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ORIGINAL ARTICLE

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Mild skin photosensitivity in cancer patients following injection of Photochlor (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a; HPPH) for photodynamic therapy

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Abstract *Purpose*: To measure skin photosensitivity in cancer patients infused with the new second-generation photodynamic sensitizer Photochlor (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a). A major disadvantage of using the clinically approved photosensitizer Photofrin is potentially prolonged and sometimes severe cutaneous phototoxicity. *Patients and methods*: Forty-eight patients enrolled in Phases 1 and 2 clinical trials underwent two or more exposures to four graded doses (44.4, 66.6, 88.8 or 133.2 J/cm^2) of artificial solar-spectrum light (SSL) before and after administration of Photochlor at a dose of 2.5, 3, 4, 5 or 6 mg/m^2 . *Results*: The most severe skin response, experienced by only six of the subjects, was limited to *erythema without edema* and could only be elicited by exposure to the highest light dose. Conversely, eight subjects had no discernible reaction to SSL at any light dose. For nearly all the patients, the peak skin response was obtained when the interval between sensitizer injection and exposure to SSL was 1 day and, generally, their sensitivity to SSL decreased with increasing sensitizer-light interval. For example, a 2-day sensitizer-SSL interval resulted in less severe reactions than those obtained with the 1-day interval in 79% of the subjects, while 90% of the subjects exposed to SSL 3 days after Photochlor infusion had responses that were less severe than those obtained with either the 1- or 2-day sensitizer-SSL interval. *Conclusions*: Photochlor, at clinically effective antitumor doses, causes only mild skin photosensitivity that declines rapidly over a few days.

Keywords HPPH · Photochemotherapy · Photodynamic therapy · Photofrin · Phototoxicity · Solar simulator

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Introduction

Photodynamic therapy (PDT), sometimes referred to as photochemotherapy, is widely used as a curative and palliative treatment for a variety of solid malignancies [4, 5, 10, 12, 15]. In addition, PDT can be used successfully in combination with the surgical resection of tumors [7, 9]. PDT involves the activation of a drug by visible light, resulting in the generation of cytotoxic oxygen species [17]. Treatment selectivity is achieved by the combination of local illumination and drug activation and the somewhat preferential localization of the photosensitizer to the tumor tissue. However, photosensitizers distribute into other tissues and this can pose a risk to patients exposed to intense natural or artificial light. In the case of Photofrin (porfimer sodium), the only photosensitizer approved for cancer therapy in the United States, Europe, Japan and Canada, cutaneous photosensitivity lasting 1–3 months has been reported [5, 6, 11, 13, 18]. Prolonged skin photosensitivity has also been described for the second-generation photosensitizer Foscan (tetra[*m*-hydroxyphenyl]chlorin (mTHPC)), recently approved in Europe for palliative PDT of head-and-neck cancer [11, 16]. Indeed, the reduction of this persistent skin photosensitivity is one

of the goals in the design and development of new PDT agents [14]. In this report, we present the results of solar-simulator studies in patients injected with the photosensitizer Photochlor (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH)). Photochlor is a chlorin-based compound that strongly absorbs light at around 408 nm and 665 nm (ϵ~90,000 M^{-1}/cm^1 and ϵ ~47,000 M^{-1}/cm^1, respectively) and has several other, much weaker, absorption bands throughout the visible spectrum (Fig. 1). Photochlor is a highly lipophilic drug that is not metabolized and, like Photofrin, clears the plasma slowly [1, 2]. We are investigating the efficacy of Photochlor-PDT in several ongoing Phases 1 and 2 clinical studies in patients with Barrett's esophagus with high-grade dysplasia, obstructive esophageal cancer, early- or late-stage lung cancer or basal cell carcinoma [1]. Most of these patients were exposed to solar-spectrum light (SSL) before, and each day up to 3 days after, injection of Photochlor in order to assess their sensitivity to sunlight. The results of the phototests are described here and discussed in the context of skin phototoxicity previously reported following PDT with the regulatory-agency-approved sensitizers Photofrin and Foscan.

