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Effects of genetic polymorphisms *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* on urinary 1-hydroxypyrene levels in sugarcane workers

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Abstract

Sugarcane workers in Brazil are exposed to various genotoxic compounds, including polycyclic aromatic hydrocarbons (PAHs), derived from an incomplete combustion process of burnt sugarcane fields. The effects of the occupational exposure to sugarcane fields burning were measured in urine samples of sugarcane workers from the northwest of the State of São Paulo when exposed (harvesting) and when non-exposed (non-harvesting). The urinary levels of 1-hydroxypyrene (1-OHP) and the influence of the genetic polymorphisms *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* were evaluated. Our results showed that the 1-OHP levels were significantly higher ($P<0.0000$) in the exposed sugarcane workers (0.318 μmol mol^{-1} creatinine) than in the non-exposed workers (0.035 μmol mol^{-1} creatinine). In an unvaried analysis, no influence regarding the polymorphisms was observed. However, multivariate regression analysis showed that the *CYP1A1*4* polymorphism in the exposed group, and age and the *GSTP1* polymorphism in the non-exposed group significantly influenced urinary 1-OHP excretion levels ($P<0.10$). The same group of sugarcane workers was significantly more exposed to PAHs during the harvesting period than during the non-harvesting period.

Keywords: Sugarcane workers; 1-Hydroxypyrene (1-OHP); Genetic polymorphisms; Polycyclic aromatic hydrocarbons (PAHs); CYPs; GSTs

1. Introduction

Brazil is the largest sugarcane producer in the world with about 4.5 $\times 10^6$ ha of sugarcane fields and 320 ethanol production plants (http://www.portalunica.com.br/index.jsp). A common procedure in this country is to burn the leaves before harvesting the sugarcane. The burning leads to production of soot, suspected to increase respiratory problems in the exposed workers (Zamperlini et al., 1997). Sugarcane workers are exposed to various genotoxic compounds, including polycyclic aromatic hydrocarbons (PAHs) derived from an incomplete organic combustion process (Zamperlini et al., 1997). PAHs

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undergo metabolic activation by phase I enzymes, mainly cytochrome P4501A1 (CYP1A1), to diol epoxides which are capable of binding covalently to DNA, potentially initiating a carcinogenic process (Hall and Grover, 1990). Activated PAH metabolites can be detoxified by phase II enzymes, such as glutathione *S*-transferases (GSTs) and uridine diphosphoglucuronosyltransferase (UDP glucuronyl-transferase), which catalyze conjugative reactions of oxidative products (Nerurkar et al., 2000). In humans, a substantial variability in the biological response to PAHs is expected, since there are interindividual differences in the rate and metabolism pathways of these compounds, determined by genetic polymorphisms of phases I and II enzymes (Alexandrie et al., 2000).

The *CYP1A1* gene is characterized by several polymorphisms. Two of them, one in the 3′-noncoding region (*CYP1A1*2A*, T3801C) and the other within exon 7 (*CYP1A1*2B*, A2455G), are of interest in the biotransformation of PAHs and have been extensively studied to evaluate their possible role in DNA damage and cancer promotion (Grzybowska et al., 2000; Gaspar et al., 2004). The *CYP1A1*4* (C2453A) polymorphism also has shown to produce a variant protein that possesses different catalytic activities when compared to the wild-type protein (Schwarz et al., 2001) and was associated with an increased risk of endometrial cancer (Esteller et al., 1997). The *GSTM1*, *GSTT1* and *GSTP1* genes produce important biotransformation enzymes (Seidegard, 1990; Pemble et al., 1994; Harries et al., 1997). The lack of GSTM1 activity, caused by an inherited homozygous deletion of the *GSTM1* gene (null genotype), has been associated with an increased risk of lung cancer (Pinarbasi et al., 2003; Mohr et al., 2003). The *GSTT1* polymorphism is also caused by a deletion that results in total lack of gene product. The null genotype of *GSTT1* was reported to be associated with an increased risk of oral and lung cancer (Sreelekha et al., 2001; Sorensen et al., 2004). The polymorphism of the *GSTP1* gene, which consists in an A→G transition at nucleotide 313 in exon 5, leads to an Ile→Val substitution in the substrate-binding active site of the enzyme. This substitution has been associated with a reduced conjugating activity of the enzyme (Taningher et al., 1999) and also with higher levels of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in human lymphocytes (Miller et al., 2003).

Urinary 1-hydroxypyrene (1-OHP) is a widely used biological marker of exposure to PAHs and indicator for internal dose of activated PAHs (Jongeneelen, 1987; Adonis et al., 2003). PAH metabolites may be excreted either as free or as conjugated compounds. When 1-OHP is measured after treating the urine with deconjugating enzymes (e.g., β-glucuronidase), the sum of conjugated and deconjugated species is quantified, reflecting the total amount of hydroxylate metabolite. Although 1-OHP itself is not a carcinogen, it can be used as a marker for a major activating step in the metabolism of PAHs (Nerurkar et al., 2000).

The aim of the present study was to evaluate 1-OHP concentrations in the urine of sugarcane workers, during harvesting (when the sugarcane fields are burnt) and during non-harvesting time, as a biological marker of exposure to PAHs. At the same time, the influence of the genetic polymorphisms *CYP1A1*, *GSTP1*, *GSTM1* and *GSTT1* was also analyzed.

2. Materials and methods

2.1. Study subjects and sample collection

The study was performed on nonsmoking and healthy sugarcane workers who were resident in the northwest of the State of Sao Paulo, Brazil. They were recruited from three different companies located at a distance of about 30 km from each other. The individuals lived in small non-polluted towns close to rural areas and all of them were transported to work by bus. Urine samples of the same workers, except 5, were collected twice: during harvest time (July to September 2001, months that include winter and spring), classified as exposed group ($n=39$, mean age 33.73±1.68 years), and during non-harvest time (January to March 2002, summer), classified as non-exposed group ($n=34$, mean age 35.21± 1.89 years). During harvest time, these workers used to go to the field few hours after the burning of the sugarcane foliages, therefore being exposed to fume and particulate matter deposited on the ground and vegetation. A group of 21 individuals (mean age 44.48± 1.50 years), including a secretaries, a gardeners, a drivers and a maintenance aids, resident in a city distant at least 60 km from sugarcane plantations, were used as a reference of subjects non-occupationally exposed to PAHs. Urine samples of these individuals were collected during the work time (August and September 2002, months that include winter and spring).

The Brazilian National Ethics Committee approved this study, and written informed consent was obtained from all individuals who agreed to participate. A questionnaire was filled out for each individual, in order to obtain personal information (age, gender, health problems and smoking habits). In addition, they were instructed to follow some procedures, such as not eating fried, grilled or barbecued meat in the 24 h preceding the urine sampling. Urine samples were collected in polyethylene containers at the end of the work shift from the moment the worker arrived home until the sleep time, more than