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Photodynamic Properties of Sn-protoporphyrin: Clinical Investigations and Phototesting in Human Subjects

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Pure synthetic metalloporphyrins have been developed for experimental and clinical use as inhibitors of heme oxygenase, the rate limiting enzyme in the catabolism of heme to bilirubin. Tin (Sn)-protoporphyrin is one such compound, which potently suppresses bilirubin production and thus jaundice in animals and man. We have previously reported that this metalloporphyrin in conjunction with UVA might be useful as a treatment for psoriasis. To assess the photodynamic properties of Sn-protoporphyrin, 31 subjects were investigated with regard to photosensitivity. In all subjects, phototesting using UVB, UVA, and visible light as well as photopatch testing was performed. Our investigations revealed that 16 of the 31 individuals treated with Sn-protoporphyrin developed a mild photosensitivity, mainly erythema of the hands and face, and in some cases a mild conjunctivitis. The duration of this sensitivity, which in no case caused discomfort, was dose-dependent and ranged from several weeks to 1–3 months. After administration of Sn-protoporphyrin, lower thresholds were found for both UVA and visible light, but the sensitivity for UVB was normal and photopatch tests were negative. In summary, the photosensitivity observed during Sn-protoporphyrin administration was of limited duration and magnitude and did not occur in all subjects. Thus, the combination of photoactive synthetic metalloporphyrins and artificial light might prove to be useful as a regimen for the treatment of skin disease. ***Key words: Photosensitivity; Synthetic metalloporphyrins; Heme oxygenase inhibitor.***

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Pure synthetic metalloporphyrins have been developed for experimental and clinical use as inhibitors of heme oxygenase, the rate limiting enzyme in the catabolism of heme to bilirubin (1–6). Tin (Sn)-protoporphyrin is one such compound, which potently suppresses jaundice in animals and man. In the small doses used clinically, Sn-protoporphyrin has proved to be innocuous (3, 4, 7).

There have been to-date no published reports on clinical effects relating to photosensitivity produced by Sn-protoporphyrin in man. We have previously reported that this metalloporphyrin in conjunction with UVA might be useful as a treatment for psoriasis (8, 9). In this study we investigated in detail the clinical photosensitization phenomenon in man and report results of phototesting in individuals treated with Sn-protoporphyrin. Although Sn-protoporphyrin has been superseded by the heme analogue Sn-mesoporphyrin for clinical studies in newborn jaundice, because of its more desirable photophysical and other properties, the photosensitization which can be elicited by Sn-protoporphyrin make it of special dermatological interest.

MATERIALS AND METHODS

Subjects

Altogether 31 subjects, 21 men and 10 women, 9 of whom were normal volunteers, e.g. medical students and medical staff, were investigated. Of the 22 patients participating in the study, 11 had chronic liver disease, and 11 were psoriatics described in detail earlier (7–9). Before participation in the study, the patients underwent a full clinical examination and extensive biochemical tests as described before (7). The tests were repeated at the end of the study and no differences compared with baseline levels were observed. The study was approved by the ethical committee of Huddinge University Hospital, the Karolinska Institute, and the clinical trial department of the Swedish Drug Agency (Socialstyrelsens läkemedelsavdelning), and all subjects participating gave informed consent. Throughout the study no restrictions of normal life pattern was given. However, the participants were advised to avoid excessive sunbathing and/or artificial UV-light. Patients no. 7–16 received suberythematose UVA-treatment as part of a clinical trial for treatment of psoriasis (8). Individuals with known photosensitivity were not entered into the study, and the participating subjects were graded into skin types I–V on the basis of their recall of their reactivity to natural sunlight (10).

Experimental procedure

Fasting blood samples for measurements of various biochemical indices, including bilirubin and hematological values, were taken each morning for 3 days before entry to the study, and for 10 days after the administration of the metalloporphyrin. Sn-protoporphyrin was infused intravenously and a second identical infusion was given 8 h later as described before (7). The total dose given was 2 μmol/kg in 16 subjects and 1 μmol/kg body weight in 15 subjects. The duration, onset and body localization of the artificially induced light-sensitivity were assessed both by history and by clinical examination. The examinations were carried out by the same investigator (LE) on all occasions. The investigator was aware of the treatment of the subjects; thus this was not a blind study. Representative skin areas were documented by photography.

