Protective efficacy of Anopheles minimus CYP6P7 and CYP6AA3 against cytotoxicity of pyrethroid insecticides in Spodoptera frugiperda (Sf9) insect cells

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Abstract: Cytochrome P450 monooxygenases (P450s) are enzymes known to metabolize a wide variety of compounds including insecticides. Their overexpression leading to enhanced insecticide detoxification could result in insecticide resistance in insects. The increased mRNA expression of two P450 genes, CYP6P7 and CYP6AA3, has been previously observed in laboratory-selected deltamethrin-resistant Anopheles minimus, a major malaria vector in Southeast Asia, suggesting their role in detoxification of pyrethroids. In this study CYP6P7 and CYP6AA3 were expressed in insect Spodoptera frugiperda (Sf9) cells via baculovirus-directed expression system. Insecticide detoxification capabilities of Sf9 cells with and without expression of CYP6P7 or CYP6AA3 were evaluated using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. The results revealed that CYP6P7- or CYP6AA3-expressing cells showed significantly higher cytoprotective capability than parental Sf9 cells against cytotoxicity of pyrethroids including permethrin, cypermethrin and deltamethrin. Such cytoprotective effect was not observed for bioallethrin (pyrethroid), chlorpyrifos (organophosphate) and propoxur (carbamate). Moreover, expression of CYP6AA3, but not CYP6P7, could protect cells against lambda-cyhalothrin cytotoxicity. In MTT assays upon co-incubation with piperonyl butoxide (P450 inhibitor), cytoprotective ability of CYP6P7 and CYP6AA3 against deltamethrin was diminished, implying that pyrethroid detoxification was due to activities of P450 enzymes. Insecticide detoxification capabilities of CYP6P7 and CYP6AA3 observed from MTT assays were correlated to their pyrethroid metabolizing activities observed from in vitro reconstitution enzymatic assays. Thus MTT assays using cells expressing P450 enzymes of interest could be primarily used to determine detoxification activities of enzymes against cytotoxic insecticides.

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