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Oral Aminolevulinic Acid Induces Protoporphyrin IX Fluorescence in Psoriatic Plaques and Peripheral Blood Cells†¶

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ABSTRACT

Photodynamic therapy (PDT) with topical aminolevulinic acid (ALA) has been shown in previous studies to improve psoriasis. However, topical ALA-PDT may not be practical for the treatment of extensive disease. In order to overcome this limitation we have explored the potential use of oral ALA administration in psoriatic patients. Twelve patients with plaque psoriasis received a single oral ALA dose of 10, 20 or 30 mg/kg followed by measurement of protoporphyrin IX (PpIX) fluorescence in the skin and circulating blood cells. Skin PpIX levels were determined over time after ALA administration by the quantification of the 635 nm PpIX emission peak with *in vivo* fluorescence spectroscopy under 442 nm laser excitation. Administration of ALA at 20 and 30 mg/kg induced preferential accumulation of PpIX in psoriatic as opposed to adjacent normal skin. Peak fluorescence intensity in psoriatic and normal skin occurred between 3 and 5 h after the administration of 20 and 30 mg/kg, respectively. Ratios of up to 10 for PpIX fluorescence between psoriatic *versus* normal skin were obtained at the 30 mg/kg dose of ALA. Visible PpIX fluorescence was also observed on normal facial skin, and nonspecific skin photosensitivity occurred only in patients who received the 20 or 30 mg/kg doses. PpIX fluorescence intensity was measured in circulating blood cells by flow cytometry. PpIX fluorescence was higher in monocytes and neutrophils as compared to $CD4^+$ and $CD8^+$ T lymphocytes. PpIX levels in these cells were higher in patients who received higher ALA doses and peaked between 4 and 8 h after administration of ALA. There was only a modest increase in PpIX levels in circulating $CD4^+$ and $CD8^+$ T lymphocytes. In conclusion oral administration of ALA induced preferential accumulation of PpIX in psoriatic plaques as compared to adjacent normal skin suggesting that PDT with oral ALA should be further explored for the treatment of psoriasis.

INTRODUCTION

Photodynamic therapy (PDT)‡ is a therapeutic modality that combines the sequential administration of a photosensitizer followed by its activation by light. Psoriasis is a common skin disorder characterized by elevated, erythematous and scaly plaques. Psoriasis can be improved by psoralen and ultraviolet A (PUVA) therapy; however, PUVA therapy is carcinogenic and has been associated with an increased risk of developing skin cancer (1,2). Clinical improvement in psoriatic plaques has been reported following PDT with intravenous photosensitizers such as hematoporphyrin derivative (3), porfimer sodium, verteporfin (benzoporphyrin derivative) (4) and tin-protoporphyrin (5). Aminolevulinic acid (ALA) is a photosensitizer precursor that is metabolized *in vivo* by cells to protoporphyrin IX (PpIX) which in turn can be activated for PDT by visible light. Topical PDT with ALA has been shown to improve and even clear selected psoriatic plaques after activation with red light (6,7). However, topical ALA is associated with a number of limitations, including inhomogeneous penetration of ALA through the stratum corneum as well as impracticality of treating larger skin surfaces such as in extensive psoriasis. In order to circumvent these limitations we have investigated oral administration of ALA to psoriatic patients. The objectives of this initial study were to evaluate PpIX accumulation in the skin and peripheral blood cells of psoriatic patients after oral ALA in order to determine the pharmacokinetic profile for eventual drug and light dosimetry and timing.

