Carcinogenesis

GSTM1 and XRCC3 Polymorphisms: Effects on Levels of Aflatoxin B1-DNA Adducts

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ABSTRACT

Objective: Aflatoxin B1 (AFB1), which can cause the formation of AFB1-DNA adducts, is a known human carcinogen. AFB1-exposure individuals with inherited susceptible carcinogen-metabolizing or repairing genotypes may experience an increased risk of genotoxicity. This study was designed to investigate whether the polymorphisms of two genes, the metabolic gene Glutathione S-transferase M1 (GSTM1) and DNA repair gene x-ray repair cross-complementing group 3 (XRCC3), can affect the levels of AFB1-DNA adducts in Guangxi Population (n= 966) from an AFB1-exposure area.

Methods: AFB1-DNA adducts were measured by ELISA, and GSTM1 and XRCC3 codon 241 genotypes were identified by PCR-RFLP.

Results: The GSTM1-null genotype [adjusted odds ratio (OR) = 2.09; 95% confidence interval (CI) = 1.61–2.71] and XRCC3 genotypes with 241 Met alleles [i.e., XRCC3-TM and –MM, adjusted ORs (95% CI) were 1.43 (1.08–1.89) and 2.42 (1.13–5.22), respectively] were significantly associated with higher levels of AFB1-DNA adducts. Compared with those individuals who did not express any putative risk genotypes as reference (OR = 1), individuals featuring all of the putative risk genotypes did experience a significantly higher DNA-adduct levels (adjusted ORs were 2.87 for GSTM1-null and XRCC3-TM; 5.83 for GSTM1-null and XRCC3-MM). Additionally, there was a positive joint effect between XRCC3 genotypes and long-term AFB1 exposure in the formation of AFB1-DNA adducts.

Conclusion: These results suggest that individuals with susceptible genotypes GSTM1-null, XRCC3-TM, or XRCC3-MM may experience an increased risk of DNA damage elicited by AFB1 exposure.

Key words: Aflatoxin B1 (AFB1); AFB1-DNA adducts; GSTM1; XRCC3; Polymorphism

INTRODUCTION

Aflatoxin B1 (AFB1), a known human carcinogen produced by aspergillus fungi, is mainly metabolized by cytochrome P450 (CYP) into the genotoxic metabolic 8, 9-epoxide-AFB1 (AFB1-epoxide). This metabolite can bind to DNA, causing the formation of AFB1-DNA adducts that may be removed or repaired by detoxification enzymes and DNA repair enzymes[1, 2].

Glutathione S-transferase M1 (GSTM1) is an important detoxified enzyme and plays an important role in the second stage of biotransformation by conjugating extraneous chemicals with glutathione[3]. Previous studies[4–11] have showed that gene deficiency of GSTM1 (namely GSTM1-null) is associated with high risk of AFB1-related hepatocellular carcinoma (HCC), suggesting that this function deficiency may be able to effect the levels of AFB1-DNA adducts. X-ray cross-complementing group 3 (XRCC3) is required for the efficient repair of double-strand breaks (DSBs)[12–14]. Recently, some studies[15–18] have exhibited that a common polymorphism in the XRCC3 gene in codon 241 (Thr241Met) may be

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associated with reduced DNA repair capacity which may result in the high risk of AFB1-DNA adducts. Therefore, we specifically conducted a study to examine whether the polymorphisms of these two genes influence the levels of AFB1-DNA adducts among Guangxi population from an AFB1 exposure area.

MATERIALS AND METHODS

Study Subjects

A total of 966 healthy who adults (21−79 y) were residence of Guangxi Zhuang Autonomous Region were enrolled from affiliated hospitals of the two main medical colleges in the Southwestern Guangxi (Youjiang Medical College for Nationalities and Guangxi Medical University) between September 2004 and August 2007. All study subjects (including 720 individuals previously studied[19]) without any clinical evidence of liver diseases were randomly selected from a pool of healthy volunteers who visited the general health check-up center of the above two hospitals for routine scheduled physical exams supported by local governments.

The characteristic information of all study subjects, including sex, age, ethnicity, hepatitis B virus (HBV) infection, and HCV infection were ascertained as described previously[4]. Those with hepatitis B surface antigen (HBsAg)-positive or anti-HCV-positive in their peripheral serum were defined as infected by HBV or HCV, respectively. After informed consent was obtained, each subject donated 4 ml of peripheral blood for GSTM1 and XRCC3 genotypes, and AFB1-DNA adducts analysis. The protocol of the study was approved by the Ethic Committees of the hospitals involved in the study.

Nucleic Acid Isolation

Leukocytes from peripheral venous blood samples were isolated by standard procedures. DNA was then extracted from leukocyte samples by standard phenol-chloroform extraction and ethanol precipitation. DNA was stored at 4°C until additional analysis.

AFB1-Exposure Years

AFB1-exposure years were ascertained by our previously published methods[4]. In brief, AFB1-exposure years were defined as the length that each subject lived in AFB1exposure region and divided into two groups: short-AFB1 exposure group (<40 y) and long-AFB1 exposure group (≥40 y).

Detection of AFB1-DNA Adducts

DNA was extracted from peripheral blood leukocytes from study subjects. AFB1-DNA adducts levels of DNA samples from leucocytes were measured by competitive enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody 6A10 and 50 μg of DNA as described by Hsieh LL, et al.[20]. The percent of inhibition was calculated by comparison with the non modified heat-denatured calf thymus DNA control. DNA samples were assayed at 50 μg/well and quantitated relative to an imidazole ring-opened AFB1-DNA standard, which has a modification level of 4 adducts/10 nucleotides. Values of 10% inhibitions were corresponded to 0.25 μmol/L DNA. Each sample was measured in triplicate with a variability of less than 10%. The quality control for adduct assays was administered by blank and positive controls. AFB1-DNA adduct levels were divided into two groups: low level (≤1.00 μmol/L DNA) and high level (≥1.01 μmol/L DNA).

Genotyping

For the genotype of GSTM1, a multiplex PCR was performed to amplify the GSTM1 and p53 genes (positive control) as described[8]. The PCR amplification of GSTM1 was as follows: 94°C for 10 min, denature at 94 ℃ for 1 min, anneal at 60°C for 45 s, and extension at 72°C for 1 min for 45 cycles, followed by a 5-min final extension.

Gene polymorphism analysis of XRCC3 Thr241Met was detected by using a previously published PCR-RFLP method[21]. Briefly, PCR was performed in a 25-μl mixture containing 25 ng of genomic DNA, 5 μmol/L of each primer, 25 mmol/L of $MgCl_2$, 2.5 mmol/L of dNTPs and 2.0 unit of Taq using the running conditions: 94°C for 45 s, 60°C for 30 s and 72°C for 1 min. The PCR products were restricted overnight at 37°C with 3 units of *Nla*III, and the digestive product was then run through 2% agarose gels and stained with ethidium-bromide. The quality control for genotypic assays was administered by blank and positive controls.

Statistical Analysis

The association between GSTM1 and XRCC3