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Basal cell carcinoma – new aspects of diagnosis and treatment

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Basal cell carcinoma – new aspects of diagnosis and treatment

Ann-Marie Wennberg

PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Stenquist B, Wennberg A-M, Gisslén H, Larkö O. Treatment of aggressive basal cell carcinoma with intralesional interferon: Evaluation of efficacy by Mohs surgery. J Am Acad Dermatol 1992;27:65 – 69.

II Wennberg A-M, Lindholm L-E, Alpsten M, Larkö O. Treatment of superficial basal cell carcinomas using topically applied delta-aminolaevulinic acid and a filtered xenon lamp. Arch Dermatol Res 1996;288:561 – 564.

III Wennberg A-M, Gudmundson F, Stenquist B, Ternesten A, Mölne L, Rosén A, Larkö O. In vivo detection of basal cell carcinoma using imaging spectroscopy. Acta Derm Venereol (Stockh) 1999;79:54 – 61.

IV Wennberg A-M, Larkö O, Lönnroth P, Larson G, Krogstad AL. Delta-aminolevulinic acid in superficial basal cell carcinomas and normal skin – a microdialysis and perfusion study. Submitted for publication.

V Wennberg A-M, Larkö O, Stenquist B. Five-year results of Mohs micrographic surgery of aggressive facial basal cell carcinoma in Sweden. Acta Derm Venereol (Stockh) 1999;79:370 – 372.

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LIST OF ABBREVIATIONS

ALA	Delta-aminolevulinc Acid
ALAS	Delta-aminolevulinc Acid Synthase
BCC	Basal Cell Carcinoma
CCD	Charge Coupled Device
CMM	Cutaneous Malignant Melanoma
DFO	Desferrioxamine
DMSO	Dimethylsulphoxide
EDTA	Ethylenediamine Tetra-acetic Acid
IFN	Interferon
[I]	Interstitial Concentration
ICCD	Intensified Charge Coupled Device
i.l.	Intralesional
MED	Minimal Erythema Dose
MBCC	Morpheiform Basal Cell Carcinoma
NBCC	Nodular Basal Cell Carcinoma
MMS	Mohs Micrographic Surgery
PBGD	Porphobilinogen Deaminase
PDT	Photodynamic Therapy
Pp IX	Protoporphyrin IX
RR	Relative Recovery
SBCC	Superficial Basal Cell Carcinoma
SCC	Squamous Cell Carcinoma
TE/CCD	Thermoelectrically Cooled Charge Coupled Devise
UVA	Ultraviolet A Radiation (320 < 400 nm)
UVA1	Long-wave Ultraviolet A Radiation (340 < 400 nm)
UVA2	Short-wave Ultraviolet A Radiation (320 < 340 nm)
UVB	Ultraviolet B Radiation (290 < 320 nm)
UVB(BE)	Biologically Effective UVB
UVC	Ultraviolet C Radiation (< 290 nm)
UVR	Ultraviolet Radiation

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Basal Cell Carcinoma—New Aspects of Diagnosis and Treatment

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The incidence of basal cell carcinoma is increasing. New aspects of diagnosis and treatment are discussed in this thesis.

Interferon can be used for the treatment of BCC. In paper I, 15 patients received 13.5×10^6 IU of alfa-2b-interferon intralesionally. Four patients healed completely whereas a 75% reduction was seen in 5 cases. Intralesional alfa-2b-interferon can reduce the number of excisions during Mohs Micrographic Surgery.

Topical photodynamic therapy involves the application of ALA on the skin. In tumour cells selectively, formation of the photosensitizer Pp IX occurs. After 4 hours of occlusion of ALA the area is irradiated with light at a wavelength of 630 nm. Tumour cells are selectively destroyed during this procedure. 144/157 SBCC healed in this series and 14/18 Mb Bowen (paper II). The method is only suited for thin BCCs as the result on thicker lesions is poor (2/10 healed). The cosmetic result was generally good or excellent.

Another way of utilising the tumour selectivity of Pp IX is for diagnostic purposes. Instead of illuminating with 630 nm, 365, 366 and 405 nm are used to induce a specific fluorescence. In the present paper (III), 50% of facial BCCs with ill-defined borders could be completely visualised and another 23% partly outlined. The technique did not seem to work in 27% of the cases.

The critical factor using ALA is probably the relatively poor penetrance through the skin. In paper IV, microdialysis is used for pharmacokinetic studies of ALA for the first time. The concentration of ALA increases rapidly in lesional skin whereas there is virtually no penetration in healthy skin. Also, the blood perfusion in BCCs was investigated by means of laser Doppler Perfusion Imager. The perfusion in skin overlying a BCC was 2.5 fold higher compared to normal skin.

For BCCs with ill-defined borders Mohs Micrographic Surgery is generally recommended. Regarding Mohs Micrographic Surgery, Sweden is underserved as only 1% of BCCs are treated with Mohs Micrographic Surgery as opposed to 30% in the US. Consequently, the Swedish cases are probably more severe. The long-term results are reported in paper V. Two hundred and twenty-eight tumours were followed for at least 5 years. The rate of recurrence was 8%. This figure is slightly higher than in international materials but surprisingly low considering the type of tumours. ***Key words: basal cell carcinoma; imaging fluorescence; interferon; microdialysis; Mohs micrographic surgery; photodynamic therapy; photosensitiser.***

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1. INTRODUCTION

Basal cell carcinoma

Basal cell carcinoma (BCC) is a malignant tumour of the skin that arises from basal cells in the epidermis. Its cellular components resemble the relatively undifferentiated cells of the basal layer in the epidermis and require a stroma for proper growth. It rarely metastasises. A synonym for BCC is basalioma. Apart from BCC, squamous cell skin cancer and malignant melanoma are common forms of skin cancer.

Historical aspects and epidemiology

Jacob originally described basal cell carcinoma in 1827 (1). It has long been known that BCC is the most common malignant tumour of the skin (2). The incidence is increasing and even in Sweden it is a major dermatological problem. There is evidence that the number of patients who develop more than one BCC is increasing and the major risk factors are tumour among relatives and sun exposure (3, 4). In another study, the incidence increase was more than twofold from 1970–1986 and the age-standardised incidence rate in the southern part of Sweden was 280 per 100 000 in 1985 to 1986 (5). The incidence rates of BCC and SCC increase with decreasing latitude in Norway (6). An estimate of the incidence in Sweden today is probably around 15 000. The rates in Australia are considerably higher than in Scandinavia with 788 per 100 000 inhabitants in 1995 which is an increase of 19% since 1985 (7). BCCs occur primarily on sun-exposed areas (8, 9) and a major etiological factor is solar irradiation. Patients with BCC have a considerably increased risk of developing malignant melanoma (10, 11).

Etiology and pathogenesis

The exact etiology of BCC development is unknown but exposure to ultraviolet radiation is considered to be the most important etiological factor. UVR also increases the risk for squamous cell skin cancer and also for malignant melanoma of the skin (12–16). The relationship between sunlight exposure and BCC is not as evident as for squamous cell carcinomas (2, 15, 16). Many BCCs develop in relatively sun-protected areas such as behind the ears or the inner canthus (17).

BCC is associated with intermittent and non-occupational sun exposure (16). BCC and CMM have a similar risk pattern with increased risk for intermediate levels of cumulative exposure and a protection associated with higher levels (18). Chronic sun exposure and high levels of cumulative exposure characterises patients with SCC (18). Persons with this skin lesion often have an outdoor occupation or have been involved in outdoor activities for many years.

BCC is most common in people with a fair skin (2, 19). More than 99% of persons in whom BCC develops are white (20). The skin's ability to tan modulates the risk of BCC, subjects who tan poorly have a steady risk increase, whereas people who tan easily develop cancer only after prolonged exposure (21).

BCC is relatively rare below the age of 30 and is more common in men than in women (60:40) (5). As the exposure patterns regarding ultraviolet radiation are changing, this

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difference will probably diminish. The majority of patients are between the ages of 40 and 80 (20).

Inorganic arsenic preparations have long been recognised as an etiological factor for the development of both squamous cell skin cancer and BCCs (22–26). BCCs caused by arsenic are characteristically of the super-ficial type. Nowadays, arsenic is not used in dermatological treatment but patients in their forties may still have been exposed to arsenic medications for psoriasis in Sweden.

Scars (27, 28) and treatment with x-rays (29–31) can be associated with the development of BCC. Chronic venous stasis dermatitis and stasis ulcers may also predispose to BCC (32–37).

A relationship between the pilosebaceous follicle and BCC is well known. BCCs are more common in areas with a high density of sebaceous glands (38). However, this has been questioned by others (39). Basal cell cancers almost never arise from stratified squamous epithelium other than that of the skin. BCCs on the oral, anal or vaginal muscosa are very unusual.

Some hereditary disorders are associated with a high incidence of BCCs. Typical examples are xeroderma pigmentosum and the nevoid basal cell carcinoma syndrome. Patients with xeroderma pigmentosum have a defective capacity to repair DNA damage caused by ultraviolet radiation (40, 41). In the nevoid BCC syndrome, there is an increased sensitivity to X-rays (42). The gene responsible for the development of the nevoid BCC syndrome has recently been found (43–46).

A BCC contains both dermal stromal and epidermal cellular components. The relative amounts of these two components vary with the type of BCC but are also an interesting phenomenon in the pathogenesis of BCC. This property is probably involved in the low capacity of BCC to metastasise (47, 48).

