

Activation of Complement in the Skin after PUVA Therapy

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Suction blister fluid from the abdominal skin of 14 healthy subjects was examined for the presence of split products of complement C3 (C3-split) as an indicator of complement activation before and after treatment with either UVA or PUVA. Following PUVA a significant increase in C3-split was found ($p < 0.05$) in both irradiated and non-irradiated skin areas. Following UVA the increase in C3-split was less pronounced. The activation of complement may play an important role in the maintenance of the delayed erythema that appears after irradiation with long-wave ultraviolet light. *Key words: Complement activation; Healthy volunteers; Photochemotherapy; Suction blister.* (Received February 24, 1983.)

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Photochemotherapy with psoralens and long-wave ultraviolet light (PUVA) is used increasingly as a dermatological therapy. The PUVA treatment may induce a delayed erythema within 24 to 48 hours after a therapeutic UVA dose (1). The mechanisms leading to this erythema are not fully understood. Mediators of low molecular weight, prostaglandins for instance, have been implicated in the pathogenesis, but they have so far not been recovered from the erythematous skin (2).

Recently, complement activation was suggested to be responsible for the inflammatory skin reaction of patients with erythropoietic protoporphyria following UVA exposure (3). The complement split products C3a and C5a are known to possess vasoactive capabilities. The present study was therefore undertaken to examine whether an activation of complement might be involved in the skin response to PUVA treatment. The results of the study indicate that complement is activated following PUVA given in therapeutic dosages, and that the activation products might participate in the induction of the delayed erythema.

MATERIALS AND METHODS

Volunteers

After giving informed consent, 14 healthy male volunteers were enrolled in the study. Their mean age was 24 years (range 19 to 31 years). They had not exposed themselves to the sun within 3 months of the study.

Light source

UVA irradiation was carried out in a stand-up unit equipped with 76 Philips fluorescent tubes (Philips TL 40 W/09) emitting mainly in the long-wave ultraviolet region with a peak at 355 nm, and producing an UVA energy of 7 mW/cm² at a distance of 50 cm.

Irradiation

The volunteers were divided into two groups. Seven subjects received PUVA (8-methoxypsoralen approximately 0.5 mg/kg followed by UVA 4 to 5 J/cm², 2 hours later) on two successive days.

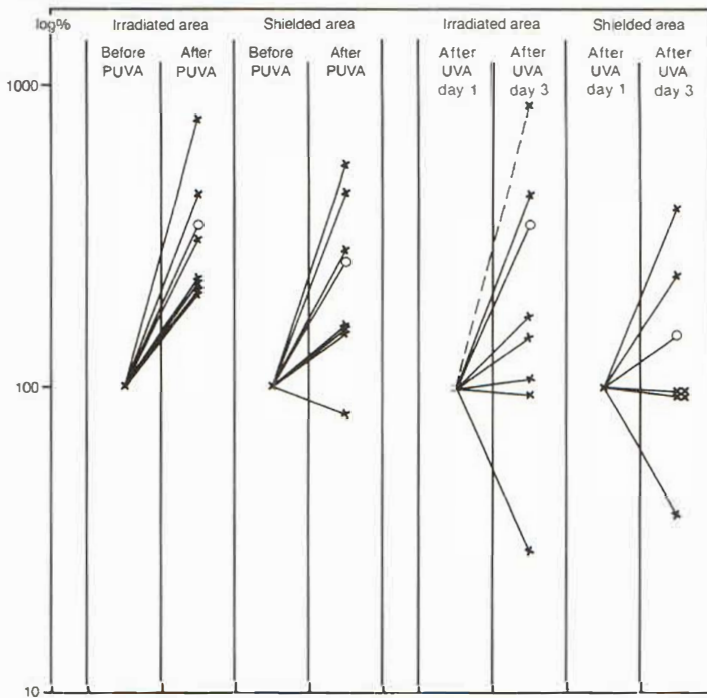


Fig. 1. The variation (expressed in percent) between the complement activation index (i.e. the ratio between C3-split and C3) in suction blister fluid before and after PUVA and after one vs. 3 days following UVA treatment of irradiated and shielded skin areas. x, individual patients; o, mean value. The end point of the broken line is at 1.16%.

Another 7 subjects received one treatment with UVA (10 J/cm²). During the irradiation an abdominal area of 13×18 cm² was shielded with lead-rubber.

Suction blisters

In the PUVA group, suction blisters (4) were produced before irradiation and 3 days after the first irradiation. In the UVA group, suction was carried out on the first and third day after irradiation. Six transparent plastic suction cups, each with five holes, were placed symmetrically on the abdominal skin, three cups on the irradiated and three cups on the shielded area, the latter cups at least 3 cm from the margin of the irradiated skin. Suction blisters developed after approx. 2 hours of suction with a negative pressure of 200 mmHg. The suction blister fluid (SBF) from irradiated and non-irradiated skin, collected separately in a syringe by puncture with a Mantoux needle, was transferred to EDTA-containing tubes and stored at -80°C. At the same time plasma samples were collected in EDTA-containing tubes and stored at -80°C.

