Photodynamic therapy of foot & hand warts

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Stender I-M, Renhua Na, Lock-Andersen J and Wulf HC. Protoporphyrin IX fluorescence in warts is not a predictor for the outcome of photodynamic therapy.

Stender I-M, Molke Borgbjerg F, Villumsen J, Lock-Andersen J and Wulf HC. Pain induced by photodynamic therapy with 5-aminolevulinic acid in foot and hand warts.

Preface

Rigshospitalet, the National University Hospital of Copenhagen and the Danish Research Academy, Aarhus financed my employment as a research fellow. Financial support to buy a fluorescence spectrometer was given from A.C. Bangs Foundation, which support is gratefully acknowledged.

I owe my greatest thanks to my chief and supervisor, Hans Christian Wulf, MD, D.Sc, who is unrelenting in elevating the standards and practice of photodynamic therapy (PDT) in dermatology. I am grateful for his scientific guidance and for educating me in the field of PDT.

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1.0 Introduction

5-aminolevulinic acid (ALA) is an endogenously occurring amino acid that is enrolled in the synthesis of hem. When ALA is applied to the skin it is absorbed via defect stratum corneum into the epidermal cells where it is enzymatically converted to the photosensitizer Protoporphyrin IX (PpIX). Illumination of cells loaded with PpIX can lead to cell death. This relatively new treatment modality is called photodynamic therapy with 5-aminolevulinic acid (ALA-PDT). ALA-PDT is rapidly expanding in dermatology. Clinical experience with ALA-PDT is primarily based on treatment of basal cell carcinomas (BCC) and actinic keratoses (AK) (Cairnduff et al. 1994, Calzavara-Pinton et al. 1995, Peng et al. 1997a), however new experimental indications for ALA-PDT show promising effect for some non-oncological proliferative dermatological diseases such as psoriasis (Boehncke et al. 1994a, 1994b, Stringer et al.1996, Collins et al. 1997) and human papilloma virus induced skin tumors such as, foot and hand warts (Frank et al. 1995, Stender et al 1999a, 1999b).

At Department of Dermatology, Bispebjerg Hospital H:S in Copenhagen we have worked experimentally for years with ALA-PDT of cutaneous tumors including non-melanoma skin cancer and benign human virus induced warts.

This thesis concentrates on the effect of ALA-PDT of foot and hand warts, the PpIX accumulation and the pain induced by PDT in warts.

1.1 Human papilloma virus infection in skin.

Etiology

Human papilloma virus (HPV) is a double stranded DNA virus of the papova virus family that is replicating within nuclei producing hyperproliferative lesions. More than 70 genotypes of HPV have been identified (Hausen 1996). Hand and foot warts are mainly caused by HPV 1, 2, 4 (Lever et al. 1990)

Prevalence

The exact prevalence of warts is not known. In a recently published Australian survey, 22% of school children had warts but two thirds were not aware of having them (Kilkenny et al. 1998). As many as 25-59 % of renal transplant patients developed warts one year after transplantation, rising to 77 %-95 % after 5 years (Glover et al. 1994).

Clinical appearance

Warts can be classified by clinical appearance, location, by histology and by type of virus. Warts are pleomorphic and can affect both skin and mucosa. HPV may appear as papules or nodules with a horny surface that range in size from millimeter to several centimeters and may confluence to large masses (Lever et al. 1990). Palmo-plantare warts are often associated with pain and may result in functional disturbances. Hand warts represent cosmetic as well as a functional problems and bothersome bleeding from finger warts is reported.

Diagnostics

Cinical judgement is often sufficient for the correct diagnosis of a wart especially when performed by an experienced dermatologist. A blind comparison cross-over experiment of experienced dermatologists using either intuitive clinical diagnosis or a diagnosis building on standardized diagnostic criteria showed that the standardized diagnostic criteria reduced the diagnostic accuracy (Young et al. 1998).

Histology

Hand and foot warts are characterized by hyperplasia of all layers of the epidermis presenting as achantosis, papillomatosis, hyperkeratosis with parakeratosis as well as dilated capillaries in dermal papillae. Viral replication takes place in the differentiated keratinocytes in stratum granulosum or above. Vacuolated cells, called koilocytotic cells are located in mid to upper dermis.

Papillomas caused by HPV are primarily benign tumors, but HPV is increasingly recognized as an important human carcinogen and papillomas may progress to dysplasia or neoplasm as squamous cell carcinomas (Hausen. 1996, McKenna et al. 1997, Favre et al. 1998, Harwood et al. 1999). HPV 16, 18 and 56 play a prominent role in the pathogenesis of Bowen disease, cervical, rectal and laryngeal verrucous carcinomas (de Villiers et al. 1994, 1997). Immune suppression often leads to HPV expression and skin cancer in organ transplant recipients on long-term immune suppressive drugs and in patients immune suppressed by malignant diseases or AIDS (Glover et al. 1994, Moy et al. 1994, de Villiers et al. 1997).

Transmission

Hand and foot warts are transmitted by direct or indirect cutaneous infection with human papilloma virus (HPV). A defect stratum corneum may be an entrance for HPV. Indirect contact with desquamated infected keratinocytes as well as auto inoculation are possible routes for infection (Cobb et al. 1990).

Therapeutic challenges

Two thirds of all hand and foot warts are never acknowledged by the patient (Kilkenny et al. 1998) and may like many other viral infections disappear without any therapy. A study performed on 1000 institutialized mentally retarded children with warts left untreated, demonstrated that two thirds of warts regress spontaneously within two years (Massing et al. 1963).

HPV lesions can however be resistant to current therapies and then represent a therapeutic challenge. A wide range of treatment modalities are reported in the literature and numerous treatments are available, probably as a consequence of a demand from wart patients to have their warts removed. As the saying goes: "*The number of available therapies is often greatest for conditions that do not predictably respond to treatments*". The reasons for wanting warts removed are mainly of functional, cosmetic and psycho social character. The fear of getting more warts and the fear of transferring the warts to other persons are also common reasons.

Although there is no reliable cure for virus infections as warts, simple treatments such as daily application with salicylic and lactic acid combined with cryotherapy cured 60-80% of patients after 3 months (Bunney et al. 1976). In some people the warts persist and due to the mentioned concerns, new therapies documented in controlled trials are needed (Begg et al. 1996).

1.2 Photodynamic therapy with 5-aminolevulinic acid (ALA-PDT)

Photosensitizer

A photosensitizer is a molecule that upon light exposure can induce cell damage due to various photochemical reactions, if present in biological tissue. Examples of photosensitizers are: hæmatoporphyrin derivatives (HPD), phtalocyanine derivatives, benzoporphyrin derivatives (BPD), meso tetrahydroxyphenyl chlorins (mTHPC) and 5-aminolevulinic acid (ALA) (Fisher et al. 1995, Calzavara-Pinton et al. 1996).

Photodynamic therapy (PDT) using photosensitizers has been used for several years in treatment of various tumors. In PDT selective tissue damage is achieved by the action of light on a photosensitizing agent. Systemic administration of the sensitizer dihematoporphyrin ether (DHE), known as the commercial preparation Protofrin, has been used with success in the treatment of internal cancer. However in spite of promising results the clinical use has been restricted because of prolonged cutaneous photosensitivity (Dougherty et al. 1978).

PDT with topical application of 5-aminolevulinic acid (ALA), a precursor for the photosensitizer protoporphyrin IX (PpIX), has during the last 10-12 years been increasingly used to treat non-melanoma skin tumors and other hyperproliferative diseases (Kennedy et al. 1990, Peng et al. 1997a).

Mechanism

When ALA is applied to the skin it is absorbed into pathological epidermal cells through defect stratum corneum. In the cells it is enrolled in the biosynthesis of hem via synthesis of porphyrins, especially protoporphyrin IX (PpIX) (**Figure 1**). Incorporation of iron into PpIX by ferrochelatase is necessary for synthesis of hem. Because of a limited

capacity of ferrochelatase, PpIX accumulates when exogenous ALA is applied (Gawkrodger, 1990). The PpIX accumulation in tumor tissue may be explained by an increased permeability of ALA through pathological skin as well as by a higher rate of ALA-derived porphyrin synthesis in neoplastic cells (Walters et al. 1963).

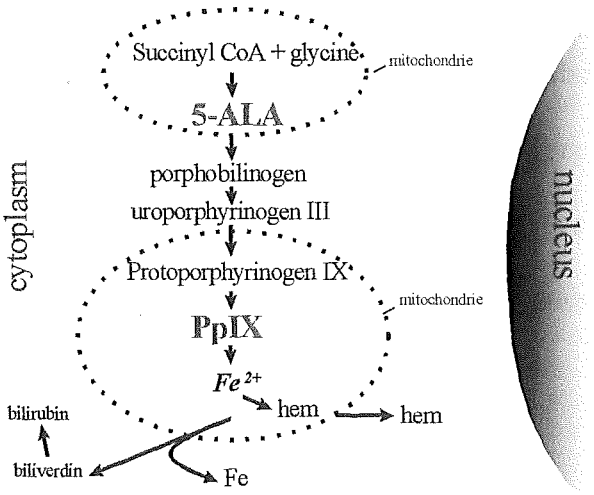

Figure 1. Metabolism of ALA to PpIX

PpIX accumulates in cellmembranes, mitochondria, endoplasmatic reticulum and lysosomes. When these PpIX loaded organelles are excited with light of an appropriate wavelength, a photochemical reaction will lead to oxygen dependent release of cytotoxic substances such as singlet oxygen followed by a destruction of the organelle (Henderson et al. 1995, Ward et al. 1996). Vascular effect may be caused by direct damage to the endothelial cells (Wyld et al. 1997) and stasis of the small vessels may induce trombosis, necrosis and apoptosis that all may be part of the tumor destruction (Webber et al. 1996, Noodt et al. 1996). The influence of PDT on the immune system is still unclear. It has been demonstrated that PDT is able to induce immune modulatory effects by a decrease in interleukine (IL-1 beta), (IL-6) and tumor necrotizing factor (TNF alfa), thus indicating a potential for treatment of psoriasis (Boehncke et al. 1994b).

Light and light sources

An appropriate light source for ALA-PDT is emitting light in a wave length or wave length range including the absorption peaks of PpIX. Lasers as well as nonlaser light sources can be used. Light sources used for PDT vary from slide projectors, xenon, halogen and tungsten lamps to advanced lasers. PpIX absorbs light in bands at 430, 579, 630 nm. PpIX has high absorption in the Soret band at 430 nm, however the penetration depth of the excitation light is limited in this emission area and deeper lesions may therefore not be reach by wave lengths in this range. Although absorption is minimal at 630 nm this wave length is routinely used because light penetration in tissue is deeper than at the shorter wavelengths (Fisher et al. 1995).

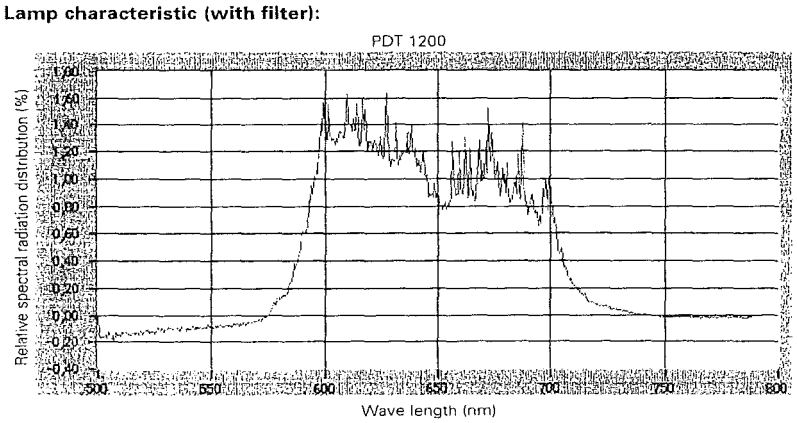

Figure 2: Spectral output from the PDT 1200 lamp
(*Information brochure for Waldmann PDT 1200 lamp.)*

In the studies reported in this thesis we used a Waldmann PDT 1200 lamp emitting light in the range from 590 nm to 700 nm including the PpIX absorption peak at 630 nm (**Figure 2**). Light doses for PDT have still not been optimized and may vary from 60-250 J/cm^2 for laser light and for non-lasers from 30-540 J/cm^2 (Peng et al. 1997a). The

optimal therapeutic effect of light, whether it is applied as a low dose for a relative long exposure time or as a high dose for a short exposure time, is not verified.

1.3 Clinical use of topical ALA-PDT

ALA can be applied topically, subcutaneously, systemically (i.v., i.m., orally, intraperitoneally), intralesionally, by instillation, inhalation and gurgling (Loh et al. 1993). Topical application of ALA is easy to handle and has a relative low risk profile and consequently ALA-PDT has become widely used for dermatological diseases.
After applying an emulsion of ALA in concentrations that may vary from 2-40 % to the target lesions and 10mm - 20mm of surrounding normal skin, the lesions are covered with a hydrocolloid dressing to enhance the absorption.
After an application time that vary from 3-12 hours, the remaining ALA is removed and the area is irradiated with a coherent or incoherent light source. The treatment can be repeated up to several times and the effect is best evaluated after 1 to 2 months.

Non-melanoma skin cancer

An overview of uncontrolled studies reporting cure rates of ALA-PDT for non melanoma skin cancer has been performed by (Peng et al. 1997a). The weighted average cure rate for complete responding non-melanoma skin tumors is shortly summarized in **Table 2.**

Table 2: *ALA-PDT cure rates for non-melanoma skin cancer, Bowen disease and actinic keratoses (Peng et al. 1997a)*

Lesion	No. of papers	No. of lesions	Cure rate % (range)	Weighted %(average)
sBCC	12	826	34-100	87
nBCC	12	208	10-100	53
Bowen	5	82	50-100	85
AK	5	116	85-100	92

sBCC: superficial basal cell carcinoma, nBCC: nodular basal cell carcinoma, AK: actinic keratoses.

In spite of more than ten years of research with ALA-PDT, only three randomized clinical controlled trials have so far been reported. For Bowens disease, PDT resulted in clearance of 15 of 20 lesions after one treatment and cryo therapy resulted in clearance of 10 out of 20 lesions. The probability that a lesion was cleared after one treatment was greater with PDT than cryotherapy ($p < 0.01$). Overall complete response rate after 1 year was 90% in the cryotherapy group and 100% in the PDT group (Morton et al. 1996). Treating basal cell carcinomas (BCC) with ALA-PDT was found to be comparable with cryo surgery considering remission (93% for PDT versus 98% for cryo ($p>0.05$) and recurrence rates within one year (26 % for ALA-PDT and 15% for cryotherapy ($p = 0.40$) (Wang et al. 1999a). In the third controlled trial one application of ALA-PDT was as effective as 3 weeks with 5 fluouracil for actinic keratoses at the dorsum of hands (Kurwa et al. 1999).

Topical ALA-PDT is not an effective treatment for deeper lesions such as nodular BCC because ALA is a hydrophilic molecule that penetrates poorly into the tissue. The average weighted clearance rates for nodular BCC and hypertrophic actinic keratoses were around 50 % (Martin et al. 1995, Peng et al. 1995, 1997b, Morton et al. 1998).

ALA-PDT may as such be regarded as a treatment only for superficial lesions. ALA-PDT has in uncontrolled case reports shown effect in cutaneous T lymphomas as well (Ammann et al. 1995a, Eich et al. 1999).

Other dermatological applications

The use of ALA-PDT for non-malignant disorders is based upon the selective uptake and retention of a photosensitizer by proliferating cells. Promising results have been reported for hæmangioma (Xiao-xi et al. 1997), psoriasis (Boehncke et al. 1994a), acne (Sigurdsson et al. 1997), nevus sebaceous (Dierickx et al. 1999), condylomata (Frank et al. 1995) and warts (Stender et al. 1999a, 1999b). It was demonstrated in preliminary in vitro and in vivo trials that ALA-PDT can treat viral infections such as herpes simplex, molluscum contagiosum and cells infected with HIV (Smetana et al. 1994, 1997). Selective accumulation of PpIX in condylomas compared to normal surrounding skin has been demonstrated (Fehr et al. 1996, Ross et al. 1997).

Advantages using ALA-PDT for non-melanoma skin cancer

Topically applied ALA followed by light exposure has several advantages. ALA and PpIX are naturally occurring compounds that are rapidly cleared from normal tissue resulting in a local photosensitivity lasting a few days (Kennedy et al. 1992, Jeffes et al, 1997).

Although ALA-PDT has not demonstrated higher clearance rates in BCC, Bowens disease and actinic keratoses than current therapies, ALA-PDT still has the advantages in being non-invasive, easy to handle and cosmetic excellent. In using ALA-PDT, it is possible to treat multiple and large areas of tumors at the same time. Due to the selectivity of ALA for pathological tissue, ALA-PDT induces minimal damage to surrounding normal tissue.

Lesions located in anatomical regions, e.g. eyelids, that are difficult to reach by surgery and cryotherapy, can be treated with ALA-PDT (Wang et al. 1999b). ALA-PDT can also be used as an adjuvant treatment to curettages. Patients with multiple tumors and larger dysplastic areas including nodular tumors at the body inclusive the scalp can benefit from ALA-PDT prior to excision. Deeper nodular tumors may not be cleared, however the removal of superficial dysplasiae will reduce the excision area and a possible skin transplantation. This "cleaning up" indication for ALA-PDT is both skin and hair saving and skin transplantation may in the future be reduced in size as well as in frequency by using ALA-PDT as pre-treatment. Besides, ALA-PDT can be used with wide margins around the visible tumor including sub clinical pathological tissue. ALA-PDT produces no plume and no blood and therefore ALA-PDT reduces the risk of transferal of e.g. HIV, hepatitis and HPV. ALA-PDT can take place as an out-patient treatment and can be repeated several times without toxic side effects. Post treatment infection, shorter healing time, scar formation and the general cosmetic outcome was in favor for PDT when compared to cryotherapy (Morton et al. 1996, Wang et al. 1999a).

Improvement of the treatment

Several avenues for improvements of the treatment outcome treating non-melanoma skin cancer have been reported: Multiple treatments (Svanberg et al. 1994, van der Veen et al. 1994), addition of EDTA, DMSO, iron chelators for inhibition of ferrochelatase activity, preparation of the lesions by paring or by superficial curettage as well as by increasing the application time for ALA (Malik et al. 1995, Berg et al. 1996, Morton et al. 1998, Soler et al. 1999). Methyl ester ALA is a lipophilic derivate of ALA and due to its lipophilicity it has an increased diffusion across cell membranes which then lead to a deeper penetration and thus may be a more effective treatment of deeper lesions (Gaullier et al. 1997, Kloek et al. 1998). Ongoing studies with light exposure schedules

and doses as well as studies with lipophilic methyl ester ALA all give promise of an increased cure rate for especially nodular lesions (Robinson et al. 1998). Oral administration of ALA has been demonstrated to improve the cure rate of deep BCC (Tope et al. 1998).

