Cutaneous side effects from laser treatment of the skin: Skin cancer, scars, wounds, pigmentary changes, and purpura

– use of pulsed dye laser, copper vapor laser, and argon laser

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LIST OF ABBREVIATIONS AND DEFINITIONS

Laser systems:

AL	Argon laser; continuous wave, 488 nm/514 nm (blue-green), 514 nm (green).
CO_2 laser	Carbon dioxide laser; 10,600 nm (infrared).
CVL	Copper vapor laser; quasicontinuous, 578 nm (yellow), 511 nm (green).
PDL	Flashlamp pumped pulsed dye laser. Initially the PDL was used at 577 nm (yellow), later it was changed to 585 nm (yellow).

Ultraviolet radiation:

MED	Minimal Erythema Dose in an individual.
SED	Standard Erythema Dose. SED is today the recommended unit to express the erythemogenic potential of a UV dose (Diffey et al. 1997).
Solar UV	Broad spectrum simulated solar ultraviolet radiation.
UV	Ultraviolet radiation (200-400 nm).
UVA	Ultraviolet radiation in the A range (321-400 nm).
UVB	Ultraviolet radiation in the B range (281-320 nm).
UVC	Ultraviolet radiation in the C range (200-280 nm).

Others:

Chromophore	Light-absorbing molecule.
Dose	The amount of energy delivered per unit area. The unit is J/cm^2.
Fluence	Is used synonymous with dose.
HYL	The amino acid hydroxylysine.
HYP	The amino acid hydroxyproline.
NA	Nicotinic acid.
PRO	The amino acid proline.
PWS(s)	Port-wine stain(s).
Scar	A clinically visible or palpable mark remaining after healing of a wound. A scar may range from a shiny epidermal appearance without dermal involvement to atrophic or hypertrophic scarring with dermal involvement. *Atrophy*, a visual or palpable depression of skin texture, *hypertrophy*, a visual or palpable protrusion of skin texture.
Textural change:	Any clinical or subclinical change of skin appearance or skin consistency. The term textural change includes a broader range of skin reactions than scarring because both clinically visible changes and subclinical changes, which are only detectable with objective methods, are included.
Thermal relaxation time (Tr)	The time required for a target object to cool to 50% of the initial temperature achieved immediately after laser exposure. $Tr \cong (d^2/16k)$; d denotes diameter of the targeted structure, k thermal diffusivity (1.3×10^{-3} cm^2/s).
Threshold dose/threshold intensity	The highest laser dose/intensity that does not induce a definite skin reaction.
Visible light	400-760 nm.
Watt (W)	The amount of power delivered during a certain time. One W is one Joule per second (W = J/s).

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V Hædersdal M, Bech-Thomsen N, Poulsen T, Wulf HC. Ultraviolet exposure influences laser-induced wounds, scars, and hyperpigmentation: A murine study. Plast Reconstr Surg 1998; 101: 1315-1322.

VI Hædersdal M, Efsen J, Gniadecka M, Fogh H, Keiding J, Wulf HC. Changes in skin redness, pigmentation, echostructure, thickness, and surface contour after 1 pulsed dye laser treatment of port-wine stains in children. Arch Dermatol 1998; 134: 175-181.

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CONTENTS

PREFACE

This thesis is based on experiments performed at the Departments of Dermatology, Bispebjerg Hospital and Rigshospitalet, University Hospitals of Copenhagen, during the years from 1991 through 1998.

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1. BACKGROUND

1.1 INTRODUCTION AND AIM

The use of laser light for treatment of skin diseases was introduced in 1963 by Leon Goldman who reported preliminary results on the effects of a ruby laser (Goldman et al. 1963). In 1964 the AL and CO_2 laser were developed and soon became the most commonly used dermatological lasers (Arndt et al. 1982). Since that time, technical improvements have expanded the clinical use of laser light significantly and laser therapy is now considered the treatment of choice for conditions that were untreatable or badly treated 30 years ago (Geronemus 1995; Spicer et al. 1996; Wheeland 1995).

Several laser types have been designed for the treatment of specific dermatological affections. The laser wavelengths must be carefully selected and applied exclusively for their specific indications in order to obtain optimal treatment results without side effects. This specialization has resulted in specific and minimally destructive skin reactions, leading to improved treatment outcomes for vascular lesions, melanocytic lesions, and tattoos. Moreover, the specialization has expanded the application of laser light to other skin conditions such as undesired hair growth (Bjerring et al. 1998; Wheeland 1997), wrinkled, photoaged facial skin (Apfelberg 1997), verrucae vulgares (Tan et al. 1993), psoriasis plaques (Bjerring et al. 1997), and erythematous hypertrophic scars (Dierickx et al. 1995a).

It is well-known that patients with PWSs may be stigmatized (Harrison et al. 1988; Lanigan et al. 1989a; Troilius et al. 1998) and affected individuals have been searching for effective treatment modalities all their lives. Some patients have undergone unsuccessful treatments with previously used methods such as surgery, cryosurgery, electrocautery, tattooing, dermabrasion, and x-ray therapy – all resulting in a high occurrence of side effects (Cosman 1980; Goldman et al. 1994). Treatment results were improved with the introduction of lasers, especially when laser systems were introduced, which emit yellow light: Continuous wave dye laser (Lanigan et al. 1989b), PDL (Greenwald et al. 1981), and CVL (Pickering et al. 1990). An overall satisfactory treatment outcome after laser treatment of cutaneous vascular lesions requires a high degree of lesional lightening together with a low occurrence of side effects. Traditionally, it has been the degree of clinically assessed lightening that describes the efficacy of a laser system and notably clinical lightening has been reported for the majority of patients treated with the AL (Noe et al. 1980), the CVL (Pickering et al. 1990), and the PDL (Garden et al. 1993). Only a few studies have been designed specifically to describe laser-induced complications and side effects and, so far, no controlled studies have compared the side effect profiles of specific laser types. Nevertheless, the incidences of reported side effects vary within the different laser systems (Nanni et al. 1998), suggesting that the optimal laser tool may depend more on the side effect profile than on the degree of lightening that can be achieved (Sheehan-Dare et al. 1996).

It has been the intention of this thesis to increase the knowledge of dermatological side effects from treatment with the AL, the CVL, and the PDL, which represent technical developments within laser systems used for treatment of vascular lesions. The investigations focused on the importance of patient and lesional characteristics (skin pigmentation, skin redness, and epidermal thickness) and on the importance of UV irradiation before and after dermatological laser treatment. The aspect of UV irradiation was added because vascular lesions frequently involve the face and, therefore, may be exposed to sunlight in relation to laser treatment and, moreover, no data were available on this clinical topic. Finally, risk assessments were performed on clinically visible side effects in order to improve the preoperative information to incoming patients concerning their individual risks of obtaining side effects from dermatological laser treatment. The laser-induced side effects were evaluated by systematic clinical assessments, by histological and biochemical examinations, by skin reflectance measurements, optical profilometry, and ultrasonography. This thesis, therefore, represents the first systematic and experimental approach to selected side effects from laser treatment of the skin with the PDL, the AL, and the CVL.

1.2 LASER LIGHT

Laser is an acronym for light amplification by stimulated emission of radiation. Laser light is characterized as i) monochromatic, ii) coherent, iii) collimated, and as iiii) having a high amount of energy. Monochromatic light means narrow band of wavelenghts, coherent light waves are aligned with each other both temporally and spatially, collimated waves are narrow, concentrated light waves with little beam spreading. These properties of laser light allow high-energy radiation of a specific wavelength to be delivered to a small area (Bailin et al. 1987; Spicer et al. 1996).

Laser systems are characterized by their wavelengths and their pulse modes. The laser systems in this thesis operate in the visible part of the electromagnetic spectrum and consist of continuous (AL), quasicontinuous (CVL), and pulsed (PDL) lasers.

1.3 LASER-TISSUE INTERACTIONS

The interaction of laser light with skin is complex and depends on the laser equipment and the skin characteristics (Anderson et al. 1981; Anderson 1994). Laser light is able to alter cutaneous tissue by thermal injury, either in a nonspecific but highly controlled way or with a high degree of specificity for selected skin components. It is the optical characteristics of skin that make possible a selective destruction of intracutaneous skin components. The skin contains chromophores that absorb specific wavelengths of light from different parts of the electromagnetic spectrum. Hemoglobin and melanin are the two major endogenous chromophores of the skin, besides the skin may contain exogenous tattoo inks that are also light absorptive (Hædersdal et al. 1996). The energy absorption by hemoglobin and melanin depends on the spectral composition of the incoming light (Anderson et al. 1981; Anderson 1994; Spicer et al. 1996). Hemoglobin has three absorption peaks at wavelengths 418 nm, 542 nm, and 577 nm, whereas the absorption by melanin increases toward shorter wavelengths over a broad spectrum from the near infrared range where absorption by melanin is basically negligible to the UV range where the absorption is very high (Fig. 1).

In the treatment of vascular lesions, oxyhemoglobin is the target chromophore whereas the epidermal melanin is an overlaying, competitive chromophore that constitutes a limitation for a beneficial outcome. Three reasons leave a potential advantage to target oxyhemoglobin with yellow laser wavelengths (CVL, PDL) compared with green or blue-green wavelengths (AL): i) The energy absorption by oxyhemoglobin is higher for yellow light than for especially blue-green light; ii)

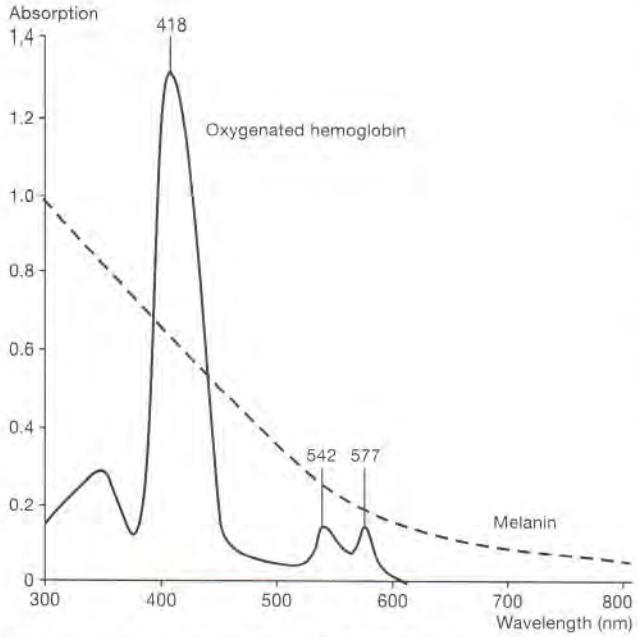

Fig. 1. *The absorption spectra of the principal skin chromophores; oxyhemoglobin and melanin. Hemoglobin has absorption peaks at 418 nm, 542 nm, and 577 nm. (From Herd et al. Dermatol Clin 1997; 15: 359, with permission).*

less energy is absorbed of yellow light by the competitive epidermal melanin; and iii) the depth of penetration is higher for yellow light due to a reduced amount of optical scatter (Anderson et al. 1981; Anderson 1994; Walsh et al. 1986).

Today the most precise targeting of vascular lesions is based on selective photothermolysis, which is responsible for a selective destruction of target tissue (Anderson 1983). Selective photothermolysis constitutes the basis of the PDL that today operates at a wavelength of 585 nm and with a pulse duration of 0.45 ms (Dover et al. 1995; Kauvar et al. 1995; Troilius et al. 1995). The PDL was initially used at 577 nm, but changing the wavelength to 585 nm increased the penetration depth from 0.5 to 1.2 mm from the dermal epidermal junction while maintaining the same degree of vascular selectivity (Tan et al. 1989b). The PDL is, therefore, preferred at 585 nm (Tan et al. 1990), although the absorption theoretically is reduced compared with the 577 nm wavelength (Fig. 1). The pulse duration of the PDL was initially set at 0.30 ms (Tan et al. 1986), subsequently at 0.36 ms (Tan et al. 1989b), and now at 0.45 ms (Onizuka et al. 1995); this prolongation has been made to approach the Tr for the lesional vessels (Nelson et al. 1995b; Garden et al. 1988). During the years it has been possible to enlarge the spot size of the PDL, thereby increasing the penetration depth and fastening the treatment time, which particularly has been an advantage in the treatment of PWSs (Seukeran et al. 1997; Tan et al. 1988). The fundamental idea of selective photothermolysis in the treatment of vascular lesions is that the wavelength must be well absorbed by oxyhemoglobin and the pulse duration must be chosen equal to or less than the Tr of the targeted vessels in order to confine the delivered energy to the vessel walls (Anderson 1983). Neither the AL, nor the CVL meet the requirements for selective photothermolysis. The AL deviates from selective photothermolysis by operating in the blue-green spectrum (combined wavelengths of 488 nm and 514 nm) or in the green spectrum (514 nm), which does not coincide with one of the three absorption peaks of oxyhemoglobin (Fig. 1). Moreover, both the AL and the CVL deviate from selective photothermolysis by their pulse modes. The AL operates in a continuous mode and the CVL in a quasi-continuous mode, which emits pulses in a train of ultrashort pulses of 20 nsec each; this repetition rate being so high that the beam appears continuous (Dinehart et al. 1993; McBurney 1993; Spicer et al. 1996). The pulse lengths of the continuous AL and the quasi-continuous CVL may be mechanically shuttered into the range of hundreds of milliseconds which, however, theoretically is too long compared with the ideal range in the short milliseconds for treatment of dermatological vascular lesions (Dierickx et al. 1995b).

1.4 VASCULAR LESIONS

The most common vascular lesions susceptible to laser treatment are PWSs, telangiectasias, and to a smaller extent hemangiomas (Garden et al. 1997; Ross et al. 1997; Spicer et al. 1996). PWSs are congenital capillary malformations, which may involve any area of the skin, but most often are located on the face (Mills et al. 1997). PWSs occur in approximately 0.3% of neonates and consist of ectatic blood vessels, surrounded by a single layer of nonproliferating, endothelial cells. With increasing age PWSs gradually darken, thicken, and develop nodules, which are due to a characteristic enlargement of the involved vessels. PWSs are present at birth and, unlike hemangiomas, they never involute spontaneously (Dover 1996; Requena et al. 1997a). Hemangiomas usually develop within the first few weeks of life and are characterized by a period of rapid proliferation of the endothelial cells of the capillary walls, followed by a period of slow involution, leading to complete resolution in the majority of cases. However, approximately 40% of patients are left with some residual abnormality of the skin; including telangiectasias, atrophy, hypopigmentation, or scarring. Complicating ulceration, bleeding, or infection may develop in about 20% of patients. Hemangiomas may occur as superficial dermal lesions, as deep subcutaneous, or mixed lesions. The prevalence is approximately 1%–3% in newborns and about 10% in 1-year-olds (Dover 1996; Requena et al. 1997b). Telangiectasias are usually acquired and represent ectasia of a preexisting venules, capillaries, or arterioles (Requena et al. 1997a).

1.5 LASER TREATMENT OUTCOME

Argon laser

The AL was the first laser used to treat large numbers of patients with PWSs (Noe et al. 1980) and the majority of clinical studies has been published during the eighties. These studies were traditionally performed as non-comparative studies that evaluated the treatment response by subjective clinical assessments. Generally, the authors report good clinical results with notable lightening for the majority of adult patients treated for PWSs and telangiectasias. It is estimated that about 60–85% of the patients obtain good to excellent lightening after treatment of PWSs (Apfelberg et al. 1977; Cosman 1980; Dixon et al. 1984; Landthaler et al. 1984; Noe et al. 1980). Five studies have used skin reflectance measurements to objectify AL-induced lightening (Malm et al. 1988b; Neumann et al. 1991d; Sheehan-Dare et al. 1993; Sheehan-Dare et al. 1996; Tang 1983). The results from these studies are not directly comparable since dif-

ferent reflectance methods were applied. In the study by Neumann et al., the average lightening was 46.5%, and 43.3% of 60 patients experienced more than 50% lightening. Unfortunately, no information is given about the number of treatment sessions (Neumann et al. 1991d). Histological studies have shown that a preoperative high percentage of vessels with intraluminal erythrocytes is associated with a good treatment outcome (Finley et al. 1981; Malm et al. 1988a; Noe et al. 1980). In spite of the fact that several papers have reported good clinical results with the AL and several attempts have been used to improve the treatment outcome such as cooling the lesion before treatment (Dréno et al. 1985; Gilcrest et al. 1982) and using automatic scanner systems (Mordon et al. 1989), the occurrence of side effects remains among the highest reported for a dermatological laser system (Apfelberg et al. 1983; Dixon et al. 1984; Vedung et al. 1992). The AL is, therefore, today limited to carefully selected adult patients with telangiectasias, small vascular lesions, or small, dark, nodular PWSs, whereas all other patients, and especially children, ought not to be treated with the AL (McBurney 1993; Smith et al. 1984; van Gemert et al. 1993; Wheeland 1993).