An especially interesting cell marker seems to be stromelysin 3. The expression of this is increased in human basal cell carcinoma (49–52). Stromelysin 3 is produced in the stroma of various malignant tumours. It is probably one of the components responsible for the local invasion of the stroma by BCCs. However, in addition to being a marker for malignant disease, stromelysin 3 is produced by fibroblastic cells associated with benign fibrosis (51).

Some natural and synthetic compounds are known to affect the growth and differentiation of BCCs. Retinoids cause BCC-cells to undergo terminal differentiation. Retinoids have been demonstrated to cause regression of BCC-growth (53–55). Also, several authors have demonstrated an effect of interferons on basal cell carcinomas (56–69).

Immunosuppression can lead to an increased risk of skin cancer development (70, 71). This is typically seen in some organ transplant recipients (70, 72–74).

Clinical manifestations

BCCs are most common on the face and 85% of all BCCs appear in the head and neck region. Around 25% occur on the nose alone, the most common site (20, 75).

We have chosen to divide the BCCs into three main forms:

Nodular basal cell carcinomas (the most common type, ~60%) have well-defined borders and are often located on the face. A typical tumour is characterised by a smooth-surfaced nodule arising in actinically damaged skin. The border is often translucent and "pearly". Teleangiectatic vessels may be present. Hair growth is seldom seen on a BCC. Ulceration may occur (noduloulcerative BCC). BCC can be cystic but this is relatively rare. Pigmented forms of BCC are seen and are a differential diagnostic problem vis-a-vis malignant melanomas. Nodular/micronodular BCCs are histologically characterised by a relatively circumscribed tumour with tumour nodules of variable size infiltrating the stroma.

Nodular BCCs are often excised. In special regions such as the nose, ear and eyelid, cryotherapy is an alternative treatment (76, 77). In some instances, especially among older people, radiotherapy is advocated.

Nodular tumours can become very large and destroy deeper structures.

Superficial BCCs (~25%) usually occur on the trunk. They are often multiple. Clinically, the tumour is superficial, erythematous and often with small crusts. Central portions of the tumours are often atrophic. The tumour is usually well demarcated. It can mimic a guttate psoriasis. Histology reveals tumour nests attached to the epidermis which do not infiltrate the dermis. Treatment consists of curettage and electrodessiccation, cryotherapy and sometimes excision. Recently, photodynamic therapy has evolved as a therapeutic alternative as the cosmetic results seem very favourable (78–81).

Aggressive BCCs (~15%) are of either **intermediate or morpheiform type** (~2%) and grow diffusely. These tumours are often much larger than is expected from the clinical impression. An average of 7.2 mm of subclinical tumour extension was found in 51 morpheiform BCCs, as compared with 2.1 mm of extension in 138 well-circumscribed nodular lesions (82). Morpheiform BCCs are often located on the face, especially on the nose, forehead and temple. This is generally called the "H-zone", with a higher risk of recurrence after therapy than other regions of the face (75). Especially troublesome areas are the embryonic fusion planes such as the nasolabial fold, medial canthus and the area behind the ears. BCCs in these regions can grow as "icebergs", with only the top of the tumour visible. Clinically, the tumour consists of a plaque with diffuse borders. Teleangiectasias can occur but are less prominent than in nodular BCCs. Histologically, the intermediate type has a higher mitotic activity and a more spinocellular differentiation with larger cells and pronounced atypia. The morpheiform BCC shows diffusely infiltrative smaller strands of tumour cells surrounded by a desmoplastic stroma reaction. Aggressive BCCs have a higher risk of recurrence than other types of BCCs (75). Treatment is generally surgical. Mohs micrographic surgery has been advocated since this technique offers the best possible evaluation of the excision margins.

There are other types of histopathological classifications i.e. the classification proposed by Glas (83).

Basal cell nevus syndrome

This is a familial autosomal-dominant disorder caused by a loss of heterozygosity of the 9q chromosome, also called Gorlin's syndrome (43, 45, 84).

The syndrome is characterised by BCCs, cysts of the jaw,

bony abnormalities, palmar and plantar pits, epidermal cysts and other neoplasms.

Metastasis of basal cell carcinoma

The ability of basal cell carcinomas to metastasise is low. Metastatic BCC shows a male predominance of 2:1 and it is more common when the tumour size is large. Metastatic sites include lymph nodes, lung, bone, skin, and liver (47, 85).

Potential for recurrence

The recurrence risk is related to the tumour location, histological type, tumour size and type of previous treatment (recurrent tumours). Regarding location, the nose, eye region, temples and ears (the "H-zone" of the face) are predominant risk areas (75, 86). Perhaps because of embryonic fusion planes at these locations, tumours dissect easily into deeper tissues and are resistant to standard treatment (87). The morpheiform and infiltrating type of BCC has the greatest recurrence potential (75, 88–92). Tumour size over 2 cm has been suggested to be a clinical factor predisposing to recurrence (93, 94). The recurrence rate for small, primary BCCs in low risk sites is less than 10% for most methods of treatment. Recurrent BCCs should be treated differently from primary tumours since the conditions for undertreatment could still be present. All modes of treatment have a higher recurrence rate when used for treating recurrent BCCs (93, 95–98).

All factors must be taken into account in order to initiate the appropriate therapy (94, 99).

Ultraviolet radiation and its effect on the skin

The sun emits ultraviolet radiation. This is divided into UVA_1, UVA_2, UVB and UVC. UVA gives rise to immediate pigment darkening and a tan, whereas UVB is responsible for most of the erythema formation. However, UVA contributes to some extent to the erythema (100). Maximal erythema occurs 8–24 hours after irradiation. A typical MED in Sweden in the summer is around 40 minutes.

UVC does not penetrate the atmosphere but is used for laboratory purposes.

The intensity of UVR varies with latitude. The biologically effective UVB corresponds to the erythema-producing UVB (101). UVB(BE) in the Canary Isles in mid June is typically 2.5 times as high as in Sweden whereas the intensity, even in the Canary Isles, is very low during midwinter, approximately 40% of the values encountered in Sweden during the summer time.

Clouds affect the UV-radiation received by persons. On a cloudy day, the amount of UVB that passes the clouds is 50% or less. The intensity of UVR varies during the day. By staying in doors between 11 and 13, the UVR received can be reduced by 50% (102). Thus, it is possible to receive more UVB-radiation at noon on a cloudy than during a sunny day in the afternoon.

Reflection from various surfaces also plays an important role. Snow can reflect 90% of the incident radiation whereas a calm water surface reflects less than 10% (102). However, higher reflection factors are encountered on windy days.

Optics of the skin

The optical properties of the skin are of importance for tumour induction, fluorescence studies with ALA and treatment with PDT. Incident radiation may be absorbed, reflected, scattered or transmitted. The thickness of the stratum corneum is increased after irradiation with UVR. Apart from melanogenesis, this is an important part of the natural protection against sunlight (103). It has been shown that the use of lipophilic compounds reduces reflectance of UVR (104, 105). The penetration characteristics of tumour skin are less well known.

Biological effects of ultraviolet radiation

Photochemical molecular events take place immediately upon absorption of ultraviolet radiation in the tissue. These events may be followed by changes in the biochemistry of the cells. The greatest attention has been focused on alterations of the bases in the DNA molecule although many other photochemical events of biological importance take place.

Erythema

Exposure to ultraviolet radiation, particularly with wavelengths shorter than 315 nm, may result in erythema formation. The shorter wavelengths are usually more erythemogenic than the longer (100). The MED in the UVA region can be a thousandfold higher. Maximum erythema occurs 8–24 hours after irradiation.

Pigment formation

Repeated exposure to ultraviolet radiation increases the number of melanocytes in the skin (106). This is probably the most important mechanism for the prevention of damage induced by UVR. Apart from melanogenesis, UVA gives an immediate pigment darkening (107–109).

Doses of ultraviolet radiation

Children are exposed to higher levels of ultraviolet radiation than adults (110, 111). This is probably due to the special habits of children and the summer holidays.

Vitamin D

An important source of vitamin D in human beings is vitamin D3 formed in the skin from 7-dehydroxycholesterol by the action of UVR. Insufficient exposure to UVR can lead to vitamin D deficiency. This is especially common among elderly people. The action spectrum for vitamin D induction is in the short wavelength UVB region (112).

Immunology

UVR also exerts immunological effects on the skin. Both UVB and UVA deplete Langerhans cells for some weeks (113). Also, UVR converts trans-urocanic acid into cis-urocanic acid. The latter substance is an immunosuppressant (114–117).

Action spectrum for skin cancer development

The earliest association between sunlight and skin cancer is commonly credited to Unna (8). Nowadays, the idea that sunlight is the most important factor in the induction of skin cancer is generally accepted.

The action spectrum for development of squamous cell skin cancer is probably roughly the same as for erythema (100). The risk is associated with the accumulated exposure. Various models have been suggested. It has been proposed that the action spectrum for melanoma induction is shifted more towards the UVA (118–121). Also, intense sunburns, especially during childhood, seem to play an important role. Much less is known regarding basal cell carcinomas but the risk factors may resemble those for malignant melanoma of the skin (16). Previously, the likelihood of developing BCCs was considered to be related to the cumulative exposure to UVR. More recent data have questioned this and it may be that intense exposure to solar radiation during childhood and adolescence plays a greater role. However, the relationship with solar exposure may be less clear than previously postulated (17).

Treatment of basal cell skin cancer

Excision

Excision is a common form of treatment for BCCs (122, 123). It can usually be carried out easily in parts of the face such as the cheek and other areas with mobile tissue. It is important to have appropriate margins when performing surgery (124). Excision remains the type of treatment against which all other are compared.

