

A Pilot Study of ICG Laser Therapy of *Acne Vulgaris*: Photodynamic and Photothermolysis Treatment

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Background and Objectives: The near-infrared (NIR) laser radiation due to its high penetration depth is widely used in phototherapy. In application to skin appendages, a high selectivity of laser treatment is needed to prevent light action on surrounding tissues. Indocyanine green (ICG) dye may provide a high selectivity of treatment due to effective ICG uploading by a target and its narrow band of considerable absorption just at the wavelength of the NIR diode laser. The goal of this study is to demonstrate the efficacy of the NIR diode laser phototherapy in combination with topical application of ICG suggested for soft and thermal treatment of *acne vulgaris*.

Study Design/Materials and Methods: Twenty-two volunteers with facile or back-located acne were enrolled. Skin sites of subjects were stained by ICG and irradiated by NIR laser-diode light (803 or 809 nm). One mg/ml solution of ICG was applied for 5 or 15 minutes to the cleaned skin site. Untreated, only stained and only light irradiated skin areas served as controls. For soft acne treatment, the low-intensity (803 nm, 10–50 mW/cm², 5–10 minutes) or the medium-intensity (809 nm, 150–190 mW/cm², 15 minutes) protocols were used. The single and multiple (up to 8–9) treatments were provided. The individual acne lesions were photothermally treated at 18 W/cm² (803 nm, 0.5 seconds) without skin surface cooling or at 200 W/cm² (809 nm, 0.5 seconds) with cooling.

Results: The observations during 1–2 months showed that soft acne treatment decreased the number of active elements, reduced erythema and inflammation, and considerably improved the skin state without any side effects. At high power densities (up to 200 W/cm²), ICG stained acne inflammatory elements were destroyed for light exposures of 0.5 seconds.

Conclusions: Based on the concept that hair follicle, especially sebaceous gland, can be intensively and selectively stained by ICG due to dye diffusion through pilosebaceous canal and its fast uptake by living microorganisms, by vital keratinocytes of epithelium of the canal and sebaceous duct, and by rapidly proliferating sebocytes, new technologies of soft and thermal acne lesions treatment that could be used in clinical treatment of acne were proposed. *Lasers Surg. Med.* 33:296–310, 2003.

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Key words: NIR laser irradiation; dye; pathogenic bacteria; sebaceous glands; skin images

INTRODUCTION

Acne vulgaris is the most common skin appendage disease seen in dermatological practice [1,2]. This is a follicular disorder that affects susceptible pilosebaceous follicles, primarily of the face, neck, and upper trunk, and is characterized by both non-inflammatory and inflammatory lesions. Abnormal keratin production with obstruction of the follicular opening, increased production of sebum (lipids secreted by the androgen-sensitive sebaceous glands (SG)), and proliferation of *Propionibacterium acnes* (*P. acnes*) leading to inflammation play the main role in the disease development.

The obstructed SG with accumulated sebum provides an ideal medium for proliferation of comensal bacteria, including *P. acnes*. The SG enlarges and forms a pimple, which is termed white head. When the SG ruptures, there is invasion of sebum/bacteria mixture to the surrounding matrix. Another clinical manifestation of acne is black head. Black head is an open comedo consisting of a plugged SG with melanin or oxidized melanin.

Treatment of acne may consist of treating the four underlying causes and symptoms:

1. Suppression of *P. acnes* by limiting its growth or by increasing the kill rate of the bacteria.
2. Reduction of sebum excretion rate by decreasing the proliferation, or by increasing the rate of sebocyte apoptosis or/and necrosis.

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3. Reduction or arrest of follicle ductal hypercornification.
4. Reduction or complete resolution of the inflammatory response in the dermis and epidermis surrounding the follicles affected by acne.

Until now, the main method of acne treatment is antibiotic treatment [1–3]. However, bacterial resistance is an increasing problem [4,5]. Therefore, various new therapeutic possibilities using light irradiation were intensively studied [6–15]. Therapies using UV and/or visible light were suggested. Recently, it has been shown that irradiation of *P. acnes* with UV (320–360 nm), white (halogen lamp), blue (415 nm), and/or red (660 nm) light, and corresponding light treatment of acne leads to different effectiveness of bacterial damage and improvement of acne in dependence of light wavelength and irradiation dose [8–10]. Visible light treatment is apparently attributed to photodynamic stimulation of aporphyrins stored in *P. acnes* (415 nm) and stimulation of fibroblast proliferation (660 nm).

Photodynamic therapy (PDT) is a well-known procedure used for recovery of many abnormalities including tumors and other diseases. PDT causes a repairable injury (local changes of the cell (bacteria) redox state) as well as irreversible damage leading to cell (bacteria) death [16,17]. The main toxic agents produced by PDT are singlet oxygen or other reactive oxygen species such as hydroxyl, superoxide, and hydrogen peroxide radicals that oxidize biological molecules. Oxidative damage has been shown to induce members of a family of stress proteins known as heat shock (HSP) and glucose-regulated proteins (GRPs) that are present at low constitutive levels in normal cells [18,19].