Copper vapor laser

Several authors have advocated the CVL (578 nm) for the treatment of PWSs, telangiectasias, and hemangiomas (Dinehart et al. 1993; Jonell et al. 1994; Waner et al. 1989). A clinical study designed to evaluate the outcome from CVL treatment of PWSs has described good to excellent clinical results in 52% of patients with PWSs, when evaluated from photographic documentation (Neumann et al. 1993). Inconsistency is reported concerning the importance of the initial lesional color for the treatment outcome (Neumann et al. 1993; Pickering et al. 1990). Skin reflectance measurements have been performed by one group to objectify treatment results from CVL treatment of PWSs: Comparative reflectance studies have outlined that the CVL produces significantly better fading than the AL in test treatment areas of red-purple or purple PWSs (Sheehan-Dare et al. 1993; Sheehan-Dare et al. 1996), whereas the CVL produces less clinically and reflectance spectrophotometric evaluated lightening than the PDL (Sheehan-Dare et al. 1994). Regarding telangiectasias, the CVL provides satisfactory clearance, when evaluated by clinical assessments in non-comparative studies (Key et al. 1992; Neumann et al. 1993). In a comparison study, the CVL and the PDL were found to be equally efficacious in treating discrete facial telangiectasias (Waner et al. 1993).

Flashlamp pumped pulsed dye laser

A combined description is presented of the PDL at 577 nm and 585 nm, pulse durations of 0.36 ms and 0.45 ms, since these wavelengths and pulse durations represent technical adjustments within one laser system. A combined description is reported in clinical studies as well (Kauvar et al. 1995; Levine et al. 1995; Renfro et al. 1993; Reyes et al. 1990).

Many studies have on the basis of clinical evaluations cited the PDL as an efficient treatment modality for vascular lesions. It is today considered the treatment of choice for PWSs in especially the paediatric population (Alster et al. 1994; Ashinoff et al. 1991; Geronemus 1993; Reyes et al. 1990; Tan et al. 1989c) but also in the adult population it is recommended (Onizuka et al. 1995; Tan et al. 1990). Skin reflectance measurements have objectified PDL-induced lightening of PWSs in adults (Troilius et al. 1995; van der Horst et al. 1998) and in children (van der Horst et al. 1998; Study VI, the section dealing with clinical results). The efficacy of the PDL has been associated with the anatomical location of the PWS (Nguyen et al. 1998; Onizuka et al. 1995; Renfro et al. 1993; van der Horst et al. 1998), the number of treatment sessions (Kauvar et al. 1995; Reyes et al. 1990), the preoperative vessel thickness and diameter, and the lesional depth in dermis (Fiskerstrand et al. 1996a; Fiskerstrand et al. 1996b; Hohenleutner et al. 1995; Svaasand et al. 1995). Diverging results have been published about the importance of the preoperative color of the PWS (Fiskerstrand et al. 1996a; Fitzpatrick et al. 1994) and the age at the treatment time (Alster et al. 1994; Nguyen et al. 1998; Onizuka et al. 1995; Reyes et al. 1990; van der Horst et al. 1998). However, based on skin reflectance measurements, a recently published study has found that PDL treatment of PWSs in early childhood is not more effective than treatment at later age (van der Horst et al. 1998). By clinical evaluation, overall half of the children achieve about 75% blanching after two to three treatments (Geronemus 1993; Reyes et al. 1990). However, for the individual patient the treatment outcome is unpredictable, since an indefinable subgroup of patients respond slowly and with a poor outcome (Kauvar et al. 1995; Troilius et al. 1995). In a study by Troilius et al. it has been documented by means of skin reflectance measurements that blanching after one treatment with the PDL is lower in the poor response group (average 14%) than in the excellent response group (average 47%), suggesting that skin reflectance measurements after a test treatment may be used to predict the final outcome of therapy with the PDL (Troilius et al. 1995).

1.6 UV RADIATION

Sunlight is the most common source of UV radiation in humans. UVB is the most potent radiation but UVA reaches the earth in amounts 10- to 100-fold greater than UVB (Soter 1995). The effects of UV on human skin can be divided into short-term and long-term effects. The short-term effects include erythema, oedema, melanogenesis, epidermal hyperplasia, vitamin D synthesis, and immunological alterations. The long-term effects of cumulative solar exposure involve photoageing and the development of skin cancer. Most human skin cancers fall into three categories; basal cell carcinoma, squamous cell carcinoma, and malignant melanoma (Soter 1995; Young 1990).

For patients with vascular lesions, tanning may have the advantage that their vascular lesions appear less distinct. The interaction between laser light and UV radiation is, however, an unexplored area. UV exposure before laser treatment may interfere with the laser treatment either by inducing erythema and oedema, or by increasing skin pigmentation and epidermal thickness (Soter 1995), in this way changing the optical properties of the skin. UV exposure may also interfere with the postoperative healing process (Kaiser et al. 1995; Nordback et al. 1990) and possibly alter the treatment outcome and the occurrence of side effects. Moreover, laser exposure may influence the carcinogenic potential of UV radiation, which serves as a complete carcinogen, as both tumor initiation and promotion are induced by UV (Baadsgaard 1991).

UV exposure was given in four of the studies in this thesis. In the animal studies (II, IV, and V) a broad-band simulated

solar UV radiation source was used to imitate the clinical situation, where patients are exposed to sunlight in relation to laser treatments. The erythemal efficacy of the applied UV doses ranged from suberythemogenic (studies II and VII) and slightly erythemogenic (study IV) to clearly erythemogenic (study V).

1.7 MURINE STUDIES

The hairless mouse model is internationally accepted for the examination of cutaneous non-melanoma photocarcinogenesis. The hr/hr C3H/Tif mouse has a lifespan of over 2 years, and even at high age it has a very low incidence of spontaneous skin tumors. Moreover, it has the ability to tan, thus being a suitable model to study laser-induced hyperpigmentation. In the albino hr/hr MORO/Ibm mice, UV exposure induces epidermal thickening without inducing melanogenesis, thus being suitable to study the importance of varying epidermal thicknesses to the development of laser-induced side effects.

In several ways the human response to UV resembles the rodent response: The DNA damage caused by UV exposure (eg pyrimidine dimers and (6–4) photoproducts) occurs both in human and rodent cells (Ananthaswamy et al. 1990). In vitro, there is a defect in the cellular immune response of hr/hr mice (HRS/J mice) (Morrissey et al. 1980; Reske-Kunz et al. 1979) but the UV-induced photoaging (Kligman 1995) and the UV-induced immunological alterations are similar. In both species, Langerhans' cells are inactivated by UV (Alcalay et al. 1990; Walker et al. 1994) and the development of contact hypersensitivity to chemicals is suppressed after UVB exposure (Kalimo et al. 1983; Noonan et al. 1990). Furthermore, the action spectrum of the acute vascular response to UV exposure is very similar (Cole et al. 1983) and nonmelanoma skin carcinogenesis is supposed to be the cumulative effect of long-term solar exposure in both species (de Gruijl et al. 1983; Young 1990). However, also dissimilarities exist between hairless mice and humans: Mice almost exclusively develop squamous cell carcinomas after chronic UV exposure (de Gruijl et al. 1983; Kligman et al. 1981), whereas the long-term effects of chronic UV exposure in humans include both basal cell and squamous cell carcinomas. The human epidermis (back skin) is approximately twice as thick as the murine epidermis (Bruls et al. 1984; de Gruijl et al. 1994; Sterenborg et al. 1986) and the dermal vascularization in mice is arranged differently from humans. The hairless mouse skin has stratified horizontal vascular plexi with few vertical loops (Rea 1968), whereas in humans, the terminal vascular loops are in a vertical orientation with cross shunts that can control the depth of the circulating blood. Different animal models have been used to examine the wound healing process and several wound healing experiments have been performed with rodents such as hairless mice (Kjolseth et al. 1994; Uhl et al. 1994), rats (Ågren et al. 1997), and guinea pigs (Davidson et al. 1991).

In this thesis, the hairless mouse was selected as animal model since it represents a suitable model for examining skin reactions within the fields of UV irradiation and laser exposure. Three experiments were performed with hairless mice: Lightly pigmented, hairless hr/hr C3H/Tif mice were used for investigations on photocarcinogenesis (study II) and for investigations on the importance of UV irradiation to the development of laser-induced wounds, scars, and hyperpigmentation (study V). Albino, hairless hr/hr MORO/Ibm mice were used to examine the importance of UV-induced epidermal thickening to the development of side effects from laser treatment (study IV).

1.8 ETHICAL CONSIDERATIONS

Inducing potentially long-lasting skin changes to human volunteers needs ethical considerations (studies I, III, VIII). When evaluating side effects and performing risk assessments it is a prerequisite that the examined end points are provoked. Laser treatments of healthy, human volunteers were performed on the inside of the proximal brachium in order to minimize any cosmetic inconvenience (studies I, III, VIII). Laser treatment of children with PWSs (study VI) was performed with clinically relevant fluences and the main intention was to objectify subclinical side effects. In study VI, no clinically visible complications were seen, except hyperpigmentation in 2 test areas.

The use of animals for experimental research raises another ethical concern. For clear ethical reasons experiments dealing with skin tumor induction can not be performed on humans. Moreover, experiments dealing with induction of scars, wounds, and pigmentary alterations are preferred to be carried out in animals. Good animal care and proper animal facilities are of fundamental importance for handling the animals without inducing stress. Treatments of the animals followed the guidelines from the Animal Experiments Inspectorate.

1.9 STATISTICAL METHODS

As the number of subjects in the studies was limited and the data might not be normal distributed, non-parametric tests were used for comparisons and correlations (studies I–VIII). Frequencies were analyzed with the Fisher's exact test (study V). Survival analysis was performed by means of the Kaplain-Meier method (study II), risk assessments by means of logistic regression (binary response variable, study III) and ordinal logistic regession (ordered response variable, study VIII). In the murine studies IV and V, sample sizes had to be established from a practical point of view rather than from an ideal statistical point of view, since establishing a sample size is a compromise between the ideal situation (power, significance level, and relevant difference) and the practical limitations (financial resources, manpower, and physical capacity). However, although mice were genetically identical, it was decided to use non-paired comparisons between groups, since each animal represents an individual, thus reducing the risk of a type II error.

2. INTRODUCTION TO LASER-INDUCED SIDE EFFECTS

In this thesis the term "side effects" is defined as both transient and permanent skin reactions such as purpura, wounds, textural changes, scars, pigmentary alterations, and squamous cell carcinomas. Purpura and wounds are unwanted, transient, attendant phenomenons to laser treatment which, nevertheless, may bother the patients and increase the risk of infection. "Side effects" does not include post-treatment erythema, oedema, blistering, bleeding, pyogenic granuloma, Koebner's phenomenon, bacteriological or herpetic infections that have also been reported as postoperative complications (Levine et al. 1995; Olbricht et al. 1987; Taieb et al. 1994; Wlotzke et al. 1996).

Side effects are attributed to nonspecific, thermal damage of epidermis and dermal extravascular structures, and theoretically this unspecificity may be due to three different mechanisms: i) Direct and competitive absorption by epidermal melanin, ii) thermal diffusion away from hemoglobin, resulting in thermal destruction of the perivascular tissue, and iii) scattering effects, which indirectly increase epidermal and dermal nonspecific injury (Anderson 1994; Tan et al. 1989a). Histological studies (Apfelberg et al. 1979; Finley et al. 1984; Greenwald et al. 1981; Morelli et al. 1986; Walker et al. 1989) and histochemical studies (Neumann et al. 1991a; Neumann et al. 1991b; Neumann et al. 1992) have documented the advantage of lasers that emit yellow light (CVL, PDL), since these lasers produce damage that is predominantly concentrated to the vessels, whereas the blue-green AL produces a non-specific epidermal and upper dermal coagulation necrosis. Concerning the CVL, however, histochemistry has revealed that the vessel selectivity is limited to fluences below the clinical whitening threshold since higher fluences result in an increased epidermal injury and a decreased vessel selectivity (constant intensity at 1.3 W, pulse durations ranging from 50 ms to 200 ms) (Neumann et al. 1992; Neumann et al. 1993). These results indicate that the CVL at 578 nm is not entirely selective for treatment of vascular lesions, because the recommended fluence level with the CVL implies clinical whitening (Neumann et al. 1993; Pickering et al. 1990; Sheehan-Dare et al. 1993). Nevertheless, a study based on patients with PWSs has reported that the initial epidermal damage is not permanent (Walker et al. 1989). For the PDL the specific vascular injury turns into extravascular dermal injury as the energy density increases (Tan et al. 1989b).

When laser-induced side effects are described in the literature, there is a remarkable variation in the reported incidences. This may be explained by the circumstance that the studies have not been carried out under standardized conditions: The majority of papers estimates the incidences of side effects by subjective clinical evaluations in studies that were designed primarily to examine the degree of lightening. Different physical laser parameters have been used (fluence level, treatment technique, or pulse duration) as well as patient and lesional characteristics have not been standardized (skin pigmentation, skin redness, individual scarring tendency). Finally, it may have biased the estimated incidences that the follow-up period varied and the inclusion criteria were not generally defined; i.e. some authors included localized and subtle, depressed scars as atrophic scars (Seukeran et al. 1997), while others classified depressed and atrophic scars separately (Renfro et al. 1993; Reyes et al. 1990). Based on the following presented literature, overall side effect profiles are estimated and summarized for the AL, the CVL, and the PDL in an attempt to minimize the above mentioned variables and to focus on the main points from the literature (Table I).

Table I. *Side effect profiles of the AL, CVL, and PDL. This overview is based on a summary of the presented literature (section 2). Low, moderate, and high refers to the postoperative occurrence of side effects.*

	AL	CVL	PDL
Hyperpigmentation	Moderate	Moderate	Moderate
Hypopigmentation	High	Low	Low
Atrophic scarring	High	Moderate	Low
Hypertrophic scarring	High	Moderate	Low

Argon laser

A few studies have been designed specifically to describe AL-induced complications and adverse reactions (Apfelberg et al. 1983; Dixon et al. 1984; Olbricht et al. 1987) and another few studies have been designed to explain and modify the occurrence of side effects (Bonafé et al. 1985; Gilcrest et al. 1982; Meffert et al. 1996; Mordon et al. 1989). Based on questionnaires, scarring has been reported as the most frequent side effect after AL therapy; 69% of physicians using the AL have experienced at least one case of hypertrophic scarring and 22% at least one case of atrophic scarring (Olbricht et al. 1987). Hypertrophic scarring has been reported with incidences from 1% to 38% (Apfelberg et al. 1983; Cosman 1980; Dixon et al. 1984; Mordon et al. 1989; Noe et al. 1980), the highest incidences found in areas of high risk for developing scarring (upper lip, nasolabial area) (Apfelberg et al. 1983; Dixon et al. 1984; Landthaler et al. 1984) and in young patients (Dixon et al. 1984; Noe et al. 1980). Treatment of PWSs with AL and scanning technique has reduced the incidence of hypertrophic scarring from 7% to 1% as when laser points were placed side by side without scanner (Mordon et al. 1989). Atrophy defined as cutaneous depression is frequently reported with percentages from 3% to 40% of the treated patients (Apfelberg et al. 1983; Cosman 1980; Meffert et al. 1996), whereas epidermal atrophy, defined as "cigaret paper", is reported to occur in 85% of the treated patients at room temperature but in 0% when chilling with ice is performed before laser exposure (Gilcrest et al. 1982). Moreover, ice-chilling before, during, and after laser exposure reduced the occurrence of obvious atrophy from 52% to 2% when compared with the standard procedure without chilling (Meffert et al. 1996). Clinically assessed hypopigmentation occured in 28% of the treated patients (Apfelberg et al. 1983) and 43% of the physicians have experienced at least one case of hypopigmentation (Olbricht et al. 1987). Hyperpigmentation occurred in 4%–20% of AL-treated patients (Bonafé et al. 1985; Meffert et al. 1996).

