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Cytogenetic alterations and oxidative stress in thyroid cancer patients after iodine-131 therapy

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This study aimed to assess two end-points of DNA damage, namely chromosomal aberrations and micronuclei in peripheral lymphocytes, and their possible relationship with oxidative stress (which may be related to DNA damage and repair) in thyroid cancer patients receiving therapeutic doses of ^{131}I. Nineteen patients receiving 2590 MBq (70 mCi) were studied. Chromosomal aberrations were scored using standard cytogenetic methods and micronuclei scored in cytokinesis-blocked lymphocytes. Oxidative stress was assessed by determining thiobarbituric acid-reactive substances in blood, total plasma antioxidant status and serum uric acid levels. All parameters were assessed before treatment and 1 and 6 months after ^{131}I administration. The frequency of micronucleated cells per 1000 binucleated cells scored (mean ± SEM) increased significantly from 5.21 ± 0.80 to 9.68 ± 1.22 1 month after treatment ($P < 0.01$) and to 8.42 ± 1.28 6 months after treatment ($P < 0.05$). The frequency of cells with chromosomal aberrations, excluding gaps, per 100 cells, increased significantly from 1.68 ± 0.41 to 3.47 ± 0.55 1 month after treatment ($P < 0.01$) and to 4.05 ± 0.46 6 months after treatment ($P < 0.01$). Oxidative stress parameters showed slight modifications over the time period studied, but the differences were not significant except for a decrease in thiobarbituric acid-reactive products 6 months after therapy ($P < 0.05$) and in serum uric acid concentration 1 and 6 months after therapy ($P < 0.01$). This report demonstrates slight but significant and persistent DNA damage in ^{131}I-treated patients as assessed by cytogenetic assays. There was no clear correlation between the cytogenetic findings and oxidative stress parameters studied.

Introduction

The β/γ-ray-emitting radionuclide iodine-131 (^{131}I) with a half-life of 8 days accumulates in thyroid tissue (Slater, 1990) and is, therefore, widely used for the post-thyroidectomy treatment of thyroid cancer (Gutiérrez *et al.*, 1995; Watanabe *et al.*, 1998) yielding high 10-year survival rates of ~90% (Ureles *et al.*, 1993). Usual ^{131}I doses for the treatment of thyroid cancer patients range from 1110 (30 mCi) to 3700 MBq (100 mCi) (Schlumberger, 1998).

The therapeutic use of ^{131}I, as that of any ionizing radiation, may cause genetic damage to the patients (Hall, 1994). Cytogenetic alterations in ^{131}I-treated patients have been detected by assaying chromosomal aberrations (CAs) (Baugnet-Mahieu *et al.*, 1994; Gundy *et al.*, 1996) and micronuclei (MN) (Livingston *et al.*, 1993; Gutiérrez *et al.*, 1995; Watanabe *et al.*, 1998) and using MN assays associated with fluorescence *in situ* hybridization (FISH) (Ramírez *et al.*, 1997, 1999a). Recently, Gutiérrez *et al.* (1998) applied the Comet assay to the study of ^{131}I-treated patients and found a slight increase in DNA damage after treatment.

Oxidative stress may also be involved in the DNA damage and repair caused by exposure to radiation or other genotoxic agents (Halliwell and Gutteridge, 1995). Oxidative stress is the result of an impairment of the delicate balance between reactive oxygen species (ROS) generation and antioxidant defence systems. It is therefore important in thyroid cancer patients treated with ^{131}I to study both systems and associate them with cytogenetic findings. ROS can interact with specific cellular targets, namely lipids and DNA, forming, among others, aldehydes that bind to thiobarbituric acid. Thiobarbituric acid-reactive substances (prTBA), quantified by the malondialdehyde (MDA) assay, could be important to assess potential DNA damage by ROS.

Concerning the antioxidant defence system, there are several plasma antioxidants, including α-tocopherol, ascorbic acid, uric acid, bilirubin, β-carotene and protein thiols. Each of these certainly has some biological relevance. A measure of the total antioxidant capacity, also known as total antioxidant status (TAS) could be, in this case, of even more importance, because it gives a general perspective on this matter.

