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Impaired DNA-Repair Synthesis in Lymphocytes of Breast Cancer Patients

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Abstract—*UV-C light induced DNA-repair synthesis was studied in unstimulated lymphocytes of 41 female patients (aged 33–83 years) with invasive breast cancer and of 27 healthy women (aged 37–68 years). As a parameter for the determination of the DNA-repair synthesis the incorporatioin of [^{3}H] thymidine was taken in the presence of 2 mM hydroxyurea (HU). The [^{3}H] thymidine incorporation levels were reduced in 20 of the 41 patients and in only 3 of the 27 controls. This difference between the two groups was significant. An impaired DNA-repair synthesis might be involved in the etiology of breast cancer in some patients.*

INTRODUCTION

DNA DAMAGE caused by UV light can be corrected by enzymatic repair. A defective DNA-repair capacity was first found by Cleaver [1] in fibroblasts from xeroderma pigmentosum patients. Patients with this disease often develop skin or eye cancer [2]. Other diseases have been found in which a defect in the DNA-repair system associated with a higher incidence of cancer [2–6]. Recently Pero *et al.* [7] reported that the level of *N*-acetoxy-*N*-2-fluoroenyl-acetamide induced unscheduled DNA synthesis (UDS) is decreased in mononuclear leukocytes of individuals with colorectal cancer.

In this study we have attempted to investigate whether an impairment in DNA-repair synthesis exists in patients with breast cancer.

MATERIALS AND METHODS

Persons investigated

Patients. The 41 patients with a histological diagnosis of invasive breast cancer were divided into three groups. In the first group 16 patients were included (12 were aged between 33 and 68 yr and four between 70 and 83 yr). Blood sampling was carried out shortly before the operation. One person (BL) had a high diastolic blood pressure. The 25 other patients were examined after treatment in a clinically disease-free stage: 14 of these women (second group, aged between 34 and 70 yr) had been treated only by surgery 9 months to 29 years before blood sampling. Eleven patients (third group, aged between 44 and 79 yr) had been treated by surgery and by chemo/radiotherapy. Blood samples were taken 1–14 yr after the end of therapy. At the time of the study two patients (GE and GV) were successfully treated for high blood pressure.

Controls. Blood from 27 healthy women (aged between 37 and 68 yr) was obtained from the Basel Blood Centre. Three women were being treated for high blood pressure, but they had a normal diastolic blood pressure at the time of sampling.

Measurement of the DNA-repair synthesis

UV-C induced DNA-repair synthesis was measured as described earlier [8]. The lymphocytes from fresh blood samples (20 ml) were separated and irradiated with doses of 2, 4, 8 and 16 J/m^2 in the presence of 2 mM HU (9,10]. The cells were then incubated with 10 μCi/2 × 10^6 cells methyl-[^{3}H]thymidine (Amersham, specific activity 25 Ci/mmol). The incorporation of labeled thymidine after 2 hr was taken as the parameter for DNA-repair synthesis. The radioactivity was measured in a Packard scintillation spectrometer.

The degree of DNA-repair synthesis was calculated as the difference between the amounts of incorporated ([^{3}H]thymidine (in the presence of

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HU) in the irradiated and unirradiated cells. It was expressed as counts/min (cpm) incorporated into 10^6 cells.

Statistical analysis

Linear regression analysis was performed using y (cpm/10^6 cells) as the dependent variable and x (log dose) as the independent variable. The 99% confidence range of regression was determined [11]. For comparison with the values of spontaneous and HU inhibited DNA synthesis in unirradiated cells the Wilcoxon U-test was used. For the evaluation of DNA-repair synthesis in irradiated cells the Fisher exact 2 × 2 table probability test was employed. The significance analysis was calculated one-tailed at a significance level of 2.5%.

RESULTS

In order to establish the values of incorporated thymidine after UV exposure, we measured the spontaneous and HU inhibited DNA synthesis in the unirradiated cells.

