Micronucleus Yield and Colorimetric Test as Indicators of Damage in Patients' Lymphocytes After ^{131}I Therapy

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To estimate the absorbed dose received by patients who underwent ^{131}I therapy, a modified compartmental model of the International Commission on Radiological Protection (ICRP) was used. The activity in plasma and micronucleus (MN) frequency (MN test) were measured before and after therapy. To evaluate whether a correlation exists between lymphocytes and absorbed dose, a colorimetric test, based on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT test), was used. **Methods:** Twenty patients who underwent ^{131}I therapy were studied. Activity was measured in plasma, and isolated lymphocytes were collected to perform the MN and MTT tests. **Results:** The mean MN frequency observed in unexposed patient lymphocytes was comparable with that of healthy subjects. ^{131}I therapy induces a small increase in MN, and a good correlation with the bone marrow absorbed dose was obtained ($P = 0.040$). A consistent decrease in phytostimulation observed after therapy (MTT test) correlated significantly with bone marrow absorbed dose ($P = 0.0085$). **Conclusion:** The MTT test appears to be more reliable than the MN test for evaluating lymphocyte damage induced by ^{131}I therapy.

Key Words: ^{131}I therapy; micronucleus test; colorimetric test; lymphocytes

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The lymphocytes of patients undergoing ^{131}I therapy could provide an interesting tool to assess individual radiosensitivity. The micronucleus (MN) frequency test (MN test) with the cytokinesis-block method is a well-established cytogenetic technique for the evaluation of lymphocyte damage. This assay has been used in in vitro studies to assess the variability of individual response to radioiodine (*1*) and to estimate the biologic dose received by patients after undergoing ^{131}I therapy (*2*). A colorimetric assay, based on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT test), is suitable for determining cell viability. It has been applied on lymphoblastoid cell lines to evaluate their radiosensitivity, but little information exists concerning its application to human short-term lymphocyte cultures.

The purposes of our study on lymphocytes of patients undergoing ^{131}I therapy were to compare the MN and MTT tests for measuring cellular damage induced by the therapy and to correlate MN and MTT measures with the bone marrow absorbed dose.

MATERIALS AND METHODS

We studied 20 patients who underwent ^{131}I therapy for differentiated thyroid carcinoma (n = 19) and for Plummer's disease (n = 1). ^{131}I was administered in a single dose: 796 MBq for Plummer's disease and 1998–5439 MBq for differentiated thyroid carcinoma.

Blood samples were collected before ^{131}I therapy (baseline) and 3–7 and 20–40 d after therapy. ^{131}I activity was measured. Lymphocytes were then separated by a gradient sedimentation method and submitted to in vitro treatment with phytohemagglutinin (PHA) to enter in the cell cycle (phytostimulation).

Absorbed Dose Evaluation

Plasma activity was measured with the physical ^{131}I decay taken into account. The radiation dose to lymphocytes could be equal to the average whole-body dose or to the bone marrow dose.

The radiation dose from radioiodine administration to drawing the blood sample has been calculated for 2 target organs, whole body and red bone marrow, using the MIRD method (*3*). Three source organs were considered: thyroid, rest of the body, and urinary bladder content.

The integrated activities (time integral of the activities) in the source organs are calculated using a modified International Commission on Radiological Protection (ICRP) model (*4*). Appropriate values for transfer and elimination constants to fit the model of considered pathology are used. These individual parameters were obtained for each patient by repeated trials using a computer code. Parameters that gave results most consistent with measured concentrations of radioiodine in plasma were chosen. For urinary bladder

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as the source organ, the filling–emptying model of Cloutier et al. (5) was used.

MN Test

We used the cytokinesis-block method for the MN test (6). Briefly, the lymphocytes were cultivated in RPMI medium 1640 (Dutch modification) containing 75 IU penicillin and 75 μg/mL streptomycin (Life Technologies Ltd., Paisley, Scotland), 20% fetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel), and 2% PHA HA15 (Murex Biotech Ltd., Dartford, England). One-milliliter aliquots (7×10^5 cells) of the lymphocyte suspension in culture cell tubes were placed in an incubator at 37°C (5% CO_2). Forty-eight hours after the beginning of culture, cytochalasin-B (Sigma, St. Louis, MO) was added at a final concentration of 3 μg/mL. Twenty-four hours later, slides were prepared using Cytospin 3 (Shandon, Astmoor, England), fixed in methanol (10 min), and stained in 5% Giemsa solution (15 min). Binucleated cells were scored at 400× magnification, and the MN frequency was evaluated as the ratio of the total number of MN to the binucleated cells scored.

MTT Test

Lymphocytes were cultivated using the same techniques as those used for the MN test. After 75 h of phytostimulation, 100 μL MTT solution (4 mg/mL in phosphate-buffered saline) were added to the cell suspension, which was then incubated for an additional 20 h. The formazan pellet was solubilized with 1 mL dimethyl sulfoxide, and the cell tubes were placed in a thermostatic bath at 40°C for 5 min. Optical absorbance (ABS) was measured at a 560-nm wavelength using a spectrophotometer (ULTROSPEC 3000; Pharmacia Biotech, Cambridge, England). The stimulation with PHA induces the lymphocytes to metabolize a greater quantity of MTT (ABS increases). The ABS increase induced by PHA treatment is measured as ΔABS = (ABS + PHA) − (ABS − PHA). To measure the change in lymphocyte phytostimulation activity after ^{131}I therapy, we used the ratio between the response to PHA before and after radiometabolic treatment (ABS ratio).

RESULTS

An MN frequency of 0.031 ± 0.018 (mean ± SD) from 19 unexposed patient lymphocytes (baseline) was obtained. This value is comparable with that observed in 177 healthy subjects (MN frequency = 0.027 ± 0.015) (mean ± SD; $P = 0.21$; 2-tailed t test) (7).

