

EFFECT OF CHLOROQUINE ON DNA SYNTHESIS IN THE SKIN OF DLE PATIENTS

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Abstract. Changes in the semiconservative and excision repair DNA synthesis were studied autoradiographically in the skin of 12 patients with discoid lupus erythematosus during Chloroquine treatment (500 mg/day for 8 weeks). The originally increased rate of the semiconservative DNA synthesis in the area of the skin lesions returned to the normal level simultaneous with clinical improvement. No effect on the excision repair DNA synthesis could be detected.

Key words: Excision repair DNA synthesis; Semiconservative DNA replication; Lupus erythematosus; Antimalarial agents

It has been reported that antimalarial agents (AMA) used in the treatment of discoid lupus erythematosus (DLE) have an inhibitory effect on the excision repair process induced by ultraviolet light (UVL), X-rays and other potentially mutagenic agents (6, 7, 8, 16, 17, 18). Presumably this inhibition can be explained by the affinity of these drugs for DNA (7).

According to our previous study, Chloroquine (C) administered orally in therapeutic dose to DLE patients has only a slight and incidental inhibitory effect on the UVL-induced repair incorporation in the peripheral lymphocytes derived from the patients (10). The aim of the present work was to study whether C treatment had any influence on the semiconservative and excision repair DNA synthesis in the skin of DLE patients, i.e. on the direct site of UVL effects.

MATERIAL AND METHODS

Persons investigated: 12 patients, aged 35-55, with florid clinical symptoms of DLE, and 6 controls of the same age without any light sensitivity. Patients and controls were tested for minimal erythema dose (MED) reaction on the symptom-free skin of the upper back of the trunk by means of a germicidal lamp. Then in all subjects one skin

area was irradiated with a single dose of three times the MED suitable for inducing changes in the DNA synthesis (5).

Punch biopsy specimens were obtained from the skin exposed to $\times 3$ MED 2 and 48 hours after UVL irradiation, from unirradiated control sites, as well as from the florid skin eruptions of the DLE patients. The patients were then given C orally in a 500 mg daily dose for 8 weeks.

The above investigations were repeated after 4 and 8 weeks of C administration to evaluate its effect. All biopsy specimens (consisting of the whole epidermis and the upper dermis) were cut into pieces of $1-2 \times 2-3$ mm then immediately placed into thymine-less Parker medium 199 containing $15 \mu\text{Ci/ml } ^3\text{H}[\text{thymidine}]$ (spec.act. 5 Ci/mmol). After incubation at 37°C for 4 hours, the tissue specimens were removed and washed twice with isotope-free culture medium, fixed in formalin and finally embedded in paraffin by a standard method (9). Sections $3 \mu\text{m}$ in thickness were prepared. Then the slides were coated with Ilford nuclear research emulsion type G5 (Ilford Ltd., Essex, England) by dipping method. After 2 weeks' exposure the slides were developed and stained with hematoxylin-eosin. Incorporation of the $^3\text{H}[\text{thymidine}]$ was determined autoradiographically. The nuclei of more than 2000 cells were counted in each section. Two types of nuclear labelling were observed: heavy labelling (more than 15 grains per cell) representing basal and epibasal cells in S-phase and sparse labelling (3-15 grains per cell) representing cells of the basal, malpighian and granular layers in excision repair synthesis. Results were expressed as percentages of the labelled nuclei. For statistical analysis the Student's *t*-test was employed.

RESULTS

As regards semiconservative DNA replication (Fig. 1) the numbers of heavily labelled nuclei of basal and epibasal cells in the biopsy specimens tested are shown in Fig. 2. The percentages of these cells in the unirradiated and symptom-free skin of patients were similar to those of the controls. Their values ranged from 1.6 to 4.2.

