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Photobiology

Kinetics of the superficial perfusion and temperature in connection with photodynamic therapy of basal cell carcinomas using esterified and non-esterified 5-aminolaevulinic acid

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Summary

Background Photodynamic therapy (PDT) is a local treatment modality with increasing indications for various malignant and non malignant diseases. The treatment parameters have not yet been optimized as there is a need for a better understanding of the process. The skin is an important target and serves as a good model for monitoring and evaluating the interaction of light with biological tissue.

Objectives The tissue perfusion and the temperature of basal cell carcinomas were measured in connection with PDT in order to investigate the biological mechanisms involved.

Methods An infrared camera was used during the treatment to measure skin temperature and a laser Doppler perfusion imaging device was used to image the superficial perfusion before and after treatment. Six hours after topical application of 5-aminolaevulinic acid (ALA) or methyl esterified ALA (ALA–ME), 38 basal cell carcinomas were treated using light from a diode laser at 633 nm.

Results In the lesions, the perfusion immediately after PDT was similar to that before PDT. One hour after the treatment the perfusion in the lesion was increased 50% compared with before PDT. However, in the skin surrounding the lesions the perfusion was doubled immediately after PDT and was still increasing 1 h after treatment. A temperature increase in the lesions of about 1–3 °C was observed for light fluence rates of 100–150 mW cm^{-2}. In all patients treated, a diffuse temperature increase was visible outside the lesions. In some of the patients, the outlines of the blood vessels surrounding the treated lesions became visible in the thermal images. Measurements of temperature on healthy volunteers not administered photosensitizer, but illuminated with light of the same fluence rate, showed a similar increase in temperature in the illuminated spots. However, no temperature increase was observed outside the illuminated area. No statistically significant differences were found between the measurements on patients treated with ALA and ALA–ME.

Conclusions The increased perfusion in the area surrounding the lesions after PDT, as seen by perfusion and temperature measurements, is the result of an inflammatory reaction to the PDT process. However, directly after PDT the perfusion in the lesions was the same as before irradiation. The combination of these observations suggests the presence of local blood stasis during and immediately after the treatment. The temperature measurements showed that the increased temperature was well below the temperature limit of hyperthermal damage. Furthermore, the

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measurements indicate that the increase in temperature was primarily a consequence of the heat absorbed in the tissue.

Key words: 5-aminolaevulinic acid, basal cell carcinoma, laser Doppler imaging, photodynamic therapy, temperature, tissue perfusion

Photodynamic therapy (PDT), a treatment modality for certain types of malignant tumours, is based on a photochemical reaction in which a photosensitizer is applied and within a relatively short time selectively accumulates in the tumour cells and/or the endothelium of the tumour vessels.[1–4] Illumination with light at the wavelength of the photosensitizer absorption peak results in the formation of cytotoxic oxygen radicals, leading to tumour destruction.

Excess amounts of topically applied 5-aminolaevulinic acid (ALA), a precursor to haem, result in a cellular build-up of protoporphyrin IX (PpIX), which is photochemically active in the PDT process. This modality has been used for the successful treatment of thin malignant non-melanoma skin lesions.[5] As the complex mechanisms involved in this form of treatment are not fully understood, intense research is required so that the vast number of treatment parameters can be optimized for clinical PDT.

Several pathways for the response of PDT have been identified: damage to critical structures in the tumour cells (i.e. mitochondria and plasma membranes) leading to direct cytotoxic effects on the cells;[6] damage to the vasculature of the tumour leading to hypoxia;[7] and induction of apoptosis and inflammatory and immunology reactions.[8–10] All major tumour treatment modalities, such as ionizing radiation, rely on an adequate supply of oxygen to the tissue. This is also the case for PDT. Oxygen is consumed during the photochemical reaction, while the blood constantly supplies new oxygen. It is therefore important to maintain a high level of perfusion during treatment to ensure that the oxygen supply to the cells does not limit the efficiency.[11,12] However, several of the response mechanisms, especially following systemic administration of the photosensitizer, will result in vascular stasis by causing thrombosis or vasoconstriction.[1,13–15] The release of a number of vasoactive substances has been associated with PDT.[7] An associated pertained reduction in blood flow following the treatment may lead to the death of tumour cells due to oxygen and nutrient deprivation.[16]

The mode of administration of the photosensitizer influences its location in the target. Systemic administration causes an extensive part of the sensitizer to localize in the endothelial cells of vessels and the treatment causes permanent vascular damage.[1,14] However, no sensitizer has been detected in the blood following topical application of ALA.[5,17] This indicates that the damage to the vascular bed may be limited following topical application of certain photosensitizing agents and treatment may instead rely on direct photochemical oxidation of the tumour cells.