Photodynamic therapy of hand and foot warts

The effect of ALA-PDT on warts were not promising in two preliminary case reports (Kennedy et al. 1990, Ammann et al. 1995b). However in a preliminary study of recalcitrant foot and hand warts we compared the effect of ALA-PDT using a filtered and unfiltered incoherent light source with that of cryotherapy. For the ALA-PDT treated warts we found a cure rate ranging from 23% to 73% compared to 20% for that of cryotherapy (Stender et al. 1999a). Encouraged by these results we routinely treated recalcitrant warts patients referred to our out-patient clinic with ALA-PDT. A retrospective survey on these patients demonstrated that 58% of the patients were cured (Stender et al. 1999b). In both studies the cure rates were based on repetitive treatments. It is still not known if it is the viral infected keratinocytes or PpIX on virion surface glycoproteins that causes the anti-viral destruction. Ultrastructural examination of photosensitized herpes-virus demonstrated damage to viral envelope that prevented viral adsorption and /or penetration (Smetana et al. 1994).

Encouraged by the promising results of ALA-PDT of warts, we decided to investigate the effect of ALA-PDT on warts in a double blind placebo controlled trial. This trial is the main subject of this thesis (Study 1).

1.4 PpIX fluorescence

Fluorescence is the emission of light following absorption of photons by some molecules (fluorophores). Fluorophores exited by light emit fluorescence characteristic for the specific fluorophore and its concentration in the tissue. Fluorophores may be used to characterize tissue and to discriminate pathological tissue from non pathological tissue. The fluorophores can also be used to study the pharmacokinetics of various photosensitizers including ALA induced PpIX (Andersson-Engels et al. 1997).

Fluorophores

Fluorophores are usually divided into the following three groups (Wagnieres et al. 1998):

A) *Endogenous fluorophores* occur naturally in the tissue. Examples of such fluorophores are NADH, porphyrins, collagen, elastin, melanin and tryptophan. The fluorescence emitted from these intrinsic fluorophores is called autofluorescence. Autofluorescence differs depending on the intrinsic molecules and their concentration at the site for registration (Andersson-Engels et al. 1992).

B) *Exogenous fluorophores* are administered as exogenous substances to the tissue. Examples of such are fluorescein, indocyanin green, hæmatophorphyrin derivates (HpD), phthalocyanines, chlorins and benzoporphyrin derivatives (BPD). Exogenous fluorophores are selectively absorbed in tumor tissue and are often used for photodynamic diagnostics (PDD) purposes often followed by PDT (Fisher et al. 1995).

C) *Exogenous fluorophores* can also be fluorophorers that are synthesized in the tissue after external administration of a prodrug such as ALA, that has no photosensitizing properties itself, but is metabolized to a fluorophore, namely the photosensitizer PpIX .

ALA induced PpIX fluorescence

ALA has a high selectivity for malignant tissue in which it is enzymatically converted to PpIX. A high activity of the enzyme porphobilinogen deaminase, a low activity of ferrochelatase, a low concentration of iron, rapid cell proliferation, the cell cycle phase, cellular oxygen, extracellular pH and skin temperature are all factors that may influence the conversion of ALA to PpIX in tumor tissue (Leibovici et al. 1988, Steinbach et al. 1995, Tope et al. 1998, Wyld et al.1997, Pourzand et al. 1999, Moan et al. 1999).

PpIX may fluoresce when excited by light and consequently be used as a photodynamic diagnostic modality (PDD) of pathological tissue based on the appearance and characteristics of the fluorescence (Kennedy et al. 1996). PDD identification of pathological tissue may be followed by a biopsy, surgery, cryotherapy, radiation therapy or even by ALA-PDT.

PpIX fluorescence can be detected by direct visualization or by quantitative detection methods. Irradiation of tissue with ultraviolet or illumination with visible light may induce fluorescence that may be seen (dependent of the molecules excited) by the naked eye as a colored light. Porphyrins fluoresce red when excited with blue or violet light (Stringer et al. 1996, Bissonnette et al, 1998) and may also be registered as a spectrum on a screen connected to a fluorescence spectrometer. The set up of such a system is illustrated in **Figure 1** and the spectrometer is shown in **Appendix 2.** Due to the low excitation wavelength used for fluorescence recordings (380 nm) it is only the superficial

layers from which we may get an impression of the fluorophores (af Klinteberg et al. 1999).

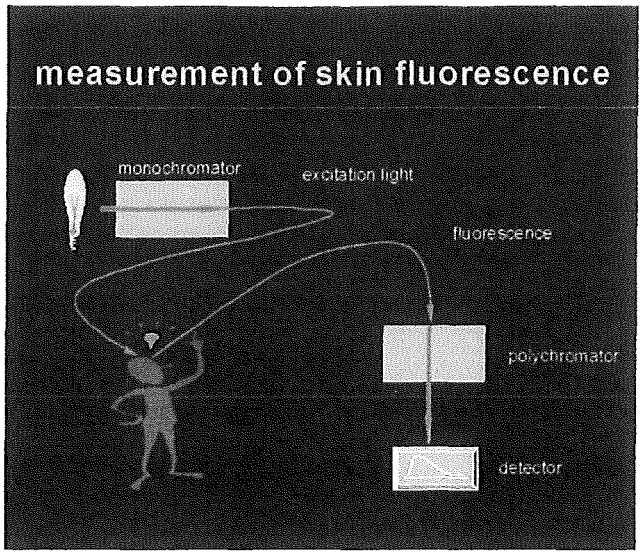

Figure 1: Schematic drawing of fluorescence recordings from the skin.

PDD with use of fluorescence is used for detection of premalignant and malignant lesions. PpIX fluorescence may be used to localize sites for biopsies (Kennedy et al. 1992, Fritsch et al. 1998), for demarcation before tumor removal as well as a control for a sufficient removal. Monitoring of PpIX by a fluorescence spectrometer may be used to investigate the absorption and conversion of ALA into PpIX and may also be used in the optimization of ALA-PDT. PDD has with success been used for photodetection of cancer and dysplastic conditions in internal organs (Kriegmair et al. 1996, Regula et al. 1995, Jichlinsky et al. 1997, Ackroyd et al. 1999). ALA-PDT as well as PDD for internal use are performed by use of flexible fibre scopes. Excitation of fluorescence with light used for PDD is often referred to as LIF (Light /Laser Induced Fluorescence) (Svanberg et al. 1994).

Whether fluorophores such as PpIX are endogenously derived or they are induced by ALA, they always have a characteristic dual-peaked fluorescence emission in

the red spectral region, with one high and narrow peak at about 630 nm and a smaller but wider peak at 690-700 nm (**Figure 3**).

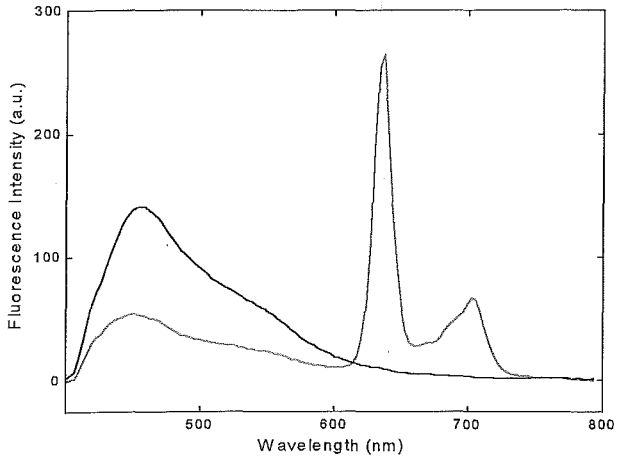

Figure 3. Representative PpIX emission spectra from an ALA treated wart (red curve) lesion and surrounding normal skin (dark curve).

Imaging based upon fluorescence characteristics is useful as a rapid, non-invasive and easy method for identification of tissue, either by the autofluorescence characteristics alone or by exogenous fluorescence. About 10^{-8} second after excitation, fluorescence is emitted and consequently fluorescence diagnostic or tissue characteristic can be used as an "in situ" investigation.

Fluorescence recordings from warts

Obtaining fluorescence spectra from hand and foot warts after ALA application make no sense for practical wart diagnostics or wart demarcation, since warts can easily be diagnosed by the naked eye (Young et al. 1998). Spectra obtained from warts may theoretically be interesting for identification of subclinical wart tissue in the wart surroundings, however it will probably never get any clinical consequences. Detection of PpIX by use of fluorescence spectrometry in warts give information about accumulation and photodegradation of PpIX and may help in choosing the optimal time for light exposure. The selectivity of ALA induced PpIX in wart tissue after consecutive ALA-

PDT treatments as well as the relation between the PpIX concentration and the outcome of ALA-PDT are the subjects for study 2 in this thesis.

1.5 Topical ALA-PDT induced side effects

The following description of adverse events related to ALA-PDT is primarily based upon own observations during the last 5 years of clinical work with ALA-PDT for non-melanoma skin cancer and warts.

Local side effects

Topical application of the cream emulsion with ALA (20%) to erosive, ulcerating and thin skin lesions may induce a momentary and short lasting scalding.

Pain during and immediately after light exposure.

When irradiating ALA exposed lesions sensations like itching, warming, burning to unbearable pain localized to the treated lesion are reported (Kennedy et al. 1992). If severe pain arises immediately after initiation of light exposure, it usually continues during the entire irradiation period. Pain vary from patient to patient and from lesion to lesion. Prior to an ALA-PDT treatment it is impossible to predict the severity of pain, however it has been suggested that patients with "Celtic" skin type are more sensitive for ALA-PDT than darker skin types (Kennedy et al. 1992). The pain is often reduced immediately after light exposure, but may continue for quite a period and can even top several hours after light exposure. We have treated more than 200 patients for hand and foot warts and some have reported shooting and radiating pain even days after ALA-PDT. We have never experienced irreversible induced pain. In agreement with Wang et al. we experienced that cold water applied with a simple spray bottle provides temporary relief. Emla cream (lidocain, prilocain) applied topically one hour before irradiation also

seemed to reduce the pain during light exposure. ALA-PDT for BCC has been reported to be slightly more painful than cryotherapy. However if the lesions were retreated, the ALA-PDT was less painful than cryotherapy (Wang et al. 1999a). Treating Mb. Bowen disease, ALA-PDT appeared to be a less painful modality than cryotherapy (Morton et al. 1996).

It has often been discussed if the burning sensation in treated lesions may be related to hyperthermia induced by the light source. It has been shown that irradiation at a power density of 100 mW/cm^2 initiates a temperature rise in ALA-PDT treated tumors and in normal skin without ALA. However, pain is only reported from the ALA treated area indicating that the pain may be a result of the photochemical process (Orenstein et al. 1995).

Based on our clinical experience pain induced by ALA-PDT may limit the use of ALA-PDT. Assessment and characterization of pain is therefore the subject for study 3 in this thesis.

Cutaneous side effect following light exposure

Cutaneous photoxic effects, as localized erythema and edema peaked 72 hours after treatment of actinic keratoses (Jeffes et al. 1997). When treating hand and foot warts, it is only ALA surrounding skin at dorsum of hands and feet, on which ALA also was applied, that appear erythematous. Edema immediately after ALA-PDT is common treating non-melanoma skin cancers. In few cases we observed local urticaria accompanied by heavy itching. It has been reported that PDT can induce histamine release from the mastcells (Yen et al. 1990).

Vesicles or bullae formation may occur during irradiation followed by superficial erosions that heal within few days.

Local cutaneous photosensitivity in the ALA applied area last up to 72 hours after light exposure. Exposure of the ALA treated area to ambient light may induce stinging and burning followed by an increased erythematous cutaneous reaction. In sunny periods it may be necessary to cover the ALA-PDT treated area for 48 hours after treatment. In sunny regions of the world it could possibly be a problem to treat sun-exposed areas due to the local but still uncomfortable light sensitivity.

Edema and erythema may be followed by desquamation or by superficial erosion followed by crusting, which may last for about a week. Infection is rare in lesions treated with ALA-PDT. Fewer infections after ALA-PDT than after cryotherapy is reported by Morton and Wang (Morton et al. 1996, Wang et al. 1999a). Sterile pustules have been observed in the treated area days after light exposure.

The response of an ALA-PDT treatment is often evaluated after 1-2 months and by that time the cosmetic outcome can be evaluated as well. Minimal scarring and a slight hyper- or hypopigmentation may be seen. A significant increase of fibrosis in the dermis years after ALA-PDT has recently been reported (Fink-Puches et al. 1998).

A case of allergic contact dermatitis (Gniazdowska et al. 1998), an induced severe flare up in inactive psoriasis, interpreted as a Koebner phenomenon (Stender et al. 1996) and a malignant melanoma in vertex previous treated with ALA-PDT (Wolf et al. 1997) are side effects reported after ALA-PDT.

Systemic side effect.

Transient mild skin photosensitivity, malaise, headache, nausea, vomiting and temperature rise after systemic administration of ALA are observed in patients given 50 mg/kg. All symptoms had settled by 48 hours (Ackroyd et al. 1999).

The symptoms described are similar to symptoms reported from patients with porphyria. After oral administration of 40 mg/kg, photosensitivity, characterized by stinging and

tingling sensation of exposed skin and transient liver enzyme elevations has been reported. No peripheral neurologic symptoms and no changes were noted in a broad examination of various other blood parameters (Tope et al. 1998).

Repetitive treatments do not seem to result in an accumulated toxicity that is clinical detectable. It has been demonstrated that 5-ALA application (up to 7 g per treatment session) has no influence on the concentrations of porphyrins and porphyrins precursors measured in various compartments (Fritsch et al. 1996).

Long term side effects

The mutagenic potential can not be entirely excluded. The alkaline comet (single cell electrophoresis) assay can visualize single strand breaks in DNA after incubation of cells with ALA followed by irradiation with visible light. The DNA damage may give an indication of the long-term side effects (Fiedler et al. 1996, van Vloten et al. 1999), however further studies concerning DNA repair must be performed. DNA is not the main site of damage, and significant transformation and mutagenesis have not been demonstrated in cell culture systems exposed to porphyrins and light (Gomer et al. 1988). Hairless mice exposed daily to a solar simulator and treated repeatedly with ALA-PDT, demonstrated a delay in skin carcinogenesis compared to control mice (Stender et al. 1997).

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2.0 The aims of the studies

Study 1

To evaluate the effect of ALA-PDT versus placebo of recalcitrant warts in a double blind randomized trial (*Photodynamic therapy with 5-aminolevulinic acid and placebo treatment of recalcitrant foot and hand warts: randomized double blind trial)*

Study 2

To examine Protoporphyrin (PpIX) accumulation in foot and hand warts treated with ALA-PDT respectively placebo-PDT *(Protoporphyrin IX fluorescence in warts is not a predictor for the outcome of photodynamic therapy).*

Study 3

To quantify and characterize pain induced by ALA-PDT (*Pain induced by photodynamic therapy with 5-aminolevulinic acid in foot and hand warts*).

Study 1

3.0 Photodynamic therapy with 5-aminolevulinic acid or placebo for recalcitrant foot and hand warts: randomized double-blind trial

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3.1 Summary

Background Photodynamic therapy (PDT) with topical 5-aminolevulinic acid (ALA) followed by irradiation with incoherent light (ALA-PDT) for recalcitrant warts have had beneficial results. Therefore, we undertook a randomized, parallel, double-blind clinical trial of ALA-PDT versus placebo-PDT for recalcitrant foot and hand warts.

Methods Recalcitrant foot and hand warts were randomly assigned to six repetitive ALA-PDT or placebo-PDT interventions combined with standard treatment encompassing paring followed by a keratolytic (Verucid®). Standardized photographs of each wart were taken before, during (week 7) and after treatment (week 14 and 18). The area of each wart compared with entry area was the primary outcome variable, measured from photographs by an evaluator unaware of treatment allocation. Pain intensity immediately and 24 hours after each intervention was assessed by a five-point scale.

Findings A total of 232 foot and hand warts in 45 patients were entered into the trial: 117 warts were allocated to ALA-PDT and 115 warts to placebo-PDT. In week 14, the median relative reduction in wart area was 98% in the ALA-PDT group (interquartile range (IR) 100%, 55%) versus 52% (IR 100%, 0%) in the placebo group ($p = 0.0006$). In week 18, the median relative reduction in wart area was 100% in the ALA-PDT group (IR 100%, 57%) versus 71% (IR 100%, 0%) in the placebo-PDT group ($p = 0.008$). Both the number of vanishing warts and the difference in relative wart area of persisting warts at week 14 and 18 were significant ($p<0.05$) in favor of ALA-PDT. Significantly more ALA-PDT warts were graded at a higher pain intensity after treatment than placebo-PDT warts.

Interpretation ALA-PDT is superior to placebo-PDT both when wart area and number of vanishing warts are considered.

3.2 *Introduction*

Foot and hand warts cause major cosmetic, functional and social problems and are a therapeutic challenge (Keefe et al. 1990). Treatment of warts usually takes place in general or dermatological practice. Simple treatments such as wart paints combined with paring of the warts, glutharaldehyde and cryotherapy may cure up to 70% of common hand and foot warts within 3 months (Bunney et al. 1976, Bourke et al. 1995). Warts being resistant to these simple "treatments" are usually dealt with by an assortment of treatments ranging from cimitidine to lasers. However, some warts remain. Patients with recalcitrant warts are frequently referred to secondary dermatological outpatient clinics, where the same interventions are reapplied, due to lack of therapeutic alternatives.

Photodynamic therapy (PDT) with topical 5-aminolevulinic acid (ALA) followed by irradiation with red light (ALA-PDT) is a well known treatment modality for non-hypertrophic actinic keratoses and superficial basal cell carcinomas. Cure rates of 90-100% have been reported in uncontrolled studies (Penq et al. 1997, Fritsch et al. 1998). Only one published randomized clinical trial comparing ALA-PDT and cryotherapy for Bowen disease has shown the same cure rate after ALA-PDT as after cryotherapy, although adverse events such as infection, healing time and scarring favored ALA-PDT overall (Morton et al. 1996). The use of ALA-PDT for viral infections such as herpes simplex, molluscum contagiosum and verrucae vulgaris has been suggested (Smetana et al. 1997).

Encouraged by our preliminary positive uncontrolled observations for efficacy of ALA-PDT for recalcitrant warts (Stender et al. 1999a, 1999b), we conducted a randomized, parallel, double-blind clinical trial to test ALA-PDT versus placebo-PDT for recalcitrant foot and hand warts.

3.3 Patients and Methods

Patients

Consecutive patients with recalcitrant (in our study defined as treatment in vain by any method for more than three months) foot and hand warts (excluding mosaic warts) referred to the outpatient clinic of the Department of Dermatology, H:S Bispebjerg University Hospital, Denmark, were considered eligible for this trial. Exclusion criteria were lack of informed consent, immunosuppressive therapy, other reasons for immunosuppression, pregnancy, breast feeding, and age below 18 years. The protocol was approved by The Ethics Committee of Copenhagen and Frederiksberg (02-092 /98).

