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Estimation of benchmark dose for micronucleus occurrence in Chinese vinyl chloride-exposed workers☆

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ABSTRACT

In this study, we estimated the possibility of using benchmark dose (BMD) to assess the dose–response relationship between vinyl chloride monomer (VCM) exposure and chromosome damage. A group of 317 workers occupationally exposed to vinyl chloride monomer and 166 normal, unexposed control in Shandong Province northern China were examined for chromosomal damage in peripheral blood lymphocytes (PBL) using the cytokinesis-blocked micronucleus (CB-MN) assay of DNA damage. The exposed group (3.47 ± 2.65)‰ showed higher micronucleus frequency than the control (1.60 ± 1.30)‰ ($P < 0.01$). Occupational exposure level based on micronucleus occurrence in all individuals was analyzed with benchmark dose (BMD) methods. The benchmark dose lower limit of a one-sided 95% confidence interval (BMDL) for 10% excess risk was also determined. Results showed a dose–response relationship between cumulative exposure and MN frequency, and a BMDL of 0.54 mg/m^3 and 0.23 mg/m^3 for males and females, respectively. Female workers were more susceptible to MN damage than male workers.

Introduction

Vinyl chloride monomer (CH_2=CHCl, VCM), a major material used in the polymerization process of polyvinyl chloride (PVC), is a human carcinogen according to the classification of International Agency for Research on Cancer (1987). Many countries, regions and organizations have established exposure guidelines for vinyl chloride in the workplace (IARC, 2008). The level of VCM in the environment (*i.e.*, air, water, food, etc.) have been summarized in Agency for Toxic Substances & Disease Registry (ATSDR). However, the human exposure of VCM at workplace is still unclear. Previous dose–response studies on VCM exposure in animals have suggested its carcinogenicity (EPA, 2000). In these studies, the end-points were based on tumor incidence or observed liver damage test results. However, the current end points are too late a biomarker that by the time they are observed in workers, the damages have already impaired their working ability significantly. In addition, chromosome damage were induced by VCM at doses much lower than those required to form tumors or cause organ damage. Therefore, the current end-points are inappropriate in assessing human cancer risks.

The frequency of micronucleus (MN) in peripheral blood lymphocytes (PBL) is extensively used as an early biomarker of chromosomal damage and genome stability in human populations. Micronucleus occurrence as an early damage end-point for benchmark dose–response research has been reported in a previous study (Chen et al., 2010). The cytokinesis-blocked micronucleus (CB-MN) assay works by causing cytokinesis inhibition by cytochalasin B (Cyt-B), thereby arresting the cell in the stage soon after completing the first *in vitro* division (the binucleate stage). This assay has facilitated MN analysis exclusively in binucleate cells after test agent treatment. CB-MN assay makes it easy to detect and predict long-term risks associated with human exposure to mutagen and carcinogen in working and non-working environment (Fenech, 2000). Previous epidemiological studies have shown that VCM exposure is associated with increased genotoxicity in human (Luo et al., 2003; Wang et al., 2010; Zhu et al., 2005).

In this study, we used the benchmark dose method to estimate the possible dose–response relationships between MN frequency and VCM exposure. The Benchmark Dose (BMD) was defined by

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Crump (1984) as a lower confidence limit corresponding to a moderate increase in risk (1–10%) above the background risk. Crump suggested that the BMD could be used to replace the no observable adverse effect level (NOAEL) or the lowest observable adverse effect level (LOAEL) for effects that proceeds tumor genesis in setting acceptable daily human exposure to potentially toxic substances. In fact, BMD has already been used as a minimal risk level assessment for damages induced by VCM in previous studies (Thornton et al., 2002). The main advantage with the BMD method is that it uses all collected dose–response data thereby increasing its accuracy and sensitivity. In 1998, Gaylor et al. (1998) introduced the concept of lower confidence limit of benchmark dose (BMDL) and suggested that it could be used instead of NOAEL or LOAEL (Filipsson and Victorin, 2003). The BMDL is typically calculated as the lower 95% confidence limit on a 1–10% risk increase above background, derived from the dose–response curve. A 10% benchmark response level (BMR) is conventionally used for dichotomous end points because it is at the low end of the observable range for many common study designs. In this experiment, we used BMD software and instruction from EPA to estimate reference concentration of VCM exposure that resulted in chromosome damage in occupationally exposed workers.

Materials and methods

Study subjects

A total of 317 workers, 257 Han Chinese men and 60 women, aged 37.16 ± 8.07 years on average, employed at a PVC polymerization plant in Shandong, China, were included in the study. upon informed consent during routine medical surveillance. The study participants had been occupationally exposed to VCM for at least one year. Their blood samples were drawn, and they were made to complete questionnaires in a one-on-one interview. A total of 136 service workers and managers from the same factory, 98 Han Chinese men and 38 women, with an age distribution similar to the exposure group but lacking direct VCM exposure were enrolled as internal controls. The subjects of the internal control group also provided a blood sample and completed questionnaires. An external control group consisting of residents from the same living area was also recruited. Subjects of this external control group, 14 Han Chinese men and 16 women, with an average age of 37.30 ± 11.43 years, had no known VCM exposure. Both age and gender were included as adjustment variables in all analyses.