Curettage and electrodesiccation

This mode of treatment is most often used on superficial and nodular BCCs on the trunk and extremities (125, 126). It is especially useful on the lower legs, due to the poor healing conditions and the tight tissue.

Curettage and cryosurgery

During recent years curettage and cryosurgery has been used more intensively (127–130). It is a safe alternative to excisional surgery in many instances. The cosmetic results vary from good to excellent on concave surfaces of the face of elderly individuals, to relatively poor on the trunk of younger persons. Curettage and cryosurgery is especially valuable for tumours on the nose, ears and eyelids, where it is considered the treatment of choice for nodular BCCs (77, 131).

Radiotherapy

Radiotherapy can be an alternative in certain instances, (132–135) especially among elderly patients with BCCs. The main indications are widespread BCCs, where other forms of therapy are not well suited (136). Brachyt-herapy represents an interesting new variant (137).

Interferon

Interferons are a group of glycoproteins with antiproliferative, immunomodulatory and antiviral functions (138, 139). There are three type I IFNs (α-, β-, and ω-IFN) and one type II IFN (γ-IFN) (140) and they all have their specific and separate cell surface receptors (141). IFN has been available for clinical use for 30 years and a number of diseases have responded well to treatment. Such malignancies are, for example, non-Hodgkin's lymphomas (142), myeloma (143) and leukemia (144) (CML and hairy cell). Patients with malignant melanoma have also to some extent responded to IFN therapy (145).

There are several theories regarding the possible mechanisms for the antitumour effects of IFN. Examples of this are the inhibition of vascularisation (146, 147) and the interaction between tumour cells and the stromal cells, surrounding or infiltrating a tumour. These stromal cells can produce factors that either stimulate or inhibit the growth of tumour cells (148). IFNs can influence this antitumour activity through effects on the stroma (149). IFNs also have effect on cell proliferation by affecting different phases in the mitotic cycle (150)

Flu-like symptoms and/or anaemia, neutropenia or thrombocytopenia are side-effects of IFN, often dose related (63, 151).

Some patients are poor or non-responders to IFN treatment and others respond well at first but do later develop antibodies and resistance to the therapy.

In dermatology, a variety of diseases have been treated with IFN (152). IFNs are today used for treatment of HIV-associated Kaposi's sarcoma (153) and cutaneous T-cell lymphomas (154). Intralesional IFN has shown activity in the treatment of noduloulcerative and superficial BCCs (56, 57, 65, 67, 155–161). IFN has also effect when treating aggressive BCCs (63).

Mohs Micrographic Surgery (MMS)

When excised tissue is prepared for histological examination, the specimen is cut vertically as a breadloaf with thick slices (162). Using this routine procedure, only 0.1% of the removed tissue is seen when reading the slides. With the technique used in Mohs micrographic surgery, the removed area is sliced horizontally from the bottom and up, giving an almost 100% view of the excised tissue. It was the American surgeon Frederic Mohs who in the 1930s introduced this technique for identification and removal of skin cancer. The tumour is fixated the first day and the second day excised in successive layers. The entire underside of each horizontally cut layer is examined microscopically until all tumour tissue is completely removed (163). This is a time-consuming procedure.

Mohs himself later simplified the manoeuvre and a description was published by his co-workers Tromovitch and Stegman in 1974 (164–166), who presented this fresh tissue technique. Excised fresh tissue sections were quickly frozen in a cryostat and examined in the same systemic way as the fixed specimens. The whole procedure could be done in one day and immediate reconstruction of the surgical wound was possible.

Another improvement of the method was introduced in the early 1990s with the single section technique (167). This

implies that the excised tissue is not divided routinely and it speeds up the procedure and makes orientation of the specimens more reliable. Mohs micrographic surgery (MMS) is used all over the U.S. for treatment of skin cancer and remains the standard against which other modes are compared. In 1986 the American College of Chemosurgery decided to use the name MMS for the procedure. The term micrographic indicates the combination of microscopic examination with graphic presentation of the wound on paper (95, 167–169). The technique is especially applicable to skin tumours on the face, where conservation of the maximum amount of healthy tissue is essential.

MMS was introduced at the Department of Dermatology, Sahlgrenska University Hospital in 1983 and it is the only place in all Scandinavia where MMS is regularly used. We have limited resources and often use other methods for treatment of skin cancer even on the face (76, 77). The most commonly accepted indications for MMS are recurrent tumours, tumours located in areas with a high risk of local recurrence or where tissue preservation is mandatory, and those with morpheiform growth (166).

Photodynamic therapy (PDT)

Photodynamic therapy implies the destruction of tumour cells achieved by a photochemical reaction producing toxic substances, mainly singlet oxygen. An exogenous photosensitiser, which can be administered topically or intravenously, is concentrated in tumour tissue. The photochemical reaction occurs when the tissue is irradiated at a wavelength matching the absorption spectrum of the sensitiser. Molecular oxygen present in the tissue is converted to cytotoxic singlet oxygen, which causes the destruction of the malignant cells, while surrounding normal tissue remains unharmed (170).

Delta-aminolevulinic acid (ALA) is being used clinically for the treatment of skin cancer (78, 79, 171–173). It can be delivered topically or systemically and it can also be used for diagnostic purposes (78, 174, 175).

ALA is the precursor in the biosynthesis of haeme. Almost all types of cells in the human body are able to synthesise haeme. ALA is formed from glycine and succinyl CoA under the influence of the enzyme ALA synthase (ALAS). This step is regulated by the amount of haeme in the cell. At the end of the synthesis, iron is incorporated into the photosensitive protoporphyrin IX molecule by the enzyme ferrochelatase and haeme is produced. The first and the last steps of the haeme biosynthetic pathway take place in the mitochondria of the cell. The formation of ALA and haeme by the enzymes ALAS and ferrochelatase are rate- limiting steps. Provision of exogenous ALA bypasses the first rate-limiting reaction. The activity of ferrochelatase in tumour tissue is lower than in normal skin (176–178). Another enzyme, porphobilinogen deaminase (PBGD), which catalyses the reaction of porphobilinogen to uroporphyrinogen, shows higher activity in tumours (179). Due to these circumstances, PpIX is accumulated in tumour tissue. Another reason is the increased ALA permeability through intact tumour skin . It is known that hydrophilic ALA poorly penetrates intact healthy skin (171, 180).

Light source:

The light source used for ALA-PDT has to deliver the right amount of light at the proper wavelength. This could be achieved either by using a laser (78) or by using a noncoherent lamp (181). Light around 630 nm is often used but at depths over 2 mm, 635 nm light may be optimal (182). Although light in the Soret band (410 nm) would give the largest cell inactivation, it is seldom used because of poor penetration (<2 mm) (183). For easily accessible skin tumours there is theoretically no need for lasers, as their unique optical properties—coherence and nondivergence—are not required (80, 171). It might even be of advantage to use a broad band light source since photosensitising photoproducts with other absorption peaks are formed during PDT and this type of lamp could cover their spectrum (184).

PDT is suitable for superficial basal cell carcinomas, actinic keratosis (171, 185–187) and early stages of squamous cell carcinomas (80, 173, 185).

Recurrence rates

There are large differences in the recurrence rates depending on whether the tumour treated is primary or recurrent. Primary BCCs that recur will do so in the first 3 years after treatment in 66% of the cases while 18% of the recurrences appear between the fifth and tenth year after treatment with most modes of therapy. The 10-year recurrence rate is double the 2-year recurrence rate (95, 188). Five-year recurrence rates for different modes of treatment of primary and recurrent BCCS are shown in table I. The cure rate for MMS on BCCs larger than 3 cm in diameter drops to 93% (166).

Topical PDT has shown promising results as a treatment for superficial BCCs, with a clearance rate of 79–100% in several studies (78, 170–172, 189). Most studies show some decrease in cure rate after long-term follow-up. A large number of lesions have been treated by Warloe et al. (~400) and these writers present a short-term cure rate of 94% and a long-term (7–18 months) cure rate of 92% (189). A few studies show a larger decrease in cure rate, exemplified by Fink-Punches et al. with an initial cure rate of 86% which decreased to 56% after a median follow-up of 19 months (95 BCCs) (190).

The PDT cure rates for nodular BCCs after a single treatment are in the majority of studies quite low, with an average cure rate below 50% (80, 172). Repeated treatments seem to improve the cure rates (78).

The use of EDTA, DFO and DMSO can increase the cure rates (186, 189).

Interferon shows a cure rate of 86–100% (56, 155) when treating superficial and noduloulcerative BCCs. Follow-up

Table I. *Five year recurrence rate for different modes of treatment (95, 188)*

Type of treatment	Recurrence rate, primary	Recurrence rate, recurrent BCC (%)
MMS	1.0	5.6
Surcical excision	10.1	17.4
Curettage and electrodesiccation	7.7	40.0
Cryosurgery	7.5	13.0*
Radiation therapy	8.7	9.8

*Follow-up less than 5 years.

studies show recurrences in 5% after one year (130 lesions) (56) and in 2% after 5 and 10 years (110 lesions) (65).

Fluorescence imaging of BCC

Chromophores such as porphyrin have the ability to absorb energy when excited by light of a certain wavelength. Positioned at their excitation levels, the molecules strive to return to their non-excited level, giving away part of the energy as fluorescence when doing so. Imaging spectroscopy using digital cameras (CCD) can be used to study such physical processes. The fluorescence imaging technique extends our ability to see patterns that cannot be perceived with the naked eye (78, 175, 191 - 193).

