Complex Mixtures and Cancer Risk
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The effect of background variables on human peripheral lymphocyte micronuclei

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Summary. Application of biological methods for assessment of occupational and environmental exposure to single agents or complex mixtures is optimized by determination of the possible influence of background factors on the biological endpoint of interest. Analysis of micronuclei in peripheral blood lymphocytes using the cytokinesis-block method was performed on healthy volunteers up to three times for each individual at intervals of approximately four months. Questionnaires were administered to ascertain recent health history and lifestyle factors such as smoking and drinking habits. Results to date indicate that age ($r = 0.45$, $p = 0.001$) and estimated number of diagnostic X-rays during the past year ($r = 0.35$, $p = 0.01$) contribute significantly to increased frequency of micronuclei. Information on the potential influence of background factors is critical for appropriate statistical analysis of data from occupational and population monitoring studies that utilize the cytokinesis block lymphocyte micronucleus assay to assess exposure to genotoxic agents.

Introduction

Micronuclei can be formed from entire chromosomes or chromosome fragments; they result from damage to the mitotic spindle and/or chromosome breakage and are considered a measure of genotoxicity (Heddle *et al.*, 1983). The cytokinesis-block modification of the lymphocyte micronucleus assay (Fenech & Morley, 1985) has previously been shown to exhibit increased sensitivity over the standard method in studies *in vitro* (Yager & Sorsa, 1987). This method has also been utilized as a biological index of occupational exposure; increased numbers of micronuclei were observed in groups of workers exposed to alkylating cytostatic drugs (Yager *et al.*, 1988). The purpose of this study is to determine the possible influence of selected intrinsic and extrinsic factors on the cytokinesis-block peripheral lymphocyte micronucleus assay. Knowledge of the impact of these factors on micronucleus frequency is critical in order to increase the ability to distinguish background effects from effects that may be due to extrinsic xenobiotic exposure(s).

Table 1. Characteristics of study subjects

Age (years)	Number	Per cent
20–30	14	29
31–40	18	38
41–50	11	23
51–65	5	10
Estimated number of X-rays during past year		
0	20	41.7
1–3	20	41.7
4–6	8	16.6
Average number of cigarettes smoked per day		
Non-smoker	22	45.8
< 10	5	10.4
≥ 10	7	14.5
≥ 20	9	18.8
≥ 30	4	8.3
≥ 40	1	2.2
Average number of alcoholic beverages per week		
Non-drinker	17	35.4
< 5	14	29.2
≥ 5	12	25.0
≥ 15	3	6.3
≥ 40	2	4.1
Average number of cups of caffeinated beverages per day		
0	5	10.8
< 5	21	45.7
≥ 5	16	34.8
≥ 10	3	6.5
≥ 20	1	2.2

Methods

Blood samples were collected up to three times from each individual at approximately four-month intervals and analysed utilizing the cytokinesis-block method as previously described (Yager *et al.*, 1988). Questionnaires were administered in order to obtain information on health history and lifestyle factors including smoking habits, intake of alcoholic and caffeinated beverages and the estimated frequency of diagnostic X-rays during the past year.

Results and discussion

Group mean micronucleus scores showed no significant difference among the three sampling times (ANOVA, $p = 0.48$) and therefore the average value for each

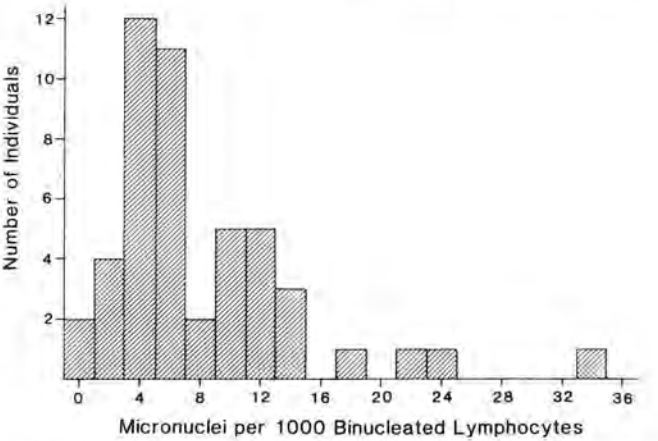

Figure 1. Frequency distribution of mean micronuclei per 1000 binucleated lymphocytes among subjects ($N = 48$)

individual was used in subsequent analyses. The frequency distribution of mean micronuclei among individuals is shown in Figure 1. Since this distribution is skewed to the right, log transformed mean micronucleus data were used to approximate the normal distribution for subsequent analyses. The distribution of characteristics of study subjects with regard to variables such as smoking and drinking habits determined to date are shown in Table 1.

Linear regressions of log mean micronuclei on the average number of caffeinated beverages per day, number of cigarettes per day and average number of

Table 2. Distribution of group mean micronuclei according to age of subjects and estimated number of diagnostic X-rays

	Number	Mean micronuclei per 1000 BN cells[a]	S.E.[b]
Age (years)			
≥20	13	4.3	0.6
≥30	19	7.8	1.2
≥40	10	11.3	2.7
≥50	6	11.5	3.3
X-rays[c]			
0	20	6.5	0.8
1–3	20	7.9	1.7
4–6	8	12.3	2.2

[a] Micronuclei were scored in 1000 binucleated (BN) lymphocytes per sample (500 per replicate culture).
[b] Standard error of the mean.
[c] Estimated number of diagnostic X-rays during the past year.

alcohol-containing beverages per week were all non-significant. However, regression of log mean micronuclei on age ($y = 0.267 + 0.01458\ x$, $r = 0.45$, $p = 0.001$) and estimated number of diagnostic X-rays in the past year ($y = 0.703 + 0.06765\ x$, $r = 0.35$, $p = 0.01$) were highly significant. Distribution of mean micronucleus values among these groups is shown in Table 2.

The effect of age on lymphocyte micronuclei has been shown previously (Amos *et al.*, 1985; Fenech & Morley, 1986). The effect of X-rays on micronucleus induction *in vitro* has been shown repeatedly (Fenech & Morley, 1986; Eastmond & Tucker, 1989). However, results are striking here in that exposure to relatively few diagnostic (as contrasted with therapeutic) X-rays was detected *in vivo*; this may be the result of increased sensitivity of the cytokinesis-block micronucleus method as well. It would be desirable to obtain medical record verification of X-ray procedures as well as an estimate of dose per procedure in a future larger population study in order to verify this result.

Conclusions

In summary, age and estimated number of diagnostic X-rays appear to have a significant influence on background frequencies of micronuclei in cytokinesis-blocked peripheral lymphocytes of humans. Other variables addressed to date in this study, including cigarette smoking, showed no detectable effect on micronucleus frequency. Additionally, micronucleus frequencies did not appear to vary significantly for the group as a whole over the study period of about one year.

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