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Role of *PON1, SOD2, OGG1, XRCC1*, and *XRCC4* polymorphisms on modulation of DNA damage in workers occupationally exposed to pesticides

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ABSTRACT

Tobacco farming has been proving to induce poor health outcomes in agricultural workers, genomic instability being the triggering one. This study evaluated influence of *PON1* (paraoxonase 1), *SOD2* (superoxide dismutase), *OGG1* (8-oxoguanine glycosylase), *XRCC1* (X-ray repair cross-complementing protein 1), and *XRCC4* (X-ray repair cross-complementing protein 4) genes polymorphisms on DNA damage in 121 subjects occupationally exposed to pesticides mixtures and nicotine at tobacco fields and 121 non-exposed individuals. Inorganic elements (Cl, P, S and Zn) and cotinine levels were found increased in farmers, confirming exposure. Results show higher frequencies of buccal micronucleus (MN), nuclear buds (NBUD), binucleated cells (BN) and damage index (comet assay), reduced telomere length (TL), and increased parameters of oxidative stress in farmers compared to non-exposed individuals. *PON1 Gln/Gln* genotype was associated with increased MN frequency. *SOD2 Val/Val* showed association with increased frequency of MN and NBUD and decreased antioxidant activity. The *XRCC1 Arg/Arg* showed protective effect for MN, BN and TL, which was also positively influenced by *OGG1 -/Cys*. MN was decreased in *XRCC4 -/Ile* farmers. These genotypes also showed a risk for antioxidant activity. Our study proposes that *PON1* and *SOD2* variants play a role in xenobiotic-metabolizing system in farmers, while base excision repair (BER) pathway could be the repair mechanism involved in genomic instability suffered by tobacco farmers.

1. Introduction

Natural and man-made chemical pesticides are widely used in agriculture with the purpose of controlling pests, resulting in contamination of the environment and living organisms. There is a growing body of epidemiological and experimental evidence on the link between exposure to pesticides and increased occurrence of different health disorders in human beings, including various cancers (Kim et al., 2017).

As expected, the jeopardy of pesticide contamination usually increases with dosage and duration of exposure, in addition to toxicity of the chemical (Kim et al., 2017). Tobacco farming is known by its long crop season of around 10 months (Da Silva et al., 2014). Tobacco farmers apply low doses of pesticides in a chronic manner and for the whole season, undergoing a lifetime of occupational exposure to pesticides (Da Silva et al., 2014). Tobacco farming presents an additional challenge in comparison with other crops: the nicotine exposure. This alkaloid is found on the surface of tobacco leaves, acting as a natural insecticide of the plant. Although nicotine is present in tobacco leaves for the entire season, it is estimated that in only one day during harvest period, agricultural workers may be exposed to nicotine in the leaf surface equivalent to a dose of 36 cigarettes (NIOSH, 1996).

Regarding tobacco farming, studies have been investigating the

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increase in acute poisoning (Fassa et al., 2014), suicide mortality (Krawczyk et al., 2014), minor psychiatric disorders (Faria et al., 2014), biochemical alterations (Khan et al., 2010; Onuki et al., 2003), and safety practices among workers (Damalas et al., 2006). To the best of our knowledge, our group is the only one analyzing the cytogenetic and molecular damages in tobacco farmers. Our previous studies observed increased DNA damage through comet assay (Alves et al., 2016; Da Silva et al., 2012a, 2012b, 2014), buccal (Alves et al., 2016; Da Silva et al., 2012b) and lymphocyte (Da Silva et al., 2012a, 2014) micronucleus cytome assay, and decreased telomere length (Kahl et al., 2016a), with epigenetic influence (Kahl et al., 2016b) and mechanism involving oxidative stress (Alves et al., 2016; Da Silva et al., 2012a; Kahl et al., 2016a). The comet assay is a rapidly and sensitive technique for the detection of DNA damage at the level of individual eukaryotic cell and is widely accepted as a biomarker of effect (Collins, 2004). The buccal micronucleus assay is minimally invasive and considered a biomarker of effect, as it evaluates DNA damage, chromosomal instability and cell death that are stablished in an oral cell population (Thomas et al., 2009). We also observed increase in cotinine (Alves et al., 2016; Da Silva et al., 2014) and trace inorganic elements (Alves et al., 2016; Da Silva et al., 2012a; Kahl et al., 2016a) levels in blood, endorsing nicotine and synthetic pesticides exposure and absorption.

The main challenge in occupational exposure investigation arises from the variability in individual responses and genetic resilience/susceptibility, portraying differences in sensitivity to a given chemical (Dourson et al., 2013). Xenobiotic-metabolizing enzymes interact with endogenous and exogenous substrates to eliminate them directly or conjugated with endogenous cofactors via through renal or biliary excretion. Paraoxonase I gene (*PON1*) express an enzyme that hydrolyze organophosphate compounds (Humbert et al., 1993), while superoxide dismutase 2 (*SOD2*) gene codes for the MnSOD enzyme, the only known antioxidant within mitochondria (Flekac et al., 2008).

