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Role of genetic polymorphisms of *CYP1A1*, *CYP3A5*, *CYP2C9, CYP2D6*, and *PON1* in the modulation of DNA damage in workers occupationally exposed to organophosphate pesticides

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ABSTRACT

Organophosphate pesticides (OPs) are primarily metabolized by several xenobiotic metabolizing enzymes (XMEs). Very few studies have explored genetic polymorphisms of XMEs and their association with DNA damage in pesticide-exposed workers. The present study was designed to determine the role of genetic polymorphisms of *CYP1A1*, *CYP3A5*, *CYP2C9, CYP2D6,* and *PON1* in the modulation of DNA damage in workers occupationally exposed to OPs. We examined 284 subjects including 150 workers occupationally exposed to OPs and 134 normal healthy controls. The DNA damage was evaluated using the alkaline comet assay and genotyping was done using PCR–RFLP. The results revealed that the PONase activity toward paraoxonase and AChE activity was found significantly lowered in workers as compared to control subjects ($p<0.001$). Workers showed significantly higher DNA damage compared to control subjects (14.37 ± 2.15 vs. 6.24 ± 1.37 tail% DNA, $p<0.001$). Further, the workers with *CYP2D6*3* PM and *PON1* (*QQ* and *MM*) genotypes were found to have significantly higher DNA damage when compared to other genotypes ($p<0.05$). In addition, significant increase in DNA damage was also observed in workers with concomitant presence of certain *CYP2D6* and *PON1* (*Q192R* and *L55M)* genotypes which need further extensive studies. In conclusion, the results indicate that the *PON1* and *CYP2D6* genotypes can modulate DNA damage elicited by some OPs possibly through gene-environment interactions.

Introduction

Organophosphate pesticides (OPs), triesters of phosphoric acid, are widely used group of pesticides in the world. Besides their use in agriculture, diseases control and as therapeutic agents, OPs are also used in industries as solvents, plasticizers, flame retardants and in defense forces as nerve agents (Holstege et al., 1997; Mackenzie-Ross et al., 2010). OPs inhibit acetylcholinesterase (AChE) resulting in chronic harmful effects on human health e.g., neuropsychological disorders, disruption of the endocrine system, developmental anomaly, disorders of immune system and hypersensitivity (Mansour, 2004). A possible association has been reported between OP exposure and cancers of lung, prostate as well as non-Hodgkin's lymphoma and leukemia (Bonner et al., 2010; Waddell et al., 2001). Experimental studies (in vitro and in vivo) have shown that several OPs exert genotoxic activity (Bolognesi, 2003) and can induce neoplastic transformation of cells (Cabello et al., 2001; Isoda et al., 2005).

OPs are primarily metabolized by various hepatic cytochrome P450s to become an active intermediate organophosphate-oxons (OP-oxons) (Mutch and Williams, 2006; Sams et al., 2004; Tang et al., 2001). These active intermediate OP-oxons are further hydrolyzed by paraoxonase to diethyl phosphate and 4-nitrophenol (Costa et al., 1999; Mutch et al., 1999), or conjugated to glutathione, with subsequent catalysis by various glutathione S-transferases (GSTs) (Fujioka and Casida, 2007). These oxons are recognized to be the mediator of acute OP toxicity, due to its ability to bind to and inhibit acetylcholinesterase in the nervous system and at neuromuscular junctions (Timchalk, 2001).

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Cytochrome P450 (CYP) 1A1 is an important phase I xenobiotic metabolizing enzyme responsible for the metabolism of numerous carcinogens and steroidal hormones including estrogens (Gonzalez, 1990). It contributes greatly to the toxicity of chemicals, since it is responsible for the creation of the toxic intermediates which bind DNA and catalyze the first step of the metabolism of these substances to electrophilic compounds (Shimada et al., 1996). Among all allelic variants of *CYP1A1*, three variants (*m1*, *m2*, and *m4*) are known to cause variation in the activity of the enzymes and possibly alter the metabolism of carcinogens and steroidal hormones.

CYP3A5 represents at least 50% of the total hepatic cytochrome P450 and metabolizes wide range of xenobiotics including some OPs (Eaton, 2000; Foxenberg et al., 2007). An *A44G* polymorphism in the promoter region of the pseudogene *CYP3AP1* has shown to be linked with the splicing defect of *CYP3A5*3*, resulting in the absence of *CYP3A5* in the tissues of some people (Paulussen et al., 2000). *CYP2C9* is another major enzyme which belongs to the CYP2C subfamily, and constitutes 20% of the hepatic cytochrome P450 enzyme expressed in humans (Seng et al., 2003). *CYP2C9* was found to be associated with metabolization of wide range of xenobiotics including some OPs (Eaton, 2000; Foxenberg et al., 2007). Three alleles of *CYP2C9*, namely *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* are frequently identified in all ethnic populations (Lee et al., 2002). It has been reported that in individuals with *CYP2C9*2* and *CYP2C9*3* mutant alleles, the enzyme activity is significantly reduced, subsequently, and may not be able to metabolize their substrates adequately leading to toxicity (Aithal et al., 1999; Miners, 2002; Verstuyft et al., 2001). *CYP2D6* metabolizes numerous chemicals including many OPs and is polymorphically expressed. Polymorphisms in the *CYP2D6* gene influence enzyme activity leading to the phenotypic appearance of distinct types of responders to its substrates. The genetic polymorphism results in different *CYP2D6* xenobiotic metabolisms phenotypes namely extensive metabolizers (EM), intermediate metabolizers (IM), and poor metabolizers (PM) (Bertilsson et al., 2002).

