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Age-related Decrease of Ultraviolet Light-induced DNA Repair Synthesis in Human Peripheral Leukocytes[1]

Bo Lambert, Ulrik Ringborg, and Lambert Skoog

Department of Clinical Genetics, Radiumhemmet and Department of Tumor Pathology, Karolinska Hospital, S-104 01 Stockholm 60, Sweden

ABSTRACT

The capacity for ultraviolet light-induced DNA repair synthesis, studied in peripheral leukocytes from 58 healthy subjects 13 to 94 years old, was found to vary greatly between individuals. A negative, statistically significant correlation was obtained between age and this synthesis, indicating a decrease in repair capacity with age. An age-related decrease in DNA repair may increase the susceptibility of cells to agents causing DNA damage, *i.e.*, carcinogens and certain cytostatic drugs.

INTRODUCTION

Age-related changes in the genetic material have been described in higher organisms. Increased amounts of DNA strand breaks were found in aging mice (23), and in human cells the frequency of chromosomal aberrations rises with age (21). During life, there is thus an accumulation of damage in the DNA. Since enzymatic repair can be used to control the amount of damage in the DNA, investigations have been performed to relate to age the capacity for DNA repair. Only late-passage human cells displayed a decreased capacity for UV-induced DNA repair synthesis (16, 22). Hart and Setlow (15), moreover, demonstrated a difference in this synthesis between animals with different life spans, long-lived species showing a higher capacity than short-lived species.

Since reduced capacity for DNA repair may be one factor responsible for the accumulation of DNA damage and possibly also for the aging of cells, we have studied DNA repair synthesis in human cells from individuals of different ages. We used human peripheral leukocytes in short-time culture. The results presented here show a decrease with age in the capacity for UV-induced DNA repair synthesis.

MATERIALS AND METHODS

Subjects. Fifty-eight individuals 13 to 94 years old were analyzed. All individuals were considered to be healthy, except that some of the oldest were being treated with digitalis and diuretics because of cardiac failure.

Isolation and Irradiation of Cells. Peripheral leukocytes were obtained from the buffy coat of 10 to 20 ml of freshly collected, heparinized venous blood. The cells were washed in PBS,[2] resuspended in the same solution, and distributed among 7 plastic Petri dishes at a cell density of about 5 to 10 $\times$ 10^6 cells/5 ml of PBS. Dishes 2 to 6 were exposed to UV at 4 doses between 3.2 and 19.2 J/sq m during slow rocking to allow optimal dispersion of the cells. Dishes 1 and 7 were left on the bench during the irradiation but were not exposed, and they served as controls for background incorporation. The UV was delivered by 2 parallel, low-pressure Philips TUV 6W mercury vapor lamps, which produced an effect of 0.64 J/sq m/sec.

Measurement of DNA Repair Synthesis. The UV-induced DNA repair synthesis was measured essentially according to the method of Evans and Norman (12) and as described earlier (19). Immediately after irradiation, the cells were transferred to centrifuge tubes, pelleted, and resuspended in 1 ml of Parker Medium 199 (Flow Laboratories) supplemented with 25% fetal calf serum, 125 μg streptomycin, and 125 IU benzyl penicillin. Samples from each tube were taken for cell counting. HU to a final concentration of 10^{-2} M was added to 5 of the tubes, including one unexposed control. The other 2 tubes (one unexposed control and one sample, which had been given 9.6 J/sq m) did not receive any HU. All tubes were preincubated for 30 min at 37°, before the addition of [^{3}H]thymidine (5 Ci/mmol; 1 mCi/ml; The Radiochemical Centre, Amersham, England) to a final concentration of 10 μCi/ml. The incubation continued for 2 hr and was interrupted by the addition of 1 ml of cold 10% trichloroacetic acid to each tube. Extraction of free nucleotides took place for 30 min at 4°. Two more washes in cold 5% trichloroacetic acid were carried out. The pellet was then resuspended in 70% ethanol and collected on a glass fiber filter. The filters were treated with solubilizer, and the radioactivity was estimated in a Packard liquid scintillation spectrometer at an efficiency of about 30% and a background of 20 cpm.

