SHORT REPORT

Effects of chronic exposure to Formaldehyde on micronucleus rate of bone marrow cells in male mice

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Abstract

Formaldehyde (FA) is the major volatile organic chemical (VOC) present in indoor air, and a constituent known to be associated with sick building syndrome. In the present study, mice were exposed to different concentrations of FA (0, 1, 10 mg/ m3) through static inhalation for 2 hours per day for 20 weeks. The polychromatic erythrocytes/ normochromatic erythrocyte (PCE/NCE) ratio and the micronucleus rates in bone marrow cells were detected. Data indicated that the PCE/NCE ratio in two FA exposure groups were statistically significant lower than the negative control group ($P < 0.05$), and the micronucleus rate in two FA exposure groups were not significantly higher than the control group ($P > 0.05$). These results suggest that chronic static inhalation of FA can reduce the ratio of PCE/NCE in the mice bone marrow, but the effects to the mice bone micronucleus rate are not sure.

Keywords: Formaldehyde (FA), Chronic exposure, Mice, Micronucleus.

Introduction

In recent years, the health damages induced by indoor air pollution have been concerned. The US EPA listed indoor air pollution as one of the top five public health hazards. Indoor decoration materials are the main source of indoor air pollution. Formaldehyde (FA) is colourless, active and easy to be polymerized at room temperature. In China, the FA concentration in 60-94% of the newly renovated rooms exceeded the national standard (< 0.08mg/m^3) for 3-10 times average. FA can cause long-term (2 to 5 years) pollution due to the slow releasing. FA has genetic, mutagenic, carcinogenic and certain reproductive toxicity.[1-3] Our previous studies showed that acute exposure to FA induced expanded simple tandem repeats (ESTR) mutations in experimental mice.[4] FA has been identified as class I carcinogen by the International Agency for Research on Cancer (IARC). The mechanism of genetic toxicity induced by FA in vivo is complex, and there are a few studies on the genetic toxicity of FA in the whole body system. However, the genetic toxicity of chronic exposure to low concentrations of FA in the experimental animals is not clear. The mice bone marrow cell micronucleus test is a sensitive indicator for study of the genetic toxicity induced by xenobiotics. It quickly detects the effect of chromosome damage, and is one of the most common methods to detect the genetic toxicity. In the present study, micronucleus test was used to examine the genetic toxicity induced by low concentrations of FA to experimental mice.

Methods and Results

The present study was conducted in Hainan Province of China from June to December 2015, after approval by the ethical review committee of Hainan Medical University. Specific pathogen free ICR mice weighing 10-12g were provided by the Shanghai Institute of Laboratory Animals. Mice were given commercial mouse chow and bottled water following the standard procedures of the animal center. All animal procedures were approved by the Hainan Medical University Laboratory Animal Center following the Guidelines of the Chinese Council on Animal Care. The research protocol was approved by the ethical review committee of Hainan Medical University.

Forty male mice were randomly divided into 2 FA exposure groups, a negative control and a positive group, each with 10 mice per group. FA exposure mice were placed in 220-L airlocked plexiglass cabinets and exposed to FA (1, 10 mg/m^3 respectively) for 2 h/d for 20 weeks, the treatment of negative control mice was the same as that of the FA exposure mice, except that there was no formaldehyde. The positive control mice were administered with cyclophosphamide (CP) by intraperitoneal injection (40mg/kg body weight, once daily for 2 days).

After 24h of post-exposure, mice were sacrificed and the bone marrow of sternum were squeezed into a drop of calf serum in a clean glass slide, then mixed uniformly and a smear was made. After drying in the air, the smear was fixed with methanol for 15 minutes, and dyed in Giemsa

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Table-1: Comparison of polychromatic erythrocytes/normochromatic erythrocyte ratio in different groups.

Groups	Numbers of mice	Observed cell numbers	PCE/NCE
Negative control group	10	10×200	0.65±0.06
Low dose FA group (1mg/m^3)	10	10×200	0.36±0.05*
High dose FA group (10mg/m^3)	10	10×200	0.23±0.04*
Positive control group (CP)	10	10×200	0.68±0.06

*P < 0.05, compared with the negative control group. FA: Formaldehyde.

Table-2: Comparison of micronucleus rate in different groups.

Groups	Numbers of mice	Observed cell numbers	Micronucleus rate(%)
Negative control group	10	10×1000	0.00(0.00, 0.00)
Low dose FA group (1mg/m^3)	10	10×1000	0.00(0.00, 0.00)
High dose FA group (10mg/m^3)	10	10×1000	0.05(0.00, 0.10)
Positive control group (CP)	10	10×1000	20.50(18.75, 26.00)*

*P < 0.05, compared with the negative control group. FA: Formaldehyde.

stain for 15 minutes.