The chemical structure of the sensitizer also influences its penetration and pharmacokinetics.[2] Lipophilic photosensitizers accumulate in the membranes of the cell and its organelles.[18] Hydrophilic and aggregated forms of photosensitizers are more likely to be localized in lysosomes and endosomes.[19,20] It has been shown that the probability of cell death per quantum absorbed light is higher for lipophilic photosensitizers, indicating that membrane structures are more vulnerable.[21] Using topical applications to the skin, lipophilic photosensitizers may penetrate faster and deeper, possibly yielding a larger treatment volume. Previous studies have shown a higher build-up of PpIX after the application of methyl esterified ALA (ALA–ME) than ALA in normal mouse skin[22] and in human basal cell carcinoma (BCC).[23,24] However, conflicting results have been reported after topical application of ALA and ALA–ME on subcutaneously implanted adenocarcinoma tumours in mice.[25]

Previous studies conducted by our research group have indicated that the perfusion in lesions increases immediately after PDT following topical application of ALA[17,26] and that this perfusion decreases immediately after PDT using ALA–ME.[24] However, it was difficult to evaluate the perfusion in the illuminated area surrounding the lesion in these earlier investigations. Perfusion is obviously a very important parameter in explaining the treatment mechanisms, and thus requires further attention.

It is important to consider a possible interaction with laser-induced hyperthermia when PDT is performed. An elevated temperature for a certain period of time may cause tissue damage. Tumours are more sensitive to temperatures in the region of 41–47 °C than normal surrounding tissue,[27,28] which perhaps can give addi-

tional therapeutic effects. In most PDT studies, light doses that do not induce a sufficient temperature increase to cause damage to the tissue are used, in order to differentiate between the treatment mechanisms. Svaasand[29] showed that there are no hyperthermic effects using fluence rates below 150 mW cm^{-2} at a wavelength of 514 nm. Warloe *et al.*[30] measured the skin surface temperature during PDT using fluence rates of 100–150 mW cm^{-2} at a wavelength of 630 nm. A temperature rise to 39–40 °C was detected in the illuminated area using a thermocouple in contact with the skin. However, little work has been performed to map spatially the temperature rise during PDT. The contributions from heating by direct light absorption and an increased blood flow must be differentiated.

In this study we employed two different methods to indirectly study possible PDT mechanisms in connection with the treatment of BCCs following topical applications of the haem precursors ALA and ALA–ME. The aim was to clarify the change in perfusion during and after the treatment. Superficial perfusion was measured using a laser Doppler perfusion imager and the temperature during PDT was monitored with an infrared camera.

Materials and methods

Patients and control group

The patients treated had been referred to the Departments of Dermatology and Oncology at the Lund University Hospital, Lund, Sweden, for PDT of BCCs. This study, approved by the local Ethics Committee at the Lund University Hospital, included 38 BCCs in 19 patients (skin types I–II, age 32–82 years, mean 61) with no other ongoing skin diseases. The lesions were localized on the trunk (68%), the extremities (8%) and the head and neck region (24%). Perfusion measurements were performed on 18 lesions (72% trunk, 11% extremities and 17% head and neck region) and the skin temperature during treatment was measured in 28 lesions (71% trunk, 7% extremities and 22% head and neck region). Thus, both temperature and perfusion measurements were performed in eight lesions.

We also performed reference measurements on nine healthy Caucasian volunteers during the winter time, six with pale non tanned skin (skin types I–II) and three with more pigmented skins (one with skin type II, well-tanned in a solarium; one with skin type III and one with skin type IV). They were not given any sensitizer and were illuminated with 50 and 100 mW cm^{-2} in 3-cm diameter spots on the shoulder. The perfusion was measured before and after illumination and the temperature was measured before, during and after illumination.