Treatments

When a patient agreed to participate in our trial, all warts were consecutively numbered and the treatments were allocated blindly to intervention by an independent centralized, computer generated block randomization. A block size of two (unknown to the clinical investigators) was chosen, ensuring the application of both treatments for patients with more than one wart.

All warts had the horny layer pared with a scalpel by a dermatologist to visualization of blood vessels. The warts received under occlusive hydrocolloid dressings either topical

application of 20% ALA cream or placebo cream base that looked and smelt the same. The cream was applied in a relative thick layer (0,2 g /cm^2). Four hours later all warts were irradiated with a red light source (Waldmann PDT 1200,Waldmann-Medizin-Technik, Villingen-Schwenningen, Germany), ranging in wavelength from 590 nm to 700 nm. The warts were exposed to a fluence rate of 50 mW/cm^2 for 23 minutes 20 seconds corresponding to a total dose of 70 J/cm^2. Multiple warts could be irradiated at the same time (Picture appendix 1). The ALA-PDT and placebo-PDT interventions were repeated after one and two weeks. Then followed one month of observation (week 7) and if the warts still persisted application of ALA-PDT or placebo-PDT were repeated three times with one week intervals. Follow-up was performed one (week 14) and two months (week 18) after the sixth and final treatment.

Patients were instructed to pare all their warts with a scalpel twice a week during the whole study period and then apply a keratolytic (Verucid® (salicylic acid 10% and lactic acid 11 %)).

Evaluations

Photographs of each consecutively numbered wart were taken by a professional photographer at entry and after 7 (just before the fourth treatment), 14 and 18 weeks. A Nikon F601 camera was used with Fuji 200 ASA film. An overview photograph was taken to identify the position of the warts. Close-up photographs of each wart together with a plastic ruler were taken for wart area evaluation. The distance, position and light were standardized for each photo by use of the previously taken photographs.

The four close-up photographs of each wart were placed in a plastic file with four pockets. The area of the wart was measured by a dermatologist unaware of treatment allocation.

Wart area was measured on each of the photographs with a divider transferred to the ruler located on the same picture. The product of the longest and widest diameter from each wart photographs was considered the wart area. Intraobserver variation was estimated of 22 photographs, which showed that 90% of the measurements varied by 2 mm or less.

Efficacy was measured as the relative change in wart area compared to wart area at entry (primary outcome measure), number of warts that vanished and change in wart area of persisting warts.

Adverse events were registered prospectively and patients filled in a pain questionnaire concerning pain in the individual warts immediately following the PDT and 24 hours later. Pain was assessed by a five-point scale (Ohnhaus et al. 1975).

Statistical analyses

With a type-1 error of 5%, a type-2 error of 20%, and an expected cure rate in the placebo-ALA of 30%, not overlooking a minimum relevant difference in cure rate of 20%, it was calculated that 103 warts per intervention arm were needed.

All data were computer-analyzed with SAS (version 6.12). Only blinded intention-to-treat analyses were done. The relative change in wart area was analyzed with Wilcoxon rank-sum test, the number of vanishing warts was analyzed with the Chi-square test, and the change in relative area of warts that persisted was analyzed by two-way ANOVA with treatments and patients as prognostic factors. The interaction between duration of warts before entry and wart area at entry on one hand and intervention on the other was analyzed by Cox proportional-hazards regression analysis. The hazard rate ratios of the Cox analyses are expressed as $\exp(\hat{\beta})$. Pain intensity was analyzed with the Mann Whitney test. $p < 0.05$ was regarded as significant.

3.4 Results

From May, 1998 to November, 1998, 45 (70%) patients out of 64 consecutive patients fulfilled our inclusion criteria and none of the exclusion criteria. Nineteen (42%) were men and 26 (58%) were women. Median age was 37 years (range 20-84 years). Forty-three (96%) patients were referred from private dermatology practice and two (4%) from general practice. Nineteen patients were excluded because of immunosuppression, pregnancy or breast-feeding, or lack of informed consent (figure 1).

Entry characteristics of the patients and warts of the two intervention groups, which were similar, are shown in table 1. The median duration of warts at entry was 55 months (range 5-240 months). The patients had received wart treatment for a median time of 36 months (range 5-240 months). The groups were similar in regard to prevalence of foot warts (79% versus 83%) and median wart area (16 mm^2 versus 24 mm^2). There was no significant difference between entrance areas in the two interventions arms ($p = 0.15$). Compliance with treatment was excellent. Two patients dropped out of the trial after week 7. One patient with 3 warts treated by ALA and two by placebo did not want to continue because of pain. The other patient, who dropped out had one placebo wart and did not want to continue after week 7 because of lack of time. Another patient with seven warts treated by ALA and seven by placebo deviated from the planned trial design because she could not endure light treatment for more than 5 minutes in each of the 6 treatments. However, she took part in all treatments and follow-ups. Five patients with a total of 29 warts had no follow-up week 14 due to reasons unrelated to the trial. Six warts treated by ALA-PDT and four by placebo

were treated three times only because they were cured at week 7. The remaining warts were all treated six times.

Table 2 shows the distribution of the relative change in wart area at each follow-up compared to the wart area at entry. The wart area decreased significantly in the ALA-PDT treated warts compared with the placebo-PDT treated warts at week 14 (46%; p = 0.0006) and at week 18 (29%; p = 0.008), but at week 7 (21%) no significant difference was observed.

The number of vanished warts was significantly ($p<0.05$) higher in the ALA-PDT treated warts compared with the placebo-PDT-treated warts at week 14 and 18, but not at week 7 (table 3). Among the warts that did not vanish at weeks 7, 14 and 18, the relative reduction of wart area compared with entry area was significantly higher in the ALA-PDT arm compared with the placebo-PDT arm at week 14 ($p<0.0001$) and week 18 ($p=0.015$) (table 4).

Cox regression analyses confirmed the therapeutic efficacy of ALA-PDT versus placebo-PDT (exp ($\hat{\beta}$)= 0·63 (95% CI 0·42 - 0·97); p=0·034). No significant effect on intervention efficacy was observed by including wart area at entry (0·64 (0·42-0·98); p = 0·041) or wart area at entry and duration of warts at entry (0·64 (0·42 -0·98); p = 0·042) into the analyses. Wart size at entry (increments of 1 mm) significantly determined the probability of cure (0·95 (0·90-1·00); p = 0·043), but duration of warts had no significant effect on cure rate (0·81 (0·61-1·07); p = 0·133).

No local or systemic adverse events occurred in any patient apart from pain. Patients were asked to report level of pain immediately and 24 hours after each PDT-intervention for up to the first four warts treated. Immediately after each treatment the pain intensity was

significantly higher in the ALA-PDT treated warts ($p < 0.05$). Twenty four hours after light exposure to light the pain intensity was still significantly higher in the ALA-PDT warts at the second, third and sixth treatment. Pain will in details be described in study 3.

3.5 Figures and Tables

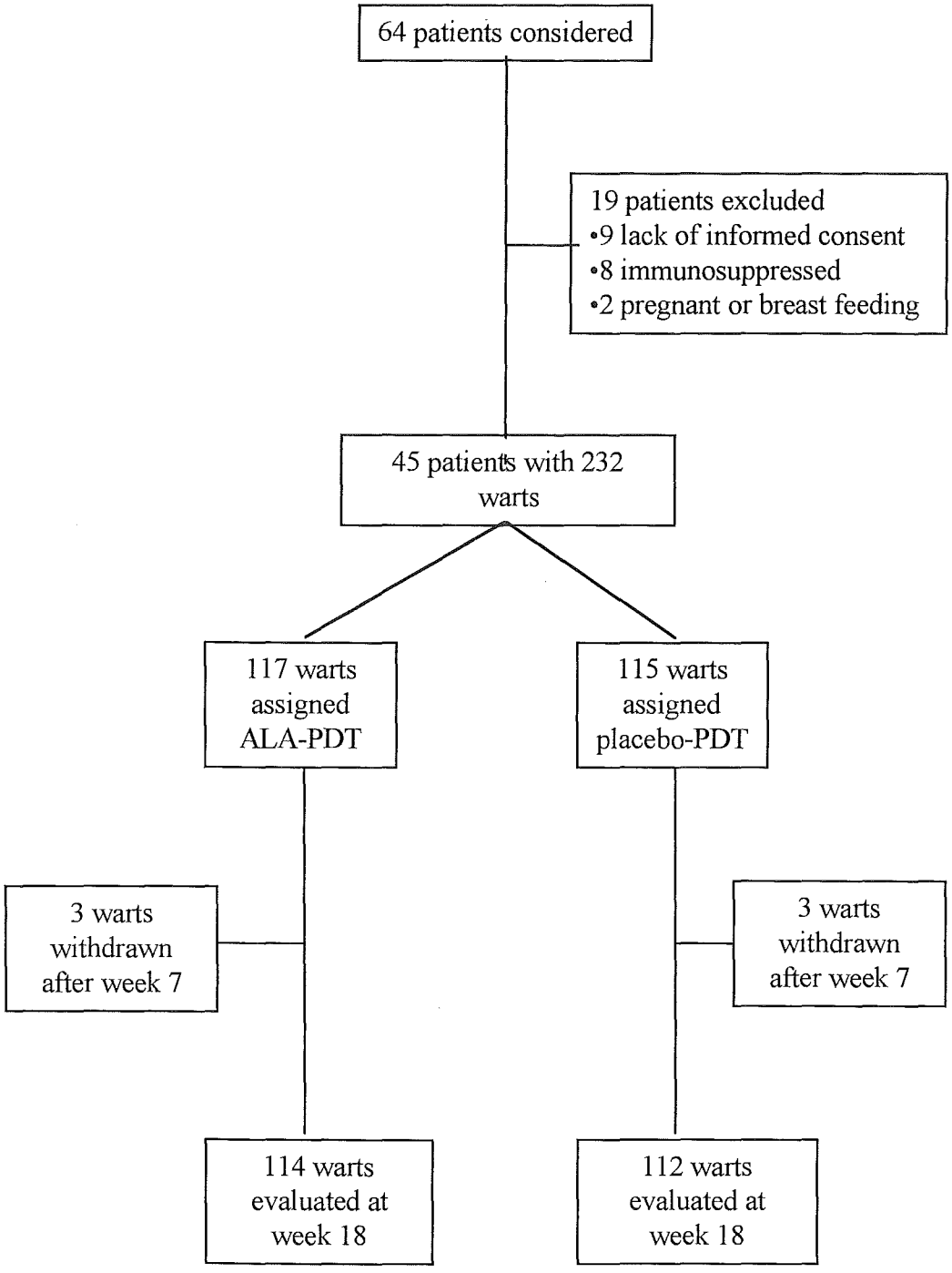

Figure 1: Trial profile

The median number (range) of warts per patient	5 (1-19)
Number (%) of patients with hand warts	6 (13)
Number (%) of patients with hand and foot warts	8 (18)
Number (%) of patients with foot warts	31 (69)

	ALA-PDT n=117	**placebo-PDT n=115**
Median (range) wart size (mm^2) at entry	16 (2-525)	24 (2-510)
Number (%) foot warts	93 (79)	95 (83)
Number (%) hand warts	24 (21)	20 (17)

Table 1: Characteristics of patients and warts at entry

Week	**ALA-PDT (%)**	**Placebo-PDT (%)**	**p for difference**
Week 7			
Median	-33	-12	0.07
Quartiles	(- 74, 0)	(- 60, 0)	
Range	(- 100, 483)	(- 100, 100)	
Week 14			
Median	- 98	- 52	0.0006
Quartiles	(- 100, - 55)	(-100, 0)	
Range	(-100, 56)	(-100, 25)	
Week 18			
Median	-100	-71	0.008
Quartiles	(- 100, -57)	(- 100, 0)	
Range	(- 100, 56)	(- 100, 60)	

Table 2: Relative change in wart area (%) compared to area at entry in ALA-PDT and placebo-PDT arms

	ALA-PDT		Placebo-PDT		
Week	**Persist**	**Vanish**	**Persist**	**Vanish**	**p-value**
0	117 (100)	0 (0)	115 (100)	0 (0)	-
7	98 (85)	18 (16)	96 (84)	19 (17)	0.835
14	49 (50)	49 (50)	64 (65)	34 (35)	0.030
18	50 (44)	64 (56)	65 (58)	47 (42)	0.033

Table 3: Number (%) persisting and vanishing warts in the ALA-PDT and placebo-PDT groups

Week	**Treatment**	**Mean change(se) (%)**	**Difference (95% CI) (%)**	**p**
7	**ALA**	-16.4 (6.1)		
	Placebo	-11.2 (6.1)	-5.2 (-19.4, 9.0)	NS
14	**ALA**	-45.3 (5.5)		
	Placebo	-16.7 (4.8)	-28.6 (-15.9, -41.4)	0.0001
18	**ALA**	-38.2 (6.3)		
	Placebo	-20.1 (5.3)	-18.1 (-3.6,-32.6)	0.015

Table 4: Relative change in area of persisting warts at week 7, 14 and 18 compared with area at entry in the ALA-PDT and placebo-PDT groups. NS: not significant.

3.6 Discussion

This trial shows that ALA-PDT is better than placebo-PDT in reducing the number and the area of recalcitrant foot and hand warts when the interventions were combined with regular paring followed by a topical keratolytic. However, ALA-PDT was associated with more pain, especially in the hours after the intervention.

Selective accumulation of protoporphyrin (PpIX) after topical application of ALA in human-papilloma virus (HPV) infections as condylomata compared with normal surrounding skin - as well as successful treatment of condylomata with ALA-PDT described in uncontrolled reports - have opened a potential for ALA-PDT treatment of lesions caused by HPV (Fehr et al. 1996, Frank et al. 1996, Ross et al. 1997). This randomized trial confirms our results of uncontrolled observations in smaller patient series with warts (Stender et al, 1999a, Stender et al, 1999b). We could not find any interaction between intervention efficacy of ALA-PDT and duration and area of warts at entry. The last PDT intervention in this study was done at week 9 and the last follow-up at week 18. No vanished warts recurred. From a previous uncontrolled study no recurrences were reported 12 months after warts had vanished (Stender et al. 1999b).

HPV infection is common, but the exact frequency is often underestimated and not well recorded. HPV can present on skin as foot and hand, plane and genital warts. The prevalence of common plane and plantar warts among Australian school students was 22% (95 % confidence interval (CI) 20.1-20.7) and almost 40% of these did not know they had warts (Kilkenny et al. 1998).

On a single predetermined day in 1978 in Denmark all dermatologists and a random sample of all general practitioners recorded that 18 % of the consultations in dermatological practice, 18 % of dermatological hospital out-patient clinic consultations, and 8% of skin-related consultations at the general practice were about warts. Sixty two

per cent were foot warts, 26 % hand warts, 6 % both hand and foot warts, 2 % plane and 4 % warts located on other locations including genital area (Christophersen. 1993). When ALA penetrates the altered stratum corneum of abnormal skin it is absorbed in the keratinocytes and is converted enzymatically into the endogenous photosensitizes PpIX. Illumination of cells containing PpIX with light of appropriate wavelength releases cytotoxic radicals. A wide range of light sources and doses has been reported effective in ALA-PDT (Kennedy et al. 1990, Svanberg et al. 1994, Peng et al. 1997b). We chose a commercial available light source for PDT (Waldmann PDT 1200) emitting wavelengths from 590 nm to 700 nm specifically to include the absorption peaks of PpIX at 630 nm and 690 nm and at the same time excite possible photoproducts generated during the illumination. We decided to use a light dose that has often been reported effective in tumor studies (Peng et al. 1997a).

The main reasons patients gave for wanting treatment of their warts were unsightly appearance, pain, and the concern that the warts might spread (Keefe et al. 1990). Many of the patients recruited claimed that, for physical and cosmetic reasons, they could not be in a study for 4 months if they could not pare their warts, so we made paring a part of our treatment. Furthermore, regular paring followed by topical application of a keratolytic is described as an excellent treatment for common warts (Bunney et al. 1976), and, in addition, paring of the superficial horny layer enhances the penetration of ALA.

No serious local or systemic adverse events occurred in any of the patients after six ALA-PDT treatments given within 9 weeks. No scarring or skin abnormalities were seen and no disturbance of function was reported after treatment. A slight, transient hyperpigmentation was observed after removal of some warts on the dorsum of the hand. ALA-PDT may, when used for treatment of non-melanoma skin cancer, induce an intense pain which occurs in some patients and at certain anatomical regions. Significantly more ALA treated warts gave severe to unbearable pain immediately after

treatment with light than placebo-PDT- treated warts. Accordingly, pain should be considered a relative contraindication, particularly when treating children, who were not included in this trial.

Surprisingly, up to 13 % of the placebo treated warts gave severe pain and we saw a higher than expected wart-cure rate (42% versus an expected 30%) in the placebo-PDT arm of the trial. It has been reported that illumination of normal skin and tumor skin with ALA applied only induced pain in the ALA treated tumor (Orenstein et al. 1995). ALA induced tissue PpIX can be seen with a fluorescence spectrometer giving a characteristic signal with a peak at 630 nm and a smaller peak at 690 nm. A physician, who was not involved in the clinical part of the trial, examined fluorescence. Four hours after the first cream application 38 ALA and 42 placebo warts were measured. Eighty two percent of the ALA treated warts showed the characteristic PpIX peak and, surprisingly, 60% of the placebo treated warts showed similar, but smaller peaks at 630 nm. To see if the peaks in the placebo treated warts were due to systemic absorption of ALA, we recorded fluorescence spectra from 39 warts in 12 patients, who had never been treated with ALA. Fifty-five per cent of these warts showed the characteristic, but small PpIX peak. It is therefore less likely that the PpIX in the placebo warts had been supplemented by systemic transfer of PpIX from the ALA treated warts. The higher than expected cure rate in the placebo-PDT treated warts could be due to natural existing PpIX and its photodegradation products.

Our results offer promise for ALA-PDT as a safe and effective therapy for patients with recalcitrant foot and hand warts. Future randomized clinical trials should try to discover whether ALA-PDT is efficient in curing new warts, mosaic warts, and condylomata. Furthermore, it is important to find out whether an esterified liphophilic derivative of ALA, such as methyl ester ALA instead of the hydrophilic ALA used in our trial, improves the cure rate (Kloek et al. 1998).