This study aimed to assess two end-points of DNA damage, namely chromosomal aberrations and MN in peripheral lymphocytes, and their possible relationship with oxidative stress, which may be related to DNA damage and repair, in thyroid cancer patients receiving therapeutic doses of ^{131}I. Simultaneous assessment of the two cytogenetic indicators was done in order to compare their validity as complementary indicators of DNA damage, since some reports have shown low persistence of MN when compared with chromosomal aberrations, e.g. dicentrics (Gantenberg *et al*., 1991; Ramírez *et al*., 1999b). Oxidative stress was assessed by determining prTBA in blood (Bartoc *et al*., 1993; Konukoglu *et al*., 1998) and plasma TAS (Miller *et al*., 1993). As a natural antioxidant, uric acid in serum was also determined (Ames *et al*., 1981). This work was carried out on 19 patients suffering from thyroid cancer, all treated with the same dose of ^{131}I (2590 MBq) and assayed immediately before treatment and 1 and 6 months afterwards.

The evaluation of the genetic risks from iodine therapy are of interest not only with regard to thyroid cancer patients but also, by way of extrapolation, to those given radioactive iodine

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for diagnostic purposes and to those exposed to an accidental release of radioactive material from nuclear installations.

Materials and methods

Patients

Nineteen patients (16 women and three men) suffering from thyroid cancer were treated at the Nuclear Medicine Department of the Portuguese Oncology Institute of Lisbon with 2590 MBq (70 mCi) of sodium [^{131}I]iodide orally following surgical thyroidectomy. The patient's ages varied between 32 and 67 years (mean 52). Fourteen patients had papillary and five follicular carcinomas. None of the patients had clinical evidence of metastasis at the time of sampling. In addition, none of the patients had been exposed to potentially confounding factors, such as exposure to other ionizing radiations or cytostatica. All patients had expressively agreed to participate in this study. Blood samples were taken from each patient at three sampling times, before the administration of ^{131}I and 1 and 6 months after treatment.

Reagents and culture media

Foetal calf serum, RPMI medium, Ham's F-10 medium, cytochalasin B, L-glutamine, penicillin, streptomycin and phosphotungstic acid were purchased from Sigma (St Louis, MO). Acetic acid, butanol, methanol, Giemsa dye and 2-thiobarbituric acid were obtained from Merck (Darmstadt, Germany). Phytohaemagglutinin (PHA) (HA 15) was purchased from Murex (Dartford, UK) and reconstituted in 5 ml of sterile water. Heparin was obtained from Braun (Melsungen, Germany). Colchicine was purchased from Fluka (Buchs, Switzerland). The Total Antioxidant Status colorimetric assay kit and the TAS control serum were obtained from Randox Laboratories (Ardmore, UK). The uric acid colorimetric assay kit was obtained from Beckman Synchron Cx Systems (Brea, CA).

Cytokinesis-blocked human lymphocyte MN

Aliquots of 0.5 ml of heparinized whole blood were cultured in 4.5 ml Ham's F10 medium supplemented with 24% foetal calf serum, penicillin (100 IU/ml), streptomycin (100 µg/ml), 1% L-glutamine and 1% heparin (50 IU/ml). Lymphocytes were stimulated using 25 µl of PHA and incubated at 37°C. Cytochalasin B was added after 44 h at a final concentration of 6 µg/ml (Fenech and Morley, 1985). After a total of 72 h culture, cells were harvested by centrifugation, treated twice with 5 ml of a mixture (pH 7.2) of RPMI 1640:deionized water 4:1, supplemented with 2% foetal calf serum, for 7 min. The cells were again centrifuged and submitted to a mild hypotonic treatment in a mixture (pH 7.2) of RPMI 1640:deionized water 1:4, supplemented with 2% foetal calf serum, for 5 min. The centrifuged cells were placed on dry slides and smears were performed. After air drying the slides were fixed with freshly prepared and cold methanol:acetic acid (3:1) for 20 min. One day later the slides were stained with 4% Giemsa in 0.01 M phosphate buffer, pH 6.8, for 8 min.

For each patient, 1000 binucleated lymphocytes with well-preserved cytoplasm were scored. MN were identified according to the criteria of Caria *et al.* (1995) using a 500× magnification for detection and a 1250× magnification for confirmation. The cytokinesis-blocked proliferating index (CBPI) was calculated according to the following formula: CBPI = [MI + 2MII + 3(MIII + MIV)]/*N* as proposed by Surrallés *et al.* (1995), where MI–MIV represent the number of human lymphocytes with one to four nuclei and *N* the total number of cells scored.

