Influence of Light Exposure on the Kinetics of Protoporphyrin IX Formation in Normal Skin of Hairless Mice After Application of 5-Aminolevulinic Acid Methyl Ester

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The rates of protoporphyrin IX (PpIX) photodegradation and reappearance after light exposure at 420 and 632 nm were measured in mouse skin at different times after 1 h topical application of 5-aminolevulinic acid methyl ester (ALA-Me). After ALA-Me application (1 h) and removal, the fluorescence of PpIX increased for about 1 h, and then reached a maximum and started to decrease. Reappearance of PpIX fluorescence after exposures (degrading 60%–80% of the PpIX) was faster for exposures 0.5 h after ALA-Me application than for exposures 3 h. The bleaching rate was largest in the former case. This indicates that PpIX is located deeper in the skin after 3 h than after 0.5 h, whereas the pool of ALA-Me in the skin is largest at 0.5 h. In all cases, the reappearance was faster at a skin temperature of 35°C than at 23°C. Reappearance of PpIX fluorescence was faster after exposure to light at 420 nm than at 632 nm. The rate of elimination of PpIX from the volume of detection was faster after 420 nm light irradiation than that after 632 nm. These findings are discussed in view of penetration depths of light and ALA-Me, and diffusion of PpIX.

Key words: 5-aminolevulinic acid methyl ester/kinetics/light exposure/photodynamic therapy/protoporphyrin IX
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5-aminolevulinic acid (ALA), as well as 5-aminolevulinic acid methyl ester (ALA-Me), is being used in the treatment of human skin cancers (Gardlo and Ruzicka, 2002; Morton, 2002; Szeimies and Landthaler, 2002; Taylor and Brown, 2002). These drugs can penetrate the skin and produce protoporphyrin IX (PpIX) with some selectivity in tumors. Photodynamic therapy (PDT) then works via singlet oxygen formation when PpIX is exposed to light. Singlet oxygen has very strong cytotoxic effects, whereby tumors can be destroyed (Fuchs and Thiele, 1998; Sharman *et al*, 2000).

Fractionation of the fluence, or applications of different fluence rates, are topics frequently discussed in connection with PDT (Gibson *et al*, 1990; Messmann *et al*, 1995; de Bruijn *et al*, 1999; Iinuma *et al*, 1999; Robinson *et al*, 2000, 2003). Delivery of a single, high fluence may deplete the oxygen level in the irradiated volume (Foster *et al*, 1993; Babilas *et al*, 2003). This will reduce the efficiency of the treatment. The use of low fluence rates (Gibson *et al*, 1990; Foster *et al*, 1993; Veenhuizen and Stewart, 1995; Sitnik *et al*, 1998; Robinson *et al*, 1998; de Bruijn *et al*, 1999; Iinuma *et al*, 1999), or fluence fractionation (Foster *et al*, 1993; Messmann *et al*, 1997; Muller *et al*, 1998; Iinuma *et al*, 1999; Robinson *et al*, 2000, 2003), may improve the situation, although one has to consider that during long exposure times, vascular damage may occur. Such damage will also reduce the oxygen level in the tumor and thus impair both the rate of PpIX appearance and the quantum yield of cell destruction. Since singlet oxygen has a very short radius of action because of its short lifetime in tissue (Moan, 1990), the localization pattern of PpIX in the tissue is of crucial importance for the processes mentioned above. Furthermore, the effects are dependent on the wavelength of the exposure light, because of the fact that the penetration depth of light into tissue is strongly wavelength dependent (Moan *et al*, 1998).

In this work, we applied ALA-Me topically on normal mouse skin *in vivo* for 1 h, and then exposed the skin to different fluences of either blue or red light at different times after the application. The kinetics of production of PpIX was then studied, which would be of great importance to know for fluence fractionation. It was shown that the rate of PpIX reappearance is strongly dependent on the wavelength of the light, as well as on the time interval between drug application and light exposure.