Photodynamic therapy procedure

The sensitizer PpIX is synthesized by the haem cycle in the cells to highly elevated levels due to the amounts of ALA or ALA–ME applied.[31] The ALA/ALA–ME powder (5-amino-4-oxopentoanoic acid/5-amino-4-oxopentoanoic acid methyl ester, Sigma Chemical Co., St Louis, MO, U.S.A.) was dissolved in water and mixed with Essex cream (Schering Corp., Kenilworth, NJ, U.S.A.) to yield a 20% concentration. The cream was applied topically with a 1-cm margin surrounding the lesion. An occlusive dressing (Tegaderm™, 3M, U.K.) covered the cream to prevent it from smearing and an ordinary dressing shielded the lesion from ambient light until treatment commenced. No local or general anaesthetics were used.

PDT was performed about 6 h after the application of ALA/ALA–ME. The same concentration and application time were used for both ALA and ALA–ME to allow direct comparison. The lesion and a 5–10-mm margin of surrounding normal tissue were illuminated with light from a diode laser (Ceralas™ PDT, wavelength 633 ± 3 nm, CeramOptec, Bonn, Germany). The light was guided through a 400-μm optical silica fibre with a microlens attached at the distal end (MR4-65S-XY-B0, Rare Earth Medical Inc., W. Yarmouth, MA, U.S.A.), yielding a uniform light distribution over the skin surface. Light fluence rates in the range of about 20–300 mW cm^{-2} were used and the total energy delivered was 30–100 J cm^{-2}.

Perfusion measurements

A laser Doppler perfusion imaging device (Lisca Development AB, Linköping, Sweden) was used for the tissue perfusion measurements.[32] The instrument consists of a scanning head with computer-controlled mirrors that during a 90-s period scan a low-power (about 1 mW) red diode laser across the skin. Moving blood cells scatter some of the light, yielding a small frequency shift in the backscattered light detected by the instrument. Owing to measurement geometry and the small penetration depth of light, the perfusion is only measured down to a depth of about 0·2 mm.[33]

Perfusion measurements were performed before the application of ALA or ALA–ME, before PDT, directly

after PDT and 1 h after PDT. The patients had been at rest for at least 5 min before each measurement. The temperature in the examination room was 22 ± 2 °C. The scanning head was placed 16 ± 1 cm above the surface, yielding a scanned area of about 4 × 4 cm. As the images generated contain 40 × 40 pixels, the spatial resolution was about 1 mm. The light in the room was turned off during the measurements, to reduce the potential influence of ambient light.

Temperature measurements

During PDT, the temperature was measured with an uncooled infrared camera (AGEMA 570 Elite, Flir Systems Inc., Stockholm, Sweden). The camera detects the blackbody radiation at wavelengths between 7·5 and 13 μm and calculates the temperature. The emissivity of skin was set to 0·98.[34] The temperature resolution is 0·1 °C at 30 °C and the full measurement range is −20 °C to 500 °C. Temperature images were captured and stored every 10 s during treatment. A 3-cm sand-blasted aluminium plate was used as a ruler in the images.

Evaluation methods

The laser Doppler perfusion images were analysed using Matlab (MathWorks Inc., Natick, MA, U.S.A.) routines especially developed for this task. The perfusion was evaluated by calculating the average perfusion in the tumour and in the area surrounding the lesion. The average in the lesion was calculated in a circular area covering the tumour. For the surrounding area, the average was calculated for the larger area including the lesion and the contribution from the lesion was subtracted. Ratios were calculated between the perfusion in the lesion (P_{les}) after and before PDT (P_{les} post-PDT)/(P_{les} pre-PDT), and correspondingly for the surrounding areas (P_{sur}) (P_{sur} post-PDT)/(P_{sur} pre-PDT). In addition, the ratios between the perfusion in the lesion and the area surrounding the lesion were calculated for images before PDT, immediately after PDT and 1 h after PDT: (P_{les} pre-PDT)/(P_{sur} pre-PDT), (P_{les} post-PDT)/(P_{sur} post-PDT), (P_{les} 1 h post-PDT)/(P_{sur} 1 h post-PDT). Unless otherwise stated, unpaired Student's *t*-test was performed to determine statistical significant differences. $P < 0{\cdot}05$ was considered significant.

