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DNA repair and recovery of RNA synthesis in uremic patients

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DNA repair and recovery of RNA synthesis in uremic patients. A high frequency of cancer appears among uremic patients. As depressed DNA repair ability is thought to be one of the causes for malignancy in cancer prone diseases, the present study was undertaken to examine DNA repair in uremic patients. Unscheduled DNA repair synthesis in peripheral lymphocytes was measured after both ultraviolet (UV) and gamma irradiations. In hemodialysis (HD) patients the repairs were normal, but in chronic renal failure (CRF) patients not yet on dialysis treatment, both UV- and gamma-induced DNA repair abilities were depressed to about 60% of the control. Recovery of RNA synthesis after UV irradiation followed the same pattern: it was reduced in CRF but normal in HD cells. When CRF lymphocytes were incubated in normal plasma, the UV-stimulated DNA repair improved to a nearly normal level, whereas incubation of normal cells in CRF plasma depressed their repair capacity to 70% of the initial level. These results suggest that a plasmatic substance such as the carcinogenic heterocyclic amines may be involved in the impairment of DNA repair in chronic renal failure.

The present investigation of DNA repair ability in uremic patients was initiated by the observation that malignancies are more common in these patients than in the general population [1–4]. In some cancer prone diseases a close connection was found between the depressed ability to repair ultraviolet (UV)-induced DNA lesions and the high incidence of cancer [5–7]. Impaired DNA repair capacity was also reported in several malignancies [8–10]. In two previous studies [11, 12] carried out in our laboratory it was found that the UV-induced DNA repair ability was actually depressed in uremics. The highest depression was observed in chronic renal failure (CRF) patients who had not yet started dialysis treatment; in patients on continuous ambulatory peritoneal dialysis (CAPD) the depression was smaller, whereas patients on hemodialysis (HD) had a nearly normal DNA repair. These results were rather unexpected because the uremic state of the HD patients is neither improved, as compared to that of CRF patients, nor does HD enhance the immune system [13, 14].

The present study was undertaken in order to reassure the previous results. A further aim was to check whether the failure to repair DNA damage caused by UV is exhibited also in a depressed ability to repair a different kind of lesion caused by another agent. Ionizing gamma radiation was chosen for this purpose. An attempt was made to prove that a plasmatic factor was involved in these DNA repair discrepancies. Unscheduled DNA synthesis capacity in nonstimulated short term cultures of lymphocytes was the method of choice because it is the most suitable for large scale screening [15]. Finally, measurements of the recovery of RNA synthesis after UV irradiation were employed to determine the transcription capacity of the DNA [16], which demonstrates the repair of the active part of the DNA rather than the repair of the whole genomic DNA.

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Methods

Patients

For the determination of DNA repair, two groups of patients were investigated: 10 HD patients and 11 patients with CRF shortly before starting dialysis treatment. Sixteen apparently healthy blood donors from the local staff and blood bank served as controls. Details about the patients are presented in Table 1. Recovery of RNA synthesis was measured in 10 samples of each group. For the examination of plasmatic influence on the DNA repair, the test groups were composed of six CRF patients and six controls.

Cells

DNA repair ability was measured in circulating lymphocytes after exposure to UV or to gamma irradiation, using modifications of well established procedures [15, 17–19]. Lymphocytes were separated from freshly drawn heparinized peripheral blood on a Ficoll Paque (Pharmacia, Uppsala, Sweden) gradient [20], and suspended in isotonic phosphate-buffered saline (PBS) at a concentration of 4×10^6 cells/ml. Portions of 0.5 ml (2×10^6 cells) were applied to each of 8 to 10 Petri dishes (50 mm diameter, Miniplast, Einshemer, Israel) for UV repair and to 8 to 10 screw cap test tubes (Sterilin, Hounslow, GB) for gamma repair measurements, and were kept cold.