Copper vapor laser

Estimation of the incidences of side effects from the CVL is traditionally based on subjective assessments in clinical studies that were designed primarily to evaluate the degree of blanching, subsidiary to evaluate the incidences of side effects (Jonell et al. 1994; Neumann et al. 1993; Pickering et al. 1990; Sheehan-Dare et al. 1993; Sheehan-Dare et al. 1994): The first publication dealing with side effects from CVL treatment reported low occurrences of scarring (3.5% of patients), hyperpigmentation (1.4%), and hypopigmentation (1.4%) (Pickering et al. 1990). Subsequently Neumann et al. reported that temporary hyperpigmentation was induced in 23% of patients treated for PWSs and facial telangiectasias, whereas slight atrophic scarring was induced in as many as 46% of the patients with PWSs and in 21% of the patients with telangiectasias; none of the patients developed hypertrophic scarring (Neumann et al. 1993). Jonell et al. reported that the incidences of scarring and hyperpigmentation were 19% and 3%, respectively (n = 31); half of the patients experiencing scarring developed atrophic scarring and the other half developed hypertrophic scarring (Jonell et al. 1994). In 3 studies based on test treatment areas (n = 31–43), Sheehan-Dare et al. found the incidences of textural changes to vary between 3%–9% and the

incidences of hyperpigmentation to vary between 3%–10% in patients that were treated with the CVL for PWSs using minimal blanching doses; no patients developed hypopigmentation (Sheehan-Dare et al. 1993; Sheehan-Dare et al. 1994; Sheehan-Dare et al. 1996).

Flashlamp pumped pulsed dye laser

Five clinical studies have been designed specifically to describe the adverse reactions from PDL treatment (Boixeda et al. 1997; Fiskerstrand et al. 1998; Levine et al. 1995; Seukeran et al. 1997; Wlotzke et al. 1996). Transient hyperpigmentation has been the most frequently reported side effect, occurring in 1–57% of patients treated for PWSs (Alster et al. 1994; Boixeda et al. 1997; Fiskerstrand et al. 1998; Levine et al. 1995; Renfro et al. 1993; Reyes et al. 1990; Tan et al. 1989c; Wlotzke et al. 1996) and in 5%–18% of patients treated for facial telangiectasias (Gonzalez et al. 1992; Ross et al. 1993). Hypopigmentation has been reported with incidences from 1–8% (Achauer et al. 1990; Boixeda et al. 1997; Fiskerstrand et al. 1998; Fitzpatrick et al. 1994; Garden et al. 1988; Levine et al. 1995; Renfro et al. 1993; Reyes et al. 1990; Seukeran et al. 1997; Sheehan-Dare et al. 1994; Wlotzke et al. 1996); sometimes being reported as transient (Levine et al. 1995; Renfro et al. 1993; Reyes et al. 1990) and sometimes as permanent beyond 6 months (Wlotzke et al. 1996). Atrophic scarring (including reports on cutaneous depression) has been reported in several studies with incidences up to 8% (Boixeda et al. 1997; Fiskerstrand et al. 1998; Fitzpatrick et al. 1994; Garden et al. 1988; Levine et al. 1995; Renfro et al. 1993; Reyes et al. 1990; Seukeran et al. 1997; Tan et al. 1989c; Wlotzke et al. 1996). Hypertrophic scarring following PDL treatment is considered extremely rare with only three reports in the literature; Swinehart et al. reported hypertrophic scarring in one patient following treatment with 6.5 J/cm^2 (Swinehart 1991), Wlotzke et al. reported one case in a series of 100 patients (Wlotzke et al. 1996), and Seukeran et al. reported hypertrophic scarring in 0.7% of 701 treated patients with a predisposition for the neck (Seukeran et al. 1997). In a study by Kauvar et al. the occurrence of side effects did not increase when up to 25 repetitive treatments were performed of PWSs with the PDL (5.75 J/cm^2–8 J/cm^2) (Kauvar et al. 1995). Selective epidermal cooling by cryogen spray represents a relatively new methodology that reduces the pain associated with PDL-treatment (Waldorf et al. 1997) and protects the epidermis from thermal injury, thereby, additionally reducing the occurrence of postoperative purpura, blistering, and eschar formation (Nelson et al. 1995a; Fiskerstrand et al. 1997).

3. SIDE EFFECTS FROM THE ARGON LASER AND THE COPPER VAPOR LASER

The AL and the CVL are described together since none of these lasers meet the requirements for selective photothermolysis, which represents the most selective delivery of energy to cutaneous vessels.

3.1 WOUNDS

The formation of wounds is an unwanted skin reaction attending cutaneous laser therapy. It is considered an adverse reaction as it may cause inconvenience to the patient either due to an itching sentation or for cosmetic reasons. Moreover, the formation of wounds may increase the risk of infection due to an injured skin surface.

It is well-known from clinical experience and from clinical studies that treatment with AL and CVL induces transient crusting. Greenwald et al. reported in 1981 a dose-dependent development of crusts within 24 hours after exposure of normal human skin to the AL (Greenwald et al. 1981). In PWSs, AL – induced crusts required 3 to 4 weeks to heal (Tan et al. 1986). Regarding CVL-treatment of PWSs and other vascular malformations, Pickering et al. described in 1990 that the crusts separate over a period of 10 days to 2 weeks (Pickering et al. 1990). CVL-induced crusts were in PWSs and facial telangiectasias induced at non-selective fluences and separated between 8–12 days postoperatively (Neumann et al. 1993). Waner et al. found in 1993 that punctate crusts from CVL treatment of facial telangiectasias appeared on day 3 and cleared by day 7 postoperatively (Waner et al. 1993). The above differences in wound parameters may be explained by the application of different laser doses.

AL- and CVL-induced wounding and tissue healing have been investigated experimentally in only a few studies. For CVL-induced wounds, it has been reported that systemic administration of antiinflammatory drugs delayed the wound healing (Hædersdal et al. 1993a). Irradiation with simulated solar UV both before and after CVL treatment decreased the wound surface areas and reduced the wound healing time (Hædersdal et al. 1993b). Moreover, darkly pigmented human volunteers obtained more heavy wounding than fair-skinned human volunteers from AL and CVL treatments (Hædersdal et al. 1994).

Own Investigations

Laser-induced wounds were examined in studies IV and V.

In study IV hairless albino mice were exposed to simulated solar UV in a daily dose of 1.4 J/cm^2, equivalent to 7.2 SED. Irradiations were performed on 0 (control), 8, or 22 consecutive days before laser exposure in order to induce epidermal thickening. Mice were exposed once to the CVL after a delay of 96 hr ±4 hr from the last UV irradiation (Table II). The delay was introduced to minimize possible UV-induced inflammation. A dose-response relationship was observed between the intensity of the laser light and the maximum size of the laser-induced wound surface areas. Generally, the maximum wound surface areas were reduced when laser treatments were performed on epidermal-thickened skin. Negative correlations were found between the maximum wound surface areas and the thicknesses of stratum corneum, cellular epidermis, and entire epidermis.

In study V lightly pigmented hairless mice were exposed once to the CVL (Table II). UV irradiations were performed separately before (3 consecutive days, daily erythemal doses of 2.48 J/cm^2, corresponding to 12.5 SED) and after (4 times weekly in 4 weeks, daily doses of 1.66 J/cm^2, corresponding to 8.4 SED) the laser treatment. Preoperative UV exposure enlarged the laser-induced wound surface areas (0.8 and 1.0 W), whereas no effect was seen on the wound healing time. In contrast, postoperative UV exposure tended to decrease the maximum size of the wound surface areas (1.0 W) and to retard the wound healing time (0.6 and 0.8 W).

Table II. *Overview of physical laser parameters, study subjects, intervening variables, and end points within the studies included in this thesis.*

	Laser-type	Intensity W	Pulse Duration	Fluence*	Beam Diameter	Study subjects	Intervening variables	End-points
Study I	AL-CVL	0.7-1.0-1.3	200 ms	14.1-20.2-26.2 J/cm^2	1 mm	Human adult volunteers	Preoperative constitutional skin pigmentation	Pigmentary changes Scars
Study II	CVL	0.5-1.0-1.4	250 ms	15.9-31.8-44.6 J/cm^2	1 mm	Lightly pigmented hairless mice	Simulated solar UV	Carcinogenesis
Study III	AL-CVL	0.7-1.0-1.3	200 ms	14.1-20.2-26.2 J/cm^2	1 mm	Human adult volunteers	Preoperative constitutional skin pigmentation	Pigmentary changes Scars
Study IV	CVL	0.6-0.8-1.0	250 ms	19.1-25.5-31.8 J/cm^2	1 mm	Albino hairless mice	Epidermal thickness	Wounds Scars
Study V	CVL	0.6-0.8-1.0	250 ms	19.1-25.5-31.8 J/cm^2	1 mm	Lightly pigmented hairless mice	Simulated solar UV	Pigmentary changes Wounds Scars
Study VI	PDL		0.45 ms	from 3.25 to 6.25 J/cm^2 (increments 0.75 J/cm^2)	7 mm	Children with PWSs	-	Pigmentary changes Textural changes
Study VII	PDL		0.45 ms	from 3.0 to 6.5 J/cm^2 (increments 0.5 J/cm^2)	7 mm	Human adult volunteers	Epidermal thickness	Purpura Pigmentary changes
Study VIII	PDL		0.45 ms	from 3.0 to 8.0 J/cm^2 (increments 1.0 J/cm^2)	7 mm	Human adult volunteers	Preoperative constitutional skin pigmentation. Preoperative redness	Wounds Pigmentary changes Textural changes

* Fluences are for the AL and the CVL stated as calibrated hexascan fluences. These fluences are 1.3 times smaller than the calculated fluence in a single spot since the calibrated hexascan fluence is an average fluence of 127 spots distributed over an area of 1.26 cm^2. Due to interjacent unexposed skin areas the average fluence becomes lower than the spot fluence.

Discussion

The acute effects of UV on the skin are erythema, oedema, epidermal hyperplasia, and melanogenesis. These UV-induced tissue effects may interfere with the severity of laser-induced wounds. Epidermal thickness has been quantified by several methods, which, however, are not comparable due to differences in the applied preservation and staining methods and due to differences in the measuring techniques (Bruls et al. 1984; Holbrook et al. 1974). In this thesis, the epidermal thickness was measured by a standardized method on cryostatic sections stained with haematoxylin-eosin. Negative correlations were found between the thicknesses of the epidermal layers and the maximum size of the wound surface areas, indicating an attenuation of laser light with increasing epidermal thickness (study IV). In contrast, preoperative application of 3 consecutive UV irradiations in erythemal doses (study V) resulted in increased laser-induced wound surface areas whereas no effect was seen on the wound healing time. These results may be explained by UV-induced erythema and an increased content of the target chromophore, hemoglobin (Soter 1995), which may lower the threshold dose to induce a specific skin reaction. Moreover, from controlled experimental animal studies (guinea pigs and rats) it is known that repeated irradiations with UVA and UVA/UVB before acute traumas reduce the wound contraction, perhaps contributing to the increased wound areas in the preoperative UV-irradiated mice in study V (Davidson et al. 1992; Özcan et al. 1993). In contrast, Kaiser et al. found in a controlled porcine study that 2 preirradiations with combined UVA/UVB in erythemal doses (2 MED) enhanced the rate of epidermal healing of partial-thickness wounds (Kaiser et al. 1995).

In study V the effect of postoperative UV irradiation was examined on laser-induced wounds. This approach has not previously been investigated. A few controlled studies have, however, focused on the importance of postoperative UV radiation to wound healing from surgically induced wounds: Nordback et al. observed a dose dependent reduction of wound size in broad-spectrum UV-irradiated rats whereas the time to complete wound closure was not affected (Nordback et al. 1990). The wound healing time was neither affected by UVC exposure in a porcine study by Basford et al. (Basford et al. 1986), whereas postoperative UVC exposure reduced the healing time of rabbit ear wounds (El-Batouty et al. 1986). When considering the results in this area, it is difficult to understand why postoperative UV irradiation in study V resulted in diminished, slowly healing wounds, indicating a deep constricted skin damage with a small surface area. It may be a question of different wounding techniques, different animals, or different UV sources, since none of the wounds in the above cited studies were thermally induced and since none of the UV sources permit conclusions on solar simulated UV that was used in study V.

3.2 PIGMENTARY CHANGES

Disturbances in pigmentation from AL- and CVL- treatment manifest clinically as either hyperpigmentation or hypopigmentation. Any change in skin pigmentation may seem remarkable to the patient although concern in particular is raised when the laser-induced skin pigmentation differs clearly from that in the adjacent, non-laser treated skin. Pigmentary changes may have an even or uneven distribution, they may have a spotted manifestation or an appearance with attenuation at the border zone.

Both hyperpigmentation and hypopigmentation have been reported as complicating skin reactions from AL (Olbricht et al. 1987) and CVL (Pickering et al. 1990) therapy. Light- and electronmicroscopic examinations have shown that AL-induced hyperpigmentation is due to an increase in the normal melanin production, which declines spontaneously 4 to 6 months postoperatively (Bonafé et al. 1985). Clinical studies have described pigmentary changes as temporary as well (Dinehart et al. 1993; Neumann et al. 1993). However, none of the clinical studies have based the concept of transiency on well-defined follow-up periods and on systematic descriptions of laser-induced pigmentary changes.

Patients are generally recommended to avoid sun exposure or to use high-factor sunscreens before, between, and after laser treatment in order to avoid an increase in pre- and postoperative skin pigmentation (Bailin et al. 1987; Dinehart et al. 1993; Jonell et al. 1994; Landthaler et al. 1984; Pickering et al. 1990). This recommendation is based on clinical experience, as it has not been experimentally investigated whether UV irradiation aggravates laser-induced pigmentary alterations. The importance of the preoperative skin pigmentation degree has neither been examined in details.

Own investigations

AL and CVL-induced pigmentary alterations were examined in studies I, III, and V.

In study I the relation between preoperative skin pigmentation and the occurrence of postoperative clinically evaluated pigmentary changes was assessed in normal-skinned human volunteers with varying degrees of constitutional skin pigmentation (Table II). Significant correlations were found 1, 2, and 6 months postoperatively between preoperative skin pigmentation and postoperative laser-induced pigmentary changes, meaning that dark-pigmented persons react with more heavy pigmentary changes than fair-pigmented persons. The threshold intensities to induce pigmentary changes were lower than the threshold intensities to induce scarring.

In study III the 6-month results from study I were used to assess the risk of inducing pigmentary changes from treatment with the AL and the CVL. Preoperative skin pigmentation and laser intensity were significant risk factors, whereas it was unimportant whether the AL or the CVL were used. Contour lines of 2.5, 5, 10, 25, and 50% risk levels of inducing pigmentary changes were calculated (Fig. 2).

