**Abstract**

**Background** Based on news about hypericin (HYP) as a potent photosensitizer with promising photobiological activities, we decided to try it as a topically applied photosensitizer in photodynamic therapy (PDT).

**Objective** The aim of this single-centre, prospective study was to investigate the effectiveness of topical HYP-PDT treatment for non melanoma skin cancer.

**Methods** The study was carried out on 34 patients (20 male and 14 women in the 32 to 83 years age range) with actinic keratoses (AK), basal cell carcinoma (BCC) and Bowen's disease. The patients were treated once a week with HYP in a glycerol solution and a red light dose of 75 J/cm$^2$ for 6 weeks on average.

**Results** The percentage of complete clinical response was 50% for AKs, 24% in patients with superficial BCC and 40% in patients with Bowen's disease. Complete clinical response did not occur in the group with nodular BCCs. A complete histological response rate was found out in 12% of patients with superficial BCCs and 80% in the patients with Bowen's disease. No control biopsies were performed after the treatment of AKs. All patients complained of burning and pain sensations during irradiation.

**Conclusion** The topical HYP-PDT is a promising treatment for non melanoma skin cancer although the results of our study were less successful in comparison with PDT using one of the most often photosensitizers (ALA, MAL). The advance of the therapy is a question of improvement of hypericin delivery into pathological cells.

## Introduction

Hypericin ($C_{30}H_{16}O_8$, molecular weight 504.44, CAS Number 548-04-9) is a polycyclic, aromatic phenanthroperylenedione with photosensitizing activity isolated from *Hypericum perforatum* plants, commonly known as St. John´s wort (Fig. 1).[1]

The first detailed report of the isolation of hypericin from *Hypericum perforatum* was published in 1939 by Brockmann et al.[2] The first correct chemical formula of hypericin was reported in 1942, also by the same author.[3]

In recent years increased interest in hypericin as a potential clinical anticancer agent has arisen since several studies established its powerful *in-vivo* and *in-vitro* anti-neoplastic activity upon irradiation. Photodestruction of cells charged with hypericin is dependent on the presence of oxygen and light of a distinct wavelength.[4] Investigations of the molecular mechanisms underlying hypericin phototoxicity in cancer cells have revealed that this photosensitizer can induce both apoptosis and necrosis in a concentration and light dose-dependent fashion.[5,6]

Here, we investigated the effects of photodynamic therapy (PDT) with a topically applied hypericin in a glycerol solution under occlusion for non melanoma skin cancer.

## Material and methods

Thirty-four patients (20 men [59%] and 14 women [41%] ranging in ages 32 - 83) with actinic keratoses (AKs), superficial or nodular basal cell carcinoma (sBCC, nBCC) and Bowen´s disease were included in the study (Table 1). 8 patients in the AKs group (age range: 63-83 years; mean age: 75 ± 6.72 years), 21 patients in the BCC group (age range: 32-82 years; mean age: 66 ± 13.35 years) and 5 patients in the Bowen's disease group (age range: 57-76 years; mean age: 67.2 ± 7.32 years) underwent treatment. Of these, three subjects in the BCC group had diagnosed nBCC and the rest sBCC. All clinical diagnoses, actinic keratoses excluded, had been verified by histology before starting PDT treatment with hypericin. Individual AK lesions designated for treatment were clinically graded as grade 1 (lesions slightly palpable and more easily felt than seen) or grade 2 (moderately thick AKs, easily seen and felt) or grade 3 (very thick and/or hyperkeratotic AKs).[7]

In the nBCC group no debulking of the tumor was done before treatment.

The above mentioned lesions were treated by photodynamic therapy in weekly intervals, the average period of treatment was 6 weeks.

As photosensitizer, we used hypericin product obtained by extraction with an addition of glycerol. The product was prepared by extraction of 25g of a dry drug (aerial parts of

Hypericum perforatum, 7% loss on drying, Hyperici Herba sc. from MEGAFYT, Czech Republic) by 500 ml of 95% ethanol (Penta, Czech Republic) in a Soxhlet extractor. After 4 hours of extraction the ethanolic extract was concentrated to 50 ml by vacuum distillation. The raw concentrate was roughly purified by column chromatography on Kieselguhr Hyflo-Super Cel (John Mansville Co., New York, USA) with 3.5 cm diameter and 6 cm height and 95% ethanol as a mobile phase. Then 3 ml of glycerol was added and 95% ethanol was removed under reduced pressure in a rotating evaporator. Subsequently, the hot product was transferred into a storage tube. The product was black-brown, homogenous,[8] very viscous, had density 1.2 g/ml and consisted of 36% glycerol, 17% water and 47% total solids (by freeze drying). The concentration of hypericins was estimated by spectrophotometry at 590nm (scan 450-800nm) in methanol (Fig. 2) after exposure to daylight to let convert protohypericin and protopseudohypericin to hypericin and pseudohypericin, respectively. Main absorbing compounds were hypericins and chlorphyll degradation products (Fig. 3).[9] Fluorescence spectroscopy revealed the main photoactive compounds at 590-670 nm, the emission light of the used excitation lamp, are hypericin and pseudohypericin. TLC analysis[10] with hypericin (Sigma) as a standard showed that the products contained 1.5-2.5 mg/ml of the hypericin (32.5%) and pseudohypericin (67.5%). Distinct products were mixed up to give the same final concentration of 2 mg/ml. Average yield of hypericins was 17 mg (0.07%) per 25 g of a drug. This product was protected from evaporation, shielded from light and stored at room temperature. Subsequent tests of this product gave the same spectra for periods up to 12 months.

