Genotoxicity Biomarkers in Occupational to Formaldehyde in Pathology Anatomy Laboratories

C LADEIRA[1,2], M.C. GOMES[3], M BRITO[1]

1 Escola Superior de Tecnologia da Saúde de Lisboa – IPL
2 CIESP - Centro de Investigação e Estudos em Saúde Pública – ENSP / UNL
3 Faculdade de Ciências da Universidade de Lisboa

INTRODUCTION

FORMALDEHYDE

Formaldehyde (FA) the most simple and reactive of all aldehydes, is a colorless, reactive and readily polymerizing gas at normal temperature *(Zang et al., 2009)*. It has a pungent, suffocating odour that is recognized by most human subjects at concentrations below 1 ppm *(Speit et al., 2007)*.
According to the Report on Carcinogens, FA ranks 25th in the overall U.S. chemical production with more than 11 billion pounds (5 million tons) produced each year (NTP, 2005). Is an important industrial compound that is used in the manufacture of synthetic resins and chemical compounds such as lubricants and adhesives *(WHO, 1989)*. It has also applications as a disinfectant, preservative and is used in cosmetics. Estimates of the number of persons who are occupationally exposed to FA indicate that, at least at low levels, may occur in a wide variety of industries *(Kauppinen et al., 2000)*. The occupational settings with most extensive use of formaldehyde is in the production of resins and in anatomy and pathology laboratories *(IARC, 2006)*.
Several studies reported a carcinogenic effect in humans after inhalation of FA, in particular an increased risk for nasopharyngeal cancer. Nowadays, the International Agency for Research on Cancer (IARC) classifies FA as carcinogenic to humans (group 1), on the basis of sufficient evidence in humans and sufficient evidence in experimental animals *(IARC, 2006)*. Manifold in vitro studies clearly indicated that FA is genotoxic. FA induced various genotoxic effects in proliferatin cultured mammalian cells. A variety of evidence suggests that the primary DNA alterations after FA exposure are DNA-protein crosslinks (DPX). Incomplete repair of DPX can lead to the formation of mutations *(Speit et al., 2007)*.

CYTOKINESIS-BLOCKED MICRONUCLEUS ASSAY

The cytokinesis-block micronucleus cytome assay (CBMN) is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity. DNA damage events are scored specifically in once-divided binucleated cells and comprise micronucleus (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD). This assay has been applied successfully for biomonitoring of *in vivo* genotoxin exposure, *in vitro* genotoxicity testing, and in fields like nutrigenomics and pharmacogenomics (Fenech, 2007; Mateuca et al., 2006; Serrano-García et al., 2001). MN originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division and are not included in the main daughter nuclei. Thus MN provide a measure of both chromosome breakage and loss and it has been shown to be at least as sensitive an indicator of chromosome damage a classical metaphase chromosome analysis (Iarmarcovai et al., 2008; Fenech et al., 1999). The analysis of NPB was validated as biomarker of DNA damage in human WIL2-NS cells treated with hydrogen peroxide, superoxide or after co-incubation with activated human neutrophils (Umegaki et al., 2000). NPB should be scored because they provide a measure of chromosome rearrangement, which is otherwise not assessed if only MN are scored (Fenech et al., 1999) . This event occurs when centromeres of dicentric chromosomes are pulled to opposite poles of the cell at anaphase. NPB are therefore biomarkers of dicentric chromosomes resulting for telomere end-fusions or DNA misrepair (Fenech, 2006; Thomas et al., 2003). NBUD are characterized by the same morphology as the MNi, except that they are linked to the nucleus by a narrow or wide stalk of nucleoplasmatic material, depending on the stage of the nuclear budding process. They are classified as biomarkers of the elimination of amplified DNA and/or DNA repair complexes (Fenech et al., 2008; Fenech, 2006; Tolbert et al., 2001).

AIM OF THE STUDY

Compare means of MN in lymphocytes and buccal cells, NPB and NBUD in 56 workers exposed to FA in pathology anatomy laboratories with 85 non-exposed controls using the multi-endpoint cytokinesis-blocked micronucleus assay.

MATERIAL AND METHODS

This study was carried out in Portugal, in 56 workers occupationally exposed to FA and in a control group of 85 non-exposed subjects (controls). All subjects were provided the protocol and consent form, which they read and signed.
Heparinized venous blood was collected between 10 and 12 a.m., from each subject and was processed by the application of CBMN assay. The slides were stained with May-Grünwald Giemsa. Buccal cells were collected with an endobrush and the slides were stained by Feulgen technique without counterstain. All slides were coded and analysed under blind conditions. The criterion of scoring the cells was the described in "The Human MicroNucleus Project" (Fenech el al., 1999) and Tolbert et al., 2001.

RESULTS

Table 1. General characteristics of exposed and control subjects

Characteristics	Exposed	Controls
N.º of subjects	56	85
Gender Females Males	 66% 34%	 54% 36%
Age Range	39.45±11.5 20-61	32.42±8.1 20-53
Smoking habits Smokers Non-smokers	 19.6% 80.4%	 29.4% 70.6%

Table 2. Descriptive statistics of the genotoxicity biomarkers in study

	Mean, MN lymphocytes S.E. (range)	Mean, NPB S.E. (range)	Mean, NBUD S.E. (range)	Mean, MN buccal cells S.E. (range)
Controls	0.81 0.172 (0-7)	0.18 0.056 (0-3)	0.07 0.028 (0-1)	0.16 0.058 (0-2)
Exposed	3.96 0.525 (0-14)	3.04 0.523 (0-15)	0.98 0.273 (0-13)	0.96 0.277 (0-9)
p-value*	<0.001	<0.001	<0.001	0.002

*** Mann-Whitney test**

Table 3. Results of binary logistic regression concerning the association between FA and genotoxicity biomarkers

	OR	CI 95%	p-value
MN PBL	9.665	3.81-24.52	<0.001
NPB	11.97	4.59-31.20	<0.001
NBUD	9.631	3.12-29.70	<0.001
MN BC	3.990	1.38-11.58	0.011

CONCLUSIONS

Long exposures to FA, as those to which some workers are subjected for occupational reasons, are suspected to be associated with genotoxic effects that can be evaluated by biomarkers.
In this study all biomarkers showed statistical significant association with FA exposure therefore we can conclude that is chemical agent is a risk factor in subjects exposed occupationally to FA in pathology anatomy laboratories. The nuclear abnormalities found in lymphocytes, expressed in the presence of MN, NPB and NBUD can be explained by the fact of FA escape from the local of first contact, such as the mouth, direct contact place, where MN was found too. Our findings in blood lymphocytes can be an indication that cytogenetic effects can be seen in tissues distant from the area of initial contact (nasopharyngeal) and even reach the bone marrow and cause toxicity, giving relevance to the thesis of Zang and colleagues *(2009)*.
Suruda *el al.* (1993) claim that although changes in oral and nasal epithelial cells and peripheral blood cells do not indicate a direct mechanism leading to carcinogenesis, they do indicate that DNA alterations took place. It thus appears reasonable to conclude that FA is a risk factor for those that are occupationally exposed in pathology anatomy laboratories (IARC, 2006).

In conclusion, the population studied is exposed to high peak concentrations of FA and a long-term exposure and these two aspects cumulatively can be the cause for the effects observed (increase in MN PBL). The association of these cytogenetic effects with FA exposure gives important information to risk assessment process and may also be used to assess health risks for exposed groups. These results suggest that must be applied preventive and protective measures aim to reduce occupational exposure to this chemical agent in these occupational settings in Portugal.

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