The comparison of the frequency (‰) of micronucleated cytokinesis-blocked lymphocytes for the three different periods studied was carried out using the paired *t*-test (dependent samples) after verifying non-significant deviation from normality by the Kolmogorov–Smirnov test (with the Lilliefors significance correction).We have also applied a non-parametric test, the Wilcoxon signed ranks test, to compare the results, reaching the same conclusion and the same significance levels. The statistical analysis was performed with SPSS 8.0.

Chromosomal aberrations

Aliquots of 0.5 ml of heparinized whole blood were cultured as described above for 48 h. Colchicine (0.5 µg/ml) was then added and culture was continued for a further 3 h (Swierenga *et al.*, 1991). Cells were harvested by centrifugation, submitted to hypotonic treatment, fixed twice with cold methanol:acetic acid (3:1), placed on slides and stained with Giemsa for 10 min.

One hundred metaphases per patient were scored at 1250× magnification for the different types of aberrations according to the criteria described by Rueff *et al.* (1993). Multi-aberrant cells, defined as cells containing >10 chromosomal aberrations, were included in the scores of the percentage of aberrant cells. The mitotic index (MI) was estimated by counting 2000 cells.

The comparison of the frequency (%) of aberrant cells excluding gaps (CAEG) for the three different periods studied was carried out using a non-parametric test, the Wilcoxon signed ranks test, after verifying significant deviation from normality by the Kolmogorov–Smirnov test (with the Lilliefors significance correction). The statistical analysis was performed with SPSS 8.0.

Oxidative stress parameters

Uric acid was determined according to Fossati *et al.* (1980). This method is based on the oxidation of uric acid by uricase (240 IU/l) followed by reaction of the hydrogen peroxide formed with 4-aminoantipyrine (0.85 mM) and 3,5-dichloro-2-hydroxybenzene sulfonate (3.4 mM) in the presence of peroxidase (961 IU/l). The absorbance of the coloured product formed was read at 520 nm versus a reagent blank in a Beckman Synchron CX Systems spectrophotometer.

TAS was determined following the manufacturer's instructions (Randox Laboratories Ltd) based on the technique described by Miller *et al.* (1993). The principle assay consists of the reaction of 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (0.61 mM) with metmyoglobin (6.1 µM) and hydrogen peroxide (0.25 mM) in order to produce the corresponding radical cation, which has a relatively stable blue-green colour at 600 nm. The absorbance was determined in a Pye Unicam spectrophotometer maintained with a thermostat at 37°C. All samples were analysed in duplicate with blank and standard control serum being assayed for each set of determinations.

prTBA in blood were determined as described by Yagi (1976). Briefly, the principle of this method, which uses 50 µl of whole blood, is isolation of the lipids and serum proteins by precipitation with 10% (w/v) phosphotungstic acid followed by the thiobarbituric acid (TBA) reaction. The TBA reagent consists of a mixture of 0.67% aqueous TBA solution and glacial acetic acid 1:1 (v/v), which was added to the formed pellet and heated for 1 h at 95°C. After this reaction, *n*-butanol was used to extract the prTBA. The *n*-butanol layer was employed for fluorimetric measurements using a Hitachi F-2000 fluorospectrometer at wavelengths of 515 nm for excitation and 553 nm for emission. Standard solutions were made from 1,1,3,3-tetraethoxypropane. All samples were analysed in triplicate.

The comparison of all the oxidative stress parameters for the three different periods studied was carried out using the paired *t*-test (dependent samples) after verifying non-significant deviation from normality by the Kolmogorov–Smirnov test (with the Lilliefors significance correction). The statistical analysis was performed with SPSS 8.0.

Results

Table I presents the results of the MNCB assay in the 19 patients studied before and 1 and 6 months after ^{131}I therapy. Table I also includes data on the histological type of the tumor (follicular or papillary carcinoma) as well as on the gender and age of the patient. The mean values (± SEM) from all patients for each parameter are also shown in Table I. The yield of micronucleated cytokinesis-blocked human lymphocytes in the pooled data from all patients, considering the number of micronucleated cells per 1000 binucleated cells scored, increased significantly from 5.21 ± 0.80 to 9.68 ± 1.22 1 month after treatment ($P < 0.01$) and to 8.42 ± 1.28 6 months after treatment ($P < 0.05$). The binucleated index and the CBPI did not change markedly over the period studied.

