

● PERSPECTIVE

Glial plasticity after hexahydrobenzene exposure

The organic solvents are not only utilized in industrial processes, but also as drugs of abuse. In fact, solvent consumption represents the fourth option for drug users just after alcohol, tobacco and marijuana. In humans, intentional inhalation of volatile drugs impairs the function of central nervous system, provokes cardiac arrhythmias and increases the risk of suicide or death. Neuroimaging studies in the human brain have shown morphological abnormalities after chronic inhalation of organic solvents. These anomalies include ventricular enlargement and diffuse atrophy that affects neocortex, basal ganglia, thalamus, cerebellum and brainstem. Additionally, solvent users show a demyelination pattern of white matter tracts in the corpus callosum and internal capsule (Yücel et al., 2008).

Organic solvents can trigger the production of oxygen-free radicals into the brain. Under physiological conditions, the cellular metabolism produces several reactive oxygen species that help maintain tissue homeostasis, such as: superoxide anion, hydroxyl radical, nitric oxide radical and hydrogen peroxide. However, solvent consumption can produce pro-oxidative status that strongly increases oxidative stress in tissues. The expression levels of oxidative stress vary according to the antioxidant defense system in every tissue. Thus, when the expression level of oxidative stress is high, alterations in cellular metabolism can occur, *i.e.*, DNA breakage, membrane lipid peroxidation, protein degradation, mitochondrial dysfunction and apoptosis (Ojo et al., 2015). Exposure to volatile substances, such as: toluene, 1-1-1 trichloroethane, dichloromethane, 1-bromopropane and, recently, hexahydrobenzene can trigger glial response in several brain regions (Subramanian et al., 2012; Campos-Ordonez et al., 2015). The recreational inhalation of hexahydrobenzene increases the levels of apurinic/pyrimidinic endonuclease 1 (APE1), an enzyme overexpressed in response to high levels of oxidative stress (Campos-Ordonez et al., 2015). This evidence suggests that hexahydrobenzene is a harmful substance that may damage the human brain.

Hexahydrobenzene and hippocampus: Hexahydrobenzene, also referred to as cyclohexane, is a lipophilic hydrocarbon commonly used as less toxic replacement of harmful solvents (n-hexane, toluene and benzene) in several products including: adhesives, gasoline, paints, pesticides, *etc.* Regrettably, there is scarce information about the effects of hexahydrobenzene recreational consumption on the central nervous system. This solvent enters into the blood brain barrier and rapidly reaches several cerebral regions such as neocortex, basal ganglia and hippocampus. The adult hippocampus has a complex network of neural projections, which integrates multimodal sensory information from olfactory, visual, auditory and somatosensory cortices. In the hippocampal CA1 and CA3 regions, pyramidal neurons contain a number of glutamate receptors (mGluR, NMDA and AMPA). Thus, if deleterious changes occur in the microenvironment, the neurotransmitter glutamate can act as a neurotoxin and provoke tissue damage, an event commonly referred to as neuroexcitotoxicity.

The hippocampus has a critical role in the development of episodic and declarative memory. This structure contains a number of neuron-glial interactions that maintain the functional balance of this region. Hippocampal astrocytes help regulate

ionic influx within the synapse and regulate local levels of glutamate (Sofroniew and Vinters, 2010; Ojo et al., 2015). Organic solvents appear to disrupt glutamatergic balance by decreasing astroglial glutamate uptake and altering the redox status in cells, which in turns produces excitotoxicity and neuronal death (van Thriel et al., 2007; Verkhatsky et al., 2014).

A recreational dose of hexahydrobenzene can induce the overexpression of APE1 and promote reactive gliosis and microglial activation in the CA1 and CA3 hippocampal regions, which suggest an ongoing brain injury (**Figure 1A**) (Campos-Ordonez et al., 2015). APE1, also known as redox effector factor 1 (Ref-1), acts as a reductive activator of transcription factors responsible for DNA repair and cell recovery during oxidative damage. This evidence suggests that hexahydrobenzene causes an accumulation of ROS and cellular dysfunction in the CA1 and CA3 regions. Nevertheless, more studies are needed to confirm these hypotheses.

Hexahydrobenzene and glial response: The presence of oxidative stress in the neuronal microenvironment activates astrocytes and microglia, which help maintain local homeostasis and preserve the brain cytoarchitecture. Under normal conditions, these cells have an important role in neuronal nourishment, lipid synthesis, growth factors release, blood-brain barrier maintenance, ionic homeostasis, neuronal excitability, energy support for neurons, neurotransmitter uptake and synapsis transmission (Gonzalez-Perez et al., 2015). Under pathological conditions, astrocytes and microglia cells regulate the synthesis and secretion of trophic and inflammatory factors, which contribute with brain repair and tissue regeneration (Ojo et al., 2015). This effect is mediated by a combination of abnormal levels of neurotransmitters, cytokines, adhesion molecules and growth factors that determine the astrocytic response in damaged area (Sofroniew and Vinters, 2010). In consequence, reactive astrocytes change their morphology, increase their proliferation and overexpress the glial fibrillary acidic protein (GFAP) (Ojo et al., 2015). GFAP overexpression is observed after any brain insult and is considered a reliable and dynamic sign of reactive gliosis (Sofroniew and Vinters, 2010). Interestingly, recreational doses of hexahydrobenzene increase the number of astrocytes and induce morphological changes in hippocampal cellularity (**Figure 1B**) (Campos-Ordonez et al., 2015). This glial response is probably triggered by changes in the levels of glutamate and pro-inflammatory cytokines, which in turn regulate the activity of microglia cells (Sofroniew and Vinters, 2010).