Spontaneous DNA synthesis and its inhibition by hydroxyurea

Table 1 compares the levels of spontaneous DNA synthesis and HU inhibited DNA synthesis in lymphocytes of the patients with invasive breast cancer and the controls. In the first group the values of spontaneous and HU inhibited DNA synthesis were significantly higher than in the controls ($P < 0.05$). Four women had extremely high levels of incorporation (range of values of spontaneous DNA synthesis: 4113–6343 cpm/10^6 cells and range of values of HU inhibited DNA synthesis: 4438–5606 cpm/10^6 cells). Measurements 4 months after surgery showed that in two out of these four patients the high values were reduced by 30–69% (Table 4).

In the second and third groups there were no significant differences in spontaneous and HU inhibited DNA synthesis from the controls.

The 2 mM HU depressed the spontaneous DNA synthesis to a similar extent in all three groups: residual activity of 65–63–66.5% (n.s. in comparison with controls: residual activity 49%).

DNA-repair synthesis. [^{3}H]thymidine incorporation after UV-C irradiation

To determine the degree of repair synthesis the cpm values of HU inhibited, unirradiated samples were subtracted from the cpm values of the irradiated samples. To determine this HU background activity, two HU samples were used independently and the average was taken. We used only a final concentration of 2 mM HU instead of the usual 10 mM, so that the UV-induced DNA-repair was influenced as little as possible [10].

Figure 1 presents each value of the incorporated thymidine for both 41 breast cancer patients and 27 female controls. As we have shown the thymidine incorporation is dose-dependent for 2, 4, 8 and 16 J/m^2 (doses plotted logarithmically). The saturation was not reached, although the result curves became progressively less steep, they did not flatten completely [8]. Comparing Figs. 1A and 1B it can be observed that the values of the patients (1B) are generally lower than the values of the controls (1A).

To establish the individual variation of DNA-repair synthesis in the dose range of 2–16 J/m^2, the regression line for each investigated person was calculated. In order to compare the regression lines

Table 1. The effect of hydroxyurea on spontaneous DNA synthesis in unirradiated unstimulated lymphocytes of breast cancer patients and female controls

Group	Incorporation of [^{3}H]thymidine (cpm/10^6 cells, mean ± S E M)	
	No HU	2 mM HU
First investigation before operation (n = 16)	2825 ± 455*	2046 ± 403*
Second investigation 9 months to 29 years after operative treatment (n = 14)	1744 ± 266	1018 ± 230
Third investigation 1–14 years after operative and chemo/radio-therapeutic treatment (n = 11)	2087 ± 466	1442 ± 398
Controls (n = 27)	1699 ± 138	816 ± 170

HU = hydroxyurea.
* $P < 0.05$ in relation to the controls.