Table II displays the individual aberrations scored as well as the total frequencies (%) of cells with CAs, including (CAIG) and excluding (CAEG) gaps, and the MI. With regard to the pooled data from all patients, the yield of CAEG (%) increased significantly from 1.68 ± 0.41 to 3.47 ± 0.55 1 month after treatment ($P < 0.01$) and to 4.05 ± 0.46 6 months after treatment ($P < 0.01$). A significant increase in the frequency of dicentric chromosomes (%) was also observed from 0.16 ± 0.09 to 0.58 ± 0.18 1 month after treatment ($P < 0.05$) and to 1.05 ± 0.39 6 months after treatment ($P < 0.05$), however, the slight increase in the frequency of CAEG and dicentric chromosomes from 1 to 6 months after treatment was not statistically significant ($P = 0.50$ and $P = 0.47$, respectively). It should be noted that the background rate of dicentric chromosomes before treatment with ^{131}I is higher than that reported in the literature for

Table I. Induction of MN in peripheral blood lymphocytes of 19 thyroid cancer patients immediately before and after ^{131}I treatment

Patient	Histology	Age	Before treatment (0 mCi)					Post-treatment 70 mCi (1 month)					Post-treatment 70 mCi (6 months)				
			BN (%)	Poli (%)	Met (%)	CBPI (%)	MNCB (‰)	BN (%)	Poli (%)	Met (%)	CBPI (%)	MNCB (‰)	BN (%)	Poli (%)	Met (%)	CBPI (%)	MNCB (‰)
D1	P	35	57.0	15.5	2.1	1.88	1	54.4	19.9	3.2	1.91	7	51.4	4.1	1.5	1.63	8
D2	P	62	51.2	25.2	3.6	1.98	0	50.6	20.1	2.1	1.89	4	52.6	11.7	1.0	1.75	4
D3	P	43	54.4	13.4	2.0	1.79	5	48.0	28.5	3.4	2.02	6	49.7	12.5	1.2	1.73	6
D4	F	36	55.2	17.3	1.4	1.88	6	52.5	27.3	4.2	2.03	4	60.4	13.6	1.0	1.87	3
D5	P	62	48.9	23.0	5.2	1.90	0	47.5	31.0	1.5	2.08	17	57.2	10.1	0.3	1.77	7
D6	P	63	52.2	18.5	1.9	1.87	8	55.0	21.5	6.3	1.92	23	55.0	14.4	1.9	1.82	10
D7	P	32	51.5	19.9	2.6	1.89	6	51.4	20.7	3.9	1.89	13	58.2	8.1	2.9	1.71	2
D8	P	49	53.9	11.2	1.9	1.74	3	52.4	18.4	6.1	1.83	14	62.0	12.5	1.3	1.86	5
D9	F	62	56.4	16.7	1.0	1.89	7	61.1	6.6	0.7	1.74	11	60.5	15.9	1.2	1.91	17
D10	F	55	56.6	9.9	2.0	1.74	11	54.0	22.0	1.9	1.96	4	56.3	11.4	2.2	1.77	8
D11	P	52	54.7	10.5	1.0	1.75	5	67.3	14.4	2.0	1.94	10	51.6	7.7	1.9	1.65	12
D12	P	46	60.9	6.7	0.8	1.73	6	61.0	18.1	1.9	1.95	8	54.8	14.8	2.9	1.81	10
D13	F	67	53.3	2.6	1.9	1.57	12	58.1	5.0	0.9	1.67	11	63.8	10.8	1.6	1.84	7
D14	P	60	57.6	4.9	1.9	1.65	10	58.9	5.8	1.5	1.69	13	51.8	10.6	1.6	1.71	11
D15	P	58	53.7	6.7	1.4	1.66	5	48.3	5.0	1.6	1.57	15	46.9	5.6	2.0	1.56	26
D16	P	61	48.8	2.5	0.7	1.53	5	46.7	4.3	1.5	1.54	9	50.6	7.7	1.3	1.65	7
D17	P	54	59.7	12.6	2.2	1.83	4	50.4	13.7	0.8	1.77	5	55.5	14.3	1.3	1.83	4
D18	F	36	57.2	15.0	1.8	1.85	5	55.6	13.2	1.2	1.81	2	43.6	7.2	1.2	1.57	9
D19	P	49	57.0	4.3	1.9	1.64	0	52.0	4.8	1.0	1.61	8	58.5	10.2	1.0	1.78	4
Mean		51.7	54.75	12.44	1.96	1.78	5.21	53.96	15.80	2.40	1.83	9.68[a]	54.76	10.69	1.54	1.75	8.42[b]
SEM		2.5	7.57	1.56	0.23	0.03	0.80	1.24	2.00	3.86	0.04	1.22	1.22	0.76	0.14	0.02	1.28