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Study 2

4.0 Protoporphyrin IX fluorescence in warts is not a predictor for the outcome of photodynamic therapy

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4.1 Summary

Photodynamic therapy (PDT) with topical application of 5-aminolevulinic acid (ALA) followed by light exposure (ALA-PDT) is used experimentally for various skin diseases. When ALA is applied to diseased skin it is absorbed via defect stratum corneum into the epidermal cells where ALA is enzymatically converted to porphyrins, especially protoporphyrin IX (PpIX). Because PpIX exhibits a characteristic fluorescence spectrum with peaks at 630 nm and 690 nm, it is possible to verify the presence and the concentration of PpIX in skin lesions by non-invasive fluorescence spectroscopy. In this study fluorescence spectra were obtained from recalcitrant foot and hand warts enrolled in a double-blind randomized placebo-controlled trial with ALA-PDT. A total of 137 spectra from ALA treated warts and 156 spectra from placebo treated warts and their adjacent skin were obtained. Fluorescence spectra were obtained 4 hours after ALA or placebo cream application. We found a selective PpIX accumulation in clinical visible wart tissue compared to normal but ALA applied surrounding skin. There were no significant differences in PpIX intensity between successful and unsuccessful outcome of the intervention. Nor did we find any correlation between relative change of wart area and PpIX fluorescence intensity. Surprisingly, we found that half of the spectra obtained from placebo treated warts demonstrated low, but visible PpIX peaks at 630 nm. We found no relation between the presence of PpIX findings and cure rate of the placebo treated warts.

Conclusion: PpIX fluorescence in warts does not seems to be a predictor for the outcome of photodynamic therapy.

4.2 Introduction

Photodynamic therapy (PDT) with topical application of 5-aminolevulinic acid (ALA) followed by irradiation with light (ALA-PDT) is a new treatment for superficial basal cell carcinomas and actinic keratoses (Morton et al. 1996, Penq et al. 1997). After topical application of ALA to the skin lesion, ALA is absorbed via defect keratin into the epidermal cells. In the cells ALA is enzymatically converted to porphyrins, especially protoporphyrin IX (PpIX) via the biosynthesis of hem. When cells loaded with PpIX are irradiated with light, cytotoxic substances, such as singlet oxygen and free radicals are generated (Penq et al. 1997).

We recently demonstrated that ALA-PDT was effective in the treatment of papilloma virus induced tumors as recalcitrant foot and hand warts. The number of completely cured warts as well as the relative change in wart area from inclusion to end of experiment were significantly ($p< 0.05$) in favor of ALA-PDT (Stender et al. 2000).

Illumination of skin accumulated with PpIX causes the skin to fluoresce with peaks at 630 nm and 690 nm. PpIX exhibits characteristic fluorescence peaks and therefore it is possible to identify PpIX in skin lesions. If the PpIX intensity is weak, the peak at 630 nm respective at 690 nm may be masked by background fluorescence and may not be visible before spectral analysis has been performed. In this study we deal with *visible PpIX peaks* that are the peaks directly identified on the screen of the computer connected to the fluorescence spectrometer. The intensity of the PpIX peaks represents calculated intensities of *visible PpIX peaks* as well as PpIX peaks that were too weak to be identified without spectral analysis.

The purpose of this study was to relate the frequency of *visible PpIX peaks* and the intensity of PpIX peaks to the effect of ALA-PDT and placebo-PDT on warts. The fluorescence spectra described in this paper were recorded within a double-blind

randomized study comparing ALA-PDT with placebo-PDT in the treatment of recalcitrant foot and hand warts.

4.3 Patients and Methods

Fourty-five patients were included in a randomized placebo controlled trial to verify the effect of photodynamic therapy with 5-aminolevulinic acid (ALA-PDT) on warts. After inclusion of patient number twelve, the patients were asked for permission to register fluorescence from their warts before light exposure. Patients were not participating in the fluorescence measurements before each of the six interventions. A total of 137 spectra were recorded from ALA treated, and 156 spectra from placebo treated warts and adjacent skin during the six treatment **(Table 1)**. The spectra obtained from one patient was excluded because the patient could not follow the study design because of pain during light exposure.

Chemical ALA was obtained as hydrochloride in 98% pure powder from Sigma (St. Louis, Missouri. United States). It was dissolved in sterile water and mixed in a cream base. The placebo cream was an identically looking and smelling cream base. The cream was kept in a refrigerator and was used within one week after production.

Pre-treatment of warts Before applying ALA or placebo cream the horny layer of the warts were pared with a scalpel by a dermatologist to visualization of the blood vessels. Caution was paid to avoid bleeding.

Photodynamic treatment of warts Warts were randomly assigned to repetitive ALA-PDT or placebo-PDT interventions. A thick layer of ALA or placebo cream ($0.2 g/cm^2$)

was applied to the wart lesions and surrounding clinically normal skin. The lesions were covered with a hydrocolloid film to enhance the absorption of ALA. Four hours later, ALA and placebo treated warts were illuminated with light ranging in wavelength from 590 nm -700 nm by a light source, Waldmann PDT 1200 (Waldmann-Medizin-Technik, Villingen-Schwenningen, Germany); 50 mW/cm^2 for 23 minutes 20 seconds to a total dose of 70 J/cm^2.

The ALA-PDT and the placebo-PDT interventions were repeated after one and two weeks. If the warts persisted at week 7, ALA-PDT or placebo-PDT were applied again three times at 1-week intervals. Follow-up was at one and two months (week 14 and 18) after the sixth and final treatment. The efficacy was evaluated by a dermatologist, blinded to interventions, as the relative change in wart area compared with wart area at entry (primary outcome measure), number of warts that vanishing and change in wart area of persistent warts (Stender et al. 2000).

Fluorescence recordings Fluorescence spectra were recorded 4 hours after cream application, prior to light exposure and after wiping off the remaining cream with plain tap water. Fluorescence spectra were recorded from the center of the wart lesion and its adjacent normal skin which also was applied with ALA or placebo cream. All measurements were performed at room temperature in a dark room by a physician, who was not involved in the clinical part of the trial unaware of treatment allocation. The fluorescence spectrometer system was a FL3095, J & M, (Analytische Mess-and Regeltechnik GMBH, Germany). The system consists of a high-pressure xenon lamp (Ozone-free, 75W) with a monochromator (band width 8 nm), and a polychromator MMS (Zeiss, Germany) with a photodiode array (256 elements; pixel resolution: 3,2 nm/pixel). An optical Y-fiber was used to deliver excitation light to the skin and collect fluorescence light (Picture appendix 2).

The fluorescence spectrometer was calibrated before each measurement. The fiber end with a diameter of 3 mm was placed gently on the wart and the surrounding skin during measurements. Pressure on the skin may alter the local blood content and so distort the spectrum. The time for each measurement was 5 seconds. The fluorescence was excited with 370 nm light and a long wave pass filter (420 nm) was inserted to minimize scattered and reflected light from entering the detector. The detector output was displayed on a computer screen as intensity versus wavelength. Spectra were stored for further analysis (Software FL3095, version 2.0). The precision of the fluorescence measurements was evaluated by performing 10 consecutive recordings on normal forearm skin. The coefficient of variation was about 10%.

Data analysis Curve fitting was applied for spectra analyses to assess the true PpIX fluorescence intensity related solely to PpIX in all spectra recorded, including spectra where the PpIX peaks are not visualized directly on the screen (Opus spectroscopic software, version 3,0, Bruker Analytische Messtechnnik GMHP, Germany). Curve fitting is often used in spectral analysis to decompose various overlapping spectra and /or to describe spectral line shape by mathematical functions (Pelikan et al. 1994). An individual peak can be characterized by parameters such as position of the peak, half-height bandwidth, maximal height of the peak. In this study we used the position of the 630 nm peak maxima as an objective parameter for PpIX intensity.

Statistical analysis Comparison of the number of *visible PpIX peaks* in the ALA versus the placebo treated warts, as well as comparison of cured and not cured warts was performed using the Chi-squared test (Fishers exact test). Comparison of the average intensities in the ALA- versus the placebo-cream treated warts, as well as comparison of the intensities in cured versus not cured warts was analyzed by use of the unpaired t-test

(Welch correction). Comparison of the average intensities in the cured versus not cured ALA treated warts excluding warts that demonstrated visible PpIX peaks in their surrounding, was analyzed by use of the un-paired t-test.

Correlation analysis (Pearson) was used to evaluate the relation between the relative change in wart area and PpIX intensity. $P < 0.05$ was considered significant.

4.4 Results

It was often possible to identify PpIX peaks directly on the screen during excitation. **Figure 1(a)** demonstrates a representative fluorescence spectrum obtained from an ALA treated wart and its nearby adjacent normal skin. The PpIX fluorescence spectrum from the ALA treated warts demonstrates the characteristic high PpIX peak at 630 nm and a lower but broader peak at 690 nm. *Visible PpIX peaks* were also identified from adjacent skin to few of the warts (**Table 1**). The autofluorescence from the ALA treated wart lesion and its surrounding skin ranged from about 430 nm (we had a filter at 420 nm inserted) to 650 nm. The autofluorescence peak was higher in surrounding skin than in the wart lesions.

Figure 1(b),(c) show representative spectra recorded from placebo-cream treated warts and adjacent placebo-cream treated normal skin. In 51% of spectra obtained from placebo treated lesions, low but *visible peaks* at 630 nm and a broader peak at 690 -700 nm were observed **(Figure 1b)**. In the remaining 49% of the placebo treated warts *visible PpIX peaks* could not be demonstrated as seen in **(Figure 1c).** PpIX fluorescence peaks were demonstrated in less than 5 per cent of spectra obtained from skin surrounding the warts (**Table 1**). Autofluorescence peak from placebo cream treated wart lesion and adjacent skin ranged in wavelength region from 430 nm to 630 nm and also here the peak from adjacent normal skin was higher than from the wart lesion.

Frequencies of visible PpIX peaks The percentage of spectra demonstrating *visible PpIX peaks* is shown in **Figure 2**. As expected significant more ALA treated warts demonstrated *visible PpIX peaks* than placebo treated warts. There were no significant

differences in the frequencies of *visible peaks* between cured or not cured warts after ALA as well as placebo interventions.

PpIX fluorescence intensity The average PpIX intensity from spectra obtained from ALA and placebo treated warts in each of the sixth treatments tended to increase during the first three treatments. The PpIX intensity in the fourth treatment almost returned to the same intensity level as in the first treatment but followed by an increase from the fourth to the sixth treatment. The intensity of the PpIX autofluorescence calculated in the placebo treated warts were stable during all 6 treatments (**Figure 3**). The PpIX intensity was not higher in cured warts than in not cured warts whether it was in the ALA or placebo treated lesions (**Figure 4**). There was no significant difference between cured versus not-cured ALA treated warts when excluding the warts that demonstrated visible PpIX fluorescence in their surroundings ($p > 0.175$).

We found no correlation between PpIX intensities and the relative change in wart areas (ALA-PDT warts: correlation coefficient; $r = 0.01$, $p= 0.93$, placebo warts: correlation coefficient; $r = 0.09$; $p = 0.61$).

4.5 Figures and Tables

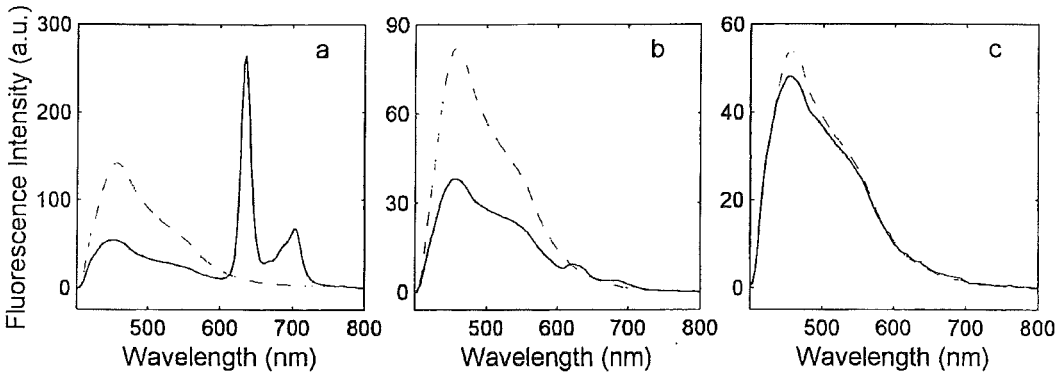

Figure 1. Representative spectra recorded 4 hours after ALA and placebo cream application. Characteristic PpIX fluorescence peaks at 630 nm and at 690 nm obtained from ALA-PDT treated warts **(a)**. 51 % of the obtained spectra from the placebo treated warts demonstrated a *visible PpIX peak* at 630 nm **(b)**. 49% of the spectra obtained from the placebo cream treated wart demonstrated no PpIX peak at 630 nm **(c)**. The full line is the spectra obtained from the wart lesions and the dotted line demonstrates the surrounding normal but also cream applied skin. *Note: different scaling of the y-axis.*

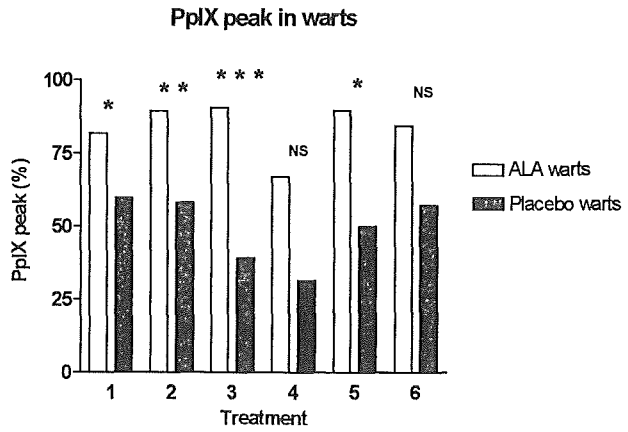

Figure 2. Number of recorded warts (%) that demonstrated *visible PpIX peaks* in the ALA and the placebo treated warts. (NS: not significant, * : $p < 0,05$; ** : $p < 0,01$; ***: $p < 0,001$). The x-axis gives number of the 6 consecutive treatments.

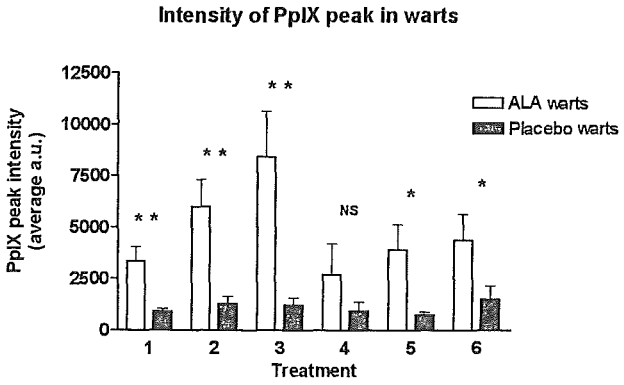

Figure 3. Average PpIX intensity (a.u.; arbitrary units) in ALA and placebo treated warts. (NS: not significant. *: $p < 0,05$; **: $p < 0,01$).
The x-axis gives number of the 6 consecutive treatments.

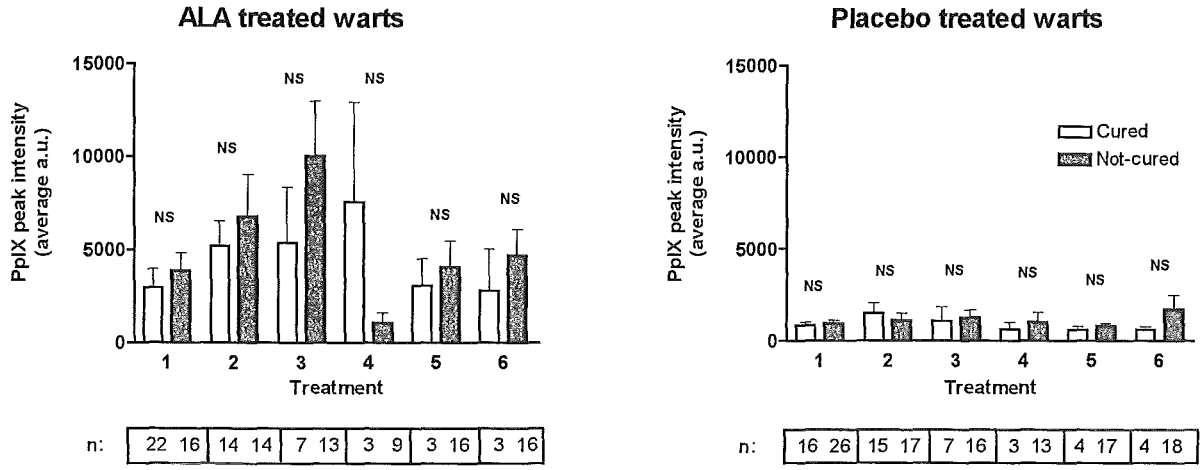

Figure 4. Average PpIX intensity (a.u.) in cured/not cured ALA and placebo treated warts. (NS: not significant).

Treatment	Week	Patients	Warts recorded		Visible PpIX peaks			
			ALA	Placebo	ALA		Placebo	
NO		n	n	n	wart	surrounding	wart	surrounding
					n (%)	n (%)	n(%)	n(%)
1	1	11	38	42	31 (82)	4 (11)	25 (60)	1(2)
2	2	8	28	32	25 (89)	3 (11)	18 (56)	0
3	3	5	21	23	19 (95)	3 (14)	9(39)	0
4	7	4	12	16	8 (67)	5 (42)	5(31)	0
5	8	5	19	21	17 (89)	5 (26)	10(50)	1 (5)
6	9	5	19	22	16 (84)	6 (32)	12(57)	1 (5)

Table 1: Fluorescence spectra recorded from ALA and placebo treated warts and their surrounding. n = number

4.6 Discussion

In this study we recorded fluorescence in warts 4 hours after repetitive applications of ALA and placebo cream. In ALA treated warts we found a selective accumulation of PpIX in wart tissue compared to surrounding clinical normal skin. We found no relation between the PpIX intensity and a successful outcome of ALA-PDT. The sub-grouping of spectra into cured and not cured warts make the number for analyzes small so that only large differences can be detected.

Surprisingly about 50 % of spectra obtained from placebo cream treated warts demonstrated low, but *visible autofluorescence PpIX* peaks at 630 nm and at 690 nm. There was no correlation between successful PDT outcome and the presence or the intensity of PpIX in the placebo treated warts.

The dual peaked fluorescence signal with PpIX peaks at 630 nm and at 690 nm after ALA application is not specific for malignant and premalignant skin lesions but can, as in this study, be demonstrated in hyperproliferative lesions as well (Wagnieres et al. 1998). Distribution of PpIX in skin depends on ALA permeability through the stratum corneum into the keratinocytes as well as the conversion of ALA to PpIX in the cells.

A changed stratum corneum in the warts as well as paring of the wart lesions preceding cream application may have enhanced the permeability of ALA. The presence of PpIX in adjacent skin of some of the warts may be explained by subclinical wart tissue, this has however not been verified by histology. Paring of the wart lesion and surrounding skin prior to cream application may facilitate ALA penetration into normal skin as well (Meijnders et al. 1996). There was a tendency of more *visible PpIX peaks* in spectra obtained from adjacent skin after an increased number of ALA-PDT treatments.

