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Micronuclei in cytokinesis-blocked lymphocytes of patients following iodine-131 radiotherapy

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The micronucleus assay in cytochalasin-B cytokinesis blocked peripheral blood lymphocytes in 10 patients with hyperthyreosis and various types of thyroidal carcinomas was investigated. Patients received 259 - 5180 MBq I-131 sodium iodide perorally. Micronucleus (MN) frequencies were measured before and after I-131 administration. Pre treatment MN results were regarded as controls. Our results show considerable variability concerning age and activity applied. Also, the number of MN and the number of binucleated cells with micronuclei (BNMN) showed Poisson regression adjust for within subject correlation overdispersion. Log dose alone was not significant. The interaction of time and dose was significant at higher doses,while the rate of MN changing was slower. Relative risk time was calculated for the lowest dose (259 MBq). By doubling the dose, the rate of daily increase in the number of MN and BNMN decreases by approximately 5% (Relative risk MN =0.955; Relative riskBNMN = 0.954).

Key words: hyperthyroidism-radiotherapy; thyroid neoplasms-radiotherapy; iodine radioisotopes-adverse effects; lymphocytes; micronucleus test

Introduction

Radioiodines are often used for experimental purposes, diagnosis and therapy in clinical practice. The ionizing energy of radioiodine affects not only the thyroid with its uptake points, but other tissues as well, especially lymphocytes during their circulation through and around the gland containing the radioisotope. Therefore, it seemed to be of interest to investigate the cytogenetic alterations in blood lymphocytes of patients treated with iodine-131.

In order to determine the genotoxic risk associated with the diagnostic and therapeutic exposure to iodine-131, we conducted a follow-up study on the frequency of micronuclei in cytochalasin-B blocked blood lymphocytes.

Until now the scoring of chromosome aberrations has been considered the most relevant method for the cytogenetic dosimetry. Nevertheless, in the last few years, the

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micronucleus assay in human peripheral blood lymphocyte cultures using the cytokinesis-block method has been demonstrated to be a fast and sensitive cytogenetic technique and has been receiving increased attention for biological monitoring of radiation exposure.[1,2]

Counting micronuclei in cells which have undergone one cell division after the clastogenic insult is deemed to be a simpler and more sensitive method than conventional chromosome aberration assay.[3] Micronuclei originate from acentric fragments or from whole chromosome aberration assay and may serve as a measure for both chromosome breakage and loss.[1,3]

The aim of this study was to identify the relation between the number and distribution of micronuclei, using diagnostic and therapeutic doses of radioiodine-131. The frequency of micronuclei in cultured and cytokinesis-blocked circulating lymphocytes was determined before the treatment and at several short intervals after it. The quantity of perorally administrated iodine-131 depends on what the clinical practice determines to be a sufficient therapeutical dose for either hyperthyroidism or thyroid carcinomas. The doses applied in our study ranged between 259 and 5180 MBq.

Materials and methods

Lymphocyte cultures and micronucleus test

Venous blood was taken from 10 patients, 2 males and 8 females between 22 and 83 years of age, with Basedow-Graves disease (hyperthyreosis) and thyroid carcinomas, treated with ^{131}I (Table 1). First we quantified the micronuclei in the samples taken before treatment, and then we repeated the measurement on samples taken at short intervals (day 1,2 and 4) after treatment. Duplicate lymphocyte cultures in F-10 medium (Gibco, GB) supplemented with 20% of new-born calf serum (Biological Industries, Israel), penicillin (10 000 IU/ml) and streptomycin (10 000 μg/ml) were set up. Lymphocytes were stimulated by phytohaemagglutinin (PHA-Murex, GB). The cultures were incubated for 72 hours at 37°C, and at 44 h after the initiation of cultures cytochalasin-B (Sigma, Germany) dissolved in dimethylsulphoxide was added to arrest cytokinesis. The cells were collected by centrifugation, and instead of hypotonic treatment, we used 0.9% sodium chloride for 5 minutes at room temperature, fixation in methanol: glacial acetic acid, 3:1. Air dried slides were stained with 5% Giemsa solution. Each sample was analyzed only for binuclear cytochalasin B blocked cells with well-preserved cytoplasm. In each sample 1000 binucleated cells were analysed. The slides were scored at 1000x magnification.