One thousand polychromatic erythrocytes (PCE) were counted for each bone marrow smear under optical microscope, and the micronucleus rates were calculated. Ratios of PCE and normochromatic erythrocyte (NCE) in 200 cells were calculated.

Data was analyzed using SPSS19.0 software. P values less than 0.05 was considered as statistically significant, and data were analyzed by one-way analysis of variance (one-way ANOVA) or Kruskal-Wallis test followed by all pairwise multiple comparisons.

The data of PCE/NCE accorded with the one-way ANOVA, and the difference was statistically significant (One-way ANOVA, F=154.258, P <0.01) for the PCE/NCE among groups. Using LSD method for two comparisons, the PCE/NCE in high dose group and low dose group were significantly lower than that of the negative control group (P <0.01). The PCE/NCE ratio between the negative control group and the positive control group had no significant difference (Table-1).

The results of micronucleus rate showed that the difference among 4 groups were statistically significant (Kruskal-Wallis test, H=29.191, P <0.01). Using Kruskal-Wallis test followed by all pairwise multiple comparisons for two comparisons, there were no statistically significant differences between the high dose group, low dose group and the negative control group. The micronucleus rate in the positive group was significantly higher than the negative group and 2 FA exposure groups (P < 0.01) (Table-2).

Discussion and Conclusions

Micronucleus is a kind of abnormal structure in eukaryotic cells, which is often caused by the abnormal changes of the cells by the radiation or chemical drugs. The micronucleus test is a fast method for the detection of chromosome damage and interference of cell mitosis.

The PCE/NCE indicates the ratio of immature red blood cells to mature red blood cells. In the present study, the PCE/NCE ratio had no significant difference between negative and positive control group. It indicated that at present CP exposure dose and time, did not significantly affect the red blood cell formation. The results of this study showed that the PCE/NCE ratio in low dose and high dose group were decreased, and the PCE/NCE ratio decreased gradually with the increase of exposure doses, which indicated that chronic inhalation of FA had certain inhibited effects on the haematopoietic system of mice.

At present, whether or not FA can induce the increase of micronucleus rate in animal cells is still controversial.[5] It was reported that short time inhalation of FA can induce the increase of micronucleus rate in bone marrow cells of exposed mice.[6-8] Our recent studies showed that the micronucleus rate of bone marrow cells in the experimental mice was increased by acute oral administration of FA.[9] In the present study, the male mice were chronically exposed to FA through inhalation. The results showed that the frequency of micronucleus in high and low dose groups was a little higher than the negative control group, but the difference was not statistically significant. The present study was a long-term chronic toxic test (exposure for 20 weeks), the mice were exposed to FA for a long time, so the body's self-protection mechanism may be triggered, and the metabolism and elimination of FA maybe increased, thereby reducing the body's sensitivity for FA. If the exposure dose of FA is still in the range of self compensation, it will not induce significant effects on the body. Only if the exposure does exceed the range of self compensation, the adverse effects can be observed. Finally, the genetic toxicity induced by FA is diverse in different animal cells, which reflects that the sensitivity to FA is different in various animal cells.

Chronic static inhalation of FA can reduce the PCE/NCE ratio in the mice bone marrow, but the effects to the mice

bone micronucleus rate are not sure. Further studies are needed to confirm it.

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Conflict of Interest: None.

References

1. Speit G., Schmid O. Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat Res 2006; 613: 1-9.
2. Merk O, Speit G.. Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. Environ Mol Mutagen 1999; 33: 167-72.
3. Speit G., Merk O. Evaluation of mutagenic effects of formaldehyde in vitro: detection of crosslinks and mutations in mouse lymphoma cells. Mutagenesis 2002; 17: 83-7.
4. Liu YR, Zhou Y, Qiu W, Zeng JY, Shen LL, Li AP, et al. Exposure to Formaldehyde Induces Heritable DNA Mutations in Mice. J Toxicol Environ Health 2009; 72: 767-73.
5. Chen WJ, Dai H. Research progress on the genetic toxicity of formaldehyde and its mechanism. Acta Academiae Medicinae Neimongol 2011; 33: 519-22.
6. Ma YP, Wu ZC, Yuan FS. Determination of the genetic toxicity of formaldehyde in indoor air of newly decorated room. J Health Toxicol 2002; 16: 192.
7. Li YL, Yuan FS, Li M, Liang RF, Zhao WH. Joint genotoxicity of formaldehyde and trichloroethylene inhalation to bone-marrow cells of mice. J Environ Health 2013; 30: 953-6.
8. Wu YL, Zhang BJ, Wu XH. The toxicity of pollutants of benzene and formaldehyde in indoor decoration on micronucleus in bone marrow PCE and teratospermia of mice. J Nanjing Xiaozhuang Uni 2002; 6: 73-6.
9. Liu YR, Ma YP, Xu QJ. Effects of intragastric administration of formaldehyde on mice bone marrow cell micronucleus rate. J Hainan Med Coll 2014; 7: 881-3.