Patients and methods

Photosensitizer

Photochlor was made by Ravindra K. Pandey (Photodynamic Therapy Center, RPCI) at the University of California Davis under good manufacturing practice conditions; preparation and characterization of Photochlor were as described previously [3]. The injectable drug was formulated in 5% dextrose in sterile water containing 2% ethanol and 0.1% polyethylene sorbitan monooleate (Tween 80) and kept frozen until use. All injectable Photochlor underwent sterility- and pyrogenicity-testing prior to its use in patients.

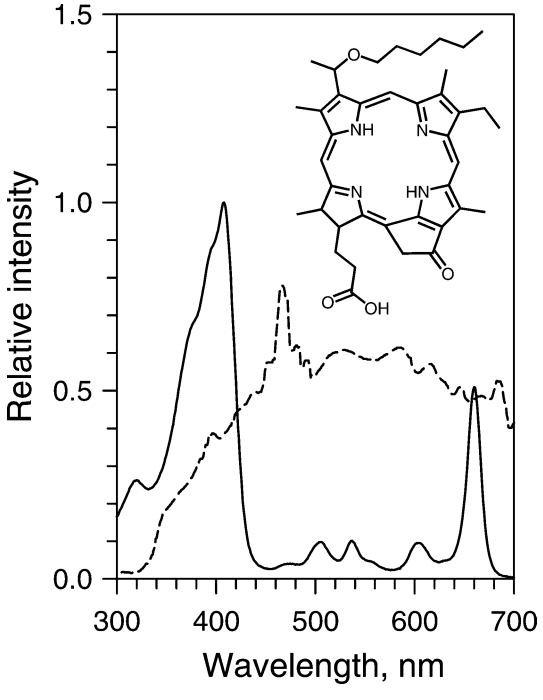

Fig. 1 Absorption spectrum (*continuous lines*) and the chemical structure of the photosensitizer Photochlor (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH)); included is the spectrum of the solar simulator with AM1.5 filter (*dotted lines*)

Patients

Forty-eight patients (40 males and 8 females), enrolled in one of seven PDT clinical trials using Photochlor as the photosensitizer, received illumination from a solar simulator. Two patients were injected intravenously with a Photochlor dose of 2.5 mg/m^2 body surface area, 13 with 3 mg/m^2 Photochlor, 20 with 4 mg/m^2 Photochlor, 3 with 5 mg/m^2 Photochlor and 10 with 6 mg/m^2; drug doses were administered over 1 h at an infusion rate of 110 ml/h. Photochlor exhibits dose-linear pharmacokinetics [1]. None of these patients had any history of photosensitivity or were taking medications that describe photosensitivity as a significant complication. Eight patients belonged to Fitzpatrick [8] skin phototype (SPT)1, 12 to SPT2, 8 to SPT3, 6 to SPT4, and 2 patients were African-Americans (SPT6); information about SPT was (unintentionally) not collected for the remaining 12 patients. These studies were approved by the Institute Review Board and the Food and Drug Administration, and informed consents were obtained from each patient before solar-simulator testing and infusion of Photochlor.

Solar-simulator illumination

Each patient was exposed to a simulated solar spectrum (350–2,500 nm; AM1.5 direct filter; the AM1.5 spectrum approximates the solar spectrum on an average sunny day on the Earth's surface; see Fig. 1) produced by a 300-W solar simulator (model 81250; Oriel Corporation, Stratford, CT, USA). In order to avoid potential site-specific variations in response, the volar forearm near the wrist (Fig. 3A) was used for phototesting for all our patients regardless of tumor diagnosis; the forearm was chosen over other anatomical locations because many of our skin cancer patients have multiple tumors on alternate test sites (e.g., chest, back, outer arm, etc.). For each test, four 1 cm^2 spots were exposed to SSL for 10, 15, 20 or 30 min at a light dose rate (fluence rate) of 74 mW/cm^2 to provide light doses (fluences) of 44.4, 66.6, 88.8 or 133.2 J/cm^2, respectively. The patients were exposed to SSL immediately before Photochlor infusion and 1 day (47/48 patients), 2 days (38/48 patients) and 3 days (20/48 patients) after; two patients were tested at additional sensitizer-light intervals (one patient at 6 days, and one patient at 30 days).

