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Original article

Interaction of benzo[*a*]pyrene with other risk factors in hepatocellular carcinoma: a case-control study in Xiamen, China

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ABSTRACT

Purpose: Large epidemiologic studies about the relationship between benzo[*a*]pyrene (B[*a*]P) and hepatocellular carcinoma (HCC) have been limited. B[*a*]P diol epoxide (BPDE) is a highly reactive metabolite of B[*a*]P that binds covalently to form DNA adducts. We evaluated the interaction between B[*a*]P exposure with other risk factors in HCC, in a case-control study of 345 HCC and 961 healthy controls.
Methods: Concentration of BPDE-DNA adducts in blood was determined by enzyme-linked immunosorbent assay. The interaction between BPDE-DNA adducts and other risk factors on HCC were evaluated by multivariate logistic regression analysis.
Results: Mean concentration of BPDE-DNA adducts in blood of cases was significantly higher than that of the controls. The risk of HCC increased with elevated concentration of BPDE-DNA adducts ($x^2 = 203.57$, $P_{trend} < .001$) and the odds ratio was 7.44 (95% confidence interval, 5.29–10.45) for the first versus fourth quartile of adduct levels. The relative excess risk due to interaction between BPDE-DNA adducts and hepatitis B virus surface antigen and drinking was 34.71 and 54.92, and the attributable proportion due to interaction was 41.53% and 75.59%, respectively.
Conclusions: The high level of BPDE-DNA adducts in blood is associated with HCC and that environmental exposure to B[*a*]P may increase the risk of HCC, especially among drinkers and populations with hepatitis B virus infection.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms worldwide, with rates that show considerable geographic variation. Along the southeastern coastline of China, the provinces of Jiangsu, Zhejiang, Fujan, Guangdong, and Guangxi Autonomous Region are well known to have a high incidence of HCC [1], and Chinese cases of HCC contribute to about 55% of the total number worldwide [2,3]. Over the past few decades, epidemiologic studies have suggested that the well-documented multiple etiologic factors associated with the development of HCC are chronic hepatitis B virus (HBV) infection, aflatoxin B1 (AFB1), alcohol abuse, smoking, diabetes mellitus, oral contraceptives, sex, and genetic susceptibility [3–15]. However, viral infection cannot explain the overall risk of developing HCC, and AFB1-lysine albumin adducts (biomarkers of AFB1 exposure) can only be detected in the serum of a small number of HCC [16–18]. In general, 15%–50% of HCC cases remain idiopathic, which suggests that additional unidentified factors may be responsible for an increased risk for HCC.

Studies have reported that an elevated HCC risk is associated with exposure to a class of ubiquitous environmental contaminants known as polycyclic aromatic hydrocarbons (PAHs) [19,20]. PAHs are formed during the incomplete combustion of coal, oil, gas, wood, or other carbon-containing organic substances in gas-burning motor vehicle, wood-burning furnaces, cigarette smoke, industrial smoke or soot, and charcoal-broiled foods and are thus widely present in polluted air, water and soil, and the diet [21–23]. PAHs comprise a wide range of different compounds with different

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aromatic ring numbers. B[*a*]P is the most thoroughly studied PAH and is a procarcinogen in animals and humans [21]. It exhibits its biological effects through metabolic activation by cytochrome P450 (CYP450) enzymes and epoxide hydrolase to the highly reactive metabolite, B[*a*]P diol epoxide (BPDE), which is an electrophilic species that can bind covalently to DNA and irreversibly damage DNA by the formation of DNA adducts [21]. So, BPDE-DNA adducts in human body reflect both exposure to B[*a*]P and the body's metabolic capacity. In addition, the formation of DNA adducts is considered as a crucial step in the initiation phase of carcinogenesis [22]. Studies have shown that exposure to high levels of environmental B[*a*]P is associated with increased risk of lung, breast cancer, hepatic angiosarcoma, and HCC [23–29].However, most studies have been small epidemiologic studies with no more than 100 cases with BPDE-DNA adducts examined in specific tumor tissue or cells [24–29]. So far, no large case-control studies have been conducted on the association between the BPDE-DNA adducts in peripheral blood and HCC in humans.

Xiamen City is located on the Southeastern coastline of China and is an area of high HCC incidence. The specific aims of our study were to assess whether B[*a*]P exposure, as determined by BPDE-DNA adduct levels in peripheral blood of subjects, is related to HCC risk; assess the interaction between BPDE-DNA adducts and other risk factors on HCC; and shed light on mechanisms implicated in the etiology of PAH-related cancers.