The maximum temperature in the illuminated area of each temperature image was evaluated and plotted vs. time. The average temperature increase, measured as the mean value between 200 and 300 s of illumination, was also calculated and plotted vs. fluence rate.

Results

Perfusion measurements

Figure 1 shows a series of images of the perfusion in tissue in connection with PDT using ALA–ME. The perfusion in the lesion was slightly increased immediately after compared with before PDT, while the perfusion in the surrounding tissue had more than doubled. Ratios (mean ± SE) between the perfusion post-PDT and pre-PDT as well as 1 h post-PDT and pre-PDT are shown for all lesions and the surrounding skin in Figure 2. On average, the perfusion in the lesion remained almost the same immediately after PDT but after 1 h, it had increased by 50% compared with the perfusion before PDT. The perfusion in the surrounding skin was doubled directly after PDT and continued to increase 1 h after PDT. There is a tendency for the perfusion ratios to be higher for lesions treated with ALA than with ALA–ME, but this is not statistically significant ($P > 0{\cdot}22$). Figure 3 shows the ratios (mean ± SE) between the perfusion in the lesion and in the surrounding skin pre-PDT, post-PDT and 1 h post-PDT. Additionally, there is a tendency for the perfusion ratios to be higher for lesions treated with ALA than ALA–ME, but this is not significant ($P > 0{\cdot}09$). There is a highly significant decrease ($P < 0{\cdot}001$) in this ratio between post-PDT and pre-PDT, mainly due to the fact that the perfusion in the surrounding tissue increases. There was no difference between the perfusion before and after light illumination of the volunteers.

No correlation was found between perfusion results and age of the patients or the location of the lesions.

Temperature measurements

The temperatures prior to treatment were slightly higher in the lesions (34·8 ± 1·2 °C; mean ± SD) than in the surrounding skin (34·5 ± 1·1 °C). The paired *t*-test comparing the lesion temperature with the surrounding temperature in each patient was statistically significant ($P = 0{\cdot}012$). There was no difference between the surrounding skin temperature of the patients and the skin temperature of the volunteers (34·3 ± 0·8 °C; $P = 0{\cdot}47$). The lesion temperature increased slightly during PDT (Fig. 4a,b) and blood vessels surrounding the illuminated area became

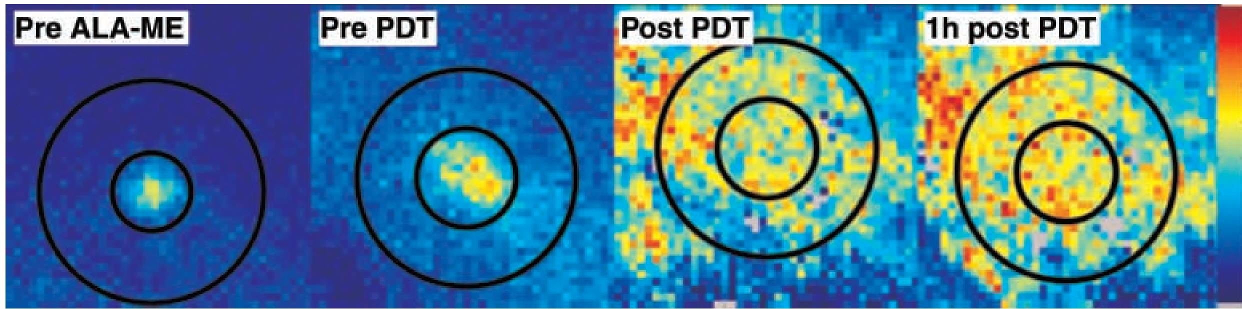

Figure 1. Laser Doppler perfusion images from a basal cell carcinoma (1-cm diameter) located on the lower back. The measurements were performed before the application of ALA–ME, pre-PDT, immediately post-PDT, and 1 h post-PDT. The circles indicate the areas used for the evaluation of perfusion in the lesion and the surrounding tissue. The grey pixels represent areas with poor signal (pen markings). PDT, photodynamic therapy; ALA, 5-aminolaevulinic acid; ALA–ME, methyl esterified ALA.

visible during treatment (Fig. 4b). The temperature also increased somewhat in the illuminated areas of the non sensitized volunteers but no vessels became visible (Fig. 4c).