In study V skin pigmentation was assessed by a semiquantitative technique in lightly pigmented hairless mice (Hansen et al. 1995). The influence of laser exposure and pre- and postoperative UV irradiations was examined. A dose-response relationship was observed between the intensity of the laser light and the postoperative skin pigmentation degrees. Postoperative UV exposure increased skin pigmentation additionally, whereas preoperative UV exposure did not influence the degree of skin pigmentation significantly.

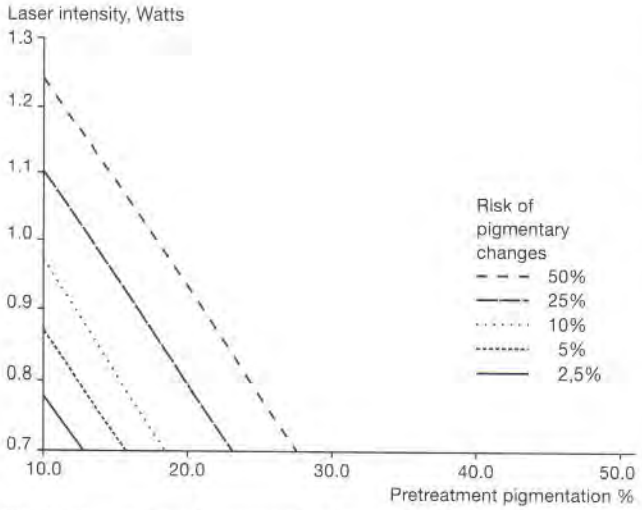

Fig. 2. *Contour lines for given risks of inducing pigmentary changes from treatment with the AL/CVL. The lines were calculated from the estimated logistic model: Ln (P/(1–P)) = –12.599 + 0.249 • X + 8.222 • Y, where P is the risk of inducing pigmentary changes, X the preoperative pigmentation %, and Y the laser intensity (W). The numerical coefficients were determined by the logistic regression analysis. (From Hædersdal et al. Lasers Surg Med 1997; 20: 84–89, with permission).*

Discussion

Hyper- and hypopigmentation from AL- and CVL treatments are well-known side effects that have been reported in several studies. The evaluation of pigmentary changes by means of a clinically graded score system is, however, for the first time reported in study I. Utilizing this score system, it was found that i) a monotonous correlation exists between the preoperative constitutional skin pigmentation% and the degree of laser-induced pigmentary changes and ii) preoperative pigmentation% and laser intensity were significant risk factors for inducing postoperative pigmentary changes, whereas it was unimportant whether the AL or CVL were used. These findings are in agreement with the circumstances that it is impossible to achieve vascular selectivity without epidermal damage in CVL treatment of PWSs in Korean patients (skin types III, IV, and V) (Chung et al. 1996) and that temporary hyperpigmentation is induced in almost all CVL treated Korean patients with PWSs (Chung et al. 1997).

In the literature no previous attempts have been made to compare the risks of inducing pigmentary changes and scarring. In study I and III the threshold intensity to induce pigmentary changes was lower than the threshold intensity to induce scarring 6 months postoperatively, indicating that pigmentary changes are induced at a lower intensity level than scarring for skin with identical constitutional skin pigmentation. For instance, a person with a preoperative pigmentation of 20% has approximately 15% risk of scarring and more than 50% risk of obtaining pigmentary changes after laser treatment with 1.0 W (both AL and the CVL), whereas a pale person with a preoperative pigmentation of 10% has no significant risk of scarring and about 15% risk of obtaining postoperative pigmentary changes (Fig. 2 and Fig. 3). These results indicate that laser-induced pigmentary changes and scarring are a continuum of skin reactions and that the induction of pigmentary changes is the first sign that a too high laser fluence has been applied to the skin. The laser-induced hyperpigmentation in study I included a broad spectrum of skin reactions from just visible, barely perceptible hyperpigmentation to intense hyperpigmentation with a clear border-hyperpigmentation, the hyperpigmentation sometimes exceeding the treatment area. The pathogenesis of postoperative pigmentary changes is not completely understood. Postoperative hyperpigmentation is, however, ex-

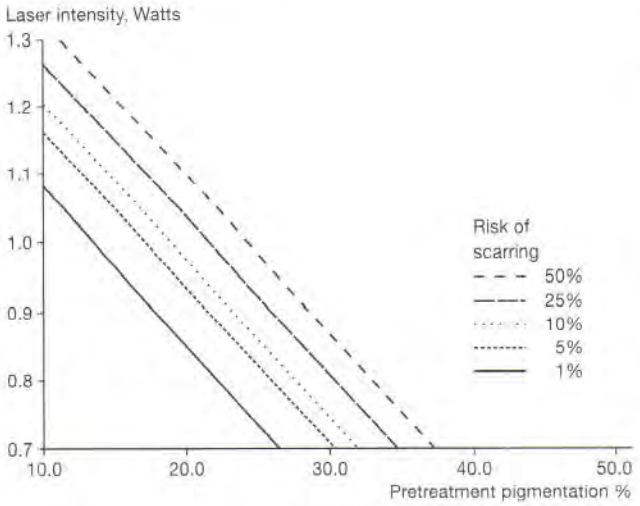

Fig. 3. *Contour lines for given risks of inducing scarring from treatment with the AL/CVL. The lines were calculated from the estimated logistic model: Ln (P/(1–P)) = –29.016 + 0.427 • X – 18.735 • Y, where P is the risk of inducing scarring, X the preoperative pigmentation %, and Y the laser intensity (W). The numerical coefficients were determined by the logistic regression analysis. (From Hædersdal et al. Lasers Surg Med 1997; 20: 84–89, with permission).*

pected to be explained by thermal injury to the epidermis, since it is well-known that any tissue injury may heal with epidermal postinflammatory hyperpigmentation. On the other hand, the laser-induced hypopigmentation, which only occurred at the highest laser intensity level in darkly pigmented volunteers, may be explained by thermal destruction of melanosomes, thus representing a more extensive skin reaction as compared with hyperpigmentation.

The circumstance that several authors report pigmentary changes as transient suggested me to reevaluate the data from study I (unpublished data). It appeared that the clinical scores (median values) within each laser intensity were at similar levels on the 1, 2, and 6 month assessments. This means that, unfortunately, there was no decline in the degree of laser-induced pigmentary changes from 1 to 6 months postoperatively and, therefore, the results from study I were not able to confirm the concept of transiency for clinically evaluated laser-induced pigmentary changes.

The suggestion to protect healing wounds from sunlight in order to prevent postoperative pigmentary changes is a routine part of postoperative laser instructions (Bailin et al. 1987; Dinehart et al. 1993; Jonell et al. 1994; Landthaler et al. 1984; Pickering et al. 1990). However, only sporadic knowledge exists on the topic of UV radiation and hyperpigmentation in healing wounds (Wiemer et al. 1983). No controlled experiments have been presented concerning laser- and UV-induced hyperpigmentation. Study V was therefore the first study to document that the laser treatment itself induces hyperpigmentation in a dose dependent way and that postoperative UV exposure increases the hyperpigmentation additionally.

3.3 SCARS

When laser treating vascular lesions, it is of great concern to avoid scarring since the postoperative clinical appearance may be aggravated if an irregular skin texture develops and it may become even more difficult to cover the vascular lesion with concealing creams. There is a discrepancy between on the one side the clinical relevance of laser-induced scarring and on the other side the degree of attention that has been paid to this field.

Own Investigations

AL and CVL-induced scars were examined in studies I, III, IV and V. The main emphasis was on the macroscopic descriptions (studies I, III, IV, and V) but also histological (studies IV and V) and biochemical (study V) examinations were included to assess and compare the development of postoperative scarring.

In study I the relation between preoperative skin pigmentation and clinically evaluated scarring was assessed in normal human skin with varying degrees of constitutional skin pigmentation (ranging from skin type I to V). Laser-treatments were performed once with the AL and the CVL on the inside of the upper brachium (Table II). Significant correlations were found 1, 2, and 6 months postoperatively between the preoperative skin pigmentation degrees and the postoperative scores of clinically evaluated scars. At the 6-month assessment the AL induced higher scores of scarring than the CVL.

In study III, the binary results from the 6 months-follow-up in study I (yes / no scarring) were used to calculate the risk of inducing scarring from treatment with the AL and the CVL. Preoperative skin pigmentation and laser intensity were significant risk factors, whereas it was unimportant whether the AL or the CVL were used. Contour lines of 1, 5, 10, 25, and 50% risk levels of inducing scarring were calculated (Fig. 3). When comparing Fig. 2 and Fig. 3, it was found that textural changes occurred at higher intensity levels than pigmentary alterations.

In study IV the macroscopic and histological appearances of laser-induced scars were studied in hairless albino mice with different preoperative epidermal thicknesses (Table II). In general, the surface areas with textural changes were smaller when laser treatments were performed on epidermal-thickened skin (0.6 and 0.8 W). Less fibrosis was induced in epidermally thickened skin (0.6 and 0.8 W). Negative correlations were found between preoperative epidermal thicknesses and postoperative scarring, evaluated clinically by maximum areas with textural changes and histologically by score of fibrosis.

In study V laser-induced scars were evaluated by macroscopic, histological, and biochemical examinations in lightly pigmented hairless mice. The influence of pre- and postoperative UV irradiations was examined (Table II). Preoperative UV exposure enlarged the surface areas with textural changes at one intensity level (0.8 W). Generally, postoperative UV exposure tended to decrease the maximum surface areas with textural changes (0.8 W), to increase the histologically evaluated fibrosis (0.6 W), and to decrease the concentration of hydroxylysine (0.8 and 1.0 W), which may indicate a deep constricted skin damage. Moreover, postoperative UV-irradiated mice developed a higher incidence of bulging infiltration compared with non-irradiated mice.

Discussion

In study I the macroscopic evaluation of scarring was made from clinical score systems that had been established on the basis of a preceeding pilot study. Histological analyses might have provided additional objective information but no skin biopsies were taken from the human volunteers. It was found that a monotonous relation exists between the preoperative consti-

tutional skin pigmentation degrees and the scores of laser-induced scarring 1, 2, and 6 months postoperatively, meaning that dark-pigmented persons react with more heavy scarring than fair-pigmented persons. This finding is in accordance with previously published data regarding the subacute response from AL and CVL treatments (Hædersdal et al. 1994). The fact that the epidermal melanin is associated with postoperative scarring is explained by competitive absorption of incoming photons (Anderson et al. 1981). However, the fact that a high amount of genetically determined melanin increases the occurrence of scarring, rises the question whether UV-induced melanogenesis has a similar importance. This aspect is clinically relevant when considering if there is any difference in the risk of inducing side effects from laser treating a winter-pale skin or a summer-tanned skin. However, today no knowledge exists on this topic.

Study III is the first study that concentrates on risk assessment of side effects from AL- and CVL treatment. The aspect of risk assessment is clinically relevant for several reasons. First of all it is important to inform patients about their individual risks of obtaining a suboptimal treatment outcome due to scarring. Secondly, for the laser surgeon it is important preoperatively to consider whether the incoming patient is at high or low risk for obtaining scarring and to predict the highest laser intensity or laser fluence that can be applied to the skin before scarring might be induced.

In the murine experiments, scarring was determined by macroscopic and histological examinations (studies IV and V) as well as by biochemical analyses (study V). Increasing scores of histologically evaluated fibrosis reflect increasing skin-injury, the skin reactions ranging from no visible histological changes to heavy full-thickness fibrosis with damaged accessory structures. Quantification of laser-induced fibrosis by the means of histological examinations indicated, generally speaking, that epidermal thickening and postoperative UV irradiation influenced the laser-induced fibrosis in opposite ways: Epidermal thickening had a tendency to reduce the scores of fibrosis compared with non-irradiated control mice (study IV), whereas postoperative UV irradiation had a tendency to increase the scores of fibrosis (study V). These findings reflect that the degree of skin injury may be reduced by epidermal thickening but increased by postoperative UV irradiation. The results are mainly in accordance with the macroscopically evaluated parameters although it is difficult to understand that the postoperative UV-irradiated mice developed smaller surface areas with textural changes. This may be explained by a deep constricted skin damage, since also the wound parameters had a reduced size as well as a prolonged healing time. The mechanisms for these counteracting skin reactions have not been investigated. Attenuation of laser light is considered a possible explanation for the reduced fibrosis in the epidermally thickened skin although, in normal human epidermis, melanin is the main responsible for attenuation of wavelengths in the visible spectrum (Anderson et al. 1981; Bruls et al. 1984). The importance of postoperative UV irradiation to the appearance of laser-induced scarring has not been addressed in the literature. A few studies have, however, investigated the influence of UV exposure on tensile strength in surgically produced wounds (Basford et al. 1986; Nordback et al. 1990). Both these studies have outlined that postoperative UV exposure had no significant influence on the wound tensile strength, neither in rat skin (Nordback et al. 1990), nor in swine skin (Basford et al. 1986).

3.4 SKIN CANCER

Patiens frequently request information about the long-term risk of malignancy from laser treatment. The potential of laser light for causing skin cancer or for interfering with UV-induced skin cancer has, nevertheless, only been sparsely examined. In 1983, Nakajima et al. studied the cytogenetic effects of AL light on hamster fibroblasts and found a significant increase in sister chromatid exchange (Nakajima et al. 1983). Apfelberg et al. in 1984 exposed mouse fibroblasts to AL and CO_2 laser and subsequently the cells were examined for malignant transformations. In this study, neither the AL nor the CO_2 laser induced a significant degree of malignant transformations (Apfelberg et al. 1984). In 1991 Neumann et al. presented a case story in which abnormal epidermal changes similar to actinic keratoses were seen 10 months after three AL treatments of a 26-year-old woman having a PWS (Neumann et al. 1991c). The authors suggested that photothermal effects might be responsible for the changes. The PWS was, however, located on the forehead and, although the lesion had been protected with sunscreens for the last 8 years, UV irradiation may be responsible for the induced changes. Marjorlin discovered in 1873 the association between thermal burn scars and neoplasia. Afterwards several authors have described the phenomenon that thermal burn scars possess the potential of malignant transformation into carcinomas after a latency of several years (Abbas et al. 1988; Lawrence 1952; Treves et al. 1930). No in vivo experimental studies have been performed to clarify the aspect whether the photothermal influence of laser light may be carcinogenic.

Own Investigations

In study II the potential of the CVL for causing carcinogenesis was examined and the effect of CVL treatment before UV irradiations was investigated on the development of UV-induced photocarcinogenesis.

Hairless hr/hr C3H/Tif mice were exposed once to the CVL (Table II). Subsequently, the mice were exposed to simulated solar UV radiation from one Phillips TL 12 and five Bellarium-S SA-1-12 tubes. UV irradiations were performed 4 days a week from the day after the laser treatment and during the entire experimental period of 18 months. The daily UV dose of 1.3 J/cm^2 was suberythemogenic, equivalent to 6.6 SED. One laser treatment with the CVL did not have a malignant potential. Preoperative treatment with the CVL at the highest intensity level (1.4W) delayed the UV-induced photocarcinogenesis.

Discussion

The question whether visible laser light is carcinogenic has not directly been addressed. No action spectra have been established for the induction of erythema, melanogenesis, and skin carcinogenesis in the visible range of the electromagnetic spectrum. Several action spectra have been performed in the UV range (Anders et al. 1995; de Gruijl et al. 1993; McKinlay et al. 1987; Parrish et al. 1982). These have outlined that the induction of erythema, melanogenesis, and skin cancer declines with longer UV wavelengths. One of the most recent skin carcinogenesis action spectra (SCUP spectrum), published in 1993 by de Gruijl et al., was based on the assumption that no carcinogenicity appears in the visible part of the spectrum (de Gru-

ijl et al. 1993). From a molecular- and cellularbiologic point of view, Peak et al. have investigated the effects of visible light on DNA and summarized that no reports of mutagenesis have been published whereas single strand breaks from blue and green light have been detected (Peak et al. 1991). The association between thermal burn scars and neoplasia has been recognized for years (Abbas et al. 1988; Lawrence 1952; Treves et al. 1930). Study II was the first in vivo study to establish that the photothermal effect from the CVL in a broad range of applied fluences does not have a malignant potential, neither in fluences that caused no visible skin reactions, nor in fluences that induced thermal burn scars.