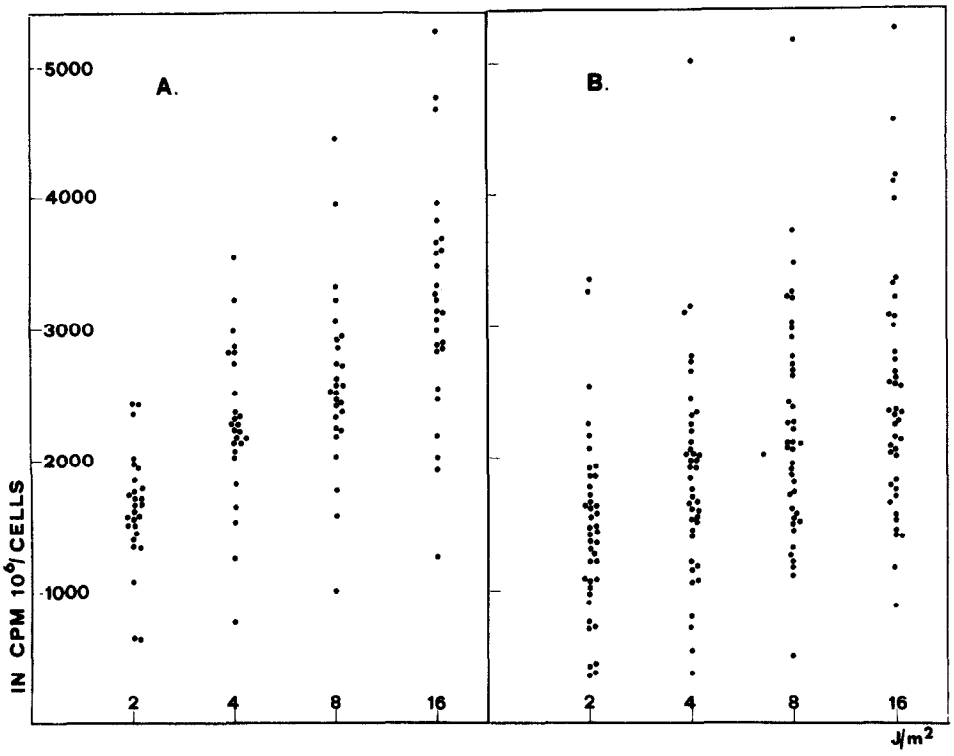

Fig. 1. DNA-repair synthesis in the 27 control women (A) (aged 37–68 years) and in the 41 breast cancer patients (B) (aged 33–83 years). Unstimulated lymphocytes were preincubated for 30 min with 2 mM hydroxyurea, irradiated with various UV doses and subsequently incorporated for 2 hr with 10 μCi 2 × 10^6 cells methyl-[^{3}H]thymidine. Each point represents the value of the incorporated thymidine after subtraction of hydroxyurea values.

of the breast cancer patients with those of the controls, we have taken the following measures: the criterion for a reduced or increased DNA-repair synthesis was if the value of the incorporated thymidine on the individual regression line at 6 J/m^2 (median dose) lay beyond the 99% confidence range of the regression line from the controls. For statistical analysis we used the Fisher exact 2 × 2 table probability test. For justification of 6 J/m^2: the median dose was taken, since the effect of discriminating between patients and controls is expected to be largest at the median dose and the error is lowest in this range. This type of evaluation considers the response to UV exposure within the definite dose range (2–16 J/m^2). Table 3 represents the measured values of the controls and the breast cancer patients with decreased or increased thymidine incorporation. It can be seen that in most cases the values for doses of 4 J/m^2 and above lie on or beyond the 99% confidence limit.

Figure 2 presents the regression lines of the 27 controls (A) and the 41 breast cancer patients (B–D) with a reduced or increased DNA-repair synthesis. The DNA-repair synthesis was reduced in 20 out of 41 breast cancer patients and in three out of the 27 control women. The number of patients having reduced levels was significantly different from the number of controls having reduced levels ($P = 0.3\%$) (see Table 2). An increased DNA-repair synthesis was found in two controls and in two patients.

Among the 16 patients of the first group (Fig. 2B, investigated before the surgical treatment) the DNA-repair synthesis was reduced in eight women: the number of patients and of controls with a reduced repair capacity was significantly different ($P = 0.72\%$).

Among the 14 patients operated for breast cancer (Fig. 2C) a decreased level was found in four women. This is not significantly different either from normal controls ($P = 22.5\%$) or from the first group ($P = 25.2\%$).

In the 11 by surgery and radio/chemotherapy treated breast cancer patients (Fig. 2D) a decreased DNA-repair was found in eight cases. This is significantly different from the controls ($P = 0.07\%$). If the values of this group are estimated at the 95% confidence range instead of 99%, all of them had reduced [^{3}H]thymidine incorporation values.

The clinical data showed that — according to information — about 70% of the patients had first and/or second degree relatives with cancer (12 patients had one or more family members with breast cancer). The tumor stage varied in the first and third group between I and III, in the second

