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Disposition and acute toxicity of imidacloprid in female rats after single exposure

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ABSTRACT

Single dose of imidacloprid (IMI-20 mg/kg bodyweight) was orally administered in female rats. Its disposition along with two metabolites 6-chloro nicotinic acid (6-CNA) and 6-hydroxy nicotinic acid (6-HNA) was monitored in organs (brain, liver, kidney, and ovary) and bodily fluids (blood, urine) at 6, 12, 24 and 48 h and faeces at 24 and 48 h. Maximum concentration (C_{max}) of IMI and metabolites in each organ and bodily fluid occurred after 12 h. Area under curve (AUC) of IMI ranged from 35 to 358 μg/ml/h; 6-CNA: 27.12–1006.42 μg/ml/h and 6-HNA: 14.98–302.74 μg/ml/h in different organs and bodily fluids. Clearance rate of IMI was maximum in ovary followed by kidney, liver, brain, faeces, blood and urine. Percent inhibition of acetyl-cholinesterase (AChE) was comparable in brain and Red Blood Cells (RBC) at 6–48 h which suggests the RBC-AChE as valid biomarker for assessing IMI exposure. It is evident that IMI was absorbed, metabolized, and excreted showing increased level of serum enzymes like Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT) and biochemical constituents like billirubin and Blood Urea Nitrogen (BUN) at 48 h. These data suggest that IMI is widely distributed, metabolized and induced toxicology effects at 20 mg/kg bodyweight to female rats.

1. Introduction

Imidacloprid (IMI; *N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-dihydroimidazol-2-yl}nitramide) a chloronicotynl neonicotinoid compound is a member of new class of systemic insecticide. It is highest selling insecticide worldwide used to control insect pests and also for seed treatment. Despite its use in agriculture it is also used in veterinary medicines for parasitic treatment in cattle (Abar et al., 2011).Global increasing trend for use of IMI is due to its low soil persistence and high insecticidal activity at a very low application rates (Council Directive 91/414/EEC). Therefore, IMI continues to be human health concern due to worldwide use and documented occupational and environmental exposure (Wu et al., 2001; Proenca et al., 2005; Yeh, 2010).

Neonicotinoids and its metabolites are known to regulate nAChR (nicotinic acetyl choline receptors) expression in mammals, and exhibited toxicity in mammalians, as indicated in human suicidal cases (Wu et al., 2001; Proenca et al., 2005). The studies on insects have revealed the involvement of several metabolites of IMI particularly olefine metabolites. These metabolites are more toxic than parent compound. However desnitro metabolite is less toxic to insects but is more toxic to mammalian nervous system. The soil metabolite ethylene–urea induces tumors in combination with nitrate and causes genetic damage. These metabolites could act either alone or synergistically with residues of the parent compound. Moreover, to understand the toxicity of IMI it is essential to know the binding interaction of parent compound and its metabolites to the same or different receptors. The nature of binding receptors reflects the toxicity of IMI, metabolites alone or the combined action of IMI and its metabolites. The metabolism of IMI has not been extensively studied in mammals which encourage to know its metabolic fate in whole body including organs and excreta. This insecticide is metabolized by human cytochrome P450 isozymes involving two major pathways: first imidazolidine hydroxylation and desaturation to give 5-hydroxyIMI and the olefin respectively, and second nitroimine reduction and cleavage to yield the nitrosoimine, guanidine and urea derivatives.

The main IMI metabolites identified in mammals are 5-hydroxy-IMI, 6-hydroxy-IMI (6-hydroxy nicotinic acid), 4,5-dihydroxy-IMI, 6-chloronicotinic acid (6-CNA) and olefin, guanidine and urea derivatives. (Proenca et al., 2005). Detection of the parent compound in blood would represent the most accurate method for

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assessing a specific pesticide exposure (Needham et al., 2005) .Biological markers, such as urinary metabolites, provide a direct objective to assess the exposure and absorbed dose. Studies that incorporate biomarker analyses will provide the most useful estimates of risks of cancer or neurobehavioral effects associated with pesticide exposures. IMI and its metabolite 6-CNA have been analyzed in urine to monitor IMI exposure (Harris et al., 2010; Carretero et al., 2003; Suchail et al., 2001; Cinner et al., 2006).