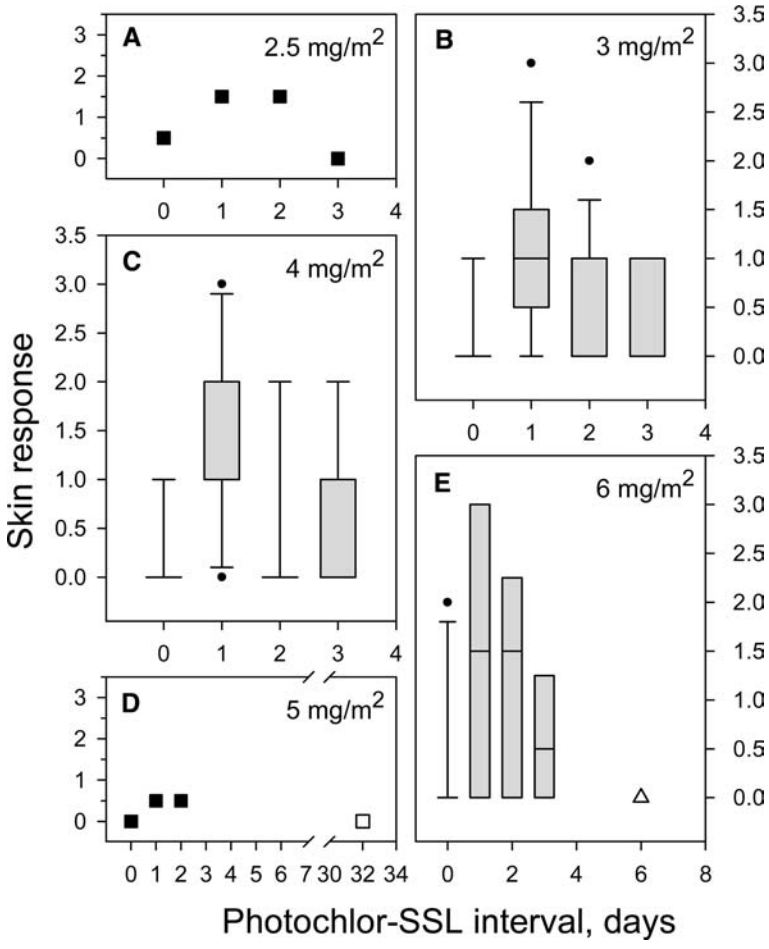

Fig. 2 Summary of skin responses as a function of interval between Photochlor infusion and exposure to solar-spectrum light (SSL). The volar forearm of each patient was exposed to 44.4, 66.6, 88.8 or 133.2 J/cm^2 SSL following infusion of Photochlor at a dose of 2.5, 3, 4, 5 or 6 mg/m^2 body surface area. For every patient the greatest skin response was always obtained with the highest light dose, so data are shown for 133.2 J/cm^2 SSL exposures only. Data are summarized as quartile plots in *panels* B, C and E as described in the text. Quartile plots could not be constructed for *panels* A and D due to an insufficient number of data points; therefore, *solid squares* represent median values. The *open triangle* in *panel* E and the *open square* in *panel* D are single data points

Clinical assessment of skin response

Skin responses were graded by two independent observers the day after each illumination and scored according to the following scale:

0*No reaction*
1*Minimal perceptible erythema, blotchy areas of faint erythema confined to the illuminated site*
2*Minimal erythema with sharp borders*
3*More pronounced erythema without edema*
4*Marked erythema with edema*
5*Marked erythema with edema and vesiculation*