The temperature increase induced during the treatment of four typical lesions and during the illumination of healthy skin is plotted in Figure 5. The temperature increased rapidly for the first 100–150 s and after 200 s it remained almost constant. This was observed in all treated lesions except one, in which the temperature continued to increase during the entire treatment. This lesion was a BCC located on the ankle of an 82-year-old woman where the overall perfusion including the venous status might be impaired due to location. The perfusion measured in the normal skin outside this lesion before the application of ALA was very low.

No correlation was found between age and temperature increase; however, both of the two lesions located on the extremities exhibited a significantly higher ($P < 0{\cdot}001$) temperature increase than the lesions located elsewhere. The temperature increase in lesions located on the trunk was similar to that in the head and neck region.

The average temperature increase vs. fluence rate is shown in Figure 6. The temperature increase is averaged over the interval 200–300 s after the start of illumination. Temperature changes vary greatly from area to area and there was no significant difference between the temperature of lesions treated with ALA or ALA–ME and that of the illuminated healthy skin. Again, the lesion on the ankle of the 82-year-old patient attained a higher temperature than the others. The average of the maximum skin temperature increase between 200 and 300 s was $2{\cdot}2 \pm 1{\cdot}2$ °C (mean ± SD), which is well below the level for hyperthermia.[27,35]

Discussion

In this study, the superficial perfusion and temperature were measured during PDT of BCCs. A scanning laser Doppler instrument was used for measurements of

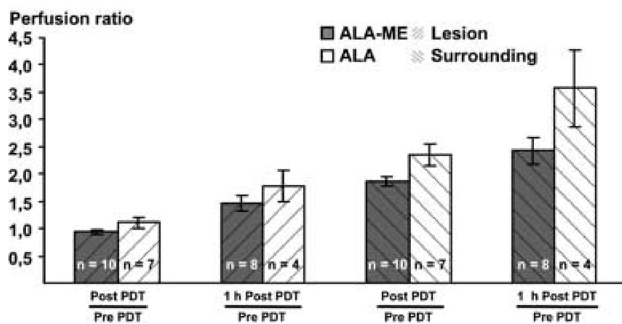

Figure 2. Perfusion ratios (mean ± SE), post-PDT and pre-PDT, and 1 h post-PDT and pre-PDT, for the lesions and the surrounding tissue. PDT, photodynamic therapy; ALA, 5-aminolaevulinic acid; ALA–ME, methyl esterified ALA.

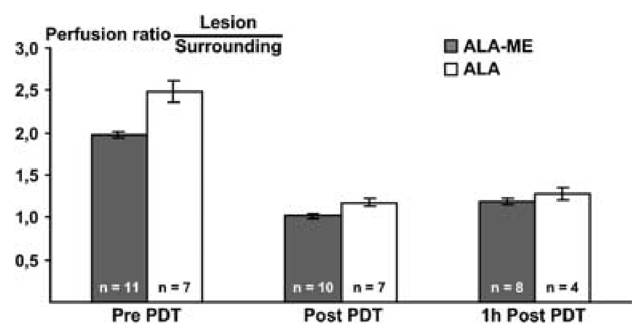

Figure 3. Ratios (mean ± SE) between the lesional perfusion and the perfusion in the surrounding tissue, pre-PDT, post-PDT and 1 h post-PDT. PDT, photodynamic therapy; ALA, 5-aminolaevulinic acid; ALA–ME, methyl esterified ALA.

