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Photodynamic diagnostics of skin and mucosal lesions

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Data of this study were in part presented at the 4th Baltic Congress of Oncology, Tartu, Estonia, 2006 (14).

Background: Porphyrin-enriched tumour tissue irradiation with a fluorescence excitation system leads to emission of pink-red fluorescence. This principle is used as a diagnostic procedure and is called photodynamic diagnosis (PDD). Complete visualization of skin and mucosal lesions may represent a diagnostic challenge; therefore PDD with different photosensitizers could give the benefit. The aim of this work was to investigate the possibilities of PDD in skin and mucosal lesion diagnostics and compare two different photosensitizers.

Materials and methods: Photodynamic diagnostics measurements were performed in 68 patients with malignant, premalignant and benign skin and mucosal lesions for detection of foci of squamous cell carcinoma, basal cell carcinoma, primary and metastatic adenocarcinoma, chondrosarcoma. The evaluated fluorescence data were correlated with cytological and / or histopathological tissue examination data.

Results: Red or red-pink fluorescence was observed in 72 malignant epithelial tumours. The most intensive red fluorescence was detected in thin superficial malignant lesions. From 165 benign lesions, very slight fluorescence was detected in a few haemangiomas, paratracheal papillomas, one fragment of herpes zoster and some superficial open wounds with very intensive capillarity. Nevi, papillomas, keratosis, scars and foci of psoriasis had no fluorescence.

Conclusions: Photodynamic diagnostics can be used for complete visualization of malignant lesions after the topical or systemic application of a tumour-selective photosensitizer. It has been shown to be highly effective in malignant superficial skin and mucosal lesion diagnostics. Fluorescence detection following i. v. injection of HpD or the topical application of ALA provides no difference.

Key words: photodynamic diagnosis, 5-aminolevulinic acid, protoporphyrin IX, hematoporphyrin derivative, fluorescence imaging

INTRODUCTION

Porphyrin-enriched tumour tissue irradiation with a fluorescence excitation system leads to emission of pink-red fluorescence. This principle is used as a diagnostic procedure and is called photodynamic diagnosis (PDD), also known as fluorescence diagnosis (FD) which is a more precise denomination (1). The term PDD is not actually correct, since reactive oxygen species are not involved in fluorescence diagnosis techniques. However, most authors use this term because it has already been widely used in the literature (2). In PDD, porphyrin fluorescence is detected under irradiation with different lights (under blue or near-UV excitation in range 300–450 nm) (3): a Wood light (370–405 nm) (2), xenon lamp (375–400 nm) (4), 370 nm (5), 410 nm (5, 6), 405 nm (7, 8), light diodes (405 nm).

In patients with advanced or small recurrent skin and mucosal cancer, PDD can improve the efficacy of treatment (9). These lesions may represent atherapeutic and diagnostic challenge because of special subtypes, location, previous therapy or accompanying diseases. Fluorescence can help in effective detecting and delineating of neoplastic areas. Recently, the use of photodynamic diagnosis and therapy has been proposed for the management of cancer (10–13).

The aim of the present work was to investigate the possibilities of PDD with different photosensitizers in skin and mucosal lesion diagnostics. Despite the easy accessibility mechanism to adequately screening and detecting premalignant changes and early lesions in the upper aerodigestive tract and skin, fluorescence diagnosis is being attempted as a diagnosis modality with the potential to bridge the gap between clinical examination and invasive biopsy (5). In order to further enhance tumour demarcation, exogenous sensitising agents can be administered. Several groups are carrying out research to develop fluorescence diagnosis methods for early detection of premalignant lesions in the upper aerodigestive tract, most of them using as a photosensitizer ALA-induced PpIX. Aminolevulinic acid has been shown to be the drug with most experimental and clinical use (1, 10). No generalized photosensitivity has been reported following topical