However, when DNA damage is established, the role of DNA repair mechanisms is to deal with different classes of lesions, using either base excision repair (BER) or non-homologous end joining (NHEJ) pathways. Some highly important enzymes are X-ray repair cross-complementing protein 4 (XRCC4), which belongs to NHEJ pathway (De Ruyck et al., 2005); 8-oxoguanine DNA glycosylase (OGG1), belonging to BER pathway; and X-ray repair cross-complementing protein 1 (XRCC1), which is a versatile player at both BER and NHEJ pathways (Rihs et al., 2012). Defective or nonexistent DNA repair is linked to increased susceptibility of cells to toxic, mutagenic and carcinogenic effects of environmental and/or occupational exposures (Langie et al., 2015). Therefore, in the current study we focused on influence of two metabolizing gene polymorphisms (*PON1* and *SOD2*) and three DNA repair gene polymorphisms (*XRCC1, XRCC4* and *OGG1*), on DNA damage evaluated by comet assay, micronucleus test and telomere length, in tobacco workers occupationally exposed to pesticides.

2. Materials and methods

2.1. Study population and design

The study involved 242 individuals from the municipalities of Santa Cruz do Sul, Sobradinho and Venâncio Aires (East Central region of Rio Grande do Sul state, Brazil). Out of these, 121 were agricultural workers occupationally exposed to pesticide mixtures and nicotine at tobacco fields (exposed group), and 121 were not occupationally exposed to any known genotoxic agents (non-exposed group). Tobacco farmers were exposed to over 20 different synthetic pesticides. The main active ingredients listed by farmers were: glyphosate, flumetralin, clomazone, imidacloprid, sulfentrazone, dithiocarbamate, magnesium aluminum phosphide, and fertilizers. According to the Brazilian Agency Sanitary Surveillance (ANVISA, 2016), those ingredients toxicity are classified in two classes: class I) extremely toxic: flumetralin, sulfentrazone and magnesium aluminum phosphide; and class III) moderately toxic: glyphosate, imidacloprid, clomazone and dithiocarbamates. However, it is important to highlight that such classification is based in one-acute exposure event and, therefore, does not represent the exactly toxicity of a long-term exposure (Anvisa, 2016). All individuals were asked to answer a questionnaire adopted from the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988). Individuals were sampled from August of 2014 to November of 2016.

All tobacco farmers were regularly exposed to mixtures of pesticides about three times per week from May to January. These pesticides consisted of complex mixtures, including carbamates, organophosphates, pyrethroids and organochlorines as the main classes used. Additionally, agricultural workers were also occupationally exposed to nicotine the entire season, although the leaves exhibit higher levels of nicotine on the surface during the harvest period (from October to January). The non-exposed individuals consisted of office and retail employees living in the same region as exposed individuals, but at least, 10 km away from tobacco farms. Blood samples were collected during the same period from both groups.

Blood samples were obtained by venipuncture using vacutainers. Buccal samples were collected using a cytobrush. Biological samples were transported to the laboratories at or below 8 °C and processed within 10 h of collection. This study was approved by the Brazilian National Ethics Committee for Research – CONEP (CAAE 35639814.5.1001.5349). Participants who were smokers or presented a chronic health condition (diabetes, metabolic, kidney or heart diseases, and cancers), were excluded. Only individuals above 18 years old were enrolled.

2.2. Comet assay

The comet assay was performed as described by Collins (2004), with slight modifications by Da Silva et al. (2014). Briefly, 5 µL of blood cells were embedded in 95 µL of 0.75% low melting point agarose. After agarose solidified, slides were placed in lysis buffer (2.5 M NaCl, 100 mM EDTA and 10 mM Tris; pH 10.0–10.5) containing freshly added 10% (v/v) DMSO and 1% (v/v) Triton X-100 for 24 h. When lysis buffer treatment finished, slides were incubated in a freshly prepared alkaline buffer solution containing 300 mM NaOH and 1 mM EDTA, pH > 13, for 20 min. Next, DNA was submitted to electrophoresis for 20 min at 25 V (0.75 V/cm) and 300 mA, after which the buffer solution was neutralized with 0.4 M Tris (pH 7.5). The DNA was stained with silver nitrate solution and scored under blind code analysis. Fifty cells from each duplicate slide were analyzed, totalizing 100 cells per individual, in order to set damage index (DI). Cells were classified visually from category 0 (cells with no damage) to category 4 (maximum damaged cell), according to tail size and shape. The values obtained for each individual can range from 0 (0 × 100) to 400 (4 × 100) arbitrary units (Collins, 2004).

2.3. Buccal micronucleus cytome (BMCyt) assay

Buccal cells were obtained by gently scrape the inside of the cheeks (right and left side) with a cytobrush. The cytobrush was immersed in 8 mL of Saccomano's fixative until processing at the laboratory (Thomas et al., 2009). Samples were spun for 10 min at 1500 rpm. Next, supernatant was removed and 5 mL of buffer (1.6% Tris-HCl, 1.2% EDTA, 37.2% NaCl; pH 7.0) was added. This process was repeated three times. Subsequently, samples were fixed with Carnoy fixative solution for 10 min. Slides were prepared in duplicate per individual by spreading 150 µL of fixed cells on each slide. The staining process consisted of dipping slides in 50% ethanol solution for 1 min followed by 1 min in 20% ethanol solution, washed in MilliQ water, treated with HCl 5 M for 30 min and washed in running water. Next step consisted of staining with Schiff's reagent for 1 h, washing in MilliQ and running tap water; and then counter-stained with Fast Green 0.2% for 5 s. Slides were