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Association of genetic polymorphisms of telomere binding proteins with cholinesterase activity in omethoate-exposed workers

Mingcui Ding[a], Yongli Yang[b], Xiaoran Duan[a], Sihua Wang[c], Xiaolei Feng[a], Tuanwei Wang[a], Pengpeng Wang[a], Suxiang Liu[d], Lei Li[d], Junling Liu[d], Lixia Tang[d], Xinhua Niu[d], Yuhong Zhang[d], Guoyu Li[d], Wu Yao[a], Liuxin Cui[a], Wei Wang[a,*]

[a] Department of Occupational and Environmental Health, College of Public Health, Zhengzhou University, Zhengzhou, China
[b] Department of Epidemiology and Biostatistics, College of Public Health, Zhengzhou University, Zhengzhou, China
[c] Department of Occupational Health, Henan Institute for Occupational Medicine, Zhengzhou, China
[d] Department of Zhengzhou Institute of Occupational Health, Zhengzhou, China

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ABSTRACT

Omethoate, an organophosphorous pesticide, can cause a variety of health effects, especially the decrease of cholinesterase activity. The aim of this study is to explore the association of genetic polymorphisms of telomere binding proteins with cholinesterase activity in omethoate-exposed population. Cholinesterase activities in whole blood, red blood cell and plasma were detected using acetylthiocholine and dithio-bis-(nitrobenzoic acid) method; Genetic Genotyping of *POT*1 rs1034794, *POT*1 rs10250202, *TERF*1 rs3863242 and *TERT* rs2736098 were performed with PCR-RFLP. The cholinesterase activities of whole blood, red blood cells and plasma in exposure group are significantly lower than that of the control group ($P < 0.001$). Multivariate analysis indicates that exposure group ($b = -1.016$, $P < 0.001$), agender ($b = 0.365$, $P < 0.001$), drinking ($b = 0.271$, $P = 0.004$) and *TERF1*rs3863242 ($b = -0.368$, $P = 0.016$) had an impact on cholinesterase activities. The results suggest that individual carrying AG + GG genotypes in *TERF*1 gene rs3863242 polymorphism were susceptible to damage in cholinesterase induced by omethoate.

1. Introduction

Organophosphorous pesticides (OPs) are wildly used in the world due to high efficiency, broad spectrum and low residue, which in turn results in environmental pollution and adverse health effects (Duan et al., 2017; Parker et al., 2017). Inhibiting cholinesterases (ChEs) activity is the primary toxicity mechanisms of OPs, which leads to immediate neurotoxic effects and delayed effects, including neurodegenerative diseases and effects on children exposed in utero (Peres et al., 2016; Quandt et al., 2015; Ridano et al., 2017). DNA damage and ChEs activity were used as biomarkers of acute and chronic effects of exposure to OPs.

Telomere binding proteins (TBPs) locate at the end of the chromosomes and are closely related to chromosome stability. Genomic stability is mainly mediated by protein complexes recruited to telomeric sequences by specific TBPs (Cervenak et al., 2017). DNA damage is one of the major molecular events in malignant transformation. Telomere DNA is in the chromosome end. Therefore, telomere is likely more vulnerable to attack from external intruders. Due to the problem of DNA replication to the ends of linear chromosomes, telomere DNA shortens at a speed of 50–200 bp during the cell replication process (Zhao et al., 2009). TBPs might involve in the DNA shortening process due to binding to telomere DNA. TBPs related genes include telomeric repeat binding factors (*TERF*1 and *TERF*2), protection of telomeres protein 1(*POT*1), human repressor activator protein 1 (*RAP*1), and other proteins (Mucciardi et al., 2014). *TERF1* can directly identify the telomeres TTAGGG repeats, and its main function is to negatively adjust telomere DNA length by inhibiting telomerase activity (Patel et al., 2015). *POT1* gene plays an important role in telomere homeostasis through extending the telomere by activating telomerase, and it also prevents telomerase-dependence telomere extension by isolating the telomeres 3′ hanging end and maintaining telomeres T loop structure with the cooperation with *TERF1* and *TRF2* (Loayza and de Lange, 2003; Yang et al., 2005). *TERT* is one of the subunits of telomerase and evidence has shown that somatic gain-of-function mutations in the *TERT* promoter promote the transcriptional activity of TERT which subsequently maintain telomere length in human cancers (Gao et al., 2016). Telomeres pathway genes *POT1*, *TERF1* and *TERT* play a key

* Corresponding author.
E-mail address: ww375@126.com (W. Wang).

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role in maintain chromosomal stability.

TBPs participate in cell differentiation, senescence and death (Hosokawa and Arai, 2018). There are possibly two main pathways for the changes in ChEs. Firstly, tissue homeostasis requires an orchestrated balance between cell proliferation and cellular senescence (Schmitt et al., 2007). An appropriate telomere maintenance system is necessary for cell replicative potential (Patel et al., 2015), critically short telomeres elicit a DNA damage associated with cell cycle arrest and replicative senescence (O'Sullivan and Karlseder, 2010). DNA-damage reparation genes and telomere maintenance genes are involved in the DNA repairing process. Therefore, the TBPs could affect the quantity and quality of peripheral red blood cells which could influence the ChEs (van der Harst et al., 2012). Secondly, the TBPs can affect chromosomal stability, and then change the expression levels of cholinesterase-related genes, and thus affect the production and degradation of cholinesterase.

