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Assessment of the inhibitory activity of the pyrethroid pesticide deltamethrin against human placental glutathione transferase P1-1: A combined kinetic and docking study

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ABSTRACT

Deltamethrin (DEL), which is a synthetic pyrethroid insecticide, has been used successfully all over the world to treat mosquito nets for the control of malaria. Glutathione *S*-transferases (GSTs; EC 2.5.1.18) catalyze the conjugation of reduced glutathione (GSH) to a variety of xenobiotics and are normally recognized as detoxification enzymes. Here, we used a colorimetric assay based on the human placental GSTP1-1 (*hp*GSTP1-1)-catalyzed reaction between GSH and the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) as well as molecular docking to investigate the mechanistic and structural aspects of *hp*GSTP1-1 inhibition by DEL. We show that DEL is a potent, noncompetitive inhibitor of *hp*GSTP1-1 with an IC_{50} value of 6.1 μM and K_i values of 5.61 ± 0.32 μM and 7.96 ± 0.97 μM at fixed [CDNB]–varied [GSH] and fixed [GSH]–varied [CDNB], respectively. DEL appears to be accommodated well in an eccentric cavity located at the interface of the *hp*GSTP1-1 homodimer, presumably causing conformational changes to the enzyme's substrate-binding sites such that the enzyme is no longer able to transform GSH and CDNB effectively. Correspondingly, considerable maternal exposure to and subsequent accumulation of DEL may interfere with the proper development of the vulnerable fetus, possibly increasing the risk of developing congenital defects.

1. Introduction

Toxic electrophiles are certainly a prominent one on a long list of assaults and insults to the human body. One key source of these electrophiles is pesticides, chemical substances commonly used to control disease vectors (Hernández et al., 2013). Humans are exposed to pesticides mostly through residues in food, water and air (which result from extensive pesticide use in modern agricultural practices to enhance food production), applications to public areas (which aim at controlling disease vectors to ensure public health), careless handling (which results from pesticide use in gardens and lawns to improve the growth of ornamental plants), and occupational exposures to workers in production plants (Hernández et al., 2013; Alavanja et al., 2004). Although pesticides have been highly beneficial to modern agriculture and public health, their impact on human health has attracted substantial attention only in recent years (Hu et al., 2015). The mechanism of toxicity of various pesticides, including organophosphates (OP), organochlorines (OC), *N*-methylcarbamates (NMC), pyrethroids, neonicotinoids, triazines, paraquat, and dithiocarbamates (DTC), has been chiefly through oxidative stress by which many disease conditions are induced (Hernández et al., 2013). Perhaps more worrisome, fetuses and babies could be relatively prone to the toxic effects of pesticides as there exists evidence in the relevant scientific literature of pesticide residues present in placenta, fetal organs, subcutaneous fat tissues, umbilical cord blood, and other body fluids (Martı´nez et al., 1993; Waliszewski et al., 2000; Perera et al., 2003; Souza et al., 2005). Even more so, it has been well established that the enterohepatic clearance system of the fetus is immature (Myren et al., 2007) and that the bodily defense system of neonates is not fully developed (Beath, 2003; Grijalva and Vakili, 2013).

Glutathione transferases (EC 2.5.1.18; also referred to as glutathione *S*-transferases, GSTs) are ubiquitous and promiscuous enzymes that catalyze various kinds of reactions involving a wide variety of substrates that are toxic and chemically reactive (Angelucci et al., 2005). The primary function of GSTs, particularly in higher organisms, is the detoxification of both endobiotics and xenobiotics through their conjugation to reduced glutathione (GSH) for the maintenance of normal redox homeostasis (Armstrong, 1991). GSTP1-1, one of the cytosolic GSTs, in addition to its primary function of detoxification regulates cell survival and apoptosis by interacting with c-Jun N-

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terminal kinase-1 (JNK1), maintaining it in an inactive form, thereby protecting the cells against hydrogen peroxide-induced cell death (Adler et al., 1999). A recent study revealed that GSTP1-1 overexpression interferes with prostate cancer cell motility and viability by interacting with MYC and shutting down the MEK–ERK1/2 pathway (Wang et al., 2017). Typically, cytosolic GSTs are homodimeric proteins consisting of two 22–30 kDa-sized subunits (Board and Menon, 2013; Turk et al., 2015). Each of the GST subunits has its own active site which is made up of a GSH-binding site (G-site) and an electrophilic substrate-binding site (H-site) (Prade et al., 1997).

Deltamethrin (DEL) is a common name for the synthetic pyrethroid insecticide *S*-cyano-3-phenoxybenzyl-*cis*-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate. It is also known by the trade names Decis®, Decasyn®, Butox®, K-Othrine®, Kordon®, and Sadethrin®. Pyrethroids are the only class of insecticides recommended by both the Centre for Disease Control and Prevention (CDC) and the World Health Organization (WHO) to treat protective nets against mosquitoes (Pennetier et al., 2008). Mosquito nets impregnated in DEL have been used successfully all over the world to control malaria (Joshi et al., 2003). Molecular targets of pyrethroids in mammals are much the same as those in insects and consist of voltage-gated ion channels for Na^+, Cl^-, and Ca^{2+}, GABA-gated chloride channels, nicotinic receptors for acetylcholine, and intercellular gap junctions. The modulation of these channels/receptors by pyrethroid insecticides induces axonal hyperexcitability, leading to prolonged depolarization and burst firing (Gupta and Crissman, 2013). Intriguingly, there exists an increased risk of pyrethroid neurotoxicity in developing mammals, which results, at least in part, from the differential expression pattern of voltage-gated sodium channel isoforms in neonates *versus* adults (Wakeling et al., 2012). It may be worth mentioning in this context that authorities on teratology recommend caution, stating that occupational, environmental and medicinal exposures to pyrethroid products should be minimized during pregnancy (Barlow et al., 2015). Although in general DEL and other pyrethroids are considered safe for human use (Rehman et al., 2014), experimental data concerning the effects of DEL on human enzymatic systems are quite lacking. The aim of this study, therefore, is to elucidate the interaction of human placental GSTP1-1 (*hp*GSTP1-1) with DEL.