To enhance photodynamic bacteria killing and to provide effective modification of SG apparatus, exogenous or inductive-exogenous, like aminolevulinic acid (ALA), were used [9,12,13]. Other dyes activated by visible and NIR laser irradiation might be also used to kill *P. acnes* and to modify SG. Such optical range is preferable due to high penetration depth of light within a tissue. For example, in paper [14] effective photoinactivation of *P. acnes* stained by methylene blue (MB) and irradiated in red spectral range is described.

Indocyanine green (ICG) and near infrared laser irradiation were successfully used to destroy some kinds of tumors and cancer cell cultures due to wavelength selected thermal and photodynamic effects [20–22]. ICG is tricyanocyanine dye with strong absorption bands between 600 and 900 nm [23,24]. Recently, application of ICG and diode laser irradiation for acne treatment was described as a new approach based on selective photothermolysis of the SG [15].

The photodynamic properties of ICG have been investigated in vitro [25–27]. Based on these studies and accounting that local hyperthermia may induce cell apoptosis [28], we may hypothesize that photodynamic and/or local photothermal reactions kill the pathogenic bacteria as *P. acnes* and modify SG apparatus functioning. Indeed, to interact with NIR light deeply penetrated within tissue SG should

be effectively targeted by ICG. Fortunately, it was recently shown that topically applied ICG is effectively accumulated into SG [15,29,30].

Lasers have been used to correct a wide variety of congenital vascular disorders under the skin surface [13,31,32]. The absorption of light by hemoglobin, melanin, and water are typically used for the particular opto-thermal treatment (photothermolysis). To enhance the effectiveness and selectivity of photothermolysis method of selective targeting of skin appendages by various dyes, absorbing and magnetic particles incorporated in lotions, oils, nanoemulsions, and microspheres was recently designed in the framework of hair removal and skin phototherapy [12,13,29,30,33–37]. It was shown that some dyes and absorbing compositions, including MB and ICG, are well concentrated within a SG of the human skin at topical administration and can be used for effective thermolysis or photodynamic damage of *P. acne* or glands. ICG has a number of advantages due to its low toxicity, extraordinary absorption within a therapeutic window around 805 nm, where powerful diode lasers are available, and rather high efficiency of photodynamic action [25–27,38,39]. Therefore, ICG application for acne treatment is preferable.

To optimize light delivery to the target many studies on control of tissue optical properties were recently performed [40–46]. The selective translucence of the upper tissue layers is a key method for phototherapy techniques requiring optical access into underlying tissue layers. The marked reduction of scattering by matching the refractive indices of scattering centers and ground matter by means of intratissue administration of appropriate chemical agents was demonstrated.

The ICG dye lotion penetrates along a hair shaft to the soft non-keratinized tissues, in particular to SG. The depth and time of dye penetration for terminal hairs are about 1 mm and 5–15 minutes, respectively [30]. SG accumulate dye for some time interval τ before it will be washed out by blood circulation. This time interval can be estimated using the diffusion coefficient of ICG glycerol–water–ethanol solution in the living dermis ($D = 2.6 \cdot 10^{-6} \text{ cm}^2/\text{s}$), $\tau \approx l^2/D \approx 1.5$ hours, where l is the mean distance from SG to blood vessels [30,44,47]. In general, similar behavior is expected for the beard, vellus, and sebaceous follicles. For example, for a vellus follicle, which has rather small pore and thin miniscule hair incubated in it, such small pore may be sufficient to have the same rate of staining like for a terminal hair follicle [30]. Sebaceous follicles have very large pores, long canal free of hair, two or more sebaceous ducts, therefore, much higher staining efficiency is expected.

At acne, there are expected additional targets and paths for ICG staining of acne lesions, like black head (an open comedo consisting of a plugged SG with melanin or oxidized melanin) or the SG rupture (invasion of sebum/microorganisms mixture to the surrounding matrix). ICG can be effectively bound by melanin due to melanin's unique high-affinity sites for the binding of a large number of organic molecules, including dye-like materials [48].

We conducted this study to test methods of soft and photothermal action of NIR laser irradiation on SGs stained by ICG for the treatment of *acne vulgaris*.

MATERIALS AND METHODS

Methods

The present study is based on a few biophysical phenomena: (1) the selective targeting of skin appendages by dyes incorporated in a lotion, (2) reduction of scattering properties of skin by refractive index matching of scatterers (cell components, collagen fibrils, etc.) and ground (interstitial) media, and (3) optically induced bacteria and/or tissue cell damage and/or killing via apoptosis and/or necrosis (photodynamic and photothermal effects).