ALA application, and ALA-induced PpIX appears to be almost completely cleared from the body within 24 h of its induction (8). Topical ALA application does not provide prolonged generalized photosensitivity. The most often used agent in the past was the hematoporphyrin derivative Photofrin, originally developed for photodynamic therapy (PDT) by intravenous administration. In therapeutic doses it exhibits an unwanted side-effect of transient skin sensitisation, which lasts for at least 1–2 weeks. Low-dose injection can be used for tumour diagnostic purposes, provided that a sensitive detection equipment is employed (7). Initialy, hematoporphyrin derivative was the photosensitizer firstly applied in diagnostics and treatment, though its use was limited because of the subsequent prolonged generalized photosensitivity (6). Currently it is used only for diagnostic purposes. Topically active agents are preferable for PDT and PDD, and most experience to date has been gathered with ALA (3).

In our study, we were able to make a comparative analysis of two photosensitizers because hematoporphyrin derivative in our clinic is used for treatment both of advanced malignant and metastatic tumours. So it is permissible for treatment purposes. In parallel, for these patients the skin and mucous malignant and benign PDD is applied, because tissues are saturated with porphyrines.

MATERIALS AND METHODS

Photodynamic diagnostics measurements were performed, using data on 68 patients with malignant, premalignant and benign skin and mucosal lesions, for detection of the foci of squamous cell carcinoma, basal cell carcinoma, primary and metastatic adenocarcinoma, chondrosarcoma. The study "Photodynamic diagnostics of skin and mucosal lesions" was approved by the Lithuanian Bioethics Committee (No. 62). Two different photosensitizers – intravenous injection of hematoporphyrin derivative (HpD) and the topical application of 5-aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX) – were used.

As a fluorescence excitation system we used a simple and friendly light system based on blue light emitting diodes that allow an easy switching from the conventional white-light mode to an ALA or HpD-induced violet-blue-light (405 nm) mode (Fig. 1). Diagnostic illumination lasted 5–30 s. A digital camera was used for taking pictures of the fluorescenting area. For patients with an advanced malignant disease, HpD (2.5–5 mg/kg Photohem, Moscow, Russia) was injected i. v. and 12–24 hours after the injection malignant lesions were illuminated with blue light for cancerous tissue detection. PDD for patients with T1-2 was carried 3–6 hours after topical ALA (20% cream of 5-aminolevulinsauerhydrochlorid MEDAC GmbH Hamburg, Germany, on Exsipiale basement) application (Table 1). After the blue-light inspection, biopsy samples of the tumour foci were taken. The evaluated fluorescence data were correlated with a cytological and / or histopathological tissue investigation.

RESULTS

Visible tumour nodules were found to be fluorescent, whereas no fluorescence was observed in normal skin and mucosa. In the blue-light mode, there is a background blue fluorescence in normal tissue and red fluorescence in malignant areas (Figs. 2–5). Red or red-pink fluorescence was observed in 72 malignant epithelial tumours; 43 of them were fluorescent sharp, 27 were less intensive, 2 malignant tumours – nasopharyngeal area chondrosarcomas – had no fluorescence. The most intensive red fluorescence was detected in thin superficial malignant lesions. In 10% a tumour focus was identified with the blue light in an area that initially appeared normal when examined with the conventional light. All the tumour foci were histologically confirmed a carcinomas by biopsy.

Fig. 1. Fluorescence excitation system based on blue light emitting diode

From 165 benign lesions, a very slight fluorescence was detected in a few haemangiomas, paratracheal papillomas, one fragment of herpes zoster and some superficial open wounds with a very intensive capillarity. The fluorescence of these benign lesions was different from malignant and was not so red, bluer and less intensive. We noticed the fluorescence of the tongue mucosa in one of 5–6 patients after a systemic application of the photosensitizer. This occurred because of saprophytic bacteria. Chewing gum or lollipops induced a very intensive, strongly raspberry fluorescence of the central part of the tongue as well. Naevi, papillomas, keratoses, scars and foci of psoriasis had no fluorescence. Patients did not note any subjective symptoms such as pain, itching, burning sensation, etc. during the diagnostic procedure. These observations confirm a good efficacy and tolerance of PDD in a cancer problem patient cohort.