MATERIALS AND METHODS

Patients and ALA administration. Oral ALA was provided by DUSA Pharmaceuticals (Valhalla, NY) in powder form and was administered to a total of 12 patients with plaque psoriasis. Informed

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‡*Abbreviations:* ALA, aminolevulinic acid; ALT, alanine amino transaminase; AP, alkaline phosphatase; AST, aspartate amino transaminase; FITC, fluorescein isothiocyanate; GGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; MPD, minimal phototoxic dose; PDT, photodynamic therapy; PE, phycoerythrin; PpIX, protoporphyrin IX; PUVA, psoralen and ultraviolet A.

consent was obtained from each patient, and the study received approval from the University of British Columbia Clinical Research Ethics Board. Three groups of four patients received one of the following ALA doses: 10, 20 or 30 mg/kg. Baseline complete blood count, aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (AP), lactate deshydrogenase (LDH), γ-glutamyl transferase (GGT), bilirubin, creatinine and urea were obtained at the screening visit in order to assess preexisting renal, hepatic or hematologic problems for every patient. Patients were all asked to eat breakfast before coming to the dermatology clinic. Oral ALA was diluted in 250 mL of orange juice immediately prior to administration in the morning, and patients were instructed to drink this mixture in less than 2 min. On the day of ALA administration patients remained indoors for at least 12 h following ALA drug dosing. The first eight patients studied (10 and 30 mg/kg) were kept in a room where fluorescent lighting was turned on while the last four patients who received 20 mg/kg were kept in subdued light in the same rooms. AST, ALT, AP, GGT, LDH and bilirubin were repeated at 24 and 72 h after the administration of ALA. Any abnormal values at 72 h for these latter tests were repeated weekly until they returned to normal values.

Macrospectrofluorometry. *In vivo* fluorescence emission spectra were obtained serially from normal and psoriatic skin before and after ALA. The detailed configuration of the clinical fluorescence spectrometer has been described previously (8). Briefly, a 442 nm HeCd laser was connected to a hand-held probe with a bundle of 6 × 200 μm optical fibers for illumination. The fluorescent light was collected with another 200 μm optical fiber at the center of the probe and analyzed by a spectrometer connected to a desktop computer. *In vivo* fluorescence spectra of normal and psoriatic skin were recorded in triplicate (three different areas of the same plaque) before and at 5, 10, 20, 40, 60 and 90 min and 2, 3, 4, 5, 6, 8, 12, 24 and 72 h after oral administration of ALA. When possible a plaque of any size that did not exhibit endogenous PpIX autofluorescence was selected for macrospectrofluorometry. The same plaque was used for each measurement. Patients were asked not to use topical treatment for psoriasis for 2 weeks. Emollients were allowed but patients were told not to apply emollient on the day of ALA administration and on days 1 and 3 when macrospectrofluorometry was performed. PpIX was quantified by integrating the area under the emission spectral curve between 630 and 640 nm after subtracting the autofluorescence background. As endogenous PpIX can sometimes be present in psoriatic scales (8) care was taken, when possible, to select plaques with no emission peak around 635 nm before ALA administration. These same plaques were used for all subsequent macrospectrophotometric measurements.

Flow cytometry. Venous blood (3–4 mL) was taken before and at the following times after ALA administration: 5, 10, 20, 40, 60 and 90 min; and 2, 3, 4, 5, 6, 8, 12, 24 and 72 h. Erythrocytes were immediately lysed in an ice-cold solution of ammonium chloride (9). Using standard flow cytometry procedures the leukocytes (resuspended in Hank's balanced salt solution with 0.01% bovine serum albumin) were then kept on ice (0°C) and stained with fluorescein isothiocyanate (FITC)-labeled anti-human CD4 and phycoerythrin (PE)-labeled anti-human CD8. Purified mouse immunoglobulin G_1 was used for blocking nonspecific staining and as an appropriate isotype control. All the antibodies were purchased from PharMingen (San Diego, CA). Flow cytometric measurements were performed with a Coulter Epics Elite ESP (Coulter Electronics Ltd., Hialeah, FL) with at least 10^4 cells analyzed per sample. The EPS Elite was configured with two lasers providing blue light excitation at 488 nm and UV excitation in the 350–360 nm range. The laser beams were displaced such that the cell sample sequentially passed through the visible and then through the UV beam. This configuration, used in conjunction with the 'gated amplifier' capabilities of the instrument, allowed us to 'electronically' select data from the various excitation/emission combinations hence providing time-based as well as optically filtered signal selection. In our case the FITC and PE signals were acquired as the cell passed through the primary (488) beam using 525 and 575 nm band-pass filters, respectively, and the protoporphyrin signal was acquired through a 610 nm long-pass filter when excited by the UV beam. Since the UV laser excites neither FITC nor PE the electronically gated signal from the second laser thus represented protoporphyrin exclusively. Forward and side light scatter were also recorded and used for the identification of monocytes and neutrophil populations. Cellular PpIX levels were determined according to our previous experience with the measurement of cellular ALA-induced PpIX (9) as well as other photosensitizers (10–12) in mouse tumor models. *In vitro* experiments performed with normal human blood incubated with ALA showed that no significant loss of cellular PpIX occurred during the sample preparation time (including erythrocyte lysis) under these experimental conditions. The measurement of PpIX accumulation was based on the increase in >610 nm fluorescence per cell in selected leukocyte populations from the blood of individual patients collected consecutively during the observation period. The results depict relative changes in cellular PpIX level in helper T lymphocytes, cytotoxic T lymphocytes, monocytes and neutrophils.