2. Materials and methods

2.1. Chemicals and reagents

Glutathione transferase P1-1 (GSTP1-1) from human placenta was purchased from Sigma-Aldrich (UK). Ethylenediaminetetraacetic acid (EDTA) and absolute ethanol were purchased from AppliChem and Riedel-de Haen (Germany), respectively. Potassium phosphate (monobasic and dibasic), L-glutathione reduced (GSH) and 2-mercaptoethanol (2-ME) were all obtained from Sigma-Aldrich (Japan). Methanol and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma-Aldrich France and USA, respectively. Deltamethrin (DEL), with the trade name Jetsis 2.5 EC®, was procured from Agrobest Group (Turkey) as an emulsifiable concentrate consisting of 25 g DEL (≥98% purity; CAS number: 52918-63-5) in 1 l of xylene. A 0.75-mM stock solution of DEL was prepared by diluting the commercially-obtained sprayable liquid with absolute ethanol, and the solutions used in all the *hp*GSTP1-1 inhibitory experiments were prepared by serial dilution from the stock one with absolute ethanol. CDNB was also dissolved in absolute ethanol, while GSH was dissolved in distilled water.

2.2. Enzyme preparation

The *hp*GSTP1-1 enzyme was commercially obtained as a lyophilized powder. It was prepared by dissolving 1 mg (48 U mg^{-1} solid) in 1 ml of 100 mM potassium phosphate buffer, pH 6.5, containing 1 mM EDTA and kept at −20 °C. The purity of the prepared enzyme was checked using native as well as sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (Laemmli, 1970). Prior to use, the prepared enzyme was diluted five times with 200 mM potassium phosphate buffer, pH 6.5, containing 2 mM EDTA.

2.3. hpGSTP1-1 activity assay

The activity of *hp*GSTP1-1 was assayed according to the method of Habig and Jakoby (1981) with slight modifications using a Perkin Elmer LAMBDA 25 UV/VIS Spectrophotometer. The activity of *hp*GSTP1-1 was determined by monitoring the increase in absorbance due to the conjugation of the natural substrate GSH to the artificial substrate CDNB (which resulted in the formation of the GS–DNB complex) at 340 nm for 20 s. The reaction was always initiated by the addition of CDNB. The total reaction volume was 500 µl and consisted of 100 mM potassium phosphate buffer at pH 6.5, 1 mM EDTA, 1 mM CDNB, 1 mM GSH, and 2 µg ml^{-1} *hp*GSTP1-1. A non-enzymatic reaction (blank) was run, which contained all constituents of the reaction mixture except *hp*GSTP1-1. The reading provided by the non-enzymatic reaction was subtracted from that for the corresponding enzymatic reaction. All measurements were taken at 37 °C and in triplicates. Experiments ensured the reproducibility of readings was within 10%. Calculated average activities (in U ml^{-1}) were converted to specific activities (in U mg^{-1} protein) using the GS–DNB extinction coefficient 9.6 mM^{-1} cm^{-1}, and the resulting specific activities were accordingly plotted to depict various graphs (Segel, 1975).

2.4. Concentration-dependent inhibition of hpGSTP1-1

The inhibition of *hp*GSTP1-1 was measured by including in the aforementioned reaction mixture different concentrations of DEL (ranging from 0.5 to 15 µM). The data were obtained in triplicates for each DEL concentration and graphed as a so-called Hill plot of $\log(V_i / (V_o - V_i))$ *versus* log[DEL] to determine the half-maximal inhibitory concentration, IC_{50} (Segel, 1975).

2.5. Kinetic studies of hpGSTP1-1 inhibition

The initial velocities for the *hp*GSTP1-1-catalyzed reaction with GSH as the variable substrate ([CDNB] constant) were determined in a total reaction volume of 500 µl consisting of 100 mM potassium phosphate buffer (pH 6.5), 1 mM EDTA and different concentrations of DEL (0 µM, 0.5 µM, 1.0 µM, 2.0 µM, 4.0 µM or 8.0 µM). The same experiment was repeated with CDNB as the variable substrate ([GSH] constant). The final concentrations of GSH and CDNB in the reaction mixture in each case were 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM or 1.6 mM (for the varied substrate), and 1 mM (for the fixed substrate). The data obtained were used to depict different plots, namely the Lineweaver–Burk and secondary plots, for the purpose of obtaining preliminary information on the mode of inhibition (Segel, 1975).

2.6. Statistical analysis

The enzyme kinetic experimental data were curve-fitted to different mathematical kinetic models encompassing the options competitive, noncompetitive, uncompetitive or mixed-type inhibition. The corresponding kinetic parameters, maximum velocity (V_m), Michaelis constant (K_m) and the enzyme–inhibitor complex dissociation constant (K_i), were estimated using STATISTICA '99 Edition for Windows (StatSoft, Inc., Tulsa, OK, USA). All computations were presented as means and standard error of the means (SEM).

2.7. Target/ligand selection and preparation

The crystal structure of recombinant human GSTP1-1 in complex with GS–DNB at a resolution of 1.9 Å (PDB: 18GS) was downloaded