Todays imaging spectroscopy is a field gaining importance mainly due to the use of digital cameras and the possibility of handling large quantities of information by computer technology. The equipment needed for fluorescence imaging is a light source for excitation, a digital camera for detection and the software for data processing. Many different types of digital cameras are available on the market. Examples of CCD-cameras for different fluorescence imaging experiments are; CCDs based on video technology, slow scan CCDs, back illuminated and intensified CCDs. The special range of a camera determines what parts of the spectra that can be recorded. The CCD chip has a spectral response, which is in itself limited by the band gap in silicon and is thus limited to the visible part of the spectral range, 400 - 800 nm. Outside this spectral range one has to use other techniques. By coating the CCD a possibility to extend the sensitivity to UV is given. In experiments with fluorescence imaging it is often desirable to record a smaller part of the incident spectrum. A way of doing this is to use glass filters. There are a variety of long-pass glass filters with relatively unsharp cut-off. Then there is also the possibility to choose high pass and band pass glass filters.

When using fluorescence imaging to determine spatial variation of the fluorescent species it is important either to control the light distribution or to use high light intensities in order to saturate the fluorescent species. One way of doing this is to ensure a homogenous illumination of the investigation-area. By using optical filters that select the exciting light for the simultaneously recorded normalisation image, the variation of the light distribution can be imaged.

Pp IX has the ability to fluoresce when illuminated with photoactivating light. This property is used for detection of tumour tissue. It is of importance to choose the proper light source and detection device so that a clear signal from the Pp IX is given as opposed to other fluorescing substances in the skin. Svanberg and co-workers have developed a system for this (175).

Microdialysis

Microdialysis, initially described in 1966 (194) is an established technique in neuro-physiology (195). The technique has more recently been extended to studies involving the gastrointestinal tract, heart,eye,adrenal gland, blood skeletal muscle and skin. It has been further developed for measurements in human tissue by Lönnroth et al (196).

Anderson et al. introduced it for dermatological use (197, 198). Here it has mainly been used for investigation of drug-penetration and to analyse the histamine concentration in the skin. Evaluation of the microdialysis methodology for the measurement of dermal interstitial fluid in humans has recently been studied by Krogstad et al (199).

A semipermeable dialysis catheter is placed in the tissue and perfused by a solution. Depending on the concentration gradient, molecules in the surrounding extracellular space which are present at a higher concentration diffuse into the dialysis fibre and the opposite takes place regarding molecules of lower concentration. Consequently, the catheter is being perfused with a fluid which equilibrates with the outside. Since the perfusate has a certain velocity, the concentration in the dialysate is not equal to the true interstitial concentration but represents only a fraction. This fraction is known as relative recovery and is defined as the relative concentration of a particular molecule in the dialysate. Several factors influence the recovery, including the physical and chemical properties of the dialysis compounds, the dialysis membrane, the composition of the perfusate, the flow rate of the perfusate and time dependence, among others.

Laser doppler perfusion measurments

Development of the laser made it possible to use the Doppler effect for assessing blood flow. The laser Doppler was introduced in the early seventies and later studied further (200, 201). Laser light has a high intensity, low divergence and is monochromatic. As laser light penetrates the skin some photons are absorbed and others backscattered. The back-scattered light from red blood cells is Doppler shifted in proportion to the velocity. This is detected by a collecting fibre and transformed into an electric output signal. Other factors, such as moving vessel walls also affect the readings.

A He-Ne laser Doppler measures the circulation in the reticular and superficial parts of dermis. There may be large spatial differences in blood flow (201). Accordingly, measurements with the old "one-spot" laser Doppler are subject to considerable variation. With the development of the Laser Doppler Perfusion Imager it is possible to record blood flow over a large surface and estimate a representative mean perfusion (202). Also, a colour-coded image is presented. This technique is now well established and has been used in a number of studies concerning cutaneous circulation.

2. AIMS OF THE INVESTIGATION

The aims of the thesis were:

- to study the effect of intralesional interferon on aggressive BCCs
- to evaluate the efficacy of photodynamic therapy of superficial BCCs
- to study the ability of ALA-induced fluorescence to delineate BCCs
- to study the pharmacokinetics of ALA penetration through skin
- to assess the results of MMS in Sweden in relation to international results

3. PATIENTS

Paper I

In our study one patient represents one BCC. Fifteen patients, 8 women and 7 men, with a mean age of 70 years (range 58–81), were treated with α-2b interferon. They all had histologically proven BCCs and were referred for Mohs micrographic surgery. Seven of the BCCs were primary (six morpheiform, one infiltrating) and eight were recurrent (four morpheiform, two superficial, two nodular), all located on the face. The tumours measured 7 to 25 mm in diameter. Before treatment, a complete history was obtained and a physical examination carried out. Routine baseline laboratory samples were taken.

Paper II

Thirty-seven patients were treated once with PDT. The median age was 65 years (range 28–83) and the patients represented 190 BCCs. Ten nodular BCCs in 6 patients with a median age of 75 years (range 62–75) and 18 Mb. Bowen in 5 patients with a median age of 81 years (range 61–83) were also treated. Some of the patients had multiple BCCs although not being patients with Gorlin's syndrome. All lesions were located on the trunk and histologically verified before treatment. The area of the lesions was determined from their length and width. The mean values for the areas of the different lesions were SBCCs 173 (20–1600) mm^2, NBCCs 1130 (100–6600) mm^2, and Mb. Bowen 103 (20–220) mm^2.

Paper III

Twenty-two patients, 14 women and 8 men, with a mean age of 65 years (range 45–87) with a total of 30 BCCs were investigated. All clinically visible lesions were measured before treatment. All tumours were histopathologically verified. There were 13 SBCCs, 11 MBCCs and 6 NBCCs. One patient represents one BCC except for four patients. Of these four patients, one had 6 SBCCs and one had 2 SBCCs, all located on the back. Another patient had two SBCCs, one on the cheek and one on the thigh, and the last patient had an MBCC and an SBCC located in the eye region. The fluorescence imaging of the initial 5 patients (lesions 1–12) was performed with an intensified charge coupled device (ICCD) and the following 17 patients (lesions 13–30) were imaged with a thermoelectrically cooled charge coupled device (TE/CCD).

Paper IV

The investigation group consisted of two women and six men with a mean age of 68 years (range 61–78 years). Altogether 18 areas were investigated, i.e. 14 SBCCs (2 BCCS on the arm, 11 on the trunk and 1 BCC on the thigh) and four normal skin areas.

Paper V

A total of 265 non-melanoma skin cancers from 1983 to 1992 were investigated. Thirty-seven of these were excluded from the material (5 squamous cell carcinomas, 3 Paget's disease, 1 recurrence after MMS, 8 tumours not radically excised and 20 tumours (7.5%) completely lost to follow up). Not counting the excluded tumours, 228 BCCs remained. The 221 patients (96 men and 125 women) presenting these tumours had a mean age of 68 years (range 24–90 years). Eighty-seven of the tumours (38%) were primary and 141 (62%) were recurrent BCCs. The recurrent tumours had previously been treated with cryosurgery, electrodesiccation and surgical excision. The patients all had BCCs with aggressive growth, i.e. infiltrative or morpheiform tumours and/or recurrent tumours. All tumours except 4 were located on the face.

4. METHODS

Paper I

A number of baseline punch biopsies were taken to verify the extent of each tumour. Altogether nine intralesional injections of 1.5×10^6 IU α-2b interferon were administered three days a week (Monday, Wednesday, Friday) during three weeks. Each tumour was injected with 0.15 ml, with the last five lesions (patients 11–15) given an increased volume of 0.50 ml keeping the same total dose. The first day of treatment the patients were observed for 2 hours after injection for adverse symptoms. After the following treatments they were observed for at least 1 hour. The efficacy evaluation was performed 1, 2, 3 and 6 weeks and 2 months after treatment by measuring the two largest perpendicular diameters of the tumour and comparing with baseline. The same investigator made all the measurements. The results were assessed as either 100% or 75% tumour reduction and everything below was considered lack of effect.

Laboratory samples were taken during and after treatment. Two months after treatment, MMS was carried out to determine the presence of residual tumour.

Paper II

ALA treatment

Cellulose gel with 20% ALA was applied topically over the tumour area with a margin of 10–20 mm. Then the treatment site was covered with plastic film for increased penetration. After 3 hours the area was irradiated with filtered light. The urine of the patients was examined for traces of porphobilinogen and porphyrin.

All lesions were clinically examined after 3 months and if there was any trace of the lesion a punch biopsy was taken. The lesions were also examined 6 months after treatment but only clinically. A yearly check-up for 5 years was initiated.

Grading for cosmetic results was based upon visual signs of erythema, skin texture and depigmentation. Lesions with no change in these parameters were graded as having "no side effects" and those with changes as having "side effects".

