

Photodynamic Therapy (PDT) and Photodiagnosis (PD) Using Endogenous Photosensitization Induced by 5-Aminolevulinic Acid (ALA): Mechanisms and Clinical Results

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ABSTRACT

5-Aminolevulinic acid (ALA), when added to many tissues, results in the accumulation of sufficient quantities of the endogenous photosensitizer protoporphyrin IX (PpIX) via the heme biosynthetic pathway, to produce a photodynamic effect when exposed to activating light. Therefore, ALA is the only photodynamic therapy (PDT) agent in current clinical development that is a biochemical precursor of a photosensitizer. Topical ALA application, followed by exposure to activating light (ALA PDT), has been reported effective for the treatment of a variety of dermatologic diseases including cutaneous superficial and nodular basal cell carcinoma, Bowen's disease, and actinic (solar) keratoses. Local internal application of ALA has also been used for selective endometrial ablation in animal model systems and in human clinical studies has shown selective formation of PpIX within the endometrium. PpIX induced by ALA application has also been used as a fluorescence detection marker for photodiagnosis (PD) of cancer and dysplastic conditions of the urinary bladder and other organs. Systemic, oral administration of ALA has been used for ALA PDT of superficial head and neck cancer, various gastrointestinal cancers, and the condition known as Barrett's esophagus. The current state of knowledge of the mechanisms of endogenous topical and systemic photosensitization using ALA, the results of published clinical trials, and possible methods of increasing the efficacy of endogenous photosensitization for ALA PDT are reviewed in this paper.

INTRODUCTION

Most forms of photodynamic therapy (PDT) involve the administration of photosensitizing molecules that are manufactured outside the body and then injected as preformed compounds. In contrast, endogenous photosensitizers are synthesized within the body from nonphotosensitizing precursors, the chemical conversion being carried out by normal metabolic pathways. The major endogenous photosensitizers (protoporphyrin IX, uroporphyrin I, uroporphyrin III, coproporphyrin I, and coproporphyrin III) are either products or by-products of heme biosynthesis (Fig. 1). They are all metal-free porphyrins with strong and characteristic fluorescence emission spectra. A few relatively rare endogenous metal-containing porphyrins are

fluorescent photosensitizers also, but by far the most common metal-containing porphyrin in the body (heme) neither fluoresces nor photosensitizes.

Since mammalian cells require heme-containing enzymes for aerobic energy metabolism, all nucleated cells must have at least a minimal capacity to synthesize heme. The endogenous photosensitizer protoporphyrin IX (PpIX) is the immediate precursor of heme in the biosynthetic pathway for heme (Fig. 1), and cells therefore must be able to synthesize the equivalent amount of PpIX also. However, heme biosynthesis normally is under such close feedback control that only a tiny amount of free PpIX is present in most cells at any given time.

Photosensitizing concentrations of one or more of the endogenous porphyrins may accumulate under abnormal condi-

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tions. The porphyrias are a group of metabolic diseases that is characterized by an inherited or acquired abnormality of the pathway for heme biosynthesis (Fig. 1). A decrease in enzyme activity at either of two specific sites in the pathway (ferrochelatase or protoporphyrinogen oxidase) may lead to the accumulation of photosensitizing concentrations of PpIX.¹ Increased synthesis of coproporphyrin III (CpIII) may be associated with decreased activity of coproporphyrinogen oxidase, uroporphyrin III (UpIII) with uroporphyrinogen decarboxylase, and both uroporphyrin I (UpI) and coproporphyrin I (CpI) with uroporphyrinogen III cosynthase. The uroporphyrins and coproporphyrins are not in the direct line of heme biosynthesis, but are merely by-products of no known value to the cell. However, like PpIX, they are efficient cell and tissue photosensitizers, and they can cause severe phototoxic tissue damage if present at high concentration. Of the endogenous porphyrin photosensitizers, only PpIX is being used clinically at present for the detection and/or destruction of abnormal cells.

Induction of increased porphyrin biosynthesis in vivo

The porphyrias are diseases that may serve as model approaches that might be taken to induce cells to synthesize abnormally large quantities of one or more of the endogenous photosensitizing porphyrins.² Each of these metabolic diseases is characterized by abnormally low enzyme activity at a characteristic site in the biosynthetic pathway for heme (Fig. 1). In functional terms, this causes a partial constriction that impedes (but does not completely block) the normal flow of heme biosynthesis. Such an abnormality may cause no problem so long as the demand for heme remains relatively low. However, if the demand for heme increases, the system will respond by increasing the synthesis of 5-aminolevulinic acid (ALA), the first committed precursor of heme. The increased concentration of ALA forces all subsequent steps in the pathway to operate at higher capacity, and under such conditions the presence of a constriction in the pathway at any site downstream from ALA may lead to the accumulation of the intermediate that lies immediately upstream of that constriction. For example, a partial block at the level of ferrochelatase may lead to the accumulation of PpIX, which is the only photosensitizing intermediate in the direct biosynthetic pathway for heme. Partial blocks at other sites may lead to the accumulation of protoporphyrinogen, coproporphyrinogen, uroporphyrinogen, or porphobilinogen. Although none of these compounds is either fluorescent or photosensitizing, when protoporphyrinogens are present in excess they are converted into the corresponding porphyrin by processes that lie outside the normal heme biosynthetic pathway. PpIX is of special interest, since it may accumulate if there is a partial block at the level of either protoporphyrinogen oxidase or ferrochelatase. The porphyrias corresponding to constrictions at these sites are variegate porphyria and protoporphryia (Fig. 1).

It is possible to mimic the enzyme defects that characterize the various porphyrias by administering inhibitors of specific enzymes.³⁻⁶ If the inhibition is complete, the intermediate that lies immediately upstream of the block will accumulate. However, a complete block would prevent the biosynthesis of any heme-containing enzymes and would therefore be toxic. If the inhibition is only partial, there will be no accumulation of

the intermediate unless the demand for heme exceeds the maximum biosynthetic capacity of the partially blocked enzyme. Any intermediate that accumulates following the partial inhibition of a specific step in the biosynthetic pathway for heme must reflect a partial failure of the cell to meet its requirements for heme, and some degree of cellular toxicity therefore will be inevitable if inhibitors of specific enzymes are used as single agents to induce the accumulation of specific intermediates.

A conceptually related technique involves interference with iron metabolism. Since the conversion of PpIX into heme requires the addition of iron, anything that interferes with this process may lead to the accumulation of PpIX. The concentration of available iron can be reduced by the use of iron chelators.^{7,8,51,52} Once again, some degree of toxicity is inevitable if the chelator blocks heme biosynthesis to the extent that PpIX accumulates. Since ferrochelatase can add zinc instead of iron to PpIX under conditions of iron deficiency, some of the accumulated PpIX may be lost by conversion into the fluorescent compound Zn-protoporphyrin.⁹

Finally, with certain types of cells it is possible to induce the synthesis and accumulation of endogenous porphyrins merely by exposing the cells to exogenous ALA. Under normal conditions, the rate of synthesis of heme in these cells is closely regulated by a feedback control mechanism in which free heme (heme that has not yet become bound to protein) suppresses the synthesis of ALA.¹⁰ Such a feedback control system offers an opportunity for manipulation of the pathway; the control mechanism can be bypassed by supplying enough exogenous ALA to saturate the biosynthetic capacity of the pathway. Under such conditions, heme and all of its intermediates will be produced at the maximum rate permitted by the enzyme profile of the cell in question. A specific intermediate will accumulate if the rate at which that particular intermediate is being synthesized is greater than the rate at which it can be converted into the next compound in the pathway. Since both the relative and the absolute capacities of the various steps in the biosynthetic pathway for heme may be different for different types of cells, the specific intermediates that accumulate in the presence of exogenous ALA may vary with the cell histology. With certain enzyme profiles, more than one intermediate may accumulate.

Any toxicity that might be caused by the administration of exogenous ALA will not be associated with a deficiency of heme, but with an excess of either ALA, or heme, or one or more of the intermediates in the biosynthetic pathway for heme. An excess of heme can be toxic,¹¹ and an excess of some of the porphyrins may cause problems also.¹² However, the body has developed quite efficient techniques for dealing with smaller amounts of these compounds under normal circumstances, and any such imbalance will be only transient.