Irradiations

UV irradiation was produced by a Philips TUV 15 W G1598 lamp. The intensity was measured by an UVX digital radiometer (UVP Inc., San Gabriel, California, USA). Four or five replicate plates were opened one at a time and UV irradiated (250 erg/mm^2 at 254 nm) while kept on ice to avoid premature repair, and were covered from daylight to avoid photoreactivation. Four or five other replicates of each sample were kept cold without irradiation. Gamma irradiation was produced by a ^{60}Co source. Half of the tubes of each sample were exposed to 100

Table 1. Features of the three study groups (mean ± SEM)

	Hemodialysis	Chronic renal failure	Control
No. of patients	10	11	16
Age *years*			
Range	29–73	26–78	31–73
Mean	62.0 ± 4.4	52.6 ± 4.5	54.7 ± 3.8
Gender *F:M*	6:4	6:5	10:6
Urea *mg/dl*	169.8 ± 7.7	107.0 ± 9.7	31.6 ± 2.9
Creatinine *mg/dl*	12.1 ± 0.8	5.0 ± 0.4	0.9 ± 0.04
Hemoglobin *g/dl*	9.8 ± 0.5	12.0 ± 0.7	13.1 ± 1.0
Dialysis period *years*	3.9 ± 0.7	—	—

Table 2. DNA repair ability of lymphocytes after UV irradiation

	Hemodialysis (HD)	Chronic renal failure (CRF)	Control (C)
DNA repair[a]	750	458	753
N	10	8	16
SEM	92.3	42.8	62.1
Analysis of variance			
HD		+	−
CRF	+		+
C	−	+	

Posthoc comparison by Duncan procedure: F = 4.17; $P = 0.025$; CRF < HD; CRF < C.

[a] The difference between irradiated and unirradiated lymphocytes of ^{3}H-thymidine incorporation (mean cpm/10^6 cells), in the presence of hydroxyurea

GY gamma irradiation [19, 21] while being kept in an ice bath, whereas the other half were kept cold without irradiation.

DNA repair synthesis

Two ml cold RPMI 1640 medium, supplemented with 15% heat-inactivated fetal calf serum, 1% glutamine and 1% antibiotic solutions, containing 10,000 U/ml penicillin, 10 mg/ml streptomycin and 25 μg/ml amphotericin B (Biological Industries, Beit Haemek, Israel) were added to all the irradiated and unirradiated samples. Hydroxyurea in PBS solution (Sigma Chemical Co., St. Louis, Missouri, USA) was added to a final concentration of 10^{-2} M to avoid scheduled DNA repair synthesis [15, 19, 22] followed by thymidine (^{3}H-methyl, 10 μCi/ml, 49 Ci/mmol; Nuclear Research Center, Negev, Israel). Incubation at 37°C in an atmosphere of 5% CO_2 in air took place for four hours to enable unscheduled DNA repair synthesis, and then the cells were ice cooled.

In several samples kinetics of irradiation time and of incubation time were performed to demonstrate irradiation dose curve and incubation time curve for optimal conditions. The procedure was finished as mentioned elsewhere [11]. Shortly, contents of plates were quantitatively transferred to plastic tubes. All the tubes were centrifuged and the pellets washed with cold PBS. The washed pellets could be stored for several days at −20° if needed. The thawed pellets were washed again, then hydrolyzed for 30 minutes in 0.5 ml 0.5 N NaOH at 37°C, cooled, neutralized with 0.5 N HCl, precipitated with an equal volume of cold 20% trichloroacetic acid, kept on ice for at least one hour, filtered through glass microfiber filters (Whatman, GF/C-0.25 mm, Tamar, Jerusalem, Israel), washed thoroughly with cold 10% trichloroacetic acid and finally with cold 95% ethanol. The dry filters were counted for ^{3}H in a liquid scintillation counter (1217 Rackbeta, LKB, Wallac, Finland). The yields of the counts were 46 to 48%.

Recovery of RNA synthesis

RNA recovery after UV irradiation was measured according to the method of Lehmann [23] with modifications which made it suitable for lymphocytes. Cells were prepared as above and portions of 250 μl (1 × 10^6 cells) were applied to each of 8 to 10 plates. After irradiation of half of the plates, incubation in 2 ml whole medium took place for one hour to allow DNA repair. Then ^{3}H-uridine (5 μCi/ml; 15 Ci/mmol; Nuclear Research Center) was added and incubation continued for another four hours. Lysis of the washed cells was performed in mild conditions to avoid RNA decomposition as following: 1 ml N/10 NaOH was added to one tube at a time, and after thorough mixing for 30 seconds the solution was neutralized with 1 ml N/10 HCl. The RNA was precipitated with 200 μl of 100% trichloroacetic acid solution in the presence of bovine serum albumine (15 μl, 10 mg/ml solution). Filtration, washing and counting of ^{3}H-uridine was as in DNA repair.