P, papillary carcinoma; F, follicular carcinoma; BN, binucleated cells; Poli, polinucleated cells (tri- and tetranucleated cells); Met, metaphase; CBPI, cytokinesis-blocked proliferating index; MNCB, binucleated cells that present MN; SEM, standard error of the mean. 1000 binucleated cells were scored for each patient at each sampling time. All patients received a dose of 70 mCi. All patients were non-smokers except patient D1 and all of them were female except patients D14, D20 and D23.

[a]$P < 0.01$ when compared with before treatment.

[b]$P < 0.05$ when compared with before treatment.

Table II. Induction of CAs in peripheral blood lymphocytes of 19 thyroid cancer patients immediately before and after ^{131}I treatment

Patient	Before treatment (0 mCi)										Post-treatment 70 mCi (1 month)										Post-treatment 70 mCi (6 months)									
	Ctg	Ctb	Int	Chg	Chb	Dic	MA	CAIG (%)	CAEG (%)	MI (%)	Ctg	Ctb	Int	Chg	Chb	Dic	MA	CAIG (%)	CAEG (%)	MI (%)	Ctg	Ctb	Int	Chg	Chb	Dic	MA	CAIG (%)	CAEG (%)	MI (%)
D1	2	2	0	0	0	0	0	3	1	0.80	0	1	0	0	0	2	0	3	3	1.15	2	5	0	0	0	0	0	7	5	0.95
D2	1	0	1	0	0	0	0	2	1	1.65	0	1	0	0	1	0	0	2	2	2.75	1	4	1	0	1	1	0	8	7	2.10
D3	2	0	0	0	0	1	0	3	1	2.60	1	3	0	0	0	0	0	4	3	2.50	0	3	0	0	2	0	0	4	4	1.60
D4	1	1	0	0	0	0	0	2	1	1.95	0	0	0	0	0	0	0	0	0	3.90	0	1	0	0	3	6	0	7	6	1.95
D5	2	0	0	0	1	0	0	3	1	2.05	0	3	1	0	0	0	0	4	4	4.50	1	3	1	0	0	0	0	5	4	3.35
D6	0	3	0	0	0	0	2	5	5	2.85	0	1	0	0	0	1	0	2	2	2.10	1	2	0	0	0	0	0	3	2	1.10
D7	0	3	0	0	0	0	0	3	3	2.35	0	5	0	0	1	1	1	5	5	1.85	1	2	0	0	0	0	0	3	2	0.60
D8	0	1	0	0	0	0	0	1	1	1.55	1	1	0	0	0	2	0	4	3	1.30	0	1	0	0	1	0	0	2	2	0.45
D9	2	3	0	0	2	0	0	7	5	1.95	2	7	1	0	1	2	0	12	10	1.30	6	4	0	0	0	3	0	12	7	2.60
D10	0	1	0	0	1	1	0	3	3	1.60	1	5	0	0	0	1	0	7	6	1.40	1	4	0	0	0	1	0	4	3	2.75
D11	0	0	0	0	1	0	0	1	1	0.90	0	0	0	0	1	0	0	1	1	1.55	3	2	0	0	0	1	0	6	3	1.55
D12	0	3	0	0	0	0	0	2	2	0.45	0	2	0	0	0	0	0	2	2	1.15	1	2	0	0	0	1	0	4	3	1.40
D13	1	1	0	0	0	0	0	2	1	0.70	2	3	0	0	2	1	0	8	6	0.70	1	0	0	0	1	3	0	4	4	1.50
D14	0	0	0	0	0	0	0	0	0	0.80	0	2	0	0	0	0	0	2	2	0.50	1	2	0	0	1	0	0	4	3	1.55
D15	1	1	1	0	0	0	0	3	2	0.55	2	2	0	0	1	0	0	5	3	0.40	1	2	0	0	2	4	0	6	5	1.60
D16	1	0	0	0	0	0	0	1	0	0.65	0	1	0	0	1	0	0	2	2	1.10	3	3	0	0	1	0	0	6	4	1.40
D17	1	0	0	0	0	1	0	2	1	1.15	3	5	0	0	1	1	0	10	7	1.75	2	1	0	0	0	0	0	3	1	5.05
D18	1	2	0	0	1	0	0	4	3	0.90	2	2	0	0	1	0	0	5	3	1.00	2	5	0	1	0	0	0	7	5	1.65
D19	1	0	0	0	0	0	0	1	0	0.55	0	1	0	0	1	0	0	2	2	0.95	1	6	0	0	1	0	0	8	7	1.30
Mean	0.84	1.11	0.11	0.00	0.32	0.16	0.11	2.53	1.68	1.37	0.74	2.37	0.11	0.00	0.58	0.58[a]	0.05	4.21	3.47[b]	1.68	1.47	2.74	0.11	0.05	0.68	1.05[a]	0.00	5.42	4.05[b]	1.81
SEM	0.18	0.27	0.07	0.00	0.13	0.09	0.10	0.37	0.41	0.17	0.23	0.44	0.07	0.00	0.16	0.18	0.05	0.71	0.55	0.25	0.32	0.36	0.07	0.05	0.20	0.39	0.00	0.55	0.46	0.24