Cell and tissue specificity of the uroporphyrins and coproporphyrins

The uroporphyrins and coproporphyrins would only be useful for photodynamic therapy if they can be induced to accumulate preferentially within the target cells or tissues. In principle, preferential accumulation might be on the basis of either preferential biosynthesis and retention of the porphyrin by the target cells, or preferential accumulation of the porphyrin by target cells at a site distant from the site of biosynthesis of the por-

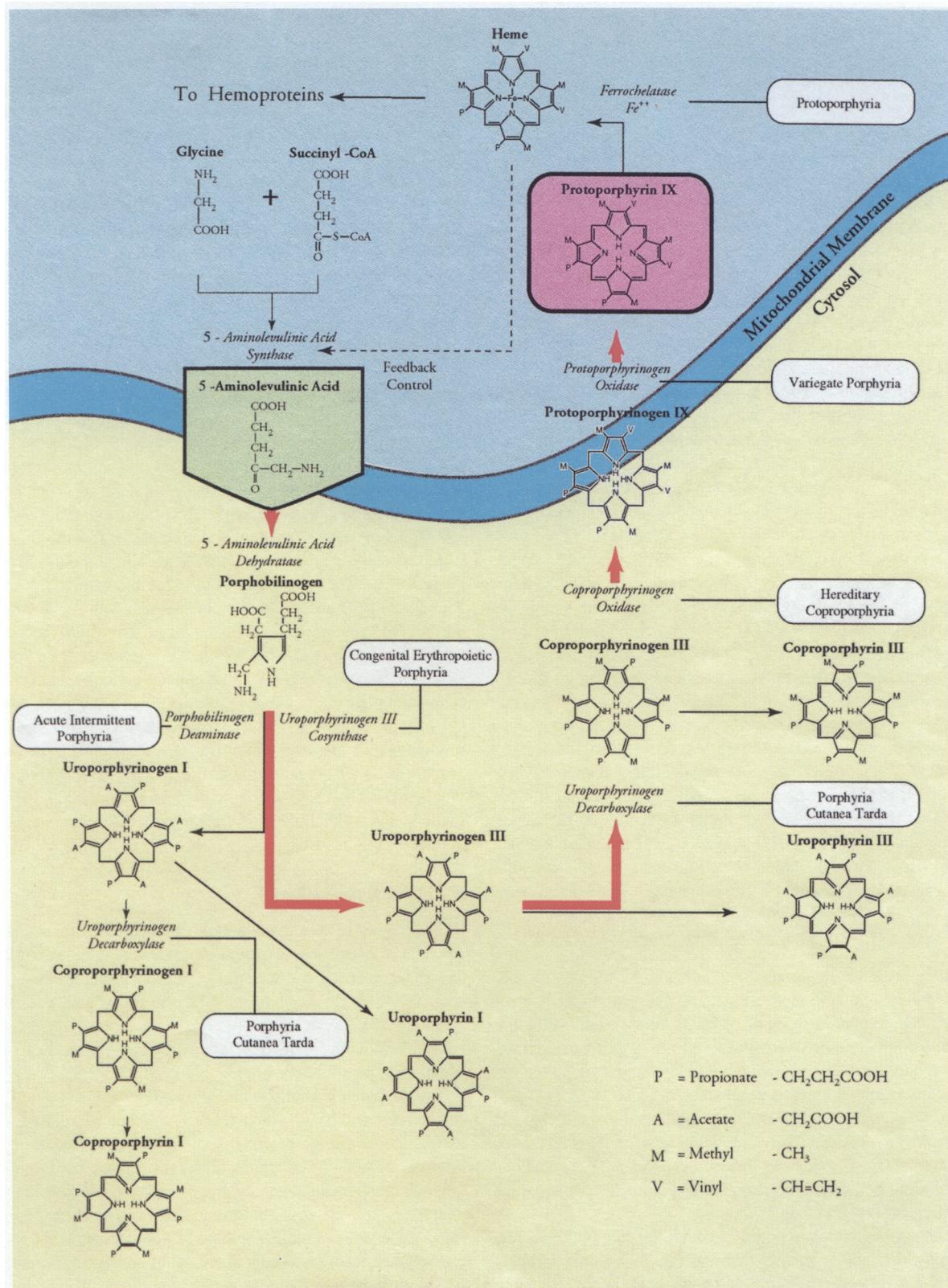


FIG. 1. The heme biosynthetic pathway.

phyrin. However, the uroporphyrins and coproporphyrins are quite soluble in water, and they quickly diffuse away from any cells or tissues in which they are synthesized.

Once they enter the circulation, endogenous uroporphyrins and coproporphyrins behave as though they were of exogenous origin. Exogenous photosensitizers normally are injected intravenously and reach the target cells and tissues via the blood. The mechanisms ultimately responsible for the specificity of exogenous photosensitizers involve interactions between the physicochemical properties of the photosensitizers and the physiology or biochemistry of the malignant cells and/or tissues. For example, the pH of the extracellular fluid in malignant tumors usually is lower than the extracellular pH in adjacent normal tissues.¹³ However, the pH of the intracellular fluid usually is comparable in both normal and malignant cells,¹⁴ and the pH gradient across the plasma membrane of malignant cells within the tumor therefore may be quite different or even reversed from that of cells adjacent to the tumor. Since the relative solubilities of ionizable photosensitizers in the lipid of cell membranes and in the aqueous environments of the extracellular and intracellular fluid (oil/water partition coefficients) may vary as a function of pH,¹⁵ any pH gradient across the plasma membrane (or mitochondrial or lysosomal membrane) may act as a "pump." This pump, which is driven by differences in the oil/water partition coefficients on the opposite sides of the membrane, may cause the photosensitizer to accumulate preferentially on one side of the membrane if the pH gradient is in one direction, but on the opposite side if the pH gradient is reversed. Such a mechanism may determine whether the photosensitizer will tend to localize primarily within the walls of the blood vessels within the tumor, or at specific sites within the malignant cells (in the plasma membranes, in the intracellular fluid, or within specific organelles). The uroporphyrins and coproporphyrins are so soluble in water that any change in pH within the physiological range has little effect upon their solubility in lipid, and these porphyrins therefore show little tendency to accumulate in any particular tissue. PpIX thus remains our most reasonable candidate for an endogenous porphyrin photosensitizer.

Cell and tissue specificity of protoporphyrin IX

In the presence of an adequate concentration of exogenous ALA, fluorescing and photosensitizing concentrations of PpIX accumulate in certain types of cells and tissues, but not in others. Substantially more ALA-induced PpIX appears to accumulate in malignant and premalignant cells than in the nonmalignant cells from which they were derived. Since PpIX is almost insoluble in aqueous solutions at physiological pH, much of it is retained by the cells in which it accumulates until it is converted into heme. This provides a physical basis for the observed cell and tissue specificity. However, the biochemical basis for such specificity is not yet clear.

PpIX will accumulate within a cell if the rate at which it is being synthesized is greater than the rate at which it is being lost by conversion into heme, by catabolism, and/or by diffusion from the cell. If malignant transformation is associated with either a significant increase in the rate of synthesis of ALA-induced PpIX or a significant decrease in the rate at which PpIX is lost from the cell, then PpIX may accumulate preferentially within the transformed cell.

When the presence of an excess of exogenous ALA forces the biosynthetic pathway for heme to operate at maximum capacity, the maximum rate at which PpIX can be synthesized will be limited by the maximum capacity of the slowest step in that segment of the pathway that lies between ALA and PpIX. ALA-induced PpIX will not accumulate if the maximum capacity of this rate-limiting step is so low that PpIX cannot be produced faster than it is lost. This may explain why certain types of cells accumulate little or no PpIX when exposed to exogenous ALA. In contrast, if the maximum capacity of the rate-limiting step allows PpIX to be produced faster than it can be lost from the cell, then PpIX will accumulate. Such a situation can occur with certain types of normal cells, as well as with premalignant or malignant cells.

ALA-induced PpIX might accumulate preferentially in malignant and premalignant cells if malignant transformation is associated with a significant decrease in the capacity of the cells to convert PpIX into heme. A decrease might involve the inhibition of ferrochelatase itself, or inhibition of iron transport or metabolism. Defects in iron metabolism are commonly found in association with cancer,^{16,17} but it is not yet clear that such defects are responsible for the observed ALA-induced PpIX phenotype of malignant and premalignant cells.

In summary, if exogenous ALA is present at a concentration sufficient to saturate the capacity of the biosynthetic pathway for heme, PpIX will accumulate in any cells in which the rate at which PpIX is being synthesized is greater than the rate at which it is being converted into heme, catabolized, and/or lost by diffusion from the cell. Malignant and premalignant cells often accumulate more ALA-induced PpIX than do the normal cells from which they were derived because malignant (and premalignant) transformation may be associated with alteration(s) in the enzyme profile for the pathway. It is not yet clear whether this change in the enzyme profile leads to an increase in the rate at which PpIX is synthesized, or a decrease in the rate at which PpIX is lost.

Practical implications

The goal of PDT is the complete destruction of specified target cells without causing a clinically unacceptable level of damage to normal cells and structures that lie within the same treatment field. To do so there must be significant cell and tissue specificity, as shown by a difference in photosensitizer accumulation. Two conceptually different clinical approaches make use of this specificity.

The degree of phototoxic damage produced in a cell or tissue can be regulated by varying either the concentration of the photosensitizer or the dose of photoactivating light. One approach involves the use of a relatively high concentration of the photosensitizer, to ensure that the target cells accumulate enough photosensitizer to cause phototoxic death if they are exposed to an adequate dose of light. The dose of light is regulated to avoid causing a clinically unacceptable level of damage to normal tissues within the treatment field. There are two difficulties with this approach. First, the ratio of the photosensitizer concentrations (abnormal cells/normal cells) decreases as the concentration of the photosensitizer increases (Gen Li, unpublished observations). Second, it may be very difficult technically to give a lethal dose of photoactivating light to every part of the target

tissue without causing severe damage to part of the adjacent normal tissues. This is a particularly difficult problem for complex shapes such as the ear, oral cavity, uterus, and urinary bladder. If the light dosimetry is calculated to avoid causing damage to the normal tissues within the treatment field, then it may not be possible to give every part of the abnormal tissue a lethal dose of light. However, if all of the abnormal tissue is given a lethal dose, some of the normal tissues within the treatment field may be destroyed also. In principle, it might be possible to use an extremely complex light delivery system to minimize the problems caused by complex shapes, but such a system may not be feasible for routine clinical practice.