Effect of uremic plasma

For this purpose the incubation medium was supplemented with human plasma instead of fetal calf serum. In each experiment plates containing lymphocytes from a CRF patient were UV-irradiated and then supplemented either with medium containing 15% of their own plasma or with a control plasma. Cells from the same control were supplemented either with their own plasma or with the CRF plasma. Nonirradiated cells were treated in the same way. This kind of experiment was repeated with six CRF patients and six controls.

Statistical analysis

Data are exhibited in cpm. The datum for each condition is a mean of four to five replicates. DNA repair ability is expressed as the difference between cpm of irradiated and nonirradiated samples per 1 × 10^6 cells. RNA recovery is expressed as the ratio between cpm of irradiated cells and cpm of nonirradiated cells.

Statistical comparison between the DNA repairs of the investigated groups was calculated by one way analysis of variance, posthoc comparison. The RNA recovery results were also submitted to ANOVA. $P < 0.05$ was considered significant. Significance of correlation coefficients was determined by calculating t values. The influence of plasma on DNA repair was analyzed by the Mann-Whitney U-test for nonrelated samples or by Wilcoxon matched T-test for related samples.

Results

DNA repair in HD and CRF patients

DNA repair abilities after UV and after gamma irradiations are presented in Tables 2 and 3, respectively. Following UV irradiation, the repair of the CRF lymphocytes was greatly reduced (458 ± 43 cpm/10^6 cells), whereas there was no significant difference between the repair of the cells of HD patients (750 ± 92) and of the controls (753 ± 62 cpm/10^6 cells). A similar pattern existed after gamma irradiation: The repair of

Table 3. DNA repair ability of lymphocytes after gamma irradiation

	Hemodialysis (HD)	Chronic renal failure (CRF)	Control (C)
DNA repair[a]	264	136	221
N	10	11	16
SEM	30.2	16.4	18.3
Analysis of variance			
HD		+	−
CRF	+		+
C	−	+	

Posthoc comparison by Duncan procedure: F = 7.33; $P = 0.0023$; CRF < HD; CRF < C.

[a] The difference between irradiated and nonirradiated lymphocytes of ^{3}H-thymidine incorporation (mean cpm/10^6 cells), in the presence of hydroxyurea

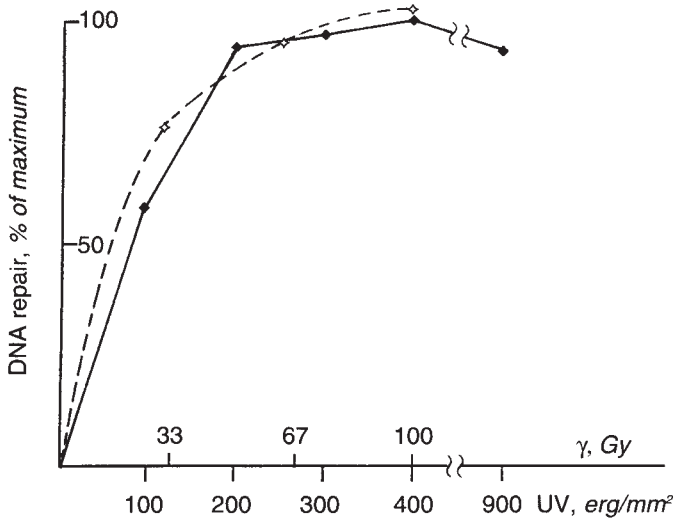

Fig. 1. *UV and gamma dose-response curves.* Results of one representative experiment. Each point stands for 5 replicates.