Measurement of dTTP Pools in Peripheral Lymphocytes. Peripheral lymphocytes were isolated from human blood by Ficoll-Isopaque centrifugation (17). The cells were pelleted at 800 $\times$ *g* for 10 min at 4° and washed with PBS. Each cell sample (approximately 2 $\times$ 10^6 cells) was extracted with 2 ml 60% (v/v) methanol at −20°. After 16 hr, the methanol-insoluble material was collected by centrifugation, and DNA was determined as described by Burton (4). The extracts were evaporated, and the residue taken up in 0.2 ml 0.05 M Tris-HCl, pH 7.5. dTTP was measured as outlined elsewhere (20).

RESULTS

Inhibition of DNA Replication by HU. The effect of HU on the incorporation of [^{3}H]thymidine into human leukocytes is shown in Table 1. HU depressed the spontaneous DNA synthesis to the same extent in the age groups 13 to 59 years and 60 to 94 years (10.0 *versus* 9.9%). The difference in spontaneous DNA synthesis in the 2 groups was not statistically significant.

Individual Variation of UV-induced DNA Repair Synthesis.

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[2] The abbreviations used are: HU, hydroxyurea; PBS, phosphate-buffered saline (KCl, 0.2g-KH_2PO_4, 0.2g-NaCl, 8g-Na_2HPO_4 $\times$ $2H_2O$, 1.15g in 1000 ml H_2O).

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In order to estimate the capacity for DNA repair synthesis, the dose dependence was investigated. Leukocytes were irradiated with 4 different UV doses and incubated with [^{3}H]thymidine in the presence of HU. Chart 1 shows dose-response curves for 4 individuals in the age range of 20 to 39 years. The curves level off at UV doses above 9.6 J/sq m. Maximal incorporation varies greatly among the individuals.

The mean value for UV-induced DNA repair synthesis at 9.6 and 19.2 J/sq m, after subtracting the background of HU-treated, nonirradiated cells, has been used as a measure of the DNA repair capacity. Table 2 compares the UV-induced DNA repair capacity as measured in a single individual on 10 different occasions and the values obtained for the group of 58 individuals. The S.D. was calculated to be 12.4% of the mean for DNA repair synthesis as measured in the single individual and 27.5% of the mean for the group of 58 subjects. This difference in S.D. indicates that the biological variation between individuals is greater than the methodological variation.

Table 1

The effect of HU on the incorporation of [^{3}H]thymidine into nonirradiated human leukocytes in individuals 13 to 59 and 60 to 94 years old

	Incorporation of [^{3}H]thymidine (cpm/10^6 cells)	
Subjects	No HU	10^{-2} M HU
13–59 yr (n = 40)	838 ± 726[a]	84 ± 45
60–94 yr (n = 18)	614 ± 341	61 ± 37

[a] Mean ± S.D.

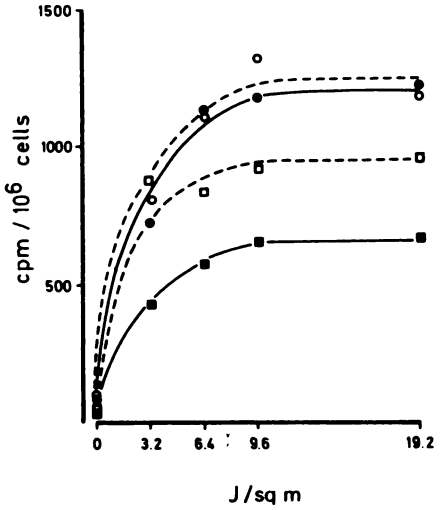

Chart 1. Dose-response curves of UV-induced DNA repair synthesis in human peripheral leukocytes from 4 individuals ages 20 to 39 years. The cells were irradiated with different UV doses, preincubated for 30 min with 10^{-2} M HU, and subsequently incubated for 2 hr in the presence of [^{3}H]thymidine (10 μCi/ml) and HU.