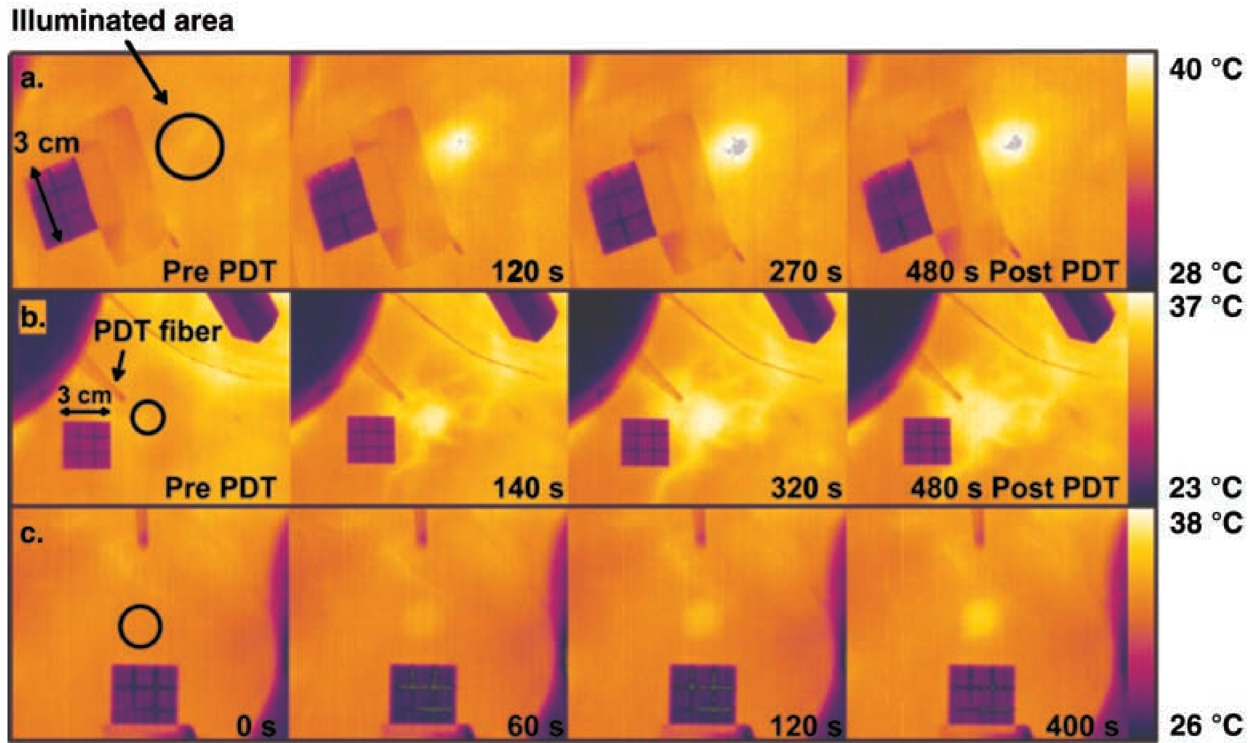

Figure 4. Temperature images during photodynamic therapy (PDT) of basal cell carcinomas located on (a) the abdomen, and (b) the chest. The lesions were treated with a fluence rate of 127 mW cm^{-2}. The temperature images during 100 mW cm^{-2} illumination of healthy skin without any application of sensitizer are shown in (c).

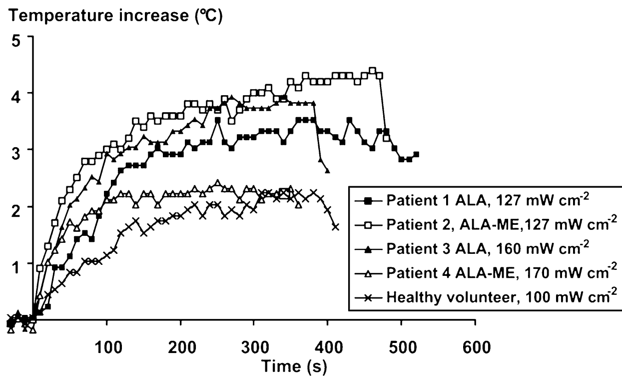

Figure 5. The maximum temperature in the illuminated area during photodynamic therapy is shown for four patients. The temperature increase during illumination with 100 mW cm^{-2} of a 3-cm spot on the shoulder of a healthy volunteer is also shown. ALA, 5-aminolaevulinic acid; ALA–ME, methyl esterified ALA.