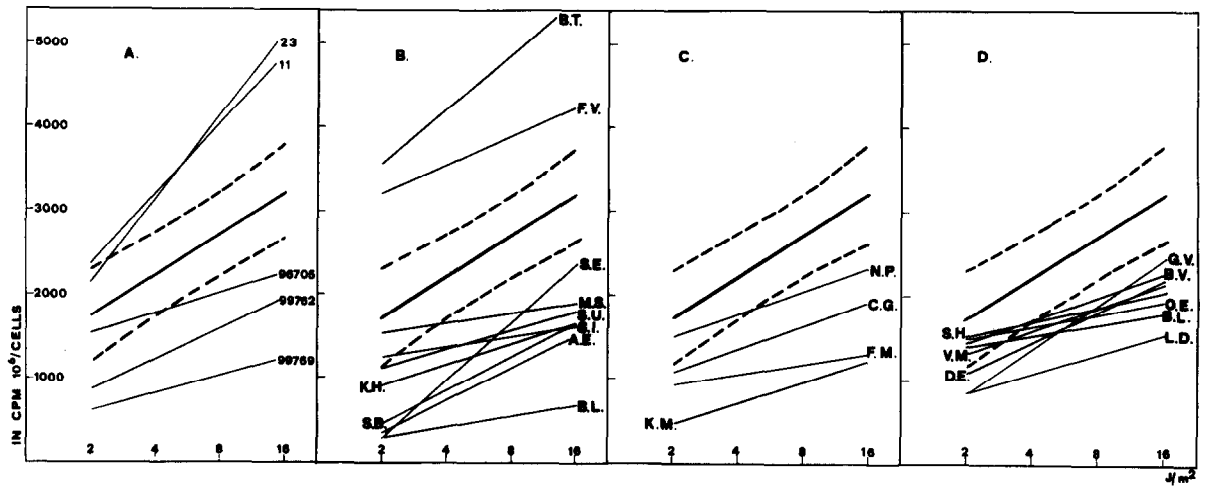

Fig. 2. Reduced or increased DNA-repair synthesis in the 27 control women (A) and in the 41 breast cancer patients (B–D). The solid line connects the regression values for each UV dose (doses plotted logarithmically); the broken lines represent the 99% confidence range of the controls. The regression line for each person was calculated and evaluated according to the controls range. The criterion for a reduced or increased DNA-repair synthesis was that the value of the incorporated [³H]thymidine on the individual regression line at 6 J/m² lay beyond the 99% confidence range. The numbers present the register number of the control women, the letters are the initials of the patients.

Table 2. Reduced DNA-repair synthesis in breast cancer patients and in control women

Group	Decrease of [³H]thymidine incorporation below the 99% confidence limit	
First investigation before operation ($n = 16$)	$n = 8$	($p = 0.72\%$)*
Second investigation 9 months to 29 years after operative treatment ($n = 14$)	$n = 4$	($p = 22.5\%$)
Third investigation 1–14 years after operative and chemo/radio-therapeutic treatment ($n = 11$)	$n = 8$	($p = 0.07\%$)*
Total ($n = 41$)	$n = 20$	($p = 0.3\%$)*
Controls ($n = 27$)	$n = 3$	

* Significantly different compared to the controls (Fisher exact 2 × 2 table probability test).

group between I and II. The number of patients in the tumor stage I was higher in the second group than in the first, although not significant. The proportion of pre- or postmenopausal patients were similar in the three groups.

Information on cancer case history could not be obtained from the families of the 27 controls.

Table 2A. 2 × 2 Contingency table

	Number of persons with decreased thymidine incorporation	normal thymidine incorporation
Patients	20	19
Controls	3	22

Repeated measurements in three patients from the second group were carried out 6 months after the first investigation. Two patients from the first group were investigated 4 months after the operative treatment. The rate of the DNA-repair synthesis remained always in the same range. For the patients BL the measured values differed from the investigation before surgery (Table 4).

DISCUSSION

Deficiencies in the DNA-repair are typical for autosomal recessive genetic disorders associated with some kinds of cancer [5–7]. A correlation of DNA-repair synthesis with age [8,12] and blood pressure [13] has been described.

Table 3. UV-C induced DNA-repair synthesis in controls and in breast cancer patients (investigation before surgery)

[^{3}H]thymidine incorporation	Register number of controls or initials of patients	age	Incorporation of [^{3}H]thymidine (cpm/10^6 cells) at UV doses (J/m^2) 2	4	8	16
Decreased*	96705	65	1063	1269	2028	2071
	99762	40	660	1515	1583	1900
	99769	38	654	772	999	1269
	MS	76	1614	1719	1818	2033
	SI	59	1224	1432	1548	1582
	KH	54	970	1138	1324	1751
	AE	68	773	1051	1562	–
	SB	62	708	800	1167	2087
	BL	70	335	382	522	857
	SU	43	1073	1531	1590	1782
	SE	60	374	542	1953	2332
Increased*	11	54	2413	3207	3951	4684
	23	38	1972	3547	4461	5289
	BT	48	3348	5020	5190	5243
	FV	38	3275	3788	3712	4125

Values after subtraction of hydroxyurea values.
* Beyond the 99% confidence limit.