Ctg, chromatid gap; Ctb, chromatid break; Int, tetraradial; Chg, chromosome gap; Chb, chromosome break; Dic, dicentric; MA, multi-aberrant cell; CAIG, aberrant cells including gaps; CAEG, aberrant cells excluding gaps; MI, mitotic index; SEM, standard error of the mean. 100 metaphases were scored for each patient at each sampling time. 2000 cells were scored for each mitotic index.

[a]$P < 0.05$ when compared with before treatment.

[b]$P < 0.01$ when compared with before treatment.

Table III. Uric acid, TAS and prTBA, expressed in terms of MDA concentration, in the 19 thyroid cancer patients before and after ^{131}I therapy

Patient	Uric acid (mg/100ml)			TAS (mmol/l)			MDA (μmol/l)		
	Before treatment	Post-treatment 1 month	Post-treatment 6 months	Before treatment	Post-treatment 1 month	Post-treatment 6 months	Before treatment	Post-treatment 1 month	Post-treatment 6 months
D1	–	4.0	4.1	–	1.11	1.71	0.08	0.11	0.03
D2	–	6.3	7.1	1.46	1.36	1.34	0.10	0.11	0.09
D3	4.8	4.5	4.1	1.09	1.04	0.98	0.14	0.19	0.30
D4	–	5.4	4.3	1.55	1.27	1.94	0.09	0.20	0.13
D5	–	4.2	5.4	1.23	1.00	1.53	0.10	0.15	0.12
D6	7.3	4.4	5.7	1.45	1.04	1.90	0.01	0.16	0.06
D7	4.5	3.8	2.8	0.92	0.82	1.27	0.13	0.10	0.05
D8	6.9	7.0	6.7	1.78	1.84	2.87	0.17	0.15	0.08
D9	6.8	4.3	4.3	1.80	1.52	1.39	0.18	0.11	0.10
D10	4.1	4.6	4.4	2.02	0.94	0.88	0.19	0.11	0.11
D11	5.2	4.4	4.2	1.90	1.15	1.33	0.09	0.13	0.10
D12	6.7	6.4	5.9	2.12	1.57	1.39	0.08	0.10	0.08
D13	7.2	5.5	7.5	1.42	1.85	1.28	0.12	0.22	0.11
D14	6.2	3.5	3.9	1.44	1.72	1.17	0.15	0.06	0.05
D15	–	3.1	3.6	0.57	0.46	0.54	0.20	0.19	0.13
D16	–	8.2	11.0	1.63	1.55	1.58	0.17	0.19	0.12
D17	–	3.6	2.8	1.58	–	0.81	0.13	0.12	0.06
D18	4.1	3.4	2.3	0.93	0.89	0.79	0.13	0.07	0.07
D19	5.9	5.3	4.7	1.77	1.92	1.26	0.15	0.09	0.08
Mean	5.8	4.8[a]	5.0[a]	1.48	1.28	1.37	0.13	0.14	0.10[b,c]
SEM	0.3	0.3	0.6	0.10	0.10	0.12	0.01	0.01	0.01