Fluorescence photography. Photographs were taken with a Nikon F-601 camera equipped with a 60 mm micro AF lens set at f/2.8. The skin was illuminated in the dark with a Wood's lamp (UVP, Model B 100 AP, Upland CA), and photographs were taken using an ASA 1600 Fujichrome film with exposure times ranging between 1/30 and 1/4 s.

Statistical analysis. Flow cytometry results were analyzed with an unpaired Student's *t*-test. The ANOVA for repeated measures was used with Bonferroni correction to analyze macrospectrophotometric data.

RESULTS

Psoriatic plaques exhibit bright visible fluorescence after ALA administration

Two hours after the administration of ALA at 30 mg/kg bright red fluorescence was visible on psoriatic skin with Wood's lamp illumination (Fig. 1a). This fluorescence was not visible on adjacent normal skin of the trunk and limbs but was readily observed on nonpsoriatic facial skin (Fig. 1b).

PpIX fluorescence intensity is higher in psoriatic plaques than adjacent normal skin

The level of PpIX fluorescence intensity on psoriatic skin increased slightly after 10 mg/kg of ALA although this increase was not statistically significant (Fig. 2a). From 1 h after administration of 20 mg/kg of ALA skin PpIX fluorescence intensity was higher on psoriatic skin as compared to normal skin ($P < 0.05$) (Fig. 2b). PpIX levels increased to reach a maximum at 3 h after ALA administration (Fig. 2b). PpIX fluorescence intensity on normal skin also increased for patients who received 20 mg/kg and reached a maximum at 4 h (Fig. 2b). For one patient who received 20 mg/kg the PpIX fluorescence level on psoriatic skin before ALA administration was 100-fold higher than the other patients, and a characteristic PpIX emission peak was present on the baseline spectra. The PpIX levels measured 4 h after ALA administration for that patient was three-fold higher than the average value observed for patients who received 30 mg/kg. For that patient the PpIX fluorescence level increased at 24 h to reach the same level as the peak PpIX level measured at 3–5 h after ALA administration (not shown). Because of these differences this patient was excluded from the 20 mg/kg analysis. This patient was not investigated for porphyrin metabolism disturbances as high levels of PpIX are known to occur in the scales of many patients with psoriasis (8). PpIX fluorescence intensity after 30 mg/kg of ALA gradually increased from baseline to reach a maximum at 5 h for psoriatic and normal skin followed by a decrease to baseline level at 24 h (Fig. 2c). PpIX levels were higher on psoriatic