Age Dependence of UV-induced DNA Repair Synthesis. Table 3 compares the UV-induced DNA repair synthesis for individuals younger and older than 60 years. For UV doses of 6.4, 9.6, and 19.2 J/sq m, individuals 60 to 94 years old showed a lower DNA repair synthesis than those ages 13 to 59 years, the differences being statistically significant.

A negative, statistically significant correlation was found between age and UV-induced DNA repair synthesis (r = −0.38; $p < 0.005$) (Chart 2). The average DNA repair capacity decreases about 30% from 20 to 90 years of age. For several individuals, the mean value of repeated determinations has been used.

Peripheral leukocytes are a heterogeneous cell population, and it has been shown earlier that lymphocytes are capable of DNA repair synthesis, while granulocytes have very little or no DNA repair synthesis after exposure to UV (8, 25). For each individual studied, a differential count of the leukocytes was performed. In Table 4, it is shown that the proportions of different types of WBC are about the same in individuals 13 to 59 and 60 to 94 years old. Thus, differences in lymphocyte counts cannot explain the differences found in DNA repair synthesis.

One explanation for decreased uptake of [^{3}H]thymidine with age may be increasing pools of endogenous DNA thymidine precursors, which dilute out the exogenously added [^{3}H]thymidine. Therefore, the dTTP was determined in 6 individuals with different ages and DNA repair synthesis. As seen in Table 5, no evidence for this hypothesis was obtained.

DISCUSSION

When studying UV-induced DNA repair synthesis, it is necessary effectively to block the replicative DNA synthesis, since the UV both induces repair synthesis and inhibits the replication of DNA. This was done with HU, which has been found to inhibit

Table 2

The variation in DNA repair synthesis among individuals

	UV-induced DNA repair synthesis (cpm/10^6 leukocytes)
Mean ± S.D. for 58 subjects	739 ± 203
Mean ± S.D. for 10 repeated measurements on one individual	893 ± 111

Table 3

Comparison of UV-induced DNA repair synthesis in human leukocytes from individuals 13 to 59 and 60 to 94 years old

		Incorporation of [^{3}H]thymidine (cpm/10^6 leukocytes) at different UV doses				
						9.6 J/sq m + 19.2 J/sq m
Age (yr)	No.	3.2 J/sq m	6.4 J/sq m	9.6 J/sq m	19.2 J/sq m	2
13–59	40	590 ± 192[a]	683 ± 206	772 ± 226	798 ± 205	787 ± 210
60–94	18	526 ± 161	573 ± 206	601 ± 192	660 ± 211	631 ± 187
t test		1.23	1.88	2.80	2.35	2.71
p		>0.10	<0.10	<0.01	<0.05	<0.01

[a] Mean ± S.D.

Table 4

Differential counts of peripheral leukocytes from individuals 13 to 59 and 60 to 94 years old

	Granulocytes					
Age (yr)	Rod-shaped	Neutrophiles	Eosino-philes	Basophiles	Lymphocytes	Monocytes
13–59	3.0 ± 1.4[a]	47.0 ± 12.0	2.5 ± 1.4	0.8 ± 0.8	42.0 ± 13.0	4.0 ± 2.0
60–94	2.0 ± 1.3	53.0 ± 11.5	2.0 ± 1.2	0.5 ± 0.9	38.0 ± 12.0	4.6 ± 1.9

[a] Mean ± S.D.

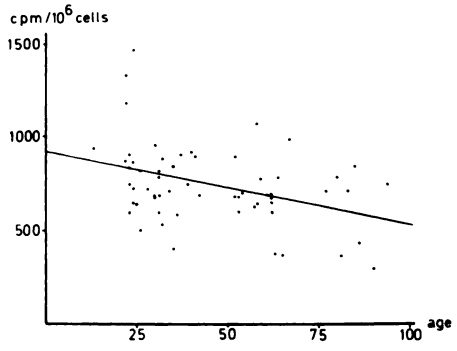

Chart 2. A correlation analysis of UV-induced DNA repair synthesis in human peripheral leukocytes and donor age. The cells were irradiated, preincubated for 30 min with 10^{-2} M HU, and subsequently incubated for 2 hr in the presence of [^{3}H]thymidine (10 μCi/ml) and HU. Each *point* represents the mean value for incorporation of [^{3}H]thymidine for UV doses of 9.6 and 19.2 J/sq m (plateau value) when the HU-insensitive background, obtained from nonirradiated cells, has been subtracted ($r = -0.38$; $n = 58$; $p < 0.005$).

