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In vitro evaluation of neurotoxicity potential and oxidative stress responses of diazinon and its degradation products in rat brain synaptosomes

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HIGHLIGHTS

- Diazoxon increases antioxidant enzymes activities and MDA level in dose-dependent manner.
- 0.1 mM diazoxon inhibits synaptosomal Na^+/K^+-ATPase and ecto-ATPase almost completely.
- Although known as non toxic, IMP induces SOD stimulation up to 30%.
- Neither diazinon nor its metabolites noticeably affects synaptosomal integrity.
- Synaptosomal biochemical parameters may be used for monitoring organophosphates toxicity.

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ABSTRACT

Although primary toxic action of organophosphorous insecticides is associated with acetylcholinesterase inhibition, later studies suggest that oxidative stress may be responsible for induced organophosphates toxicity. These studies mostly include thio forms, while the effects of their metabolites/degradation products have been less investigated. Therefore, this paper studies the toxic effects of diazinon degradation products, diazoxon and 2-isopropyl-6-methyl-4-pyrimidinol, and compares them with the toxic potential of the parent compound. The toxicity induced by various concentrations of the investigated compounds was *in vitro* evaluated by the activities of acetylcholinesterase, ATPases, antioxidant defense enzymes and lactate dehydrogenase, and malondialdehyde level in rat brain synaptosomes. Diazinon inhibited acetylcholinesterase and Na^+/K^+-ATPase in dose-dependent manner, while the inhibition of ecto-ATPase activity was less than 15% at all investigated concentrations. It did not demonstrate noteworthy prooxidative properties causing increase (up to 10%) in antioxidant enzymes activity and malondialdehyde level, as a marker of lipid peroxidation. Diazinon oxidation product, diazoxon was found as the most toxic investigated compound. Beside the expected strong inhibitory effect on acetylcholinesterase, it induced dose-dependent and almost complete inhibition of Na^+/K^+-ATPase and ecto-ATPase at the highest investigated concentration (0.1 mM). Increasing diazoxon concentrations activated catalase (up to 30%), superoxide dismutase (up to 50%), glutathione peroxidase (up to 30%), and significantly increased malondialdehyde level (up to 50%). The investigated hydrolysis product of diazinon, 2-isopropyl-6-methyl-4-pyrimidinol did not remarkably alter the activities of acetylcholinesterase, Na^+/K^+-ATPase, catalase, glutathione peroxidase and lipid peroxidation level (up to about 10%). Although this diazinon metabolite has been known as non toxic, it induced superoxide dismutase stimulation up to 30%. Finally, even high concentrations of both diazinon and its metabolites did noticeably affect lactate dehydrogenase activity as a marker of synaptosomal integrity. The changes in

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investigated biochemical parameters in rat brain synaptosomes could serve as indicators of toxicity due to the exposure to thio organophosphates and/or their break-down products.

1. Introduction

Organophosphorus pesticides (OPs) have been the most widely used insecticides for last several decades, which undergo metabolic transformations in animals and humans, and microbial and chemical degradation in the environment (Wu and Jans, 2006; Fujioka and Casida, 2007; Li et al., 2007). The primary mechanism of OP toxicity is the inhibition of acetylcholinesterase (AChE) in the central and peripheral nervous system, leading to a variety of short-term and chronic effects such as nausea, headache, confusion, depression, memory loss and chronic fatigue syndrome (Sultatos, 2006; Ma et al., 2013; Nachon et al., 2013). Although OPs are known primarily as AChE inhibitors, oxidative stress and hyperglycemia has been reported as one of the adverse effects in poisoning by OP in both humans and animals (Akhgari et al., 2003; Altuntas and Delibas, 2002; Ghafour-Rashidi et al., 2007; Ranjbar et al., 2002). Investigations have shown that pesticides can damage the balance between prooxidants and antioxidants in body and lipid membrane resulting in lipid peroxidation (Karademir Catalgol et al., 2007; Mohammad et al., 2004). Lipid peroxidation is a complex process resulting from free radical reactions in biological membranes, which are rich in polyunsaturated fatty acids. It forms lipid hydroperoxides which decompose double bonds of unsaturated fatty acids and destruct membrane lipids. Both the increased production of reactive oxygen species (ROS) and attenuation of the antioxidant barrier of the organism are likely to induce oxidative stress. ROS may be produced as the result of the metabolism of OP by cytochrome P450s (Lukaszewicz-Hussain, 2010). The other way of ROS generation in OP toxicity is disturbance in cell redox system, which is caused by high energy consumption coupled with inhibition of oxidative phosphorylation (Lukaszewicz-Hussain, 2010; Milatovic et al., 2006). The key enzymes for the detoxification of ROS in all organisms are superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC 1.11.1.9). Enzymatic degradation of superoxide (O_2^-) to H_2O_2 is ensured by SOD. CAT primarily occurs in peroxisomes and detoxifies H_2O_2 to O_2 and water. GPx is the most important peroxidase for the detoxification of hydroperoxides (Lackner, 1998).