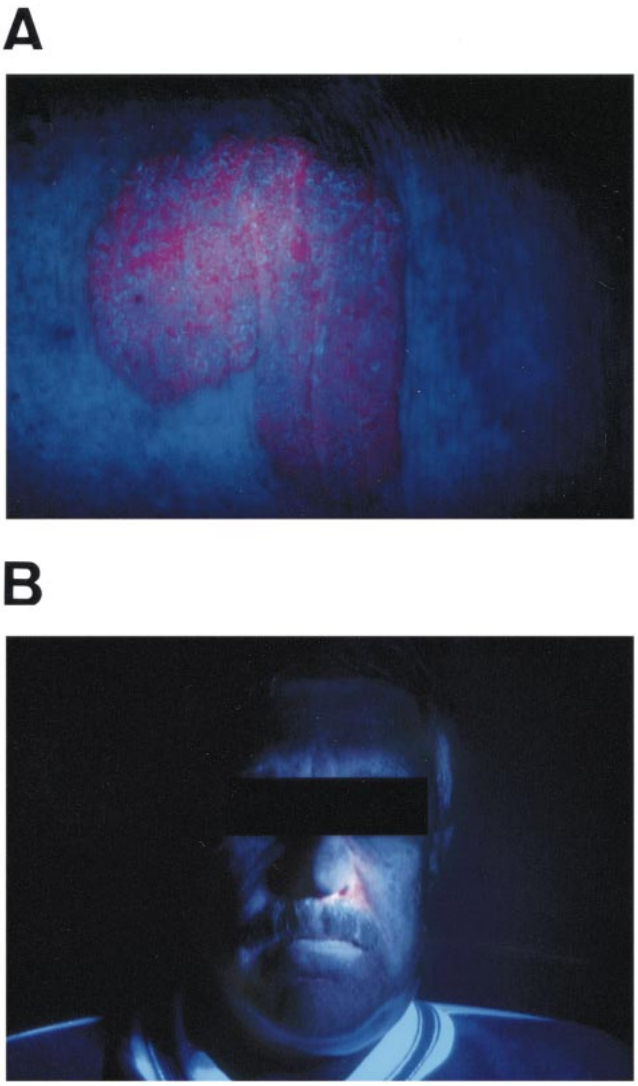

Figure 1. (a) Bright red fluorescence is observed on a psoriatic plaque of the leg with no visible fluorescence on adjacent normal skin 4 h 45 min after oral administration of ALA at 30 mg/kg. (b) Red fluorescence is also seen on nonpsoriatic areas of the face at the same time after oral administration of ALA at the same dose.

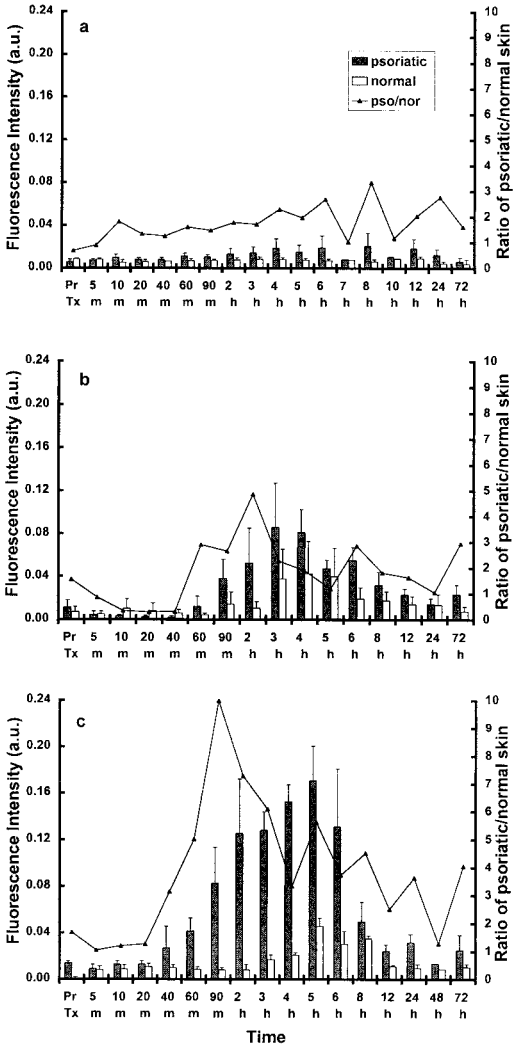

Figure 2. PpIX levels in normal and psoriatic skin at different times after oral administration of ALA at (a) 10, (b) 20 and (c) 30 mg/kg. PpIX fluorescence was measured with *in vivo* fluorescence spectroscopy and is reported as average for four (a, c) and three (b) patients. Psoriatic: normal skin PpIX fluorescence ratios (triangles) are also reported according to time after ALA administration. Bars indicate SEM.

