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INVESTIGATIVE REPORT

Phototherapy Reduces the Number of Epidermal and CGRP-positive Dermal Nerve Fibres

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The purpose of this study was to gain an understanding of why phototherapy relieves itching. Skin samples (3 mm punch biopsies) from non-inflamed gluteal skin of 10 patients undergoing phototherapy were compared before and after 20 treatments. All the cutaneous nerve fibres were visualized by antibodies against PGP 9.5, sensory nerve fibres by antibodies against calcitonin gene-related peptide (CGRP) and capsaicin-sensitive primary nociceptive afferents by antibodies against VR1-receptor. Following treatment, the number of PGP 9.5-positive nerve fibres in the epidermis was reduced from 193 ± 52 to 102 ± 34 ($p < 0.0001$) and the number of CGRP-immunoreactive nerve fibres, which occurred only in dermis, was reduced from 28 ± 15 to 22 ± 7 ($p = 0.04$). The VR1-immunoreactive nerve fibres, some of them containing immunoreactivity to CGRP, were not affected. The success of phototherapy in combating itch may at least partly be linked with the reduction in the number of epidermal nerve fibres. The reduction in the number of CGRP-immunoreactive nerve fibres in the dermis may contribute to the beneficial effects of UV irradiation on the inflammatory process. *Key words: phototherapy; itch; nerve fibres; CGRP; PGP 9.5; vanilloid receptor.*

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More than 3000 years ago, the Greeks used sunlight for therapy. In 1890, Niels Finsen initiated the use of artificial UV light from a carbon arc in treating lupus vulgaris and other skin disorders, being awarded the Nobel Prize for this in 1903. Goeckerman was the first to thoroughly document the effectiveness of crude coal-tar application followed by exposure to UV radiation in treating psoriasis (1). It later became obvious that UV radiation alone could clear up psoriasis, UVB having been used for this during the past 70 years (2). Conventional broadband UVB (290–320 nm) has been refined and partly replaced by narrowband UVB (314 nm), TL01 (3, 4). Atopic dermatitis, often treated by a combination of UVA and UVB, has been shown recently to respond to TL01 (5, 6). Photochemotherapy comprising UVA in combination with psoralen has been found to be useful in the treatment of all severe skin disorders (7).

Many skin disorders are associated with itch which parallels the course of dermatitis. As inflammation in the skin clears, the itch disappears. Sometimes, pruritus occurs in apparently normal skin.

Phototherapy has been used to control the itch in primary biliary cirrhosis and uraemia, and the intense pruritus of the acquired immune deficiency syndrome, in which the skin is not inflamed (8–10). The success of UV light in treating itch has been attributed to its effect on the distribution of mast cells, their degranulation and the release of histamine (11, 12). A single exposure to UVB, which induces an erythema, has been shown to cause mast cell degranulation within an hour. Immediately after the onset of erythema, the histamine content of the suction blister fluid rises approximately fourfold compared with control values, returning to baseline within 24 hours (11). The fact that repeated UV exposure produces no erythema can be explained by mast cell degranulation and by the tachyphylaxis of the histamine effects. Such effects on the mast cells may also contribute to the reduction in itching. Phototherapy of pruritus is often useful, however, when antihistamines have failed. Thus the effectiveness of UV in the treatment of itch cannot be explained on the basis of the suppression of mast cell histamine alone, although there is often a close morphological relationship between the mast cells and nerve fibres. The obvious question is whether phototherapy changes the occurrence and distribution of cutaneous nerve fibres. To investigate this, we quantified the nerve fibres in punch biopsies of the gluteal skin of patients with different skin disorders that had been treated by phototherapy three times a week for about 6 weeks. The biopsies were taken from non-inflamed skin so as to avoid any contribution of neurogenic inflammation of the tissue (13, 14).