In the biopsy specimens obtained from the florid clinical eruptions of DLE patients the number of S-phase cells was significantly higher than in the

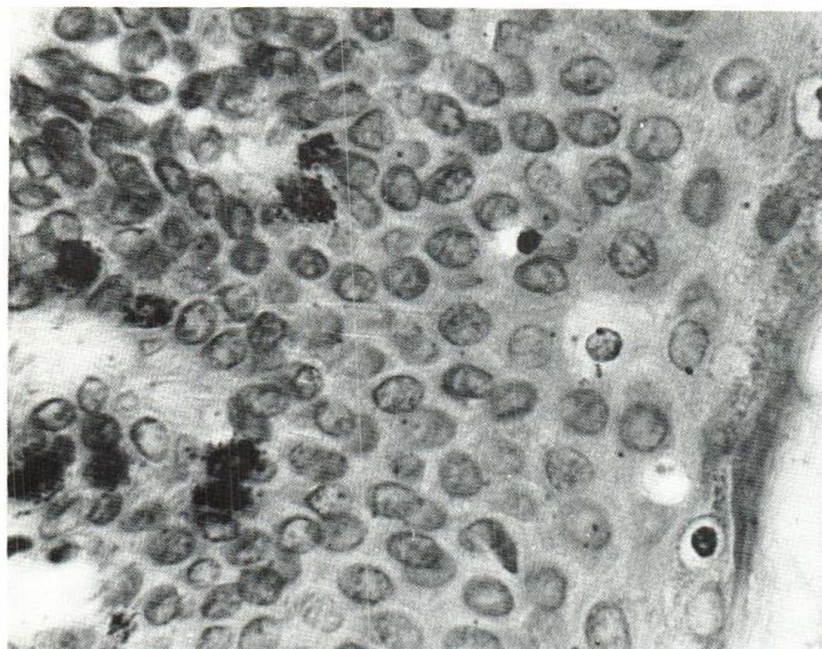


Fig. 1. Autoradiogram of healthy human epidermis (P. G., male, aged 38). Heavily labelled basal and epibasal cells representing semi-conservative DNA synthesis 48 hours after UVL irradiation with $\times 3$ MED (hematoxylin-eosin, $\times 400$).

control sites ($p < 0.01$). In biopsy specimens from the skin irradiated with $\times 3$ MED 2 hours after UVL radiation a slight decrease in the percentage of the heavily labelled cells can be observed in patients and controls. 48 hours after the irradiation, a significant increase occurred in the semi-conservative DNA synthesis.

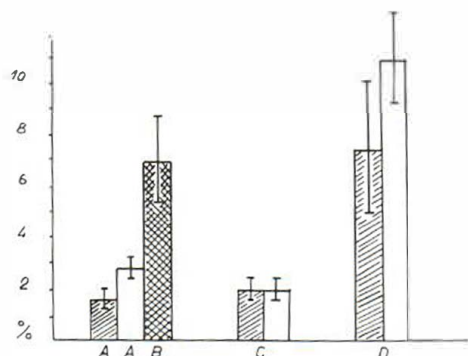


Fig. 2. Percentages of S-phase cells in the epidermis of skin obtained from persons without light sensitivity, ▨; from patients with DLE, ▤; from the clinical eruptions of DLE, ▩.

(A) unirradiated control site.

(B) from the clinical eruption of DLE,

(C) 2 hours and (D) 48 hours after UVL irradiation with $\times 3$ MED.

Range bars indicate standard deviation.

Changes in previous parameters during C treatment are shown in Fig. 3. The intensity of semi-conservative DNA replication did not differ in the unirradiated symptom-free skin of the patients after 4 and 8 weeks of C administration. Changes induced by UVL irradiation with $\times 3$ MED were also similar to those before treatment. However, the originally

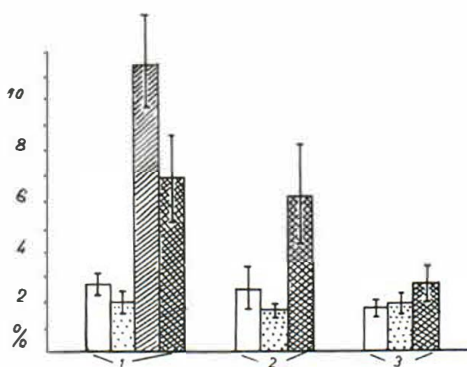


Fig. 3. Percentages of S-phase cells in the epidermis of skin obtained from DLE patients. (1) before C treatment, (2) after 4 weeks of C treatment and (3) after 8 weeks of C treatment. ▨, Clinical eruption; ▤, unirradiated control site; ▩, 2 hours after UVL with $\times 3$ MED; ▩, 48 hours after UVL with $\times 3$ MED. Range bars represent standard deviation.

