benzene and as a solvent. Exposure to toluene may occur from breathing ambient or indoor air affected by such sources. The ubiquity of toluene creates a high potential for societal cost if long-term exposure results in toxicity (ATSDR, 1994).

Occupational and recreational exposure to toluene and other VOCs has been shown to produce persistent toxicity, especially in the nervous system (Win-Shwe and Fujimaki, 2010), which is the primary target organ for toluene toxicity in both humans and animals for both acute (short-term) and chronic (long-term) exposures (EPA, 1994; Filley et al., 2004; Formazzari et al., 1983; Greenberg, 1997; Wang and Chen, 1993). Following acute exposure in humans, adverse effects included headaches, fatigue, eye irritation, increased tendency to sleep, and memory impairment (Echeverria et al., 1991). Whereas such effects are associated with repeated exposure in occupational settings, a recent study showed that the accuracy of detecting subtle visual stimuli was reduced in healthy volunteers after a single acute exposure to 200 ppm toluene (Kobald et al., 2015). In chronic toluene abusers, cerebellar dysfunction and cerebral and hippocampal atrophy as well as loss of brain volume have been observed (Deleu and Hanssens, 2000; Kamran and Bakshi, 1998). Chronic abusers exposed to high levels of toluene also have been shown to have significant neuropathological abnormalities including lesions in white and gray matter, which were associated with neurological and cognitive deficits (Rosenberg et al., 2002; Aydin et al., 2009).

In rats, concentrations of 1000–4000 ppm of toluene reliably induce robust neurophysiological and behavioral effects during exposure (Boyes et al., 2007; Bushnell et al., 2007; Oshiro et al., 2011). A meta-analysis of dose–response functions for toluene and three other solvents suggested that acute exposures to low levels of these VOCs may lead to increased reaction times, altered visual evoked potentials, and altered performance of cognitive and behavioral tasks (Benignus et al., 2009) in both rats and humans. Physiologically-based pharmacokinetic models (Kenyon et al., 2008) demonstrated that brain solvent concentration accurately predicts acute changes in behavior and neurophysiological functions (Boyes et al., 2007; Bushnell et al., 2007). In contrast to the significant effects on motor activity, anxiety-related behavior in the elevated plus maze, trace fear conditioning, acquisition of an appetitively-motivated visual discrimination, or performance of visual detection task seen during acute exposure, neurobehavioral effects of subchronic (90-day) exposure resulted only in a delay of appetitively-motivated acquisition of a lever-press response (Beasley et al., 2010).

Other reports from the literature do indicate that subchronic exposure to toluene can impact behavioral endpoints. For example, repeated exposure to toluene (1000–5000 ppm) was associated with hearing loss (Pryor, 1995). Acute high concentrations (8000–12,000 ppm), representing abuse-level exposures, resulted in neurotransmitter changes in adult animals, an effect not observed in adolescents (O'Leary-Moore et al., 2009). Huerta-Rivas et al. (2012) reported that both acute and chronic toluene inhalation impaired learning, short-term and long-term memory in an object-recognition test and in an inhibitory avoidance task in adolescent and young adult rats at doses as low as 1000 ppm. Low dose (80 ppm) subchronic toluene inhalation exposure was also found to cause statistically significant impairment in acquisition and retention of spatial learning task (von Euler et al., 1993, 2000). Berenguer et al. (2003) reported that subchronic toluene exposure at 40 ppm for 16 weeks resulted in sensitization to toluene-induced narcosis, a decrease in rearing activity, and alterations in dopamine and serotonin transmissions.

Although there is some debate over behavioral effects following subchronic exposure, behavioral and functional changes following toluene exposure are well documented yet the mechanisms underlying these effects are not well understood. Studies have reported that toluene exposure results in altered dopaminergic neurotransmission (von Euler, 1994), inhibition of nicotinic acetylcholine receptors (Bale et al., 2002) and inhibition of the function of excitatory receptors such as N-methyl-D-aspartate (NMDA), glutamate (Cruz et al., 2000). In contrast, the function of inhibitory receptors such as gamma-aminobutyric acid ($GABA_A$) and glycine was enhanced (Meulenberg and Vijverberg, 2003; Beckstead et al., 2001). Toluene can activate microglia in the hippocampus, which in turn secrete proinflammatory cytokines, reactive oxygen species, and other toxic products like nitric oxide (Win-Shwe and Fujimaki, 2010; Win-Shwe et al., 2012). Reports over the past indicated that oxidative stress (OS) might play a key role in the neurotoxicity of several solvents, including toluene (Bondy et al., 1995; Mattia et al., 1993a,b; Myhre et al., 2004; Kodavanti et al., 2011). OS occurs in the cell when there is an imbalance in the production of oxidants or reactive oxygen species (ROS) and the ability to remove them by endogenous antioxidants. The resulting accumulation of oxidative free radicals can directly damage proteins, DNA, and lipids, leading to cellular dysfunction. OS is also considered as a possible mode of action for a number of diverse toxic chemicals (Bondy, 1994; Kodavanti, 1999).

In the present study, we investigated several OS measures in the frontal cortex, cerebellum, hippocampus and striatum of male Long–Evans rats following acute and subchronic toluene exposure in an attempt to understand the role OS plays in toluene-mediated effects on the nervous system. We selected these brain regions in order to correlate changes in OS parameters to the neurobehavioral effects related to these brain regions. Since ROS are short lived with a half-life in milliseconds (Kodavanti, 1999), we selected two assays to indicate ROS production (NQO1 and UBIQ-RD). We also selected several assays related to antioxidant homeostasis in response to different OS reactants to understand cell defense mechanisms against ROS damage (TAS, GPX, GRD, GSG, GST and SOD). If ROS production exceeds antioxidants, the result will be oxidative damage and we have measured total aconitase and protein carbonyls in this respect. In addition, we have addressed the differential effects of toluene on OS parameters between acute and subchronic exposure.

2. Materials and methods

2.1. Animals and acclimation before toluene exposure

Male Long–Evans rats were received from Charles River Laboratories (Portage, MI) at 64 days of age and were allowed to acclimate for 12 days in the vivarium, a facility that follows the NIH guidelines for animal care and is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures were approved by the NHEERL Institutional Animal Care and Use Committee, which ensures conformance with the 1996 NRC "Guide for the Care and Use of Laboratory Animals", the Animal Welfare Act and Public Health Service Policy on the Humane Care and Use of Laboratory Animals. To implement exposures to toluene, each rat was transported from its home cage in the vivarium and placed in one of four Hazelton 2000 Inhalation chambers (for figures and additional details, see Beasley et al., 2010) every Monday morning, and remained in the chamber until Friday afternoon. Each rat was