Light source (figure 2)

A xenon lamp that gave the required power level over a 30 mm diameter spot was used. It was an ILC 402 engineering prototype equipped with a CERMAX 300 W xenon lamp with an internal elliptical reflector. An interference filter with a pass-band of 620–670 nm was used for the output light. The irradiance was adjustable, with a maximum value of 300 mW/cm^2 at 40 mm target distance from the source. By

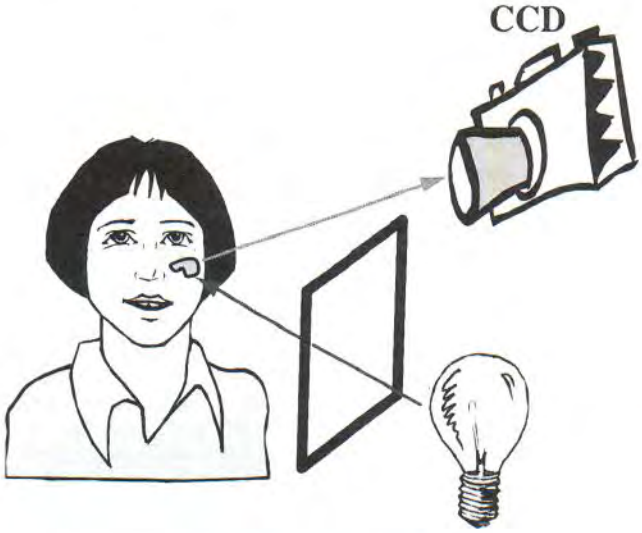

Fig. 1. Overview of the spectroscopic technique.

Fig. 2. The Xenon lamp used for PDT.

scanning the cross-section of the output beam, using a small silicon photodiode with a 1 mm active area, the brightness distribution at the target site was checked. An acceptable distribution over the circular treatment area of 700 mm^2 was found. An International Light radiometer with a "321" silicon detector head examined the irradiance at the target. The lamp was placed 40 mm away from the treatment area.

The light doses given were 75 or 100 J/cm^2 delivered over 10 min in one exposure, producing an irradiance of 125 mW/cm^2 and 166 mW/cm^2.

Paper III

ALA application (figure 3)

A cellulose gel containing 20% delta-aminolevulinic acid was applied to the skin area of investigation 4 hours before fluorescence imaging. The site was covered with plastic film for occlusion.

Digital fluorescence imaging (figure 4)

A bandpass glass filtered mercury lamp was used as a light source with three peaks as emission spectrum. These three peaks at 365, 366 and 405 nm are depicted in figure 5.

The CCD camera recording the fluorescence images had a longpass filter letting only wavelengths longer than 590 nm through (figure 6).

The light source and the CCD device were fitted with the filters to match the emission and absorption of Pp IX. The fluorescence images were analysed with a computer program

Fig. 3. Application of ALA-gel.

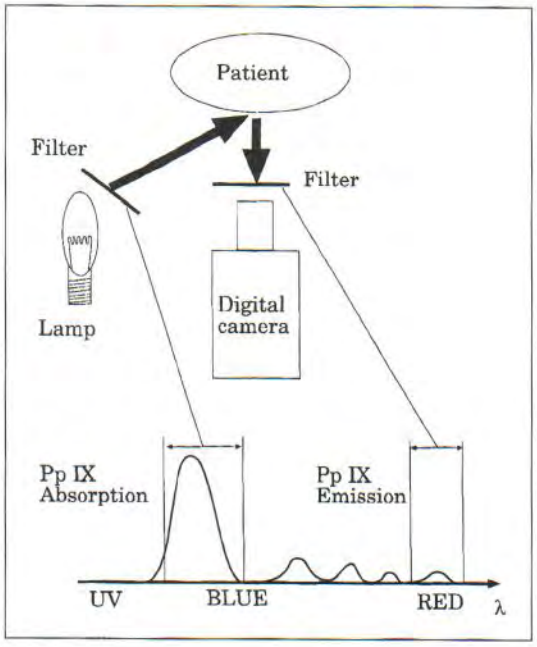

Fig. 4. Schematic set-up for recording fluorescence images.

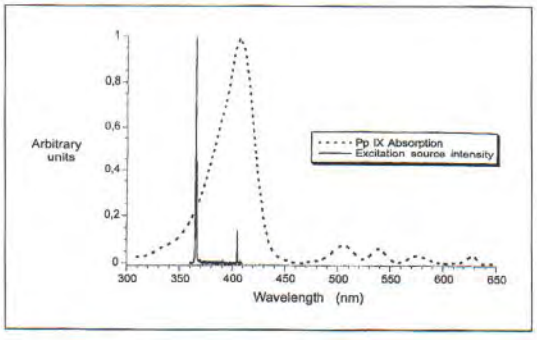

Fig. 5. Emission spectrum of the filtered mercury lamp together with the absorption spectra of Pp IX.

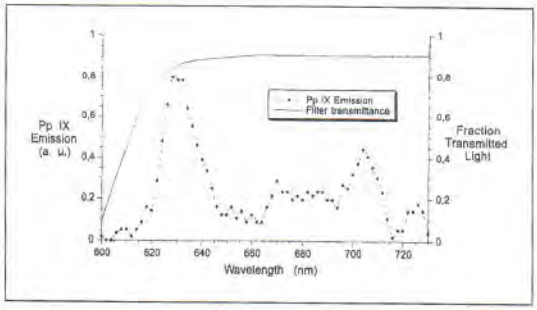

Fig. 6. Transmission characteristics of the long-pass filter together with the emission spectrum of Pp IX.

using the threshold criterion for tumour fluorescence as 40% or more of the mean fluorescence in the ALA area treated. The first 12 lesions were examined using an intensified CCD and for the following 18 tumours we used a slow-scan CCD. We achieved much better imaging properties with the non-intensified CCD and the fluorescence intensity was strong enough. The imaging quality of this camera facilitated the interpretation of the images, as the fluorescence images resembled black and white photographs.

Histological mapping

Lesions 13 to 30 were excised using MMS without preceding knowledge of the fluorescence results. The lateral extensions of the tumour were determined and a histological map was drawn. According to this map, the tumour extent was marked with a black line on the photograph taken before surgery and this was later compared with the tumour extension as given by the fluorescence image.

Lesions 9 to 12 were also mapped microscopically after excision. Mohs fresh frozen technique was not used; instead, each specimen was fixed and the tissue cut vertically.

Paper IV

Microdialysis procedure (figures 7 and 8)

Catheters for microdialysis were inserted into the skin. No local anaesthesia was used. The catheters were all placed in the dermis and the depth was checked by ultrasound measurements. Each end of the catheter was glued to a polyethylene tube and to one end of the tubes a pump was

Fig. 7. The microdialysis catheter inserted into the skin.

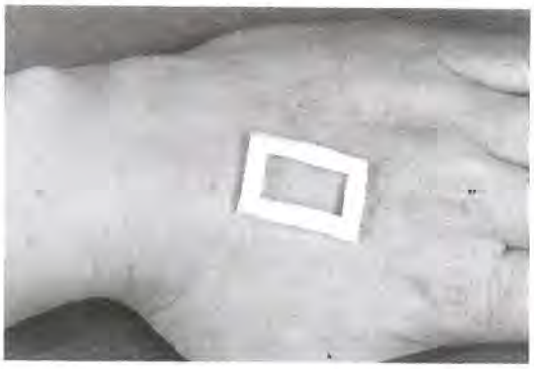

Fig. 8. The Duoderm frame used for ALA microdialysis.

connected, perfusing the system at a constant rate with isotonic saline containing 1.5 mmol/l glucose. "Light" curettage of the basalioma skin was performed before insertion of the catheters using a Thiemann curette, causing no bloodshed.

Eighty to 90 min after insertion, the catheters were flushed and perfused for 10 minutes before the dialysate collection started. The first microdialysis sample was collected during the time interval from 80 to 110 min after the needle insertion. Self-adhesive frames were cut out of a hydrocolloid dressing (Duoderm) and fastened to the skin between the inlet and outlet of the microdialysis membrane. Into these frames, the cellulose gel containing 20% ALA was applied after sampling of the first microdialysate. The gel was occluded for 210 to 240 minutes throughout the period of sample collection. The investigation was performed with the patients in a supine position after a 30 min acclimatisation period in a room kept at 27°C, humidity 70%.

The dialysates were stored immediately at −20°C until analysis. ALA and amino acid concentrations were analysed with an ion exchange high performance chromatograph where the detection limits for ALA was 5 μmol/l.

Two sets of experiments

First a pilot experiment was carried out to investigate the effect of illumination of ALA-treated basal cell carcinomas. Two BCC areas received standard PDT. A third BCC served as a control and here ALA was applied but no illumination took place. The ALA concentration before and after PDT was investigated. The perfusion rate was 1 μl/min and a xenon arc lamp was used for illumination.

In the other experiment the ALA concentration in 11 BCCs and 4 normal skin areas was examined. The perfusion rate was 2 μl/min and samples were collected every 30 minutes.

Calculation of recovery

In order to calculate the interstitial dermal ALA concentration as correctly as possible, certain assumptions had to be made. The catheter was calibrated in situ based on the hypothesis that the relative dialysis recoveries (RR = dialysate concentration/interstitial concentration) of two substances are constantly and proportionally related regardless of the ambient medium. In in vitro measurements in plasma at 37°C to which known concentrations of ALA were added, the concentrations of ALA and amino acids were calculated. The

relative recovery was calculated to 0.18 for ALA and 0.23 for amino acids, respectively. The interstitial concentration of each substance was then calculated using the formula:

$$\text{RR (Urea) in vivo} = \frac{\text{Dialysate urea concentration}}{\text{Plasma urea concentration}}$$

$$\left.\frac{\text{RR (ALA, aa)}}{\text{RR (Urea)}}\right\} \text{in vitro} = \left.\frac{\text{RR (ALA, aa)}}{\text{RR (Urea)}}\right\} \text{in vivo}$$

$$\text{I (ALA, aa)} = \left.\frac{\text{Dialysate (ALA, aa) concentration}}{\text{RR (ALA, aa)}}\right\} \text{in vivo}$$

Laser Doppler perfusion imager

Four healthy skin areas and eleven BCCs were investigated with a laser Doppler- scanning perfusion imager. The skin perfusion data were gathered directly before the microdialysis investigations and before curettage.