The intensity of a PpIX fluorescence peak is correlated to the concentration of the photosensitizer in the skin. The higher the concentration, the higher the peak (Kennedy et al. 1992). The average PpIX fluorescence intensity tended to increase with number of treatments up to the third treatment in the ALA treated warts. Repetitive treatments with ALA-PDT may have lead to a facilitated penetration of ALA through the stratum corneum.

The findings of PpIX after ALA application in condylomata and in psoriasis have often brought authors to conclude a potential effectiveness of PDT (Ross et al. 1997, Bissonnette et al. 1998). According to our findings, the accumulation of PpIX verify that an increased synthesis of PpIX has taken place, but that do not garantee a successful treatment outcome. These findings are in agreement with Klinteberg *et al.* who recently found that PpIX fluorescence intensities in basal cell carcinomas after ALA application did not tend to be significantly higher in cured lesions than in not cured lesions (af Klinteberg et al. 1999). Iinuma et al. found that there was no correlation between PpIX cellular content and ALA-induced phototoxicity in different cell lines. He found that one cell line, which synthesized the least among three cell lines, was killed most efficiently by PDT. They explained the lack of correlation of cellular PpIX content and phototoxic damage by different intracellular localization of PpIX that may have lead to different susceptibility to PDT. By fluorescence microscopy they found different submitochondrial localizations of PpIX (Iinuma et al. 1994).

Something else than PpIX, such as photodegradation products, may possibly turn out to be a predictor of the successful outcome of ALA-PDT. Kønig et al. showed by fluorescence measurements carried out during PDT that the fluorescence intensities at 630 nm decreases, whereas the fluorescence intensity in the spectral region around 670 nm increased to a maximum and then slowly decreased. At the end of the PDT no

fluorescence in the red spectral region could be measured as a result of complete photobleaching. The possible photosensitizing capacity of the photoproducts may be a part of the clearing effect of the PDT treated lesion (Konig et al. 1993, Gudgin Dickson et al. 1995).

In the present study we found after first cream application that 23 % of spectra obtained from placebo treated finger warts and 76 % of spectra from placebo treated foot warts demonstrated low intensity PpIX peaks. The presentation of PpIX peaks in placebo cream treated warts was unexpected. Initially we suspected that a leakage of our bandages resulted in ALA dissemination to the placebo treated warts. However, the findings of peaks in two warts located on left respective right foot made us to believe in systemic absorption of ALA. We therefore examined fluorescence spectra from warts in patients never treated with ALA and in these we found visible PpIX peaks from 64% of foot warts but none from finger warts. The more frequent appearance of PpIX peaks in foot warts compared to finger warts may be explained by a bleaching of PpIX in finger warts exposed to daily light. The finding of this endogenous PpIX may help us to explain the high cure rate of placebo treated warts (42%), by still unknown photodestructive processes.

We conclude that presence of PpIX in warts treated with ALA cream verify a selective accumulation of PpIX in warts tissue, but neither selective accumulation nor high intensity peaks of PpIX in wart tissue are predictors for a successful wart removal after ALA-PDT.

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Study 3

5.0 Pain induced by photodynamic therapy with topical 5-aminolevulinic acid in warts.

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5.1 Summary

Photodynamic therapy (PDT) with topical 5-aminolevulinic acid (ALA) followed by irradiation with red light (ALA-PDT) is used experimentally for non-melanoma skin cancer and other proliferative dermatological diseases. Pain during and after light exposure is a well-known adverse advent that may be a limiting factor for the use of ALA-PDT. To assess the pain induced by ALA-PDT we asked 45 patients enrolled in a randomized, placebo controlled trial with 6 consecutive ALA- and placebo-PDT treatments for recalcitrant foot and hand warts, to fill in questionnaires about pain immediately and 24 hours after each treatment. Immediately after each of the six treatments, pain intensity was significantly higher in warts treated with ALA-PDT than in warts treated with placebo-PDT ($p < 0.028$). Twenty four hours after light exposure the pain intensity was still significantly higher after ALA-PDT in treatment 2, 3, 6. Severe or unbearable pain was reported from a median of 17 % (6%-31%) of the ALA treated warts and from a median of 2% (0%-15%) from the placebo treated warts immediately after the treatments. According to the number of the six consecutive treatments no significant change in pain intensity was observed ($p \geq 0.06$) as well as no significant relation was found between the pain intensity and the relative change in wart area ($p \geq 0.03$). The pain was primarily characterized as *burning* and *shooting*. The patients became painfree after an average of 30 hours (range: 1-96 hours). We conclude that pain induced by ALA-PDT is of such an intensity in about one fifth of the lesions that pain relief is indicated. According to the description, the pain is most likely neurogenic.

5.2 Introduction

Photodynamic therapy (PDT) with topically applied 5-aminolevulinic acid (ALA) followed by irradiation with light (ALA-PDT) has recently shown effect in the treatment of recalcitrant foot and hand warts (Stender et al. 1999).

The penetration of ALA into the wart is facilitated due to a destruction of the keratin layer over the wart. When ALA is absorbed in the pathological changed epidermal cells it is transformed via the biosynthesis of hem, to the photodynamically active protoporphyrin IX (PpIX). The PpIX synthesis seems to be accelerated in proliferative tissue because of changes in enzyme activity. When cells loaded with PpIX are irradiated with red light, cytotoxic substances such as singlet oxygen are released and may lead to cell death (Kennedy et al. 1990, Kennedy et al. 1996).

During and after light exposure many patients report pain. It is our impression that pain vary significantly and that some patients have severe pain not only during but also several hours after light exposure.

In this study we assessed pain in foot and hand warts that were treated with six repetitive treatments with ALA-PDT and placebo-PDT. The pain was monitored to determine pain intensity, quality and duration - to help to decide on the choice of therapy.

5.3 Patients and Methods

Patients

The patients were recruited from May to November 1998. Forty-five patients with a total of 232 foot and hand warts were enrolled in a randomized double-blind placebo controlled trial in order to examine the effect of six successive treatments of ALA-PDT on recalcitrant warts (Stender et al. 2000). All patients filled in the questionnaires (**Table 1**) about pain intensity and characteristics immediately (0 hours) and 24 hours after each of the six treatments. Patients were told to grade pain intensity of up to four treated warts. A total of 61 ALA (11 hand and 50 foot) and 63 placebo (9 hand and 54 foot) treated warts were included for pain intensity grading. One patient with three warts in the ALA group dropped out after the third treatment because of pain and another patient in the placebo group with one wart dropped out because of personal reasons.

Study design

The warts were random numbered and the treatments were allocated blindly to topical application of a 20% ALA cream or to a cream vehicle without ALA by an independent, centralized, computer generated block randomization. A block size of two was chosen, ensuring the application of both treatments for patients with more than one wart. Four hours after ALA or placebo cream application, all warts were irradiated with Waldmann 1200 PDT lamp (Waldmann Medizintechnik, VS-Schwenningen, Germany) emitting light in the 590 - 700 nm range. The warts received 70 J/cm^2 at a fluence rate of 50 mW/cm^2 (Peng et al.1997). The ALA-PDT and the placebo-PDT treatment was repeated once a week three times (week 1, 2, 3), referred to as session one. After one month of observation (week 7) the warts were treated three more times, again with a one week

interval (week 7, 8, 9), referred to as session two. Follow-up was performed one (week 14) and two months (week 18) after the sixth treatment. The relative change in wart area from entrance to week 7, 14 and 18 was evaluated from clinical photographs by a dermatologist unaware of allocation and patient identification. PpIX fluorescence intensities were measured immediately before light exposure from a random selection of the warts with a fluorescence spectrometer (J & M, Fl 3095, Analytische Mess-and Regeltechnik GMBH, Germany).

In order to identify the warts that should be pain rated, the patients received a drawing of the position of up to the first four warts numbered. The questionnaires were returned to the out-patient clinic at the following consultation. The pain intensity was assessed by a verbal rating scale (VRS) also called a 5 point scale (Table 1) (Ohnhaus et al. 1975, Carlsson et al. 1984, Drewes et al. 1995).

Statistical Analyses

Pain intensity in ALA-PDT versus placebo-PDT treated warts: The comparisons of pain intensity were done immediately and 24 hours after light exposure for each of the three treatments in the first session (treatment 1, 2 and 3) and in the second session (treatment 4, 5 and 6). The warts were regarded as being independent of each other. The ALA and the placebo treated warts were compared using Mann-Whitneys test at all treatments, controlling for the three comparisons by adjusting the level of significance to 0.05/3 = 0.017.

Pain intensity related to the number of treatment: Assessment of whether the number of the 6 consecutive treatments influenced the pain intensity was done for both

interventions groups as the concordance of pain intensity immediately after the first and third treatment in the first session as well as after the fourth and sixth treatment in the second treatment session. The concordance was calculated as Kendalls τ_B. This measure of association was used because it reflects the ordinal nature of the pain score and gives a possibility for testing equal associations for the treatment groups. The measure is between -1 and 1 and is a special case of correlation between pain score at the two visits. A large positive value indicates that the pain scores at the two visits are strongly associated.

Pain intensity and PpIX fluorescence intensity: The association between pain intensity immediately after light exposure and the PpIX fluorescence intensity assessed by fluorescence spectrometry at treatment 1, 2, 3 was performed using Spearmans rank correlation. The level of significance was adjusted for the three comparisons by adjusting the level of significance to 0.05/3 = 0.017.

Pain intensity and change in relative wart area: The association between pain intensity immediately after the first light exposure in the first treatment session and the relative change in wart size during the following seven weeks as well as the fourth treatment (the first treatment in the second session) and the relative change in wart size during the following seven weeks (week 14) was assessed by Spearmans rank correlation. The level of significance was adjusted for the two comparisons by adjusting the level of significance to 0.05/2 = 0.025.

Comparison of pain intensity and pain characteristics was performed by Fishers exact test.

5.4 Results

Pain intensity in ALA-PDT versus placebo-PDT treated warts

The pain intensity graded immediately (0 hours) and 24 hours after each of the six ALA-PDT and placebo-PDT treatments are shown in Table 2. Immediately after each of the six treatments (except treatment 4 ($p = 0.028$)) the pain intensity was significantly higher in the ALA than in the placebo treated warts ($p < 0.017$). Twenty-four hours after light exposure there was still a higher pain intensity in ALA-PDT warts than in placebo treated warts in treatment 2 ($p = 0.003$), 3 ($p= 0.008$) and 6 ($p = 0.001$).

"No pain" was graded from a median of 49% (range 23%-59%) of the ALA treated warts and from a median 70% (range: 56%-81%) of the placebo warts. The number of warts that had *no pain* immediately after light exposure was significantly lower in the ALA than in the placebo treated warts in all six treatments ($p < 0.01$), except for the fourth treatment ($p = 0.066$) (Fishers exact test) (Figure 1).

Immediately after light exposure severe to unbearable pain was reported from a median of 17 % (range: 6% - 31%) in the six ALA-PDT treatments and from a median of 2% (range 0 %-15 %) in the six placebo-PDT treatments (Figure 1).

The average duration of pain, whether mild or severe, was 32 hours for the ALA treated warts (range: 1- 89 hours) and 29 hours for the placebo treated warts (range: 1-96 hours) (unpaired t-test: $p = 0.32$).

Pain intensity related to treatment number

There was no significant change in pain intensity in the ALA and the placebo treated warts from treatment 1 to 3 in the first treatment session (τ_B (standard error (SE))); (ALA-PDT: 0.50 (0.08), placebo: 0.39 (0.11), p = 0.43) nor from treatment 4 to treatment 6: (ALA-PDT : 0.48 (0.11), placebo: 0.13 (0.15), p = 0.06). Between treatments 1 and 4 there was no significant change in pain grading (ALA-PDT: 0.33 (0.11), placebo: 0.36 (0.13), p = 0.84).

Pain intensity and PpIX fluorescence intensity

No correlation was found between PpIX intensity recorded from a random selection of the warts and their corresponding pain intensities at treatments 1, 2 and 3. Treatment 1: ALA: R = 0.49 (p = 0.03), placebo: R = -0.23 (p = 0.33). Treatment 2: ALA-PDT: R = -0.06 (p = 0.83), placebo: R = -0.29 (p = 0.27). Treatment 3: ALA: R = 0.59 (p = 0.12), placebo: R = 0.12 (p = 0.75).

Insufficient number of recorded fluorescence intensities made it impossible to make analyses of the correlation between fluorescence intensity and pain for treatment 4, 5 and 6.

Pain intensity and change in relative size of wart

No significant correlation was found between pain intensity immediately after the first treatment and the relative change in wart area from trial entrance to week 7 (ALA-PDT: R = 0.28 (p=0.03), placebo: R = 0.08 (p = 0.54). Nor was there a significant correlation between the pain intensity immediately after treatment four and the relative change in

wart area from trial entrance to week 14 (ALA-PDT: R = 0.06 (p = 0.69), (placebo: R= -0.05 (p = 0.72)).

There was no significant difference in pain intensity rating immediately after the first ALA-PDT treatment between men and women (p = 0.275), between patients younger than 30 years, between 30 and 50 years, and older than 50 years (p = 0.11), between small warts (< 60mm^2) and larger warts (≥ 60mm^2) (p = 0.315) nor between patients with one, two, three or four warts (p = 0.62).

Only few hand warts were included in our trial, however the hand warts were significantly more painfull immediately after the first ALA-PDT than foot warts (p = 0.013).

Characterization of pain

There was no significant difference in the distribution of *local* and *diffuse* pain registration in ALA treated and placebo treated warts (p > 0.63: Fishers exact test). If the warts had pain, the pain was characterized as local from more than 75 % of the ALA- and placebo-treated warts immediately after the first light exposure. There was no significant difference in the distribution of *superficial* or *deep* pain reported from ALA-PDT and placebo-PDT treated warts immediately after light exposure (p > 0.48: Fishers exact test). There was an even distribution between superficial (about 50%) and deep pain (about 50%) reported from the ALA-PDT treated warts immediately after light exposure.

Table 3 demonstrates that pain characteristics as shooting and burning were dominant.

5.5 Figures and Tables

Pain Intensity	
no pain **or**	☐
light pain **or**	☐
moderate pain **or**	☐
severe pain **or**	☐
unbearable pain	☐
Pain Character	
local pain **or**	☐
diffusē pain	☐
superficial pain **or**	☐
deep pain	☐
burning pain	☐
shooting pain	☐
cramping pain	☐
squeezing pain	☐
drilling pain	☐
pulsating pain	☐
no pain after ____ *hours*	

Table 1. Questionnaire for grading pain intensity and for characterization of pain.

	ALA-PDT					placebo-PDT					
Intervention No.	**No**	**Light**	**Moderate**	**Severe**	**Unbearable**	**No**	**Light**	**Moderate**	**Severe**	**Unbearable**	**p**
Pain immediately after light exposure											
1	43	28	13	15	2	71	19	8	2	-	0·001
2	36	11	21	26	5	68	13	5	13	2	0·001
3	23	21	36	15	6	56	21	19	4	-	0·001
4	59	15	20	4	2	76	16	5	0	2	0·028
5	55	13	21	7	4	81	9	9	2	-	0·002
6	54	20	10	12	4	80	14	6	-	-	0·003
Pain 24 hours after light exposure											
1	67	17	15	2	-	81	10	8	2	-	0·078
2	56	16	18	5	5	81	6	8	5	-	0·003
3	50	23	21	4	2	75	15	7	4	-	0·008
4	76	19	6	0	-	84	13	2	2	-	0·32
5	77	13	5	5	5	86	7	5	2	-	0·21
6	68	20	2	10	-	96	4	-	-	-	0·001

Table 2: Per cent of warts exhibiting the pain assessed using a 5-point scale immediately and 24 hours after each of the six interventions ($p < 0.017$ is significant).

Treatment		n	Pain					
			Burning	Shooting	Pulsating	Drilling	Squeezing	Cramping
No.	intervention	No.*	%					
1	ALA	46	37	35	22	7	0	0
	Placebo	28	39	32	21	7	0	0
2	ALA	70	36	34	9	14	4	3
	Placebo	34	35	32	9	18	6	0
3	ALA	51	45	29	14	4	4	4
	Placebo	35	40	26	20	9	3	3
4	ALA	28	46	39	7	7	0	0
	Placebo	16	44	31	0	13	13	0
5	ALA	37	49	24	6	22	0	0
	Placebo	15	40	40	0	20	0	0
6	ALA	32	47	22	13	13	6	0
	Placebo	7	71	29	0	0	0	0

* = number of responses. Subjects were allowed to indicate more than one sensation.

Table 3. Characterization of pain after repetitive treatments with ALA-PDT and placebo-PDT (% responses)

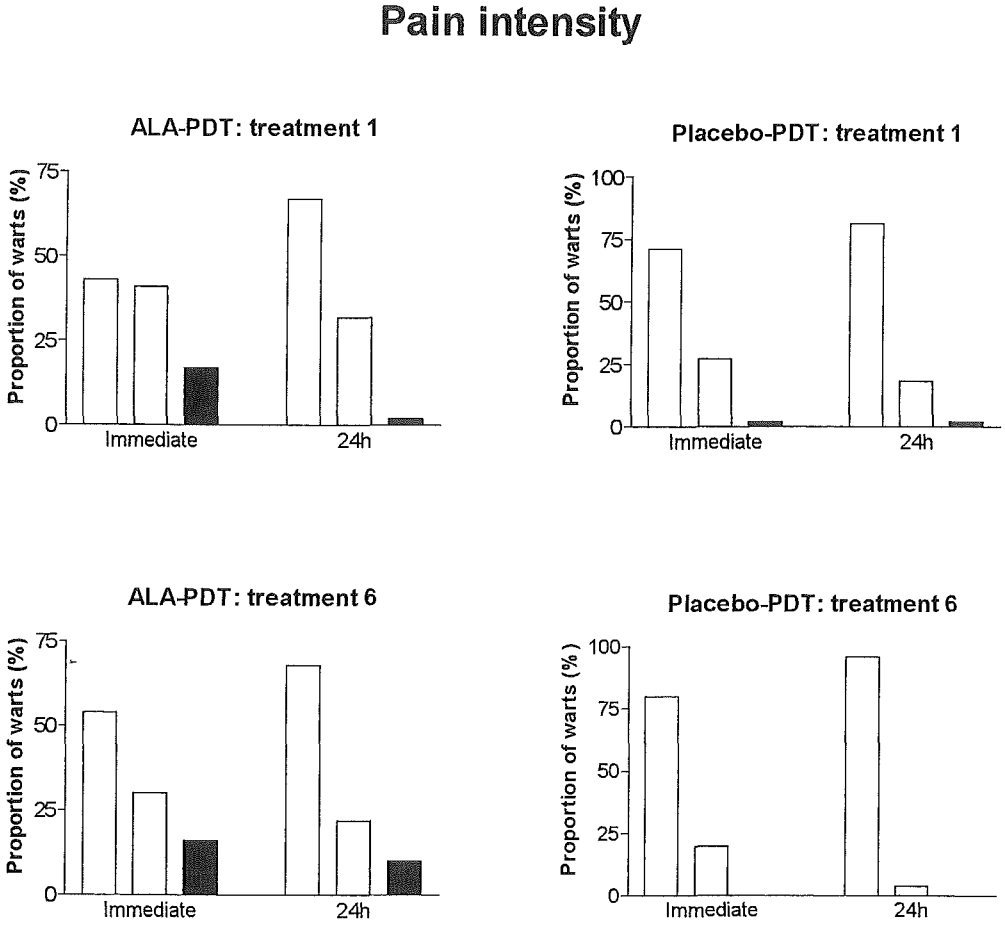

Figure 1: Proportion of warts (%) grading pain intensities immediately and 24 hours after first and sixth ALA-PDT (left) and placebo PDT treatment (right). *White bars:* no pain; *gray bars :* light and moderate pain and *black bars:* severe and unbearable pain.