skin as compared to normal skin after administration of ALA at 30 mg/kg ($P < 0.005$). The psoriasis to normal skin PpIX fluorescence ratios were low before ALA administration and increased to reach a maximum of 10 at 90 min after 30 mg/kg of ALA and 4 at 2 h after 20 mg/kg of ALA.

Patients

Nonspecific skin photosensitivity characterized by facial erythema was apparent at 24 h for three patients who received 30 mg/kg, one patient who received 20 mg/kg and none of the patients who received 10 mg/kg. Patients did not complain of burning sensation or pruritus following ambient light exposure. One patient who received 30 mg/kg fainted approximately 2.5 h after ALA administration followed by nausea and vomiting. Detailed clinical evaluation of this patient was normal except for mild to moderate prolonged hypotension which lasted for approximately 6 h after ALA administration. Transient mild increases in serum transaminases of up to 12% of normal values were seen in two patients who received 30 mg/kg. Transaminase levels returned to normal at 72 h for one patient and 10 days for the other.

PpIX levels are higher in circulating monocytes and neutrophils as compared to CD4 and CD8 lymphocytes

Four blood cell populations were analyzed for PpIX fluorescence with flow cytometry: $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, monocytes and neutrophils. PpIX was quantified as fluorescence intensity per cell relative to the fluorescence levels for the respective cell populations before ALA. As expected there was considerable variation in cellular PpIX levels among patients. These variations did not seem to correlate with levels of PpIX measured in the skin by

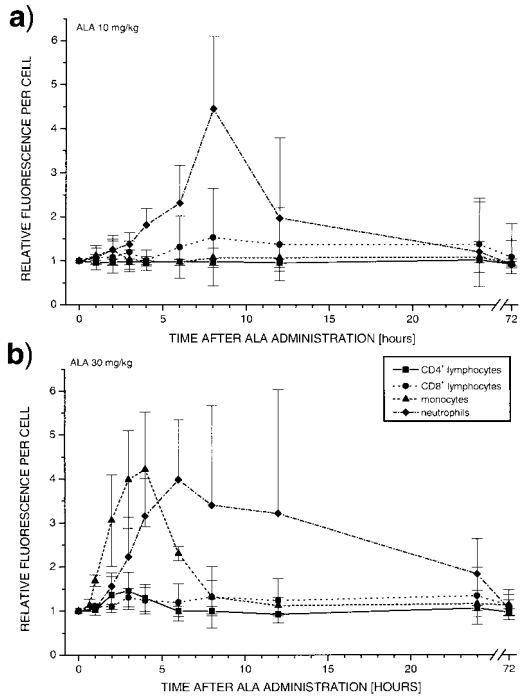

Figure 3. PpIX levels in blood leukocytes of psoriatic patients. Immediately before and at different time intervals after the oral administration of ALA samples of venous blood were withdrawn from the patients. The cellular PpIX levels were determined in $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, monocytes and neutrophils using flow cytometry. The cellular content of PpIX is expressed as fluorescence intensity >610 nm normalized by those recorded for particular cell populations before the ALA administration. The results shown are average values for groups of four patients given either (a) 10 mg/kg ALA or (b) 30 mg/kg ALA. Bars indicate SD.