The method gives a non-invasive measurement of the microcirculatory blood flow and the principle is to measure the Doppler shift of the red blood cells. A computer-controlled optical scanner directs a low power He-Ne laser beam (0.6 mm wide), which moves over the skin surface at a distance of 17 cm. It is assumed that the emission light penetrates the skin to a median depth of 0.2–0.3 mm. The reflected light is related to tissue per-fusion and the mean perfusion was calculated from a 7×7-pixel area where the blood flow was at its maximum with the distance between impending pixels being 0.8 mm.

Statistical evaluation

Calculations of the ALA concentration were based on data from 14 investigated superficial basal cell carcinomas and 4 healthy areas. Perfusion values were expressed as percentage changes relative to baseline (control = 100%). The statistical significance of differences was tested with the two-tailed Student's t-test for unpaired data.

Differences in ALA concentration between sequential measurements were tested with ANOVA followed by Duncan multiple range tests. P-values <0.05 were considered statistically significant.

The data were checked for normality before performing parametric tests.

Paper V

Skin tumour clinic

All dermatological patients referred with advanced non-melanoma skin cancer on the face are seen by our MMS team. These patients are inhabitants from the western and southern parts of Sweden. The team includes one general surgeon, one dermatologist, one dermatopathologist, one nurse, one nurse's aide and two histotechnicians. A number of patients are seen but only about 30 patients were operated upon per year initially. Since 1994, when we introduced the single section technique, which speeded up the procedure, we operate upon roughly 60 patients a year. Because of our limited resources for MMS, a hard selection has to be made. That is why only complicated forms of BCC i.e. recurrent tumours, infiltrative and/or morpheiform-growing tumours, many of which are located in the H-zone of the face, come into question.

MMS technique

Our MMS technique is modified such that the surgeon and the pathologist are different persons. MMS is usually an outpatient procedure with the patient being able to leave the hospital at the end of the day. Surgery is performed under local anaesthesia using long-acting bupivacaine 0.5% (Marcain®) with adrenaline for infiltration. The tumour area is carefully inspected and a margin of 1–2 mm round the tumour is imprinted with ink. Debulking of the tumour is done when necessary. The area is then excised holding the scalpel at an angle of 45°, resulting in a bowl-shaped piece of tissue. For orientation, marks are made at the 3, 6 and 9-o'clock position both in the removed specimen and in the remaining wound. A suture is placed at the 12-o'clock position in the resected tissue and a corresponding stitch is left in the normal skin of the patient some centimetres above for direction if more excisions are necessary. Computerised bipolar diathermy is used for haemostasis.

At the adjacent Mohs laboratory, the epidermal margin is pushed down and brought into the same level as the deep margins before sectioning. When necessary, the specimen is divided into pieces suitable for frozen sectioning and all edges are colour-coded for alignment (Davidson Marking System, Bradley Products, Inc. Bloomington MN, USA). The frozen tissue is cut in 5–7 μm horizontal sections from the bottom and the slides are stained with hematoxylin and eosin. The pathologist notes residual tumour areas on the hand-written map. Repeated layers are excised at the precise location. The stepwise procedure is continued until all cancer is removed.

All patients are followed for 10 years, more frequently in the beginning. When there is a need for dermabrasion or intralesional steroid injections, this is performed within 2–3 months.

Statistical evaluation

The Kaplan-Meier model was used for the statistical analysis of the recurrence rate (203). All patients were followed for 5 years after surgery with considerations taken to the deaths that occurred during this period (26 patients) and to the patients that were not followed for the whole period (13 patients). For detecting possible risk factors, Fisher's exact test was carried out. The parameters tested for differences in recurrence rate after MMS were: different regions (temple, nose, forehead), primary or recurrent BCC, age (below or above 70) and number of excisions (≤ 2 vs. ≥ 3).

5. RESULTS

Paper I

The histological mapping indicated that in 9 out of 15 (60%) patients there was a total or 75% effect of interferon. Four patients showed no remaining tumour and 5 patients had a tumour reduction of 75%. Six patients had only a small or no

effect of interferon. The most common adverse effects were mild flu-like symptoms with fever and myalgia. Five patients presented swelling and/or erythema at the injection site. Three patients had a slight but reversible leukopenia during treatment.

Paper II

When treating with PDT once, we achieved a 92% clearance rate for SBCCs, a poor effect of 20% for the thicker NBCCs and 78% clearance for Mb. Bowen.

The patients were checked after 3 and 6 months and during this time 157 skin areas were controlled after SBCC treatment. With a dose of 75 J/cm^2, 88% (74/84) of SBCCs cleared and with a dose of 100 J/cm^2, 96% (70/73) of the lesions cleared. Combining the two dose regimens, 92% (144/157) of the lesions cleared.

Of the NBCCs, only 22% (2/9) cleared with a dose of 75 J/cm^2 and at 100 J/cm^2 none cleared (0/1). The two doses combined gave 20% (2/10) clearance. None of the above figures indicated any statistically significant difference.

Mb. Bowen showed 43% (3/7) clearance at 75 J/cm^2 and at 100 J/cm^2 100% (11/11) clearance. Here there was a statistically significant difference. Both dose regimens together gave 78% (14/18) clearance.

The cosmetic outcome was generally very good.

No local anaesthesia was given during treatment and only four patients complained of stinging in the lesion during irradiation.

Urine samples from 3 patients indicated reversible traces of porphyrins.

Paper III

A histological map was made of a total of 22 lesions and a good correlation between the fluorescing tumour area and this map was found in 11 BCCs (50%). Of these, 3 were primary SBCCs (1 chest, 1 thigh, 1 back), 4 were primary MBCCs (1 nose, 1 cheek, 2 eye region), one was recurrent MBCC (ear) and 3 were recurrent NBCCs (2 eye region, 1 nose). In 5 (23%) of the tumours there was a partial correlation. In this group there was one primary SBCC (cheek), one primary MBCC (nose), 2 recurrent MBCCs, (1 eye, 1 nose) and one recurrent NBCC (nose). Finally, in 6 (27%) BCCs there was no correlation at all. These tumours were 1 primary SBCC (eye), 3 primary MBCCs, (2 nose, 1 cheek) and 2 recurrent NBCCs (1 eye, 1 cheek).

Eight primary SBCCs on the back were verified by punch biopsies after fluorescence imaging. Two of these were diagnosed by the fluorescence method itself and could not be seen clinically prior to investigation.

Paper IV

The ALA-concentration was rapidly increased in tumours treated with ALA. Within 15 minutes of local application, the tumour interstitial ALA concentration had risen to 3.1 mmol/l from the original zero. In healthy skin no measurable amounts of ALA were found. The interstitial concentration of amino acids was the same in tumour tissue and in normal skin.

The two BCCs that were illuminated after ALA treatment showed a decrease in concentration of interstitial ALA after illumination. The concentration fell to one third of its maximum level, while it was stable in the non-illuminated BCC. The amino-acid levels did not change at all.

The investigation with the laser Doppler perfusion imager revealed a 2.5-fold blood perfusion increase in basal cell carcinomas.

Paper V

After MMS, 18 out of 228 BCCs recurred during the 5-year follow-up period. Five of the recurrences were in primary BCCs, which gave a 6.5% recurrence rate when applying the Kaplan-Meier model. According to the same statistical method, there was a 10% recurrence rate regarding the recurrent BCCs. A tendency to more recurrences was seen in the temple region. There was no significant difference when age (above or below 70) was considered. In respect of recurrences and number of Mohs layers, there was no significant difference. The cosmetic and functional result was assessed, 12 months after surgery indicating that 8 (3.5%) out of 17 (7%) wounds that healed with a skin defect required plastic surgery for reconstruction.

6. DISCUSSION

Methodological considerations

Diagnostic procedures

Paper III

There are many pitfalls when using fluorescence spectroscopy.

In order to enhance ALA-penetration, the treatment site was covered with plastic film for a number of hours. The plastic film was covered with a thick, homogeneous white dressing in order to let as little light as possible through the Band-Aid. A better way of doing this would have been to cover the area with aluminium foil.

The filters on the CCD-camera were chosen to match the excitation and emission wavelengths of Pp IX. The cut-off point for collecting fluorescent light was at 590 nm. The sensitivity of a CCD-camera is low above 750 nm.

The exposure time used for the images was 2 seconds and with the patients lying on a bed there were no problems with unsharp pictures (figure 9).

The area has to be evenly illuminated and, especially in the face, curvatures etc can affect the result. Often, fluorescence is at its maximum on the vertex of an investigated area. This is

Fig. 9. Fluorescence imaging recording.

important on the cheek, the eyelids and the nose. Of course, this plays a minor role on the trunk. The uneven illumination is important in the normalisation process.

In our work we used the mean fluorescence intensity in the entire ALA-treated area as the normalisation value. This is a simple way to normalise but it supposes that the tumour area is small compared to the whole ALA-treated area. If the tumour is large relative to the total area applied with ALA, the sensitivity of the method declines. Another approach can be to compare the fluorescence of the tumour area with that from a healthy area using the same angle of incident light. This method may have a higher degree of precision.