Number of warts in ALA-PDT: treatment 1: 61; treatment 6: 46

Number of warts in placebo-PDT: treatment 1: 63 ; treatment 6: 49.

5.6 Discussion

Pain induced in recalcitrant warts by ALA-PDT is of high intensity and long lasting in about one fifth of the treated warts. Pain characteristics as *shooting* and *burning* were dominant (Table 3) and according to that characterization, the pain was classified as neurogenic (Cousins et al. 1999).

A pain scale such as verbal rating scale (VRS) is used easily and convenient for the assessment of pain intensity and therefore we used VRS for pain intensity rating in our trial (Melzack et al.1999). The pain intensity was constant in consecutive treatments, and no relations were found between pain intensity and cure rate, nor between pain intensity and PpIX fluorescence intensity.

Pain induced by ALA-PDT has been reported in a few studies. In Bowens disease, pain was present in 55% of the ALA-PDT treated lesions and was reported as mild in 54% and as moderate in the remaining. In the same study ALA-PDT appeared to be significantly less painful than cryotherapy (Morton et al. 1996). Wang et al. demonstrated in a randomized clinical trial of ALA-PDT versus cryotherapy for basal cell carcinomas, that the average pain score was slightly but not significantly higher in ALA-PDT than in cryotherapy treated patients. An average score during treatment was about 40 mm on a 115 mm visual analogue scale (VAS) (Wang et al. 1999). Patients treated with ALA-PDT for multiple actinic keratoses at the dorsum of the hand experienced during treatment mild to moderate pain graded on a 4 - point scale (0 = none, 3 = severe) (Kurwa et al. 1999). The relatively high incidence of severe pain reported from warts located on feet and hands in the present study, may be related to the rich nerve innervation of these regions.

Pain reported as severe and unbearable are clinically important, since pain at these high levels must be relieved. If no relief can be offered, patients must carefully be informed

about the possibility of long lasting and severe pain and that further treatments can be expected to be of similar pain intensity.

The ALA induced PpIX fluorescence intensities that were measured before light exposure, differ significantly from lesion to lesion and are dependent of ALA penetration and absorption as well as of PpIX synthesis in the cells. Surprisingly, we found that 51% of the placebo treated warts demonstrated PpIX fluorescence peaks of low intensity, indicating an intrinsic accumulation of PpIX in warts. Intrinsic PpIX in placebo treated warts may lead to photosensitization when exposed to light and that may explain the high cure rate (42%) as well as the pain in the placebo treated warts.

In patients with acute intermittent porphyria (AIP), cutaneous lesions are absent because the precursors that accumulate are ALA and phorpobilinogen. None of these are photosensitizes. Patients with AIP have neurovisceral symptoms and other neurological complications such as paraesthesia and peripheral neuropathy that lead to pain and weakness, because of accumulation of ALA (Gawkrodger, 1990). Topical applied ALA to the skin for several hours does not induce pain itself unless the skin area is exposed to light. Therefore a local neurotoxic reaction induced by ALA is less likely.

The *"burning"* pain has been suggested to be induced by hyperthermia from the lamp. In a study performed by Orenstein et al. 1995 temperature monitoring during ALA-PDT with 100 mW/cm^2 from a non-coherent light source was performed in patients with non-melanoma skin cancer. Most of the patients complained of an intense sensation during irradiation of the area treated with ALA cream. The pain sensation was pronounced when the temperature was $>39^0$ C and moderate when the temperature was below $39\ ^0$C. There were no complaints of pain in the normal skin areas heated even over 44-45 ^{0}C and it was concluded that the light could not itself induce the pain. Green light has been

reported to be less painful than red light as well as exposure with light outside the absorption peak for PpIX generated lesser pain (Fritsch et al.1997).

It has been stated that PDT with methyl ester ALA, a lipophilic derivate of ALA, may be less painful in the treatment of basal cell carcinomas than ALA-PDT because of differences in the up-take of methyl-ester ALA in peripheral nerve endings (Gaullier et al.1997). Hypothetically the use of methyl-ester ALA instead of ALA followed by light exposure may result in a less painful PDT treatment.

Pain is strongly individual and difficult to quantify, describe, and most of all to compare. Pain is a complex subjective experience and not only a condition that can be object for a one-dimensional assessment. Many variables associated with pain origin, transmission, perception and response must be understood to provide the best pain relief (Wall et al. 1999).

In the present study the pain was primarily characterized as a *local, burning and shooting pain.* These characteristics lead us to suggest that pain induced by ALA-PDT is neurogenic and may be compared with acute pain reported from herpes simplex and herpes zoster lesions (Cousins et al. 1999). Pain caused by local inflammation induced by PDT can not be excluded as an additional pain sensation.

We conclude, that pain induced by ALA-PDT is of such a frequency, intensity and duration that pain relief is needed. We suggest that future randomized trials should examine the efficacy of pre-emptive and post-treatment analgesia, maybe as a combination of suitable doses of non steroid anti-inflammatory drugs and tricyclic antidepressant for pain induced by ALA-PDT (Sindrup et al. 1999, McQuay et al. 1996, McQuay et al. 1999).

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6.0 Summary of Results

Photodynamic therapy with topical applied 5-aminolevulinic acid of recalcitrant long-lasting warts has shown efficacy over placebo-PDT in both reducing the area and the number of long-lasting recalcitrant foot and hand warts.

Warts demonstrate a selective accumulation of ALA induced PpIX in warts compared to surrounding normal skin, however the intensity of ALA induced PpIX in wart lesions can not be directly related to the cure rate. Placebo treated warts demonstrated endogenous PpIX, which may help us to explain the high cure rate observed in the placebo treated warts.

Severe and unbearable pain was induced by ALA-PDT in about one fifth of the ALA-PDT treated warts. There was no relation between pain intensity, PpIX intensity, and the relative wart area reduction. The pain was not changed significantly during the six consecutive treatments, the pain lasted in average 30 hours after light exposure and was characterized as a local, shooting and burning pain.

7.0 General discussion

The biological mechanisms behind PDT.

Our study did not make any attempt to understand the mechanisms behind ALA-PDT but aimed to prove if ALA-PDT has the effects of wart removal and wart size reduction. The fact that ALA-PDT has these effects, makes the basic sciences around the underlying mechanisms even more interesting.

The findings of selective accumulation of PpIX after topical application of 5-aminolevulinic acid (ALA) in condylomata gave promise for ALA-PDT as a potential therapy for HPV induced skin tumors (Ross et al. 1997, Fehr et al. 1996, Frank et al. 1995). The accumulation of PpIX in warts after topical application of ALA is particular believed to be caused by a change of the stratum corneum, facilitating the absorption of ALA. A change in the synthesis of PpIX by rapidly proliferating epithelial cells also seems to influence the accumulation of PpIX (Svaasand et al, 1996).

Since ALA-PDT is a rather new treatment modality, the biological mechanisms behind ALA-PDT induced cell death and ALA-PDT as an anti-viral agent is not yet clarified. The mechanisms for cell death in warts is believed to be the same as in the case of non-melanoma skin tumors. The localization of the PpIX photosensitizer determines the site and type of damage of the cells. By fluorescence microscopy PpIX fluorescence was primary found to be localized in the mitochondria, in the cytoplasma and in the plasma membranes (Iinuma et al. 1994, Malik et al. 1987, Peng et al. 1992, Steinbach et al. 1995, Fisher et al. 1995). Electron microscopic findings of damage of the mitochondria but not of the lysosomes or Golgi after PDT, further support the hypothesis that the

most direct effect after light exposure is mitochondrial phototoxicity (Iinuma et al, 1994).

Damage of cells and tissue by PDT is induced by formation of singlet oxygen and free radicals that are able to generate cytotoxic damage to the cellular organelles (Salet et al. 1990, Moan et al. 1982, Berg et al. 1997). This is supported by the findings that the cytotoxic effect is limited by lack of oxygen (Moan et al. 1985, Henderson et al. 1989).

Destruction of membranes on mastcells and macrophages by ALA-PDT leads to release of vasoactive agents and cytokines that may cause the inflammatory reaction that may be seen after ALA-PDT treatment (Penq et al. 1992). Photosensitization of PpIX accumulated in endothelial cells of the vessels leads to vascular changes and to tumor necrosis (Wyld et al. 1997, Henderson, 1989, Henderson, 1995, Fingar et al. 1992).

Ultrastructural changes after ALA-PDT in normal but tape-stripped skin demonstrated recently that PDT mainly affected the keratinocytes and some of the Langerhans cells, which became apoptotic, whereas the melanocytes were unresponsive to standard PDT (Bartosik et al. 2000). Apoptosis induced by ALA-PDT is reported by other authors (Dougherty et al. 1998, Webber et al. 1996, Agarwal et al. 1991, Noodt et al. 1996).

It is unclear how PDT influences the immune response (Kobelik, 1985, Henderson et al. 1995), but immunosuppression in mice was observed by inhibition of contact hypersensitivity of dinitrofluorobenzene after PDT (Gomer et al. 1988, Elmets et al.1986, Henderson et al. 1989).

The role of PDT as an anti viral agent is most likely that PDT induces destruction of the HPV infected keratinocytes as reported above, but direct viral inactivation has also been demonstrated (Gaspard et al. 1995, Ross et al. 1997). When PpIX binds to the

surface of the virus, photoactivation at this site may change the lipids and the proteins leading to a change in the ability of the virus to adhere (North et al. 1992).

Light and ALA penetration

A sufficient penetration of light as well as of ALA into the wart tissue is necessary to induce phototoxicity. The penetration depth of ALA as well as light may be a limiting factor for treatment of thick lesions such as warts. The callus of hand and foot warts was removed before ALA cream application and the papillomatosis it-self may give excellent possibility for penetration of the ALA cream and light. Nodular basal cell carcinomas often penetrate deep in dermis and may therefore be difficult to reach by the cream and by the light (Martin et al. 1995). The virion containing keratinocytes are located in the upper epidermis as stratum granulosum or above and do not invade deeper than epidermis. The localization of virus infected keratinocytes in epidermis is equal to the localization of superficial basal cell carcinomas that are treated successfully with ALA-PDT. Despite warts are thick epidermal lesions, the infected keratinocytes may be reached by light as well as by ALA.

Light in the visible spectrum can be used for PDT. The penetration of light decreases however with decreasing wavelength (Wilson et al. 1985, Martin et al. 1995) and therefore the commonly used wavelength is around 630 nm even though the porphyrin absorption band at 630 nm is weak. A high porphyrin absorption is found at 370-410 nm, but because of a low light penetration, light in this area is primarily used to induce PpIX fluorescence (Szeimies et al. 1995, Moan, 1996) or to treat superficial tumor lesions.

During illumination of PpIX photoproducts as the photosensitizer photoprotoporphyrin is generated. Photoprotoporphyrin has an absorption peak at 670nm and therefore a broad-band light source, with an emission spectrum that include this action spectrum, may be advantageous in the process of cell death.

Cryotherapy versus ALA-PDT

Patients enrolled in our ALA-PDT trial had all prior to inclusion been treated unsuccessfully with a variety of different treatments including repetitive cryotherapy combined with local application of keratolytics for longer periods. The patients had all been consulting highly trained and experienced dermatologists several times before referral to hospital. It is therefore unlikely that the warts included in our trial could benefit from additional cryotherapy.

Cryotherapy combined with local application of a keratolytic agent is indeed an effective treatment for many warts. In a randomized study, wart clearance occurred in about 45% of patients that completed 12 treatments of cryotherapy, regardless of whether these were performed at weekly, fortnightly or 3-weekly intervals (Bourke et al. 1995). Double freezing on foot warts improved the cure rate from 41% to 65% whereas it did not improve the cure rate for hand warts (Berth-Jones et al. 1994). More than 3 weeks interval between the freezing reduced the cure rate significantly from 75% to 40% (Bunney et al. 1976) and two minutes of freezing was significantly better than 15 seconds (Hansen et al. 1986). A combination of cryotherapy and salicylic acid with or without paring prior to cryotherapy demonstrated that the cure rate was improved from 39% to 75% by paring the foot warts. No additional effect was observed from paring of the hand warts (Berth-Jones et al. 1992).

In spite of optimal cryotherapy some warts are left in the group of more or less therapy resistant warts. These recalcitrant warts often lead to therapeutic as well as to social problems. The intense desire in patients to be wart free is often sought honored by doctors by offering a variety of more or less documented treatments. Examples of such are topical retinoids, glutaraldehyde, laser destruction, oral cimetidine, ultrasound and superficial x-rays.

Spontaneous cure

Patience will help the many wart patients better than anything else, however some warts are resistant even that.

It is difficult, if not impossible to predict the course of warts for any individual. In our study it cannot be excluded that some warts might have cleared due to chance during the 4 months our trial lasted. However, as patients in the ALA-PDT study had had warts for about 5 years and previous treatments of different nature had failed, it seems unlikely that any notable resolution would be spontaneous within our 4 months trial period.

The observation that a treatment of one or several warts in an immunocompetent host may led to resolution of all warts is often stated. It has however never been documented. As suggested by (Purhonen et al. 1975) it could be interesting to know whether wart-virus antigens from destroyed cells could be capable of attaching to other cell membranes on adjacent tumor cells and there trigger immune destruction of the cell and thus being responsible for the above statement. If this still undocumented phenomenon may have influenced the cure of the placebo treated warts in our PDT trial can only be hypothesized. The number of patients with only one wart that cleared after placebo could be compared to the number of cleared placebo warts in patients with two warts, of which one of the two was cleared by ALA , but in our study, this number is to small for comparative analysis. So we can not from our ALA-PDT study conclude anything relating to above phenomenom.

Patients included in our experiment were more than 18 years old and were referred from private dermatological clinics because of recalcitrant warts. The patients reported an average of about 35 consultations prior to referral. It seems therefore difficult to compare our results with cure rates reported in randomized studies in which relative young and untreated warts as well as children are included.

In general, introducing a new therapy it is important to verify that the level of cure is higher than the spontaneous resolution within the same period (Begg et al. 1996).

Controlled trials

Therapeutic studies ought to be studied in randomized trials with a proper inclusion and randomization technique. Patients ought to be included consecutively and at least 80% of the included ought to have fulfilled the treatment. In the result analysis, all randomized patients ought to be enrolled (Begg et al, 1996).

Randomized trials using different treatment modalities for hand and foot warts are summarized in Table IA. The references are found in EM-base (1980-2000), Med-line(1966-2000) and Cochrane Library with seach words: "hand and foot warts" or "palmar and plantar warts" or "verrucae vulgaris" and "randomized". Futhermore, some references found in cited references are included as well. Few examples of uncontrolled but well known and often used wart treatments are summarized in Table IB.

Even within the randomized studies the success rates are difficult to compare. They are influenced by several factors, that may complicate interpretation of the results. Differences in treatment regimens, variation in the extent and duration of infection, differences in biological age of the study population, the high rate of spontaneous remission and different inclusion criteria for warts as well as for patients are all factors, that makes it impossible to draw any meaningful comparisons between the different studies.

Table 1A: Randomized studies on hand and foot warts from (*EM-base* (1980-2000), *Med-Line* (1966-2000), *Cochrane Library*).

Intervention	control	inclusion	age	duration warts	duration of treatment	trial design	number cured warts(w);patients(pt) (%)	conclusion *(significant)	reference
acyclovir cream 5%	placebo cream or N_2	plantar warts	> 8 years	not stated	once daily - 6 weeks, N_2: at entrance, week 2, 4, 6. Evaluation: week 8	randomized	acylovir : 7/18 pt (39%) placebo: 5/18 pt (28%) N_2 : 1/11pt (9%)	acyclovir no better than placebo or liquid nitrogen	Gibson, 1984
anthralin 2%	Verucid	not stated	6-45 yrs 5-58 yrs	not stated	twice a day-2 months follow: 2-9 months	randomized	anthralin: 19/27 pt (56%) verucid: 9/31 pt (26%)	anthralin* better than verucid	Flindt-Hansen et al. 1984
bleomycine	placebo (saline)	hand and foot, recalcitrant warts	> 18 years	> 6 months	2 injection with 2 weeks interval	randomized, placebo-controlled, double-blind, cross over	bleomycin: 123/151 w (81%) placebo: 0/55 w (0%)	bleomycin* better than placebo	Shumer et al. 1983
bleomycine	placebo (saline)	pairs of hand warts, recalcitrant	32 years (15-59)	3 years	3 injections with 3 weeks interval, evaluation: 6 weeks	randomized, placebo, controlled, double-blind	bleomycin: 34/59 w (58%) placebo: 6/59 w (10%)	bleomycin* better than placebo	Bunney et al. 1984
bleomycine	placebo, left (saline)	pairs of hand and foot warts, recalcitrant	15-35 years	> 6 months	2 injection - 2 weeks interval, evaluation: 12 weeks	controlled, placebo	bleomycin: 97/143w (68%) placebo: 1/35 w (3%)	bleomycin* better than placebo	Amer et al. 1988
cimetidine	placebo	multiple, treated and untreated > 4 warts	15 years, (not male > 16 years)	2,5 years	3 months	randomized, placebo controlled, double-blind	cimetidine: 9/28 pt (32%) placebo: 8/26 pt (31%)	cimetidine no better than placebo	Yilmaz et al. 1996
cimetidine	placebo	recalcitrant, not flat and mucosal warts	mean:34 years (16-59)	> 2 years	3 months	randomized, placebo controlled, double-blind	cimetidine: 5/19 pt (25%) placebo: 1/21pt (5%)	cimetidine no better than placebo	Rogers et al. 1999
cimetidine	placebo	treated /not treated	3-14 years	not stated	2 months	randomized, placebo controlled	cimetidine: 2/6 pt (33%) placebo: 3/7 pt (43%)	cimetidine no better than placebo	Baumann et al. 1996
cimetidine	placebo	recalcitrant warts	12-57 years not < 16	> 6 months	3 months	randomized, placebo, controlled, double-blind	cimetidine: 10/27 pt (37%) placebo: 4/16 pt (25%)	cimetidine no better than placebo	Karabulut et al. 1997
cryo (N_2) with 1 week interval (keratolytic nightly)	2 and 3 weeks interval	hand and foot warts treated/ not treated	mean: 23 to 27 years	24 months	3 months up to 12 treatments	randomized	*3 months* *12 treat* 1 week: 43% pt 43% pt 2 weeks: 37% pt 48% pt 3 weeks: 26% pt 44% pt	no better cure rate, but faster cure with 1- and 2- than 3 weeks interval.	Bourke et al. 1995
cryo (N_2) single (keratolytic daily +/- occlusion)	double cryo (N_2)	hand and foot warts, treated/ not treated	20-24 years	not stated	3 weeks interval 3 months	randomized	*hand (pt) foot* single: 41/63 (65%), 16/39 (41%) double:46/72 (64%), 33/51 (65%)	double freeze* better than single on foot warts, not on hand warts	Berth-Jones et al. 1994
cryo (N_2) with 2 weeks interval	3- and 4 weeks interval	hand warts	median 21 years (4-74)	median from 9 months to 12 months	maximum 6 treatments, 6 months follow-up	randomized	2 weeks: 63% w 3 weeks: 70% w 4 weeks: 63% w	identical cure rates with 2-, 3-, 4-week interval	Larsen et al. 1996
cryo (N_2) and keratolytic daily	cryo and keratolytic and paring	hand and foot warts treated /not treated	5-85 years, 54% 11-25 years	median: 12 months (1-360)	daily salicylic, cryo and paring with 3 weeks interval, 3 months duration	randomized	*hand foot* par: 68/ 147 pt(46%), 45/60(75%) not: 74/ 147pt (50%), 23/60(39%)	better cure rate with paring of foot warts*, not better with hand warts	Berth-Jones et al. 1992
cryosurgery 2 minutes	15 seconds	plantar warts solitary < 6 mm	5-40 years	< 1 year	maximal 3 times with 3 week interval, evaluation : 9 weeks	randomized	2 minutes: 8/33 w (24%) 15 seconds: 3/27 w (11%)	better cure rate with 2 min freeze* than with 15 seconds freeze	Hansen et al. 1986
cryo(N_2)	histofreezer	hand warts	not stated	not stated	2 times / 2-4 times	randomized	N.. 67% pt	N..	