UVA-testing

In 25 subjects UVA threshold values were measured with a Waldmann UVA 800 unit (Waldmann, Schwenning, Germany) before administration of Sn-protoporphyrin (1 μmol/kg body weight) and for 2–4 days after the infusion. Six of the subjects given 2 μmol/kg body weight had no predetermined UVA threshold value, and in those the UVA-testing was done 4–6 weeks after the injections (nos. 1–6 in Table I). Each subject's back was illuminated through a green cloth in which had been cut standardized open squares (5 × 5 cm). 5, 10, 15, and 25 J/cm^2 of UVA were given to each square. The test areas were evaluated 24 h after the irradiation. A positive minimum erythematous dose was defined as the lowest dose of UVA to produce an erythematous reaction in shape of a square (at least three corners had to be visible to be regarded as a positive reaction). The output of the lamps was measured weekly with a Waldmann meter type 585 100.

Table I. *Clinical photosensitivity and results of phototesting in subjects receiving a high dose of Sn-protoporphyrin (2 μmol/kg), n = 16*

Subjects no.	Sex	Age (yrs)	Diagnosis	Skin type (ref. 10)	Month of injection	Localization of photosensitivity				Dose of UVA (J/cm²) producing MED[1]	
						Conjunctiva	Face	Neck	Hands	Before Sn-PP	After Sn-PP
1.	M	33	Control	II	May	X	X	X	X	nd[2]	10
2.	M	33	Control	II	Apr	X	X	X	X	nd	5
3.	M	55	Hemochromatosis	II	Apr	X	X	–	X	nd	10
4.	F	67	Hemochromatosis	III	Apr	X	–	X	X	nd	5
5.	M	25	Control	II	May	X	–	–	–	nd	10
6.	F	23	Control	III	Apr	–	X	–	X	nd	20
7.	M	54	Psoriasis	III	Apr	–	–	–	–	>25	10
8.	M	61	Psoriasis	III	Feb	–	–	–	–	>25	15
9.	F	60	Psoriasis	III	Oct	–	–	–	X	>25	5
10.	M	59	Psoriasis	III	Apr	X	–	–	–	>25	10
11.	M	48	Psoriasis	III	Mar	X	–	–	–	25	10
12.	M	62	Psoriasis	III	Apr	–	–	–	–	>25	5
13.	M	58	Psoriasis	I	Dec	–	–	–	–	>25	10
14.	M	34	Psoriasis	III	Feb	X	–	–	X	>25	10
15.	M	42	Psoriasis	II	Mar	X	–	–	X	>25	5
16.	M	41	Psoriasis	III	Apr	X	–	–	–	>25	5

[1] MED: minimal erythemal dose; [2] nd: not done.

UVB-testing

In 8 subjects, UVB threshold values were measured in a similar manner as for UVA threshold, but here a XBO 150W high pressure xenon lamp (Zeiss, Oberkocken, Germany) and a WG 295 Schott filter (Schott, Mainz, Germany) were used.

Photopatch testing

Sn-protoporphyrin (1.4, 2.8, 5.6, 8.4, 11.2 and 56 mM) in distilled water was applied in duplicate on the lower back using Finn chamber discs, and the skin areas were subsequently covered with light-proof black paper. The covers were removed 6 h later in dim light; one skin area was immediately covered, and the corresponding area was exposed to 50% of the predetermined UVA threshold dose, i.e. 2.5–10 J/cm² UVA was used. Evaluation was performed 48 h after the irradiation.

Visible light testing

Testing for sensitivity to visible light was performed as described above for UVA. Here a lamp in an operating theatre (Angénieux 42570 type AX14 300 × 24 W, France) was used at a distance of 1 m and the open squares of the green cloth were 10 × 10 cm. The test areas were evaluated 24 h after the irradiation.

Table II. *Clinical photosensitivity and results of phototesting in subjects receiving a low dose of Sn-protoporphyrin (1 μmol/kg), n = 15*

Subjects no.	Sex	Age (yrs)	Diagnosis	Skin type (ref. 10)	Month of injection	Localization of photosensitivity				Dose of UVA (J/cm²) producing MED[1]	
						Conjunctiva	Face	Neck	Hands	Before Sn-PP	After Sn-PP
17.	F	70	Primary biliary cirrhosis	III	May	–	–	–	–	>25	20
18.	M	24	Hemochromatosis	III	Dec	–	–	–	–	>25	5
19.	M	27	Primary biliary cirrhosis	III	Dec	–	–	–	–	>25	15
20.	M	41	Control	III	Apr	X	X	–	–	25	10
21.	M	42	Primary biliary cirrhosis	II	Apr	X	–	–	X	>25	10
22.	F	55	Primary biliary cirrhosis	I	Apr	–	–	–	–	>25	>25
23.	F	49	Psoriasis	IV	Apr	–	–	–	–	>25	15
24.	M	32	Control	II	Oct	–	X	–	–	>25	15
25.	F	65	Alcoholic cirrhosis, hypersplenism	III	Nov	–	–	–	–	>25	5
26.	M	27	Control	II	Oct	–	X	–	X	>25	15
27.	M	42	Primary biliary cirrhosis	III	Nov	–	–	–	–	>25	10
28.	F	51	Primary biliary cirrhosis	III	Jan	–	–	–	–	>25	10
29.	M	38	Hemochromatosis	III	Nov	–	–	–	–	>25	10
30.	M	38	Control	II	Dec	–	–	–	–	>25	15
31.	F	71	Primary biliary cirrhosis	III	Oct	–	–	–	–	>25	>25