DNA damage is considered as an important biomarker and is the underlying cause of mutations leading to cancers (Bernstein, 2009). Very few studies have explored genetic polymorphisms of xenobiotic metabolizing enzymes (XMEs) and their association with DNA damage in pesticide-exposed workers (Abhishek et al., 2010; Da Silva et al., 2008; Liu et al., 2006; Rohr et al., 2011; Wong et al., 2008). Previous studies have revealed that genotypes of *CYP3A5*, *GSTP1* (Liu et al., 2006), and *GSTT1* (Abhishek et al., 2010) are associated with increased risk of DNA damage in pesticide-exposed subjects. Recently, we reported the association of *PON1 QQ* and *MM* genotypes with increased risk of DNA damage in occupational workers exposed to some OPs (Singh et al., 2011b). The present study was designed to determine the role of genetic polymorphisms of *CYP1A1*, *CYP3A5*, *CYP2C9*, *CYP2D6*, and *PON1* in the modulation of DNA damage in workers occupationally exposed to OPs. AChE and paraoxonase (PONase) activities were also analyzed as the biomarkers of toxicity due to long term exposure to some OPs.

Materials and methods

Study population. This cross-sectional study was approved by the Institutional Ethical Committee, National Centre for Disease Control, New Delhi, India. Informed consent was obtained in English and local language Hindi, from each individual prior to blood sample collection. The study involved 284 subjects divided into two groups. The first group (workers) consisted of 150 occupational workers of Northern Indian ethnicity, employed for spraying OPs for community health programs (control of vector-borne diseases) in Delhi. The average age of the workers was 46.84 ± 5.36 (mean ± S.D.) years. These workers were exclusively handling OPs that were pirimiphos methyl, chlorpyrifos, temephos and malathion. The duration of exposure to OPs ranged from 10 to 35 years, the average being 22.61 ± 6.11 (mean ± S.D.) years. The workers were not using any kind of personal protective measures, such as gloves, covering arms, face mask, etc., while mixing or handling the stock of pesticides formulation and application. Thus, there was direct exposure of various OPs through inhalation, skin, and eyes. The second group (control subjects) consisted of 134 normal healthy volunteers, guardian or an accompanying person of a patient (not their blood relatives) who came for routine checkup at the National Centre for Disease Control, Delhi, India with no records of occupational or any other exposure to pesticides or any particular toxic or carcinogenic environmental agent. The average age of control subjects was 45.48 ± 4.70 (mean ± S.D.) years. None of the subjects from both groups were suffering from any chronic disease like tuberculosis, diabetes, thyroid malfunction, heart diseases, or malignancy. It was also ensured that both control subjects and workers were not taking any kind of medicine or not exposed to any other carcinogen for the last 10 days nor had they been exposed to any kinds of radiation (diagnostic and therapeutic) within the last 12 months before blood sampling. In India, pesticide spray workers are usually males, while female workers in this occupation are negligible. Therefore, only male workers were included in the present study and the same number of gender, age and ethnicity matched controls were recruited to avoid discrepancies.

In the present study, 230 subjects (115 workers and 115 control subjects) were those who were previously enrolled to evaluate the effect of *PON1* genotypes and phenotypes on DNA damage (Singh et al., 2011b). In the current study, sample size was increased to acquire sufficient statistical discriminatory power to detect a difference in the level of DNA damage and other parameters.

Epidemiological information. All subjects were asked to complete a face-to-face questionnaire that included standard demographic data (age, gender, body weight and height), medical (exposure to X-ray and medication), lifestyle (smoking, alcohol and diet), illness (any kind of acute and chronic illness), symptoms (toxicities due to chronic exposure of OPs), and occupation-related information (working hours/day, years of exposure, use of protective measures, type of exposure, type of pesticide used, etc.). The medical history was recorded with the help of qualified personnel. Subjects were assigned to one of three groups (heavy smokers, mild smokers, and non smokers) on the basis of smoking history. Heavy smokers (HS) were those who smoked more than five cigarettes or *bidis* (a type of low cost local product smoked in India) per day for at least one year and were currently active smokers. Mild smokers (MS) were those who currently smoked, yet had not reached the duration or consumption level to fit into the heavy smoking category. While, nonsmokers (NS) either had never smoked tobacco or had quit smoking more than one year before collection of their blood sample. Alcoholics were habitual drinkers and consuming at least two units of alcohol per day at least for one year. In India, traditionally *bidis* are smoked, which are made by rolling active tobacco in betel leaves.

Duration of exposure. The workers were applying pesticides for a minimum of five hours/day, six days a week with one day weekly off. Thus, these workers were continuously exposed to OPs without repair/treatment plan. An exposure index was calculated for each interviewed subject according to the following formula: hours/day × days/year × years (Farrow et al., 1989). Subjects with an exposure index of more than 2400 h (duration of exposure) were considered to be "exposed". The 2400-hours cutoff value was chosen on the basis of previous reports indicating that this figure represents heavy exposure due to genotoxic agents (Dich et al., 1997; Potti et al., 2003). In the present study, the exposure index value was found to be more than two folds higher than 2400-hours cutoff value.

Sample collection. Blood sample (10 ml, twelve-hour fasting) was collected from each subject by venopuncture and transferred to sterile heparinised vacutainers (4 ml) for lymphocyte separation. Another 4 ml was transferred to plain vacutainers to obtain a serum for enzyme