the CRF lymphocytes (136 ± 16 cpm/10^6 cells) was much depressed as compared with healthy cells (221 ± 18) while HD lymphocytes showed a normal repair (264 ± 30 cpm/10^6 cells). A very significant positive correlation existed between the DNA repairs after UV and after gamma irradiations within the controls and within the HD groups ($r = +0.874$, $P < 0.001$ and $r = +0.970$, $P < 0.001$, respectively), whereas in the CRF groups the correlation between UV and gamma repairs was lower and not significant ($r = +0.703$, P = NS). UV and gamma irradiation-dose response curves were performed in several samples in order to demonstrate optimal irradiation conditions (Fig. 1). An irradiation dose of 100 Gy reached the plateau and was in agreement with the results of other investigators [19, 21] for double strand break production. UV irradiation dose was 250 erg/mm^2, which is above the maximal UV energy which saturates the UV effect [24], and was used in other repair studies [11, 15] as well.

Incubation time of four hours was found to be optimal for the combined repair experiments and was generally accepted to be in the plateau of unscheduled DNA synthesis. The following parameters were checked for possible relationship with DNA

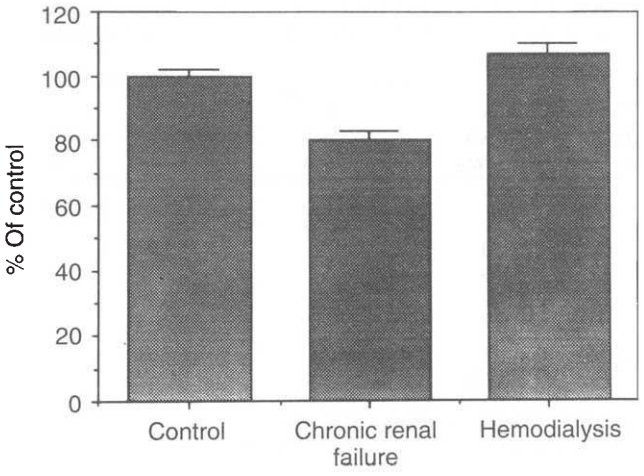

Fig. 2. *Recovery of RNA synthesis in lymphocytes from control, hemodialysis and chronic renal failure patients after UV irradiation.*

repair ability: plasma urea or creatinine levels, hemoglobin level, the period of time on maintenance HD, age, and gender. None of them had any correlation with DNA repair, although some investigators claim that it decreases with age [25, 26] whereas others disagree [27, 28].

RNA recovery in HD and CRF patients

Recovery of RNA synthesis in lymphocytes from CRF and HD patients and from controls after UV irradiation is presented in Figure 2. RNA recovery of CRF cells was reduced to 80% of the recovery of control cells (0.46 ± 0.014 vs. 0.57 ± 0.013 respectively, $P < 0.0005$), whereas the HD cells did not differ much from the control (0.61 ± 0.021; P = NS).

In DNA repair measurements the ^{3}H-thymidine counts of nonirradiated cells never exceeded 100 cpm/10^6 cells because DNA synthesis rarely takes place in nonstimulated lymphocytes and starts only after UV irradiation for repair purposes. In RNA recovery tests the ^{3}H-uridine incorporation into the nonirradiated cells varied from 600 to 3000 cpm/10^6 cells and were 1.4 to 2.9 times higher than incorporation into the irradiated ones, thus representing the transcription activity, which was reduced by the UV irradiation.

Effect of normal and CRF plasma on DNA repair

In some experiments the UV irradiated cells were incubated in the presence of plasma during the repair process. The repair ability of CRF lymphocytes in the presence of normal plasma as compared with incubation in their own plasma is presented in Table 4, and the effect of the CRF plasma on normal cells is demonstrated as well. These results can be concluded as following: (1) The DNA repair ability of CRF cells in their own plasma (594 ± 20) was only one half the ability of normal cells in their plasma (1154 ± 114, A vs. C; $P < 0.001$). (2) Incubation of the CRF cells in normal plasma elevated their repair ability from 594 ± 20 to 831 ± 58 cpm/10^6 cells (A vs. B; $P < 0.025$). (3) Incubation of the normal cells in plasma of CRF patients decreased their repair ability from 1154 ± 114 to 811 ± 84 cpm/10^6 cells (C vs. D; $P < 0.025$).