[1] MED: minimal erythemal dose.

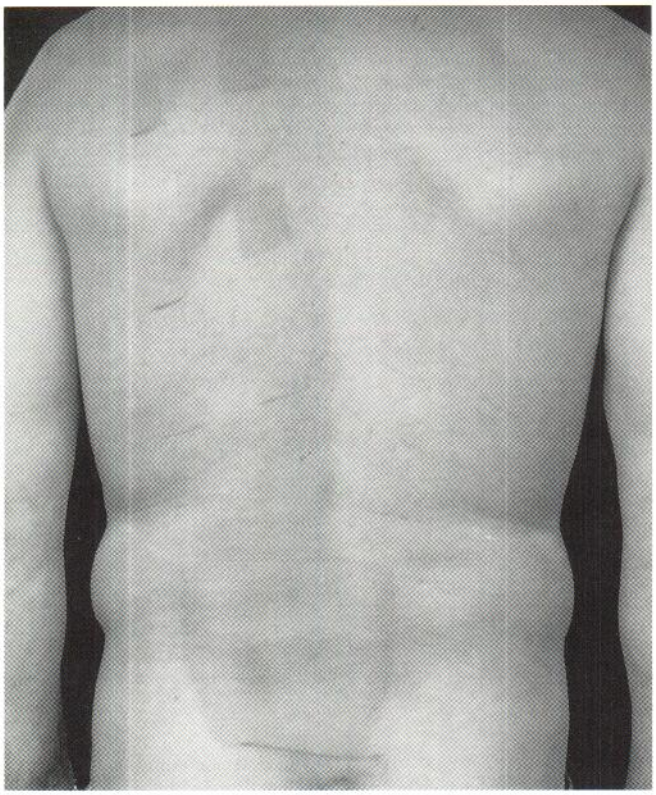

Fig. 1. Results of phototesting a subject after the administration of 2 μmol/kg of Sn-protoporphyrin. 48 h after the injections the upper left squares have been irradiated by 25, 15, 10 and 5 J/cm^2 UVA light and the lower square on the lumbar region by visible light for 30 min.

RESULTS

Clinical data and the results of the UVA-testing are found in Tables I and II. A moderate photosensitivity, with a sudden onset, and in 3 subjects also itch, were clinically apparent among 16 of the 31 Sn-protoporphyrin treated subjects after the injections. Clinical photosensitivity occurred in 8 of the 9 subjects with a skin type II and in 6 of 19 subjects with a skin type III. This light sensitivity was also demonstrated in the objective light test performed in the study. Mild erythemal reactions were noted in the face and on the hands, as well as a slight conjunctival injection. As seen in Tables I and II, the severity of the sun sensitivity was found to be dose-dependent. The persons receiving Sn-protoporphyrin at a dose of 2 μmol/kg body weight showed a relatively higher sensitivity compared to those receiving the lower dose of the metalloporphyrin (1 μmol/kg body weight). The duration of the sensitivity was 2–3 months in the high dose group, but only 4–6 weeks in the low dose group; during these weeks the photosensitivity steadily declined. In no case was the sensitivity troubling enough to cause the subjects to refrain from work. In a few cases, anamnestic reports on reaction to sunlight passing through window-glass and light from ordinary light-bulbs were obtained, indicating an action spectrum of the drug in the visible light region of the solar spectrum. There were no skin reactions at the injection sites.

UVA-sensitivity and UVB-sensitivity

In all persons except 2 the UVA-thresholds were below 25 J/cm^2 after the injections with mean UVA-threshold of 10 J/cm^2 (5–25 J/cm^2) (Fig. 1; Tables I–II). All except 2 of the 15 individuals not developing a light sensitivity still had markedly lowered UVA-thresholds. All persons tested had normal threshold values for UVB after the injections.