Statistical methods

For the statistical analysis we used Poisson regression adjusted for within subject correlation. The data were analysed using the SAS 612.PROC.GENMOD statistical package.

Results

Table 1 shows age, sex, diagnosis, the applied activity for each patient, the total number of micronuclei (MN) in cytochalasin B cytokinesis blocked peripheral human blood lymphocytes in 1000 binucleated cells, and the number of binucleated cells with micronuclei (BNMN) in patients before and after radioiodine application, in order to show the MN distribution among cells. In the group studied, there was a considerable variability concerning age and activity applied.

In some cases the cytogenetic study was not able to be carried out in all samples due to low stimulation of the cultured lympho-

Table 1. Dose, age, sex, diagnosis, total number of micronuclei (MN) in 1000 binucleated cells, number of binucleated cells with micronuclei (BNMN) in the hyperthyroidism and carcinoma patients treated with radioiodine-131

Subject	Sex	Age (years)	Diagnosis	Aplicated activity (MBq)	Pretreatment		Post-treatment (24 hours)		Post-treatment (48 hours)		Post-treatment (96 hours)	
					MN	BNMN	MN	BNMN	MN	BNMN	MN	BNMN
1	F	53	Hyperthyreosis	259	56	45	78	64	81	68	119	100
2	F	29	Hyperthyreosis	262	24	18	-	-	84	72	62	57
3	F	56	Hyperthyreosis	309	26	24	-	-	74	60	100	91
4	F	40	Toxic adenoma glandulaae thyroideae	1176	22	22	37	34	37	31	44	39
5	F	57	Papillary thyroid carcinoma	2960	22	18	28	23	32	29	32	30
6	F	67	Follicular thyroid carcinoma	2960	22	19	35	34	52	46	94	83
7	F	83	Papillary thyroid carcinoma	2960	70	56	69	57	62	53	-	-
8	M	22	Mixed follicular/papillary carcinoma metastases	3700	20	16	15	14	20	16	25	23
9	F	64	Papilary thyroid carcinoma	3700	25	24	12	12	29	26	19	17
10	M	71	Follicular thyroid carcinoma - distant metastases	5180	102	82	156	130	100	84	117	98

Table2. Results of statistical analysis

Variable	Parameter	Relative risk	Lower confidence limit	Upper confidence limit	p
1	MN time (day)	1.27494	1.04487	1.55566	0.0000
2	MN time x dose	0.95530	0.93518	0.97585	0.0000
3	MN age (10 years)	1.22708	1.09297	1.37763	0.0005
4	BNMN time (day)	1.29153	1.05895	1.57520	
5	BNMN time x dose	0.95426	0.93418	0.97477	
6	BNMN age (10 years)	1.21817	1.08966	1.36184	

cytes. We observed binucleated cells with 1-4 micronuclei (Figure 1)

Poisson regression adjusted for within subject autocorrelation was used to compare the variation in the frequency of MN and BNMN in the four sampling times. The results in Table 2 show that the number of micronuclei increased in time. For both MN and BNMN the models showed overdispersion. For MN the deviance was 473.5 and Pearson χ^2 515.7 with 33 degrees of freedom. For BNMN the deviance was 364.5 and Pearson,s χ^2 was 399.5 with 33 degrees of freedom. We have introduced a scaled in the final model to adjust for overdispersion (for MN scale was 3.7878 and for BNMN 3.3234).

Log dose alone was not significant. The interaction of time and dose was significant at higher doses, the rate of the MN changing was slower. A relative risk for time was calculated for the lowest dose (259 MBq). By doubling the dose, the rate of daily increase in the growth of number of MN and BNMN decreased for about 5% (Relative $risk_{MN}$=0.955; Relative $risk_{BNMN}$= 0.954).