The second approach involves the use of a concentration of photosensitizer low enough to prevent the normal tissues within the treatment field from experiencing irreversible phototoxic damage even when exposed to very large doses of light. Phototoxic damage occurs, but the rate at which it occurs does not exceed the repair capacity of the normal cells. However, since the abnormal cells contain significantly more photosensitized, it may be possible to destroy them selectively. The main advantage of this approach is that it greatly simplifies the dosimetry of the photoactivating light. If it is not possible to cause irreversible harm to the normal tissues no matter how much light is given, then the whole treatment field can be given whatever dose of light is required to ensure that every part of the target tissue has received a lethal exposure. However, the concentration of the photosensitizer in the target tissues must be high enough to cause lethal phototoxicity. Once again, adequate cell and tissue specificity is essential for successful PDT.

Another application of fluorescent photosensitizers is to increase the sensitivity of detection of abnormal cells and tissues (photodiagnosis or PD). The best fluorescence differential usually is produced by relatively low doses of photosensitizer (J.C. Kennedy and Gen Li, unpublished observations), although these low concentrations may or may not be sufficient to cause lethal photosensitization of the lesions.

Modulation of the concentration of PpIX

The accumulation of ALA-induced PpIX in cells and tissues can be modulated by varying the concentration of the exogenous ALA to which the cells or tissues are exposed, by chelating iron that otherwise might be used to convert PpIX into heme, and by inhibiting selected enzymes in the biosynthetic pathway for heme. The pharmacokinetics of the various processes also may have an important influence upon PpIX accumulation, and these vary with the route and timing of ALA administration as well as with the biosynthetic activity of the cells in question. Granted that it is possible to modulate the accumulation of ALA-induced PpIX, what should be our goal?

The cell and tissue specificity of ALA-induced PpIX is greatest when the normal cells and tissues contain only a little PpIX. For example, if 20% ALA in an oil-in-water emulsion is applied to the skin of a patient with diffuse T-cell lymphoma (mycosis fungoïdes) for 6 to 18 h, the "normal" skin develops so much PpIX fluorescence that it becomes difficult to identify many of the sites of localized accumulation of the malignant cells. However, if the skin is exposed to a concentration of 2% or even 1% ALA for the same period of time, the background fluorescence of the "normal" skin almost disappears and the localized

accumulations of T cells can be identified as focal areas of bright red fluorescence (J. Kennedy, unpublished observations). Some patients with basal cell nevus syndrome have a very reactive "normal" skin. Exposing such skin to 20% ALA in an oil-in-water emulsion for 3 h causes the whole skin to develop a bright red fluorescence, but exposure to 1 or 2% ALA for 6 to 18 h results in a greatly reduced background that permits the detection of occult areas of abnormality (J. Kennedy, unpublished observations). If our goal is the detection of occult malignant tissue, there would seem to be little value in using iron chelators or enzyme inhibitors in addition to exogenous ALA, since ALA by itself can induce more than enough PpIX for our purpose.

However, the concentration of ALA-induced PpIX that is optimal for the detection of occult malignant tissues may not be adequate to ensure their destruction by photodynamic action. The concentration of PpIX can be increased merely by increasing the concentration of exogenous ALA or the time that the cells or tissues are exposed to the ALA, but there may be some situations in which the resulting increase in PpIX concentration is not enough. The addition of iron chelators and/or enzyme inhibitors could then be considered. However, once again we would face the problem of maintaining adequate cell and tissue specificity. Any increase in the PpIX concentration in the normal tissues within the treatment field leads to a decrease in tissue specificity. Moreover, since the specificity of ALA-induced PpIX accumulation is dependent upon characteristic differences between the normal and abnormal cells with respect to the activity of specific enzymes in the heme biosynthetic pathway, it is not obvious that drug-induced changes in those pathways will be helpful unless they act to emphasize the preexisting differences. For example, if malignant transformation is characterized by a substantial increase in the capacity of the transformed cells to convert ALA into PpIX (but without any change in their capacity to convert PpIX into heme), then the use of an iron chelator or enzyme inhibitor to inhibit ferrochelatase activity may be counterproductive. The conversion of PpIX into heme will be blocked in both the normal and the malignant cells, but the proportional increase in the accumulation of PpIX will be much greater in the normal cells because a greater proportion of their ALA-induced PpIX is normally converted into heme. On the other hand, if malignant transformation is characterized by the development of a partial block in the conversion of PpIX into heme, then the specificity of ALA-induced PpIX is not likely to be improved by adding a drug-induced block in the normal cells at the level of either ferrochelatase or protoporphyrinogen oxidase (Fig. 1), since such a block would merely cause the normal cells to mimic the ALA phenotype of the malignant tissue. See, however, Moan *et al.*¹⁸

Pharmacokinetic studies

Pharmacokinetic studies can be used to identify conditions that would enhance the accumulation of ALA-induced PpIX in abnormal cells while maintaining or improving cell and tissue specificity. The pharmacokinetics of ALA-induced PpIX are quite different from those of preformed photosensitizers. The injection of a preformed photosensitizer into the body at a specific anatomical site is followed by dilution of the photosensitizer and several possible modes of delivery to the target cells or tissues.¹⁹ However, since ALA-induced PpIX must be synthe-

sized within the body, the overall pharmacokinetic curves involve the summation of several different processes.

ALA can be delivered to the target tissue by topical application, by oral administration, or by injection (intravenous, intraperitoneal, subcutaneous, intradermal, or intralesional). Each technique delivers a different proportion of the total dose of ALA to the target tissues, and with somewhat different timing. The topical application of ALA creates an extracellular reservoir from which ALA slowly diffuses into the adjacent tissues, while the intravenous injection of ALA may create intracellular reservoirs of porphobilinogen and other intermediates that permit the continued biosynthesis of PpIX at a time when much of the exogenous ALA has vanished from the circulation and extracellular fluid. A relatively large proportion of the ALA that is administered either orally or by intraperitoneal injection will be lost by conversion into PpIX in the liver (with subsequent excretion via the bile), while ALA in the blood can be lost by excretion through the kidneys. Once the ALA reaches the mitochondria, the rate at which PpIX accumulates within the target cells is a function of both the rate at which PpIX is being synthesized and the rate at which it is being lost by conversion into heme, by catabolism, or by diffusion out of the cells. The ALA-induced PpIX that diffuses out of the cells and enters the circulation behaves much like a preformed photosensitizer. With all of these possible variables, it might be expected that the pharmacokinetic curves for ALA-induced PpIX will be rather complex.

With the above considerations in mind, one would predict either a two or a three stage time course for PpIX accumulation in the presence of an excess of exogenous ALA. The first stage reflects the process of delivery of exogenous ALA to the mitochondria and its subsequent conversion into PpIX. For example, the time required for topical ALA to diffuse through normal human skin can cause a delay of approximately 1 h in the development of PpIX fluorescence, relative to the time required if the ALA is injected intradermally. In either case, the concentration of ALA within the mitochondria is very low initially, but increases rapidly and progressively until the maximum capacity of the pathway for heme biosynthesis is reached. The PpIX concentration vs time curve therefore will show a progressive increase in the rate of accumulation of PpIX until the biosynthetic capacity for PpIX is saturated, at which point the rate of accumulation becomes constant. Subsequent variations in the concentration of ALA will have no effect upon the rate of synthesis of PpIX so long as there is enough ALA to maintain saturation of the biosynthetic pathway. If the various mechanisms by which PpIX can be lost become saturated also, then the rate of accumulation of PpIX will remain constant.

ALA is cleared rather rapidly from the blood and extracellular fluid. In the absence of either a continuous infusion or an extracellular reservoir of exogenous ALA, the concentration of ALA eventually will drop below the level required to saturate the biosynthetic pathway for PpIX. Intracellular reservoirs of porphobilinogen and other precursors of PpIX may delay the response to the lower concentration of ALA, but eventually the rate of synthesis of PpIX will begin to decrease. During this transitional stage, PpIX continues to be synthesized but not at the maximum possible rate, and eventually the rate of PpIX biosynthesis is negligible. During this final stage of the pharmacokinetic curve, the decrease in PpIX fluorescence is primarily

a reflection of the rate at which PpIX is being converted into heme, catabolized, or lost from the cells by diffusion.