MATERIALS AND METHODS

Patients and phototherapy

Thirteen randomly assigned patients at the Department of Dermatology (7 men and 6 women, mean age 33 years, range 20–62), who were receiving phototherapy (UV given as UVA/UVB, TL01 and PUVA) for atopic eczema (5 patients),

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Table I. *Clinical details on the 10 patients who completed the study, and results of immunofluorescence analysis of nerve fibres in skin biopsies taken before and after UV therapy*

No./sex/age (years)	Diagnosis	Site	UV source (no. of treatments)	Effect of treatment	Total number of PGP 9.5-IR nerve fibres mean (range)		Epidermal PGP 9.5-IR nerve fibres mean (range)		CGRP-IR nerve fibres mean (range)	
					Before	After	Before	After	Before	After
1/M/21	Atopic eczema	Trunk and extremities	UVA/UVB (23)	Improved	237 (232–240)	210 (178–248)	151 (131–169)	99 (90–110)	14 (11–17)	28 (19–42)
2/F/21	Atopic eczema	Face, neck and trunk	UVA/UVB (19)	Improved	364 (307–394)	254 (240–269)	293 (300–323)	93 (88–97)	49 (48–51)	19 (14–25)
3/M/30	Psoriasis	Extremities	TL 01 (22)	Improved	229 (193–256)	132 (123–143)	179 (165–188)	32 (26–37)	14 (11–17)	14 (12–16)
4/F/56	Nummular eczema	Extremities	TL 01 (15)	Cleared	224 (196–271)	233 (196–259)	125 (115–144)	90 (86–105)	6 (5–7)	15 (11–17)
5/F/36	Nummular eczema	Almost all body	TL 01 (16)	Cleared	277 (266–296)	335 (319–353)	173 (153–199)	238 (229–256)	19 (16–22)	25 (15–36)
6/F/20	Atopic eczema	Face, arms	TL 01 (16)	Much impr.	264 (257–270)	240 (222–270)	157 (152–166)	95 (80–104)	26 (24–29)	21 (12–29)
7/F/28	Psoriasis	Trunk, extremities	TL 01 (15)	Improved	325 (301–349)	335 (308–344)	235 (215–259)	97 (88–108)	47 (40–55)	28 (20–36)
8/M/62	Nummular eczema	Extremities, hands	TL 01 (23)	Much impr.	384 (323–468)	263 (229–297)	113 (93–140)	88 (64–113)	12 (5–18)	18 (12–26)
9/M/20	Psoriasis	Trunk, extremities	PUVA (16)	Cleared	440 (357–527)	286 (256–311)	239 (228–250)	63 (49–84)	52 (43–64)	15 (13–19)
10/M/21	Psoriasis	Arms, back	TL01 (15)	Cleared	534 (478–574)	218 (197–248)	267 (240–291)	117 (106–125)	41 (38–45)	33 (29–40)

PGP 9.5, protein gene product 9.5; CGRP-IR, calcitonin gene-related peptide-immunoreactive. Much impr.: much improved.

nummular eczema (3 patients) or psoriasis (5 patients), were included in the study (Table I). The light was applied to the whole body.

The reason for phototherapy was the dermatitis, itch being a minor problem in all cases. Those participating were enrolled by a nurse at the phototherapy unit, informed consent was obtained in all cases. The study was approved by the Ethics Committee of Lund University Medical Faculty.

Punch biopsies (3 mm diameter) were taken from normal skin in the upper gluteal region, before phototherapy. Only patients whose gluteal skin was clear of dermatitis were selected. The initial dose was determined by skin type and was then successively increased, the speed of increase depending upon the degree of erythema produced. Irradiation was repeated three times weekly until a total of 15–22 doses had been given. The second and final biopsies were taken 48 hours after the last UV exposure.