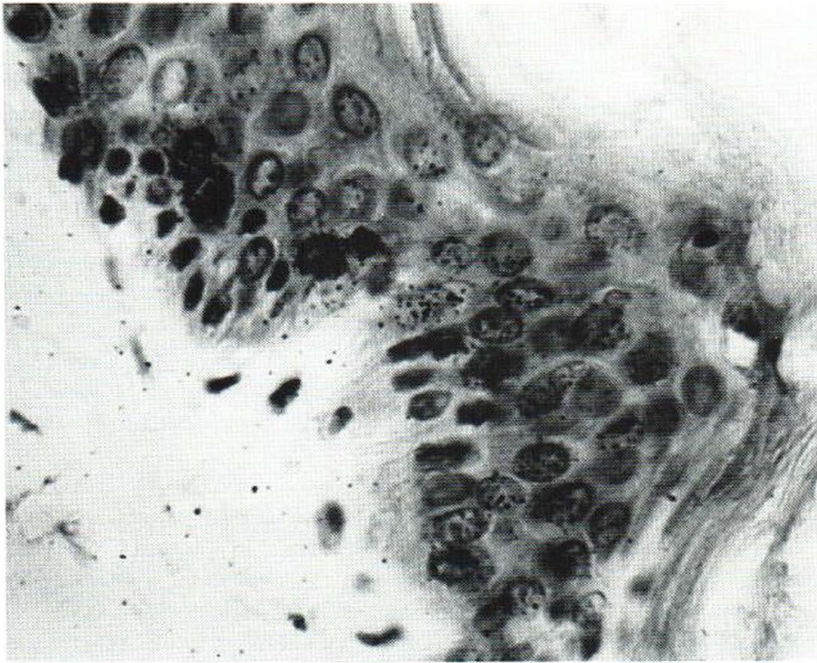


Fig. 4. Autoradiogram of healthy human epidermis (S. I., female, aged 42). Sparsely labelled epidermal cells in the basal, malpighian and granular layers of the skin exhibiting excision repair synthesis 2 hours after UVL irradiation with $\times 3$ MED (hematoxylin-eosin, $\times 400$).

increased numbers of S-phase cells in the skin of the clinical eruptions returned to normal after 8 weeks of C administration.

As for the excision repair DNA synthesis (Fig. 4) the percentage of the repairing cells did not differ in the skin of DLE patients and the controls 2 hours after UVL irradiation with $\times 3$ MED (Fig. 5). Sparse labelling of the nuclei indicating repair incorporation can be seen in 70–80% of the cells in the basal, malpighian and granular layers of the epidermis. According to the dose-response curve (Fig. 6) max-

imum repair incorporation occurs following UVL irradiation with $\times 3$ MED.

The intensity of the repair synthesis, i.e. the average grain count per cell, is significantly lower in the skin of DLE patients than in the controls. Repair incorporation during C administration is demonstrated in Fig. 7. The percentage of repairing cells as well as the intensity of repair synthesis were unchanged.

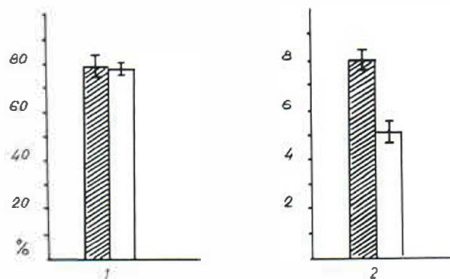


Fig. 5. Percentages of cells in DNA repair synthesis (1) and DNA repair synthesis rate (average grain count per cell) (2) in epidermis 2 hours after UVL irradiation with $\times 3$ MED for controls, ▨; for DLE patients, □. Range bars indicate standard deviation.

