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Photodynamic therapy using topically applied hypericin: Comparative effect with methyl-aminolevulinic acid on UV induced skin tumours

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ABSTRACT

Photodynamic therapy (PDT) is a treatment option particularly well-suited for superficial (pre)malignant skin lesions due to the skin's accessibility to light. In the present study, the efficacy of topical hypericin-PDT was evaluated using a mouse model for actinic keratosis. For comparison, similar experiments were conducted with methyl-aminolevulinic acid (Me-ALA). Small skin tumours (1–2 mm) were induced in hairless mice by chronic UV irradiation. After topical application of hypericin (0.1% in gelcream for 24 h) or Me-ALA (Metvix® for 4 h), the lesional/non-lesional skin surface fluorescence ratio was determined and fluorescence microscopy was used to study the skin penetration of the photosensitizers. The antitumour activity of topical PDT (20 mW cm^{-2}, 40 J cm^{-2}) was evaluated by measurement of the lesional diameters. Moreover, biopsies were taken at various time points after PDT for histological evaluation of the therapy.

Our results demonstrate that after topical application of hypericin and Me-ALA, tumour selectivity is limited in mouse skin. The microscopic distribution of hypericin fluorescence showed an accumulation in the stratum corneum and low fluorescence levels in the rest of the lesions, whereas the distribution of PpIX in the skin was more homogenous. Topical hypericin-PDT was found to be less efficient (44% total lesional clearance) as compared to Me-ALA-PDT (80% total lesional clearance). Full lesional necrosis was observed in responsive lesions, and the atypical cells of actinic keratosis were replaced by normal keratinocytes 3 weeks later, both after hypericin-PDT and Me-ALA-PDT.

1. Introduction

Non-melanoma skin cancers (NMSCs) are among the most common malignancies in the Caucasian population. The incidence of NMSC has been steadily increasing worldwide at a rate of 3–8% per year since 1964 and has reached epidemic proportions [1,2]. Generally, NMSCs have a favourable prognosis when treated early. Yet, squamous cell carcinoma (SCC) can be life threatening since it is capable of metastasis [3]. It is therefore equally important to treat precancerous lesions of Bowen's disease and actinic keratosis (AK) that can transform into SCC [4,5].

Photodynamic therapy (PDT) is a treatment option particularly well-suited for such skin diseases [6]. PDT is a relatively new treatment modality that combines the administration of a photosensitizer and subsequent targeted irradiation with visible light to generate an oxygen dependent destruction of the diseased tissue [7,8]. The number of cases of superficial (pre)malignant skin lesions treated with PDT has undergone a huge expansion in the last decade as this alternative to conventional therapy is non-invasive, safe and minimizes the risk of scarring [9,10].

Topically applied photosensitizers are preferred for dermatological PDT because of the reduced risk for prolonged skin photosensitivity, which is often the case after systemic administration [11]. Most experience with dermatological PDT to date has been with aminolevulinic acid (ALA). This is an early precursor in the haeme biosynthesis pathway, in which the endogenous photosensitizer protoporphyrin IX (PpIX) is formed. Exogenous administration of ALA can increase the intracellular concentration of PpIX to therapeutically useful concentrations by bypassing the cell's feedback control [12–14]. To increase the skin penetration of the hydrophilic ALA, esters with increased lipophilicity were developed. The only one currently clinically used is ALA methyl ester (Me-ALA) which has recently become licensed widely in Europe.

Although the use of ALA-induced PpIX as a photosensitizer is attractive, there are some concerns about the amount of PpIX that accumulates, especially in deeper skin lesions, and the occurrence of rapid photobleaching. In contrast to PpIX, hypericin, a photo-ac-

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tive dianthraquinone dye with a broad action spectrum originating from the herb *Hypericum perforatum* (St. John's wort) [15,16], is very photostable [17] and laboratory studies have indicated that it has a pronounced photo-dependent antitumour activity [18–20]. Furthermore, our previous experiments investigating the topical application of hypericin have shown promising results as the compound was found to penetrate into the hairless skin and skin lesions of mice [21,22]. These data together with the finding that a photosensitizing activity after application of hypericin on mouse ears could be observed [23], suggest the potential of the compound for the photodynamic treatment of skin disease.

In the present study, we evaluated the efficacy of topical hypericin-PDT in an animal model for AK/SCC that involves the induction of small skin lesions on hairless mice by chronic UV irradiation [24,25]. The model was chosen since the lesions show a high resemblance with skin lesions of AK and SCC observed in humans, which are also closely associated with chronic, repeated exposure of the skin to solar UV radiation [26]. The mouse model was used to study the penetration characteristics of hypericin in detail, to gain knowledge about the tumour selectivity and to evaluate the efficacy of topical hypericin-PDT. Additionally, all parameters were compared to Me-ALA which is typically used in the clinic for the treatment of AK [6,27].