Processing of biopsies

The specimens were fixed by immersion overnight in a mixture of 2% formaldehyde and 0.2% picric acid solution in 0.1 mol/l phosphate buffer (pH 7.2) and were then thoroughly rinsed in a Tyrode solution containing 10% sucrose. They were frozen on dry ice and were serially sectioned at 10 μm thickness in a cryostat. The sections were processed for immunocytochemistry, using antibodies against the neuropeptide CGRP and a pan-neuronal marker (protein gene product 9.5, PGP 9.5). The antibodies against CGRP were raised in guinea pig (working dilution 1: 1200, Euro Diagnostica, Malmö, Sweden) and were used to demonstrate C- and A δ-fibres. The PGP 9.5 antibodies (working dilution 1:200, Ultraclone, Cambridge, UK) were used to visualize all the cutaneous nerve fibres. Importantly, PGP 9.5 is a cytoplasmic constituent present in all parts of the neurone – the cell body as well as all processes. Data on the specificity of the antibodies employed have been presented (15). Antibodies against the vanilloid receptor, VR1, were raised in rabbit (working dilution 1: 1200, Euro Diagnostica) and were used to identify capsaicin-sensitive primary nociceptive afferents (16). Vanilloid capsaicin, the main agent contained in hot pepper, is known to release neuropeptides from sensory nerve fibres (17).

Double staining for CGRP and VR1 was performed by simultaneous incubation with the primary antibodies overnight at 4°C, followed by incubation with the secondary antibodies (anti-guinea pig IgG labelled with FITC and anti-rabbit IgG labelled with Texas Red) for 1 hour at room temperature. Three consecutive sections of all biopsies were studied. The microscope used was a Leica Aristoplan (epifluorescence microscope). The numbers of immunoreactive nerve fibres in the epidermis and the dermis were assessed visually at $\times 250$ magnification. All immunopositive nerve fibre fragments in the whole biopsy section were counted. As a single undulating and branching nerve fibre may present itself more than once, the numbers reported are referred to as nerve fibre profiles. Blind evaluation of the biopsies was performed by a single observer. Micrographs were taken using Kodak TriX film.

Statistical analysis

The statistical evaluation is based on the mean of the counts in three sections, from each of the 10 patients. Results are expressed as mean $\pm$ SEM. Student's paired *t*-test was used for comparing the skin biopsies before and after the UV irradiation.

RESULTS

Details of the patients, treatments and skin biopsy results are summarized in Table I. Two patients who failed to appear for the second biopsy, and one patient who received only 8 treatments, are omitted from Table I. The statistical evaluation concerns 10 patients altogether. The inflammatory skin disease improved considerably in all the patients during treatment, and any itch that was present, ceased.

PGP 9.5-positive nerve fibres were found to be densely distributed in the epidermis, in the subepidermal layer as free nerve endings and in the upper dermis around blood vessels (Fig. 1a). The mean number of all PGP 9.5-positive nerve fibre profiles found in the biopsies was 328 ± 85 before treatment. After treatment, the number of positive fibres were reduced to 249 ± 50 ($p = 0.002$) (Fig. 1b, Fig. 2a).

After phototherapy the remaining nerves appeared thicker. The most striking reduction was observed in the innervation of the epidermis, the number of PGP 9.5-positive nerve fibre profiles being reduced from 193 ± 52 to 102 ± 34 ($p < 0.001$) (Fig. 1b, Fig. 2a). The intraepidermal reduction in the number of PGP 9.5-immunoreactive nerve fibre profiles accounted for the whole reduction in these fibres found after treatment, the number of intradermal nerve fibres remaining unchanged.

The CGRP-immunoreactive (CGRP-IR) profiles were found as single nerve fibres, most of them just beneath the epithelium, and sometimes also occurring around blood vessels in the upper dermis (Fig. 1c). The mean number of CGRP-IR nerve fibre profiles found in the biopsies was 28 ± 15 before treatment. After treatment, the number of CGRP-IR nerve fibre profiles was reduced to 22 ± 7 ($p = 0.04$) (Fig. 1d, Fig. 2b).

VR-IR was found in the thin nerve fibres just beneath the epidermis but was totally absent in the epidermis (Fig. 3a). The mean number of VR1-carrying nerve fibres, which varied markedly between individuals, was identical before (47 ± 23) and after (47 ± 33) treatment. Some VR1-IR structures were found to co-exist with CGRP (Fig. 3b).