We investigated the usefulness of ALA-induced porphyrin fluorescence in preoperative demarcation of ill-defined clinical tumour margins and as a control after PDT. There was a strong correlation between the clinical extension and fluorescence pattern of the tumours. In addition, all fluorescent areas were proved to be neoplastic by histopathological examination.

Table 1. **Doses and types of application of photosensitizers**

Photosensitizer	Dose	Application	Time till PDD
5-aminolevulinic acid (ALA)	20% ALA cream	Topical application (on the skin or mucosa)	2–8 hours
	0.4% ALA solution	Rinse the mouth (20 min.)	1–2 hours
Hematoporphyrin derivative (HpD)	2.5–5 mg/kg	Intravenous	3–72 hours

Table 2. **Fluorescence intensity according to morphology**

Morphology	Fluorescence			Total
	Sharp	Less intensive	No	
Basal cell carcinoma (BCC)	36	20	-	56
Squamous cell carcinoma (SCC)	5	7	-	12
Chondrosarcoma	-	-	2	2
Adenocarcinoma	2	-	-	2
Total	43	27	2	**72**

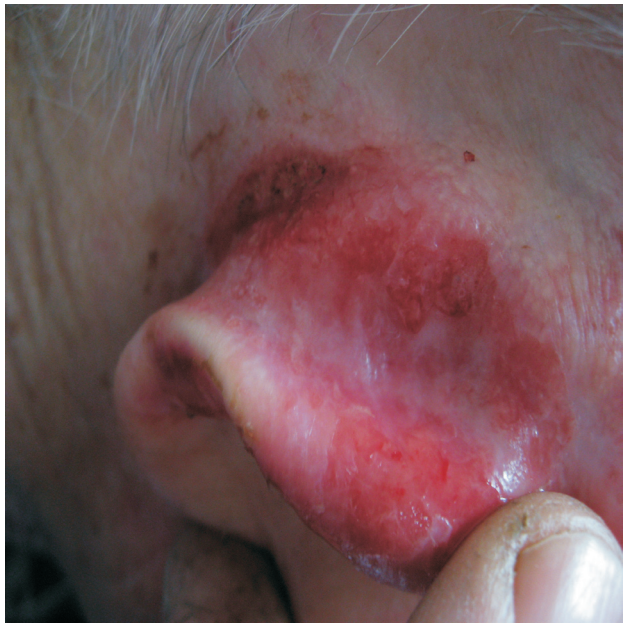

Fig. 2. Squamous cell carcinoma

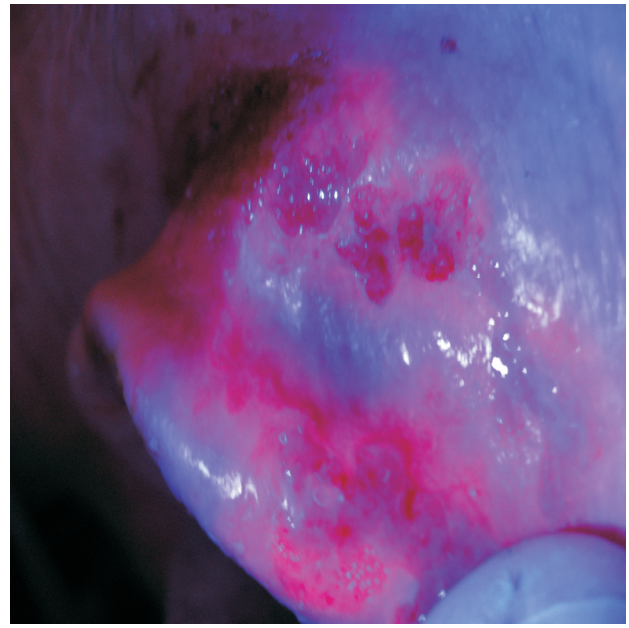

Fig. 3. Fluorescence after 5-aminolevulinic acid (ALA) application

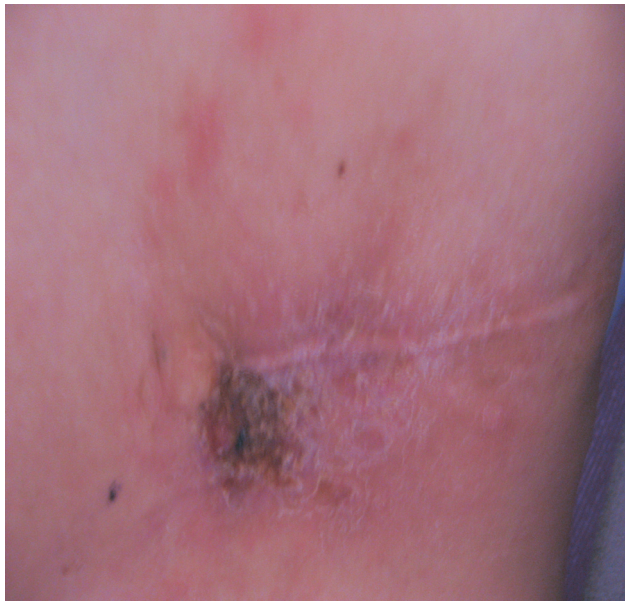

Fig. 4. Metastatic carcinoma in postmastectomy scar

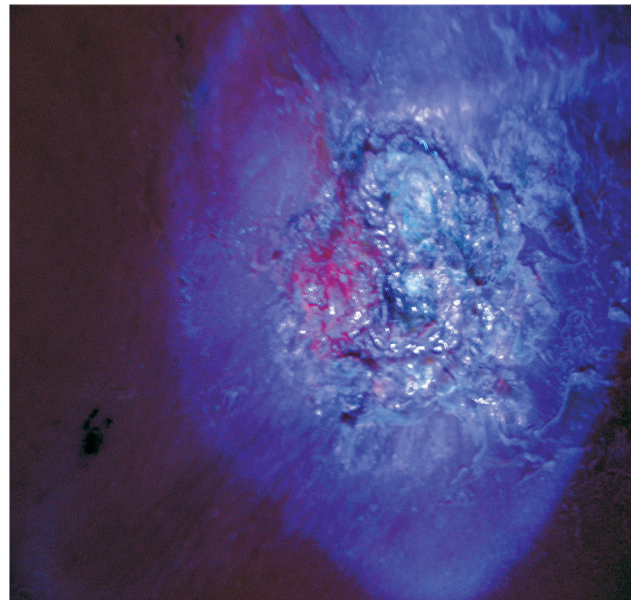

Fig. 5. Fluorescence after intravenous injection of hematoporphyrin derivate (HpD)

DISCUSSION

Advanced or recurrent cancer tissue may be difficult to differentiate from abnormalities induced by previous surgical procedures, such as granulomas and scars. In addition to functioning as a novel therapeutic tool, photodynamic sensitisation of skin and mucosal cancer cells is increasingly used for photodynamic diagnosis. The fluorescence of induced porphyrins is effective in detecting and delineating neoplastic areas (1), it helps to recognize the appearance of small tumour foci. Attention should be paid to lesions showing only moderate fluorescence in the mucosa, as this might already indicate the onset of carcinoma *in situ* (4). The additional information we have obtained from this method may be of great significance.

The use of PDD allowed delineation of clinically ill-defined tumours and detection of tumour relapses or new tumours that were not clinically detectable (2).

After the early studies using HpD, the majority of clinical studies have involved the topical application of ALA to skin lesions (15), without their comparison. The same efficacy of PDD has been observed for HpD and ALA in this study. Therefore, if the patient is treated with PDT using HpD, it is completely enough to diagnose skin and mucosal lesions and their spreading. Additional application of ALA is not necessary in this particular case. That is the explanation why HpD is being replaced by ALA only for inducing photosensitivity, but it isn't a worse photosensitizer than ALA used for PDD.