macrospectrofluorometry. The pattern of PpIX accumulation in blood cell populations was somewhat dependent on the administered ALA dose (Fig. 3). For patients who received the lowest ALA dose (10 mg/kg) high PpIX levels were observed exclusively in neutrophils and peaked around 8 h post ALA administration. For patients who received 20 or 30 mg/kg elevated PpIX levels were detected in monocytes as well as neutrophils. After 30 mg/kg ALA PpIX fluorescence levels were similar in monocytes and neutrophils, peaking around 4 and 6 h, respectively (Fig. 3). The levels of PpIX detected in $CD4^+$ and $CD8^+$ T cells were considerably lower than in myeloid cell populations. The relative increase in PpIX fluorescence in monocytes and neutrophils was several times greater than in lymphocytes. The increased PpIX levels in monocytes at 1, 2, 3, 4 and 6 h after ALA administration and in neutrophils at 3, 4, 6, 8 and 12 h after ALA administration were significant as compared to baseline ($P < 0.01$) while increases in $CD4^+$ and $CD8^+$ lymphocytes were not statistically significant. These differences in PpIX accumulation are illustrated in Fig. 4 where the results for separate cell types are shown for three individual patients who received 30 mg/kg. The data indicate that relatively modest PpIX levels accumulate in both lymphoid cell types, and this is generally most prominent at 2–4 h after ALA administration. The exception was one patient who showed persistently elevated PpIX levels in $CD8^+$ cells up to 1 day after receiving ALA.

DISCUSSION

To our knowledge this is the first report of oral ALA administration to psoriatic patients. Preferential accumulation of PpIX was observed in psoriatic plaques as compared to the normal skin of the trunk and limbs. The 635 nm emission peak detected with our macrospectrophotometer in normal

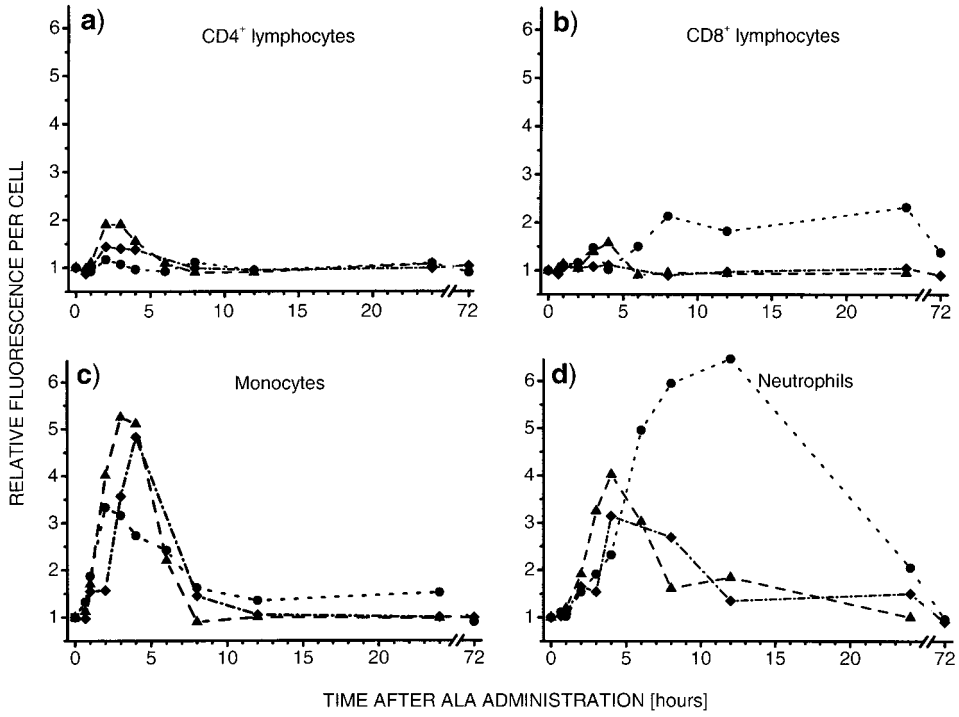

Figure 4. ALA-induced PpIX accumulation according to time in blood leukocytes of three individual psoriatic patients who received ALA at 30 mg/kg. Fluorescence levels are presented for (a) $CD4^+$ (b) and $CD8^+$ lymphocytes as well as (c) monocytes and (d) neutrophils.