Sensitivity to visible light

The investigation of sensitivity to visible light was made in 2 subjects (patients no. 14 and 16). One reacted with a brisk erythema and edema 24 h after a 45 min exposure to the operating theatre lamp, and the other after a 30 min exposure (Fig. 1). In normal, not sensitized individuals such a lamp is used for up to 6–8 h during surgery without causing any skin reactions.

Photopatch-testing

Five individuals were tested and no immediate or late positive reactions were recorded.

DISCUSSION

Our investigations have revealed that 16 out of 31 individuals treated with Sn-protoporphyrin developed a moderate photosensitivity, including a slight conjunctivitis. The duration of this sensitivity was dose-dependent, ranging from 1 to 3 months, with onset after the injections. Lowered thresholds for both UVA and visible light were observed. The 14 of the 15 subjects who did not develop clinical photosensitivity also had a lower threshold for UVA sensitivity following the injections. Eight of the 16 individuals without an apparent light sensitivity were treated during the winter, with small amounts of natural sunlight. One would expect a higher number of subjects with clinical photosensitivity if the drug was given during April to September.

The action spectrum of Sn-protoporphyrin is in the long-wave range of UVA and/or in the visible light region of the solar spectrum, which is consistent with the previously described absorption spectrum of the compound, showing a peak at 408 nm (11, 12). Our findings of erythema and edema following the illumination of the sensitized patients with an ordinary operating lamp are consistent with previous reports on patients treated with porphyrins (13, 14) or patients with erythropoietic protoporphyria (15) developing photosensitivity after general surgery.

Photopatch tests were negative in all 5 individuals tested, despite the fact that concentrations up to 10-fold above the therapeutic serum concentration were applied in the test. This argues against a phototoxic mechanism, although this presently cannot be completely ruled out. Phototoxicity in general should be confirmed in *in vitro* and animal models.

It should be emphasized that the described photosensitivity induced by Sn-protoporphyrin in the patients treated is mild, dose-dependent, and reversible. The substance can be given in single small doses intramuscularly or intravenously. It is not enzymatically metabolized to bile pigments *in vivo* and it has

no other known side-effects in the doses used clinically. The drug has been safely used in the treatment of hyperbilirubinemia of newborns (16), as well as in patients with liver diseases (17). The results of this study are consistent with the notion that photodynamic effects are obtained in the combined treatment with synthetic porphyrins and UV-light. These effects could be beneficial in the treatment of skin disease, and partly equivalent to PUVA-treatment. We have with some success treated psoriasis patients with Sn-protoporphyrin and UVA (8, 9). When compared to the standard regime of PUVA in these patients (18), Sn-protoporphyrin treatment offers certain advantages including the fact that no patient treated with Sn-protoporphyrin has suffered from nausea which is common in PUVA-treatment. Another potential advantage of the use of synthetic metalloporphyrins instead of psoralens for the treatment of skin disease could be that the target site for cell killing most likely is the cell membranes rather than DNA (19–21). Therefore, little or no mutagenesis would be expected from this mode of phototherapy. Recently, an increased incidence of genital invasive squamous cell carcinoma has been reported among male patients exposed to high levels of PUVA (22). In a large-scale, epidemiological study we have confirmed previous reports of a dose-dependent increase in the incidence of squamous cell cancer in patients treated with PUVA; significant increases of some internal cancers have also been observed (23).

Dihematoporphyrin ether, also known as Photofrin-II, is currently used in the treatment of a variety of epithelial neoplasms, in a modality known as photodynamic therapy. A major drawback of these porphyrins for photodynamic therapy is their ability to evoke prolonged and intense cutaneous photosensitization (for review, see 13). This photosensitization is similar to that of patients with erythropoietic protoporphyria (24). By the use of Sn-protoporphyrin in the doses of the present study this drawback may be circumvented. Newly synthesized derivatives of tin-protoporphyrins, i.e. Sn-mesoporphyrin and Sn-diiododeuteroporphyrin, have recently been shown to have reduced photoactive properties *in vitro* compared to Sn-protoporphyrin (28), and may thus offer other advantages in clinical use. Other routes to administer the synthetic porphyrins, such as the development of topical hematoporphyrin derivatives (29) or targeting of heme oxygenase inhibitors to the spleen (30), may minimize the potential problem of phototoxicity. Recently, the protoporphyrin precursor 5-aminolevulinic acid (ALA) has successfully been applied to superficial skin cancers followed by photoactivating light, selectively destroying tumors (28, 29).

In conclusion, the photosensitivity observed during Sn-protoporphyrin administration was of limited duration and magnitude and did not occur in all subjects. Thus, the combination of photoactive synthetic metalloporphyrins and artificial light might prove to be useful as a regimen for the treatment of skin disease.

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