For the attainment of good demarcation of ALA, we kept it for 3–6 h, mostly for 4 h (80% of cases). Here we referred to Marica B. Ericson and others who indicate the strongest fluorescence after 4 h (8).

A review of similar works has shown that results are very similar and the best fluorescence was observed in superficial basal cell carcinoma and squamous cell carcinoma while benign lesions had no fluorescence (7, 8, 16). The fluorescence contrast is of great importance for the reliability of PDD technique when demarcating tumour tissue from normal skin. There was an exclusive possibility to compare the use of two photosensitizers for PDD in this study.

PDD is a simple diagnostic method. This technique has found a practical role in everyday clinical practice. Despite some previous doubts (17) that it will not be a clinically useful method, it is clear that sometimes it is impossible to manage without PDD (e. g., to determine the radicality of treatment after a surgical operation and especially after PDT); also large cutaneous malignancies can present a diagnostic challenge. Fluorescence imaging is an attractive diagnostic technique for skin and mucosal tumour demarcation with a potential to move into clinical use.

CONCLUSIONS

Photodynamic diagnostics can be used for a complete visualization of malignant lesions after the topical or systemic application of a tumour-selective photosensitizer. It has been shown to be highly effective in malignant superficial skin and mucosal lesion diagnostics. A very slight fluorescence, if any, was observed in benign lesions. PDD may be required to optimise detection of lesions in post-PDT patients and to guide tumour therapy. Fluorescence detection following i. v. injection of HpD or topical application of ALA shows no difference. This method is applicable for detecting early superficial tumours, margins of tumours and in follow-up after therapy.

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ODOS IR GLEIVINĖS NAVIKŲ FOTODINAMINĖ DIAGNOSTIKA

S a n t r a u k a

Įvadas. Toks metodas, kai stebima porfirinais prisotintų ir tinkamo bangos ilgio šviesa apšviestų navikinių audinių fluorescencija, vadinamas fotodinamine diagnostika (FDD). Ja diagnozuojami navikai. Aprašyto darbo tikslas – įvertinti fluorescencijos priklausomybę nuo histologinio tipo, ištirti skirtingų fotosensibilizatorių sukeltą švytėjimą.

Metodai. Vilniaus universiteto Onkologijos instituto Lazerinės ir fotodinaminės terapijos laboratorijoje FDD tirti 68 ligoniai, sergantys gerybiniais, ikivėžiniais ir piktybiniais odos bei gleivinių navikais. Naudoti du fotosensibilizatoriai: hematoporfirino darinio intraveninė injekcija ir po 5-aminolevulininės rūgšties aplikacijos susidaręs protoporfirinas IX. Fluorescencija buvo sužadinta melsvai violetinės (405 nm) šviesos diodu. Iš fluorescuojančių sričių buvo paimta medžiaga morfologiniam ištyrimui ir šie duomenys palyginti su fluorescencijos rezultatais.

Rezultatai. Apšvietus 405 nm šviesa stebėta rausvai avietinė navikinių audinių fluorescencija sveikų, nefluorescuojančių, audinių violetiniame fone. Užfiksuota 72 epitelinių navikų fluorescencija, 43 iš jų fluorescavo labai ryškiai, 27 – silpniau, 2 piktybiniai navikai – nosiaryklės chondrosarkomos – nešvytėjo. Tiriant 165 gerybinius darinius, ryškios fluorescencijos nebuvo matyti.

Išvados. Fotodinaminė diagnostika naudinga nustatant odos ir gleivinės piktybinių navikų tikslias ribas prieš ir po gydymo, diagnozuojant ankstyvas navikų stadijas ir recidyvus. Fluorescencijos skirtumų, naudojant aminolevulininę rūgštį ir hematoporfirino darinį, nepastebėta.

Raktažodžiai: 5-aminolevulininė rūgštis, hematoporfirino darinys, fotodinaminė diagnostika, selektyvi navikų fluorescencija, protoporfirinas IX