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Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study

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Abstract

Oxidative stress and DNA damage have been proposed as mechanisms linking pesticide exposure to health effects such as cancer and neurological diseases. A study of pesticide applicators and farmworkers was conducted to examine the relationship between organophosphate pesticide exposure and biomarkers of oxidative stress and DNA damage. Urine samples were analyzed for OP metabolites and 8-hydroxy-2′-deoxyguanosine (8-OH-dG). Lymphocytes were analyzed for oxidative DNA repair activity and DNA damage (Comet assay), and serum was analyzed for lipid peroxides (i.e., malondialdehyde, MDA). Cellular damage in agricultural workers was validated using lymphocyte cell cultures. Urinary OP metabolites were significantly higher in farmworkers and applicators ($p<0.001$) when compared to controls. 8-OH-dG levels were 8.5 times and 2.3 times higher in farmworkers or applicators (respectively) than in controls. Serum MDA levels were 4.9 times and 24 times higher in farmworkers or applicators (respectively) than in controls. DNA damage (Comet assay) and oxidative DNA repair were significantly greater in lymphocytes from applicators and farmworkers when compared with controls. Markers of oxidative stress (i.e., increased reactive oxygen species and reduced glutathione levels) and DNA damage were also observed in lymphocyte cell cultures treated with an OP. The findings from these *in vivo* and *in vitro* studies indicate that organophosphate pesticides induce oxidative stress and DNA damage in agricultural workers. These biomarkers may be useful for increasing our understanding of the link between pesticides and a number of health effects.

Keywords: Organophosphate pesticides; Lymphocytes; Azinphos methyl (AZM); AP endonuclease (APE); Base excision repair (BER); Malondialdehyde (MDA); 8-Hydroxydeoxyguanosine (8-OH-dG); Comet assay; Reactive oxygen species (ROS); Glutathione (GSH); Dialkylphosphate metabolites

Introduction

Multiple studies have reported associations between exposure to agricultural chemicals and various health outcomes including cancer, Parkinson's disease and other neurological diseases. However, these investigations are often limited by self-reported exposures and do little to advance our understanding of the biological mechanisms linking exposure to pesticides and the observed health outcome. DNA damage and oxidative stress have been proposed as mechanisms that could mechanistically link pesticide exposures to a number of the health outcomes observed in epidemiological studies.

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify the reactive intermediates or easily repair the resulting damage. Toxic effects are caused through the production of peroxides and free radicals that damage all the components of the cell, including protein, lipids, DNA and RNA. In addition to damaging macromolecules, ROS influence molecular and biochemical processes and disrupt the function of DNA repair

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proteins (Martignoni et al., 1999; Shimura-Miura et al., 1999; Sofic et al., 1992). In contrast, an adaptive cellular response to oxidative stress occurs in many pathological conditions and this is characterized by increases in DNA repair proteins (e.g., APE/Ref1) (Deganuto et al., 2007; Kelley et al., 2001; Moore et al., 2000; Shaikh et al., 2002). Cells possess two major defense mechanisms to counteract ROS-induced damage—antioxidants and DNA repair processes. The efficiency of these defense mechanisms is reportedly influenced by various environmental factors, resulting in an accumulation of oxidative DNA lesions (e.g., 8-hydroxy-2′-deoxyguanosine). Levels of oxidative DNA lesions in biological fluids (e.g., urine, serum, cerebrospinal fluid) and tissues have been reported to be reliable biomarkers of oxidative stress (Anwar, 1997; Kadiiska et al., 2005; Rahman and Biswas, 2004; Sato et al., 2005).

Oxidative damage is thought to be an important mechanism of damage for organophosphate pesticides (Bagchi et al., 1995; Banerjee et al., 2001; Delescluse et al., 2001; Flessel et al., 1993; Halliwell, 2002). There is compelling evidence from whole-animal and tissue culture studies that pesticides, especially organophosphate pesticides, induce oxidative stress (Bagchi et al., 1995; Lodovici et al., 1997; Nag and Nandi, 1987; Yang and Sun, 1998). Increased serum and urinary levels of lipid peroxides and altered blood levels of glutathione (GSH) and antioxidant enzymes have been detected in several cases of pesticide poisoning (Banerjee et al., 1999; Blasiak et al., 1999; Maroni and Fait, 1993; Peluso et al., 1996). Moreover, DNA damage comparable to that seen after oxidative stress has been detected after exposure to pesticides (Lebailly et al., 1998a,b; Lieberman et al., 1998; Lodovici et al., 1997; Peluso et al., 1996; Shah et al., 1997). Organophosphate pesticides have also been reported to reduce antioxidant enzyme activity, enhance the production of lipid peroxides and reduce the level of cellular antioxidants (Julka et al., 1992). These non-specific mechanisms of oxidative damage and disrupted repair may explain in part the link between pesticide exposure and adverse health outcomes.