The photosensitizer was applied to lesions and 10mm of surrounding skin in a 1-mm thick layer under occlusive dressing (Tegaderm, 3M, St. Paul, Minnesota, USA) for 2 hours, and then removed with saline and non-woven gauze. Before its application, the surface of treated areas was cleaned of possible crusting and flaking skin. After the period of incubation the site was irradiated with non-coherent red light with an emission spectrum of 580 to 680 nm wavelength (Medeikonos PDT, Model 200, Medeikonos AB, Gothenburg, Sweden). A total light dose of 75 J/cm$^2$ was delivered at all times. Before the irradiation an accumulation of hypericin in treated area was verified by a diagnostic lamp.

The clinical development of the treated manifestation was photographically documented. Dermatological medical history of all data concerning the duration of the disorder and treatment up to that point were obtained from each patient before starting the PDT treatment. Controlled biopsies were performed from the 3$^{rd}$ to the 8$^{th}$ week after completion of the PDT in the cases of patients with BCCs and Bowen's disease.

The protocol of this prospective study was approved by the local ethics committee and all of the subjects gave informed consent. Exclusion criteria of this study included a history of cutaneous photosensitization, hypersensitivity to St. John´s wort, photodermatoses, use of photosensitizing drugs, pregnancy or lactation and active infectious disease.

**Results**

In the group of patients with AKs a total of 8 people were treated (grade 1: 3/8, 37.5%; grade 2: 4/8, 50%; grade 3: 1/8, 12.5%). The manifestation was localized on the scalp and face. Four patients (4/4, 50%) showed complete clinical recovery, the remaining 4 (4/4, 50%) only showed improvement of clinical findings with lasting light infiltration of the treated area. The lesions that cleared completely were clinically graded as grade 1 in three cases and grade 2 in one case. Complete clinical recovery was seen in all patients with healed lesions (4/4, 50%) during follow-up at 3 months after the cessation of PDT treatment. In the remaining patients, clinical findings were aggravated. In the follow-up control at 6 months, only 3 patients, who had shown complete clinical response, were present. Two patients (2/3, 67%; 2/7, 29%) showed healed lesions, and one showed recurrence. A grade 2 patient with complete clinical recovery of AK was not present for the follow-up control after 6 months because of his death from an unrelated internal disease to the cutaneous disorder.

In the group of patients with BCCs, a total of 21 patients, 18 with sBCC and 3 with nBCC, were treated. The tumors were located on the trunk, extremities, scalp and face. In the sBCC group, of the 5 patients (5/18, 28%) showing complete clinical recovery, only 2 (2/18, 11%) showed complete histological recovery. In 3 patients, who were considered to have recovered clinically, signs of lasting basal cell carcinoma were histologically detected. Seven patients (7/18, 39%) showed partial improvement of clinical findings following treatment while the treated tumours of 6 patients (6/18, 33%) failed to respond to PDT treatment with hypericin. At 3 months following PDT treatment, 15 patients appeared for follow-up monitoring; the remaining 3 patients were started on a new therapeutic modality. Clinical healing persisted in 4 patients (4/15, 27%; 4/18, 22%) while signs of persisting tumour were clinically found in the remaining 11 patients (11/15, 73%; 11/18, 61%). At 6 months following PDT treatment, only 7 patients appeared for follow-up monitoring, 4 of whom (4/7, 57%; 4/18, 22%) still showed clinical healing. The remaining 8 patients were started on a new therapeutic modality before the 6-month follow-up.

None of the patients in the nBCC group showed complete clinical recovery. In 2 cases (67%), however, there was an improvement of the clinical picture; a reduction of the tumour mass and a decrease of infiltration. All patients were started on a new treatment.

