The Accumulation of Protoporphyrin IX in Plaque Psoriasis After Topical Application of 5-Aminolevulinic Acid Indicates a Potential for Superficial Photodynamic Therapy

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The success reported for the treatment of superficial skin carcinomas by photodynamic therapy with topical application of the photosensitizer precursor 5-aminolevulinic acid has therapeutic implications for the treatment of other skin disorders. This paper describes the accumulation of the photosensitizing agent protoporphyrin IX in areas of plaque psoriasis by monitoring of the fluorescence emission induced by low-intensity laser excitation at 488 nm. We present results from 15 patients with a total of 42 plaques and show that the characteristic fluorescence emission of protoporphyrin IX increases in intensity within the 6-h period following application of 5-aminolevulinic acid, suggesting that there is a potential for superficial photodynamic therapy. The rate of increase and maximum intensity of fluorescence emission was not directly related to the applied quantity of the precursor. The variability of the fluorescence intensity was as great between plaques at different sites on the same patient as between different patients. Also, the effect of plaque occlusion following application appeared insignificant. Although there was only limited enhancement of emission from areas of skin surrounding the plaque, a significant build-up of sensitizer was detected after several days in some areas of psoriasis that received no application. Key words: fluorescence/photosensitization. J Invest Dermatol 107:76-81, 1996

Since Kennedy et al (1990) first described the treatment of superficial skin carcinomas by photodynamic therapy (PDT), using topical application of the photosensitizer precursor 5-aminolevulinic acid (ALA), other centers (Wolf et al, 1993; Cairnduff et al, 1994; Svanberg et al, 1994; Fijan et al, 1995) have reported the effectiveness of this type of treatment. The perceived advantages of ALA-PDT, notably the localized and short-term photosensitivity, have led to consideration of the technique for other sites of malignancy or premalignancy such as Barrett’s esophagus† (Barr et al, 1995) and for nonmalignant conditions that involve only the most superficial tissue layer, such as the endometrial surface of the uterus (Gannon et al, 1995).

The use of photodynamic therapy to treat psoriasis was first reported in 1937; using systemic administration of hematoporphyrin in combination with ultraviolet radiation, Silver reported improvement in seven patients (Silver, 1937). Subsequent workers have also reported the efficacy of hematoporphyrins administered systemically followed by 630-nm illumination (Berns et al, 1984) or topically using ultraviolet or visible radiation (Meffert et al, 1989). The resultant prolonged photosensitivity was recognized as the major limitation of systemic administration for this form of treatment. Recently, Boehncke et al (1994a) reported the decrease in fluorescence emission (photobleaching) of the photosensitizer Photosan-3 induced by illumination at 630 nm after topical application to psoriatic plaques. The same author (1994b) has subsequently demonstrated the possibility of topical ALA-PDT in therapy by achieving clearance comparable to that with dithranol in chronic plaque-stage psoriasis in three patients. It had previously been observed (Kennedy and Pottier, 1992) that topical ALA-induced protoporphyrin IX (PpIX) was confined to areas of psoriasis, but the clinical response to illumination was variable.

In order to maximize PDT effectiveness after application of ALA, illumination of the treatment area should occur at a time of sufficiently increased concentration of the endogenous photosensitizer. The accumulation of intracellular PpIX is governed by the heme biosynthetic pathway and is therefore dependent upon the diffusion rate of exogenous ALA throughout the abnormal epithelium and by the rate of heme formation. It has been shown (Loh et al, 1993) that different types of tissue metabolize the sensitizer at different rates and to different degrees. In the treatment of superficial skin carcinomas reported to date, time intervals of 3 to 20 h have been adopted between ALA application and illumination. With ALA in combination with the iron chelator desferrioxamine, favorable results were reported (Fijan et al, 1995), particularly for thicker lesions such as nodular basal cell carcinomas that were
ascribed to the inhibition of heme formation and a concomitant enhancement of PpIX concentration.

The purpose of our study was to (i) monitor the rate of accumulation and persistence of PpIX in plaque psoriasis; (ii) determine the effect of different quantities of applied ALA upon the rate and level of PpIX accumulation; (iii) determine the effect of occlusion of the plaque, following ALA application, upon the rate and level of PpIX accumulation; (iv) investigate the degree of

Figure 1. The extraction of photosensitizer fluorescence from the autofluorescence background. The shape of the autofluorescence curve was recorded upon areas of plaque not applied with ALA, enabling a background subtraction that yields the emission due to PpIX. The height of the 635-nm peak is plotted against time after application, to indicate the progress of PpIX synthesis.