The first noninvasive monitoring of the bioconversion of ALA into PpIX was carried out 10 years ago, in mice.²⁰ Following the ip injection of a bolus of ALA into nude mice, surface-detected PpIX fluorescence was monitored for a 24-h period. A two-stage pharmacokinetic curve was obtained for ALA doses ranging from 16 to 250 mg/kg of body weight. The initial phase of increasing PpIX fluorescence lasted from 2 to 4 h, with a linear relationship between the time of maximum PpIX fluorescence and the log of the ALA dose (these may be termed "fluorokinetic" parameters). There was a linear relationship also between the maximum intensity of the PpIX fluorescence and the log of the ALA dose. Once the maximum PpIX fluorescence had been achieved, there was a rapid monofunctional reduction in PpIX fluorescence. The clearance time (defined as 3 times the 1/e lifetime) of PpIX injected directly into nude mice has been reported to be 7.8 h,²¹ which is in good agreement with the second phase clearance pharmacokinetics of ALA-induced PpIX reported by Pottier *et al.*²⁰

When ALA was dissolved in an oil-in-water emulsion and applied topically to the skin of normal human volunteers, the fluorokinetics of PpIX accumulation was quite different from that observed following the ip injection of ALA into nude mice. As shown in Figure 2, there was an initial rapid rise in PpIX fluorescence during the first 3 to 5 h, and during the terminal phase there was a rapid fall. These parts of the curve are quite similar to the pattern observed in the mice that had been injected intraperitoneally. However, the ALA-induced PpIX fluorescence in human skin persisted much longer, primarily because the fluorokinetic curves showed a long segment that linked the initial and terminal phases. This intermediate phase was not observed in the mice. The intensity of the PpIX fluorescence in this region showed a slowly oscillating pattern. As previously suggested, this may be due to a dynamic and somewhat unstable balance between the rate of production of PpIX and its rate of clearance.

In a controlled fluorokinetic study involving the administration of ALA in an oil-in-water emulsion, the surface detected PpIX fluorescence was monitored over 48 h on patients with actinic keratosis, basal cell carcinoma and psoriasis. The ALA

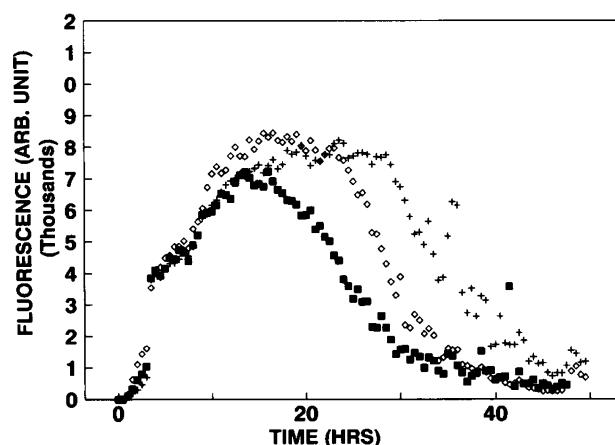


FIG. 2. Typical ALA-induced protoporphyrin IX fluorescence vs time curves for normal human skin. ALA concentrations (■, 9.7%; +, 19.2%; ◇, 29.3%).

was applied topically under occlusion for the first 3 h, then wiped off, and PpIX fluorescence intensity measurements made every 15 min. There was considerable patient-to-patient variation in the time to maximum fluorescence, with no apparent systematic difference in timing between the normal and the abnormal tissues. However, the abnormal tissues developed much stronger fluorescence. The fluorescence intensity in all cases appeared to be independent of the concentration of ALA over the range tested (10, 20, or 30%) (Kennedy *et al.* unpublished observations). Similar results have been reported for psoriatic plaques by Stringer *et al.*²²

The results of clinical trials are given below.

TOPICAL ALA PDT FOR DERMATOLOGIC INDICATIONS

Superficial basal cell carcinoma (sBCC) and nodular basal cell carcinoma (nBCC)

Results reported using topical ALA PDT for the treatment of sBCCs are summarized in Table 1. All studies reported used 20% ALA (w/v) in various cream formulations. Light application was performed from 3 to 8 h topical ALA application, and PpIX fluorescence was detected through direct visualization^{23–25} or quantitative²⁶ fluorescence detection methods. Kennedy and Pottier²³ irradiated lesions with broadband (600–800 nm) red light using a wide light dose range from a Kodak® slide projector with a red filter, and reported a 90% complete response (CR) rate at the relatively short follow-up period of 3 months. Wolf *et al.*²⁴ also used a slide projector (Leik Pradovit®), but with unfiltered white light or filtered red light to a total dose of 30 J/cm² to treat 37 sBCCs and reported a 97% CR rate with follow-up periods ranging from 3 to 12 months, with 1/36 lesions recurring during that

time. Cairnduff *et al.*²⁵ irradiated 16 sBCC lesions in 4 patients with 630 nm light from a copper vapor/dye laser to a light dose of 125–250 J/cm², and had an 88% CR, but found a 50% (8/16) tumor recurrence rate with a relatively long median follow-up of 17 months (range 4–21 months). Svanberg *et al.*²⁶ used an Nd:YAG-pumped dye laser to generate 630 nm light to a total dose of 60 J/cm² to treat 55 lesions in 21 patients. All of the 55 treated lesions showed a CR after 3 weeks, and no recurrences were reported over a follow-up period of 6 to 14 months. Warloe *et al.*²⁷ treated a large number of sBCC and used 630 nm laser light from a copper vapor-pumped dye laser at a power density <150 mW/cm² and a total fluence which ranged from 40 to 200 J/cm² with excellent results. The addition of 2–20% DMSO and 2–4% EDTA to ALA in cream did not appear to improve efficacy in this population of patients, and neither did treatment of the lesion with 50–99% DMSO applied 15 min prior to cream application (data not shown).

Calzavara-Pinton²⁸ used ALA PDT in a manner different from the other reports; treatments were repeated every other day "until the entire area appeared uniformly eroded, without clinically evident tumor, after gentle removal of blistering, crusting and/or necrosis."⁸ From 1 to 3 treatments (average 2) were used to treat sBCC by the author.²⁸ In most sBCC treatment studies, after ALA PDT treatment the lesions became erythematous and edematous, later forming a crust which fell off within 2 to 6 weeks, with complete healing by reepithelialization reported in 8–12 weeks.

Most investigators reported patient discomfort (described as mild to strong itching, stinging, prickling, or burning sensations) during the period of light irradiation, and two reports^{25,26} used topical local anesthetic in order to prevent the discomfort. The discomfort was noted to last only during light treatment. No other adverse events were noted.

Results of the topical ALA PDT treatment of nBCCs are sum-

TABLE 1. RESULTS REPORTED FOR TREATMENT OF SUPERFICIAL BASAL CELL CARCINOMA USING 20% ALA

Paper	Number of patients (number of lesions)	Wavelength (nm)	CR No. (%)	Follow-up (months)	Tumor regrowth
Kennedy and Pottier ²³	(80)	600–800	72 (90)	3	0/72
Wolf <i>et al.</i> ²⁴	4 (37)	Broadband	36 (97)	3–12	1/36
Cairnduff <i>et al.</i> ²⁵	14 (16)	630	14 (88)	17 (med)	8/16
Svanberg <i>et al.</i> ²⁶	21 (55)	630	55 (100)	6–14	0/55
Warloe <i>et al.</i> ²⁷	ALA (141) ALA +DMSO/ EDTA (125)	630	130 (92) 114 (91)	>12	6/130 1/114
Calzavara-Pinton ²⁸	(23)	630	23 (100) CCR 10/11	24–36	2/12
			biopsy-proven		

marized in Table 2. Wolf *et al.*²⁴ reported treating 10 nBCCs, with only 1/10 showing a complete response (PR), the extent of response was not specified. Svanberg *et al.*²⁶ however, treated 25 nBCCs and reported 64% (16/25) CRs after a single treatment, with the rest PRs. Retreating the partially responding lesions 6–8 weeks after the first treatment converted all PRs to CRs. Duration of response or recurrence rates for nBCCs, however, were not reported. Warloe *et al.*²⁷ treated a total of 187 nBCCs that were >2 mm thick, and the results obtained using ALA alone or ALA + DMSO + EDTA are shown in Table 2. Only 19/56 (34%) showed a complete response, and this increased to 25/45 (55%) if DMSO + EDTA were used in the emulsion with ALA. Treatment of 86 lesions with 50–99% DMSO applied 15 min prior to ALA cream application yielded 41 CRs (48%)(data not shown). Calzavara-Pinton²⁸ reported that nBCCs required an average of four treatments with ALA PDT (range 2–8), and 2 of 6 nBCCs that appeared to be CRs developed recurrences at 2 and 19 months. Tumor remnants in biopsies that had completely healed and appeared as CRs, when present, were found in the medium and deep dermis and were covered by normal skin.²⁸

Bowen's disease (squamous cell carcinoma *in situ*)

This completely intraepidermal disease appears to respond well to topical ALA PDT^{25,26,28} (reported results are given in Table 3). Cairnduff *et al.*²⁵ reported that 35/36 (97%) of lesions completely responded at 2 months after treatment, and only 3/35 (9%) recurred after 18 months of follow-up. Similar results were reported by Svanberg *et al.*²⁶ Calzavara-Pinton²⁸ that treated a small number of Bowen's disease lesions (6) that required an average of 2 ALA PDT treatments (range 1–3) and complete, biopsy-proven or durable responses were seen in all lesions.