Table 4. Repeated measurements of the DNA-repair synthesis in five patients

Group	Initials	Date of the investigation	Incorporation of [^{3}H]thymidine in cpm/10^6 cells: No HU	2 mM HU	at various UV doses (J/m^2): 2	4	8	16	[^{3}H]thymidine incorporation
	SB	6.02.84							
		(before surgery)	4112	4246	708	800	1167	2087	Decreased
First		6.06.84	2948	2541	587	934	999	1779	Decreased
	BL	6.02.84							
		(before surgery)	5725	5605	335	382	522	857	Decreased
		6.06.84	2640	1730	732	895	1170	1240	Decreased
	FM	19.01.84	3136	1720	900	1089	1200	1254	Decreased
		15.06.84	2120	980	870	1250	1340	1410	Decreased
Second		15.12.83	3803	1037	1622	2671	3117	3371	Normal
	RA	20.06.84	2687	815	2002	2980	3570	4285	Normal
	TT	12.10.84	1310	836	1369	2348	3231	4114	Normal
		16.06.8	2314	1105	1650	1910	2780	3880	Normal

HU = hydroxyurea.

In lymphocytes the absence of normal levels of unscheduled DNA synthesis is evidence for a systemic defect in DNA-repair. In this study half of the 41 breast cancer patients had reduced DNA-repair capacity.

The DNA-repair mechanism is dependent on cell type, cell cycle, dose and the duration of exposure to the DNA-damaging agent. Furthermore the measurement of DNA synthesis may also be influenced by individual variation in the uptake of the radioactive precursors into the cells. In an earlier study it has been shown that the [^{3}H]thymidine incorporation is dose-dependent for 2,4,8 and 16 J/m^2 and the interindividual variation is higher than the methodological variation (the methodological variation in our investigations lay between 13.4 and 15.1%) [8].

When studying induced DNA-repair using [^{3}H]thymidine incorporation the replicative synthesis was to be inhibited by HU. For this purpose 2 mM HU was added to the cell cultures. In this concentration HU does not completely inhibit the spontaneous DNA synthesis.

In order to estimate the repair capacity, the values of the unirradiated HU inhibited samples were subtracted from the values of the irradiated

samples. A decreased [^{3}H]thymidine incorporation found in individual cases of both controls and patients could be due (1) to the high rate of spontaneous DNA-repair or (2) to the high rate of spontaneous DNA synthesis in the presence of HU. In the first case, because of the high rate of spontaneous DNA-repair the cells might have a lower capacity to repair the additional UV-C induced damage. The high rate of spontaneous DNA synthesis in the presence of HU could be explained by either a lower uptake of HU by the cells or an altered inhibition of the ribonucleoside diphosphate reductase.

The values of spontaneous and of HU inhibited DNA synthesis were significantly higher in the patients investigated before surgery than in the controls and other women. This significance is due to the very high values obtained only in four women: measurements 4 months after surgery showed that in two out of these four patients the high values were reduced (in the other two patients no repeated measurements were carried out). These results do not indicate that the percentage of lymphocytes in the S-phase is higher in breast cancer patients investigated before surgery.

In an additional investigation there was also no difference in B, NK, T_s or T_h cells between controls ($n = 3$) and breast cancer patients investigated before operation ($n = 3$) (the phenotype distribution of cultured cells was determined by using monoclonal antibodies).

The proportion of separated lymphocytes in the cell cultures lay over 95%, determined in 10 controls and 12 patients.