Collection and treatment of samples

A detailed questionnaire was completed by each study participant followed by the collection of a 10-ml anticoagulated wholeblood sample. Blood samples were stored at room temperature in an insulated container and were delivered to the laboratory within 12 h of collection. Cytokinesis-blocked micronucleus assays (CB-MN) were performed on the blood samples.

Assessment of vinyl chloride exposure

The VCM plant had been monitoring ambient air VCM concentration at different worksites since the beginning of its establishment. We estimate the cumulative exposure dose (CED) of each worker using the following equation:

$$\mathrm{CED}\,(\mathrm{mg/m^3\text{-}year}) = \sum C\,(\mathrm{mg/m^3}) \times T\,(\mathrm{year})$$

where C is the geometric mean of VCM exposure concentration (in $\mathrm{mg/m^3}$) for each month in a given workplace (calculated for all worksites). By this method, personal cumulative exposure doses (CED) in the VCM exposure group ranged from $0.09\ \mathrm{mg/m^3}$ to $27.02\ \mathrm{mg/m^3}$. We then grouped the VCM exposed subjects into four groups by the quartile cumulative doses ($0\ \mathrm{mg/m^3}\sim$, $0.48\ \mathrm{mg/m^3}\sim$, $1.13\ \mathrm{mg/m^3}\sim$, $6.36\ \mathrm{mg/m^3}\sim$).

CB-MN assay

The CB-MN assay was performed according to standard methods as described by Fenech (1993). Briefly, 0.5 ml heparin-anticoagulated whole blood was added to 4.5 ml of cell culture medium (RMPI1640) and incubated at 37 °C in 5% CO_2. Cytochalasin-B (Cyt-B, Sigma) was added to each cell culture after 44 h at a final concentration of 6 μg/ml to prevent cytokinesis. Twenty-eight hours after the addition of Cyt-B, cells were harvested by cytocentrifugation and fixed with methanol and acetic acid at a ratio of 3:1. Slides were air-dried and stained with Giemsa. For each subject, CB-MN in 1000 binucleated lymphocytes with well-preserved cytoplasm were scored in a blinded fashion by the same reader. MN frequencies are the number of micronucleus observed per 1000 lymphocytes, expressed as a count per thousand (‰).

Statistical methods

Unadjusted MN frequency was reported together with sample characteristics and VCM exposure. The frequency of micronucleus was estimated by computing frequency ratios ($\mathrm{FR} = e\beta$, $e = 2.71828$, β: regression coefficient); an increase/decrease in FR suggests proportional change of micronucleus frequency. The statistical analyses were done using the software SAS (SAS Institute).

Benchmark dose estimation

Benchmark Dose Software (BMDS) Version 2.0 (U.S. EPA) was used for calculating BMD and BMDL. After fitting the Poisson dose–response model to the MN frequency as outlined in the previous section, a BMD was determined as the dose at which exposure would result in a specified level of increase in adverse response above that of the controls. This specified level of increase in adverse response, typically between 1 and 10%, is called the benchmark response (BMR) level. Alternatively, a lower confidence limit to the BMD estimate, *i.e.* BMDL, is often used to account for uncertainty in the BMD. In the present study, we chose BMR to be 10% above the control adverse response and a 95% confidence level for BMDL.

Results

Subject characteristics and demographics and lifestyle factors

Table 1 presents the gender, age, smoking and drinking status of the study population, along with their associations with MN frequency. There was a small, yet statistically insignificant increase in MN frequency in women compared to men (FR = 1.12, 95% CI: 0.97–1.30). No significant increase in MN was detected in smokers and regular alcohol consumers. However, the older age group (>35 years) exhibited generally a higher MN frequency than the younger age group (≤35) (FR = 1.32, 95% CI: 1.17–1.50; $P < 0.01$).

MN frequency and VCM exposure dose

The exposed group had a higher MN frequency (3.47 ± 2.65)‰ than the external controls (1.60 ± 1.30)‰ ($P < 0.01$) based on simple Poisson regression. Simple Poisson regression also showed an increasing MN frequency in each of the four exposure levels ($0\ \mathrm{mg/m^3}\sim$, $0.48\ \mathrm{mg/m^3}\sim$, $1.13\ \mathrm{mg/m^3}\sim$, $6.36\ \mathrm{mg/m^3}\sim$) compared with the external controls (FR = 2.07, 95% CI: 1.61–2.83; $P < 0.01$); (FR = 1.80, 95% CI: 1.36–2.41; $P < 0.05$); (FR = 2.43, 95% CI: 1.86–3.23; $P < 0.01$); (FR = 2.88, 95% CI: 2.21–3.82; $P < 0.01$), respectively (Table 2).