Actinic (solar) keratoses

These dysplastic, epidermal lesions found on sun-damaged skin were also treated with topical ALA PDT (Table 4). Wolf *et al.*²⁴ treated a small number of lesions, but 9/9 completely responded to the treatment. A single-center Phase I DUSA-sponsored protocol was carried out in which lesions were exposed to varying concentrations of ALA in an oil-in-water

TABLE 3. REPORTED RESPONSES OF BOWEN'S DISEASE TO ALA PDT WITH 20% ALA AND 630-nm LIGHT

Paper	Number of patients (number of lesions)	CRs No. (%) at 2 months	CRs No. (%) at 18 months
Cairnduff et al. ²⁵	14 (36)	35 (97)	32 (89)
Svanberg et al. ²⁶	3 (10)	9 (90) (6–14 months)	
Calzavara- Pinton ²⁸	(6)	6 (100) [24–36 months (3 lesions) or biopsy-proven (3 lesions)]	

emulsion under occlusion for 3 h and then treated with 630-nm laser light doses ranging from 10 to 150 J/cm². Jeffes *et al.*²⁹ in a preliminary report, stated that using 30% ALA, 19/32 (59%) of lesions responded completely at 16 weeks posttreatment, with an additional 34% giving a partial response (>50% improvement in lesion area or depth). Calzavara-Pinton²⁸ treated 50 actinic keratosis lesions, all on the face, which required an average of 2 treatments (range 1–3) for 100% apparent clinical complete responses. Of 17 lesions that were subjected to biopsy in this latter study, 4 showed foci of dysplasia of epidermal keratinocytes. Of the remaining 33 actinic keratoses that were followed for 24 to 36 months, 5/33 (15%) developed recurrences at 3, 6, 15, 19, and 32 months. All of the 5 recurrent actinic keratoses were retreated with ALA PDT; 4 responded to a second treatment cycle and one to a third treatment cycle.

INTRAUTERINE ALA FOR ENDOMETRIAL ABLATION

The two-horned (didelphic) rat uterus was used by Yang *et al.*³⁰ in a series of *in vivo* experiments showing that intrauterine ALA injection (4–16 mg in 0.1 ml) followed by exposure to

TABLE 2. REPORTED RESPONSES OF nBCCs TO TOPICAL ALA PDT TREATMENT

Paper	Number of patients	Number of lesions	CR No. (%)	PR No. (%)
Wolf <i>et al.</i> ²⁴	5	(10)	1 (10)	9 (90)
Svanberg <i>et al.</i> ²⁶				
First treatment	21	(25)	16 (64)	9 (36)
Second treatment			25 (100)	0
Warloe <i>et al.</i> ²⁷				
ALA		(56)	19 (34)	35 (63)
ALA + DMSO/ EDTA		(45)	25 (55)	16 (36)
Calzavara- Pinton ²⁸		(30)	24 (80) 15 (50) biopsy- proven	15 (50)

TABLE 4. REPORTED RESPONSES OF ACTINIC KERATOSES TO TOPICAL ALA PDT

Paper	Number of patients (number of lesions)	CRs No. (%)	PRs No. (%)
Wolf et al. ²⁴ (20% ALA, broad band light)	3 (9)	9 (100)	0
Jeffes et al. ²⁹ (30% ALA, 630 nm)	7 (32)	19 (59)	11 (34)
Calzavara-Pinton ²⁸ (20% ALA, 630 nm)	(50) by biopsy	50 (100) 13/17 (76)	4 (24)

broadband red light (150 J/cm² total light dose) from a slide projector, 3 h after injection, caused selective photodynamic ablation of endometrial tissue. By treating one of the two uterine horns with ALA PDT the authors were able to show that a single treatment with ALA PDT could completely prevent fetal implantation in the treated horn when animals were mated as long as 60–70 days after treatment, although normal implantation occurred in the untreated control horn. Atrophic endometrium persisted in the ALA PDT-treated uterine horn despite the strong hormonal stimulus of multiple pregnancies occurring within the contralateral horn. Similar results have been obtained with rat and rabbit uteri using intrauterine ALA instillation and 630-nm laser light delivered to the uterus via a cylindrical diffuser-tipped fiber optic delivery system.³¹

Instillation of ALA into human uteri *in vivo* and *in vitro* has recently been shown to cause selective endometrial fluorescence from PpIX accumulation.³² ALA (100–200 mg) was placed in the cavity of 4 human uteri after hysterectomy and uterine perfusion performed for 4 h. In the *in vivo* study, ALA (25–900 mg) was placed in uteri of 8 women for 2 to 5 h prior to hysterectomy, and the selectivity of endometrial PpIX accumulation in endometrium and myometrium examined using fluorescence microscopy as well as direct porphyrin extraction of the exposed tissue. The *in vitro* perfused uterus model exhibited a 10-fold greater PpIX concentration (mean 0.31 ng/mg) than the myometrium (mean 0.03 ng/mg tissue). *In vivo* concentrations of PpIX extracted from the endometrium (mean 1.39 ng/mg tissue) were 9-fold higher than in the myometrium (mean 0.17 ng/mg). Fluorescence photomicrographs indicate that the bulk of PpIX accumulation occurred specifically within the superficial and deep uterine glands, with most glands in the ALA-treated uteri exhibiting fluorescence.

FLUORESCENCE DETECTION (PHOTODIAGNOSIS) USING ALA

Fluorescence detection of nonmelanoma skin and head and neck cancers

While ALA is a simple molecule that does not demonstrate fluorescence, PpIX has a high fluorescence yield.³³ The high

fluorescence yield of PpIX has allowed visual verification of the accumulation of PpIX synthesis in skin lesions using simple hand-held UV lamps (PpIX fluorescence is seen as a pink-red color in a background of blue or violet light).²³ Under conditions used for ALA PDT of skin lesions, normal skin usually does not produce sufficient PpIX to cause fluorescence.²³ Wang *et al.*³⁴ used laser-induced fluorescence (LIF) to document an increase in fluorescence 4–6 h after the application of topical 20% ALA to 24 lesions (12 sBCC, 7 nBCC, 3 Bowen's disease, and 5 cutaneous T-cell lymphoma) in 17 patients. A nitrogen laser-pumped tunable dye laser, was used to provide an activating wavelength of 405 nm and the fluorescence emission spectra of lesions was obtained by point monitoring. Tissue autofluorescence was evaluated at 490 nm emission, and the specific PpIX related fluorescence peak at 635 nm was also monitored. Using PpIX-related fluorescence without correcting for autofluorescence but subtracting background fluorescence, all lesions showed fluorescence ratios of tumor:normal skin varying from a low of 9:1 (for Bowen's disease) to a high of 15:1 (cutaneous T-cell lymphoma), with the BCCs falling in between the two extremes. Incorporating autofluorescence into the study provided a further enhancement of 7 to 10-fold for cutaneous tumors as compared with normal skin (ratios varying from 47:1 to 127:1).¹³ These findings support the concept that PpIX accumulation from topical ALA application to skin tumors is quite selective as compared with normal skin.

Svanberg *et al.*³³ examined fluorescence in various types of head and neck cancers and precancerous conditions from approximately 30 patients given ALA by oral administration. Fluorescence quantitation was determined over varying periods of time using LIF as described by Wang *et al.*³⁴ PpIX-specific fluorescence was observed in lesions as early as 30 min following ingestion, with maximum fluorescence occurring 5–8 h after ingestion. In a report on 7 patients, the ratio of fluorescence intensity at 635 and 490 nm appeared elevated for advanced tumors, but not for nonmalignant or dysplastic tissue, in most cases. The authors reported that an ALA dose of 5.0–7.5 mg/kg of body weight was optimal for tumor demarcation.³³ No adverse events of oral administration of ALA were reported at this dose range.

Intravesical ALA for diagnosis of bladder cancer and dysplasia

The recurrence rate of superficial bladder cancer after endoscopic resection may be as high as 50–75%; this high rate of recurrence is thought to be due to precancerous (dysplastic) areas or foci of remaining cancer that are not visible during routine cystoscopic procedures.³⁵ Baumgartner *et al.*³⁶ carried out studies in rat bladder tumor model systems and showed that intravesically instilled ALA produced PpIX fluorescence in bladder tumors that was 20-fold as intense as that in healthy urothelium. In a clinical study, 3% ALA was instilled into the bladders of 68 patients with bladder cancer. In this procedure, 50 ml of 3% (w/v) ALA was instilled into patient bladders and held there between 0.5 and 4.0 h. Endoscopic examination was carried out after at least 3 h of exposure to ALA. After a median of 204 min of ALA exposure, fluorescence cystoscopy was carried out using activating violet light from a krypton laser or white light; 299 lesions visible under white or violet light (nonfluorescent or fluorescent, respectively) were subjected to biopsy and histo-

logic examination.³⁵ Tumor borders were found to be sharply demarcated by red fluorescence (Fig. 3). Correlation of fluorescent and histologic examination revealed that ALA fluorescence-guided biopsies showed a sensitivity of 100% (all fluorescent lesions were abnormal) and a specificity of 68.5%.³⁵ Of note was the finding of 26 malignant or dysplastic lesions detected only by ALA-induced fluorescence, and missed by white light cystoscopy.³⁵ No false negative findings or adverse events attributable to ALA were reported.