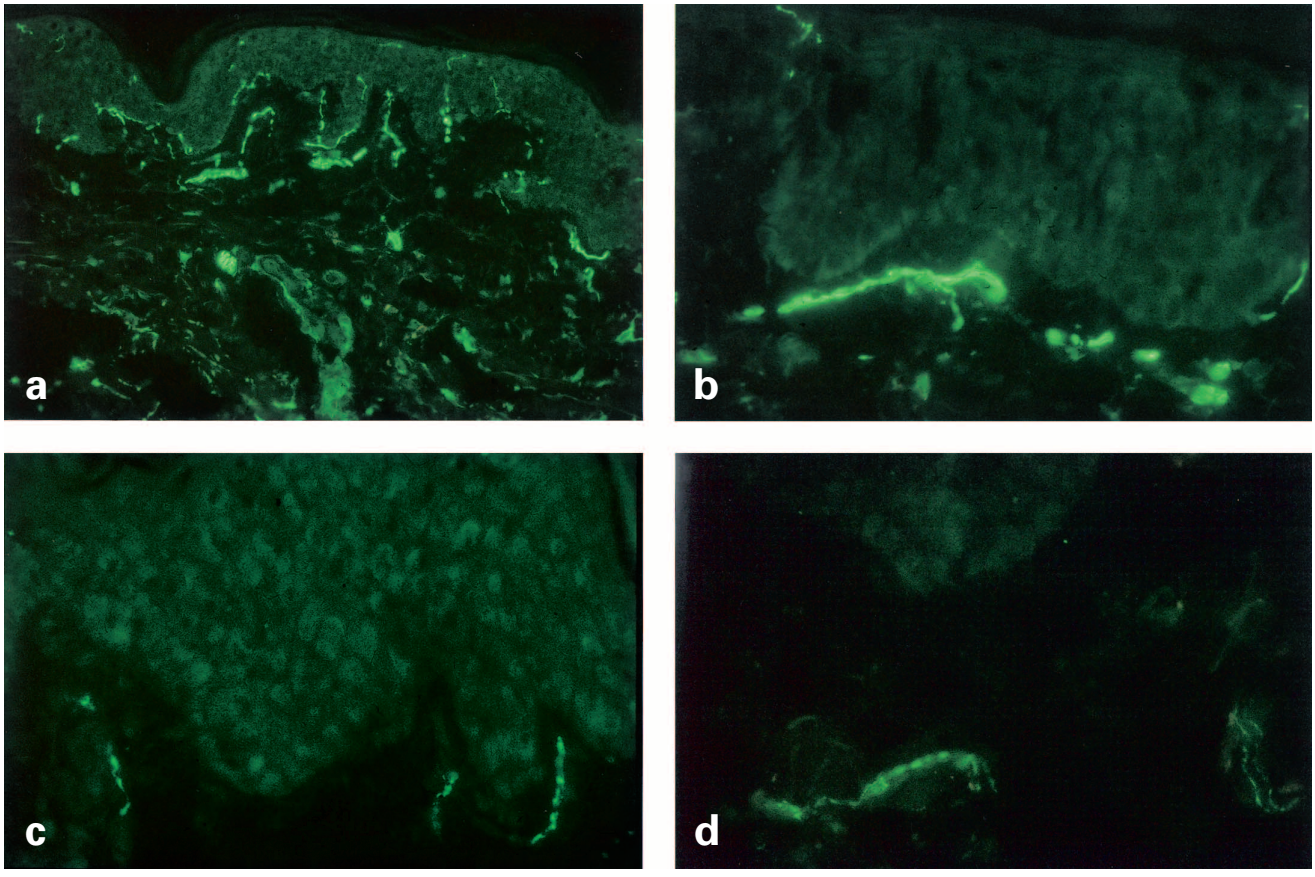

Fig. 1. Skin biopsies taken before (a, c) and after (b, d) phototherapy. Immunostaining for PGP-9.5 (a, b) shows the total cutaneous innervation. Before therapy the PGP-immunoreactive nerve fibres are distributed both in the epidermis and the dermis. (Magnification $\times 175$.) After phototherapy, there is an absence of intraepidermal nerve fibres and an apparent thickening of subepithelial nerve fibres. (Magnification $\times 250$.) Immunostaining for CGRP-immunoreactive nerve fibres (c, d). Before therapy CGRP-immunoreactive nerve fibres are distributed superficially in the dermis. (Magnification $\times 250$.) After therapy the nerve fibres are fewer in number but appear to be thicker as compared with those in the skin biopsy taken before phototherapy. (Magnification $\times 250$.)