Abbreviations: ALA, 5-aminolevulinic acid; ALA-Me, ALA-methyl ester; PDT, photodynamic therapy; PpIX, protoporphyrin IX

Results

After 1-h application of the cream with ALA-Me, the fluorescence of PpIX in the skin increased for about 1 h, remained constant for another hour, and then decreased (Fig 1). The PpIX fluorescence was similar at 0.5 and at 3 h. Therefore, these two time points were chosen for further experiments.

The fluence rates of the two light sources were adjusted so that the exposure times needed to reduce the PpIX

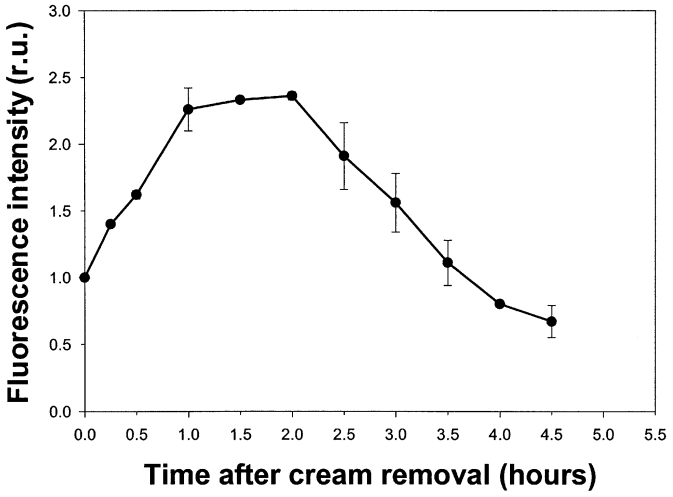

Figure 1
Production of protoporphyrin IX in normal mouse skin at different times after 1 h topical application of 20% 5-aminolevulinic acid methyl ester. The cream with ALA-ME was removed from the skin after the 1-h application. Error bars represent the standard errors for groups of three mice.

fluorescence in the skin by 50% were in the same range (Fig 2). For the blue light lamp (420 nm), 1.5 and 2.6 min exposures were needed for 50% reduction 0.5 and 3 h, after cream removal, respectively (Fig 2*A*). For the light emitting diodes (LED) light (632 nm), the corresponding exposure times were 2.1 and 4.2 min (Fig 2*B*). In both cases, the photodegradation rate of PpIX was larger 0.5 h after cream removal than 3 h after removal (Fig 2). The rate of PpIX appearance after light exposure was significantly faster 0.5 h after cream removal than 3 h after cream removal. This was true both for exposure to blue and to red light (Figs 3 and 4). Furthermore, the appearance of PpIX 0.5 h after cream removal was faster after blue light exposure than after red light exposure (Figs 3 and 4). PpIX reappearance was significantly faster at a skin temperature of 35°C than at a temperature of 23°C (Fig 5). In all cases, the shape of the fluorescence excitation spectra was similar before and after light-induced degradation of PpIX in the skin (data not shown).

Moreover, the reactions of skin to PDT with blue and red light were documented by photos (pictures not shown). In general, for blue light-PDT after 0.5 h, the skin reaction was negligible for light exposures of 1 and 3 min, whereas the skin became pale and significant edema developed for an exposure of 4 min. This reaction was less expressed when