To evaluate whether radiometabolic treatment can influence the yield of MN, we measured MN frequency 3–7 d after the beginning of therapy. Comparison between these data and baseline values appears to suggest that ^{131}I therapy induces a small increase in MN frequency (ΔMN = MN frequency after therapy − baseline). The ΔMN frequency versus the ^{131}I administered, the whole-body–absorbed dose, and the bone marrow–absorbed dose was plotted. The regression line in the 3 correlations showed Ps of 0.054, 0.113, and 0.040. Figure 1 shows the relationship between ΔMN frequency and the bone marrow–absorbed dose.

The biologic dose estimated in patients (mean ± SD), using the DOSIME program (7), was 0.345 ± 0.179 Gy. Comparison with the mean patient bone marrow dose estimated with the compartmental model (0.239 ± 0.068 Gy) shows a P of 0.035 (paired, 2-tailed t test). Therefore, the biologic dose estimated with MN should be considered with caution because the high variability of baseline values could modify the biodosimetric evaluation.

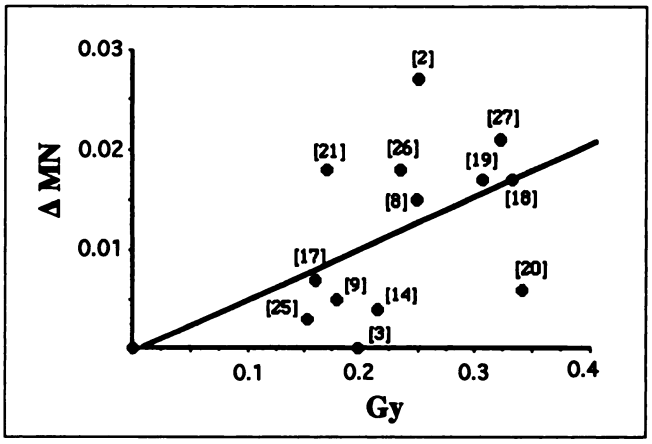

FIGURE 1. ΔMN frequency plotted vs. dose absorbed by bone marrow. [ ] = patient number.

To evaluate the capability of lymphocytes to respond to the mitogenic PHA stimulus (phytostimulation) after ^{131}I therapy, the MTT test was used. A consistent decrease in lymphocyte response to phytostimulation was observed. Figure 2 shows the decrease correlated with the bone marrow–absorbed dose: A significant correlation was found. The linear regression slope with a value of −2.85 ± 0.82 (mean ± SE) and the good statistical significance (P = 0.0085) of regression indicate a strong biologic signal and the higher sensitivity of this method, respectively.

The MN and MTT data obtained 20–40 d after therapy were comparable with the baseline values.

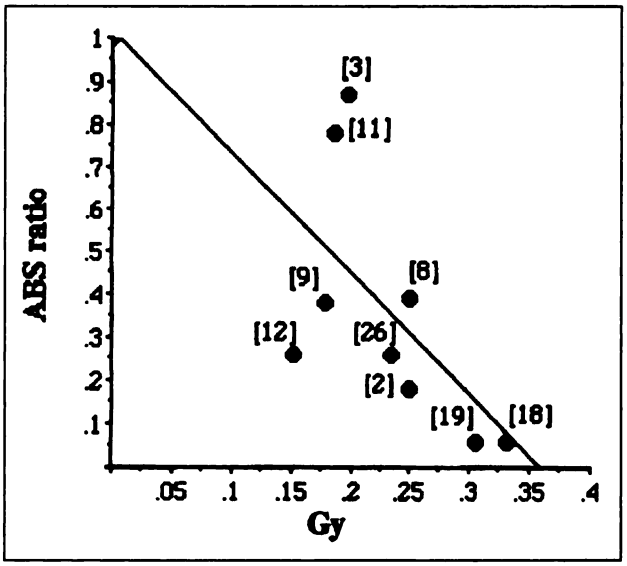

FIGURE 2. ABS ratio as function of dose absorbed by bone marrow after ^{131}I therapy. [ ] = patient number.

DISCUSSION

To estimate each patient's absorbed dose, radioactivity can be measured in plasma drawn at different times after ^{131}I treatment. Our modified ICRP compartmental model shows that evaluation of the absorbed dose by bone marrow is possible and that the findings are correlated with the damage observed in lymphocytes.

The cytogenetic assay, applied to each patient's lymphocytes, is used to measure the biologic dose after ^{131}I therapy (*2*) and to determine the individual radiosensitivity (*7*). The moderate increase observed in individual cytogenetic responses related to the absorbed dose was used to evaluate the biologic dose but was not suitable to discriminate individual variability of response to ^{131}I therapy.

To investigate more sensitive biologic assays we used the colorimetric MTT test. The ABS ratio, determined by the MTT test, is proposed as a better measurement assay than the MN frequency to evaluate the individual response to ^{131}I therapy and may be a good candidate to evaluate the usefulness of continuing ^{131}I treatment in patients who need to repeat various courses of therapy.

These investigations should be considered preliminary because of the small patient population and the lack of an MTT calibration curve. In future studies, a dose–effect curve will be produced, which is related to the degree of lymphocyte phytostimulation after in vitro radiography and exposure to different ^{131}I doses. Comparison of these data with those obtained after ^{131}I therapy in vivo may be useful to better understand the quality of the differences between individuals.

CONCLUSION

The MN and MTT tests appeared to offer good biologic methods to measure the damage induced in lymphocytes after ^{131}I therapy. The MTT test is more sensitive and easier to perform than the MN test to evaluate the individual lymphocyte stress associated with therapy.

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