Assessment of complement C3-split products (C3-split) was detected by crossed immunoelectrophoresis as described by Teisberg (5). In brief: High voltage electrophoresis with undiluted SBF was performed in the first dimension (10 V/cm for 2 hours). The second dimension electrophoresis was carried out at low voltage (4 V/cm for 18 hours) into an agarose gel containing monospecific antibodies against C3 (beta-1-A, beta-1-C, DAKO immunoglobulins, Copenhagen). The agarose gel slides were stained with Coomassie brilliant blue. The precipitates were photographed. After planimetry of the magnified photographs the complement activation index (CAI), i.e. the ratio between the areas of C3-split and C3, was calculated for each gel slide. The amounts of C3 and C3-split in SBF showed wide individual variations. Quantitative determinations of C3 were not performed, however, as the aim of the study was to demonstrate relative changes. The deviation of the ratios is therefore given in percent of the pre-irradiation values (Fig. 1). A standard lipopolysaccharide-activated normal plasma was applied throughout the study as an identity control for the C3-split precipitates.

Quantitative determinations of plasma C3 were performed before and after irradiation (rocket immunoelectrophoresis).

Statistics

Wilcoxon's rank sum test for paired observations was applied for statistical evaluation.

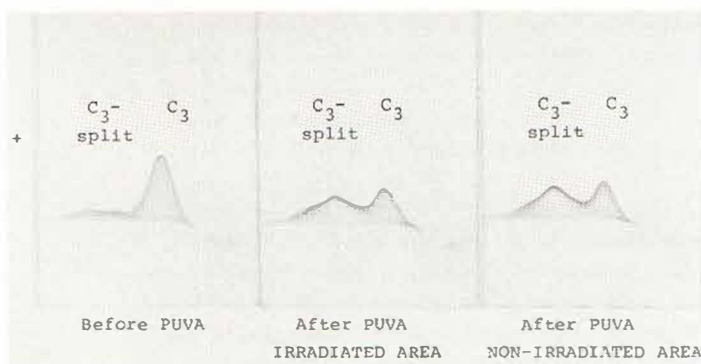


Fig. 2. Crossed immunoelectrophoresis of suction blister fluid obtained from a shielded and an irradiated skin area before and after PUVA. Activation of complement (C3-split) increased in the blister fluid from both shielded and PUVA treated skin areas. + indicates the position of the anode.

RESULTS

Most of the subjects in the PUVA group developed mild erythema of the irradiated skin and a moderate pruritus. No such symptoms were recorded after UVA. The plasma C3 was normal in all individuals, both before and after irradiation. C3-split was not found in any of the plasma samples. In most individuals a slightly increased level of C3-split was found in SBF on the first examination (Fig. 2).

After PUVA treatment, CAI increased significantly ($p < 0.05$) in SBF from both irradiated (in 7 out of 7 subjects) and shielded (in 6 out of 7 subjects) areas (Fig. 1). CAI increased from day one to day 3 after UVA in SBF from irradiated areas in 5 out of 7 individuals, but only in 2 subjects from the shielded area (Fig. 1). These variations are not statistically significant. No differences were found between the PUVA and the UVA groups as regards the slightly increased levels of C3-split in SBF on the first determination.

DISCUSSION

Though the suction blister technique is gentle, an activation of the complement system assessed by the increase in C3-split is to be expected. An *in vitro* activation of complement in undisturbed plasma has been described (6), and some individual variations in the degree of activation could therefore be expected after identical stimuli. Actually, we found a minor and individually varying increase in C3-split in the SBF before light exposure. The experimental procedure, however, was rigorously standardized throughout the study in order to ensure the validity of the relative rather than the absolute deviations.

The amount of C3-split in SBF increased after PUVA, which seems to imply that the PUVA treatment rather than the suction itself caused the splitting of C3. Coburn et al. (7) found granular deposits of C3 along the dermo-epidermal junction, and C3 in the upper dermal vessel walls in non-lesional skin of patients receiving PUVA therapy. The significance of these findings is uncertain. The increase in C3-split indicates that the complement system had been activated. A simple degradation of C3 without activation of the complement cascade, mediated by enzymes released secondary to tissue injury from the erythema, is unlikely, since the increased C3-split was found also in SBF from the non-erythematous, shielded areas.

In humans it has been shown that both PUVA and UVA irradiation can induce an increased leakage of plasma proteins in the skin vessels (8, 9). In pig skin an increased microvascular permeability was demonstrated after treatment with anthracene and UVA (10). If the demonstrated complement activation is the cause of the increased vascular permeability after PUVA, one would expect the activation to take place within the vessels

and to be accompanied by circulating C3-split. However, we were unable to demonstrate measurable C3-split in any of the plasma samples either before or after irradiation. Accordingly, we suggest that the increased vascular permeability is an event primary to an extravascular complement activation, which might increase or sustain the microvascular leakiness to macromolecules. The mechanism of complement activation, however, remains obscure.

The finding of an increase in C3-split after irradiation in SBF from the shielded areas supports the hypothesis put forward by Staberg et al. that humoral factors may play a major role in the increased microvascular permeability demonstrated after both UVA and PUVA (8, 9). In the present study, the complement activation was greater in the PUVA group than in the UVA group. This difference is not readily explained, but might be due to quantitative or qualitative differences of the assumed extravascular complement activation. The patients who received PUVA developed a mild erythema, whereas those receiving UVA did not. Rosario et al. (11) showed both qualitative and quantitative differences between equally erythemogenic doses of UVA and PUVA in various histological changes in the skin following irradiation. Both treatments induced tissue damage in the epidermis (dyskeratosis, spongiosis, intracellular oedema) and dermis (inflammatory cell infiltrate, endothelial swelling, nuclear dust) but the changes after PUVA, were more severe and persisted longer than after UVA. The significance of C3-split in SBF after long-wave UV light is uncertain, but we propose that the leakage of plasma proteins after UVA and PUVA is followed by a subsequent activation of complement components. This activation might perpetuate the vicious circle responsible for the delayed erythema after PUVA treatment.

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