Statistics

When there were a sufficient number of data points the skin responses are summarized as box (quartile) plots in Fig. 2 (box boundaries = 25th and 75th percentile, line within box, or lower box boundary if no line within box = median, whiskers = 90th and 10th percentile, solid squares = outliers). Groups with either a small number of data points or a single data point are represented by their median value only. Graphing and analysis were performed using SigmaPlot software (version 8.02; Systat Software Inc., Point Richmond, CA, USA). The possible relationships between dose of Photochlor, skin type, or gender with phototoxic response were examined with either the Cochran–Armitage Trend test or the Fisher Exact test. To do this, a phototoxic binary (yes/no) endpoint for each patient was arbitrarily defined as having at least one skin response reading of two or higher (endpoint = yes) or a skin response never >1 (endpoint = no) after phototesting. An additional phototoxic binary endpoint for each patient was defined as having at least one skin response reading of three or higher (endpoint = yes) or a skin response never >2 (endpoint = no) after phototesting. These exact tests were run with StatXact (version 4.0; Cytel Software Corp., Cambridge, UK) as 2-tailed tests, with a type I error rate of 0.05.

Results and discussion

Photochlor plus SSL did not elicit clinically significant skin responses

Most of the patients in our Phases 1 and 2 clinical studies at RPCI were exposed to graded-doses of SSL (44.4, 66.6, 88.8 or 133.2 J/cm^2) before and up to three consecutive days after receiving Photochlor (Fig. 1) at a

dose of 2.5, 3, 4, 5 or 6 mg/m^2. The responses were scored on the day after SSL exposure and are summarized in Fig. 2. Eight subjects had no response to Photochlor plus SSL at any sensitizer dose, light dose or sensitizer-light interval. Only six of the subjects had a skin response grade of 3, defined as *more pronounced erythema without edema*. This was the strongest reaction obtained in the study, elicited by only the highest light dose of 133.2 J/cm^2 (approximately equivalent to 30 min of average mid-day sunlight irradiance in the northeast United States) following a Photochlor dose of either 6 mg/m^2 ($n = 3$), 4 mg/m^2 ($n = 2$) or 3 mg/m^2 ($n = 1$).

Moderate-to-severe phototoxicity is a widely recognized risk in patients injected with Photofrin. Unfortunately, there is just a single published account of photosensitivity testing in patients receiving this photosensitizer. In that study, *erythema with urticarial swelling* developed in 25% of Japanese cancer patients administered 2 mg/kg Photofrin and exposed to only 9 J/cm^2 light from a slide projector [13]. However, there are numerous reports of phototoxicity in cancer patients that were given Photofrin and who then unintentionally exposed themselves to intense sunlight or artificial lights [5, 6, 11, 18]. Some of these exposures have resulted in clinically significant reactions (e.g., Fig. 3C; note that this response would have received a score ≥5 in our present study) that have required medical therapy. Foscan is a second-generation photosensitizer that has been approved for palliative PDT in Europe. Like Photochlor, Foscan is a chlorin-based compound with strong absorbance maxima in the violet (˜415 nm) and red (∼652 nm) regions of the visible spectrum. A study of the effect of Foscan- and light-dose, as well as interval between drug-administration and light exposure, was performed in patients undergoing PDT. The maximum skin response following SSL-irradiation testing was reported as *papular erythema*; however, the authors noted that reactions such as *blister formation* and *necrosis* were deliberately avoided via their study design for ethical reasons [16]. However, in a different study comparing the efficacy of Foscan, ALA-induced protoporphyrin IX and Photofrin, *facial erythema*, *swelling* and *blistering* was reported for a patient, who exposed herself to sunlight for 30 min after receiving Foscan a week earlier [11].