Materials and methods

Subject enrollment and sample collection

A total of 407 HCC cases were newly diagnosed histopathologically at the Department of Hepatobiliary Surgery, Xiamen Hospital of Traditional Chinese Medicine and the 174th Hospital of People's Liberation Army from March 2007 to December 2009. All the HCC cases were clinical patients who had not suffered any other types of cancer and were recruited at the time of diagnosis. A total of 1173 controls were selected simultaneously from healthy non-blood relatives (especially the patient's spouses) of all hospitalized patients except of those who suffered from smoking-related diseases (such as HCC, gastrointestinal, lung or head and neck cancers, and so forth). The controls and cases were matched by age (±5 years), gender, and ethnicity (Han Chinese), and so forth. All participants had been living at least 10 years in Xiamen and signed the written consent form to voluntarily provide 10 mL blood sample at the time of enrollment and participate in a structured questionnaire survey that collected demographic information through in person interviews by trained interviewers. Finally, the participation rate of cases and controls were 84.8%(345/407) and 81.9% (961/1173), respectively. The other 62 new patients of HCC and 212 controls were failed to be complete the survey, due to the following reasons: refusing the interview accounted for 69%; language barrier accounted for 13%; and interview in improper time accounted for 18%. A total of 1306 subjects were enrolled and there were no significant difference between the subjects of participation and of nonparticipation in age, gender, education level, marital status, and ethnicity etc. The samples of whole blood with anticoagulant were separated and stored at −70°C for the determination of BPDE-DNA adducts. This study was approved by Institutional Review Board, School of Public Health, Xiamen University, Xiamen City, Fujian province, China.

Variables and definitions

The structured questionnaire included demographics (sex, age, income, educational attainment, occupation, and marital status), lifestyle factors (cigarette smoking, alcohol, and tea and coffee intake), contaminated water drinking, and family history of cancer. The main definitions of risk categories were as follows: (1) a cigarette smoker was a person who had smoked 1 or more cigarette per day and for at least 6 months; (2) an alcohol drinker was a person who had consumed beer, wine, or hard liquor at least once weekly for at least 6 months during their lifetime; (3) a tea drinker was a person who had consumed tea twice weekly for at least 6 months during their lifetime; and (4) a drinker of contaminated water was a person who did not drink tap water, but did drink pond, ditch, or well water in daily life.

Quantification of BPDE-DNA adducts in peripheral blood

For detecting BPDE-DNA adducts in peripheral blood, the DNA was first extracted from 10 mL whole blood according to the protocol of the DNA isolation kit for mammalian blood (Roche, Penzberg, Germany). The extracted DNA was checked by agarose gel electrophoresis for integrity and quantified by ultraviolet spectrophotometer. PAH-DNA was measured by an immunoperoxidase method described previously [30]. Briefly, the detection of BPDE-DNA adducts of extracted DNA was conducted using the BPDE-DNA adduct enzyme-linked immunosorbent assay kit (OxiSelect; Cell Biolabs, San Diego, CA) following the recommended protocol. The quantity of BPDE adduct in DNA samples was determined by relative comparison of a known BPDE-DNA standard curve. BPDE-DNA standards or unknown DNA samples were adsorbed onto a 96-well DNA high-binding plate. The BPDE-DNA adducts present in the sample or standard were probed with an anti-BPDE-I antibody, followed by a horseradish peroxidase–conjugated secondary antibody.

Statistical analysis

SARS software version 9.1 (SAS Institute, Cary, NC) was used for the statistical analyses. *t* and χ^2 tests were conducted to analyze the distribution of variables between cases and controls and estimate the relative risk as the odds ratio (OR) of HCC in relation to BPDE-DNA adducts. Based on the fourth quartile of BPDE-DNA adducts in the controls, quartile division was applied to divide the subjects into four subgroups (quartiles 1–4) for trend analysis and elucidation of the dose response for HCC risk. Unconditional logistic regression analysis was performed to evaluate each possible risk factor for HCC. The unconditional multivariate logistic regression was performed when the exposed factor was significantly associated with HCC in the univariate analysis. The attributable proportion due to interaction (API) and the relative excess risk due to interaction (RERI) were used to evaluate the interaction of risk factors. The Mann-Whitney *U* test was used to test the significance of RERI. The significant criteria in the tests were two tailed at significance level $P = .05$. The adjusted OR of age, sex, education, marital status, and the other HCC risk factors and 95% confidence interval (CI) were calculated by the maximum likelihood approach. The assessment of two factors' interaction was done using the following equations:

$$\begin{aligned} \mathrm{API(AB)} &= [\mathrm{RR(AB)} - \mathrm{RR(AB_0)} - \mathrm{RR(A_0B)} + 1]/\mathrm{RR(AB)RERI} \\ &= \mathrm{RR(AB)} - \mathrm{RR(AB_0)} - \mathrm{RR(A_0B)} + 1, \end{aligned}$$

where API (AB) is the API of factor A and factor B, and RERI is the relative excess risk of interaction. RR is the relative risk. AB means exposure to factor A and factor B; AB_0 means exposure to factor A but not to factor B; A_0B means exposure to factor B but not to factor A.