Omethoate is one of the OPs, which is widely used in China. Our previous study shows that the relative telomere lengths in the omethoate exposure group were significantly longer than that in the control group. The genetic polymorphisms in cell-cycle-regulating genes including p53 and p21 may influence the DNA damage (Duan et al., 2017). Recently, there are a large body of studies suggested that polymorphisms of *POT1* rs1034794, *POT1* rs10250202, *TERF1* rs3863242 and *TERT* rs2736098 are closely related to chromosome stability and cancers (Cheng et al., 2017; Yuan et al., 2017). TBPs gene polymorphisms influence the stability of chromosome. Genetic susceptibility is different in individuals who are exposed to OPs. So far, there are few studies on the correlations between the polymorphisms of *POT1*, *TERF1, TERT* gene and ChEs activities.

Therefore, the relationship between ChEs activity and polymorphisms in *POT1*, *TERF1, TERT* genes were explored in order to screen susceptible biomarkers in this study.

2. Materials and methods

2.1. Study population

Total 180 subjects exposed to omethoate for more than 8 years were recruited as exposure group. These people are from an OP Manufacturer omethoate packaging workshop. Because omethoate can be absorbed through respiratory tract and skin, the exposure group included workers from packing, screwing, filling and corking sections. The concentration of pesticides in the working environment was detected by specialized health institutions every year, and found lower than occupational exposure limits prescribed by the state in type of work. Total 115 unexposed controls without a history of exposure to OPs or other toxicants were recruited from a company organization in the same city. Written informed consent was issued from each subject, and to collect occupational history, basic situation and biological samples. The study protocol was subject to approval by the Ethics Committee in Zhengzhou University, China.

2.2. Methods

2.2.1. DNA extraction

Genomic DNA from peripheral blood was extracted according to the instruction from Blood DNA Kit (Beijing BioTeke Corporation), and the DNA purity and concentration was determined.

2.2.2. The determination of cholinesterase activity

ChEs activity was determined in strict accordance with the occupational health standards of the People's Republic of China (GBZ52-2002). The formula as follows:

$$\frac{\text{Absorbance}}{1.36 \times 10^4} \times 10^3 \times 2.6 \times \frac{1}{6} \times \frac{10^3}{10} \times \frac{10}{4} = \text{Absorbance} \times 7.97$$

1 μM thiocholine produced by per mL of whole blood (plasma or red blood cells) per minute was regarded as 1 unit of ChEs activity measurement value. In the formula, 1.36×10^4 represents the absorbance of nitrobenzoate ions formed in the enzyme assay. The number of nitrobenzoate ions is the same with that of thiocholine.

2.2.3. The detection of genetic polymorphisms

Four polymorphic loci of *POT1* rs1034794, *POT1* rs10250202, *TERF1* rs3863242 and *TERT* rs2736098 were detected. Among them, *POT1* and *TERF1* genotyping, primer sequences, and restriction endonucleases were performed as previously reported (Wang et al., 2017, 2016a, 2016b), *TERT* genotyping was detected by PCR-RFLP, the primer sequences were listed below: forward primers: (5′-GACCGAGT GACCGTGGTTTC-3′); reverse primers: (5′-CCAGAAAGATGGTCTCCA CGA-3′).

2.2.4. The PCR and enzyme reaction

The PCR was carried out in a volume of 15 μL containing 50 ng of genomic DNA, 2 × PCR Master Mix (Shanghai Lifefeng Biotech Co., Ltd.) and 3 pmol each primer. PCR reaction conditions were as follows: DNA was denatured at 95 ℃ for 5 min; and thermal cycles were set at 95 ℃ for 30 s, annealing temperature for 30 s, 72 ℃ for 20 s for 30 cycles; followed by a final extension at 72 ℃ for 10 min. Annealing temperature was performed as previously reported (Wang et al., 2017, 2016a, 2016b). PCR products were electrophoresed on 2–4% agarose gels and visited with ethidium bromide staining and ultraviolet illumination to determine the success of PCR.

Enzyme digestion was conducted in a 20 μL final volume using 5 U enzyme (the Thermo Scientific Co., LTD.) and 10 μL of PCR product. Enzyme reaction was carried out according to the enzyme reaction temperature for more than 4 h or overnight. The digested products were visualized using gel imaging analyzer after electrophoresis.

2.3. Statistical analysis

The basic data were analyzed using SPSS21.0 software in this research. Methods of representation and examination were based on the distribution of quantitative data. The spearman rank correlation was utilized to analyze the correlation for abnormal distribution data. Multiple linear regression was performed to analyze the influencing factors of cholinesterase activity. All statistical tests were two-sided, and the level of statistical significance was set at $\alpha = 0.05$.

3. Results

3.1. Population-based data

Consistent with the results from our previous study (Duan et al., 2017), the differences are significant in the agender, age, smoking and drinking between the two groups ($P < 0.001$).

3.2. The determination of cholinesterase activity

Consistent with the results from our previous reports of this study (Duan et al., 2017), we found that ChEs activities of whole blood, red blood cells and plasma in the exposure group were lower than that of control group ($P < 0.001$), respectively. Three sets of data were normally distributed and had the same changing trend, and red blood cells ChEs activity were selected as a representative to damage induced by omethoate in the following analysis.

3.3. The effects of agender, age, smoking, drinking and working duration on cholinesterase activity

The results in Table 1 showed that ChEs activities were associated with agender, smoking and drinking in exposure group ($P < 0.05$), the age and working duration had no effect on the ChEs activities in