Kriegmair *et al.*³⁷ recently extended their clinical report on detection of early bladder cancer by ALA-induced PpIX fluorescence compared with white light cystoscopy to 106 patients with a suspicion of primary or recurrent bladder cancer. All patients received 50 ml of filter-sterilized 3% ALA solution (neutral pH) intravesically via a 14 F catheter. Patients retained the ALA intravesically before voiding for a mean of 140 ± 90 min (range 15–420 min). All activating light for fluorescence detection used in this study was produced by the krypton ion laser. The interval between ALA voiding and cystoscopy ranged from 10 to 400 min (average 150 ± 80 min). Of the 106 patients, 77 had been treated previously for bladder cancer. A total of 449 biopsies was obtained, 433 of which were evaluated. The sensitivity of the fluorescence cystoscopy (96.9%) was significantly ($p < 0.0001$) greater than that of white light (72.7%). Specificities for white light and fluorescence cystoscopy were similar (approximately 70%). Fluorescence cystoscopy detected 35 urothelial neoplasms that could not be detected under white light (12 dysplasias, 2 CIS, 20 superficial and 1 muscle-infiltrating carcinoma). Tumor grades were not specified. Of the lesions which were only detected by fluorescence cystoscopy, 14 were from normal-appearing urothelium and 21 were from papillary tumors. In this study, the authors noted that false-positive fluorescence findings occurred more frequently in the region of the trigone, bladder neck, and anterior bladder wall than in the remainder of the bladder. Of the 102 false-positive (fluorescent areas found to be histologically benign), areas of florid cystitis, hyperplastic urothelium, or hyperemic mucosa were more frequently falsely positive than those taken from areas of normal, chronically inflamed, or edematous urothelium.

No serious adverse events were reported in this study³⁷; after ALA instillation 7/104 (7%) of patients complained of suprasymphysis pain, alginuresis, or urgency. Patients (9) who could not retain ALA for 60 min all had bladder capacities of <250 ml due to prior procedures, and they suffered pollakiuria and nocturia prior to entry into the study. After fluorescence cystoscopy these symptoms worsened in 4 patients. Gram-negative bacteriuria was detected in 3/107 ((2.8%) of patients following fluorescence cystoscopy but resolved with appropriate antibiotic therapy. Phototoxic reactions were not detected in any of the 106 patients. ALA and various products of porphyrin metabolism (porphobilinogen, uroporphyrin, coproporphyrin, protoporphyrin) were reportedly found in the urine, feces, and erythrocytes from 14 patients in whom the ALA solution was retained intravesically for at least 1 h (levels were not given nor the duration for which they could be detected).

Jichlinski *et al.*³⁸ recently reported similar results using intravesical ALA-induced fluorescence for detection of bladder cancer in 34 patients. Thirteen patients (38%) had primary bladder cancer and 21 (62%) had one (9 patients) or more (12 patients) prior transurethral resections of bladder carcinomas.

Thirteen (38%) patients had also received prior intravesical chemotherapy or BCG. ALA was instilled intravesically as a 3% solution buffered with 5 ml of phosphate-buffered saline and adjusted to pH 5.3 with 1N NaOH. Fluorescence activating light was provided either by a krypton ion laser or a filtered xenon arc lamp (380–450 nm wavelength output). The power of the excitation light at the end of the cystoscope was between 10 and 25 mW for the laser and between 150 and 480 mW for the filtered arc lamp source. Final power densities at the bladder wall with cystoscope illuminated using either the laser or lamp placed 2 cm from the bladder wall were calculated as about 3 and 65 mW/cm², respectively. A long-pass (>520 nm wavelength) filter was placed in the cystoscope eyepiece for visualization of fluorescence. After intravesical instillation of ALA through a 16 F Foley catheter, the catheter was clamped for 4 h (range 175 to 285 min).

Essentially identical results were reportedly obtained using either light source, and the results were combined.³⁸ From 215 biopsies, a total of 97 carcinomas were detected as fluorescence-positive lesions, 47 of which were not detected by white light cystoscopy. An 11% rate of false negatives was obtained in this study, and a 43% rate of false positives. Of the 47 "invisible" foci of carcinoma detected by fluorescence, 14 were dysplasia grade G1, 10 were dysplasia grade G2–G3, 19 were CIS, and 4 were carcinomatous chorionic lymphangitis. Of the 12 fluorescence "false-negative" lesions, 9 were dysplasia grade G1–G2, with one each diagnosed as CIS, pTaG1, and carcinomatous chorionic lymphangitis. It is interesting to note that in this study, of the 46 false-positive results seen under fluorescence cystoscopy, 33/46 (74%) were visibly or pathologically abnormal, and only 26% were diagnosed as normal urothelium. No systemic or local side effects were noted as a result of ALA intravesical instillation or of the photodiagnostic procedure. These results are quite similar to those of Kriegmair *et al.*³⁷ except for a higher percent of false-negative results. The sensitivity of the fluorescence detection procedure could not be calculated because systematic random bladder wall biopsies were not performed. However, the authors note that the use of ALA-induced fluorescence to map the mucosa in bladder cancer surveillance procedures is more efficient and rapid than that of white light cystoscopy, because of the enhanced lesion contrast seen with fluorescence, and may eliminate the necessity of "random" biopsies of normal-appearing bladder mucosa in the future.

SYSTEMIC TREATMENT WITH ALA FOR PDT OF HUMAN CANCERS

In rats, intravenous administration of 200 mg/kg ALA results in selective accumulation of PpIX in the mucosa of hollow viscera, while PpIX synthesis occurred to a much lesser extent in the muscularis layer.³⁹ The highest PpIX accumulation occurred in a colon tumor model.³⁹ Oral administration of 400 mg/kg ALA in phosphate-buffered saline to rats resulted in similarly selective PpIX accumulation peaking within 2 to 5 h of dosing in gastric, colonic, and bladder mucosa.³⁹ The level of fluorescence was found to differ from one epithelial tissue to another (gastric > colon > bladder). Extending these studies to 3 patients with colorectal cancer, the authors found that oral ad-

ministration of 30 mg/kg ALA to 2 patients did not result in selective tumor fluorescence up to 8 h after ALA administration. However, at an ALA dose of 60 mg/kg given to one patient, preferential tumor PpIX accumulation was observed, with a tumor:normal mucosal fluorescence ratio of 5:1 at 7 h post-ALA administration.

ALA PDT of patients with advanced squamous cell carci-

noma (SCC) of the mouth, using orally administered ALA, has been reported.⁴⁰ For fluorescence studies, 2 patients were given 30 mg/kg and 2 were given 60 mg/kg ALA. The PpIX tumor fluorescence profile for both groups of patients was found to peak at 6–8 h post-ALA administration, with twice the fluorescence observed in tumors from patients given the higher dose ALA compared with the lower dose group. Three patients were

