Short Communication

Micronucleus Assay of Human Lymphocytes: A Comparison of Cytokinesis-block and Human Capillary Blood Lymphocytes Methods

Ying TIAN¹, Hitoshi ISHIKAWA², Feng Yuan PIAO², Hidetaka YAMAMOTO³, Toru YAMAUCHI², Zhi Wen DUAN⁴, Yu Min ZHANG⁴, Ming Yue MA⁴ and Jin Shan CUI⁴

¹School of Public Health, Shanghai Second Medical University, China, ²Department of Public Health, ³Department of Forensic Medicine and Science, Mie University School of Medicine, Japan and ⁴Department of Toxicology, Shenyang Medical College, Shenyang, China

Key words: Micronucleus, Female, Human, Lymphocyte, Cytokinesis-block

Micronucleus (MN) expression in Peripheral blood lymphocytes is well established as a method to monitor chromosome damage in human populations¹. The use of MN as a measure of chromosome damage in peripheral blood lymphocytes was first proposed by Countryman and Heddle² and subsequently improved with the development of the cytokinesis-block micronucleus (CBMN) method³, which has been extensively used to evaluate chromosome damage in human populations. Nevertheless, the CBMN method requires more blood collected by venous puncture, and the procedure detecting MN in cultured lymphocytes is time-consuming. In 1992, a method for MN testing of lymphocytes with human capillary blood collected by skin puncture was developed⁴. The procedure was simple and fast because MNs were detected in non-cultured lymphocytes, and only 1 or 2 drops of capillary blood were needed, so it was called the human capillary blood lymphocytes MN (HCBL-MN) method⁴.

The present study gives the comparison of the results of MN frequency in human lymphocytes using the two methods in the same subjects in order to find a simple and sensitive method, to clarify the correlation between the two methods and to provide information on baseline MN frequency in human lymphocytes detected by the two methods.

Materials and Methods

The study population consisted of 95 (19–47 yr) healthy female subjects. All were non-smoking office workers

who lived in the same district. The study was approved by the Ethical Judging Committee of Mie University (Japan). Participants were informed of the study aims and asked to sign consent and complete a questionnaire. Venous blood samples were collected in coded heparinized tubes, and divided into two parts, 0.8ml for the CBMN method, and 100 μ l for the HCBL-MN method, to test MN frequency.

1. CBMN method: The human lymphocyte MN assay was processed with a modification of the Fenech method³. Briefly, 0.8ml of blood was added to medium supplemented with phytohaemagglutinin (PHA, 1%). Cytochalasin B (Cyt-B, Sigma Chemical Co., Louis, MO) was added after 44h. At the end of incubation at 37°C for 72 h, the cells were centrifuged, fixed, stained with Acridine Orange (Sigma: 40 μ g/ml) and observed by fluorescent microscopy (LEICA-MPS60). The main nucleus and MN showed bright yellow against the red cytoplasm when viewed with a yellow filter (excitation 490 nm). One thousand binucleated cells were examined per culture to ascertain the frequencies of MN.
2. HCBL-MN method: The procedure for the HCBL-MN analysis was as described by Xue *et al*⁴. Briefly, 40 μ l 0.3% methylcellulose solution was added to 100 μ l blood in tubes, at 37°C for 40 min, to separate the lymphocytes. The lymphocyte suspension was centrifuged at 100 g for 6 min. The supernatant was removed and the pellets in the sediment were carefully mixed. A small drop of the cell suspension was put on the end of a slide and spread by pulling the material. The preparations were then air dried, fixed in 100% methanol for 1 min, and stained in 7% buffered Giemsa (pH 6.4) for about 10 min. Two thousand small lymphocytes per person were scored. The main nucleus and MN showed dark blue against the light blue cytoplasm.

The differences between groups were tested by the Mann-Whitney U test. Correlation analysis was performed with Spearman's rank correlation test.

Results

The MN frequencies in binucleated cells obtained by CBMN in 72 h cultures of human lymphocytes from all subjects were about 5-fold higher than those in non-cultured lymphocytes analyzed by the HCBL-MN method for human lymphocytes in the same subjects (1.46 ± 0.15 vs. 0.33 ± 0.06 , $p < 0.01$ Table 1.)