and psoriatic skin after ALA administration is indicative of PpIX according to the known emission spectrum for PpIX in human tissue (13,14). A similar endogenous red emission peak is often present in psoriatic scales, and biochemical analysis has previously shown that it arises from PpIX (8). For macrospectrophotometric measurements performed during the course of this study care was taken to select psoriatic plaques that did not exhibit this endogenous PpIX peak. However, for one patient who received 20 mg/kg the PpIX fluorescence on psoriatic skin was so elevated before ALA administration that we decided not to include this patient in the 20 mg/kg analysis.

Preferential accumulation of PpIX in psoriasis may be related to preferential uptake of ALA, or retention of PpIX at the cellular level or to increased thickness of the epidermis in psoriatic skin as compared to normal skin. As psoriatic keratinocytes are dividing at a higher rate than normal skin the accumulation of PpIX may be related to an increased synthesis of heme required for the production of cytochromes. Maximum PpIX fluorescence in normal skin was observed at 3 and 5 h for patients who received 20 and 30 mg/kg of ALA. In another study where patients received oral ALA at 40 mg/kg macrospectrofluorometry performed with a spectrometer system revealed maximum skin fluorescence at 6.5 and 9.8 h depending on the body region studied (13). Webber *et al.* (15) also performed macrospectrofluorometry on normal skin of 22 patients and found a peak PpIX fluorescence intensity at 6 h after 60 mg/kg of oral ALA. The time differences for maximum skin fluorescence between Rick *et al.* (13) and our study could be related to the different ALA doses that were studied, variability between patients or to the small number of patients studied.

Nonspecific skin photosensitivity has been reported in patients receiving systemic ALA (16,17). Webber *et al.* (15) reported no cases of photosensitivity in 41 hospitalized patients who received either 30 or 60 mg/kg and were kept in subdued light for 48 h thereafter, suggesting that clinical photosensitivity can be avoided if patients limit their light exposure during the first 2 days after ALA administration. Rick *et al.* (13) reported two cases of clinical photosensitivity out of 11 patients who received 40 mg/kg and who were only exposed to artificial indoor lighting, whereas photosensitivity was not reported in 17 patients with head and neck tumors who received 5 or 15 mg/kg of ALA (14). Rick *et al.* (13) also reported increased PpIX fluorescence on the cheeks and lips as compared to the limbs. The clinical photosensitivity we observed for patients who received 20 and 30 mg/kg is possibly related to the increased PpIX accumulation on facial skin as suggested by *in vivo* fluorescence spectroscopy (data not shown) as well as to greater ambient light exposure on the face as compared to the rest of the body. PpIX fluorescence on the face was higher around the nose but correlation between PpIX localization and facial erythema 24 h after ALA administration was not performed. Although we did not administer ALA to nonpsoriatic patients the absence of psoriasis on facial areas where red fluorescence was visualized with Wood's lamp suggests that this PpIX accumulation is not related to facial psoriasis. PpIX is known to accumulate preferentially in sebaceous glands (18), and the increased facial PpIX fluorescence is possibly related to this phenomenon. Our findings strongly suggest that for whole body light exposure after systemic administration of ALA a reduced light dose may be required for the face if treatment of this region is necessary.