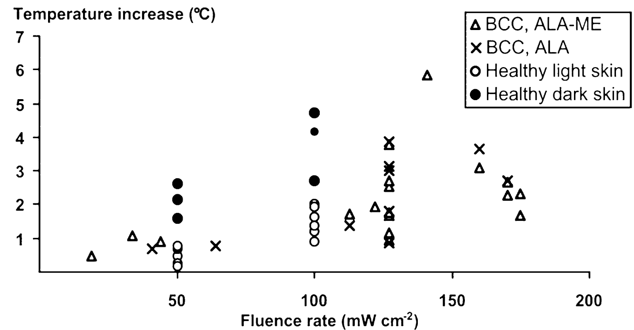

Figure 6. The average temperature increase in the interval 200–300 s after the start of the illumination is plotted vs. fluence rate. Light skin refers to healthy volunteers with skin types I–II and dark skin to people with more pigmented skin. BCC, basal cell carcinoma; ALA, 5-aminolaevulinic acid; ALA–ME, methyl esterified ALA.

superficial perfusion. The temperature at the skin surface was measured using an infrared sensitive camera.

The optical properties of tissue limit the light penetration and in order to interpret our results, the measurement depths must be evaluated. The laser Doppler instrument uses a wavelength of 632 nm and a beam diameter of 1·5 mm, resulting in an effective probing depth of about 0·2 mm, which is about the same as the average thickness of the epithelium. However, the epithelial thickness varies considerably with location on the body and in some parts it can be as

thin as 35 μm.[36] The thermal camera is sensitive to radiation in the infrared range in which water absorbs. Thus, the emission depth for the temperature measurements is also very limited, about 10 μm. However, tissue contains a high proportion of water and thus has a high heat conductivity, which permits temperature changes in deeper layers to be observed by the emission of the surface.

The treatment light is absorbed both by the natural tissue chromophores as well as by PpIX. The direct absorption of light, e.g. by melanin and haemoglobin, leads to a slight temperature increase not enough to cause hyperthermic action. No significant difference was observed between the temperature increase during treatment or during illumination of the healthy volunteers with pale skin type (Fig. 6). In addition, there was no difference in perfusion before and after illumination of the healthy volunteers. Thus, we conclude that the temperature increase in the lesions is merely a result of the direct absorption of light by the tissue chromophores as in the normal healthy skin. In addition, no difference was apparent between the temperature increase when using ALA or ALA–ME.

The variation in temperature increase in the treated tumours is due to different light fluence rates, the pigmentation of the skin and normal perfusion. Skin type is correlated to the amount of melanin content of the skin, which is significantly higher in sun-exposed skin than non-exposed skin.[37] The patient skin type and the location of the lesion result in differences in the energy absorbed and thus the increase in temperature (Fig. 6). There is a significant difference in temperature increase between the pale and dark healthy volunteers (Fig. 6). Cutaneous perfusion in normal skin acts as a regulating system, increasing the temperature when the tissue temperature is below 37 °C, and reducing the temperature when the tissue temperature is above 37 °C. Perfusion varies with patient age and the location of the lesion.[38] The temperature may rise due to low perfusion in elderly people with lesions located on the lower extremities. This was the case in one patient, whose lesion temperature continued to increase during treatment and reached 7 °C above the initial skin temperature after 424 s of illumination at a light fluence rate of 141 mW cm^{-2}. Special caution has to be taken when treating lesions located on the lower extremities in diabetes and elderly patients with reduced peripheral arterial perfusion.

In the areas surrounding the lesions, a general increase in perfusion was observed during PDT. The superficial perfusion measured by means of laser Doppler imaging had doubled in the surrounding skin directly after PDT. However, it was almost the same in the lesion directly after PDT illumination as before (Fig. 2). One hour later, the superficial perfusion had increased by approximately 50% in both the lesion and the surrounding skin. There was a diffuse temperature increase in the non-illuminated skin surrounding the lesion during the PDT treatment, suggesting an increase in perfusion in the entire region. Deeper lying, large blood vessels in the vicinity of the treated lesion appeared, to varying degrees, in the temperature images during treatment, indicating that they were dilated and perfused with blood of higher temperature from deeper regions (Fig. 4). None of the perfusion-related effects could be observed in illuminated areas on the volunteers. The increased perfusion in the area surrounding the lesions is thus not a result of the heating by absorbed light energy.