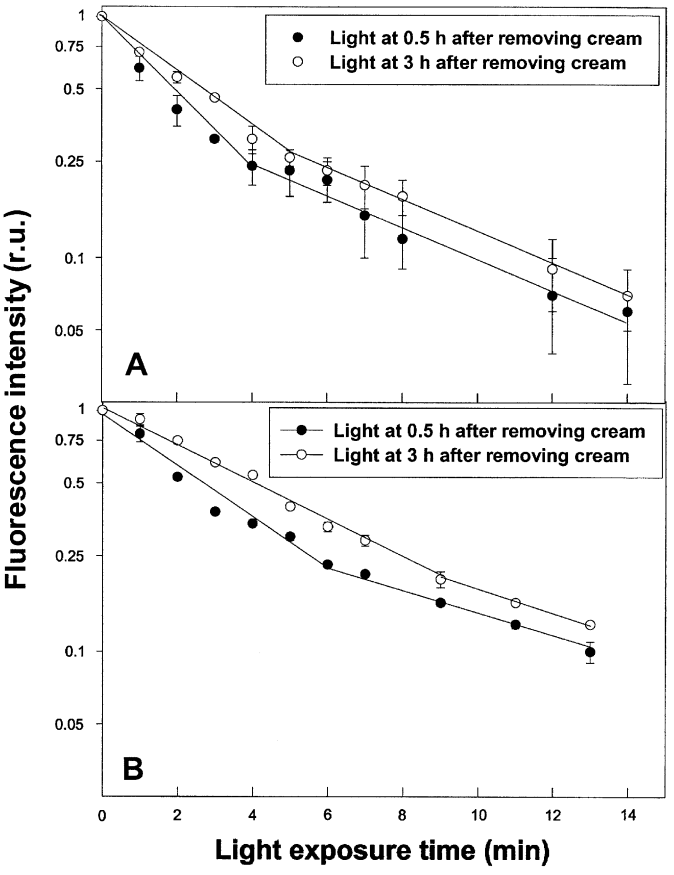

Figure 2
Decay of protoporphyrin IX fluorescence in mouse skin induced by exposure to blue (*A*) or red (*B*) light at 0.5 or 3 h after removal of cream. Twenty percent of 5-aminolevulinic acid methyl ester cream was topically applied for 1 h and then removed. Error bars represent standard errors for groups of three mice.

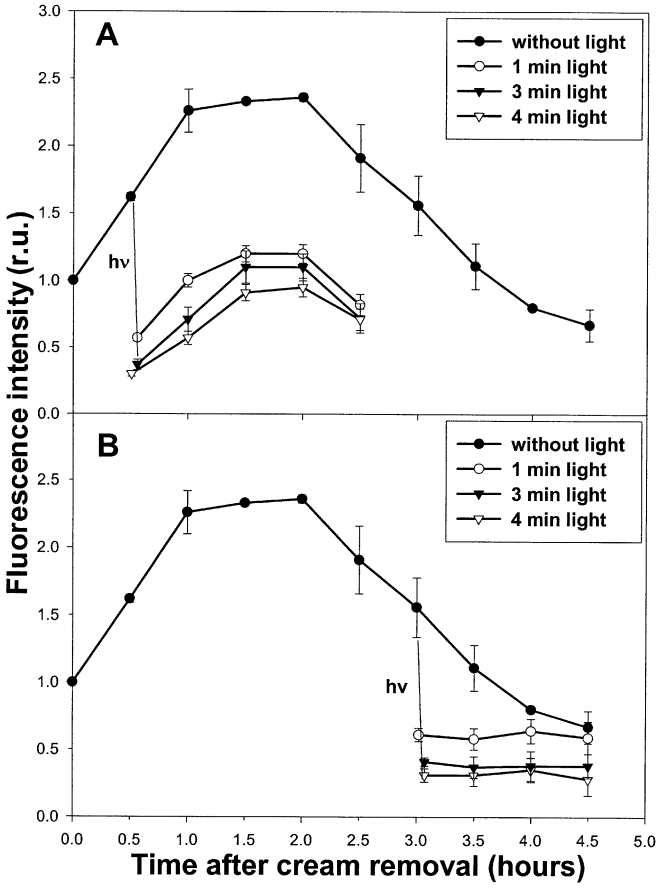

Figure 3
Rebuilding of protoporphyrin IX fluorescence in mouse skin after exposure to blue light at 0.5 h (*A*) or at 3 h (*B*) after removing the cream (20% 5-aminolevulinic acid methyl ester was topically applied for 1 h). The mice were irradiated for 1, 3, or 4 min. Error bars represent standard errors for groups of three mice.