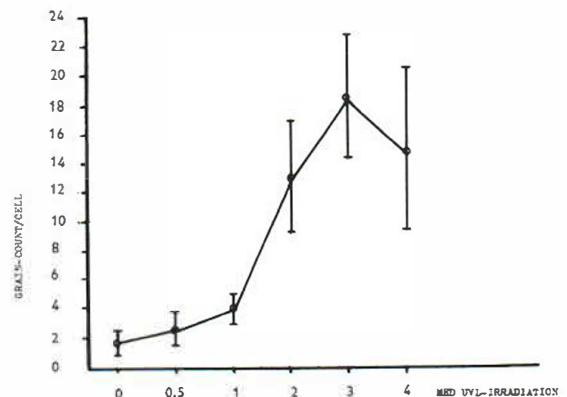


Fig. 6. Intensity of repair incorporation after various doses of UVL irradiation in healthy human epidermal cells.

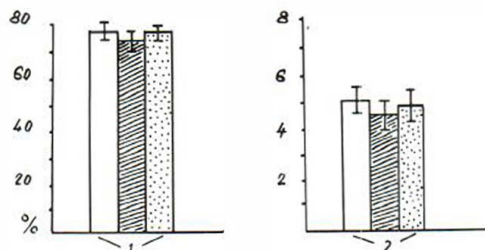


Fig. 7. Percentages of cells in DNA repair synthesis (1) and DNA repair synthesis rate (average grain count per cell) (2) in epidermis of DLE patients 2 hours after UVL irradiation with $\times 3$ MED before C treatment, $\square$; after 4 weeks of C treatment, ▨ ; after 8 weeks of C treatment, ▩ . Range bars indicate standard deviation.

DISCUSSION

It has been reported that antimalarial agents, owing to their affinity for DNA, form a complex with it (4, 12, 13, 14). In this way they change some of the biological and physical properties of DNA. According to the literary data the resulting complex formation would account for their inhibiting effect on the excision repair processes, too (6, 7, 17).

Yielding & Gaudin were the first to study this phenomenon in connection with UVL. In their experiments, C added to irradiated *E. coli* cultures reduced the survival of bacteria and inhibited the recovery from DNA damage (17). In another experiment, C caused a remarkable reduction in the repair incorporation of the cells when added to healthy human lymphocyte cultures ($6-7 \times 10^{-5}$ M concentration) (8). In our previous investigations (10) the therapeutic dose of C administered orally to DLE patients had only a slight and incidental inhibitory effect on the UVL-induced excision repair incorporation in the peripheral lymphocytes of the patients.

The present results are in agreement with the findings of others as well as our own previous ones. Cleaver demonstrated a normal excision repair capacity in cultured dermal fibroblasts of DLE patients (3). Epidermal cells of our patients also displayed a normal excision repair incorporation induced by UVL, even though the intensity of the repair synthesis was lower than in healthy controls (11). Altmann et al. (1, 2, 15) reported an altered repair incorporation accompanied by an increased semiconservative DNA replication in the systemic form of lupus erythematosus.

As for the effect of C on the DNA repair processes, neither the percentage of repairing epidermal cells nor the intensity of repair incorporation changed during the 8 weeks of C treatment. This suggests that C, when administered orally in a therapeutic dose, has no significant inhibitory effect on the excision repair processes, either in the epidermal cells or in the peripheral lymphocytes of DLE patients (10). According to some published data, C accumulates first of all in actively proliferating tissues and cells (13, 14), in the lymphoid organs and epidermal cells among others; its *in vivo* concentration attained via oral administration seems to be insufficient to effect a remarkable repair inhibition.

As regards the semiconservative DNA replication in the epidermal cells of DLE patients, changes induced by UVL developed in a normal way (5) (an early depression in the number of S-phase cells, then a significant increase 48 hours after UVL). C administration did not alter these processes. On the other hand the originally increased rate of the semiconservative DNA synthesis in the area of the skin lesions returned to the normal level simultaneous with the clinical improvement at the end of the 8 weeks of C therapy.

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