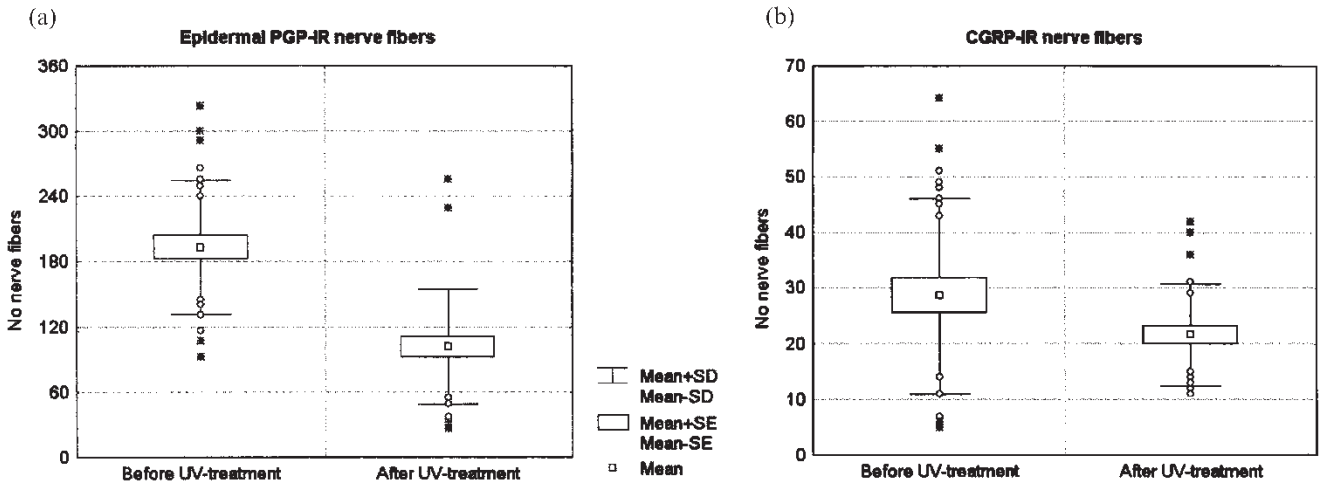

Fig. 2. The numbers of epidermal PGP-immunoreactive (PGP-IR) (a) and calcitonin gene-related peptide-immunoreactive (CGRP-IR) (b) nerve fibres found before and after phototherapy. The difference in PGP-IR fibres is statistically significant ($p = 0.04$).

DISCUSSION

In the present study, UV therapy was found to be successful in treating the inflammatory skin disease and the itch of all the patients. Numerous inflammatory mediators are involved in skin disease, histamine being the main mediator of itch (18). Mast cell histamine is known to participate in neurogenic inflammation and to stimulate sensory nerve fibres to release such neuropeptides as substance P and CGRP. The released neuropeptides will then stimulate new mast cells to degranulate. The final result reflects the combined, direct vascular effects of the histamine, neuropeptides and other bioactive agents released from the mast cells and the sensory nerve fibres (19). Administering the local anaesthetic xylocaine intracutaneously was found earlier to abolish the histamine-evoked flare, but to have no effect on the weal response (20). The flare reaction thus appears to depend upon the dermal nerve supply being intact. The itch and flare evoked by intradermal injection of the histamine liberator compound 48/80 have also been shown to be reduced by repeated UVA, UVB or PUVA treatment (21).

The number of the nerve fibre profiles in the gluteal skin in our study was approximately the same as those in a previous report on the skin of the thigh, where around 250 PGP 9.5-positive nerve fibre profiles were found per mm^2 of projected skin surface (22).