Adenosine triphosphatases (ATPases) are a group of enzymes which play an important role in intracellular functions and critical for cellular viability because they control many essential cellular functions, and are considered to be a sensitive indicator of toxicity (Čolović et al., 2010; Oruç and Usta, 2007). Na^+/K^+-ATPase (EC 3.6.1.37) is a cell membrane located enzyme that establishes and maintains the high internal K^+ and low internal Na^+ concentrations, which are essential for neurotransmission and represent a convenient driving force for the secondary transport of metabolic substrates such as amino acids and glucose (Köksoy, 2002; Wang and O'Doherty, 2012). Moreover, recent studies show that in addition to pumping ions, Na^+/K^+-ATPase interacts with neighboring membrane proteins and organized cytosolic cascades of signaling proteins to send messages to the intracellular organelles (Xie and Askari, 2002; Xie and Cai, 2003; Wang and O'Doherty, 2012). The ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases, ecto-ATPase) are plasma membrane bound enzymes that, in the presence of divalent cations (Ca^{2+} or Mg^{2+}), hydrolyse extracellular nucleotides (ATP and ADP) and represent the major part of purinergic signaling (Al-Rashida and Iqbal, 2013; Matsuoka and Ohkubo, 2004; Wall et al., 2008).

Diazinon(*O*,*O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl phosphorothionate) is a commonly used thionophosphorous OP to control a variety of insects in agriculture and household environment (Cox, 1992). Despite its low persistence in the environment, it is a nonspecific insecticide and highly toxic to animals and humans. Moreover, the toxicity of OPs is increased by their break-down products, which may be bioactivated within an organism or through exposure to the sunlight. Diazinon undergoes fast

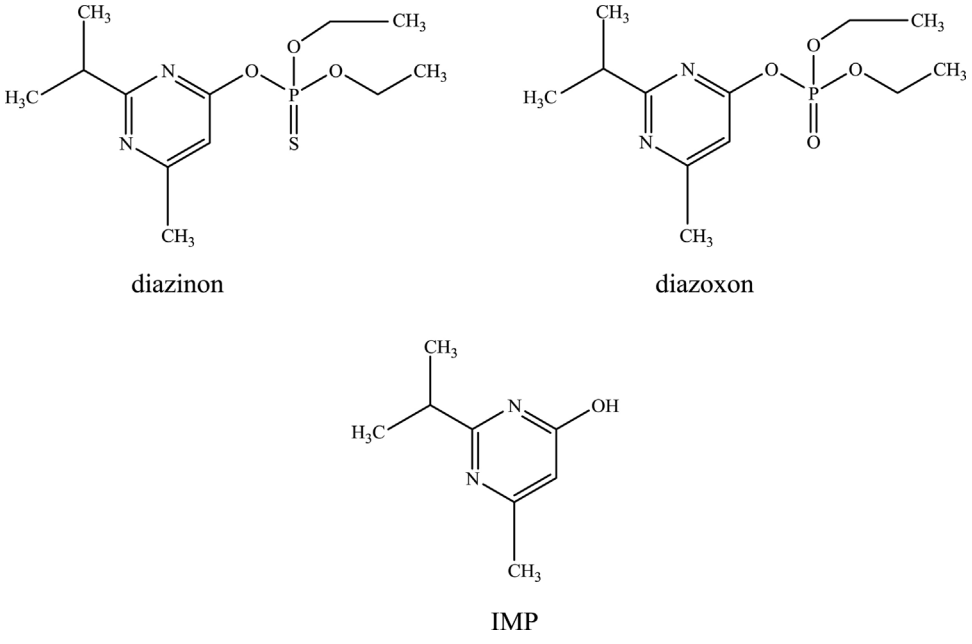

Fig. 1. Structural formulas of diazinon and its degradation products, diazoxon and IMP.