The data obtained when patients were classified into five subgroups, according to the activity received (< 300 MBq, 1176 MBq, 2960 MBq, 3700 MBq, 5180 MBq) are shown graphically in Figure 2. These values show wide variability in the baseline frequency of BNMN among patients before treatment.

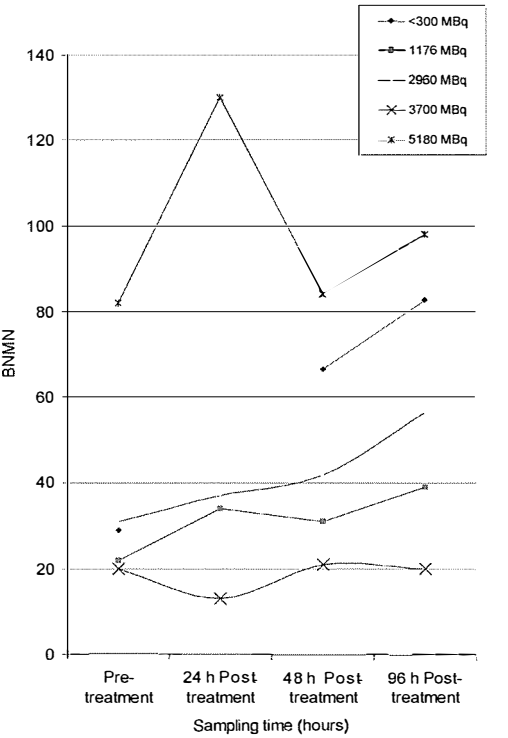

Figure 2. Frequency of BNMN before and after the treatment.

Discussion

A consequence of radiation-induced DNA damage is the formation of micronuclei, which appear similar to the main nuclei but are smaller and have a reduced DNA content.[4]

The use of micronucleus assay to evaluate radiation exposures resulting from internally deposited radioactive materials in people pre-

