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Neurotransmitter changes in rat brain regions following glyphosate exposure

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ABSTRACT

The effects of glyphosate oral exposure (35, 75, 150 and 800 mg/kg bw, 6 days) on brain region monoamine levels of male Wistar rats were examined. Glyphosate-treated rats (35, 75, 150 and 800 mg/kg bw, 6 days), had no visible injury, *i.e.,* no clinical signs of dysfunction were observed. After last dose of glyphosate, serotonin (5-HT), dopamine (DA) and norepinephrine (NE) and its metabolites levels were determined in the brain regions striatum, hippocampus, prefrontal, cortex, hypothalamus and midbrain, by HPLC. Glyphosate caused statistically significant changes in the 5-HT and its metabolite 5-hydroxy-3-indolacetic acid (5-HIAA), DA and its metabolites 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and NE and its metabolite 3-metoxy-4-hydroxyphenylethyleneglycol (MHPG) levels in a brain regional- and dose-related manner. Moreover, glyphosate, dose-dependent, evoked a statistically significant increase in 5-HT turnover in striatum and hypothalamus and in DA turnover in prefrontal cortex and hippocampus, and a statistically significant decrease in NE turnover in prefrontal cortex and hypothalamus. The present findings indicate that glyphosate significantly altered central nervous system (CNS) monoaminergic neurotransmitters in a brain regional- and dose-related manner, effects that may contribute to the overall spectrum of neurotoxicity caused by this herbicide.

1. Introduction

Glyphosate (N-phosphonomethyl-glycine) is a post-emergent, systemic and non-selective herbicide intended for use against deep-rooted perennial species, and also biennial and annual broad-leaved, grass and sedge species. Glyphosate is used in both agriculture and forestry. Fields of agricultural use include grassland renovation, horticulture, fruticulture, arable cultivation, and rice cultivation. Use in forestry includes the killing of fast growing competitors in conifer plantations or conservation areas, and the treatment of tree stumps. Glyphosate may also be used for weed killing in non-agricultural areas such as water systems, including irrigation and temporarily drained waters, parks, road verges and gardens (WSSA, 1983). Glyphosate use is the highest on any pesticide in worldwide. Glyphosate is an active ingredient in a number of commercially available herbicides, including several that are used in concert with genetically modified crops. Several concerns have been raised about the use of glyphosate and adjuvants in the eradication programs for illicit crops; these concerns range from damage to other crops to adverse effects on the environment and human health (Solomon et al., 2007). Evidence of human exposure to this herbicide has been shown through the detection of glyphosate in urine samples of people living in farm and non-farm household (Acquavella et al., 2004; Conrad et al., 2017).

The herbicidal action of glyphosate derives from its inhibition of a key plant enzyme, 5-enolpyruvyl shikimate-3-phosphate synthase, which is involved in the synthesis of aromatic amino acids (Steinrücken and Amrhein, 1980; Devine et al., 1993; Franz et al., 1997). This inhibition results in decreases in the synthesis of the aromatic amino acids tryptophan, phenylalanine and tyrosine, as well as decreased rates of synthesis of protein, indole acetic acid (a plant hormone), and chlorophyll. Since this enzyme is not present in vertebrates, it has long been believed that glyphosate would not affect non-target species. However, over the last few years, glyphosate has received significant attention by the scientific community as well as regulatory agencies around the world. Between 2012 and 2017, in the European Union, glyphosate has been thoroughly assessed by Member States, the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) to establish whether its use results in any unacceptable effects on human, animal or environmental health. In fact, further toxicological and epidemiological studies are needed to draw better conclusions about safety

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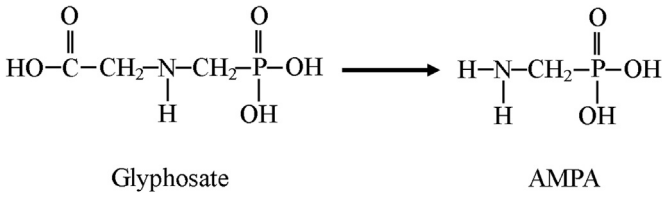

Fig. 1. Chemical structures of glyphosate and its metabolite AMPA.

of glyphosate.

Potential routes for human exposure to glyphosate include inadvertent ocular exposure during herbicide mixing and application; dermal exposure due to mixing/ application or contact with treated plants; and oral exposure through the ingestion of treated crops or contaminated water. Inhalation of glyphosate is anticipated to be minimal because of the low volatility of the chemical. Based on studies conducted in the rat, oral absorption appears to be limited, in a range of 23–36% (Brewster et al., 1991; Anadón et al., 2009). Following ingestion, the glyphosate is slowly absorbed and eliminated, plasma glyphosate concentrations peaked at around 5 h and the elimination half-live from plasma was approximately 14 h (Anadón et al., 2009). Glyphosate is poorly metabolized, its major metabolite, aminomethyl phosphonic acid (AMPA), represented 6–7% of the parent drug plasma concentrations (Anadón et al., 2009). Glyphosate and AMPA (Fig. 1) are classified in the least toxic category (category IV; practically non-toxic and not an irritant) by the Unites States Environmental Protection Agency (USEPA). There is not robust evidence of cytotoxicity, genotoxicity, DNA damage, carcinogenicity or reproductive toxicity from glyphosate and AMPA (Williams et al., 2000; Greim et al., 2015; EFSA, 2015).