Table 2 summarizes the mean MN frequencies detected by the two methods with selected host factors. The results for MN frequency in human lymphocytes from both the CBMN and HCBL-MN methods showed a similar tendency in almost all of the host factors, but only a weak positive correlation of MN frequencies with the two methods was obtained ($r = +0.17$, $p = 0.009$).

Received March 14, 2003; Accepted Sep 2, 2003

Correspondence to: Y. Tian, School of Public Health, Shanghai Second Medical University, No. 227 ChongQing Nan Road, Shanghai, 200025, China

Table 1. MN frequencies in human lymphocytes after the CBMN and HCBL-MN methods

	Subjects No.	Min-max MN/cell No.	MN frequency (‰)
CBMN method	95	0–7/1000	1.46 ± 0.15
HCBL-MN method	95	0–6/2000	0.33 ± 0.06*

*: $p < 0.01$ compared with the CBMN method by the Mann-Whitney U test. CBMN: cytokinesis-block micronucleus. HCBL-MN: human capillary blood lymphocytes micronucleus.

Table 2. MN frequencies detected by two methods with host factors

Covariates	Number of subjects	CBMN Method (‰)	HCBL-MN Method (‰)
Age at the time study (yr)			
≥38	37	1.60 ± 0.27	0.38 ± 0.09
<38	58	1.38 ± 0.17	0.29 ± 0.08
passive smoking habit			
Smokers	89	1.49 ± 0.15	0.34 ± 0.06
Non-smokers	6	1.00 ± 0.26	0.33 ± 0.21
Alcohol consumption habit			
Drinkers	16	1.63 ± 0.16	0.16 ± 0.09
Non-drinkers	79	1.43 ± 0.16	0.36 ± 0.07
x-rays examination the latest month			
examined	22	1.50 ± 0.31	0.66 ± 0.19*
Non-examined	73	1.45 ± 0.17	0.23 ± 0.05

*: $p < 0.05$ compared with the Non-examined group by the Mann-Whitney U -test.

Discussion

In the present study, we found that the mean MN frequency detected by the CBMN method was about 5-fold higher than that detected by the HCBL-MN method and suggested that the CBMN method was more sensitive in detecting MN in human lymphocytes. To explain the results, we compared the characteristics of the methods as follows: For the CBMN method, PHA was used to stimulate the lymphocytes to transform and divide in culture; Cyt-B was used to stop the cytokinesis, and MN were scored in cytokinesis-block (CB) lymphocytes. For the HCBL-MN method, only 0.3% methylcellulose was used to separate the lymphocytes from blood, and MN were scored in non-cultured lymphocytes.

Numerous authors have considered that micronuclei are the consequence of mitotic loss of acentric chromosome fragments and / or whole lagging chromosomes that appear in the cytoplasm of divided cells¹⁾. The CBMN method allowed MN to be scored in cells that had complete nuclear division. Our results confirmed that CB cells were more sensitive than non-cultured lymphocytes because they were certain to have divided and MN can be easily recognized. The present study proved that the mean MN frequency detected by the HCBL-MN method was weakly correlated with that

of the CBMN method. From the viewpoint of epidemiology, further study should be taken to ascertain the correlation of the two methods in detecting MN frequency by increasing the number of subjects.

In conclusion, the present study demonstrates that under the present experimental conditions, for quantitation of MN frequency of human lymphocytes, the CBMN method was more sensitive and reliable than the HCBL-MN method.

References

- 1) H Thierens, A Vral, R Morthier, B Aousalah, DL Ridder: Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus centromere assay. *Mutagenesis* 15, 245–249 (2000)
- 2) RI Countryman and JA Heddle: The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutat Res* 41, 321–332 (1976)
- 3) M Fenech: The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat Res* 285, 35–44 (1993)
- 4) Xue KX, Ma GJ, Wang S, Zhou P: The in vivo micronucleus test in human capillary blood lymphocytes: methodological studies and effect of ageing. *Mutat Res* 278, 259–264 (1992)