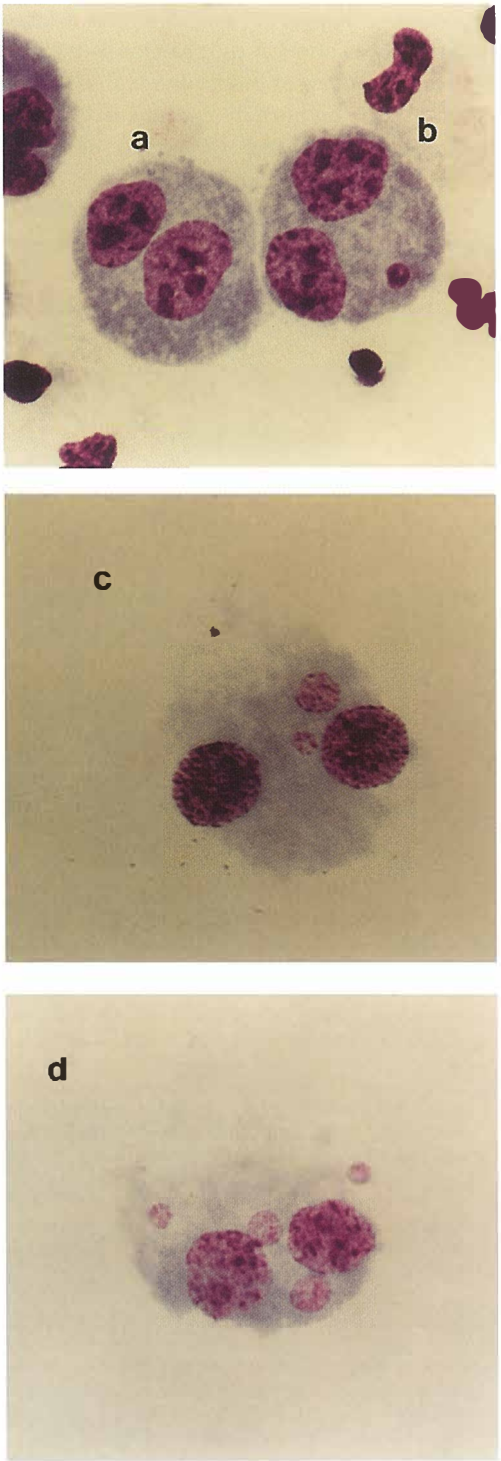

Figure 1. Photomicrograph of binucleate human blood lymphocyte without micronuclei (a) , with one micronuclei, (b), with two micronuclei (c) and with four micronuclei (d). Stained by 5% Giemsa (magnification 1000 x)

sents several specific problems. First, the deposition, distribution, and dose to individual cells are dependent on the radionuclides involved, the route of exposure, the metabolic state of the individual and the chemical and physical form of the material. Second, because of individual differences, it is impossible to make a reliable estimate of the radiation dose that the individual receives even if the exposure level, radionuclides involved, and their physical and chemical form are known.

For nonuniformly distributed internally deposited radionuclides, it is important to recognize that not only the radiation dose is nonuniform, but also the lymphocytes used to evaluate the exposure are nonuniformly distributed in blood, organs, lymph nodes and lymph follicles throughout the body.

It is well established that there is heterogenity in the repair of lesions along the DNA molecule.[5-8] The level of gene activity and repair at the level of the chromosome are critical factors that may influence the formation of chromosome aberrations and micronuclei.[9]

The obtained results indicate that the varied cell response to low doses depends on individual features of the patients, quite similar to those observed by Brown.[10] Moreover, it is important to notice a corresponding irregularity in the distribution of the incorporated radionuclides, not only in the doses, but in the lymphocytes irregularly distributed in blood, organs, lymph nodes and follicles. The obtained results as well as the referred literature [11,12,13] point out individual differences in reacting to exposure. As there is variation in the proliferation rate of the lymphocytes from different individuals, kinetic differences appear to remain a source of vari-

ability in the MN assay.[14] Keldsen and his coworkers[15] noticed considerable absorption differences between patients equally exposed to iodine-131 (here patients 6, 7 and 9). It is interesting to note that some patients have micronuclei control values within the range of those seen in healthy population.[16] This finding has been confirmed by the results of our study (patients 1, 2, 3, 6, 7, 8, and 10). It seems that the loss of lymphocytes is associated with big lesions.

The evaluation of damage immediately after the exposure is incomplete. The reason can be sought in the temporary mitotic delay as well as in the potential transfer of chromosomal instability from the exposed parental to daughter cells.[17] The comparison of all patients in the study brings forward big individual differences since the changes were noted before the exposure (patients 1, 7, and 10). Unable to determine the etiology of such changes, we came to the conclusion that the results obtained after the therapeutic doses of radiopharmaceuticals should be evaluated with respect to the results obtained before the exposure.

If we want to compare our results with the similar study of Gutierrez and coworkers,[2] it is evident that in their group of subjects there is a significant increase of 5% of MN per week, compared to pre-treatment, and after that MN is lower than in the pre-treatment period. The rate of increase was essentially lower than in our results. In the study by Gutierrez *et al.*, a time change was significant, but one could not observe the interaction between time and dose. *Log* dose and group, together, were significant.

Depending on age, our results point out an increase in MN by 20% for ten years.

Radiotherapy treatments are very toxic for lymphocytes, and could result in multilocus mutagenesis which could affect cloning efficiency of „hit" cells. Seifert *et al.*[11] calculated that each lymphocyte of a radiotherapy treated patient carried an average of six induced mutations. This group also observed a large individual variation in the reaction exposure. One of the causes of interindividual variation could be the polymorphism at the large number of loci involved in repair of DNA damage.[18] Another one is individual body size, which influences the dose to the target tissues. Finally, the differences in each individual's physical or chemical environment may be involved in the heterogeneity of response.

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