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The effect of fluence rate on the acute response of vessel diameter and red blood cell velocity during topical 5-aminolevulinic acid photodynamic therapy

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KEYWORDS
5-Aminolevulinc acid;
Vascular response;
Vessel diameter;
Flow;
Red blood cell velocity;
Intra-vital microscopy

Summary
Background: In a previous study it is shown that for topically applied ALA-PDT, PpIX concentration correlates with vascular changes including vasoconstriction and/or vascular leakage of small vessels and arterioles in the mouse epidermis and dermis. In this study we report on vascular responses induced by ALA-PDT for different fluence rates, including both changes in vessel diameter and dynamics in RBC velocity in arterioles, imaged using intra-vital confocal microscopy in skinfold chambers in hairless mice. Our interest is in the dynamics of vascular changes in the early stages of illumination.
Methods: We have determined the total PDT dose to be relatively low, 13 J cm^{-2}, and fluence rates of 26, 65 and 130 mW cm^{-2} were investigated. Local vascular effects occurred very soon after the start of the therapeutic illumination in ALA-PDT.
Results: In this study, we did not find a significant difference between fluence rates. Arterioles were particularly sensitive to vasoconstriction during low dose PDT, often resulting in complete vasoconstriction. When we observed complete vasoconstriction, this coincided with changes in RBC velocity.
Conclusion: Since the therapeutic effects of PDT are dependent on a fine balance between the need for oxygen during illumination and disruption of the vasculature, the results of the present study add to our understanding of acute vascular effects during ALA-PDT and aid our efforts to optimize PDT using porphyrin pre-cursors.

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Introduction

Photodynamic therapy (PDT) using topically applied porphyrin precursors is a treatment modality used for various (pre-) malignant skin lesions [1,2]. The precursor 5-aminolevulinic acid (ALA), and it is esterified derivatives methyl aminolevulinate (MAL) and hexyl aminolevulinate (HAL), are taken up by cells and converted into the endogenous photosensitizer protoporphyrin IX (PpIX) by means of the haem cycle [3]. The application of an excess of exogenous ALA leads to the accumulation of therapeutic concentrations of PpIX. During illumination, PpIX mediates the transfer of energy from light to molecular oxygen, resulting in the generation of reactive oxygen species (ROS). These ROS have a very short lifetime and diffusion radius and cause damage to nearby critical tissue structures through a combination of cellular, vascular and immunological pathways [4,5].

Since the formation of reactive oxygen species is the initiator of local tissue damage during PDT, tissue oxygenation and therefore the local blood supply plays an important role in determining tissue response to PDT. The role of the local blood supply during PDT is multifaceted. Some (mainly intravenous administered) photosensitizers directly target the vasculature, which upon illumination results in irreversible damage to vessels and thereby destruction of the illuminated tissue. However vascular shutdown and blood flow changes during early stages of illumination decrease the transport of oxygen, which then limits the production of singlet oxygen and therefore reduces the effect of the local cellular and immunological response. Treatment efficacy is therefore dependent on a fine balance between the positive and negative effects of vascular damage. Here, light dose and fluence rate play an important role in optimizing PDT [6,7].

Although the porphyrin-precursor ALA is usually topically applied to the skin, the precursor penetrates into the epidermis and dermis. We have recently shown accumulation of significant amounts of PpIX in vessel and arterial walls in subcutaneous tissue after topical application of ALA on skin [8]. In this circumstance the vasculature itself is a target for photodynamic therapy, and the effects of illumination on the vasculature have to be taken into account.

To overcome the problem of direct vascular shutdown and increase treatment efficacy during ALA-PDT, different illumination protocols are developed. We and others have shown that illumination with a lower fluence rate results in more PDT induced damage in normal mouse skin [7,9]. In a hypoxic environment, the synthesis of nitric oxide stagnates, leading to direct local vasoconstriction and with that only enhancing hypoxia in the area [10]. Low fluence rate PDT is coupled with lower oxygen demand and might therefore reduce vascular shutdown during early stages of illumination. An alternative illumination scheme that we and others are investigating is fractionated illumination with long dark intervals. In this approach a maximal efficacy is achieved when a small fluence is delivered first, separated from the second fluence by a dark interval of >90 min [6]. Although the mechanism(s) underlying this increased efficacy is not yet fully elucidated, an ischemia/reperfusion injury mechanism and/or increased reperfusion in the dark interval do not seem to be significant factors [11]. We have recently shown in vitro that cell kill after light fractionated PDT shows a strong dependence on the concentration of PpIX at the time of the first illumination [12]; only cells incubated with low concentrations of ALA show enhanced cell death. These findings support the hypothesis that the positive effect of light fractionation is based on a cellular mechanism that leads to sub-lethally damaged cells after the first light fraction, after which these cells become more susceptible to a second light fraction 2 h later.

In order to understand the interaction between PDT induced vasculature effects and tissue destruction, knowledge on vascular effects during and immediately after PDT is crucial. Various authors have reported on monitoring blood flow during and/or following ALA-PDT, showing little consistency in results [13–17]. However, these studies show large variation in experimental design on for example the average volume over which flow is determined and direct response vs. long-term (clinical) response. In a previous study it is shown that for topically applied ALA-PDT, PpIX concentration correlates with vascular changes including vasoconstriction and/or vascular leakage of small vessels and arterioles in the epidermis and dermis [8]. In this study we report on vascular response induced by ALA-PDT for different fluence rates, including both changes in vessel diameter and RBC dynamics in arterioles, imaged using intravital confocal microscopy in skin-fold chambers in hairless mice. While previous studies investigated variations in vessel diameter pre- and post PDT, we included measurements of vessel diameter and RBC velocity during PDT illumination. We show that situations occurred in which vessel diameter analysis showed an uninterrupted blood supply, while RBCs had come to a standstill. Furthermore, we analyzed the relation between PDT illumination fluence rate, PpIX accumulation in tissue and vascular reactions in skin-fold observation chambers.

Materials and methods

Experimental design

This study focuses on the analysis of vasculature during and following ALA-PDT using intra-vital microscopy. To facilitate this, hairless mice were equipped with a one-sided skin-fold observation chamber. ALA is topically applied on the epidermal side, while imaging was done from the subcutis, where the larger vessels were located, toward the epidermis. Red blood cells (RBCs) from a donor mouse were labeled with FITC isomer I and injected in the chamber-bearing mouse. The mouse was fixated under a confocal microscope, where high speed scanning of 1D-line perpendicular to the vessel provided information on RBC velocity. This 1D-scanning was performed during the PDT illumination and repeated 2 h after the end of the therapeutic illumination. PDT was performed using an external light source and according to different illumination parameters, which are described in detail below. The spatial distribution of PpIX and 514 nm bright-field transmission images were recorded before, directly after and 2 h after illumination.

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