In the present study, phototherapy was found to reduce the number of cutaneous nerve fibre profiles, especially the number in the epidermis. This accords with findings reported for photodamaged skin a loss of epidermal nerve fibres in skin; biopsies from sun-exposed areas as compared with sun-protected skin was demonstrated by electron microscopy (23). Our findings show that nerve fibres become fewer but thicker after phototherapy. This phenomenon may indicate a remodelling of the nerve fibres and not necessarily a degeneration. Other treatments of localized itch, such

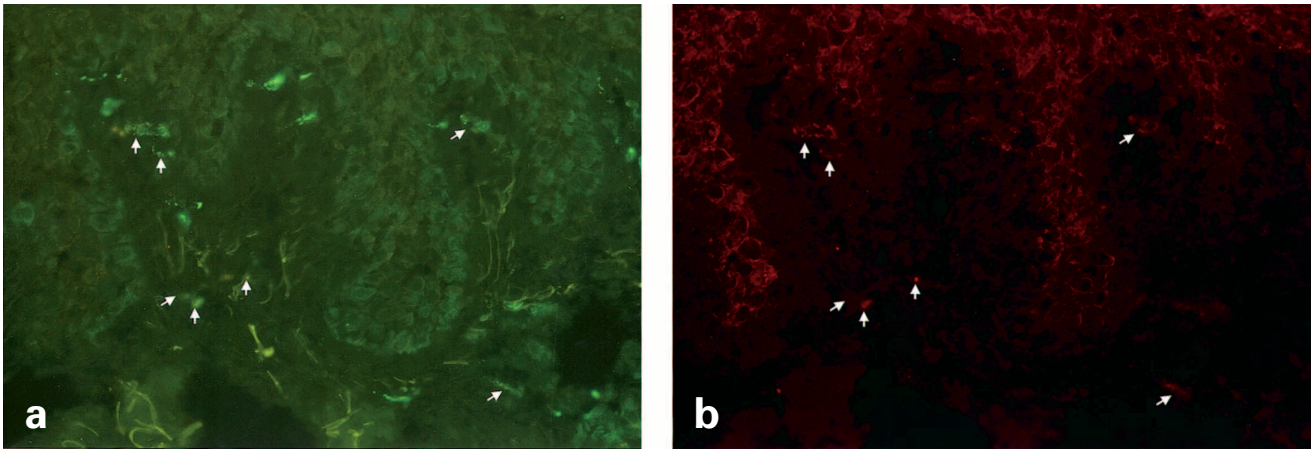

Fig. 3. Skin biopsy taken before phototherapy, immunostained for the capsaicin receptor VR1 (a) and for CGRP (b). VR1-immunoreactive nerve fibres are distributed in the subepithelial part of dermis, at the border to epidermis. Only a few VR1-immunoreactive nerve fibres are identical with CGRP-immunoreactive nerve fibres (indicated by arrows in a and b). Magnification $\times 250$.

as use of capsaicin (24) or cutaneous nerve stimulation (25) have also been shown to reduce the number of epidermal nerve fibres. In the present study, we showed that the intraepidermal nerve fibres are not carriers of the capsaicin receptor. In a recent report, VR1-IR was not found to co-exist substantially in the nerve fibres and terminals that contain substance P or CGRP (26). In the present study, VR1-IR structures were found in the subepithelial nerve fibres, occasionally co-existing with CGRP immunoreactivity. Interestingly, our study shows that UV treatments induce a reduction of CGRP-IR dermal nerve fibres, while the VR1-IR structures remain unaffected. The reduction of CGRP-IR dermal nerve fibres could explain the effects that these treatments have on inflammation, as the processes involved occur in the dermis.

In conclusion, serial phototherapy that induces a clearing up of inflammatory skin disease and itching is accompanied by a reduction of cutaneous nerve fibres. We suggest that the reduction of nerve fibres seen in the epidermis can account for the antipruritic effect of phototherapy and that the reduction in nerve fibres in the dermis can explain the reduction in inflammation following the treatment. As the VR1-expressing nerve fibres are not influenced by the UV treatment, it seems that the target of phototherapy differs from that of capsaicin.

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