The interaction between laser light and UV radiation for the induction of carcinogenesis has not been examined directly. Urbach et al. hypothesized in 1974 that unspecific damage before UV radiation might accelerate the appearance of UV-induced skin cancer due to epidermal hyperplasia (Urbach et al. 1974). The epidermal hyperplasia secondary to laser treatment might then potentially accelerate UV-induced carcinogenesis. Griffin et al. studied the effect of broad band visible light on UV-induced carcinogenesis, and their results indicated an activation of UV-induced ear tumors by white light (Griffin et al. 1955). However, a fluorescent lamp with UV emission at wavelengths shorter than 330 nm was used to emit white light and these short wavelengths may be responsible for the increased carcinogenesis. Study II was the first animal study to examine the long-term interaction between yellow laser light and UV irradiation. Pretreatment with the CVL at the highest intensity level (1.4W) delayed the UV-induced photocarcinogenesis significantly, suggesting that epidermal cell proliferation secondary to thermal damage is unimportant to UV-induced photocarcinogenesis.

3.5 CONCLUSIONS AND PERSPECTIVES

Wounds, pigmentary changes, scars, and photocarcinogenesis were evaluated as end points from AL and CVL treatment. The development of postoperative scars and pigmentary changes depended on the preoperative skin pigmentation degree (studies I and III). Significant correlations were found between preoperative skin pigmentation and clinically scored pigmentary changes and scarring 1, 2, and 6 months postoperatively. Pigmentary changes occurred at lower intensity levels than scarring. No difference was seen between the AL and the CVL concerning the risk of inducing these side effects (studies I and III). In studies I and III, volunteers were included with different preoperative degrees of constitutional skin pigmentation. No studies have been performed on the aspect of acquired skin pigmentation. It remains unknown whether constitutional and acquired pigmentation are of equal importance to the development of laser-induced side effects. Further studies may clarify this topic. However, it seems reasonable to recommend strict sunprotection to laser-patients in order to minimize the development of side effects.

In hairless, albino mice increasing epidermal thicknesses tended to reduce CVL-induced wounds and scars and negative correlations were found between the preoperative epidermal thicknesses and the CVL-induced skin reactions (study IV). In lightly pigmented, hairless mice, CVL treatment induced an increase in skin pigmentation, evaluated by a semiquantitative technique (study V). Postoperative UV irradiations with simulated solar UV increased the CVL-induced skin pigmentation additionally (study V). The size of CVL-induced wounds and scars tended to enlarge by preoperative UV irradiation but to diminish by postoperative irradiation (study V). The postoperatively irradiated wounds had a prolonged wound healing time, which may indicate a deep constricted skin damage (study V). Moreover, the histologically evaluated fibrosis and the frequency of bulging infiltration increased by postoperative UV irradiation (study V). When taking the liberty to extend the obtained results from the animal studies to humans, these results support the importance of pre- and postoperative sunprotection. It is estimated that sunprotection – by high-factor sunscreens or by clothes – needs to continue for at least a year after the laser treatment. However, the length of the required protection period remains to be clarified in further studies.

In hairless mice, one treatment with the CVL did not have a malignant potential. Preoperative treatment with the CVL at the highest intensity level, 1.4 W, delayed UV-induced photocarcinogenesis significantly (study II). In the clinical situation, patients are often laser treated several times at the same skin area before acceptable clinical results are obtained. Therefore, it remains to be clarified if repetitive laser exposures may influence the development of UV-induced photocarcinogenesis.

4. SIDE EFFECTS FROM THE PULSED DYE LASER

The PDL is described separately because it is the only laser in this thesis that fulfills the requirements for selective photothermolysis.

4.1 PURPURA

The PDL produces selective vascular damage to ectatic blood vessels within a PWS. An almost immediate development of well-defined purpura at the site of laser impact is the macroscopic evidence that dermal vessels have been targeted. The postoperative purpura is a temporary, attendant phenomenon to PDL treatment that occurs in almost all treated patients and, usually, disappears within 1–2 weeks (Spicer et al. 1996). In this thesis, purpura is considered a side effect, since it is an unwanted skin reaction that may bother the treated patients: In 1993 Waner et al. published a comparative study of the CVL and the PDL in the treatment of facial telangiectasias. Based on questionnaires, patients reported the larger purpuric macules produced by the PDL to be less cosmetically acceptable when compared with the thin linear crusting produced by the CVL (Waner et al. 1993). In a subsequent questionnaire survey based on 62 adult patients with PDL-treated PWSs, the major cause of morbidity was protracted bruising which, in 45% of treated patients, caused a significant restriction of social activities (Lanigan 1995).

The purpuric reaction has been studied experimentally in normal human skin in order to establish relations between the purpura threshold dose and laser pulse duration (Garden et al. 1986), skin type (Tan et al. 1984), and skin surface temperature (Tan et al. 1985). In addition, in the early days with the PDL, the purpuric reaction in normal skin was used to estimate the optimal treatment dose in lesional skin (Garden et al. 1988).

Own investigations

In study VII the effect of epidermal thickness on the development of purpura was investigated.

The study design included 15 fair-skinned human volunteers that were laser treated in two test regions of varying epidermal thicknesses; i) normal buttock skin, and ii) preoperative UVB-exposed buttock skin (Table II). UV radiation was obtained from Phillips TL 01 tubes that emit UVB in a narrow peak around 311–313 nm. Eight UVB irradiations were performed before laser treatment, doses increasing from 0.75 to 1.25 × the original MED. The total epidermal thickness ranged from 60 to 100 μm. No correlations were found between the epidermal thicknesses and the clinically established threshold doses to induce purpura; neither for stratum corneum, the cellular part of epidermis, nor the total epidermis (10 minutes and 1 day postoperatively). Skin reflectance measurements were performed 1 day, 6 days, and 2 weeks postoperatively: No correlations were found between the epidermal thicknesses and the laser-induced redness degrees, whereas a dose-response relationship was seen between the applied laser fluence and the skin-reflectance determined skin redness.

Discussion

From a clinical point of view, it is relevant to consider the importance of variations in epidermal thickness to skin reactions from the PDL, since i) vascular lesions are located in anatomical regions of varying epidermal thickness, ii) the thickness of the cellular part of epidermis decreases with advancing age (Lock-Andersen et al. 1997b), and iii) sunexposure is known to increase the epidermal thickness (Soter 1995). From the optical characteristics of human skin, it is known that melanin is the main responsible for attenuation of wavelengths in the visible spectrum, whereas the importance of epidermal thickness decreases with increasing wavelength and is considered of minor importance in the visible part of the electromagnetic spectrum (Anderson et al. 1981; Bruls et al. 1984). The transmission of 546 nm green light is, nevertheless, reduced by approximately one third when the epidermal thickness is increased from 10 μm to 120 μm (Bruls et al. 1984). It is, therefore, possible that the epidermal thickness might influence the PDL-induced skin reactions.

No experimental studies have previously investigated the effect of different epidermal thicknesses on PDL-induced skin reactions. In study VII, variations in epidermal thickness did not influence the clinically evaluated and skin reflectance-determined purpuric reaction from PDL treatment. On the other hand, UV-induced epidermal thickening was found to reduce CVL-provoked side effects in a murine study (study IV), probably due to attenuation of the laser light through the epidermal layers. The results from the PDL and the CVL are, however, not directly comparable, since these represent a wide range of skin reactions from respectively human and murine skin; the PDL representing a vessel-specific acute skin reaction due to the concept of selective photothermolysis and the CVL representing a chronic skin reaction from an unspecific coagulation necrosis. Although transmission increases with increasing wavelengths, it is hard to believe that different wavelengths of 578 nm and 585 nm should give an explanation for the diverging results in studies IV and VII. The same method was used to quantify the epidermal thickness in the two studies, and it seems most reasonably that different ranges of epidermal thicknesses may explain the deviating results: In the human study (study VII) the total epidermal thickness ranged from 60 to 100 μm whereas, in the murine study, it ranged from 26μm–116μm (study IV). It may thus be necessary to operate with a difference of more than 40 μm (60–100 μm) to show detectable differences on the importance of epidermal attenuation of laser light at 585 nm. The range of epidermal thicknesses from 60–100 μm approximates the anatomical variation in humans; infants and elderly people, however, experiencing epidermal thicknesses below the 60 μm (Southwood 1955).

The laser-induced purpura is connected with an inflammatory response, therefore, bringing up the question whether any relation exists between laser-induced purpura and postinflammatory hyperpigmentation. This topic has not been investigated in previous studies. In study VII volunteers were only included of skin types I and II in order to avoid interference from the epidermal melanin. By clinical evaluation, no hyperpigmentation was seen in these volunteers. Neither did skin reflectance measurements detect laser-induced hyperpigmentation (unpublished data). It is, therefore, concluded that purpura in fair-skinned volunteers is not associated with postinflammatory hyperpigmentation.

4.2 WOUNDS

PDL treatment occasionally induces crust formation secondary to epidermal damage (Garden et al. 1993; Nanni et al. 1998). Wounding and crusting is not traditionally included in the description of side effects from the PDL and there exists a considerable discrepancy in the reported presence and severity of PDL-induced crusting. In clinical studies the incidence of crusting spans from 0% (Alster et al. 1994) to 52% (Fitzpatrick et al. 1994), even though identical laser parameters (wavelenths 585 nm, 0.45 ms pulse durations, spot sizes 5 mm) and similar energy levels (between 5.5 and 7.75 J/cm^2) were used in the two studies. The most possible explanation for the disagreement may be inter- and intraindividual variations in the PWSs and uncontrolled conditions for evaluating the laser-induced crusting; in the study by Fitzpatrick et al. patients evaluated the presence of crusting by responding to a questionnaire whereas a physician assessed the presence of crusting in the study by Alster et al. A subsequent questionnaire survey published in 1995 by Lanigan et al. outlined that 48% of patients with PWSs experienced postoperative epidermal damage, manifested by either weeping or crusting of the treated area (Lanigan 1995). The PDL-induced crusts have not been investigated from an experimental point of view.

Own investigations

No data has been published in studies VI–VIII concerning the development of crusts from PDL treatment. Unpublished data are presented from studies VII and VIII.

In study VII no crusts developed in normal human skin with epidermal thicknesses ranging from 60 to 100 μm, fluences ranging from 3-6.5 J/cm^2. Skin reactions were evaluated on 10 minutes, 1, 6 days, 2 and 6 weeks postoperatively.

In study VIII laser-induced crusts were examined in healthy human volunteers with different preoperative skin pigmentation and redness degrees on 1, 2, and 6 weeks postoperatively. Skin was artificially reddened using topical application of 10% NA cream. A clinical score system spanning from 0–3 was used to grade the wounds: 0 denoted no visible wounding, 1 superficial wounding, 2 moderate wounding, and 3 heavy wounding. The development of crusts depended on the applied laser dose.

Table III. *Overview of PDL-induced crusts 1 and 2 weeks postoperatively at a fluence level of 6 J/cm². Data are presented as frequencies of test areas with crusts (%) and as median values (25th - 75th percentiles) of the scores (scores range from 0 to 3). P values are obtained when the current value is compared with the corresponding normal skin test region (Fisher's exact test was used for comparisons of frequencies, the Wilcoxon test for comparison of score levels). It it seen that the normal skin and placebo cream test regions approaches each other.*

	Frequency (%)	Median (25th - 75th percentiles)
Normal skin test region		
1 week	43%	0.0 (0-1)
2 weeks	50%	0.25 (0-1)
Nicotinic acid test region		
1 week	92% p = 0.02	1.0 (0.5-2) p = 0.008
2 weeks	62% p = 0.83	0.75 (0-1.5) p = 0.77
Placebo cream test region		
1 week	46% p = 1.00	0 (0-1.5) p = 0.13
2 weeks	54% p = 0.98	0.25 (0-1) p = 0.88

At 3 J/cm² no crusts developed; at 4 J/cm² slight crusting was seen 1 week but not 2 weeks postoperatively. At 5, 6, 7, and 8 J/cm² crusts were visible both 1 and 2 weeks postoperatively, whereas six weeks after laser exposure the skin was healed. As a result of the dose-dependency, the crust data were calculated separately at each fluence level. Data are presented only at the 6 J/cm² level, which approximates the median dose in study VIII (5.5 J/cm²) and, moreover, represents a representative clinical fluence with the 7 mm spot size (Table III). The frequencies and the score levels of the laser-induced crusts were higher in NA-reddened skin than in normal and placebo cream test regions (Table III). Similar tendencies were observed at 5, 7, and 8 J/cm². Concerning the importance of preoperative skin pigmentation and skin redness to the development of postoperative wounds, the most consistent data were found for skin pigmentation 1 week postoperatively: Significant Spearman correlation coefficients (P values ≤ 0.05) were found between the preoperative skin pigmentation and the 1-week crust scores within the normal skin test region (5–8 J/cm² analysed separately, r values between 0.64 and 0.72), the placebo cream test region (5–8 J/cm² analysed separately, r values between 0.60 and 0.80), and the NA test region (6–8 J/cm² analysed separately, r values between 0.65 and 0.77). No consistency was observed regarding correlations between preoperative skin redness and postoperative crust scores.

Discussion

Clinical studies designed specifically to describe PDL-induced side effects, focus on crusting to considerably different extents. Of these studies, the first study was published in 1995 and no comments were given on crusting (Levine et al. 1995). The next study was published in 1996 and the presence of crusting was devided into serous and impetigo-like crusting (Wlotzke et al. 1996). Post-treatment serous crusting developed in 46% and 83% of patients (based on physicians and patients observations, respectively), whereas impetigo-like crusting occurred in 25% of the treated patients. At the later 6-week follow-up visit, 27 patients (27%) experienced hyperpigmentation; 24 of these patients had previously experienced crusting, suggesting that the presence of crusting may be associated with subsequent hyperpigmentation. In study by Seukeran et al., crusting appeared as a transient event in 0.7% of the patients and usually healed without subsequent scarring (701 patients were included in the study) (Seukeran et al. 1997). The discrepancy in the reported incidences of crusting in the studies by Wlotzke et al. and Seukeran et al. may partly be explained by the use of different laser equipments and laser settings. The most recent study concentrating on side effects from PDL treatment was published in the 1998 (Fiskerstrand et al. 1998). This study focused on pigmentary changes from PDL treatment and no data were presented on crusting. No studies have previously concentrated on the importance of preoperative skin pigmentation and skin redness for the development of PDL-induced crusting. The here presented data from study VIII substantiate that crusting in particular is provoked in darker red and darkly pigmented skin types.

4.3 PIGMENTARY CHANGES

In general, the PDL is considered safe and the incidence of side effects is regarded low (Table I). Hyperpigmentation has in several clinical studies been pointed out as the most frequent side effect from PDL treatment, in newer studies occuring in 1%–27% of the patients that have been examined systematically for pigmentary alterations in a period from 6 weeks to at least 6 months after treatment (Boixeda et al. 1997; Fiskerstrand et al. 1998; Levine et al. 1995; Seukeran et al. 1997; Wlotzke et al. 1996). Several clinical studies have described pigmentary changes as temporary (Seukeran et al. 1997; Wlotzke et al. 1996). However, no studies have evaluated the transiency systematically and no studies have documented PDL-induced pigmentary alterations by objective methods.