Despite the apparent well-established safety of glyphosate for humans by regulatory agencies, it has been suggested that large term low-level exposure might lead to human chronic diseases. There are few human epidemiology studies examining the impact of glyphosate on human diseases. USEPA (2009, 2016) issued a 'Glyphosate Final Work Plan (FWP) registration review' that identified uncertainties about the toxicity of glyphosate. For example, USEPA announced its plan to require that registrants conduct acute and subchronic neurotoxicity studies. Herbicides have been suggested as an environmental risk factor for neurodegenerative disorders. In this context, it has been reported that acute and chronic exposure to glyphosate might cause Parkinsonism (Barbosa et al., 2001; Wang et al., 2011). Barbosa et al. (2001) reported a case of a 54-year-old man who accidentally sprayed himself with the chemical agent glyphosate and one month later he developed Parkinsonism. One year later, the patient presented a slow resting tremor in the left hand and arm, accompanied by impairment of short-term memory. Further, Wang et al. (2011) reported a case of Parkinsonism following chronic exposure to glyphosate in a previously healthy 44-year-old woman who worked for 3 years in a chemical factory, exclusively in the glyphosate production division. The glyphosate neurotoxicity was associated with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory. Glyphosate is a derivative of glycine. As a consequence, glyphosate could inhibit serine hydroxymethyl transferases enzyme activities, a major source of intracellular glycine. Glycine consumption is a hallmark of rapidly proliferating cell (Jain et al., 2012). Antagonizing glycine uptake and biosynthesis preferentially impaired rapidly proliferating cells. For these reasons, glyphosate has also been suggested to inhibit cellular proliferation through depleting glycine (Li et al., 2013). The fact that glycine and other amino acids like glutamate act as neurotransmitters and play an important role in brain function raises the question of the potential neurological effects of glyphosate. Cattani et al. (2014) suggested that glyphosate induces glutamatergic excitotoxicity, and important mechanism known of cell death in neurological diseases, which lead to an uncontrollable stimulation of proteolytic enzymes, lipoperoxidation and generation of reactive oxygen species (ROS) (Arundine and Tymianski, 2003).

Because major information regarding glyphosate neurotoxic effects and underlying mechanisms involved after mammalian exposures are needed for risk evaluation, and taking into account that glyphosate-containing pesticide promotes degeneration of γ-aminobutiric acid and dopamine neurons in *Caenorhabditis elegans* (Negga et al., 2012), the aim of this study was to determine in rats the effects of glyphosate on the levels of the dopamine, norepinephrine and serotonin neurotransmitters, their metabolites and the neurotransmitter rate (turnover) in striatum, hippocampus, prefrontal cortex hypothalamus and midbrain.

2. Materials and methods

2.1. Chemicals

Glyphosate [N-(phosphonomethyl) glycine], molecular formula $C_3H_8NO_5P$ CAS RN 107-83-6, purity ≥ 98%, serotonin (5-HT) and its metabolite [5-hydroxy-3-indolacetic acid (5-HIAA)], dopamine (DA) and its metabolites [3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and norepinephrine (NE) and its metabolite [3-metoxy-4-hydroxyphenylethyleneglycol (MHPG)] were purchased from Sigma-Aldrich, St Louis, MO, 63103 USA. All other chemicals were of the highest quality grade and obtained from commercial sources.

2.2. Animals and experimental design

All experiments using live animals were undertaken in accordance with the ethics requirements and authorized (protocol number 086) by the official ethical committee of our university. Male Wistar rats of 60 days old each weighting 200–210 g (Charles River Inc., Margate, Kent, UK) were used. The animals were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2 °C and 50 ± 10% relative humidity) with a 12 h light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Scientific Animal Food & Engineering, SAFE, Augy, France) and water were available *ad libitum*. Thirty male rats were assigned randomly to five groups of 6 animals each, a control group and four glyphosate treated groups. Animal treated groups received glyphosate orally at the dose of 35, 75, 150 and 800 mg/kg bw [equivalent to 1/160, 1/75, 1/37 and 1/7 of the acute oral rat LD_{50} ≈ 5.6 g/kg bw (Street et al., 1979; WHO, 1994) for 6 consecutive days. The doses were chosen taking into account the LD_{50} oral value as well as the NOAEL (no observed adverse effect level) described in the literature. The evaluation conducted by JMPR (2004) established an acceptable daily intake (ADI) for glyphosate and AMPA of 0–1.0 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100, but a NOAEL of 50 mg/kg bw per day was also observed considering several developmental toxicity studies in rabbits (EFSA, 2015). An ADI of glyphosate of 0.5 mg/kg bw per day (based on the maternal and developmental NOAEL of 50 mg/kg bw per day and applying a standard uncertainty factor of 100) has been allocated in the EFSA evaluation (EFSA, 2015). The high dose of 800 mg/kg bw of glyphosate was selected on the basis of the high acute toxicity value of glyphosate (rat, LD_{50}, oral > 5000 mg/kg bw) as well as taking into account that into the critical end-points for setting guidance values for oral exposure to glyphosate, no evidence of neurotoxicity was observed in any study conducted (JMPR, 2004).

The glyphosate treated group rats were deprived of food for 6 h before the oral administration of glyphosate, but were allowed water *ad libitum*. Glyphosate was dissolved in water and was administered orally by gavage in a maximum volume of 2 mL/rat. Control animals received the vehicle (water) on the same schedules.