2. Methods

2.1. Formulations

Hypericin was synthesized from emodin anthraquinone as described by Falk et al. [28]. The topical gelcream formulation containing hypericin (0.1%) was prepared as described by Boiy et al. [22]. This formulation was chosen as it guarantees an optimized penetration of hypericin into the skin [21,22]. For experiments with Me-ALA, Metvix® cream (Photocure ASA, Oslo, Norway) which contains 16% Me-ALA, was used.

2.2. Animals

Adult female hairless mice (NMRI – HR-HR) were obtained from Charles River (Lyon, France). Treatment groups consisted of 7 mice. The mice were fed a standard rodent diet and kept in a 12-h on–off light cycle except after administration of a photosensitizer, then they were kept in the dark. All aspects of the animal experiment and husbandry were carried out in compliance with national and European regulations and were approved by the Animal Care and Use Committee of the University of Leuven.

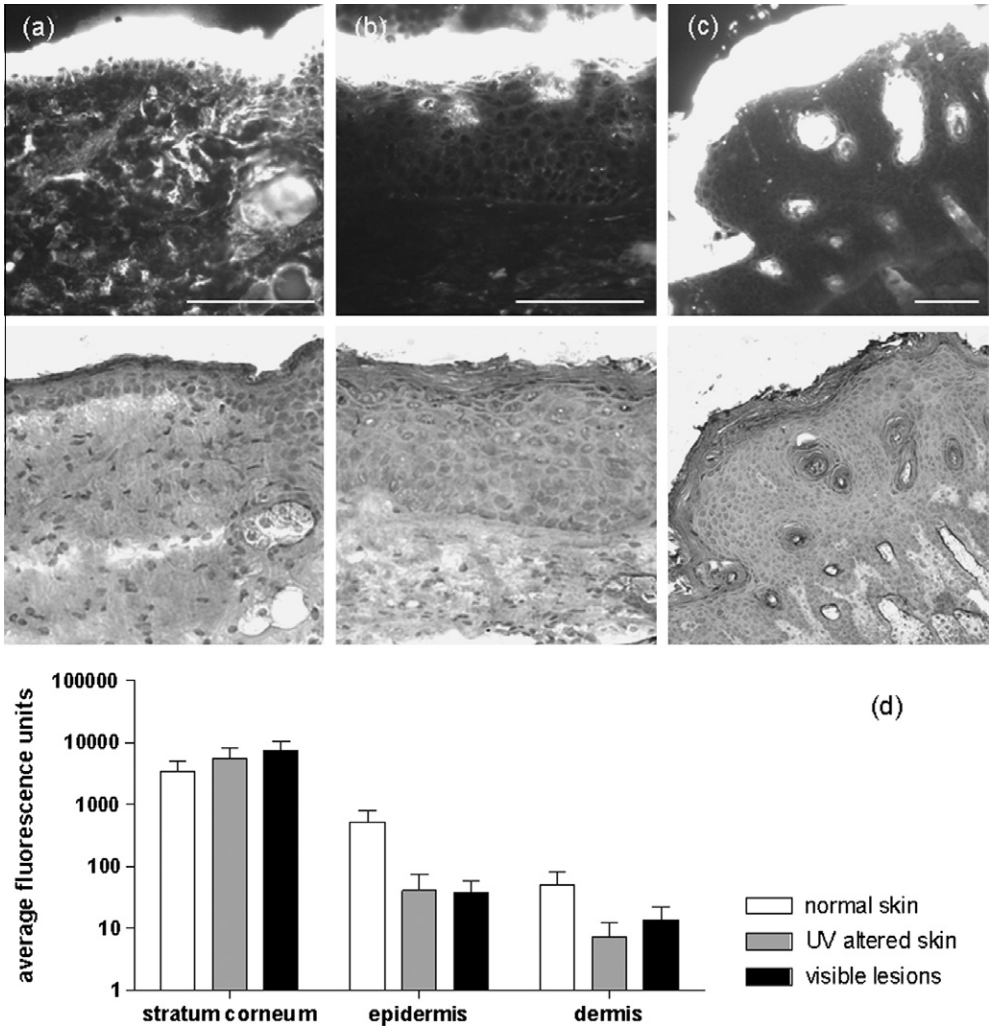

Fig. 1. Microscopic distribution of hypericin fluorescence in normal mouse skin (a), altered skin (b), and visible lesions (c), after a 24-h application, with corresponding H&E stains underneath. The scale bars represent 100 μm. Semi-quantification of the average fluorescence in the different skin layers was carried out and plotted as mean ± SD (d).