One of our patients experienced nausea, vomiting, hypotension and fainting possibly because of a vaso vagal reaction after administration of ALA. Nausea and vomiting after oral ALA administration has been reported for patients (16,19) as well as dogs (20). To our knowledge no previous episodes of fainting have been reported after ALA administration. Rick *et al.* (13) reported a case of hypotension occurring 2–8 h after administration of 40 mg/kg of ALA. The hemodynamic effects of oral ALA were studied in six patients who underwent Swan-Ganz catheterization prior to receiving 60 mg/kg of ALA as a single oral bolus (21). A decrease in systolic and diastolic pressures as well as pulmonary and systemic vascular resistances was observed. Fainting in our case may have been caused by bradycardia and hypotension (vaso vagal reaction) secondary to vomiting. It is possible that nausea and vomiting may be related to the amount of food present in the stomach at the time of ALA administration. This was not specifically studied. The exact mechanisms underlying nausea, vomiting and hypotension after ALA administration is unknown. The elevated transaminases which have been described in the past after oral administration of ALA (16,19) suggest that ALA may induce hepatocellular damage at higher doses. The absence of nausea, vomiting, photosensitivity and elevated transaminase in our patients who received 10 mg/kg suggest that lower doses of ALA may be better tolerated.

T lymphocytes probably play a pivotal role in the genesis of psoriasis as suggested by clinical improvement with the use of cyclosporine, interleukin-2-diphtheria toxin fusion protein (22), anti-CD4 (23) and anti-TNF alpha (24) monoclonal antibodies. Further evidence includes induction of psoriatic phenotypes in human skin from nonpsoriatic patients grafted onto SCID mice with the intralesional injection of immunocytes from psoriatic patients (25). Our macrospectrofluorometric data probably reflects PpIX within keratinocytes and not in lesional T lymphocytes as keratinocytes greatly outnumber lymphocytes in the skin of psoriatic patients. Moreover, systemic ALA administration is known to give rise to PpIX accumulation in keratinocytes in mouse skin (18). Because PpIX fluorescence is extracted when tissue sections are fixed it is difficult to directly quantitate the levels of PpIX in skin lymphocytes. In order to study the kinetics of PpIX fluorescence in T lymphocytes after oral ALA administration we decided to use flow cytometry on circulatory blood cells. Only low levels of PpIX were found in circulating $CD4^+$ and $CD8^+$ lymphocytes. PpIX levels were higher in monocytes and neutrophils presumably because these cells are metabolically more active. PpIX is synthesized by internal organs such as the liver suggesting that PpIX released into circulation could be delivered to target tissue and contribute, with *in situ* production, to intracellular PpIX accumulation (26). Elevated PpIX levels measured in peripheral neutrophils (with peak time delayed compared to monocyte and persisting longer than in other cell types) may reflect the uptake of circulating PpIX by these cells. Given the higher number of neutrophils in blood as compared to monocytes, levels of PpIX in neutrophils may largely dominate the overall blood PpIX content. The healthy human

plasma PpIX content was reported to peak at 6.7 h after oral administration of 40 mg/kg of ALA (13). The delay between the PpIX peak in blood leucocytes observed in this study and the plasma peak reported in the literature could be due to the production and release of PpIX into the circulation by the liver. This is further supported by an experiment where the PpIX peak was measured in monocytes from a healthy volunteer at 3 h after *in vitro* incubation of whole blood with 0.1 m*M* ALA at 37°C (data not shown). PpIX levels in this case were also largely confined to monocytes and neutrophils (peaking at around 3 h of ALA exposure for monocytes and 1–2 h later for neutrophils) with very little, if any, accumulation in $CD8^+$ T cells and no detectable PpIX in $CD4^+$ cells. Low levels of PpIX in circulating $CD4^+$ and $CD8^+$ lymphocytes do not imply that PpIX levels are low in psoriatic lesional lymphocytes. PpIX synthesis is known to be lower in resting as compared to phytohemagglutinin-activated peripheral T lymphocytes (27).

In conclusion, oral administration of ALA to psoriatic patients induced a preferential accumulation of PpIX in psoriatic plaques as compared to adjacent normal skin. Knowledge gained with this study suggests that for further development in the treatment of psoriasis lower oral ALA doses such as 10 or 20 mg/kg should be selected particularly if multiple drug and light exposures are involved. The elevated level of PpIX fluorescence observed on the face also suggests that if whole body exposures are to be performed the face should be covered. The preferential accumulation of PpIX in psoriatic skin suggests that oral ALA-PDT should be further explored for the treatment of psoriasis.

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