cryo N_2 2-week interval	3- or 4 weeks interval	hand warts	all ages	not stated	2, 3, 4 week interval , max. 6 treatments, evaluation: 12 weeks	randomized	2 weeks: 18/34pt (78%) 3 weeks: 18/31pt (75%) 4 weeks: 10/35pt (40%)	2-,3-week interval* better than 4 week interval	
cryo N_2	salicylic acid or both	hand warts	all ages	do	cryo: 3 weeks interval salicyl: daily evaluation: 12 weeks	randomized	salicylic: 64/95 pt (67%) N_2: 68/99 pt (69%) both: 78/100 pt (78%)	salicylic acid and N_2* better than salicylic alone and N_2 alone	Bunney et al. 1976
5-flurouracil 5%	placebo	bilateral warts, left and right	any age	not stated	1 month daily application under occlusion	randomized, placebo-controlled, double-blind	5-fluouracil: 29/48w (60%) placebo: 8/48w (17%)	5-FU* better than placebo	Hursthouse. 1975
glutaraldehyde	salicylic acid	mosaic warts	any age	not stated	daily, evaluation: 12 weeks	randomized	glutaraldehyd: 18/38pt (47%) salicylic acid: 19/43pt (44%)	glutaraldehyde not better than salicylic acid	Bunney et al. 1976
Heat therapy (local)	control	at least 2 warts on hands	>21 years	heat: 16 months, control: 20 months	50 degrees Celcius, average: 2 treatments	randomized, controlled	heating: 25/29w (86%) control: 7/17 w (41%)	heating* better than placebo	Stern et al. 1992
Homeopathy	placebo	children, hand warts common warts	6-12 years	not stated	once daily to once every other day in 2 months	randomized, placebo-controlled, double-blind	(reduction in wart area > 50%) homeopathy: 9/30 30% placebo: 7/30 23%	homeopathy not better than placebo	Kainz et al. 1996
Homeopathy	placebo	one or more plantar warts	6-59 years	> 3 months	application: dependent of the choice of homeopathic agent for 6 weeks	randomized, placebo controlled, double blind	*homeopathy(86 pt) placebo(88pt)* 6 weeks: 4.8 % 4.6% 12 weeks: 13.4 % 13.1% 18 weeks : 20% 24.4%	homeopathy not better than placebo	Labreque et al. 1992
Hypnosis	-salicylic acid -placebo -no treatment	hand and foot warts	18-35 years	not stated	hypnotic suggestion, "salicylic" and placebo at 2 weeks interval and no treatment, evaluation: 6 weeks	randomized	*(n = 10 pt/group)* *lost one or more warts* hypnosis: 6 pt salicylic acid: 0 pt placebo: 1 pt no treatment: 3 pt	hypnosis* better than salicylic agent, placebo and no treatment	Spanos et al. 1990
Keratolytic (promicid gel)	promicid and occlusive dressing	one to ten plantar warts on each foot	<14 years > 14 years	not stated	promicid: twice a day promicid/occlusion: every other day, paring every other day, evaluation:17 weeks	randomized	age (years): <14 >14 pro/occlusion : 78% pt 64% pt promicid: 73% pt 39% pt	*Promicid better* with* than without occlusion	*Veien et al. 1991*
monocloracetic acid and 60% salisylic acid	placebo	plantar, not mosaic, treated and untreated, < 1 cm, < 3 warts	active:18 years, placebo: 12 years	< 3 months to > 24 months	Occlusion one week followed by paring, evaluation: 6 weeks	randomized, placebo, controlled, double-blind	monocloracetic acid: 19/29pt (66%) placebo: 5/28pt (18%)	monocloracetic acid and salicylic acid* better than placebo	Steele et al. 1988
PDT with 0.1% proflavine	placebo PDT (picric acid)	symmetric, multiple hand and foot warts, treated and untreated, primary recalcitrant	7 pt < 10 years, 29-: 10-20 years, 20-: > 20 years	6 pt: < 6 months, 11 pt: 6-12 months, 39 pt: > 12 months	once a week 7 weeks, evaluation: week 8	randomized, placebo controlled, double blind, paired	10/27pt (37%) PDT and placebo warts disappeared simultaneously	PDT with proflavine 0.1% was not better than placebo	Veien et al. 1977
PDT with 0.1% neutral red	placebo PDT (color ruber)	symmetric, multiple hand and foot warts, treated and untreated	do	do	do	do	10/23 pt (42 %) PDT and placebo warts disappeared simultaneously	PDT with 0.1 % neutral red not better than placebo	do
PDT with methylene blue	keratolytic (salicyl and creosote)	> 1 wart, hand, foot	>10years 20 years (10-74)	1 months to 20 years (26 months)	PDT: once a week for 8 weeks keratolytic: once daily 8 weeks	randomized	PDT: 5/65 pt (8%) keratolytic: 8/55 pt (15%)	PDT with methylenblue not better than a keratolytic	Stahl et al. 1979
salicylic acid 15% in karaya gum patches	placebo	hand warts, < 1yr, <12mm < 4 weeks treatment, maximal 3 warts treated	active: 30 years placebo: 36 years	7-8 months	once a day, 3 months	randomized, placebo-controlled, double-blind	salicylic acid: 19/28 pt (68%) placebo : 7/25 pt (28%)	salicylic acid 15% in karayagum* better than placebo	Bart et al. 1989
verucid	traditional treatments in a clinic	hand and foot warts, adults and children	not stated	not stated	verucid: daily evaluation: 3 months	randomized	verucid: 43/84 pt (51%) traditional: 54/101pt (54%)	verucid not better than traditional treatments in a clinic	Auken et al. 1975

Table 1B: Examples of uncontrolled studies.

Intervention	inclusion	age of patient	duration of warts	duration of treatment	trial design	number cured warts(w);patients(pt) (%)	reference
Anthralin 2% (weekly paring)	mosaic warts	not stated	2 years (3 months to 6 years)	once a day, maximum 10 months	open	17/24 pt (71%)	Hjorth et al. 1966
ALA-PDT	recalcitrant hand and foot warts	16-79 years	not stated	3 treatments with 1 week interval, repeated after 1 month	retrospective	30/52 pt (58 %)	Stender et al.1999
bleomycin	recalcitrant palmar, plantar, periunquale, mosaic	35 years (15-76)	> 2 years	once monthly until cleared	open	57/62 w (92%)	Munn et al. 1996
cimetidine	recalcitrant	38 years (21-63)	> 2 years	once daily for 3 months	open	12/18 pt (67%)	Glass et al. 1996
co_2 laser	recalcitrant hand and foot solitaire and multiple	29 years (16-64)	5 years (7-20)	once evaluation: 6 months	open	10/18 pt (56%)	Logan et al. 1989
co_2 laser	solitary and multiple, primary and recalcitrant	6-68 years	not stated	1-3 times evaluation:3-6 months	survey by questionnaire	124/166w (75 %)	Mancuso et al. 1991
co_2 laser	recalcitrant hand and foot and others	not stated	median 24 months	once evaluation:12 months	retrospective	58/92w (64%)	Sloan et al. 1998
DCP	plane, hand, foot, mosaic treatment resistent	21 years	3 years	every 3 week up to 18 months (average: 5,8 months) average: 8 treatments	prospective	154/213 pt (72%)	Larsen et al. 1995
DCP	recalcitrant hand and foot warts	34 years (11-65)	3 years (3 months-14 years)	1-4 weeks interval, mean 5 (1-22) treatments	retrospective	42/48 pt (88%)	Buckley et al. 1999
DCP	recalcitrant periunquale palmo, plantare warts	15-71 years	> 1 years	once a week for 8 weeks	open	49/111pt (44%)	Rampen et al. 1996
DNCB	recalcitrant hand, foot other sites multiple warts	29 years (14-70)	5 years (14 months-30 years)	weekly, (1-6 months)	open	19/37 pt (51%)	Johansson et al. 1984
glutaraldehyde	recalcitrant periunquale, palmar, plantar warts	younger than 5 years	not stated	daily - up to 3 months	open	18/25 pt (72%)	Hirose et al. 1994
alfa-interferon	palmar and plantar warts	(7-48 years)	not stated	twice weekly, 15 (2-32) treatments, minimum 8 weeks	open	16 /22pt (73%)	Brodell et al. 1995
infrared coagulation	common hand and foot, treated and untreated warts	9-60 years	not stated	once	open	25/44 w (57%) 11/21pt (52%)	Charles et al. 1994
pulsed dye laser	primary and recalcitrant warts	3-72 years	not stated	1-2 month interval, average: 1,7 treatments	open	recalcitrant : 82/122 w (68%) primary: 16/43 w (47%)	Jacobsen et al. 1997
pulsed dye laser	treated and untreated, digital, periunquale, body warts	30 years (3-82)	6 months to 27 years	average: 3.4 treatments, 3-9 months follow-up	open	hand: 95%; foot: 84%	Kauvar et al. 1995
pulsed dye laser	recalcitrant, finger, hand , foot, verrucae planae	5-59 years	months to more than 5 years	average: 1.68 treatments	open	28/39 pt (72%)	Tan et al. 1993

In the randomized studies summarized in Tabel 1A no significant difference was found between liquid nitrogen versus histofreezer (Erkens et al. 1991), glutaraldehyde versus salicylic paint (Bunney et al. 1976) and verucid versus traditional treatments (Auken et al. 1975). Anthralin was significantly better than verucid (Flindt-Hansen et al. 1984) and in uncontrolled studies anthralin showed a cure rate of 71% (Hjorth et al. 1986). In a randomized study glutaraldehyde was not proven more effective than salicylic acid (Bunney et al. 1976) whereas glutaraldehyde had a cure rate of 72% in an uncontrolled study (Hirose et al. 1994) (Table1A and 1B).

Among the controlled randomized trials in Table 1A the sixteen placebo controlled trials are summarized in Table 2.

Table 2. Placebo -controlled trials.

Treatment	Number of studies	cure rate in intervention group (%)	Cure rate in placebo group (%)
Acyclovir cream	1	39	28
Bleomycine*	3	58-81	0-10
Cimitidine	4	25-37	5-43
5-flouracil*	1	60	17
Monocloroacetic acid*	1	66	18
Heat therapy*	1	86	41
Homeopathy	2	30	23
Hypnosis	1	lesser warts in 6 pt	lesser in 3 pt
Salicylic acid/ gum*	1	68	28
PDT(neu.red, proflavin)	1	37-43	37-43

- Significant effect proven. Pt = patients.

The cure rates for acyclovir, cimetidine, homeopathy and PDT with neutral red and proflavine were not significantly higher than in their respective placebo treatment.

Local injection of *bleomycine* in recalcitrant hand and foot warts demonstrated cure rates ranging from 58%-81% for the bleomycin treated warts versus 0%-10% for the saline treated warts (Bunney et al. 1984, Shumer et al. 1983, Amer et al. 1988). In an uncontrolled study it was demonstrated, that 52 warts out of 62 warts (92%) injected

with bleomycine were cleared (Munn et al, 1996). It has been the impression that bleomycine injections are relatively painful. Nevertheless the patients preferred treatment with bleomycin to cryotherapy (Bunney et al. 1976). It appears that bleomycin acts directly on the warts and is suggested to be particular useful when the immuneresponse is diminished or absent. Bleomycine must be injected and may therefore be difficult to perform if the patient have too many warts and warts in the plantar.

Peroral *cimetidine* versus placebo for recalcitrant warts had no significant effect after a treatment period of 3 months. The cure rates ranged in the cimetidine treated groups from 25% to 37% compared to the placebo cure rates ranging from 5% to 43 % (Rogerts et al. 1999, Yilmaz et al.1996, Karabulut et al.1997, Baumann et al. 1996). In an uncontrolled study a 67 % cure rate was reported (Glass et al. 1996).

5-Fluouracil was better than placebo with a cure rate of 50 % versus 17 % in the placebo group (Hursthouse, 1975). Patients of all ages were enrolled. 5-FU is justified and suitable for patients with multiple plantar and finger warts.

Irritants as *monocloracetic acid combined with 60% salicylic acid* demonstrated in a young population of not recalcitrant, simple warts a cure rate of 66% compared to that of a placebo treatment of 18% (Steele et al. 1988). It is easy applicable, but a rarely used method.

Heat therapy is also rarely used, despite it has been proven effective with a cure rate of 86% of hand warts versus 41% of the placebo treated warts. Treated as well as untreated warts were included in the trial. Heat treatment must be performed after local anesthesia and that may have limited the use (Stern et al, 1992).

Salicylic acid 15 % in karayagum were able to cure 68% of small and young warts within three months compared to the placebo treatment of 28 % (Bart et al. 1989). This

treatment may be relative attractive since it is easy to apply, have a high therapeutic effect, low costs and lack of adverse advents.

Photodynamic therapy studied by Veien et al. showed cure rates of 37% respective 43% treating multiple symmetrically located hand and foot warts in children and adults with proflavine, respectively neutral red, followed by weekly light illumination over a seven week period. The proflavine group was irradiated with a light bank of Westinghouse sunlamps and black lights filtered through pyrex glass with emission distributed in the UV-A. The neutral red were irradiated with an ordinary light bulb 550-650 nm emission. Warts on the placebo treated side disappeared simultaneously with that on the active treated side, indicating that cell-mediated immunity may have played a role in the destruction. An effect on the light itself could be considered the reason for the disappearance of the warts on both sides (Veien et al. 1977). Cure rates of 15% versus 8% were observed when Stahl et al. compared PDT performed with methylenblue followed by light exposure with a mecury lamp (530nm) with that of a keratolyticum. No significant difference between the two groups was observed (Stahl et al. 1979).

In our study with ALA-PDT and placebo-PDT we had an unexpectedly high placebo effect, namely 42%. In sample size calculation performed prior to the experiment we estimated a placebo effect of 30% which we expected to be a relative high estimate since patients for inclusion were referred with "recalcitrant warts".

We cannot help to speculate if the high placebo effect in our study as well as in the study of Veien et al. may be caused by the light itself due to a naturally existing content of photosensitizers such as PpIX or others in the warts. It is noticeable, that the two PDT studies reported, both have this high cure rate in the placebo treated group.

Patients enrolled in the ALA-PDT study were familiar with daily paring followed by topical application of a keratolyticum. The patients claimed that, for physical and

cosmetic reasons, they could not participate in a study for 4 months if they could not pare their warts and apply a keratolytic agent. In order to have all patients treated identically during the trial, we told all our patients to pare their warts followed by Verucid application twice a week during the four months trial period. Additionally, paring was performed by an enthusiastic doctor prior to each PDT treatment. This basic treatment might also have contributed to the high cure rate observed in the placebo group.The cure rates for non-recalcitrant warts treated with verucid once and twice a day for two months cleared 51% (Auken et al. 1975) respective 26% (Flindt-Hansen et al. 1984) of patients treated. Promicide gel containing 15% salicylic acid showed significant higher cure rate (64%) with - than without occlusion (39%) (Veien et al. 1991).

If a systemic effect of ALA-PDT should account for the high cure rate in the placebo group it would have weakened our results making the difference between placebo and ALA-PDT treated warts smaller - concluding that ALA-PDT is better than verified in this study.

The high number of wart-related consultations prior to entrance into our trial as well as the low withdrawal from the trial indicate highly motivated patients with an intense wish to be wart free, not only during but also prior to inclusion in the wart trial.

Reported side effects in relation to ALA-PDT are mainly related to reversible pain during and after the treatment. This pain seems no more serious and not longer lasting than reported after cryotherapy and laser treatment. Properly administered liquid nitrogen is a painful and unpleasant therapeutic modality, that frequently leads to blistering and ulceration. Cryotherapy is therefore often not tolerated in children aged under 10 years.

Uncontrolled studies

When conventional wart treatments have proven unsuccessful doctors often offer undocumented treatment modalities to their patients. Examples of such frequently used treatment modalities are summarized in Table 1B and include among others topical immunotherapy with the universal contact sensitizer *diphencyprone (DCP)*. This treatment is widely used for recalcitrant warts although no controlled and randomized studies have been reported. The rates of successful topical DCP of resistant warts vary from 8% -93% (Wiesner-Menzel et al. 1984, Lane et al. 1988, Naylor et al. 1988, Orecchia et al. 1988, Rampen et al. 1996, Buckley et al. 1999, Larsen. 1995). Rampen et al. treated 111 patients with hand and foot warts once a week for 8 weeks (8 treatments) and cleared 8 %, whereas Buckley et al. included 30 patients treated 1-22 times, reported a cure rate of 93%.