Figure 2. PpIX fluorescence intensity versus time after ALA application. Measurements were made on a patient (group 1) with four areas of elbow plaque psoriasis using 50, 100, and 200 mg of ALA (20%) in Unguentum Merck within 2 × 2 cm test sites.
localization of PpIX to the area of ALA application. The results are used to assess the potential for superficial PDT and establish appropriate treatment conditions.

MATERIALS AND METHODS

Experimental Apparatus. Fluorescence was induced with the 488-nm output from an argon-ion laser delivered to the surface of the skin via a 600-μm-core optical fiber at a power of 4 mW. A second fiber was used to collect the emitted light, which was filtered (OG 530, long-pass) to block the scattered laser radiation and delivered to the entrance slit of a single-channel optical spectrum analyzer (Rees Instruments Ltd, Godalming, UK). The delivery and detector fibers are arranged normal to the skin surface in a hand-held probe that analyzes a circular area of 0.1 cm², resulting in a localized irradiance of 40 mW cm⁻². The spectrum analyzer employs a revolving diffraction grating to capture a complete spectrum (450–900 nm) every 80 ms, which is registered by a red-sensitive photomultiplier tube. This ensures that nonlinearity of detector response is avoided, but in order for an adequate signal-to-noise ratio to be achieved,
Figure 5. The intensity of PpIX fluorescence across an area of plaque and the border of normal skin. The measurements were made 4.3 h after ALA application (50 mg cm\(^{-2}\)) upon a patient in group 1.

Data Analysis  Figure 1 illustrates the procedure involved in obtaining the photosensitizer fluorescence spectrum. In this example, 12.5 mg cm\(^{-2}\) of the ALA preparation was applied to a plaque upon the elbow. The fluorescence spectra were recorded at times ranging from 85 to 280 min after application and then again after 4 d. It is clear from the spectra shown that the tissue autofluorescence dominated the recorded signal. Also, although the experimental parameters remained constant for all measurements, there was considerable variation in the intensity of autofluorescence, even for repeated measurements upon the same plaque, but by analysis of the shape of an averaged autofluorescence spectrum (obtained from measurement points upon at least one area of plaque not exposed to ALA, on each patient), a background correction could be made to allow the subtraction of the autofluorescence output from the total. This yielded the photosensitizer fluorescence spectrum. The intensity of the 488-nm excitation was recorded prior to each measurement and varied by up to ± 5\% from the mean. The spectra were therefore normalized for laser power variations after the background correction.

A plot of the height of the 635-nm emission peak as a function of time after application demonstrated the build-up of PpIX within the area of analysis. The error bars represent the standard deviation of three readings taken from closely spaced points within the same plaque. The major contribution to the error is a result of subtraction of the autofluorescent background. A second measurement at the same point, performed immediately after the first, indicates that the peak fluorescence intensity of PpIX is reduced by approximately 5\%, a consequence of sensitizer photo-oxidation during the period of measurement. None of the data presented here, however, involved such a repetition, and we have not, therefore, attempted to apply a photobleaching correction to individual measurements.

RESULTS

Evidence of PpIX Accumulation  The fluorescence intensity from ALA-induced PpIX increased with time after application for all patients studied. Figure 2 shows the complete set of results from...
The Effect of These graphs include the data points from patients that exhibited 4 d after the initial measurements. After a further 3 d no signal persisted. A comparison of the fluorescence measured within an area of plaque and that upon its border illustrates the low level of photosensitizer production in the adjacent skin.

The Effect of Using Different Quantities of ALA Is Not Significant Figure 3 shows the results from 5 of the 10 patients upon which the three different quantities of ALA were applied. These graphs include the data points from patients that exhibited the greatest extremes of fluorescence intensity. The plots that are omitted (for clarity) all lie within these extremes. For each concentration of ALA used there was a significant increase in PpIX fluorescence, but the intensity of fluorescence obtained with only 50 mg of ALA preparation was much more variable between patients than that induced by the use of 100 or 200 mg.