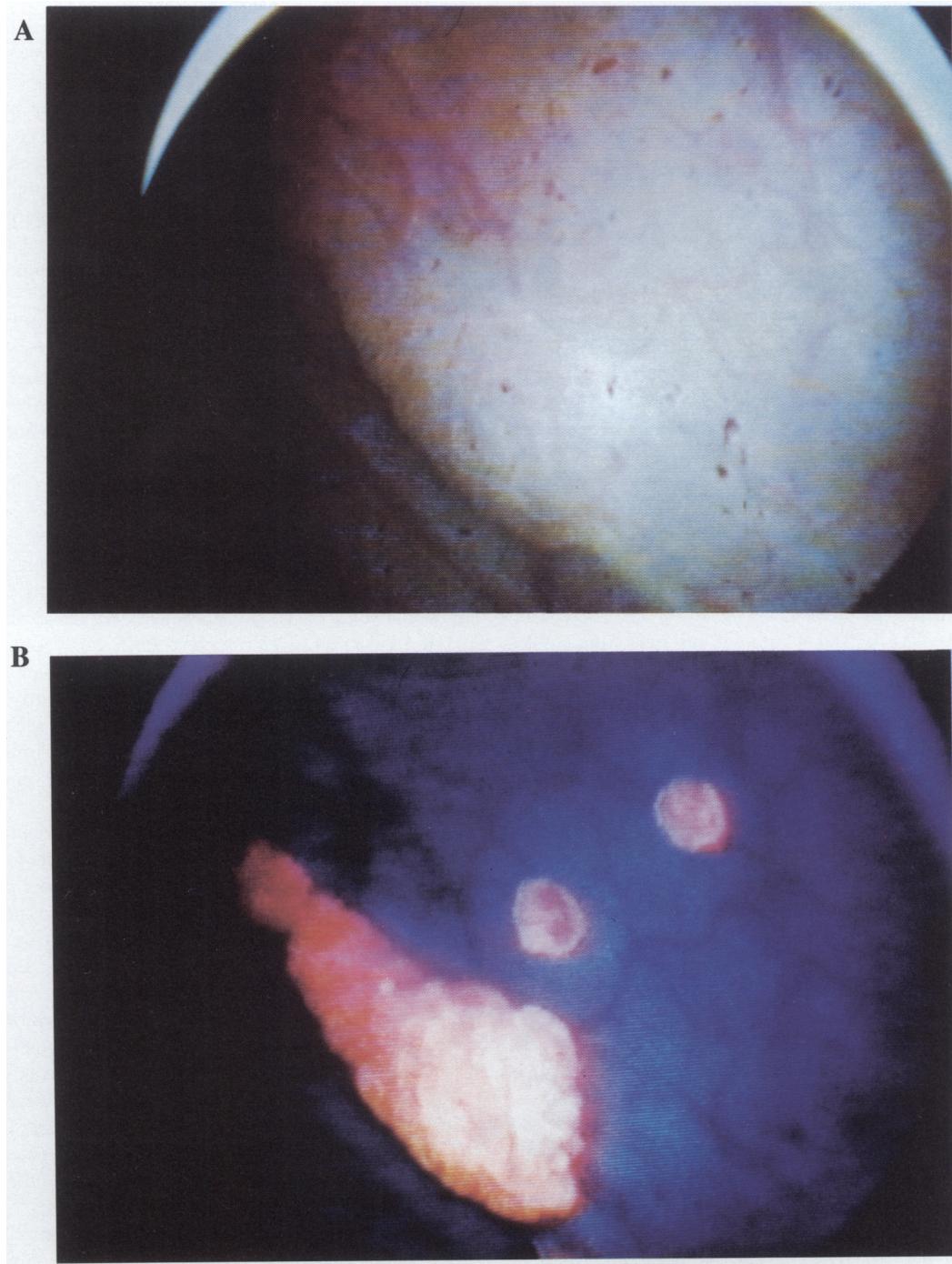


FIG. 3. Photographs of video images taken during cystoscopy of a patient after intravesical application of ALA. (A) White light image showing a recurrent bladder carcinoma. (B) Blue light image of same field showing clear delineation by red fluorescence of the large papillary tumor and two minute satellite tumors. (Courtesy: Dr. M. Kriegmair.)

then given 60 mg/kg ALA, and 4–6 h later their oral cavity SCCs were exposed to 630-nm laser light, with a total light dose of 50–100 J/cm² (dose rate not given). Local discomfort during light treatment was noted, and the surface of the treated tumors became edematous and ulcerated during the 24 h post-ALA PDT. Histologic examination of all treated sites done at 48 h after PDT showed coagulation necrosis and microvascular injury. Elevations of serum aspartate aminotransferase levels, which resolved within 3 days, were noted in 3/4 patients after ALA dosing, and a temporary rise in total bilirubin was also reported. Some necrosis of normal mucosa adjacent to tumor and exposed to laser light treatment was also observed. No cutaneous phototoxicity was observed.

A recent paper by Regula *et al.*⁴¹ reported on the kinetics of fluorescence development in tumors and normal mucosa in 18 patients with gastrointestinal tumors after oral ALA ingestion and subsequent PDT in 10 patients. The study consisted of 3 patient groups, 8 with colorectal tumors (7 adenocarcinoma, 1 adenoma), six with duodenal tumors (3 adenomas and 3 papilla of Vater adenocarcinomas), and 4 patients with esophageal carcinomas (3 adenocarcinoma, 1 squamous cell carcinoma). ALA was given orally at either 30 or 60 mg/kg body weight, with 3 patients receiving drug as a bolus and the remainder of patients receiving it as a fractionated dose in six equal portions at hourly intervals. Using fluorescence microscopy with digital fluorescence quantitation, peak values of PpIX fluorescence were found at 6 h post-ALA ingestion in colorectal tumors with a tumor:normal mucosa fluorescence ratio of approximately 5:1 only in those patients receiving 60 mg/kg ALA. The patients in the esophageal and duodenal tumor groups demonstrated similar high fluorescence intensities for tumor and normal mucosa at 30 mg/kg 6 h post-ALA ingestion, corresponding to that seen in colorectal tumors in patients given the higher ALA dose. Ten patients received ALA PDT, 9 with 628 nm light from a gold vapor laser and the remaining patient receiving 630 nm light from a copper vapor pumped dye laser. The light was delivered either interstitially using a bare fiber or intraluminally using a 1-cm-long cylinder-shaped diffuser tip. Up to 100 J/cm² of light was delivered to the surface, and interstitial light doses given at 2 to 7 spots per tumor was delivered as either 50 or 100 J. Necrosis was observed in 8/10 tumors receiving ALA PDT, with the two instances of no visible necrosis occurring at the low light dose, which was felt by the authors to be clinically insufficient.⁴¹ No complications from endoscopy were seen. Transient increases in serum aspartate aminotransferase activity was seen in six patients (in 66% of patients receiving 60 mg/kg ALA and 20% of patients who received 30 mg/kg ALA), which resolved within 72 h. Mild nausea and occasional vomiting occurred in five patients within 12 h of receiving ALA. Mild skin photosensitivity reactions were seen in two patients between 24 and 48 h after ALA ingestion, but none was observed after 48 h.

Barrett's esophagus is a metaplastic, premalignant disease entity, and patients with this condition may have a risk of esophageal cancer 30–100 times greater than that of the general population. Risk of malignant transformation is greatest when dysplasia is present, although focal dysplasia may be missed during endoscopic examination. Results from fluorescence photometry after oral ALA dosing have suggested that selective accumulation of PpIX occurs in dysplastic areas of Barrett's esophagus. Barr *et al.*⁴² treated eight patients having high grade

dysplasia with ALA PDT using 630-nm laser light delivered from a 3-cm-long cylindrical diffusing fiber at estimated 90–150 J/cm² fluence, approximately 3–4 h after ingestion of 60 mg ALA/kg body weight. During a 2–18 month follow-up period, normal squamous cell regeneration occurred in all patients, and a small area of dysplasia remained in only one patient. No systemic photosensitivity or strictures were observed.

DISCUSSION

ALA PDT using the endogenously synthesized photosensitized, PpIX, has been extended since its original use in skin cancer to a variety of internal applications, through local application to hollow organs as well as via systemic administration using the oral route. ALA has been shown to be effective for selective photosensitization whether administered topically for PDT of cutaneous lesions,^{23–29} locally for use in internal organs such as the urinary bladder^{35–38} or uterus,^{30–32} or systemically via oral or parenteral administration for PDT of internal cancers.^{39–42}

Topical ALA PDT use has resulted in a number of studies reporting efficacy in the treatment of sBCCs.^{23–28} Complete, durable responses ranging from 50 to 100% have been reported following a single ALA PDT procedure using 20% ALA in cream formulations applied for 3–8 h with various light sources. Treatment variables that may alter the efficacy of sBCC treatment include lesion selection ("superficiality" of the lesion), ALA concentration and type of formulation, and light wavelength, power density, and fluence. Szeimies *et al.*⁴³ reported that using 10% ALA in ointment formulation applied to BCCs, PpIX fluorescence 4 h after application was detectable only within sBCCs and skin appendages, but that extending the time of application to 12 h showed PpIX fluorescence within nBCC cells in the deep dermis. Thus, optimizing time of topical ALA application for each ALA formulation used may improve the efficacy of topical ALA PDT. The use of a second ALA PDT treatment 6–8 weeks after initial treatment²⁶ or the use of longer ALA topical preparation application times⁴³ may further improve efficacy for nBCC treatment. Martin *et al.*⁴⁴ recently reported a study in which PpIX fluorescence distribution in 16 BCCs of varying histologic type was measured after topical application of 20% ALA in cream formulation under occlusion for periods ranging from 3 to 18 h. Methods used included *in vivo* photography, *ex vivo* video fluorescence imaging, and digital imaging fluorescence microscopy. Visible pink-red fluorescence was seen in all tumors and fluorescence microscopy revealed strong epidermal fluorescence in all cases. However, full-thickness PpIX fluorescence was seen in 6/7 (86%) sBCCs and 4/9 (44%) of nBCCs with mean tumor depths of 0.34 and 0.75 mm, respectively.⁴⁴ There was no consistent ratio of tumor-to-epidermal fluorescence intensity, and high levels of PpIX fluorescence were consistently seen in the epidermis and epithelial appendages of photodamaged skin, with little PpIX in the dermis, cutaneous vasculature, or musculature.

It should be noted that cross-study comparisons of topical ALA PDT clinical trial reports will remain difficult as long as investigators continue to use different topical vehicles and ALA formulations as well as differing light sources and treatment protocols with differing power density, activating wavelength,

and fluence. However, the body of published work now accumulated on clinical trials suggests that even a single application of topical ALA PDT may be able to remove the great majority of truly superficial SBCCs. Thickened or nodular BCCs appear to vary in their ability to take up ALA or to convert it to PpIX and therefore produce results of varying efficacy following ALA PDT. Initial reports suggest that topical ALA PDT is most effective for treatment of epidermal dermatoses such as Bowen's disease²⁵ and actinic keratoses.^{26,28,29} The selectivity of the pilosebaceous unit in the accumulation of PpIX^{23,43,44} is being used as the scientific basis for the exploration of topical ALA PDT as a treatment of acne and for permanent hair removal.