Table 5

DNA repair synthesis and dTTP in peripheral lymphocytes from 6 individuals of different ages

Age (yr)	DNA repair synthesis (cpm/10^6 cells)	dTTP (pmol/μg DNA)
23	897	0.10
28	859	0.07
30	670	0.07
54	688	0.05
58	620	0.06
82	450	0.06

replication of DNA selectively without affecting DNA repair synthesis at all substantially (6). Although the replicative DNA synthesis of peripheral leukocytes is very low, it should be inhibited as much as possible in order to obtain a proper estimation of repair synthesis and avoid misinterpretations (25). We used 10^{-2} M HU to reduce the background as much as possible. With 10^{-3} M HU, we obtained a somewhat higher background, which may interfere with the reproducibility in the measurements. We found no indications that 10^{-2} M HU would depress the induced DNA repair synthesis.

Great variations in DNA repair synthesis were found among these 58 healthy individuals in the age range of 13 to 94 years. Repeated measurements in one individual showed the method variation (S.D.) to be 12.4% of the mean. The variation among individuals was higher, and we consider that this variation reflects biological differences between individuals. $r = -0.38$ found for the data in Chart 2 implies that 14% ($r^2 \times 100\%$) of the variations among individuals may be ascribed to an effect on repair.

The capacity for UV-induced DNA repair synthesis showed a decrease with age, the individuals who were 90 years old having values about 30% lower than those aged about 20 years. Since a dose-response curve was established for each individual, the different DNA repair levels cannot be attributed to different amounts of damage in the different individuals.

In Chart 2, 2 individuals about 25 years old show high values of DNA repair synthesis. The slope of the line was determined without these 2 individuals, and the negative correlation obtained was still statistically significant.

Some investigators have studied the relation between aging of cultured cells and DNA repair synthesis (14, 16, 22), but they have observed that cells with a low capacity for DNA repair appear only in the late passage of cultivation (16). They therefore conclude that DNA repair is probably not causal to aging. A correlation between DNA repair synthesis and life span in mammalian species was found by Hart and Setlow (15), and a decreased capacity, although still disputed (3, 24), for rejoining DNA breaks has been reported in progeria (9, 10), a disorder associated with premature aging. DNA damage caused by alkylating agents is also repaired enzymatically, probably in an analogous way. When *in vivo* repair of rat intestinal DNA damage by alkylating agents was studied, a relationship was found with age; old rats showed a lower capacity for alkylation-induced DNA repair as measured by induction and rejoining of DNA strand breaks (18). Some patients with the recessive genetic disorder xeroderma pigmentosum develop pathological aging of the nervous system. Fibroblasts, as well as epidermal cells and lymphocytes, from patients with this disorder are defective in UV-induced DNA repair synthesis (2, 5, 11), and the defect has been localized to the action of the UV-specific endonuclease (7, 26). A strong correlation has been found between the UV-induced DNA repair capacity and the pathological aging of the nervous system, the individuals with a low DNA repair capacity developing the most severe neurological symptoms (1).

We have studied peripheral leukocytes, and one could argue for the study of cultured fibroblasts, in order to work with a homogeneous cell population. Since the cells lose their capacity for cell division with age, this could give rise to a selection of cells during the cultivation, a process which is avoided by using fresh cells. On the other hand, the lymphocyte population may vary between young and old individuals, and different lymphocyte populations may differ in repair capacity. However, this was not found when lymphocytes from patients with chronic lymphatic leukemia (being essentially B-cells) were compared with lymphocytes from healthy individuals (which are dominated by T-cells) (13, 25). Further studies on fractionated lymphocyte populations may answer such questions.

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