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Review

The lymphocyte cytokinesis block micronucleus test in human populations occupationally exposed to vinyl chloride: A systematic review and meta-analysis

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ABSTRACT

Vinyl chloride (VC) is widely used in industry in the production of polyvinyl chloride (PVC), which is used to manufacture a large variety of materials. VC was classified as a known (Group 1) human carcinogen by IARC on the basis of increased risk for liver angiosarcoma and hepatocellular cancer, and the carcinogenicity of VC was shown to be mediated by a genotoxic mechanism. Following inhalation, the compound is rapidly absorbed and metabolized in the liver to the electrophilic metabolites chloroethylene-oxide and chloroacetaldehyde, which form DNA adducts that can be processed into point mutations in cancer-related genes detected in humans and rats exposed to VC. A number of genotoxicity biomarkers were applied in workers exposed to VC to detect early biological responses associated with the carcinogenesis process. The present systematic review analyzed the published studies in which the cytokinesis-block micronucleus assay in peripheral lymphocytes (L-CBMN) was applied in VC-exposed subjects. Thirteen out of fifteen retrieved studies performed in China showed increased MN frequencies (FR 1.92–3.98) associated with increased cumulative exposure or employment time. Twofold and more than threefold increases were detected in PVC-exposed workers exposed to a mean of 50 ppm of VC in the former Yugoslavia and in South India, respectively. The meta-analysis of MN frequency from six eligible studies confirmed this tendency (pooled MR 2.32 – 95% CI 1.64–3.27). The benchmark dose lower limit for 10% excess risk (BMDL 10) calculated from three studies resulted in an estimated exposure limit of 0.03–0.07 mg/m^3. Overall the results of this review showed the need for further studies, especially because PVC products from China may contain high levels of uncoupled VCM that could represent a source of exposure to workers and consumers. Moreover, the results underline the importance of re-evaluating the recommended exposure limits using new biomonitoring methods in addition to MN.

Contents

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1. Introduction

Vinyl chloride (VC) is a gas widely used in the production of polyvinyl chloride (PVC) plastics applied in manufacturing a large variety of materials and furniture, packaging, electrical wire insulation cables, transportation industries, industrial and household equipment, and medical products.

VC was evaluated by IARC [1–4] and classified as group 1, "carcinogenic to humans", based on increased risk for ASL, and hepatocellular cancer (HCC). Epidemiological evidence for the carcinogenicity of vinyl chloride in humans derives principally from two large, multicenter cohort studies carried out in USA and in Europe, including thousands workers employed for at least one year in plants that manufactured VC and PVC or PVC products [3].

VC, although regulated in many countries, still remains a cause for concern [5]. The occupational exposure to VC indeed seems to be high in some countries with some ASL cases still reported [5]. In addition, although evidence of the carcinogenic effect of VC in humans has come from groups occupationally exposed to high doses of the compound over long periods, there is no evidence of an exposure level below which no increased risk of cancer would occur in humans.

There is a strong indication that the carcinogenicity of VC is mediated by a genotoxic mechanism. The compound was reported to be genotoxic, mainly in the presence of metabolic activation in various in *vitro* and *in vivo* systems inducing gene mutations, increase of chromosomal aberrations, micronuclei, SCE and unscheduled DNA synthesis [6,3].

The compound, following inhalation exposure, was shown to be primarily metabolized in the liver to the electrophilic intermediates, chloroethylene oxide and chloroacetaldehyde, which can react with DNA bases to form main adducts: 7-(2_-oxoethyl) guanine and four cyclic etheno adducts responsible accounting for the occurrence of specific point mutations in *p53* and K-*ras* genes detected in rat and human liver tumors [3,7].

Analyses in patients with ASL or with pre-malignant angiomatous lesions [8,9] and follow up studies carried out in cohorts of VC exposed workers [10,11,3] identified mutant p53 or p21ras proteins in serum significantly associated with cumulative exposure to the compound, indicating their key role as intermediate biomarkers of VC carcinogenesis [7]. The evidence of statistically significant increase of mutant ras-p21 and mutant p53 proteins in serum of workers with cumulative exposures >10 ppm-years suggesting that the current permissible exposure limit of 1 ppm for VC (2.6 mg/m^3) [12,13] is not adequately protective. Moreover significant dose–response relationships between plasma oncoprotein expression and VC exposure with a large inter-individual variability was observed in exposed human populations [14,15].

The possible explanation for this variability was investigated through the analysis of the genetic polymorphisms in genes that encode the enzymes involved in the different steps of activation/detoxification of vinyl chloride (*CYP2E1*, *GSTT1*, *GSTM1*, *ALDH2*) and in DNA repair pathways for Base-Excision-Repair (BER) and Nucleotide-Excision-Repair (NER).The available evidence indicates that specific polymorphisms and possible interactions between polymorphisms in the metabolic pathway and/or in the DNA repair processes could contribute to the variable susceptibility to the mutagenic effects of vinyl chloride in exposed populations [16–18].

The application of genotoxicity biomarkers, as early biological responses associated with the VC exposure showed a dose-response increase of DNA damage, measured by comet assay, SCE and chromosomal aberrations in workers occupationally exposed to >5 ppm of VC [6,3,19,20]. More recently a number of studies reported the application of the cytokinesis-block micronucleus assay in peripheral lymphocytes (L-CBMN) of workers occupationally exposed to vinyl chloride addressing the dose-response relationship and the correlation with different susceptibility factors.

The aim of the present study is to review the published studies on the application of the cytokinesis-block micronucleus assay in peripheral lymphocytes (L-CBMN) of VC exposed subjects to:

- Identify the role of L- CBMN as a biomarker of effect associated with exposure to VC
- Consider the proposal of a new permissible exposure limit (PEL) or TLV-TWA based on the dose-effect relationship between MN and cumulative exposure.

2. Materials and methods

This systematic review follows the methodology described in the PRISMA statement.

A literature search through electronic databases MedLine/PubMed, TOXLINE, was carried out up to December 2016. Key search terms included "micronucleus" and "micronuclei" in combination with "vinyl chloride" supplemented by an internet-based search using Google. A manual search of the reference list of studies and review articles was subsequently performed. References of retrieved articles were also analyzed to identify any publications which may have been potentially missed in the initial search. The first author (C.B.) did the initial selection based on titles and abstracts. Eligible for the inclusion in the present review were all studies which concerned the application of L-CBMN assay in groups of subjects occupationally exposed to VC. Only studies in English where the full text was available were considered. Full text articles were assessed for the inclusion in the analysis independently by two reviewers (C.B.; M.KV.) with all the discrepancies resolved through a discussion.

The articles considered as eligible for the review were classified with respect to their quality following the criteria applied the recent review in a recent special issue of Mutation Research reviewing of the application of the L-CBMN assay in people exposed to chemical genotoxins [21]. The parameters most relevant for the L-CBMN assay were considered, each one scoring from 1 to 3 points: i) number of subjects in control and in exposed groups (1 = <20, 2 = 20–50, 3 = >50), ii) age-, gender-, smoking status-, alcohol intake-, nutritional intake- matching (1 = significantly different or no data collected; 2 = not statistically different; 3 = perfectly matched) iii) the measurement of the exposure to VC (1 = assessed by questionnaire only, 2 = measurement in ambient environment, 3 = measurement in body fluids) iv) number of cells