One may speculate on the mechanisms responsible for the changes in perfusion in connection with PDT, which is an artificially induced mechanism comparable with cutaneous porphyrias. It has been shown that the histopathological changes in light-exposed skin are qualitatively similar in all cutaneous porphyrias.[39,40] Erythropoietic protoporphyria (EPP) is a disorder characterized by acute photosensitivity caused by the increased production of PpIX resulting from decreased ferrochelatase enzyme activity.[41,42] At the molecular level porphyrin-catalysed photodynamic reactions have been shown to cause damage to proteins, lipids and DNA,[43] activate complement,[44] degranulate mast cells,[45] and enhance degradation of the dermis and basal membrane by metalloproteinases.[46] Proteolytic enzymes are released from damaged keratinocytes.[47] Once released, these mediators initiate the inflammatory reaction. However, in this initial state, there is virtually no inflammatory infiltrate.[47] The visually observed diffuse reddening surrounding the lesion indicates an axon flare response,[48] which will open up the arterioles and thus increase the perfusion in the region served by these nerves. During treatment the patients sense pain, which indicates that nerves are stimulated during PDT (unpublished data). Thus, the increased perfusion in the entire region is probably due to stimulation of the nerve ends around the lesions (normal skin) and the activation of inflammatory response where there is a release of histamine and other mediators[45] from the increased number of mast cells in the treated area.[49,50]

The perfusion in the lesion itself responds differently from that in the surrounding tissue. The blood vessels in BCC lesions grow in an abnormal disorganized

pattern, and the tissue volume occupied by the vessels is much greater than that in normal tissue.[51] Vessels in the lesion have larger diameters[51] and the vessel walls are thinner without the ability to contract actively.[52] These differences between lesions and normal tissue may cause variations in perfusion upon the stimuli induced by the treatment.

If PpIX is built up in the endothelial cells of blood vessels, vasoconstrictive effects would be induced. This has been shown in connection with systemic administration of ALA resulting in reduced superficial tissue perfusion directly after PDT.[16,17] Vasoconstriction may be caused by local release of vasoactive substances with a limited range of action, such as histamine. In addition, mechanical and chemical irritation of the skin can induce a white reaction in which capillary sphincters are contracted and there is no perfusion in the capillaries.[53,54] Other studies have shown that topical administration of ALA results in increased tissue perfusion after PDT,[17] and topical administration of ALA–ME has been found to cause decreased perfusion immediately after PDT.[24] Depending on the method of sensitizer administration, blood coagulation or damage to the endothelial or smooth muscle cells of the vasculature in the lesion do not seem to have a large influence on the perfusion. The decrease in perfusion in the lesions is also very temporary during the treatment itself. One hour after the treatment, the perfusion is also high in the lesion. Lastly, the local oedema caused by the treatment may cause increased pressure in the lesion, hampering perfusion. The oedema may also result in a smaller number of capillaries contributing to the Doppler signal, due to the limited probe depth.

The results presented in this study indicate no measurable difference between the use of ALA or ALA–ME in superficial perfusion or heating in connection with PDT. Neither of the agents led to a systematic reduction in the measured perfusion in the lesion immediately or within 1 h after the treatment.

For a better understanding it would be of interest to monitor the superficial perfusion during treatment, to avoid the influence of the kinetics of vasoactive substances after treatment. This would enable both spatial and temporal resolution of the perfusion. However, this is not possible with the present system, as it uses the same wavelength as the PDT laser. The treatment light would thus interfere with the laser Doppler system.

Our results show that the temperature increase on the surface of the skin was small enough to avoid hyperthermia. However, we have only imaged the superficial skin and the possibility remains that the temperature may be slightly higher deeper down where there is no evaporative cooling of the surface and the scattering properties of the tissue are different. Furthermore, we have shown that the increase in superficial perfusion in the treated area and the surrounding area during PDT is not a consequence of heating. Thus, we have indirectly shown that the increased perfusion is a result of the inflammatory reaction initiated by the photochemical reactions during PDT. The observations also suggest that there is a temporary vasoconstriction in the lesions during PDT, which may hamper the inflow of oxygen. Further studies on perfusion and oxygenation are needed to improve our understanding of PDT mechanisms during topical ALA–PDT.

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