Although 50% (3/6) of the grade-3 erythema responses in the present study were recorded for the patients given 6 mg/m^2 dose of Photochlor, there was no significant relationship (Cochran–Armitage Trend test) found between Photochlor dose and the binary phototoxic endpoints used in our statistical analyses. Note that in a 19 patient subset of this data, for which we had both Photochlor pharmacokinetic data and phototoxicity data, we found that neither the predicted 24-h Photochlor plasma levels nor the AUCs (area under the time–plasma concentration curve) were correlated in any obvious way with the skin phototoxicity results [1]. Our assessment of Photochlor dose-response data in our

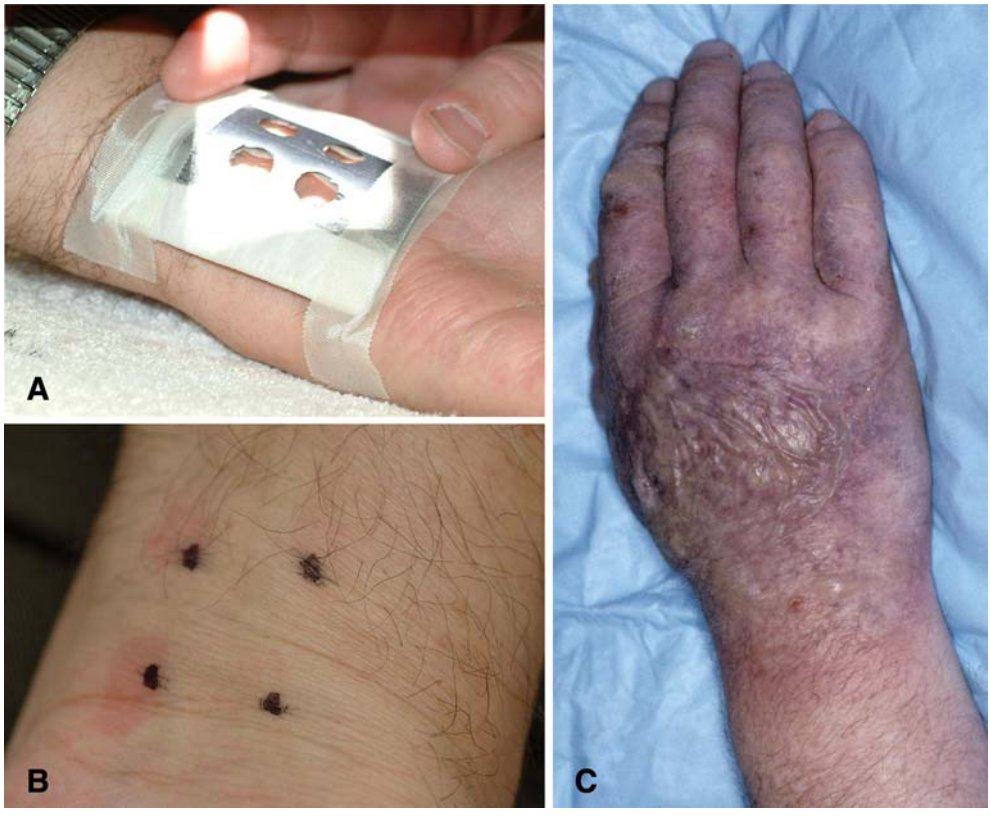

Fig. 3 **A** Setup for exposure of the skin of the volar forearm of patients to SSL. **B** Response of a representative patient exposed to SSL 1 day after receiving 4 mg/m^2 Photochlor; photograph was taken immediately after SSL exposure. The center of each exposed area was marked with black ink after the end of SSL. SSL doses were (counter-clockwise from lower right) 44.4, 66.6, 88.8 or 133.2 J/cm^2. Reactions following exposure to the three lowest SSL doses had disappeared by the 24 h evaluation interval and received scores of 0; the reaction to 133.2 J/cm^2 SSL faded over the same interval and received a score of 1(*minimal perceptible erythema, blotchy areas of faint erythema confined to the illuminated site*). **C** For comparison, cutaneous phototoxicity in a patient who was exposed to bright sunlight 8 days after receiving 2 mg/kg Photofrin. The patient was hospitalized at RPCI to receive treatment for deep second-degree burns