–, not performed; SEM, standard error of the mean.
[a]$P < 0.01$ when compared with before treatment.
[b]$P < 0.05$ when compared with before treatment.
[c]$P < 0.01$ when compared with 1 month after treatment.

normal subjects (Bauchinger, 1995). The MI did not change significantly over the periods analysed.

With regard to the oxidative stress parameters (Table III), uric acid concentration (mean ± SEM) in the pooled data from all patients decreased significantly from 5.8 ± 0.3 to 4.8 ± 0.3 mg/100 ml ($P < 0.01$) 1 month after treatment and to 5.0 ± 0.6 mg/100 ml 6 months after therapy ($P < 0.01$). TAS in the plasma decreased slightly, but not significantly, from 1.48 ± 0.10 to 1.28 ± 0.10 1 month after treatment ($P = 0.06$) and to 1.37 ± 0.12 6 months after treatment ($P = 0.30$). The prTBA, expressed as MDA concentration (μmol/l), displayed a slight, non-significant ($P = 0.61$) increase from 0.13 ± 0.01 to 0.14 ± 0.01 1 month after treatment and a slight decrease to 0.10 ± 0.01 6 months after treatment. The decrease in MDA observed at 6 months after treatment was significant when compared with values before treatment ($P < 0.05$) and at 1 month after treatment ($P < 0.01$).

Statistical analysis demonstrates that the different cytogenetic and biochemical parameters do not correlate in individual patients.

Discussion

In this study all 19 thyroid cancer patients received the same therapeutic dose of ^{131}I, 2590 MBq (70 mCi), and thus, in principle, should have been exposed to the same genotoxic burden. However, differences in the type of tumour and its ability to accumulate iodine, as well as the age and gender of the patients, might modify individual responses. Due to the small number of patients suffering from follicular carcinoma, it was impossible to separate the experimental group according to the histological type of carcinoma, but an inspection of the values in Tables I and II does not suggest important differences between the two types of cancer. With respect to gender, there are too few males in the sample to make a valid comparison, but again no difference stands out on inspection of the data. The age of the patients varied between 32 and 67 years and an increase in the frequency of certain cytogenetic end-points with age, especially of MNCB, has been observed by some authors (Yager, 1990; Fenech and Rinaldi, 1994) whereas others (reviewed in Bauchinger, 1995) reported conflicting results with regard to the influence of age on dicentric chromosomes. Ramírez *et al.* (1997) separated their results of cytogenetic assays in ^{131}I-treated thyroid cancer patients according to age and found a 2.3-fold increase in the spontaneous MN frequency in the older group. They also observed differences between both groups, not in the induction of MN due to the treatment, but in the content of those MN, suggesting that the aneugenic effects of ^{131}I are much more important in elderly patients. Separation of our results into groups of younger and older than 50 years suggested slight, but non-significant differences in MNCB and CAEG. Consequently, we pooled the results from all patients for statistical analysis.

Our data confirm that treatment with ^{131}I for thyroid cancer can cause genetic damage in circulating lymphocytes with a significant increase in the frequency of MNCB (~1.9-fold), CAEG (~2.0-fold) and dicentric chromosomes (3.0-fold). At 6 months after therapy, the results of these tests seem to diverge: CAEG/dicentric chromosomes increased further and MNCB decreased (Tables I and II). It is not clear whether the decrease in MNCB is a result of a shorter lifespan of such aberrations compared with dicentrics or of statistical variations. It should also be pointed out that a substantial part of the increase in dicentrics at 6 months after ^{131}I administration was due to patient D4, who had several cells with multiple aberrations, and that the increase was not significant if this