It is widely acknowledged that toxicokinetics can provide valuable information for risk assessment. Exposure to a chemical does not automatically mean that all of the dose will be bioavailable. The data on systemic exposures to the chemical and its metabolites, for understanding of absorption, distribution, metabolism and excretion may assist in interpretation of toxicity studies and the prediction of differences or similarities across animal species or from animal to man. Toxicokinetic data can also play an important role in selection of test species and doses for further toxicity studies (Creton et al., 2009). Several studies describe both the pharmacokinetic and pharmacodynamic responses of pesticides in both rodents and humans (Timchalk and Poet, 2008; Smith et al., 2009).

The objective of the current study was to evaluate the pharmacokinetic and pharmacodynamic response following single oral exposures to IMI in female rats. The pharmacokinetic study focused on quantifying IMI and its major metabolites 6-CNA and 6-HNA in blood, urine, faeces, liver, kidney, ovary and brain. The pharmacodynamic study herein focused on the impact of IMI on AChE activity in brain and RBC. Brain AChE inhibition may correlates with observed toxicological response while RBC AChE activities are important as biomarkers for assessing exposure in laboratory animals.(Timchalk et al., 2005). In the present study we have tried to correlate the concentration of IMI in blood and tissues with behavioral changes. The relationship between tissue concentration and behavioral effect will aid in determining biologically effective dose (Scollon et al., 2011). Thus our additional objective was to measure IMI level in different tissues to know the biologically effective dose level. Quantification of pesticides and their metabolites in bio fluids and tissues of experimental animals is an important for toxicology research as it will links target tissue dose to the biological effects caused by such chemicals (Ross et al., 2009). This study is in continuation to our earlier toxicological studies in which we have evaluated subchronic 90 days oral and oxidative stress toxicity of IMI to female rats. (Bhardwaj et al., 2010; Kapoor et al., 2010, 2011). Based on the morphological, biochemical, haematological, neuropathological, antioxidant enzymes and lipid peroxidation it was evident that IMI has not produced any significant adverse effects at 5 and 10 mg/kg/day doses but induced toxicological effects at 20 mg/kg/day. Hence 10 mg/kg/day dose was considered as No observed effect level (NOEL) for female rats. It was also observed that IMI at 20 mg/kg/day had produced significant toxicological impact on ovary of female rats as evident by pathomorphological changes, hormonal imbalance and generating oxidative stress. Therefore 20 mg/kg/day may be considered as Lowest Observed Effect Level (LOEL) of IMI for chronic study. (Kapoor et al., 2011) In continuation to previous studies, the present studyhas been undertaken for the evaluation of tissue disposition and acute toxicological effect of IMI in female rats.

2. Materials and methods

2.1. Chemicals

IMI technical 96% pure, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, [CAS No. 138261-41-3] was obtained as gift from Bharat Rasayan, India Limited, New Delhi, India. All solvents like n-hexane, acetone and ethyl acetate (HPLC grade) were purchased from Sigma–Aldrich. Co.USA, Spectrochem Pvt. Ltd. India. Sodium chloride, anhydrous sodium sulphate and anhydrous magnesium sulphate were procured from Himedia Pvt. Ltd. India. Acetylthiocholine chloride and 5,5,-dithio-bis 2-nitrobenzoic acid (DTNB) were purchased from Sigma (St Louis, MO, USA). C-18 cartridges were procured from United Chemical Technology, Bristol.

2.2. Animals and dosing

Forty adult female rats (*Rattus norvigicus Wister strain*) weighing 150–175 g of Indian Institute of Toxicology Research, Lucknow breeding colony were maintained under condition of controlled temperature (22 ± 3 °C) and humidity (30–70%) with 12:12 h of light:dark cycle. The animals were given synthetic pellet diet (M/S Provini Animal Nutrition India Pvt Ltd. India) and water *ad libitum*. Rats were acclimatized for one week prior to experimentation. The rats were placed in individual metabolic cages 1 day before experimentation and maintained until necropsy. An approval from institutional animal ethics committee was obtained for the use of animals (ITRC/IAEC/04/2012.).After acclimatization period, female rats were divided into two groups having 20 in each group. Group I served as control and was given corn oil (0.4 ml/rat) as vehicle through gavage. Group II was orally administered IMI (20 mg/kg body weight) suspended in corn oil in single dose with equal volume of oil per rats. Animals were periodically monitored at 6 h and recorded for signs of clinical toxicity such as diarrhea, salivation, dyspnea, piloerection, excessive secretions and tremors till the period of 48 h exposure.