Sebaceous glands fluoresce and this is a disturbing factor (204), especially on the nose. Actinic keratoses and Mb. Bowen also fluoresce, as do areas with sun damage. However, an experienced observer can most often tell the difference between an area of solar damage and a BCC. On the other hand, necrotic material in wounds of ulcerative BCCs does not produce any PpIX and no fluorescence is observed here. The fluorescence technique is convenient and promising but the observer needs to be trained and experienced.

A histological map was made after Mohs surgery in each patient and it was measured and compared with the size judged after fluorescence. Three different modes were used, punch biopsies (lesions 1–8), vertical (lesions 9–12) and horizontal sectioning (lesions 13–30). The specimens were re-embedded in paraffin for safer evaluation. The excised tissue shrinks after surgery and this has to be taken into account when performing the measurements. We estimate the precision of the method to be ± 3 mm. The precision of the method can also be affected if the recording is not entirely perpendicular to the surface. This can underestimate the size of the fluorescent areas.

The reproducibility of the histology can be affected by areas of inflammation in the specimen, fixation technique etc.

Paper IV

The microdialysis technique has recently been introduced in dermatology. Several factors can affect the result.

The procedure is done without using local anaesthetics. The perforation of the skin itself leads to an axon reflex and the liberation of vasoactive substances. In the present microdialysis study, however, we allowed the patient to rest for 80–90 minutes before commencing the actual study. After this, the catheters were flushed and perfused for 10 minutes before the dialysate collection started.

The depth of the membrane was estimated by means of 20 MHz ultrasound. The technique was found to be easy to use and reproducible.

The type of dialysate membrane is also of great importance. In the present investigation, a membrane with a 6 kD cut-off was used. The length of the membrane was 30 mm with an external diameter of 0.3 mm.

The tissue equilibrium is affected by the composition of the dialysate. It usually contains small amount of glucose in order not to reduce the tissue glucose concentration. Urea is regarded as a reference molecule in this case. It is probably appropriate as both urea and ALA are water soluble and of approximately the same size.

The perfusion rate is also of importance. In the present study, two different perfusion rates were used, 1 µl/min and 2 µl/min. The reason for choosing 1 µl/min was that we did not know the kinetics of ALA in the dialysate and did not want to lose any information. The different perfusion rates are probably of minor importance for the results. Using 2 µl/min makes it easier to follow rapid changes in the dialysate concentration.

As a whole, there was a rapid increase in ALA concentration in the tumour area. In one case, the ALA concentration increased in healthy skin. This was probably due to leakage from the ALA chamber.

Light curettage, causing no bleeding, was performed to mimic the clinical situation. It is reasonable to assume that this enhances penetration of ALA. The importance of this has to be studied further. Interestingly, healthy skin without curettage showed no increased penetration of ALA.

The typical diameter of a BCC in the present study was less than 1 cm. The dialysate membrane has a length of 30 mm. Consequently, the dialysate membrane collects ALA from a larger area than the BCC. However, the penetration of ALA in healthy skin is very low. Possibly, the values should be multiplied by an unknown factor to compensate for this. As the factor is unknown, we chose to publish raw data.

The detection limit of the ion-exchange chromatography was 5 µmol/l, which is very sensitive.

In one set of experiment ALA was applied over 3 SBCCs, and removed after 200 minutes. Two of the areas were illuminated with a Xenon arc source with emission maximum at 630 nm with a dose of 100 J/cm^2. One tumour served as a control and was not illuminated. Two and a half hours after irradiation the interstitial ALA concentration decreased to 1/3 of its maximum whereas the ALA concentration was stable in the non-illuminated control area. This finding indicates that extracellular ALA was consumed during and/or immediately after illumination. It is unlikely that the finding is related to tissue aberrations induced by the microdialysis catheter, since the concentration of amino acids was approximately the same in tumour and normal skin and not altered by irradiation.

Too few patients were studied in this experiment and further investigations have to be done.

The laser Doppler measurements revealed a 2.5-fold increase of the perfusion in BCCs compared to healthy skin. The technique is well established and superior to conventional laser Doppler as an entire area is measured. Measurements were made directly before insertion of the microdialysis needle.

Treatment procedures

Paper I

Interferon was injected intralesionally at a volume of 0.15 ml per BCC, not considering the fact that the lesions were different in size. The overall impression after treating 10 patients was that the volume was too small and did not reach all tumour tissue. The injected volume was therefore increased to 0.5 ml with the same total dose of IFN and administered to patients 11–15. The material is too small for statistical evaluation of the different volumes given.

One might argue that with i.l. injection of an active substance all tumour tissue would not be reached and some tumour cells left untreated as islands can later cause recurrences. Therefore, a follow-up was made after 7 years

and only 2 recurrences were documented, the first after 1 year (recurrent, morfeiform BCC in the temple) and the second after 4 years (primary, morfeiform BCC on the nose). One patient was not able to come after the 3-year check-up but showed no recurrence at this visit. Two patients died after 3.5 and 4 years, respectively, and had no recurrences just before this. Ten of the patients were followed for 7 years and no residual tumours were observed.

We also took punch biopsies before treatment and one can discuss whether or not this influences the therapeutic outcome. Others have found a high cure rate in placebo-treated lesions and this could indicate that either biopsies and/or injections with ineffective substances play a role in the healing process (205, 206).

A success rate below 75% was considered to be a failure. It is hard to differentiate between different outcomes below this level.

Paper II

Thirty-seven patients with a total of 190 SBCCs received PDT. It can be questioned whether or not the number of patients successfully treated should be counted or the number of lesions. However, we have no indication that SBCCs differ from patient to patient; other factors such as location seem to play a more important role.

The dose levels in the study were 75 and 100 J/cm^2. The dose was increased in the middle of the study. The power output of the lamp was varied. For Mb. Bowen 100 J/cm^2 seems to be better. It is not known whether or not it is the dose itself or the power output that plays a role. Other authors have used different doses but these are not necessarily comparable as the emission spectrum of the treatment lamp and the sensitivity spectrum of the meter used for determining power output influence this.

The reproducibility of the power output of the lamp was rather stable. No special delivery system was used. Thus, some areas were hard to irradiate and the patients had to be placed in uncomfortable positions.

The radiation field was rather small, with a 30 mm diameter spot. When treating larger lesions, we had perform multiple irradiations and there may theoretically be a risk of overlapping or insufficient treatment.

The effect of storage of ALA was investigated and ALA seems to be stable for at least 14 days. In all the studies, ALA was kept in the refrigerator and was used within one week.

The same person made all the applications of the ALA before treatment. Uneven uptake of ALA can play a role in areas with a defective barrier. This can occur in areas with loss of stratum corneum. In this series, no curettage was performed. Warloe has discussed the importance of curettage (189).

In Paper II many relatively thick SBCCs were treated. The exact thickness is hard to evaluate without ultrasound. The thickness was assessed clinically. The healing rate would probably be higher if light curettage had been done prior to therapy and some other therapy chosen for the thicker lesions. It seems that the penetration of ALA is the limiting factor when performing PDT, as red light has a good penetration.

Also, PDT was only performed once. In other studies, the lesions often receive multiple treatments. This leads to a higher cure rate (78).

In a 4-year follow-up of our material in this study, we found a 79% cure rate (138/175). The BCCs were clinically controlled and biopsies were performed if there was suspicion of recurrence.

Paper V

In Sweden there is very limited access to MMS. Hence, the patients referred to our skin tumour clinic are highly selected. The largest tumours are probably identified primarily and referred to plastic surgeons. In the present study, BCC patients having tumours with ill-defined borders have been treated. Both recurrent and primary tumours have been operated upon. Among the primary tumours, a morpheiform and histologically aggressive growth pattern dominates.

At follow-up we regard all new tumours in the area previously treated with MMS as recurrences. Of course, we cannot rule out that the "recurrence" is a new primary tumour. This is especially important, as we know that 40% of BCC patients develop a new BCC within 5 years.

In the present study, we have followed the patients for 5 years. The recurrence rate would probably increase when studying a 10-year period (188).

Since 1994 we usually use the single section technique. This is a safer and more rapid procedure. It minimises the risk of disorientation of the specimen.

Traditionally in MMS, the surgeon and the pathologist are the same person, i.e. the Mohs surgeon. In our clinic, we are fortunate to have dermatopathologists specialised in interpretation of horizontal sections. This probably leads to a higher quality level compared to when a surgeon reads the MMS-slides.

General discussion

Basal cell carcinoma is the most common skin cancer among white people. The cancer registry in Sweden does not record BCC but the estimated annual incidence in Sweden alone is 15.000 and the rate is steadily rising (5, 207). The treatment of BCC consumes considerable medical resources. It is therefore of great importance to have as efficient methods of treatment as possible. This makes the investigation and evaluation of new diagnostic and treatment procedures necessary.

Diagnostic procedures

Paper IV

A major problem when using ALA for PDT or fluorescence is the penetration of the substance. This seems to be the limiting factor as the penetration of red light is fairly good. Thus, measurements of the ALA concentration in tissue is of great interest. In recent years microdialysis has evolved as a new elegant way of doing this. In the present study, we have measured the ALA concentration in the tissue with the microdialysis technique. It turns out that the penetration in tumour areas is rapid and after 15 minutes the concentration is high and stable. On the other hand, virtually no ALA penetrates healthy skin. However, it should be remembered that light curettage causing no bleeding was performed in the tumour areas. This probably affects the results but, on the other hand, we mimic the clinical situation. The ALA uptake in skin after curettage would be enhanced as crusts are removed and the stratum corneum is diminished. The light

curettage would also ensure a more even uptake. The exact consequence of curettage needs to be investigated further.