The aspect, whether intra- or interindividually varying parameters such as skin redness, pigmentation, and epidermal thickness may influence the occurrence of postoperative pigmentary alterations, has not been directly addressed. Concerning preoperative epidermal pigmentation, it is well-known that the most specific vascular injury is obtained in fairly pigmented skin (Hohenleutner et al. 1995; Tan et al. 1984; Tong et al. 1987) and according to clinical experience, it is generally recommended to use high-factor sunscreens in order to avoid UV-induced melanogenesis (Pickering et al. 1990; Seukeran et al. 1997) – although the influence of acquired pigmentation compared with constitutional pigmentation remains unknown. Recently Fiskerstrand et al. published that post-treatment hyperpigmentation occurs with equal frequencies during summer and winter (23%), and facial regions do not exhibit higher occurrences of hyperpigmentation than lesions located elsewhere, suggesting a constitutional disposition to the development of postoperative hyperpigmentation (Fiskerstrand et al. 1998). Differences in preoperative skin pigmentation may have influenced these results. Moreover, a case report has described that no improvement took place in a black patient with a PWS after treatment with the PDL, whereas persistent hypopigmentation and textural changes were induced (Ashinoff et al. 1992). No studies have examined whether variations in preoperative epidermal thickness and skin redness may influence the development of postoperative pigmentary alterations from the PDL.

Own investigations

PDL-induced pigmentary changes were examined in studies VI, VII, and VIII.

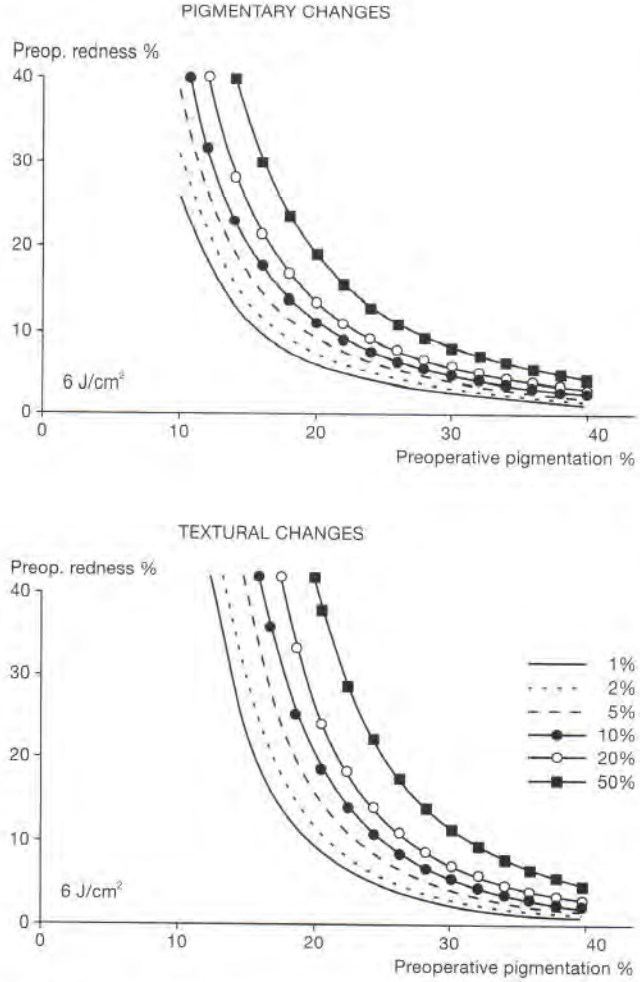

Fig. 4. *Contour lines for given risks of inducing pigmentary alterations and textural changes 6 months after PDL treatment with a fluence level of 6 J/cm². Data were analysed by ordinal logistic regression, which describes the risk of inducing side effects after laser therapy as a mathematical function of the risk variables in the model, i.e. preoperative skin pigmentation, preoperative skin redness, and laser fluence. The mathematical description of the ordinal logistic regression method is presented in study VIII.*

In study VI twelve children with macular PWSs were laser treated with the PDL (Table II). It was the intention to objectify and study treatment results and side effects systematically from one treatment with the PDL. In this thesis attention is paid to the section dealing with side effects. By clinical evaluation, hyperpigmentation occurred in 2 of 43 test sites (4.7%). Reflectance-evaluated hyperpigmentation occurred in 29 of 43 test sites (67.4%), indicating the presence of a subclinical increase in skin pigmentation.

In study VII the total epidermal thickness ranged from 60 to 100 μm (Table II). By clinical evaluation, no pigmentary changes were seen. Reflectance-measurements (unpublished data) documented that no pigmentary alterations were induced (median preoperative pigmentation 7% (25th and 75th percentiles 5–9%), postoperative pigmentation 6% (4–7%)).

In study VIII the importance of preoperative skin pigmentation and skin redness was investigated for the development of pigmentary changes after one PDL treatment of normal-skinned human volunteers (Table II). The volunteers were selected with varying degrees of constitutional skin pigmentation, skin types ranging from skin type I to V. Skin was artificially reddened using topical application of 10% NA cream. Preoperative skin pigmentation, skin redness, and laser fluence were significant risk factors for the induction of postoperative clinically visible pigmentary changes 3 and 6 months postoperatively. Risk levels of 1, 2, 5, 10, 20, and 50% for inducing pigmentary changes are illustrated for different combinations of preoperative pigmentation and redness degrees at a treatment level of 6 J/cm² (Fig. 4). The risk of pigmentary changes was higher 3 months postoperatively than 6 months postoperatively, indicating a gradual disappearance of laser-induced pigmentary alterations. Skin reflectance measurements documented that postoperative hyperpigmentation faded partially from 3 to 6 months postoperatively and that hyperpigmentation was more intense in the NA-reddened test area compared with the normal skin and placebo cream test areas.

Discussion

The influence of preoperative skin redness, pigmentation, and epidermal thickness on the development of pigmentary changes from PDL treatment was investigated in studies VI, VII, and VIII. Skin reflectance measurements (studies VI, VII, and VIII), systematic clinical evaluations (studies VI and VII), and clinical score systems (study VIII) were used to quantify and graduate the laser-induced pigmentary alterations. The skin reflectance measurements are based on 555 and 660 nm wavebands of light in which the discrimination between light absorption in melanin and hemoglobin is maximal (Fig. 1). None of the skin chromophores absorb in narrow bands. Therefore, both heavy melanin pigmentation and severe erythema will influence calculations of the other chromophore (Andersen et al. 1990). Equations for calculating pigmentation and redness independently are build into the instrument. Skin pigmentation and skin redness are quantified on relative biological scales from 0% to 100% (Wulf 1986). The reflectance method has previously been used to determine erythema and melanogenesis from UV exposure (Bech-Thomsen et al. 1994; Lock-Andersen et al. 1997a), to estimate facultative and constitutional skin pigmentation within skin types I to IV (Lock-Andersen et al. 1998), and to predict UV sensitivity in normal skinned persons and persons with cutaneous malignant melanoma and basal cell carcinoma (Lock-Andersen et al. 1997b). Moreover, a highly-significant correlation has been found in the objective evaluation of UV-induced erythema between skin reflectance and laser Doppler flowmetry (Lock-Andersen et al. 1997a). In laser studies the metod has previously been used to quantify preoperative skin pigmentation (Hædersdal et al. 1994).

In study VIII, preoperative skin pigmentation was pointed out as a significant risk factor for inducing postoperative clinically visible pigmentary changes (Fig. 4), which is in accordance with the results from the AL and the CVL (studies I and III). For the first time in literature, skin reflectance was used with success to objectify fading of laser-induced hyperpigmentation from 3 to 6 months postoperatively. However, reflectance measurements have previously been unable to quantify pigmentary alterations from AL and CVL exposure (study I). It is not clear why the reflectance-method could be used to quantify pigmentary changes from PDL treatment but not from treatment with the AL and the CVL. Uneven pigmentary changes, irregular surface texture, and small measuring areas may have

influenced the measurements relatively more in study I than in study VIII, thus explaining the discrepancy partially.

In study VIII the importance of preoperative skin redness was investigated for the development of postoperative pigmentary changes. This approach has not been examined by other groups. The skin was artificially reddened by topical application of NA and the preoperative redness degree was found a significant risk factor for inducing postoperative pigmentary alterations, since a high degree of preoperative redness was associated with a high degree of postoperative pigmentary alterations (Fig. 4). This finding was quite surprising since, in advance, it was presumed that a high amount of target chromophore might result in a high degree of specific, intravascular energy delivery and, consequently, a reduced degree of unspecific energy delivery. The results, nevertheless, correspond to the clinical situation that laser-induced purpura is more intense in dark-red lesions than in light-red lesions due to a high amount of the target chromophore. The applicability of the risk assessment model to PWSs was evaluated by reconsidering the data from study VI and, unexpectedly, it was found that no correlation existed between the preoperative skin redness and the laser-induced hyperpigmentation, assessed by skin reflectance (study VI, unpublished data). The question is, therefore, raised whether the NA model overestimates the importance of preoperative skin redness. The missing correlation between preoperative redness and postoperative hyperpigmentation in PWSs may be explained by the circumstance that the prostaglandin-induced vasodilation from topical application of NA (Morrow et al. 1992; Wilkin et al. 1985) does not correspond to the histological appearance of PWSs that are characterized by capillary ectasia in the papillary and superficial reticular dermis (Dover 1996) and sometimes by an increased blood flow (Troilius et al. 1992). Another explanation may be the circumstance that preoperative skin redness is only one among several influential parameters (i.e. dose, skin pigmentation, lesional depth) and it may not be possible to separate the individual importance of these variables from each other.

In study VII the importance of preoperative epidermal thickness was evaluated for the occurrence of PDL-induced pigmentary alterations. Neither by clinical examination nor by reflectance measurements was it possible to detect any pigmentary changes, indicating that epidermal thickness is unimportant for the development of PDL-induced hyperpigmentation.

4.4 TEXTURAL CHANGES

Several clinical studies have advocated the PDL as safe due to a low incidence of scarring. A few studies have evaluated the occurrence of textural changes systematically (Boixeda et al. 1997; Fiskerstrand et al. 1998; Levine et al. 1995; Seukeran et al. 1997; Wlotzke et al. 1996) and one study has investigated textural changes objectively (Alster et al. 1993). In this study, 2-dimensional optical profilometry describes that the surface texture remains unchanged after PDL-treatment of previously untreated PWSs (Alster et al. 1993).

Own investigations

Textural changes and scarring from PDL-treatment were investigated in studies VI, VII, and VIII.

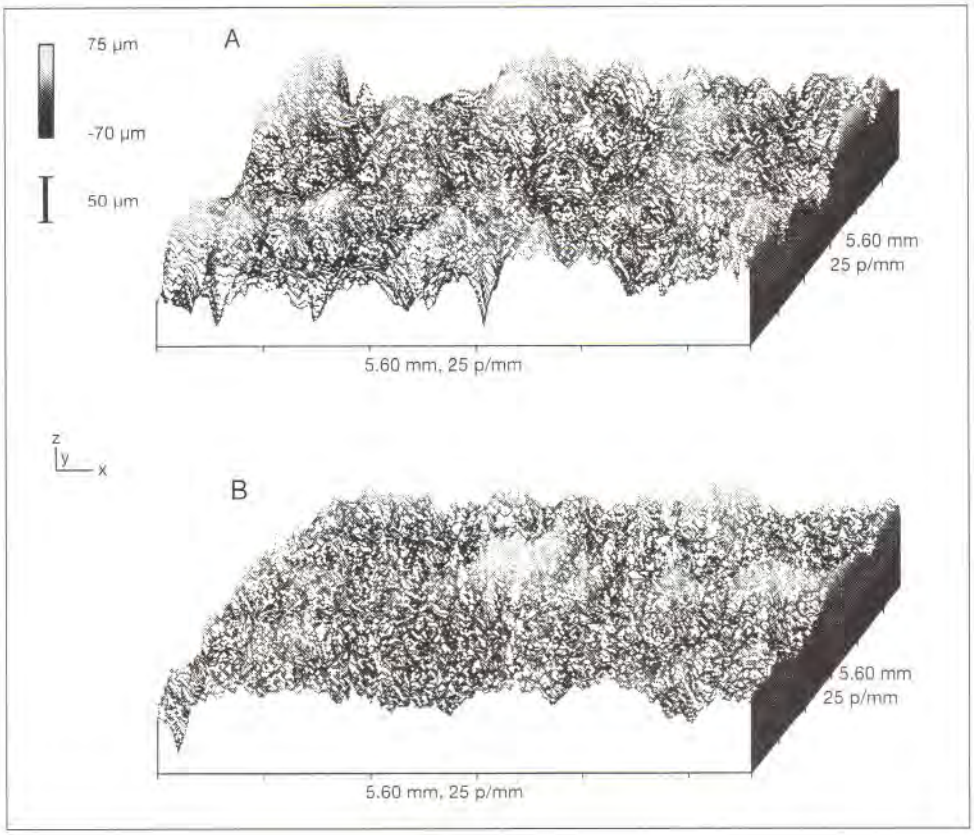

Fig. 5. *Representative three-dimensional profile plots obtained from a skin area before PDL treatment (A) and 12 weeks postoperatively (B) with values of scan area and sample density. The illustrated plots are depicted as inverted plots and give a direct impression of the skin surface topography. The surface topography appears more homogeneous and flat after laser treatment (B) than before laser treatment (A). This impression agrees with the quantitative reduction in skin contour parameters after laser treatment. (From Haedersdal et al. Arch Dermatol 1998; 134: 175–181. Copyright 1998, American Medical Association).*

In study VI children with macular PWSs were laser treated with the PDL (Table II). By clinical evaluation, no patients developed epidermal alterations, atrophy, or hypertrophy. Three-dimensional surface contour analyses revealed that the surface contour parameters (S_a, S_q, S_k S_{pk} and S_{vk}) decreased postoperatively, indicating a flattening of the skin surface (Fig. 5). By reevaluating the surface contour data, it was found that the preoperative skin redness tended to correlate with the degree of laser-induced postoperative surface contour changes (unpublished data). Correlation coefficients and corresponding p values were for the different contour parameters: $\triangle S_k$ ($r = 0.34$, $p = 0.03$), $\triangle S_a$ ($r = 0.30$, $p = 0.06$), $\triangle S_q$ ($r = 0.29$, $p = 0.07$), $\triangle S_{pk}$ ($r = 0.35$, $p = 0.03$), $\triangle S_{vk}$ ($r = -0.10$, $p = 0.5$).

In study VII the effect of epidermal thickness on laser-induced purpura was investigated (Table I). The total epidermal thickness ranged from 60 to 100 μm. By clinical evaluation no epidermal alterations, atrophy, or hypertrophy could be detected.

In study VIII the importance of preoperative skin pigmentation and skin redness was investigated for the development of textural changes from one PDL treatment of normal-skinned human volunteers (Table II). The risk of inducing clinically visible textural changes 3 and 6 months postoperatively increased with higher preoperative skin pigmentation and redness degrees, and with the application of increasing laser doses. Risk levels of 1, 2, 5, 10, 20, and 50% of inducing textural changes are illustrated for different combinations of preoperative pigmentation and redness degrees at the 6 J/cm^2 treatment level (Fig. 4). The risk of clinically visible textural changes was higher 3 months postoperatively than 6 months postoperatively, indicating a gradual disappearance of laser-induced textural changes. Textural changes were induced at a higher fluence level than pigmentary alterations for individuals with identical preoperative skin pigmentation and redness degrees. Surface contour parameters obtained preoperatively, 3 and 6 months postoperatively from NA-reddened skin, decreased after laser treatment, indicating a flattening of the skin surface (Table IV, unpublished data). Skin thicknesses (ultrasonography, mm) increased gradually during the postoperative observation period (unpublished data): Median values (25th and 75th percentiles) were in the NA-reddened skin 0.95 mm (0.89–1.05) before treatment, 1.00 mm (0.90–1.11) 3 months postoperatively, and 1.03 mm (0.99–1.16) 6 months postoperatively (0 vs 3 months postoperatively $p < 0.01$, 3 vs 6 months postoperatively p = ns, Wilcoxon rank sum test based on median values from each volunteer. A p value was considered non significant at $p > 0.05$). Untreated control skin thickness remained constant during the observation period. Significant correlations were seen between the applied laser doses and the increase in skin thickness within the NA-reddened skin 3 months ($r = 0.31$, $p < 0.02$) and 6 months postoperatively ($r = 0.59$, $p < 0.0001$).

