CHROMOSOMAL BREAKS AND LOSSES IN LYMPHOCYTES FROM WORKERS EXPOSED TO FUMES OF BITUMEN AT HIGHER PROCESSING TEMPERATURES – A CROSS SHIFT STUDY

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Introduction

At higher processing temperatures mastic asphalt workers may be exposed to several polycyclic aromatic hydrocarbons (PAH) contained in fumes of bitumen. Little is known regarding clastogenic or aneugenic effects in bitumen-exposed workers. We conducted a cross-shift study in German mastic asphalt workers and in a reference group without exposure to bitumen and determined the micronucleus (MN) frequencies in lymphocytes. The cytokinesis-blocked micronucleus (CBMN) assay detects chromosome breaks and losses.

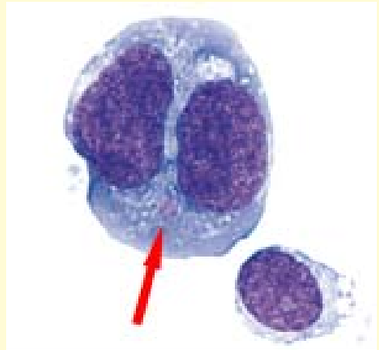

Fig. 1: Binucleated lymphocyte with one micronucleus

Materials and Methods

The study group consisted of 34 mastic asphalt workers exposed to fumes of bitumen and 14 street workers without exposure to fumes of bitumen as a reference group. Blood was collected before and after work shift to determine the MN frequencies. The concentration of fumes of bitumen in ambient air and the concentration of 1-hydroxypyrene and the sum of hydroxyphenanthrenes in urine were analysed.

The CBMN assay was done according to the method described by Fenech (2000). Lymphocytes were isolated from whole blood by Ficoll density gradient centrifugation. Duplicate lymphocyte cultures were set up and the lymphocytes were stimulated to divide by addition of phytohemagglutinin (PHA, final concentration 4,5 µg/ml). After forty-four hours of incubation, cytokinesis was blocked with 6 µg/ml cytochalasin-B. 28 hours later lymphocytes were harvested by cytocentrifugation and stained with May-Gruenwald/Giemsa staining. Standard scoring criteria for selecting binucleated cells and identifying MN were adopted. For each sample, the number of MN in 1000 binucleated cells (BNC) was measured.

Tab. 1: Characteristics of the study population

		Exposed	Reference group
Number		34	14
Age [years]	Median	41	40
	Range	17 - 53	18 - 54
Smokers		21/34 (62%)	8/14 (57%)

Results

In 34 workers exposed to fumes of bitumen a median of 8.25 MN/1000 BNC (P25-P75: 6.5-11.0) was found before shift and 7.58 MN/1000 BNC (P25-P75: 5.5-10.1) after shift ($P = 0.53$). In lymphocytes of the reference group ($n = 14$) a median of 5.75 MN/1000 BNC (P25-P75: 3.5-7.7) before shift and a median of 6.92 MN/1000 BNC (P25-P75: 5.36-9.0) after shift were found ($P = 0.60$). There was no significant difference in the MN frequencies between exposed and reference persons after shift ($P = 0.55$). The only significant difference ($P = 0.03$) in the micronucleus frequencies between exposed and controls before shift should be interpreted with caution because of the limited number of controls. External exposure to fumes of bitumen showed no association with the number of micronuclei after shift ($P = 0.93$). Also there was no association with 1-hydroxypyrene ($P = 0.89$) or the sum of hydroxyphenanthrenes ($P = 0.85$) in urine of exposed workers.

Tab. 2: Micronuclei in workers exposed to fumes of bitumen and in reference persons (number of micronuclei in 1000 binucleated cells)

	Pre work shift Median (P25 – P 75)	Post work shift Median (P25 – P75)	
		$P = 0.53$	
Exposed (n=34)	8.25 (6.5-11.0)	7.58 (5.5-10.1)	$P = 0.55$
$P = 0.027$			
Reference group (n=14)	5.75 (3.5 – 7.7)	6.92 (5.4 – 9.0)	
		$P = 0.60$	

Discussion

The MN frequencies in our study were in the middle of the range reported as background frequencies in other laboratories surveyed by Surralles and Natarajan (1997) and Bonassi et al. (2001). Järvholm et al. (1999) investigated MN in peripheral lymphocytes of 27 nonsmoking road pavers (4.1 ± 2.0 micronuclei/1000 cells (geometric mean ± standard deviation)) and 30 nonsmoking referents (4.5 ± 1.7 micronuclei/1000 cells) and did not find a significant difference between both groups. These results are in concordance with our study. In contrast to this Burgaz et al. (1998) found significant higher frequencies of MN in lymphocytes of 28 workers exposed to bitumen fumes (2.25 ± 0.42 micronucleated cells/1000 cells (mean ± standard deviation)) compared to 28 controls (1.79 ± 0.32 micronucleated cells/1000 cells). Similar to our study, Burgaz et al. (1998) did not find a correlation between the urinary excretion of 1-hydroxypyrene and the MN frequency. The MN frequency in this Turkish bitumen study was lower than in our German study group. However, Burgaz et al. (1998) and Järvholm et al. (1999) used a slightly different method (without addition of cytochalasin B).

Conclusions

- In our study group the exposure to fumes of bitumen did not result in elevated micronucleus frequencies after shift, compared to the reference group.
- There was no significant difference between micronucleus frequencies before and after work shift in exposed and unexposed persons.
- The study size should be expanded to allow a more precise estimation of possible bitumen-related genotoxic effects.

Literature

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