Phases 1 and 2 clinical trials indicates that a dose less than 6 mg/m^2, most likely in the range of 3–4 mg/m^2, will be the most effective for treating cancer patients; the use of this lower dose should limit the probability and severity of any phototoxicity reactions. In addition to the lack of a discernible Photochlor dose-photoxicity relationship, no significant relationship was found between skin type and binary phototoxic endpoints (Cochran–Armitage Trend Test) or between gender and binary phototoxic endpoints (Fisher Exact Test). It appears that the skin phototoxicity caused by Photochlor is subtle, and may be the result of a complex interplay of Photochlor dose (or skin tissue level), skin type, gender and other, as yet unknown factors; a very large number of patients may be necessary to reveal such a subtle complex multivariate relationship. When these relationships were evaluated by Moriwaki et al. [13] for Photofrin, photosensitivity was (1) greater in females than in males (at 3 weeks after injection) and (2) persisted longer in patients with SPT2 compared to SPT3/4. On the other hand, Wooten et al. [19] suggested that cutaneous phototoxicity was less severe in females than males given HPD (hematoporphyrin derivative; a slightly less-pure form of Photofrin used in earlier clinical studies).

Photosensitivity decreased rapidly after Photochlor administration

For nearly every subject, the peak responses were first obtained when SSL irradiation was performed 1 day after Photochlor injection and, generally, photosensitivity declined as the sensitizer-SSL interval was increased (Fig. 2). For example, 79% (26/33) of the subjects had less severe skin responses when SSL was delivered 48 h after Photochlor than when a 1-day sensitizer-SSL interval was used, and 90% (18/20) of the subjects that were phototested 3 days after Photochlor had lower responses with that interval than those obtained with 1- or 2-day intervals. In the remaining two patients tested at 3 days, sensitivity did not diminish with increasing interval (one subject had skin response scores of 1, and a second patient had response scores of 2, after every phototest regardless of sensitizer-SSL interval).

As described above, only 6 of 48 patients experienced skin responses of 3 (*more pronounced erythema without edema*), the highest score seen in this study. Each of these responses was elicited following a 1-day Photochlor-SSL interval and using the highest light dose of 133.2 J/cm^2. Phototesting at subsequent intervals resulted in weaker responses in five subjects. The remaining subject, who received 6 mg/m^2 Photochlor, had two consecutive skin-response scores of 3 (following 1- and 2-day sensitizer-SSL intervals); nevertheless, the response to SSL using a 3-day interval resulted in a lower score of 2 and a phototest applied 6 days after Photochlor elicited no response. Of all the cancer patients receiving Photochlor at RPCI, only one patient has complained of photosensitivity following an unintended exposure to intense sunlight (21 days after receiving 5 mg/m^2 Photochlor). He returned to RPCI 11 days later where illumination with 133.2 J/cm^2 SSL, equivalent to 30 min of sunlight exposure, could not elicit a skin reaction.

Clinical phototesting using Photofrin and Foscan show protracted cutaneous photosensitivity. In the study of Moriwaki et al. [13], 28 and 50% of male and female patients, respectively, were still sensitive to slide-projector light 3 weeks after receiving 2 mg/kg Photofrin. Solar-simulator irradiation of a single patient 13 weeks after Photofrin injection resulted in a positive skin reaction [16]. In studies where unintentional light exposures were reported [5, 6, 18], photosensitivity was found to last up to 14 weeks [11]. The decay of photosensitivity appears to be somewhat faster for Foscan than for Photofrin. Skin reactions could be elicited in cancer patients by SSL up to 5 weeks after receiving Foscan [16]. Both the duration of photosensitivity and the severity of the responses appeared to depend on sensitizer dose.

Summary and conclusion

While the number of patients receiving Photochlor is relatively small, our data show that this sensitizer elicits considerably less potential for cutaneous phototoxicity than in patients receiving Photofrin or Foscan. This was true even at short time intervals after injection, when blood- and tissue-levels of Photochlor are at their highest [1, 3]. In the long run, it will be necessary to have phototoxicity data from a larger number of patients who have received Photochlor, at doses that have been shown to give tumor responses equivalent to those with Photofrin and Foscan, in order to make a definitive comparison among these three photosensitizers.

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