patient was eliminated from the analysis. There is no correlation between these parameters in individual patients, because whole body doses and the responses are small, resulting in considerable variability. In general, however, CAs and MN are known to correlate well in short-term and *in vitro* studies under circumstances when genotoxic damage is substantial. An attempt was made to calculate whole body doses, i.e. the genotoxic damage, from the data using *in vitro* dose–effect relationships. As has been mentioned by Baugnet-Mahieu *et al.* (1994), the exposure is delivered at a low dose rate so that only the linear coefficient α of the dose–effect curve $y = B$ (background) + αD + βD^2 needs to be considered. The parameters of the mentioned curve for the *in vitro* γ-irradiation of human lymphocytes have been published. Using values of $\alpha = 0.03$ Gy^{-1} for dicentrics (Baugnet-Mahieu *et al.*, 1994) and of $\alpha = 0.06$ Gy^{-1} for MN (Hung *et al.*, 1995), such calculations done for the individual patients as well as for the group at the different times of treatment yields whole body doses of the order of 200–300 mGy from the dicentric data and ~200 mGy from the MN data. This is less than expected by dosimetric calculations. In fact, published values for whole body doses in patients treated with radioiodine were estimated at 0.181 mGy/MBq (6.7 mGy/mCi) (reviewed in Norton *et al.*, 1989), which gives a value of ~400 mGy.

The results of the present study on MN agree with those of Livingstone *et al.* (1993), who observed a 6-fold increase in MN counts 11 days after therapy with 1780 MBq persisting for 9 months, and with those of Ramírez *et al.* (1997), who, using the MN assay associated with the FISH technique, found a 2.3-fold increase in MN counts 1 week after treatment with doses ranging from 3700 to 5500 MBq. Conversely, Gutiérrez *et al.* (1995) could not find a significant difference compared with the control group for MN in thyroid cancer patients 1 and 5 years after ^{131}I treatment. These patients received different doses and numbers of ^{131}I treatments and the total dose administered ranged from 3400 to 37500 MBq. It is conceivable that MN had already been eliminated after the longer periods of time.

With regard to CAs, Baugnet-Mahieu *et al.* (1994) reported a small, but significant increase in CAs over a period of ~10 days after treatment with 3700 MBq. Also, Gundy *et al.* (1996) found that CAs were increased in patients subject to ^{131}I therapy (1734–2600 MBq). M'Kacher *et al.* (1997), using either the conventional CA assay or painting of chromosome 4, reported persistent biological damage for up to 2 years after therapeutic exposure.

In this report we also studied three different parameters that could be representative of the potential oxidative stress to which the patients may have been exposed. Whereas MDA indicates the presence of oxidative stress, TAS and uric acid content would reflect the defence mechanisms against such stress. It should be pointed that many factors could intervene in such assessments, especially in tumour patients, e.g. uric acid also reflecting nucleic acid breakdown. We found a small initial increase (~1.1-fold at 1 month) in MDA followed by a significant decrease (1.4-fold at 6 months). Bartoc *et al.* (1993) and Konukoglu *et al.* (1998) found an increase in MDA very shortly after iodine treatment. Regarding the defence mechanisms, uric acid and TAS mildly decreased (both ~1.2-fold), although only the uric acid decrease was significant. Bartoc *et al.* (1993) found a significant decrease in total plasma antioxidative capacity 1 week after ^{131}I treatment. Our sampling was much later than that of the other investigators and we may have missed the maximal period of oxidative stress and, with regard to TAS, our samples may already have entered into a period of recovery.

In conclusion, this report points to a slight but significant and persistent increase in DNA damage as assessed by both the CA and MNCB assays. Both assays are undoubtedly useful to evaluate the genotoxic effects of treatment of thyroid cancer with ^{131}I. The MNCB assay is less time consuming, but the results obtained at 6 months after therapy suggest that for longer periods of time after ^{131}I therapy the increase in the frequency of micronucleated cells seem to be less evident in comparison with the increase in CAs. Concerning the oxidative stress parameters studied, it is clear, in an overall view, that these biomarkers are not sensitive for the evaluation of the deleterious effects of this therapy when performed long after the treatment.

Finally, in agreement with the observations of other authors, it appears that the genotoxic dose to the whole body from such treatments is rather small, i.e. of the order of several hundred mGy, and that, consequently, such determinations would not be helpful for assessing the dose due to radioactive iodine from a nuclear release.

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