Table 4. Effects of normal plasma on UV-induced DNA repair in CRF cells, and of CRF plasma on normal cells (cpm/10^6 cells)

	CRF cells		Normal cells	
Exp. no.	A Own plasma	B Normal plasma	C Own plasma	D CRF plasma
1	637	934	1071	552
2	537	877	1212	897
3	650	1040	730	544
4	579	693	1585	1121
5	525	618	952	802
6	637	829	1377	942
Mean[a]	594	831	1154	811
SEM	20.5	57.9	113.8	84.0

Each figure is a mean of five replicates.
[a] The statistical analysis appears in the text

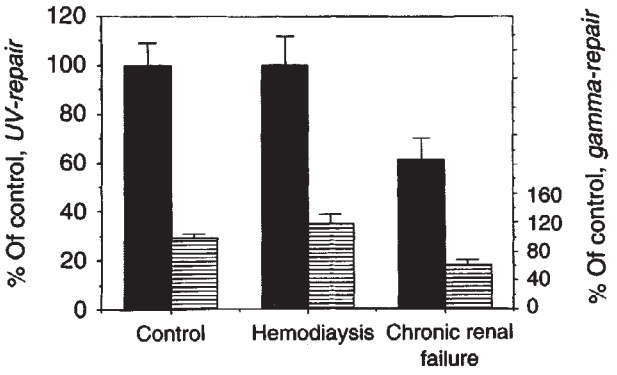

Fig. 3. *DNA repair in lymphocytes of control, hemodialysis and chronic renal failure patients after UV (■) and after gamma (▤) irradiations.*

Discussion

In the present study the repair ability of damaged DNA, induced both by UV and gamma irradiations, was found to be depressed to 60% of the normal values in peripheral lymphocytes from CRF patients with high urea and creatinine levels. On the other hand, uremic patients on maintenance hemodialysis treatment demonstrated a normal repair capacity after both types of irradiation. Depressed ability to repair DNA damage is associated with cancer and with cancer prone diseases [5–10], and is considered to be one of the causes of malignancy. As cancer in uremic patients appears more often than in others [1–4] the present study was initiated to find out whether these patients also belong to the group of high risk for cancer combined with low DNA repair ability. The first studies were performed several years ago, with only UV irradiation initiating the repair [11, 12], and their results opposed the working hypothesis which anticipated HD and CRF cells to behave similarly. The present UV repair ability of the experimental and control groups reassured the unexpected improvement of DNA repair ability in uremic patients who were on HD treatment in comparison with pre-dialysis CRF. The gamma-induced DNA repair showed the same relationships between the different groups as did the UV repair, but with lower absolute values (Fig. 3). The difference in ^{3}H-thymidine counts of the UV induced repair between the published results and the recent experiments can be attributed to changes in the procedure which had to be made in order to enable simultaneous UV and gamma repairs. The DNA repair measurement demonstrates the ability of the whole genome to repair itself after being damaged by mutagenic agents, but it does not discriminate between the actively transcribed part and the nonactive part of the DNA. Hence the recovery of RNA synthesis after UV irradiation was determined to find out whether the DNA repair process included both transcribed and nontranscribed DNA [16]. Our results show that RNA recovery in HD cells was equal to that in the normal cells, but in lymphocytes from CRF patients it was reduced to 80% of the control. Twenty percent reduction of RNA synthesis may have a major peripheral expression. Both methods together shed some light on the differential ability of the cells to repair active or inactive genes.

Screening the lists of medications, medical treatments, previous low dose radiations and exposure to metal ions or to other compounds have not provided a satisfactory explanation to the elevated DNA repair in HD in comparison with CRF patients. Yet several reasons for such findings can be raised, among which are the beneficial influence of the dialyzer on superoxide anion generation during HD [29, 30], or the presence of high concentrations of heterocyclic amines in CRF patients [31–36]. These compounds are known to interact with DNA and form adducts. High levels of heterocyclic amines in uremic blood was suggested as a hypothesis to explain the high incidence of cancer among these patients [1]. The fact that CRF patients do not exhibit more malignancies than the HD patients may be explained by the possibility that some mutational changes, which start at the CRF stage, become overt only later while undergoing HD.

In conclusion, CRF patients have an impaired DNA repair ability which is improved by HD. The reduced DNA repair may be one of the causes for the high cancer incidence among uremic patients.

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