The variation in success rates can be related to different treatment regimens (types and duration of warts included, concentrations of DCP and number of applications). However, DCP immunotherapy seems to be a promising option that justifies the respect of further exploration, namely to be documented in a larger randomized controlled study. Uncomfortable DCP side effects (4-56%) such as blistering and erythema multiformes (Perret et al. 1990), severe urticaria (Alam et al. 1999) and vitiligo (MacDonald-Hull et al. 1989, Duhra et al. 1990) and eczematous eruptions are reported and make it even more necessary to document that this treatment is more effective than a standard treatment without these side effects. A therapy applied to a relative harmless disease such as warts ought not to result in more problems to a patient than the disease it self, especially if not proven more effective than a treatment with lesser side effects.

CO_2 laser is another used but yet undocumented treatment with reported cure rate for recalcitrant and non recalcitrant warts around 65%. However, in the study of (Logan et al. 1989), 50 % of the patients felt that they were worse off after the treatment. Fourteen patients reported post-operative pain to be severe and long-lasting (3 weeks). In another study in which an overall success rate of 61% was reported, it was concluded that patient satisfaction and cure rates were impressive enough to recommend use of CO_2 laser for wart treatments (Sloan et al. 1998)

Pulsed dye laser has been reported to produce excellent responses in treatment of verrucae. New studies indicate that pulsed dye laser therapy may be effective with clearance rates for first time treated lesions and for recalcitrant lesions ranging from 0-93 % (Huilgol et al. 1996, Tan et al. 1993, Kauvar et al. 1995, Jakobsen et al. 1997). Ross et al.1999 reported a complete wart clearance of 48%, they concluded however, that pulsed dye laser is an effective treatment option for recalcitrant warts with an excellent side effect profile.

Conclusion

It is difficult to treat warts when they are resistant to traditional treatments. A lot of therapeutic challenges are available. When more treatments are to be added to the existing aramentarium of therapies, the treatment modalities introduced should be scientifically tested whenever possible. It is important to compare the effect of the uncontrolled treatment modalities with the modalities investigated in randomized studies, that give the best evidence on the effect of an intervention as they control for both known and unknown variables. Other trials are observational and open to systematic biases.

The double-blind and placebo controlled study with ALA-PDT for recalcitrant warts that fulfilled the standards concerning design and analysis for randomized trials with a very low dropout rate (Begg et al, 1996) gives promise for photodynamic therapy as a new therapy for recalcitrant warts. The treatment can possibly be optimized in future studies. The high cure rate of the placebo treated warts is an interesting objective for further investigations, whether they will include the biological mechanisms behind PDT or an evaluation of wart treatment in general.

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8.0 Summary in English.

Photodynamic therapy (PDT) with topical application of 5-aminolevulinic acid (ALA) followed by irradiation with incoherent light (ALA-PDT) is a rapidly expanding field in dermatology. Promising results with ALA-PDT of superficial non-melanoma skin cancer are reported. Lately, reports have shown that ALA-PDT may be useful in the treatment of proliferative dermatological diseases as e.g. psoriasis, verrucae vulgaris and condylomata.

When ALA is absorbed through defect keratin into epidermal cells, it is enzymatically converted to porphyrins especially the photosensitizer protoporphyrin IX (PpIX). When tissue accumulated with PpIX is exposed to light, cytotoxic metabolites are released and pain is induced.

In this thesis the effect of six consecutive ALA-PDT treatments of recalcitrant warts was examined in a randomized placebo-controlled trial. The patients with recalcitrant warts were referred to our out-patient clinic after years of treatments in the primary health sector. ALA-PDT as well as placebo-PDT treated warts were treated with paring followed by a topical keratolytic twice a week during the experimental period.

The ALA-induced as well as the endogenous Protoporphyrin IX (PpIX) intensity was recorded with a fluorescence spectrometer from some of the warts. The selectivity of PpIX accumulation in wart lesions compared to adjacent skin, the relation between the concentration of PpIX and the number of treatments as well as the relation between PpIX intensity and clearance rate were examined. Side effects such as pain were quantified and characterized immediately and 24 hours after light exposure by use of a questionnaire.

The relative wart area was significantly reduced in the ALA-PDT warts compared to the placebo-PDT warts ($p < 0.008$) and 56% of the ALA-PDT treated warts vanished

compared to 42% of the placebo treated warts. There was a selective PpIX accumulation in wart lesions compared to surrounding normal skin. The PpIX concentration varied highly from lesion to lesion, however there was no relation between the concentration of PpIX and the relative change in wart area and consecutive treatments did not increase the accumulation of PpIX.

About 17 % of the ALA-PDT treated warts had severe to unbearable pain during and after light exposure. The pain lasted in average 30 hours after light exposure and was characterized as local, burning and shooting pain, indicating a neurogenic pain.

On the basis of our results we recommend ALA-PDT to recalcitrant warts. However, we recommend restricted use of ALA-PDT for children, until a sufficient pain relief is available.

9.0 Summary in Danish. Dansk Resume

Lokal påsmøring af 5-aminolevulinsyre (ALA) efterfulgt af belysning med synligt lys (PDT) kaldes oftest for photodynamisk terapi (ALA-PDT). Anvendelse af ALA-PDT indenfor dermatologien er kraftigt ekspanderende, og der er rapporteret særdeles tilfredsstillende resultater i behandling af overfladiske basal-celle carcinomer samt af aktiniske keratoser. Igennem de senere år har kliniske undersøgelse vist, at ALA-PDT også er effektiv til andre ikke onkologiske hudlidelser, eksempelvis psoriasis, fod og hånd vorter samt kondylomer.

Efter optagelsen af 5-ALA i syge hudceller via defekter i hornlaget, omdannes ALA ved en enzymatisk proces til den lysfølsomme metabolit Protoporphyrin IX (PpIX). Lyser man på hud der indeholder forhøjede koncentrationer af PpIX, frigives nogle cytotoksiske stoffer, der kan få de syge celler til langsomt at henfalde. Under og efter belysningen kan der hos nogle patienter opstå forbigående smerter af vekslende intensitet.

Afhandlingen omhandler effekten af gentagne ALA-PDT behandlinger til terapiresistente hånd og fodvorter henvist til vores ambulatorium efter års behandling i primær sundhedssektoren. Undersøgelsen er dobbelt blindet og placebo kontrolleret. ALA såvel som placebo behandlede vorter blev under hele forløbet behandlet to gange ugentlig med beskæring efterfulgt af Verucid. Koncentrationen af PpIX i ALA og placebo behandlede vorter samt i omkringliggende normal hud blev målt fra en del af vorterne ved hjælp af fluorescens spektroskopi. PpIX koncentrationen er søgt relateret til behandlingseffekt samt til smerte induceret af belysningen. Smerter umiddelbart samt 24 timer efter belysning ved gentagne ALA-PDT behandlinger blev

kvantificeret samt karakteriseret ved hjælp af spørgeskemaer udleveret til patienterne efter hver behandling.

Den relative reduktion i vorte areal var significant større i ALA-PDT behandlede vorter end i placebo behandlede vorter ($p< 0.008$) og signifikant flere ALA-PDT behandlede vorter (56%) end placebo-PDT vorter (42%) forsvandt fuldstændigt. Af de persisterende vorter blev de ALA behandlede significant mindre end placebo vorterne. Vi fandt desuden, at vorterne selektivt ophobede PpIX i sammenligning med omgivende normal hud. Vi fandt ingen korrelation mellem vorte areal reduktion og PpIX koncentration. PpIX koncentrationen ændrede sig ikke væsentlig under de gentagne behandlinger. Fra ca 17 % af vorterne angav ALA-PDT behandlede patienter alvorlig til ubærlig smerte. Smertene blev primært beskrevet som lokale, brændende og jagende. Smerteintensiteten syntes at være nogenlunde uændret fra behandling til behandling. Vi fandt ingen relation mellem smerte og behandlingseffekt og ingen relation mellem smerte og PpIX koncentration. På baggrund af vore resultater anbefaler vi brug af ALA-PDT i behandlingen af terapiresistente vorter. Vi anbefaler dog at udvise tilbageholdendehed med at ordinere ALA-PDT til mindre børn, indtil de af behandlingen inducerede smerter kan mindskes.

10.0 Future perspectives

Since warts are affecting a significant proportion of the population and consequently is consuming a sizable proportion of patients and health care resources, any significant progress in the field of wart removal ought to have further attention.

Future studies ought to deal with:

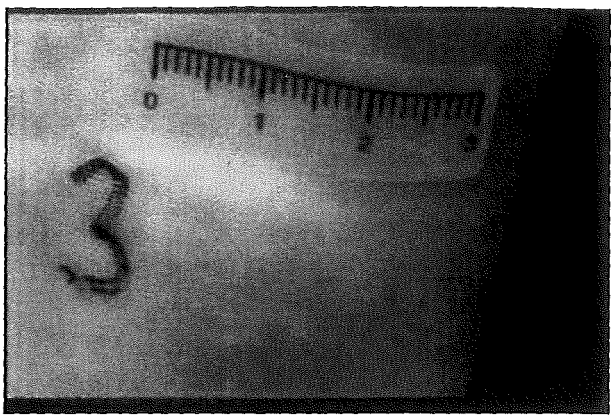
- Optimization of ALA-PDT including simplification of the treatment modality such as home application of the cream, reduction of the number of treatments, optimization of illumination time and intervals between treatment sessions as well as selection of more effective ALA-variants
- Clinical randomized trials to verify the effect of ALA-PDT on non recalcitrant hand and foot warts, plane warts, mosaic warts and genital warts.
- Studies involving pharmacokinetic of PpIX and its photodegradation products to understand the mechanism behind ALA-PDT.
- The effect of ALA-PDT on the virion
- Pain relief studies in order to find a possible relief to the twenty per cent of the patients that registered severe to unbearable pain during and after ALA-PDT.
- The effect of ALA-PDT on other virus induced diseases

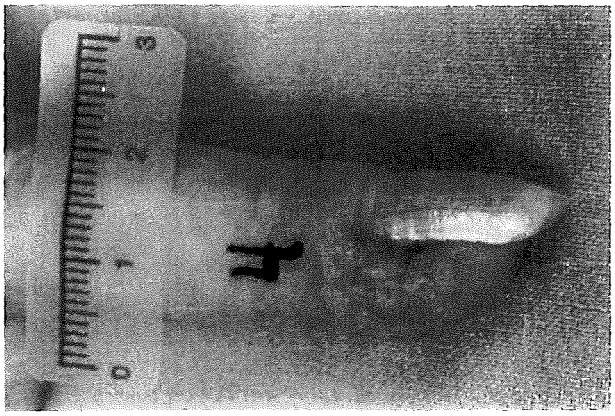
11.0 Abbreviations

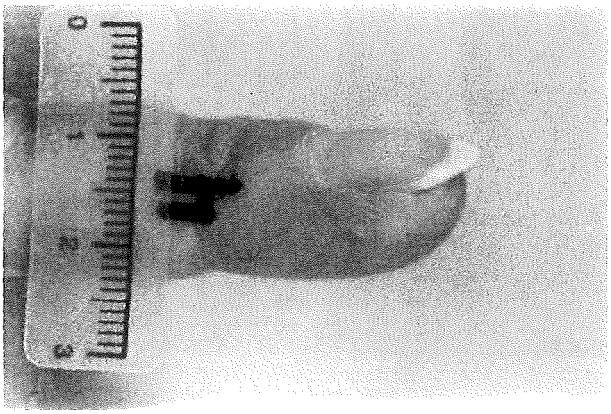
ALA:	aminolevulinic acid
PDT:	photodynamic therapy
ALA-PDT:	photodynamic therapy with 5-aminolevulinic acid
PpIX:	protoporphyrin IX
GP:	general practitioner
BCC:	basal cell carcinoma
AK:	actinic keratoses
SCC:	squamous cell carcinoma
CR:	complete response
LIF:	light induced fluorescence
nm:	nano meter
HPD:	haematoporphyrin derivative
HPV:	human papilloma virus
A.U.:	arbitrary unit
mW:	milli watt
J:	joule
H:S:	Hovedstadens Sygehusfællesskab
VAS:	Visual Analoque Scale
VRS:	Verbal Rating Scale
i.v:	intravenous
i.m:	intramuscular

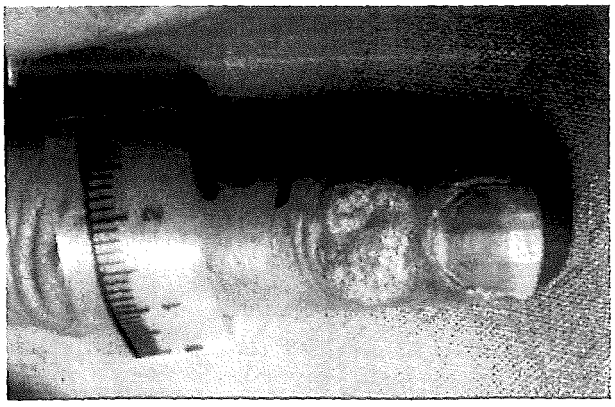
Appendix 1

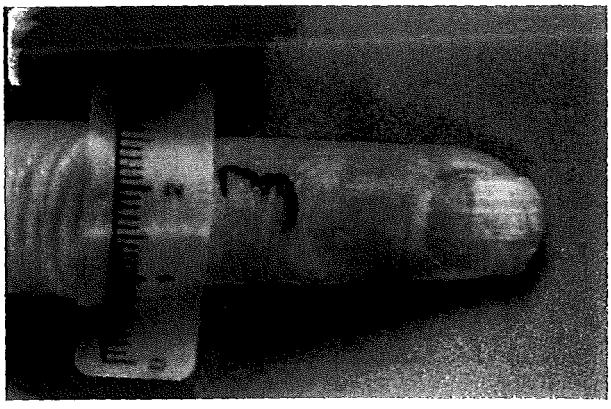
Before (left) and after (right) photodynamic therapy with 5-aminolevulinic acid





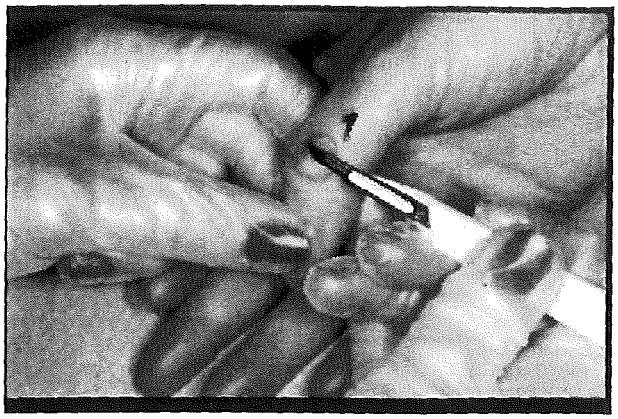




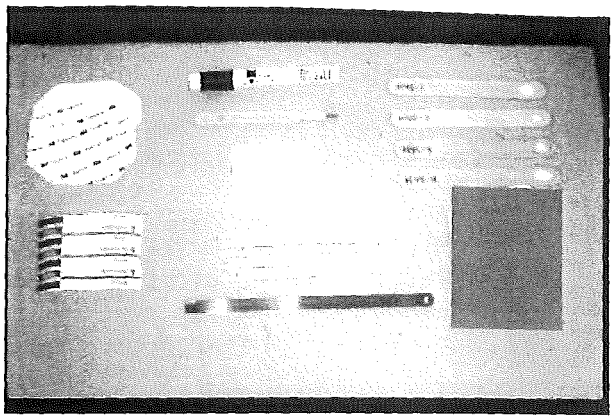


Appendix 2

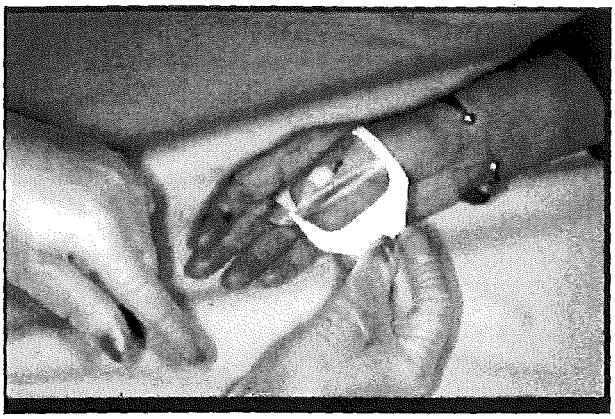
Treatment procedure



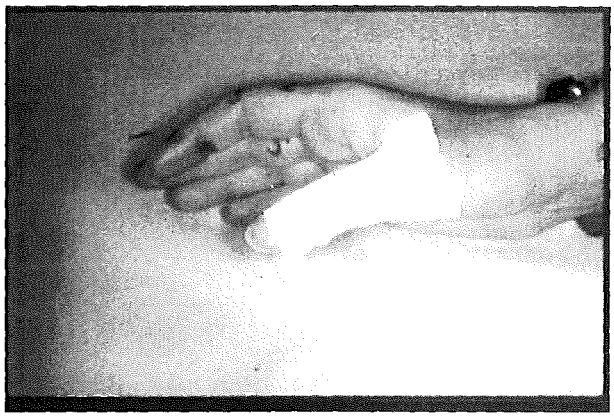
Paring of the warts before cream application



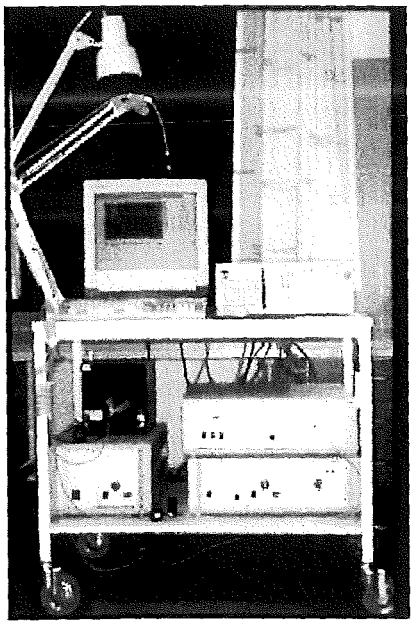
Cream application after randomisation



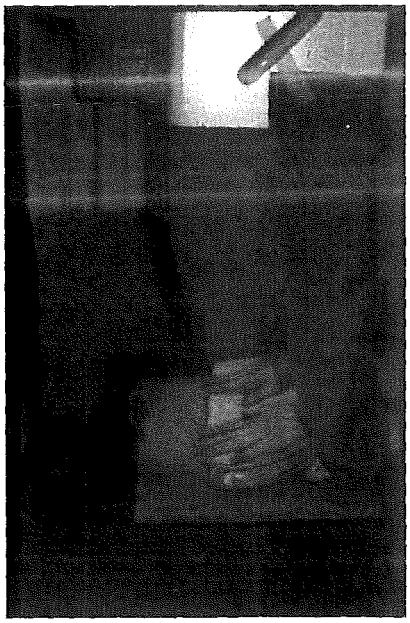
Covering with a semipermeable membrane



Covering with a bandage



Fluorescence measurements



Light exposure with red light