The Mouse Ovary has the Capacity to Convert the Neonicotinoid Pesticide Imidacloprid to Toxic Metabolites *In Vivo*

Jadesola I. Oladosu, Vasiliki E. Mourikes, Colin Lee, Megan Mahoney, Jodi A. Flaws

Imidacloprid (IMI) belongs to a class of neuro-active insecticides called neonicotinoids. Neonicotinoid pesticides are nicotine derivatives that target nicotinic acetylcholine receptors (nAChRs) in the nervous system. IMI is used in large scale agricultural systems, sold for private residential use, and is found in veterinary pharmaceuticals. Because IMI is more water soluble than other insecticides, there is tremendous potential for environmental accumulation and chronic exposure of non-target species. IMI can be converted to desnitro-imidacloprid (DNI) by the liver through phase I biotransformation; both compounds act as an agonist for mammalian nAChRs, with DNI having a significantly higher affinity than IMI for nAChRs. A previous study showed that IMI is converted to toxic metabolites *in vitro*. However, it was not known whether the whole ovary contains the metabolic enzymes required to convert IMI to toxic metabolites. Thus, this study tested the hypothesis that the ovary contains the enzymes required to metabolize IMI. To test this hypothesis, mice (6 weeks of age) were dosed orally with either vehicle control (dimethyl sulfoxide), a low dose of IMI (0.5 mg/kg body weight), or high dose of IMI (5.7 mg IMI/kg body weight for 30 days. After dosing, the ovaries were collected and ovarian RNA was used for qPCR reactions to quantify gene expression of metabolic enzymes associated with IMI metabolism. The expression of the following six genes was measured: aldehyde oxidase 1 (*Aox1*), aldehyde oxidase 2 (*Aox2*), aldehyde oxidase 3 (*Aox3*), cytochrome P450 family 2 subfamily D member 22 (*Cyp2d22*), cytochrome P450 family 2 subfamily E member 1 (*Cyp2e1*), and cytochrome P450 family 4 subfamily F member 18 (*Cyp4f18*). These genes were selected because they are known to convert IMI to toxic metabolites such as DNI in the liver. The results indicate that the ovaries expressed all six genes. However, exposure to both low and high doses of IMI did not significantly increase expression of *Aox1, Aox2, Aox3, Cy2d22, or Cyp4f18* compared to control. Interestingly, low dose IMI significantly increased the expression of *Cyp2e1* compared with control. These data indicate that mouse ovaries metabolize IMI and that IMI can induce ovarian *Cyp2e1* expression at a low dose, with IMI following a non-monotonic dose response curve. *Cyp2e1* is important in IMI metabolism because it reduces IMI to DNI and other downstream metabolites. Collectively, these data suggest that the mouse ovary has the capacity to metabolize IMI to DNI and other downstream metabolites. Supported by NIH F30 ES033914 and NIH R01 ES028861.