The penetration kinetics at various depths also need to be clarified in further studies. In the present study, some amino acids were measured. Taurine is often associated with tissue damage but the concentration of taurine was not altered after microdialysis (208–210). A reasonable interpretation is that the microdialysis technique itself does not damage the tissue significantly. The concentration of taurine was also not altered after illumination despite the fact that irradiation leads to tissue damage. Tissue damage probably occurs later in the event.

The laser Doppler-imaging technique revealed that the perfusion in BCCs is 2.5-fold higher than in normal skin. This could lead to a situation where topically applied ALA is washed away, reducing the tissue concentration of ALA and Pp IX. Due to the pain during therapy, EMLA is often applied. As EMLA causes vasoconstriction, this can possibly increase the tissue concentration of ALA and Pp IX and lead to a better therapeutic response. This has to be investigated further, though.

Wang and co-workers have investigated the perfusion in superficial basal cell carcinomas and there was an increased blood perfusion after topical PDT (211)

Paper III

Morpheiform BCCs often have ill-defined borders. So far, the best therapeutic method is MMS. However, MMS is more time-consuming than routine excision. Consequently, it would be of great help to have a technique for better delineation of this type of tumour. It has been known for a long time that applying ALA to skin tumours makes them fluoresce. However, it is not until recently that this technique has been further developed (78, 175, 212).

In the present investigation, we found good demarcation in 50% of BCCs with ill-defined margins. In another 23% there was partial agreement.

A major advantage of the fluorescence technique is of course that it can be used in sensitive areas of the face, i.e. the H-zone, as the tissue sparing is of utmost importance in these delicate areas. Fluorescence measurements can also be made before treatment with cryosurgery and radiotherapy. At present, the method cannot replace MMS but it can reduce the number of excisions necessary for complete tumour eradication.

The detection of tumour tissue by imaging Pp IX fluorescence is based on the fact that topical ALA-application results in tissue-specific photosensitisation where BCC is one type of lesion that gives rise to increased Pp IX fluorescence (170, 171, 191, 213). The question as to whether the Pp IX concentration is higher in cancer cells than in normal cells has been addressed by several authors. Martin et al. (214) reported that there was no selectivity for Pp IX fluorescence in tumour tissue vs. normal skin as seen with fluorescence microscopy. Szeimies et al. (215) observed a low fluorescence yield from MBCCs in a fluorescence microscopy study.

Also, the penetration of light into different tissues varies. It is reasonable to believe that light penetration in dense tumours such as morpheiform BCCs is less than in superficial BCCs.

The technique needs further improvement and other derivatives than ALA can possibly be more useful as the penetration of ALA is not optimal. Only the superficial extension of a BCC can be visualised. The ALA ester p-1202 seems to be more specific and penetrates more deeply.

Treatment procedures

Paper I

Regarding IFN-treatment of BCC, it would have been interesting to perform a new study investigating larger doses and volumes. In another study, eight large BCCs were effectively treated with increasing doses of IFN as a function of the surface area of the tumour (156). When giving larger doses of IFN, one must also consider the fact that there is usually an increase of adverse effects and that mild flu symptoms could develop into severe.

Cornell and Greenway et. al. (56) have looked into different dose regimens and found the optimal regimen to be the one used in our study. Smaller doses of drug and/or fewer injections reduced the cure rate. They (56) also gave i.l.α-2b-IFN to a total of 172 patients with noduloulcerative (largest dimension between 0.5–1.5 cm) or superficial BCCs (largest dimension between 0.5–2.0 cm). Forty-two of these patients received placebo. The cure rate, which was determined by investigation of punch biopsies performed before and after treatment, was 86% for the IFN-treated lesions and 29% for the placebo group. The same two groups were examined after one year and 81% and 21% remained tumour free, respectively.

IFN has a definite effect on BCCs, especially the superficial and noduloulcerative types. For more aggressive lesions, IFN is a good adjuvant to, for example, surgery, but sufficient volumes are required. A sustained-release preparation of IFN has successfully been tried but there are no data on its efficacy on aggressive tumours (158).

The treatment schedule with injections every other day for three weeks is tiring for the patients and also time-consuming. To have to endure flu-like symptoms with fever and myalgia every other day for three weeks is also a strain on the patients.

The ideal IFN-treatment for BCCs would be a sustained release preparation which primarily had a local effect, minimising systemic symptoms. In the future, IFN intralesionally could be a valuable therapy in order to reduce the number of excisions required in MMS.

Paper II

When PDT is applied to superficial and/or large BCCs the method is efficient. We also obtained an excellent cosmetic result in most cases. In many patients, it was impossible to locate the exact origin of the treatment area. This is of course a very crucial aspect in regions of the body where a scar can be quite disfiguring. Examples are keloid-prone areas such as the chest, shoulders and back, especially on patients with multiple SBCCs.

Unfortunately, the effect of PDT on thicker lesions such as NBCCs is poor. This is probably due to insufficient ALA penetration as red light penetrates into the skin sufficiently (216). For thick lesions, fractionated treatment with a partial light dose, adding ALA between each treatment session, need to be studied. One could also treat the thicker lesions repeatedly, with few side effects, instead of only once as in our

study (78, 170). The optimal light dose seems to be individual and it is clinically hard to tell which dose is optimal for which patient. Measuring the fluorescence of Pp IX immediately before treatment can be a way of optimising the therapy.

Peng and co-workers have reported enhanced ALA penetration in BCCs when treating patients with ALA cream containing DMSO and EDTA, more ALA-induced porphyrins were also seen (191). DFO, which is an inducer of porphyrin synthesis, can increase PDT cure rates (186). The penetration of ALA into deep layers of BCC lesions could be increased by prolonging the time of topical application of ALA (215). Warloe has also tried a curettage procedure to reduce tumour volume and remove the surface structure of 152 nodular tumours before PDT and achieved an 85% cure rate (81).

The PDT-group at the Radium Hospital in Norway has studied lipophilic ALA esters (217, 218). These penetrate more easily into cells and deeper into tumours than ALA. Such ALA derivatives are de-esterified by esterase in cells and tissues. Clinical trials with ALA esters show a more homogeneous distribution and a better selectivity than induced by ALA (81). ATMPn has been tried recently (219). The penetration seems to be good.

Comparing laser versus non-coherent light sources, there might be less pain involved when treating with laser (220). A laser is also a better choice when there is a need to convey the light using optical fibres, since the collimated light can easily be collected in a fibre. However, eye protection is mandatory as opposed to non-coherent light, as the high-intensity collimated laser beam can cause eye injury. There seems to be no significant difference in the therapeutic effect of PDT when using a laser and a non-coherent light source (81). The latter is also relatively cheap, simpler to use and well suited for treatment of easily accessible lesions in the skin (80).

Paper V

Sweden is underserved regarding MMS. Less than 1% of all BCCs are treated with this method. In the USA, 30% of BCCs are treated with MMS (221). Thus, MMS should be performed at more centres in Scandinavia. It has been said that MMS is time-consuming and expensive. It has recently been shown that MMS is also cost-effective. The average cost for one MMS treatment in our clinic is 7000 SEK. The price in the US and Sweden seems to be of the same order of magnitude (222).

The lack of facilities for MMS leads to a highly selected patient material. In view of this, one would expect that the recurrence rate would be higher compared to other centres. To some extent this is also the case.

BCCs on the face can be treated with several other methods, i.e. cryosurgery, conventional surgery and radiation. Treatment of skin cancer on the face is a multidisciplinary issue and good co-operation with ENT specialists, general surgeons, plastic surgeons and oncologists is advantageous.

7. CONCLUSIONS AND OUTLOOK FOR THE FUTURE

During recent years there has been a dramatic increase in the incidence of basal cell carcinomas (BCC). Most BCCs can be cured with conventional methods. Large superficial BCCs and tumours with ill-defined borders present a challenge.

1. Treatment with alfa-2b-interferon (IFN) gives a tumour reduction of 75% or more in 60% of the patients and could serve as an adjuvant to Mohs Micrographic Surgery (MMS). However, IFN treatment has troublesome side effects.
2. The results also verify that photodynamic therapy (PDT) is a very good alternative to other treatments for superficial basal cell carcinomas and Mb Bowen. The cosmetic outcome is generally very good and the side effects are few. Nodular basal cell carcinomas are not suited for PDT as the penetration of delta-aminolevulinic acid is not deep enough.
3. Our findings also demonstrate that the selectivity is high concerning ALA-induced tumour fluorescence. This implies a safer delineation of morpheiform and other BCCs with ill-defined borders before therapy. MMS procedures can be facilitated. However, the method has to be developed further as there is total agreement between fluorescence and histology in only 50% of the cases.
4. For the first time, the microdialysis technique is used to show that ALA rapidly penetrates the skin of basal cell carcinomas while the penetration through healthy skin is negligible. ALA is also consumed during irradiation. Laser Doppler Perfusion Imaging reveals that the blood flow in BCCs is 2.5 times higher compared to normal skin.
5. The use of MMS is limited in Sweden compared to other countries. As a result, more severe cases with diffuse borders are operated upon in Sweden as opposed to the USA. The recurrence rate is accordingly slightly higher in our country than in the USA. Our results clearly indicate the value of the technique.

In the future, the fluorescence technique has to be optimised. Further microdialysis investigations revealing the concentration of ALA at various depths have to be performed. New compounds with better tumour selectivity and penetration characteristics will be introduced.

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