Microglia cells are known as resident macrophages and represent the innate immune system of the brain. Microglia cells represent the first line of cellular defense in the adult brain. In normal conditions, microglial cells have stellate morphology, small somas, and long thin branches. This morphological state is commonly known as “resting microglia” (**Figure 1B**). In pathological conditions, the “active or reactive” microglia shows two major changes. First, microglia cells acquire an amoeboid shape and then turn into phagocytic cells (Kettenmann et al., 2013). A good marker for detecting morphological changes in microglia is the ionized calcium-binding adapter molecule 1 (Iba-1), which is constitutively expressed by microglia cells.

Hexahydrobenzene administration triggers microglia reactivity that is characterized by a significant increase in the number and morphology of cells in the hippocampal CA1 and CA3 regions (**Figure 1B**) (Campos-Ordonez et al., 2015). The activation of microglia has been associated with two physiological events. First, neuronal loss provoked by secretion of pro-inflammatory cytokines. Second, neuroprotective and regenerative roles that are modulated by secreting trophic factors and

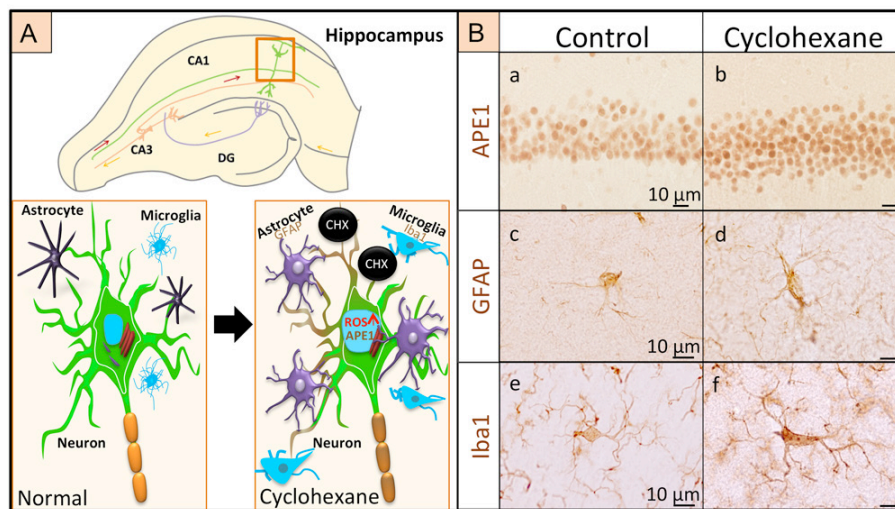


Figure 1 Glial response after hexahydrobenzene exposure.

(A) Scheme of the effects of hexahydrobenzene (cyclohexane) in the mouse hippocampus. Astrocytes and microglia (in close proximity with pyramidal neurons at CA1 and CA3 hippocampal regions) help preserve local homeostasis and neuronal communication. Hexahydrobenzene inhalation increases the production of reactive oxygen species (ROS) and intracellular apurinic/aprimidinic endonuclease 1 (APE1) that, in turns, provokes glial-cell activation to preserve the tissue microenvironment. (B) Morphological changes observed in the mouse brain after cyclohexane inhalation for 30 days: APE1 expression in the CA1 region of mouse hippocampus under control conditions (a). APE1 expression in the CA1 region mouse hippocampus after a 30-d cyclohexane inhalation (b). GFAP-expressing astrocytes under normal conditions (c). Reactive astrocyte (after cyclohexane exposure) displays cytoplasm swelling and high number of cytoplasmic processes (d). Iba1-expressing ‘resting’ microglia shows a stellate morphology: the small soma has a number of long and fine cytoplasmic processes (e). A reactive microglia cell showing hypertrophic branches after cyclohexane inhalation (f). Bars = 10 μm.

cytokines (Kettenmann et al., 2013). Therefore, the biological meaning of microglial reactivity observed near CA1 and CA3 pyramidal neurons after hexahydrobenzene inhalation remained to be elucidated.

Conclusion: Hexahydrobenzene inhalation produces important alterations on brain physiology, which are triggered by increasing oxidative stress, activating astrocyte response and recruiting microglia cells into the brain. This glial response may be associated with neuroinflammation and excitotoxicity that can culminate in the death of pyramidal neurons in the CA1 and CA3 hippocampal regions. These pathological responses can be controlled with antioxidant molecules that are able to react with oxygen-free radicals and arrest the tissue oxidation. These beneficial effects are mediated by oxygen radical scavenging, sequestration of transition metals, enzymatic hydrolysis of ester bonds and reduction of peroxides. Thus, a feasible therapeutic alternative might be the combination of antioxidants, such as alpha-lipoic acid, vitamin E and vitamin C, which can reduce or prevent the oxidative damage in several animal models (Gonzalez-Perez and Gonzalez-Castaneda, 2006). However, the efficiency of this pharmacological approach for humans needs further confirmation.

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