Compared to operative treatment, radiotherapy causes a decrease in K-cell activity [14], a reduced proliferative stimulation by PHA [15,16] and a probably reduced lymphocyte count [15–18]. In this study many years after the end of the chemo/radiotherapy the clinically disease-free patients showed deficiency in their DNA-repair capacity. This deficiency had a tendency to be higher than that of the patients before and after operative treatment. Thus the defect in the DNA-repair system from these patients could reflect a systemic repair abnormality and/or the effect of radiotherapy. To clarify this question we plan to reinvestigate those patients who had normal repair activity before surgery one year after the end of chemo/radiotherapy.

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REFERENCES

1. Cleaver JE. Defective repair of DNA in xeroderma pigmentosum. *Nature* 1968, **218**, 652–653.
2. Kraemer KM. Heritable diseases with increased sensitivity to cellular injury. In: Fitzgerald TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. *Dermatology in General Medicine*. New York, McGraw-Hill 1983, 113–142.
3. German J. Chromosome-breakage syndrome: different genes, different cancers. In: Generoso WM, Shelby MD, de Serres FJ, eds. *DNA Repair and Mutagenesis in Eurokaryotes*. New York, Plenum, 1979, 429–445.
4. Lehmann AR. Cancer-associated human genetic diseases with defects in DNA repair. *J Cancer Res clin Oncol* 1981, **100**, 117–124.
5. Lambert B, Ringborg U, Svanbeck G. UV-induced DNA repair synthesis in lymphocytes from patients with actinic keratosis. *J Invest Dermat* 1976, **67**, 594–598.
6. Ringborg U, Lambert B, Landegren J, Lewensohn R. Decreased UV-induced DNA repair synthesis in peripheral leukocytes from patients with nevoid basal cell carcinoma syndrome. *J Invest Dermat* 1981, **76**, 268–270.
7. Pero RW, Miller DG, Lipkin M *et al.* Reduced capacity for DNA repair synthesis in patients with or genetically predisposed to colorectal cancer. *JNCI* 1983, **70**, 867–875.
8. Kovacs E, Weber W, Müller Hj. Age-related variation in the DNA-repair synthesis after UV-C irradiation in unstimulated lymphocytes of healthy blood donors. *Mutation Res* 1984, **131**, 231–237.
9. Francis EA, Glecins RD, Carrier WL, Smith DP, Regan JD. Inhibition of DNA repair in ultraviolet-irradiated human cell by hydroxyurea. *Biochim/Biophys Acta* 1979, **563**, 385–392.
10. Irwin J, Strauss B. Use of hydroxyurea in the measurement of DNA repair by the BND cellulose method. *Envir Mutagen* 1980, **2**, 381–388.
11. Sachs L (ed.) *Angewandte Statistik*. Berlin, Springer, 1968, 344–347.
12. Lambert B, Ringborg U, Skoog L. Age-related decrease of ultra-violet light-induced DNA repair synthesis in human peripheral leukocytes. *Cancer Res* 1979, **39**, 2792–2795.
13. Pero RW, Bryndelsson C, Mittelmann F, Thulin T, Moroen A. High blood pressure related to carcinogen-induced unscheduled DNA synthesis, DNA carcinogen binding and chromosome aberrations in human lymphocytes. *Proc natn Acad Sci USA* 1976, **73**, 2496–2500.

14. McCredie JA, McDonald HR, Wood SB. Effect of operation and radiotherapy on antibody-dependent cellular cytotoxicity. *Cancer* 1979, **44**, 99–105.
15. Stjernsewärd J, Jondal M, Vanky F, Wigzell H, Sealy R. Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. *Lancet* 1972, June 24, 1352–1356.
16. Blomgren H, Wassermann J, Wallgren A, Baral E, Petrini B, Ideström K. Lymphocyte counts and responses to PHA and PPD following radiation therapy for breast cancer patients who develop recurrent disease and those who remain clinically disease-free. *J Rad Oncol Biol Phys* 1980, **6**, 471–479.
17. Keller StE, Joachim HL, Pearse Th, Siletti DM. Decreased T-lymphocytes in patients with mammary cancer. *Am J clin Path* 1975, **65**, 445–449.
18. McCluskey DR, Roy AD, Abram WP, Martin WMC. The lymphocyte subsets in the peripheral blood of patients with benign and malignant breast disease. *Br J Cancer* 1983, **47**, 307–309.