The Effect of Plaque Occlusion Is Not Significant The effect upon the ALA-induced PpIX accumulation of plaque occlusion by application of a polythene dressing is shown in Fig 4. Only in one patient did there appear to be evidence of a significant difference in fluorescence intensity between occluded and nonoccluded sites over the whole period of analysis.

Enhanced PpIX Accumulation in Plaque, Compared to Adjacent Skin Figure 5 shows the results of measurements made transversely across an area of skin that contained a small plaque 4.3 h after application of cream. The intensity of the 635-nm peak is shown to be almost equal across the plaque, with a much lower (< 25%) emission from the skin margin that had received ALA. Away from the area of application, the signal was reduced to less than 5% of the maximum.

Evidence of PpIX in Untreated Plaque The spectra shown in Fig 6 are derived from a patient in group 1. Measurements were made upon sites that included plaque treated with ALA, adjacent nontreated plaque, remote plaque, and areas of normal skin. These spectra show that over the first few hours, only those sites receiving ALA displayed a PpIX emission. After 4 d, however, not only had the fluorescence intensity doubled at the sites of application, but there was also a significant emission in nontreated points on the same plaque as well as in a plaque separated (> 15 cm) from those treated. The signal obtained from normal skin was smaller than the treated plaques by at least a factor of 100, and 50 times smaller than that in the remote plaque.

DISCUSSION

It should be emphasized that these are macroscopic measurements that register the average fluorophor distribution within the 0.1-cm² area of analysis. The microscopic distribution of photosensitizer and tissue fluorescence is not resolved by this technique. The use of 488-nm excitation limits the analysis to the epidermis, and so we can conclude from these measurements that the synthesis of PpIX proceeds effectively in plaque psoriasis after topical application of ALA. In all examples, there was a steady build-up in fluorescence intensity during the first 4 h, with instances of continued PpIX accumulation up to 5 h and beyond. There were considerable variations in both the rate of increase and the maximum intensity of fluorescence achieved. These variations were as great between different sites on the same patient as between different patients. The PpIX accumulation was not significantly dependent upon the applied quantity of ALA, although in the sample studied (group 1) the smallest variation in data points was obtained with a concentration of 25 mg cm⁻². When the patient and site variations are taken into account, the effect of occluding the plaque following
ALA application also appears not to be significant, implying that occlusion does not enhance ALA penetration. Such variations also preclude any accurate prediction of PpIX accumulation at different sites upon the body, although the data from this study do suggest that the most intense fluorescence is obtained from plaques located upon the elbows. The five biopsy samples are currently undergoing analysis by fluorescence microscopy in order to establish the epidermal distribution of PpIX.

There was a measurable PpIX content in the border of skin immediately adjacent to a plaque when both received ALA. Skin not covered by cream showed a much less significant enhancement. It is likely that the proximity of the point of measurement to the plaque is the dominant factor in this effect.

Results obtained from one patient show not only that those plaques treated with ALA display an enhanced PpIX content after several days, but also that remote plaques display significant fluorescence. Although the magnitude of effect in this individual is the greatest that has been measured to date, the persistence and distribution of the fluorescence has been registered in other patients and raises some important questions regarding this type of drug application. The separation of the sites involved and the supervision of the patient over the initial period after application (during which most if not all of the ALA is absorbed) indicates that the ALA is not transferred to the remote sites by direct contact, which implies a biochemical transport mechanism either for the photosensitizer or the precursor. The bloodstream is the most obvious candidate for systemic distribution of either of these agents; a program of blood sampling over an extended time period after application of ALA is now in progress.

The intensity of fluorescence emission reported in this study is comparable to, and in many cases greater than, measurements we have made upon areas of superficial skin carcinoma. This indicates that PDT has the potential to be a beneficial therapy for plaque psoriasis. Using these findings, we have established a treatment protocol that involves using 100 mg of 20% ALA in Unguentum Merck with no occlusion of the site and with the subsequent PDT illumination to proceed at a time point of 3.5 h after application. Lack of occlusion allows the possibility of unsupervised application of the cream by the patient several hours before attending the clinic. A clinical trial is underway, involving the superficial illumination of selected areas of plaque psoriasis using a broad-spectrum light source.

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REFERENCES