Organophosphate pesticides have also been shown to have cytotoxic, cytostatic and cytogenetic effects on human lymphocytic cells (Sobti et al., 1982, Braun et al., 1982), suggesting that they may also have mutagenic properties. *N*-methylcarbamate esters insecticides have also been shown to have the potential to act through mediation of epigenetic and genotoxic mechanisms respectively in the multiple stages of chemical carcinogenesis (Wang et al., 1998).

This paper describes the results of a study designed to measure urinary biomarkers of organophosphate pesticide exposures in agricultural workers and to assess the relationship between pesticide exposure and measures of oxidative stress and DNA damage. These results were validated *in vitro* by examining similar biomarkers in lymphocyte cultures treated with a commonly used organophosphate, azinphos methyl (AZM). The overall goal of this investigation was to demonstrate that exposure to organophosphate pesticides induces oxidative stress and DNA damage in agricultural workers, and these biomarkers of oxidative stress are dose dependent.

Methods

Target population. Three Hispanic populations were selected for this study conducted during the spring and summer growing seasons in 2000. Certified pesticide handlers and applicators were recruited from contacts with agricultural companies in the Willamette and Columbia River valleys in Oregon. The certified pesticide applicators were recruited based on their job classification. However, we could not assure that they were actively spraying pesticides at the time of enrollment in the study. If pesticides were not currently being sprayed in their worksite, they were engaging in general agricultural work. Farmworkers were recruited as part of a larger community based participatory research study taking place in an agricultural community located 60 miles east of Portland in the Columbia River valley (Lambert et al., 2005; McCauley et al., 2001a,b,c). The farmworkers' primary job activities included pruning, thinning and picking apples and pears. We recruited the workers during the preharvest period in orchard crops, a time that the use of organophosphates is expected to be the highest. We also recruited during the same time period a sample of non-agricultural adults as a referent population residing in urban Portland. Subjects were informed about the purpose of the study and were asked to sign a consent form. All procedures for this investigation were approved by the Oregon Health and Science University Internal Review Board. Subjects were paid $60.00 for their participation in the study.

Procedures. Questionnaires were administered to characterize their work practices, exposure history, job activities and use of protective equipment and length of time doing present work activity, proximity of home to agricultural fields. The study questionnaires have been previously used in our investigations of more than 1000 agricultural workers and families in Oregon and have been developed in Spanish to be administered by Spanish-speaking interviewers (McCauley et al., 2001a,b,c). We developed a short survey to assess smoking, alcohol intake and dietary patterns associated with antioxidant activity. The questionnaire asked weekly intake of fish, legumes and nuts. Spot urine samples were collected at the end of the workday in 4-oz. sterile specimen containers. Urine samples were marked with ID number and transported in refrigerated containers to the laboratory for analysis of organophosphate metabolites and markers of oxidative stress. Upon arrival, the samples were vortexed, pH adjusted, aliquoted into 5-ml fractions and stored at −80 °C.

Venous blood (2×10 ml) was drawn by a phlebotomist into heparinized vacutainers. Heparinized blood samples were transported on ice to the laboratory for the isolation of lymphocytes and serum. Lymphocytes were isolated by centrifugation at 400×*g* for 45 min on a Ficoll/Hypaque gradient as described by Boyum (1964) and Wottawa et al., (1974) and stored at −80 °C until analysis. Lymphocytes and serum samples were prepared within 24 h of collection to maintain the integrity of DNA and the stability of the biomarkers of oxidative stress (Wu et al., 2004).

Analysis of urine for OP metabolites. Frozen urine samples from study subjects were thawed and analyzed for organophosphate pesticide metabolites by gas chromatography with pulsed flame photometric detector (GC-PFPD) as previously described by Lambert et al. (2005). The alkylphosphate pesticide metabolites measured were dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP) and dimethyldithiophosphate (DMDTP). The limits of detection (LOD) for the five metabolites were 4.0 ng/ml (0.032 μmol/L) DMP, 2.0 ng/ml (0.013 μmol/L) DEP, 2.2 ng/ml (0.015 μmol/L) DMTP, 1.6 ng/ml (0.010 μmol/L) DMDTP and 1.6 ng/ml (0.0095 μmol/L) DETP. The average extraction efficiencies of the five metabolites were 45%, 84%, 97%, 96% and 93%, respectively. Because of less than optimal recovery, the non-thiol alkyl phosphates DMP and DEP were not included in the data analysis. Urine samples were also analyzed for creatinine concentration (mg/dL) and the values were used to normalize OP metabolites across samples.

Analysis of urine for oxidative DNA damage. 8-Hydroxy-2′-deoxyguanosine (8-OH-dG) is a promutagenic lesion in DNA that is generated in response to a number of chemicals that induce oxidative stress (Kasai et al., 2001) and it is widely used as a marker of oxidative injury (Toraason, 1999). Frozen urine samples were thawed, centrifuged, diluted 1:5 with potassium phosphate buffer (pH 4.0) and 2.0 ml of the pH-adjusted urine loaded onto a preconditioned C_{18} SFE SepPak™ cartridge (Waters Associates). The SepPak™ cartridge was