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UV-LIGHT INDUCED DNA DAMAGE AND REPAIR IN LYMPHOCYTES IN PHOTODERMATOSES

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Abstract. UV-light induced DNA damage, and its repair in lymphocytes derived from patients with photodermatoses were studied. Radioactivity was measured by means of the liquid scintillation technique. Repair incorporation could be found in the lymphocytes of all healthy controls free from light sensitivity and in those of nearly all the patients with porphyria cutanea tarda. On the other hand, in the lymphocytes of one patient suffering from xeroderma pigmentosum and the majority of patients with polymorph light eruption, no precursor uptake was detectable.

Since Rassmussen's first publication in 1964 (13) the damage caused by UV irradiation and its repair mechanism in mammalian cells have been observed in cell cultures of various origins and qualities (4, 6, 7, 16). The investigation of this phenomenon in the epidermal and corial cells of human skin yielded valuable criteria by which to recognise the early effect of UV-light (4) as well as to interpret the aetiopathogenetic problems of some dermatoses with light sensitivity, primarily those of xeroderma pigmentosum (X.P.) (1, 2, 5, 8, 11). The correlation of extreme light sensitivity with the absence of DNA repair observed in the skin of patients with X.P. suggests that the repair of DNA damage is one of the factors of protection against UV-light (3). Inability to repair is detectable not only in epidermal cells and cultured corial fibroblasts of the skin of the patients but also in their peripheral lymphocytes (10).

It seemed of interest to study whether the repair potential can be found in the other photodermatoses and whether its occasional variation bears any significance in the development of photosensitivity. The aim of our investigation was to study the DNA damage caused by UV irradiation and the repair incorporation in peripheral lymphocytes derived from patients with photodermatoses.

MATERIAL AND METHOD

Subjects

Four healthy controls free from light sensitivity (aged 20–43), 6 patients with polymorph light eruption (PLE, aged 10–60), 4 patients with porphyria cutanea tarda (PCT, aged 39–61) and 1 girl, aged 5, suffering from X.P.

Method

Blood from the cubital vein was sedimented with dextran. Two samples of 0.5 ml of the suspension rich in lymphocytes (cell count: 1–1.5 M lymphocytes/ml) was irradiated with UV-light by means of a quartz lamp, type Medicor Q 250 W, at a distance of 30 cm for 30, 60, 120 and 300 sec in a Petri dish covered with an interference filter. Its maximal transmission was 2 610 ± 130 Å. *T* max.: 19%. The output of the lamp: 2 000–2 800 Å: 47.37 $\mu W/cm^2$ at a distance of 1 m. Both the irradiated lymphocyte suspensions were incubated at 37°C in 2 ml of Parker TC 199 medium containing 10^{-3} M hydroxyurea for inhibiting the normal semiconservative DNA replication (6, 19) and 10 μCi ^{3}H-thymidine (spec.act. 5–20 Ci/mM) ac-

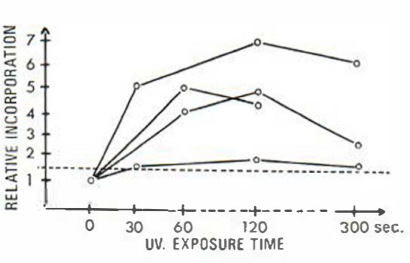

Fig. 1. Rate of incorporation of ^{3}H-thymidine in lymphocytes of healthy controls after various periods of UV irradiation.

Figs. 1–5. Abscissa: UV-exposure (seconds); *ordinate:* relative incorporation (dpm).

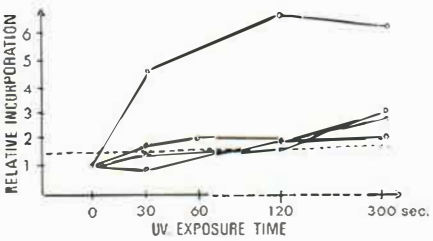

Fig. 2. Rate of incorporation of ^{3}H-thymidine in lymphocytes of patients with porphyria cutanea tarda after various periods of UV irradiation.

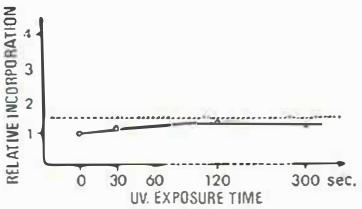

Fig. 4. Rate of incorporation of ^{3}H-thymidine in lymphocytes of patients with xeroderma pigmentosum after various periods of UV irradiation.

cording to Evans's method (6). After 4 hours the cultures were filtered on Millipore filters (SMWP09000) and treated successively with saline and 5% ice-cold TCA. To measure the repair replication after DNA damage caused by UV-light, the incorporation of labelled DNA precursor (^{3}H-thymidine) was determined. Radioactivity was measured in a toluene-based scintillation fluid by means of a Packard 3320 liquid scintillation counter, in 3–3 parallel tubes of each sample. The ratio in dpm of irradiated to nonirradiated cultures was considered to be significant above the 1.5 level. Samples were counted with $< 3\%$ counting error.

RESULTS

The results are shown in the figures. The exposure time of UV-light irradiation in seconds can be found on the abscissa, the ratio of disintegration rate of labelled thymidine in the irradiated and non-irradiated cultures can be found on the ordinate ("relative incorporation").