Discussion

The evaluation of textural changes from PDL treatment was performed by means of clinical descriptions and score systems (studies VI, VII and VIII), three-dimensional surface contour analyses (studies VI and VIII), and ultrasonography to evaluate skin thickness (study VIII).

The clinically scored data on textural changes in study VIII formed the basis of a risk assessment. No groups have previously dealt with this aspect. As for the risk of inducing pigmentary changes from the PDL, skin pigmentation, skin redness, and laser fluence were significant risk factors for inducing textural changes, meaning that dark-pigmented skin types, heavy skin redness, and high fluence levels indicate a high risk of postoperative textural changes. Laser-induced pigmentary changes were provoked at a lower fluence level than textural changes for individuals with identical preoperative pigmentation and redness degrees (Fig. 4), these results being in accordance with the AL and the CVL data (studies I and III). Moreover, the risk of inducing textural changes 3 months postoperatively was higher than the risk 6 months postoperatively, suggesting a gradual disappearance of clinically visible textural changes. This finding agrees with the clinical studies that have reported scarring as transient (Reyes et al. 1990; Seukeran et al. 1997). On the other hand, by means of non-invasive methods to objectify skin surface topography and skin thickness, it was found that a progressive subclinical remodelling of skin texture took place up to 6 months after PDL-exposure: Evaluation of the skin surface topography in children with PWSs revealed a subclinical flattening of the skin surface 3 months postoperatively (study VI). The surface flattening progressed in normal-skinned human volunteers from 0 to 6 months postoperatively as well as the ultrasonographic-evaluated skin thickness increased gradually during the 6 months postoperative observation period (study VIII).

Measuring skin thickness by means of 20 MHz ultrasonography has been described extensively (Serup et al. 1995). In the laser field, skin thickness has been suggested to be one of several factors that may account for anatomical differences in the response to PDL treatment of PWSs (Renfro et al. 1993). No detailed studies have been performed exploring the variations in skin thickness before and after PDL treatment, except from studies VI and VIII. In study VI, laser treatments were performed of PWSs and the observed decrease in skin thickness was considered a treatment response, whereas, in study VIII, normal skin was exposed to the PDL and the decrease in skin

Table IV. *The surface contour parameters before PDL treatment, 3 and 6 months postoperatively in nicotinic acid reddened human skin (median values, (25th and 75th percentiles), units in μm). The decrease in S_a and S_q indicates a reduction in the average deviation of the profile from the mean line, the decrease in S_k indicates a reduction of the height of the core material, ie the bearing area, and the decrease in S_{pk} and S_{vk} indicates a reduction in the peaks and valleys extending above and below the material core surface. The Friedman's two way analysis of variance and the Wilcoxon rank sum test were used for paired comparisons; these were based on median values from each volunteer. The P values were considered significant at $p \leq 0.05$.*

Parameter	Preoperatively	3 months postoperatively	6 months postoperatively	P value, 0 vs 3 months	P value, 0 vs 6 months
S_a	11.6 (10.3-12.1)	11.5 (9.5-12.0)	10.3 (8.8-12.0)	ns	0.01
S_q	14.6 (13.1-15.6)	14.7 (12.1-15.2)	12.9 (11.5-15.1)	ns	0.01
S_k	37.5 (33.5-38.0)	35.9 (30.3-38.6)	32.8 (27.8-37.3)	ns	0.01
S_{pk}	14.1 (13.3-16.1)	14.4 (11.6-15.9)	13.1 (11.0-15.6)	ns	ns
S_{vk}	14.9 (14.2-18.1)	13.8 (12.3-15.5)	14.1 (13.1-17.8)	0.05	0.05

thickness was considered an adverse effect. In 1992 Pickering et al. suggested optical profilometry as a suitable method to objectify changes in the surface contour from laser treatment of PWSs (Pickering et al. 1992). In study VI and VIII 3-dimensional changes in skin surface topography were detected from silicone impressions before and after laser exposure (Efsen et al. 1995). The circumstance that the 3-dimensional surface parameters decreased 3 and 6 months after laser treatment (studies VI and VIII) disagrees with the 2-dimensional analyses performed by Alster et al. in 1993. In this study, it was found that no surface contour changes followed PDL treatment of PWSs (Alster et al. 1993). However, it is a limiting condition to the 2-dimensional method that the parameters reflect the situation from a localized place at the impressions. On the contrary, the 3-dimensional method represents an accumulated image from the whole impression, therefore, reproducing the situation more realistically. The study by Alster et al. is, moreover, biased by methodological problems (one-tailed statistical comparisons, comparisons were performed from adjacent skin areas although it is a prerequisite for profilometry that comparisons are performed of exactly the same skin areas, since displacement just a few µm may alter the demography dramatically).

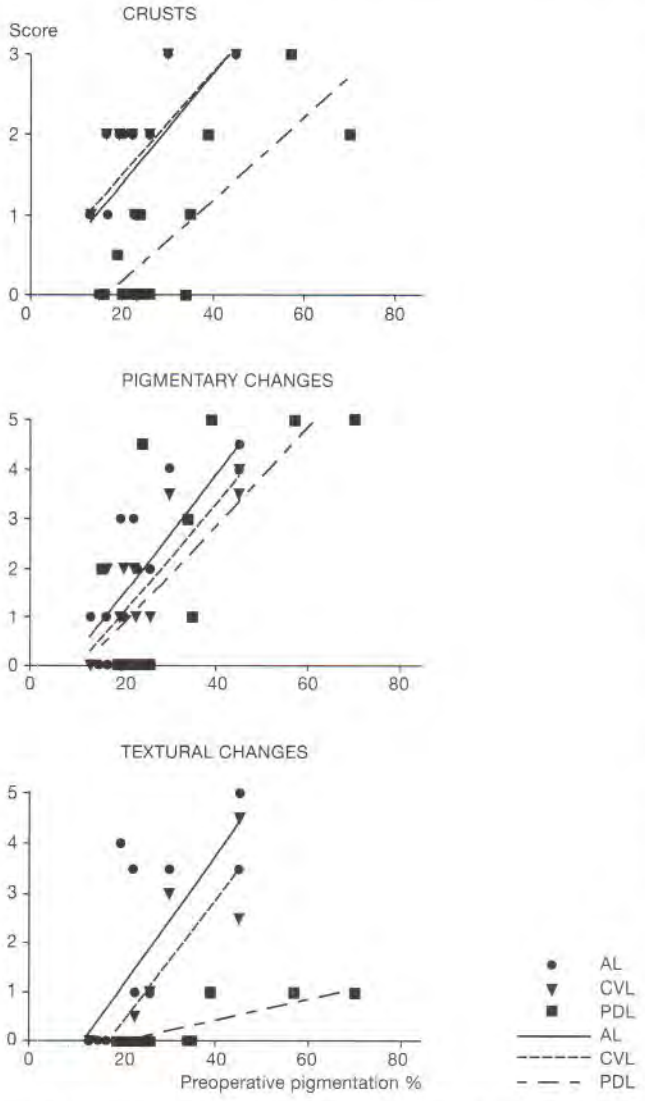

Fig. 6. *Comparative clinical scores for crusts (1 week postoperatively), pigmentary and textural changes (6 months postoperatively) from AL (1W), CVL (1W), and PDL (6 J/cm²) treatment. The PDL induced lower scores of crusts and textural changes than the AL and CVL, whereas no differences were seen for the scores of pigmentary changes. Median values (25th and 75th percentiles) of the score levels and significance levels were:* Crusts: *PDL 0 (0–1), AL 2 (1–2), CVL 2 (1–2), p = 0.022 for PDL vs AL, p = 0.017 for PDL vs CVL.* Pigmentary changes; *PDL 0.5 (0–4), AL 2 (1–3), CVL 1 (0–2), p = 0.70 for PDL vs AL, p = 0.98 for PDL vs CVL.* Textural changes; *PDL 0 (0–0), AL 1 (0–3.5), CVL 0 (0–1), p = 0.07 for PDL vs AL, p = 0.37 for PDL vs CVL.*

4.5 COMPARISON OF THE PULSED DYE LASER WITH THE ARGON LASER AND THE COPPER VAPOR LASER

Many clinical studies have advocated the PDL as a safe and effective treatment tool with a lower risk of side effects than the continuous wave AL and the quasicontinuous wave CVL. However, no comparative studies have been designed specifically to establish the relative incidences of side effects with the AL, the CVL, and the PDL.

Own investigations

No comparative data have been published in studies I–VIII regarding the relative efficacy of the AL, CVL, and PDL to provoke wounds, pigmentary changes, and scars. Unpublished data are presented in Fig. 6 from studies I (AL, CVL) and VIII (PDL, data from the normal skin test region), and from a previously published study dealing with short-term skin reactions from AL and CVL treatment (Hædersdal et al. 1994). The intention was to compare the presence of side effects from AL, CVL, and PDL treatments, using similar, clinical score systems, similar treatment models, and comparative fluence levels. The PDL and the AL/CVL were in different studies applied to healthy human skin with different preoperative skin pigmentation degrees, skin types ranging from skin type I to V. The preoperative skin pigmentation percentages were not significantly different for the PDL and the AL/CVL treated skin areas; the laser types could, therefore, be compared. All laser treatments were performed on the inside of the proximal brachium. Side effects were graded on similar clinical score systems that, however, were not identical for the PDL, respectively the AL/CVL. Therefore, the score systems were merged into new common score systems of wounds, pigmentary changes, and scars after PDL, AL, and CVL treatments, respectively: Wounds were graded 1 week postoperatively on the clinical score system that originally was used to score PDL-induced wounds (0–3). The areas of AL/CVL-induced wounds were initially quantified by depicting the outline on paper (Hædersdal et al. 1994). An area of 0 mm² was decided to correspond to a clinical score of 0, an area between 1–40 mm² to correspond to a clinical score of 1, an area between 41–80 mm² to correspond to a clinical score of 2, and an area between 81–120 mm² to correspond to a clinical score of 3. Pigmentary changes and scars were graded 6 months postoperatively on the clinical score system that originally was designed to grade AL/CVL-induced skin reactions (0–5, study I). The score levels of PDL-induced pigmentary changes (Table I in study VIII) corresponded exactly to the original score system with the only exception that score levels of 5, 6, and 7 were merged into score level 5. The PDL-induced scoring of textural changes (Table I in study VIII) corresponded to the original score system in the

way that score levels of 1, 2, and 3 were merged into a new score level of 1, PDL-score levels of 4, 5, 6, and 7 corresponded to new score levels of 2, 3, 4, and 5.

The results are presented in Fig. 6. For the 3 lasers, the presence of side effects was associated with the preoperative pigmentation degree. Regarding crusts and textural changes, it turned out that the trend lines of the PDL were located to the right compared with the trend lines of the AL and the CVL, indicating that lower scores of crusts and textural changes were induced with the PDL (6 J/cm^2) compared with the AL (1W) and the CVL (1W) for individuals with similar preoperative skin pigmentation percentages. On the contrary, no differences were seen for the scores of pigmentary changes. Median values and significance levels (the Mann-Whitney test) are stated in the legend text to Fig. 6.

In Fig. 6, it was decided to compare the intensity levels of 1W (AL, CVL, pulse duration 200 msec, spot size 1 mm, median intensity level in study I was 1W) with the fluence level of 6 J/cm^2 (PDL, pulse duration 0.45 msec, spot size 7 mm, median fluence level in study VIII was 5.5 J/cm^2), because these treatment levels are representative of the median values of the applied laser intensities/fluences in studies I and VIII. In these studies, the treatment levels were selected to include a broad range of skin reactions, spanning from no visible skin reactions to heavy skin reactions. The fluence of 6 J/cm^2 represents a slightly higher fluence level than the median fluence value. However, 6 J/cm^2 was selected in order to avoid underestimation of side effects from the PDL. Based on Fig. 6 it is, therefore, concluded that the PDL induces lower scores of crusts than the AL / CVL and lower scores of textural changes than the AL. Moreover, it is seen that the PDL tended to induce lower scores of textural changes than the CVL, whereas no differences were seen between the 3 lasers concerning the induction of pigmentary alterations.

Discussion

This is the first time that the relative efficacy is presented of the AL, the CVL, and the PDL to provoke crusts, pigmentary changes, and textural changes. Concerning the development of crusts and textural changes, these data are in accordance with the impression that the PDL has a safe risk profile due to the concept of selective photothermolysis (Table I). Regarding the presence of postoperative pigmentary changes from PDL treatment, it was unexpected to find similar results for the PDL, the AL, and the CVL 6 months postoperatively. However, the results support the overall side effect profiles for the 3 laser types that were established from a summary of the literature (Table I). Here the 3 lasers were found to have equal, moderate postoperative occurrences of hyperpigmentation, which is in accordance with the results from Fig. 6. The results in this thesis confirm that postoperative pigmentary changes from PDL treatment occur at a level that need to be taken into account. Moreover, the results confirm the importance of standardizing the variables such as physical laser parameters, biological skin variables, and observation time when side effects are compared for different laser tools. Furthermore, the results from Fig. 6 illustrate that the preoperative degree of skin pigmentation is associated directly with the presence of side effects for both the AL, the CVL, and the PDL. In darker skinned patients, the laser fluence must be kept low, possibly subtherapeutic, to avoid damage to the epidermis and subsequent development of crusts, pigmentary changes, and textural changes. Therefore, it may be relevant to operate with a benefit/risk ratio for the individual patient that is going to be laser treated.

Risk assessments were performed for the AL/CVL (study III) and the PDL (study VIII). Comparing the contour lines from studies III and VIII, one has to be careful since preoperative skin redness is included as a risk factor only in study VIII (PDL). However, comparing the risk levels to induce pigmentary and textural changes for the AL/CVL versus the PDL, it turns out that for skin with identical preoperative skin pigmentation (20%) and similar preoperative skin redness (17%, average redness in studies I and III) there was ~ 35% risk with the PDL (6 J/cm^2) and beyond 50% risk with the AL/CVL (1.0 W) for inducing pigmentary alterations 6 months postoperatively. The corresponding risk levels to induce textural changes were 10% with the PDL (6 J/cm^2) and ~ 15% with the AL/CVL (1.0 W). It is difficult to believe that the estimated risk profiles to induce textural changes from PDL and AL/CVL treatments do not vary more than described above. This may be explained by several conditions: Changes in skin pigmentation and textural changes from PDL treatment were evaluated from blown-up before-and-after slides at high magnification whereas skin reactions from the AL/CVL treatments (studies I and III) were bed-side examined, thus facilitating and possibly overestimating the risk for the PDL compared with the risk for the AL/CVL. Moreover, not exactly the same end-poins and assessment-methods were used for the PDL (study VIII) and the AL/CVL (study I and III) when estimating the risk of textural changes: For the PDL the end point was "shiny epidermal appearance", for the AL/CVL it was "just visible textural changes". Therefore, when comparing the efficacy of the AL, the CVL, and the PDL to induce side effects, it is a prerequisite that the preoperative skin conditions and the evaluated end points are identical. Consequently, the data from Fig. 6 are preferred to be used when the AL, the CVL, and the PDL are compared.

4.6 CONCLUSIONS AND PERSPECTIVES

Purpura, wounds, pigmentary changes, and textural changes were evaluated as end points from PDL treatment. Variations in preoperative epidermal thickness did not influence the development of postoperative purpura and pigmentary alterations (study VII). Preoperative skin pigmentation and laser fluence were in healthy human skin associated with the development of postoperative wounds, pigmentary alterations and textural changes (study VIII). Pigmentary changes were induced at a lower fluence level than textural changes, indicating that postoperative pigmentary changes may be the first sign that a too high laser fluence has been applied to the skin. Laser-induced pigmentary alterations and textural changes faded during the postoperative observation period, resulting in a higher risk of clinically visible side effects 3 months postoperatively than 6 months postoperatively (study VIII). The postoperative fading of laser-induced hyperpigmentation was documented by reflectance measurements. In contrast, optical profilometry and ultrasonography documented a progressive remodelling of the surface contour and the skin thickness up to 6 months after treatment (study VIII).