2.3. Analysis of IMI and metabolites

The five animals from control and treated groups were necropsied at 6, 12, 24 and 48 h. At each time point blood was collected from jugular vein of animals in Ethylene-diamine tetra acid (EDTA) tubes. Brain, liver, kidney and ovary were collected. However before necropsy urine was collected at the same intervals. The faeces were pooled for 0–24 h. and 24–48 h. All samples were stored at −80 °C until analysis. All tissues including faeces (1 g) were homogenized in 2 ml phosphate buffer (pH 7.4) and 10 ml of acetonitrile in centrifuge tube. 1 ml of urine and blood were used for analysis. The homogenates were mixed with 4 g anhydrous magnesium sulphate, and shaken for 10 min. at 50 rpm on rotospin which were further centrifuged for 10 min at 10,000 rpm. Supernatant was collected and evaporated to dryness under slow stream of nitrogen at 40 °C. The dried extracts were reconstituted with 1 ml acetonitrile. The concentrated extract was cleaned through hexane prewashed C_{18} solid phase extraction (SPE) cartridges. The SPE cartridge was then eluted with 1 ml acetonitrile @ 1drop/s. The eluates obtained were filtered through milipore filter membrane (0.22 μm). 20 μl cleaned extract was injected into HPLC-Photon Diode Array (HPLC-PDA) for the analysis of IMI and its metabolites. All samples were analyzed in triplicates.

2.4. HPLC analysis

The aliquot of final sample extracts was analyzed on HPLC system (515 series; Waters, Milford, Massachusetts, USA) equipped with *PDA* (photodiode array detector) using a reversed-phased, C_{18} ODS analytical column (75 · 4.6-mm i.d., 3.5 μ particle size), with a precolumn of the same phase (both supplied by Waters). The HPLC system consisted of a binary pump, an online degasser, thermostatic column housing, and Empower^2 chromatography manager software. The solvent system that constituted the mobile phase was acetonitrile and water (20:80, v/v). The flow rate was maintained at 1.0 ml/min in isocratic mode throughout the analysis and the injection volume was 50 μl. Chromatograms were extracted at 270 nm using PDA. Residues were further confirmed by liquid chromatography mass spectometry (LC–MS).

2.5. LC–MS analysis

Mass spectrometry (MS) detection was carried out by a Waters ZQ 2000 single quadrupole mass spectrometer with electrospray ionization (ESI) performed in positive and negative mode. Full-scan spectra were recorded from *m*/*z* 100–500, at a scan time of 0.5 s and an interscan delay of 0.1 s. The mass spectra were represented by centroid mode. The main other instrumental settings were: capillary voltage 3.5 kV; cone voltage 30 V; extractor 5 V; ion energy 0.1; source temperature 150 °C; desolvation temperature 300 °C; cone gas (N_2) flow rate 0 L/h and desolvation gas (N_2) flow rate 300 L/h. Selected-ion monitoring (SIM) of the most abundant ion was used for quantification. The detector of the mass spectrometer was tuned for the maximum sensitivity of the parent ion at *m*/*z* 256 (0.5 and of the product ions) at *m*/*z* 209 and at *m*/*z* 175.

2.6. AChE activity in blood and brain

2.6.1. Acetylcholinesterase (AChE) activity determination

Separated RBC and an aliquot of brain homogenates were used for estimation of AChE activity (Ellman et al., 1961). 2.9 ml of phosphate buffer (pH 7.4) was diluted with 40 μl of distilled water. In diluted phosphate buffer, 10 μl of tissue homogenate and 10 μl of Ellman reagent (5,5V-dithiobis-nitrobenzoic acid) (0.1 mM) was