Fig. 1 shows the rate of thymidine incorporation in the lymphocytes of 4 healthy control subjects after irradiation for 30–300 sec. The rate is most significant after an exposure time of 120 sec; it generally decreases after an exposure of 300 sec. Presumably this can be explained by the toxic, cell-destructive effect of UV-light. Though the rate of thymidine incorporation can differ from one individual to another, the lymphocytes of all the healthy persons observed were able to repair the DNA damage caused by UV-light.

Fig. 2 illustrates the radioactivity index in lymphocytes derived from patients with PCT. As with the controls, thymidine incorporation indicating repair can be found in all 4 cases, though its rate seems to be slightly lower.

On the other hand, among the patients suffering from PLE there is only one case in which a significant incorporation of labelled DNA precursor is detectable (Fig. 3). We failed to demonstrate it in 5 other cases. Neither were we able to observe repair incorporation in the lymphocytes of our patient with X.P. (Fig. 4).

Finally, Fig. 5 shows the average incorporation rates in the lymphocytes derived from each group of persons investigated. The significance of these rates after 2 minutes of irradiation was calculated by *t*-test. The data obtained were as follows: 4 650 for the controls, 3 428 for patients with PCT ($p < 0.2$) and 1 477 for patients suffering from PLE ($p < 0.05$).

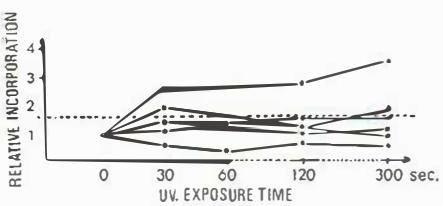

Fig. 3. Rate of incorporation of ^{3}H-thymidine in lymphocytes of patients suffering from polymorph light eruption after various periods of UV irradiation.

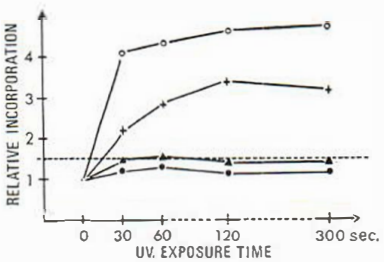

Fig. 5. The averages of rates of incorporation in lymphocytes derived from each group of persons investigated after various periods of UV irradiation. ○, healthy controls; ×, patients with porphyria cutanea tarda; ▲, patients with polymorph light eruption; ●, patient suffering from xeroderma pigm.

DISCUSSION

The epidermal cells and corial fibroblasts of the skin as well as peripheral lymphocytes of healthy persons repair the DNA damage induced by UV-light by uptake of DNA precursors (3, 4, 6, 7). Of the pathological photoproducts formed by irradiation, the thymine dimers are removed from DNA enzymatically and the deficiency is restored by taking up pyrimidine-deoxynucleosides (12, 14, 15, 18). This process can be observed by autoradiography and liquid scintillation technique: it reaches its maximum within the first 4–6 hours after irradiation, it can be observed in every phase of the cell cycle, it involves between 50% and 80–90% of the cells (6, 10), and cannot be inhibited by hydroxyurea in contrast to the semiconservative normal synthesis of DNA (16, 17).

According to the investigations of Cleaver, Jung and other authors (2, 3, 11, 1) the repair incorporation cannot be observed in patients with X.P. associated with light sensitivity. It is suggested that its cause may be the inability of the cells to remove the thymine dimers enzymatically from DNA (3, 10). We also observed the absence of repair incorporation in the peripheral lymphocytes of our patient when applying UV exposure times after which lymphocytes of healthy persons unequivocally showed enhanced thymidine incorporation as reported by Jung (10). The rate of incorporation found in the cells of healthy controls corresponds with Evans's results carried out by liquid scintillation technique (6).

We found that the repair incorporation in lymphocytes derived from patients with PCT of photodynamic pathomechanism resembled that in healthy persons. However, it is surprising that we succeeded in demonstrating it in only 1 of the 6 patients suffering from PLE, by means of liquid scintillation technique. This suggests that repair of DNA damage in lymphocytes in the majority of the patients with PLE is lacking, or that its development is unusual. It is all the more interesting because this disease is considered to be a manifestation of photoallergy mostly of a delayed type in which only the lymphocytes play a significant role. In our previous experiments (9) a blastoid transformation more intensive than normal was tentatively demonstrated after UV exposure in 5-day cultures of lymphocytes derived from patients with PLE. At the same time this phenomenon could be observed only to a lesser extent in PCT and healthy controls. Naturally the thymidine incorporation following this phenomenon cannot be mistaken for the repair incorporation which begins immediately after irradiation and is completed 24 hours later. Furthermore, its incorporation rate is different from that brought about by specific antigen stimulus indicating the semiconservative DNA synthesis following blastoid transformation.

Nevertheless, it is remarkable that the behaviour of lymphocytes derived from some cases with PLE differs from that of normal lymphocytes in the course of blastoid transformation as well as during repair incorporation following UV irradiation. Only further investigations will reveal whether the absence of repair incorporation observed in such cases is a consequence of an enzymatic disorder resembling X.P. or whether it is brought about by other causes (for example, the UV dose applied is insufficient or—on the contrary—is already too toxic for the lymphocytes). Our investigations concerning this problem are in progress.

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