Approximately 200,000 hysterectomy procedures are performed in the United States annually for treatment of excessive uterine bleeding. The results seen in animal model systems using ALA PDT for endometrial ablation^{30,31} show that complete ablation of uterine glands to the basal layer is possible, such that regeneration of endometrium is not seen even under the strong hormonal stimulus of pregnancy in the untreated uterine horn. It has recently been shown that the human uterus specifically accumulates 9- or 10-fold the amount of PpIX within the endometrium as within the myometrium (by direct tissue extraction) following intrauterine application of ALA, both *in vitro* using a perfused uterine model and *in vivo*.³² Furthermore, specificity may exceed that seen by chemical extraction as fluorescence microscopy shows that fluorescence appears mainly confined to the uterine glands and is minimal in surrounding stroma. Clinical trials in the near future will determine whether locally applied ALA followed by light treatment may allow the substitution of a minimally invasive procedure (ALA PDT) for hysterectomy.

ALA also appears to have potential for clinical use as a photodiagnostic or photodetection agent.³³⁻³⁸ Fluorescence visualized after intravesical instillation of ALA appears to show some specificity for mucosal lesions, including cancer and dysplasias³⁵⁻³⁸ (Fig. 3), although false positives caused by inflammatory areas are also seen. However, it is the improvement in sensitivity rather than specificity that suggests that ALA PD could be a useful tool for guiding biopsies. An additional potential application of ALA-induced tumor fluorescence is to use it during transurethral electroresections to clearly delineate the tumor borders as well as to detect additional tumors or dysplasias that might not be seen using white light cystoscopy.^{37,38} The consistent identification of dysplastic areas of the bladder using ALA-induced fluorescence may allow further identification of those patients and areas within the bladder at risk of recurrence. Furthermore, the ability to identify the precancerous areas may allow prospective analysis of the contribution of such areas to tumor recurrence since, at this time, treatment of or removal of areas of dysplasias is not carried out. The sensitivity of surveillance cystoscopies in patients at risk of recurrence for bladder cancer may also be improved using ALA-induced fluorescence-guided biopsies.

Oral ALA administration in humans has resulted in selective PpIX accumulation within colorectal carcinomas^{39,40} and oral SCCs,⁴⁰ sufficient in the latter case to produce a PDT response when exposed to laser light. Damage to adjacent normal oral mucosa was noted, however, suggesting that light doses might not have been optimized. Increases in hepatic aminotransferase

and total bilirubin, although transient, might be obviated if parenteral ALA administration were used, thus bypassing the first-pass effect resulting in hepatic ALA accumulation. The short period of PpIX accumulation and its rapid clearance from tissue¹ account for the lack of long-term cutaneous photosensitization seen in clinical ALA PDT using systemic ALA. The oral administration of ALA for internal ALA PDT applications is beginning to be explored.³⁹⁻⁴² At the present time this appears most useful for the treatment of thin, mucosal, dysplastic, or malignant lesions of the gastrointestinal tract. The selectivity of PpIX accumulation within the mucosa of hollow viscera after systemic administration, with little or no fluorescence developing within the muscularis layer, has been used to suggest that ALA PDT within such organs should not lead to strictures, unlike results found in clinical studies performed using preformed photosensitizers.³⁹ It is therefore interesting to note that an initial report on the use of systemic ALA PDT to treat Barrett's esophagus in 8 patients⁴² reported normal squamous cell regeneration in all patients without stricture development.

Areas for future research

An important area of present and future research into topical ALA PDT concerns the search for methods of increasing the effective depth of lesion necrosis achieved by a single treatment. For example, a high complete response rate has been reported for superficial basal cell carcinomas, but nodular basal cell carcinomas do not respond as well to topical ALA PDT.²⁸ There appear to be two components to this problem: the limited depth of penetration of the light that is used to photoactivate the PpIX, and the limited depth of penetration of ALA following its topical application.

The wavelength commonly used for ALA PDT is 635 nm, which is the peak of absorbance for the fourth Q-band of PpIX *in vivo*.⁴⁵ However, the depth of penetration of 635 nm light in tissue is rather limited. Moan *et al.*⁴⁶ used a noninvasive technique to measure the penetration of light in human tissues *in vivo*. For ALA-induced PpIX, they concluded that excitation at ~410 nm (the peak of absorbance of the Soret band of PpIX *in vivo*) would produce the greatest photodynamic effect for the first 2 mm of tissue below the surface of the skin, while at depths exceeding 2 mm the conventional 635-nm light would be optimal. They reported also that light at the argon laser wavelength of 514.5 nm is more effective than light at 635 nm for the first 1 mm depth of tissue.

It is now well established, at least for *in vitro* systems, that PpIX is rapidly converted into one or more chlorin-type photo-products that absorb at ~670 nm.^{47,48} Since it appears that photoporphyrin is produced *in vivo* during ALA PDT,⁴⁹ it has been suggested that the excitation light for dermatological and other surface applications of ALA PDT should consist of a broad band of 600 to 700 nm,⁴⁸ or even 550 to 700 nm.⁵⁰ For internal applications that require the use of optical fiber light delivery systems, a combination of laser diodes or light emitting diodes that emit at different wavelengths (perhaps 410, 635, and 670 nm) might enhance the effectiveness of ALA PDT. Light delivery systems that include on-line monitoring of tissue fluorescence and reflectance would be useful for evaluating the depth of the ALA-induced PpIX fluorescence and for measuring the response to therapy.

One relatively simple way to increase the dose of light in the depths of a large tumor is to increase the duration of exposure to the light. Since PpIX photobleaches very readily, the more superficial tissues will not experience any additional damage during this process, even though the dose of light that they receive is very much greater than required. However, there is a limit to how much deeper it will be possible to treat merely by increasing the exposure time, since reciprocity cannot hold for the very low intensities of light that are found at the greater depths.

When ALA is administered systemically (by injection or by mouth), the ALA reaches the target tissues via the blood and there is little reason to be concerned that different parts of the target tissue might be exposed to very different concentrations of ALA. However, if the ALA is applied to the surface of a relatively large tumor nodule, the more distant parts of the tumor are exposed to lower ALA concentrations. Once the concentration of ALA begins to drop below the minimum level required to saturate the biosynthetic pathway for heme, the amount of PpIX that accumulates will begin to drop also; and once the amount of PpIX drops below the minimum level required to cause a lethal phototoxic reaction, the target cells will survive no matter how great the dose of photoactivating light. The depth of kill in thick tissue then becomes a function of the diffusion characteristics of the topical ALA.

There are several possible approaches to increasing the intracellular concentration of ALA-induced PpIX in the depths of relatively large tumors. First, as shown in Figure 2, the amount of PpIX in a target tissue may be increased merely by increasing the time that it is exposed to topical ALA, up to approximately 16 or 18 h. Longer exposure times permit deeper diffusion of the ALA and a greater accumulation of PpIX, which may convert nonlethal to lethal photosensitization. However, if the normal tissues also accumulate more PpIX under such conditions, there may be a decrease in specificity. Second, it may be possible to enhance the rate of diffusion of the ALA into the target tissue. The simplest technique is to inject the ALA, either intralesionally or intradermally about the lesion, using an aqueous solution of 10 mg ALA/ml adjusted to a pH of \sim 6.5 with NaOH. Another approach involves mechanical modification of the surface of the skin by tape stripping or controlled abrasion, or chemical modification by the use of various penetration-enhancing solvents. The penetration of ALA may be enhanced also by using a modified form of ALA, such as an ester derivative. Some ALA esters appear to diffuse more readily than ALA itself through the keratinized layers of the epidermis, and free ALA will be regenerated when the ALA ester encounters the esterases of the extracellular fluid and cells in the deeper layers of the epidermis. Warloe et al. recently reported an increase in the complete response rate for basal cell carcinoma from 50 to 90% when the methyl ester of ALA was used rather than ALA itself.⁵⁰ Any of these techniques could be used to increase the rate at which ALA diffuses from the surface of the skin and into the deeper tissues, but it is not clear that the concentration of PpIX induced at a given depth would be any greater than that which might be produced merely by increasing the time that the tissues are exposed to the ALA.

A technique that certainly can cause an increase in the maximum concentration of PpIX at depth involves the use of chelators of iron in conjunction with ALA. As discussed previously, chelation of the available iron prevents the conversion of PpIX

into heme and thus blocks a major route by which ALA-induced PpIX is lost to the cells. Such a block therefore leads to an increase in the concentration of the ALA-induced PpIX. Both EDTA⁵¹ and deferoxamine⁵² have been shown to enhance the concentration of PpIX in neoplastic tissues. However, once again there may be a problem with decreased specificity, since chelation of iron will block the conversion of PpIX into heme and thus increase the accumulation of PpIX in normal cells also.

A final important area for future research involves evaluation of the clinical potential of drug and/or light dose fractionation. Messmann *et al.*⁵³ reported enhanced ALA PDT efficiency with split light doses. They speculated that light dose fractionation permits replenishment of the oxygen in the tumor tissue, with a consequent increase in efficiency of the PDT. Alternatively, the initial dose of light may damage the mitochondrial membranes enough to permit free diffusion of PpIX through the cytoplasm to the nuclear and plasma membrane, while continuous exposure to light of relatively high intensity would cause rapid photobleaching and inactivation of the PpIX before it could diffuse out of the mitochondria. Continuous light of low intensity should behave much like fractionated doses of higher intensity. Research in this area may well lead to significant improvements in ALA PDT.

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