The importance of preoperative skin redness was evaluated in artificially reddened skin by topical application of NA. It was documented that the presence of wounds, pigmentary altera-

tions, and textural changes was higher in NA-reddened skin than in corresponding normal skin and that the risk of inducing pigmentary alterations and textural changes was highly dependent on the preoperative redness degree (study VIII). Concerning risk assessments, the question is raised whether the NA model overestimates the importance of preoperative skin redness, since it was impossible to confirm the preoperative skin redness as a potential risk factor in PWSs: Preoperative lesional redness tended to correlate with laser-induced postoperative surface contour changes, quantified by optical profilometry, but not with laser-induced hyperpigmentation, assessed by reflectance measurements. In conclusion, the NA model is not optimal but, at the present time, it is the best available human model for experimental studies on laser treatment of vascular lesions. In order to be able to predict the precise individual risk of side effects from PDL-treatment of vascular skin, an additional study on PWSs may, therefore, be carried out. Hereby the preoperative information to incoming patients will be improved and the individual patient will obtain a realistic impression of the risks to dermatological laser therapy.

In children with macular PWSs, skin reflectance measurements documented a postoperative subclinical increase in skin pigmentation as well as optical profilometry documented that the surface contour relief was subclinically flattened after PDL-treatment (study VI). Except reflectance measurements, the objective, non-invasive end points are rather time-exhausting in the clinical situation. However, optical profilometry, and ultrasonography may be used as research tools to refine the description of laser-induced side effects.

In normal human skin with different preoperative skin pigmentation degrees, the PDL was found to induce less heavy crusting (1 week postoperatively) than the AL and the CVL and less heavy textural changes (6 months postoperatively) than the AL, whereas comparable results were found for the 3 laser tools concerning the development of pigmentary changes 6 months postoperatively (Fig. 6). When characterizing and comparing different laser systems, it may be relevant to operate with a benefit/risk ratio, because the treatment not only must be effective but also free of side effects.

5. SUMMARY IN ENGLISH

It has been the intention of this thesis to increase the knowledge on the development of cutaneous side effects from treatment with the argon laser, the copper vapor laser, and the pulsed dye laser, which represent technical developments within laser systems used for treatment of vascular lesions. To reach that goal, the investigations focused on patient and lesional characteristics (skin pigmentation, skin redness, and epidermal thickness) and on the importance of UV irradiation before and after dermatological laser treatment. The aspect of UV irradiation was added because vascular lesions frequently involve the face and, therefore, may be exposed to sunlight in relation to laser treatment. Risk assessments were performed on clinically visible side effects in order to improve the preoperative information to the patients about their individual risks of obtaining side effects from dermatological laser treatment. The laser-induced side effects were evaluated by systematic clinical assessments, by histological and biochemical examinations, by skin reflectance measurements, optical profilometry, and ultrasonography. The term side effects is associated with both transient and permanent skin reactions such as purpura, wounds, textural changes, scars, pigmentary changes, and squamous cell carcinomas. Lightly pigmented, hairless hr/hr C3H/Tif mice, hairless, albino hr/hr MORO/Ibm mice, human, healthy volunteers, and children with port-wine stains were included in the studies. This thesis represents the first systematic and experimental approach to selected side effects from laser treatment of the skin.

The argon laser (AL) and the copper vapor laser (CVL)

The results from AL and CVL treatments are described together because these lasers are continuous / quasicontinuous lasers that do not meet the requirements for selective photothermolysis, which represents the most selective delivery of energy to cutaneous vessels. In normal-skinned human volunteers, the postoperative development of scars and pigmentary alterations depended on the preoperative constitutive skin pigmentation degree. Significant correlations were found between the preoperative skin pigmentation and the clinically scored pigmentary changes and scarring 1, 2, and 6 months postoperatively, indicating that dark-pigmented skin types respond with more heavy skin reactions than fair-pigmented skin types. Pigmentary changes occurred at lower intensity levels than scarring. No difference was seen between the AL and the CVL concerning the risk of inducing these side effects.

In hairless, albino hr/hr MORO/Ibm mice increasing epidermal thicknesses reduced CVL-induced wounds and scars. Significant negative correlations were found between preoperative epidermal thicknesses and CVL-induced skin reactions.

In lightly pigmented, hairless hr/hr C3H/Tif mice, CVL treatment induced an increase in skin pigmentation, evaluated by a semiquantitative technique. Postoperative UV irradiations with simulated solar UV increased the CVL-induced skin pigmentation additionally. The size of CVL-induced wounds and scars tended to enlarge by preoperative UV irradiations. In contrast, CVL-induced wounds were diminished and had a prolonged wound healing time when postoperative UV irradiations were given. This may indicate a deep constricted skin damage. Moreover, the histologically evaluated fibrosis and the frequency of bulging infiltration were increased by postoperative UV irradiation. When taking the liberty to extend the obtained results from the animal studies to humans, these results support the importance of pre- and postoperative sunprotection. Regarding skin cancer, in hairless mice it was found that one treatment with the CVL did not have a malignant potential itself. Pretreatment with the CVL at the highest intensity level (1.4W) delayed UV-induced photocarcinogenesis significantly.

The pulsed dye laser (PDL)

The PDL is described separately because it is the only laser in this thesis that fulfills the requirements for selective photothermolysis. Variations in preoperative epidermal thickness did not influence the development of postoperative purpura and pigmentary changes in fair-skinned human volunteers. Preoperative skin pigmentation and laser fluence were in healthy human skin associated with the development of postoperative wounds, pigmentary alterations and textural changes. Pigmentary alterations were induced at a lower fluence level than textural changes for individuals with identical preoperative skin pigmentation and redness degrees, indicating that pigmentary alterations may be the first sign that a too high laser fluence has been applied to the skin. Laser-induced pigmentary alterations and textural changes faded during the postoperative observation period, resulting in a higher risk of clinically visible side effects 3 months postoperatively than 6 months postoperatively. Optical profilometry and ultrasonography documented a progressive remodelling of the surface contour and the skin thickness up to 6 months after treatment. The postoperative fading of laser-induced hyperpigmentation was documented by reflectance measurements.

The importance of preoperative skin redness was evaluated in healthy human volunteers, in which the skin was artificially reddened by topical application of nicotinic acid (NA). Using this model, laser fluence, preoperative skin pigmentation and skin redness were significant risk factors for inducing clinically visible side effects. Moreover, the presence of wounds, pigmentary alterations, and textural changes was higher in NA-reddened skin than in corresponding normal skin. Concerning risk assessments, the question is raised whether the NA model overestimates the importance of preoperative skin redness since it was impossible to confirm preoperative skin redness as a potential risk factor in port-wine stains: Preoperative lesional redness tended to correlate with laser-induced postoperative surface contour changes, quantified by optical profilometry, but not with laser-induced hyperpigmentation, assessed by reflectance measurements. In conclusion, the NA model is not optimal but, at the present time, it is the best available human model for experimental studies on laser treatment of vascular lesions.

In children with macular port-wine stains, skin reflectance measurements documented a postoperative subclinical increase in skin pigmentation and optical profilometry documented that the surface contour relief was subclinically flattened after PDL-treatment. Except reflectance measurements, the objective, non-invasive end points are time-consuming in the clinical situation. However, optical profilometry and ultrasonography may be used as research tools to refine the description of laser-induced side effects.

In normal human skin with different preoperative skin pigmentation degrees, the PDL was found to induce less heavy crusting (1 week postoperatively) than the AL and the CVL and less heavy textural changes (6 months postoperatively) than the

AL, whereas comparable results were found for the 3 laser tools concerning the development of pigmentary changes 6 months postoperatively. When characterizing and comparing different laser systems, it may be relevant to operate with a benefit/risk ratio, because the treatment must not only be effective but also free of side effects.

6. SUMMARY IN DANISH (DANSK RESUME)

Denne afhandling tilsigter at øge den eksisterende viden om dermatologiske bivirkninger efter laserbehandling med argon laser, kobber damp laser, og pulseret farvestof laser. Disse 3 lasertyper repræsenterer den tekniske udvikling, der er sket indenfor laserbehandling af vaskulære hudlæsioner gennem de seneste år. Undersøgelserne fokuserer på patient- og hudlæsionskarakteristika (hudpigmentering, rødme og epidermal tykkelse) samt på betydningen af UV-eksponering før og efter laserbehandling. UV-aspektet er inddraget, fordi vaskulære læsioner hyppigt er lokaliseret i ansigtet og derfor har mulighed for at blive udsat for soleksponering i forbindelse med laserbehandling. Yderligere er der foretaget en individuel risikovurdering med henblik på at forbedre den præoperative information til patienter, der skal laserbehandles. De laserinducerede bivirkninger blev vurderet ved kliniske, histologiske og biokemiske undersøgelser, ved hudreflektansmålinger, optisk profilometri samt ved ultralydsmålinger. I afhandlingen anvendes »bivirkninger« både om midlertidige og permanente hudlæsioner som purpura, sår, teksturændringer, ar, pigmentforandringer og spinocellulær hudcancer. I studierne indgik hårløse, let pigmenterede hr/hr C3H/Tif mus, hårløse, albino hr/hr MORO/Ibm mus, frivillige, raske forsøgspersoner og børn med portvinsmærker. Denne afhandling repræsenterer således en systematisk og eksperimentel indfaldsvinkel til at vurdere kutane bivirkninger efter laserbehandling af huden.

Argon laser (AL) og kobber damp laser (CVL)

Resultaterne fra AL og CVL beskrives samlet, idet disse lasertyper er kontinuerte/kvasikontinuerte lasere, som ikke opfylder kravene til selektiv fototermolyse, der i dag anses for at være den mest selektive måde at destruere kutane kar. Hos frivillige forsøgspersoner med forskellig konstitutionel hudpigmentering afhang fremkomsten af laser-inducerede ar og pigmentforandringer af den præoperative pigmenteringsgrad, ligesom risikoen for at inducere bivirkninger steg med tiltagende hudpigmentering. Signifikante korrelationer kunne påvises mellem den præoperative hudpigmenteringsgrad og de klinisk evaluerede pigmentforandringer og arvævsdannelse 1, 2, og 6 måneder efter laserbehandling. Pigmentforandringerne blev induceret ved lavere intensitetsniveauer end arvævsdannelse. Der kunne ikke påvises nogen forskel mellem AL og CVL med hensyn til risikoen for at inducere disse bivirkninger.

I hårløse, albino hr/hr MORO/Ibm mus betød stigende epidermal tykkelse, at størrelsen af CVL-inducerede sår og ar blev reduceret. Der kunne påvises signifikante, negative korrelationer mellem den præoperative epidermale tykkelse og de CVL-inducerede hudreaktioner.

I hårløse, let pigmenterede hr/hr C3H/Tif mus fandtes, at CVL behandling i sig selv inducerede en stigning i hudens pigmenteringsgrad, bedømt ved non-invasiv, semikvantitativ teknik. Gentagne postoperative bestrålinger med simuleret sollys øgede pigmenteringen yderligere i såvel den laserbehandlede hud som i den omkringliggende ikke-laserbehandlede hud. I forhold til de ubestrålede kontrolgrupper var der en tendens til, at størrelsen på de CVL-inducerede sår og ar blev forøget, når UV bestrålinger blev givet forud for laserbehandlingen. Derimod resulterede postoperative UV bestrålinger i mindre sår med forlænget helingstid, hvilket kan afspejle en dyb, kontraheret hudskade. Graden af histologisk bedømt fibrose og frekvensen af klinisk arvævsdannelse med øget konsistens var forøget i de postoperativt UV bestrålede grupper, bedømt i forhold til tilsvarende kontrolgrupper. Med det forbehold at resultaterne kan overføres fra dyreeksperimentelle studier til patienter med vaskulære læsioner, støtter resultaterne nødvendigheden af at anvende solbeskyttelse forud for og efter laserbehandling. En behandling med CVL havde i sig selv ikke et malignt potentiale. Den UV-inducerede fotokarcinogenese var forsinket, når CVL-behandling i høj intensitet blev givet forud for bestrålinger med simuleret sollys.

Pulseret dye laser (PDL)

Resultaterne fra PDL-behandling beskrives særskilt, da PDL er den eneste lasertype i denne afhandling, som opfylder kravene til selektiv fototermolyse. Variationer i præoperative epidermal tykkelse var uden betydning for fremkomsten af postoperativ purpura og postoperative pigmentforandringer hos frivillige, raske forsøgspersoner med lys hud. Derimod kunne den præoperative pigmenteringsgrad og de anvendte laserdoser associeres med graden af de laser-inducerede sår, pigment- og teksturforandringer hos frivillige, raske forsøgspersoner med varierende konstitutionel hudpigmentering. Pigmentforandringerne blev induceret ved lavere dosisniveauer end teksturforandringerne, hvilket tyder på, at pigmentforandringer efter PDL-behandling er det første tegn på, at bivirkningsgrænsen er nået. De laser-inducerede pigment- og teksturforandringer aftog i løbet af den postoperative observationsperiode, hvorfor der er en større risiko for at observere bivirkninger 3 måneder efter laserbehandling end 6 måneder efter behandling. Hudreflektansmålinger dokumenterede, at den laserinducerede hyperpigmentering aftog i løbet af den postoperative observationsperiode. Optisk profilometri og ultralydsmålinger dokumenterede derimod en progressiv remodellering af såvel overfladekontur som hudtykkelse i op til 6 måneder efter laserbehandling.

Betydningen af præoperativ hudrødme for fremkomsten af bivirkninger blev undersøgt på frivillige, raske forsøgspersoner, hvor påsmøring af nikotinsyre gjorde huden rød. Laserdosis, hudpigmentering og hudrødme var signifikante risikofaktorer for at inducere klinisk synlige bivirkninger. Desuden fandtes, at forekomsten af sår, pigmentforandringer og teksturforandringer var højere i nikotinsyre-rød hud end i den tilsvarende normale hud. Med hensyn til risikovurdering stilles der spørgsmål ved, om nikotinsyre-modellen overestimerer betydningen af præoperativ hudrøme, idet det ikke var muligt at udpege præoperativ hudrødme som en potentiel risikofaktor i behandlingen af portvinsmærker. Her var tendensen, at den præoperative hudrødme korrelerede med de laser-inducerede konturforandringer, objektiviseret ved optisk profilometri, hvorimod der ikke var nogen sammenhæng mellem hudrødme og graden af laser-induceret hyperpigmentering, bedømt ved hudreflektansmålinger. Det konkluderes, at nikotinsyremodellen ikke er optimal. Den er dog i øjeblikket den bedst tilgængelige humane model til eksperimentelle undersøgelser af vaskulære læsioner.

Hos børn med makulære portvinsmærker dokumenterede hudreflektansmålinger en subklinisk stigning i hudpigmentering efter PDL-behandling, ligesom optisk profilometri påviste en postoperativ subklinisk affladning af hudens overfladerelief. Bortset fra hudreflektans er de noninvasive, objektive parametre i den kliniske situation tidskrævende. Optisk profilometri og

ultralydsmålinger er derimod velegnede til forskningsmæssigt at objektivisere og forfine beskrivelsen af laser-inducerede bivirkninger.

Laserbehandlinger på frivillige, raske forsøgspersoner med varierende konstitutionel hudpigmentering dokumenterede, at PDL inducerer mindre sårdannelse (1 uge efter laserbehandling) end både AL og CVL samt mindre teksturforandringer (6 måneder postoperativt) end AL, hvorimod der ikke kunne påvises nogen forskel mellem de 3 lasertyper med hensyn til fremkomsten af pigmentforandringer 6 måneder efter laserbehandling. Som led i at karakterisere og sammenligne forskellige lasertyper vil det være relevant at operere med et benefit/risk begreb, idet det overordnede behandlingsresultat afhænger af både den kliniske effekt samt af forekomsten af bivirkninger.

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