In the group of patients with Bowen's disease, 5 subjects in total were treated. Manifestation was localised on the scalp, face, trunk and extremities. Of the total number of afflicted patients, 2 (2/5, 40%) had complete clinical recovery. Histologically, 4 (4/5, 80%) recovered completely. In 2 subjects, whose clinical findings following treatment were evaluated as improved yet not recovered, there were no histological signs of carcinoma. At 3 months following PDT treatment, complete healing persisted in 2 patients (2/5, 40%). In these patients recovery still persisted after 6 months (2/5, 40%) (Fig. 4). Aggravation was noted in 2 patients with improvement findings following treatment at 3 months follow-up control after the cessation of PDT treatment.

Upon irradiation all patients complained of pain and burning sensations, which spontaneously disappeared within 24 hours of terminating the therapeutic session. No other serious negative side effects were reported.

The results are summarized in bar charts and tables (Fig. 5, 6 and 7; Table 2 and 3).

**Discussion**

The term “photodynamic reaction” was first coined by Hermann von Tappeiner in early 20$^{th}$ century. At that time it was already known that PDT treatment required the simultaneous presence of a photosensitizer, light and oxygen inside the diseased tissue. Today, PDT serves as an effective treatment modality for non-melanoma skin cancers and certain other dermatoses.[11] By all appearances, PDT treatment may also have a preventive effect on premalignant lesions in immunosuppressed patients.[12]

The first PDT drugs were topically applied dyes like eosin red or erythrosine. Today, for dermatological purposes, only haematoporhyrin derivatives or protoporphyrin IX-inducing precursors such as 5-aminolevulinic acid (ALA) or methyl aminolevulinate (MAL) are of practical concern.[13]

Concurrently, however, the weight of evidence in research is directing increasingly more attention to other possible photosensitizers. The main reasons for this are the high economic costs of photodynamic therapy, both from the aspect of photosensitizers as well as that of the quality of radiation devices of varying provenance. Therefore, there have been efforts to bring into practice other new photosensitizers, which are not only chemically different from those used most frequently (ALA, MAL) in current practice, but also financially more accessible.

Hypericin would be one such example. Its stability in different formulations was proved in several studies.[14, 15]

In-vivo experiments with hypericin in cancer phototherapy were originally performed at the University of Nevada in 1990. Thomas and collaborators demonstrated that hypericin phototherapy can kill Mx1-mammary carcinoma cells in athymic mice.[16] The first local use of hypericin as a photosensitizer in photodynamic therapy for skin metastases of a malignant mesothelioma of the tunica vaginalis testis was described in 1994 by Alth and Ebermann.[17] In this case, treatment with superficially applied hypericin was combined with interstitial haematoporphyrin derivatives therapy.

In 1998 eight patients with squamous cell carcinoma and eleven patients with basal cell carcinoma were treated with intralesional injections of hypericin. In those cases, as well as in ours, hypericin displayed selective tumour-targeting (Fig. 8) and lower photobleaching when compared to ALA. Penetration in the surrounding tissues did not induce necrosis or cell loss. The effectiveness of the therapy depended on the concentration and total dose of hypericin and the frequency and duration of the therapy. The duration of therapy was from 2 to 6 weeks and was repeated 3 to 5 times per week.[18]

In our study, the average duration of the PDT treatment was 6 weeks, repeated once a week. In contradistinction we applied hypericin topically on the surface of treated lesions under occlusion for 2 hours.

Unlike ALA-PDT or MAL-PDT, hypericin therapy showed limited success in our study. This is interpreted as being due to the higher molecular weight of hypericin (504.44 Da) in comparison with the low molecular weight of 5-aminolevulinic acid (167.59 Da), which can cause a decreased penetration of hypericin in the skin tissue. For a drug to be delivered passively via the skin it is better to have a molecular weight <500 Da and also an adequate lipophilicity, which can be affected by a suitable vehicle.[19]

Various strategies have emerged over recent years to optimize skin delivery and they can be categorized into passive and active methods. The passive approach entails the optimization of formulation or drug carrying vehicle to increase skin permeability. Skin penetration enhancers as dimethylsulphoxide or carriers such as liposomes are used today. But in general, passive methods do not greatly improve the permeation of drugs with molecular weights >500 Da. In contrast active enhancement of drug delivery that normally involve physical (iontophoresis, electroporation) and mechanical methods (abrasion, ablation, perforation) or other energy-related techniques (ultrasound) has been shown to be generally superior.[20]

The present study describes diagnoses where PDT treatment with locally applied hypericin is less successful in comparison with more often used photosensitizers. However, this does not diminish the fact that hypericin is still considered to be one of the most promising photosensitizers today, especially for superficial cancers. The effecttiveness might be improved by higher concentration, light source with an emission designed for hypericin or a better vehicle to improve penetration. It seems that efficient penetration of hypericin or its derivates into the skin and its delivery into pathological cells is a critical factor for therapy effectiveness.

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