Method uses biocompatible chemical carriers and enhancers of skin and cells permeability, like ethanol, propylene glycol, glycerol, to provide maximal concentration of dye within a target tissue component (sebaceous gland) and/or bacteria within a short time period. Skin heating and massage were also used to increase dye diffusivity. Some of these agents also serve for enhancement of light penetration into skin (glycerol and propylene glycol) due to optical immersion effect.

The staining procedure includes the skin site cleaning by ethanol, followed removing of sebum and the upper layer of keratinocytes by a topical application of 3% hydrogen peroxide, deep cleaning of epidermis (particularly a keratinized epithelium of pilosebaceous canal) provided by the usage of cosmetic scrub or cream for peeling. Finally, 1 mg/ml solution of ICG in mixture with ethanol, glycerol, propylene glycol, and distilled water is applied for 5 or 15 minutes to the cleaned skin site. To minimize blocking of the irradiating light and overheating of the superficial skin layers, dye solution is carefully removed from the skin surface by ethanol immediately after staining.

Diode-Laser Systems and Irradiating Protocols for Acne Treatment

For photodynamic and photothermal treatment of acne, two laser-diode systems were used. The OPC-BO15-MMM-FCTS diode laser (Opto Power Corp., Tucson, AZ) working at 803 nm providing at a distance from a fiber tip a rather big and smooth illumination area with power density up to 50 mW/cm² was used for the low-intensity soft acne treatment. The 90 W Palomar diode-laser system ASAH 430P (Palomar Medical Technologies, Inc., Burlington, MA) with a water-cooled hand-piece, originally designed for hair removal and correction of a wide variety of congenital vascular disorders, working at 809 nm was used for medium-intensity soft and thermal acne treatment. To provide smooth and controlled power density (up to 190 mW/cm²) at large areas of the skin surface as a hand-piece, the fiber-optic-Fresnel's lens (LightCube) assembling was used (see, Fig. 1).

For photothermolysis of acne lesions, OPC-BO15-MMM-FCTS diode laser (803 nm) was used at a short distance from the fiber tip to the skin surface providing a light spot of about 5 mm. No skin cooling was applied. The

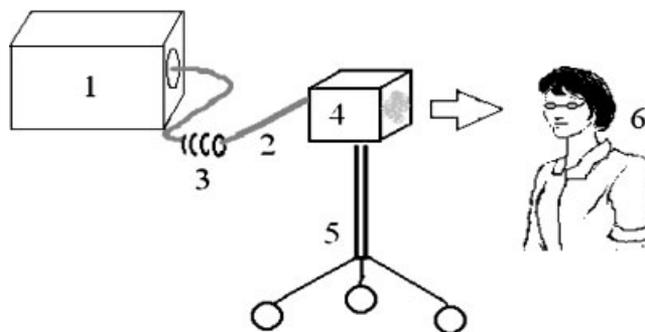


Fig. 1. Universal infrared (809 nm) diode-laser system for soft acne treatment: 1, ASAH 430P laser; 2, optical fiber; 3, fiber mode mixer; 4, Fresnel's lens (LightCube); 5, moveable support; 6, patient.

original ASAH 430P diode-laser system (809 nm) with a water-cooled hand-piece of 4 mm in diameter was also explored for lesion coagulation.

Four different irradiating protocols were tested keeping in mind future fields of laser acne treatment procedures suitable for ambulatory or home use. For soft acne treatment, two protocols were used—low-intensity and medium-intensity. Low-intensity protocol was realized at laser power densities of 10–50 mW/cm² (803 nm) on the skin surface and time exposures of 5–10 minutes, and medium-intensity protocol at 150–190 mW/cm² (809 nm) and exposure of 15 mm.

Photothermolysis was realized also using two protocols: (1) without skin surface cooling by irradiating the skin site by the laser beam (803 nm) at 18 W/cm² during 0.5 seconds; and (2) with skin surface cooling by irradiating the skin site by the laser beam (809 nm) at 200 W/cm² during 0.5 seconds. The individual acne lesions were treated.

Testing of Recovery

Treatment effects were determined using the comparison of the patient's scores from each follow-up visit to the baseline scores, which were documented using Nikon Coolpix 990 (Japan) digital camera. Clinical evaluation of changes in acne compared with the baseline was visually assessed using fixed-magnification photographs taken at standard illumination of the typical skin sites of 4 × 5 cm² or 3 × 4 cm². A linear polarizing filter (Crystal Optics, Japan) was placed on the lens to reduce specular reflection from the skin surface. To look deeper into skin, in particular to see blood supply of lesions, a cross-polarized mode of digital photography (polarized light illumination and cross-polarized detection) was used [12].

When lesion area was sufficiently big the comparison of four skin sites: ICG-light treated, only ICG stained (control), only laser irradiated (control), and untreated (control), was done. Usually these sites were chosen on the neighboring and/or symmetric areas of the skin (left and right cheeks or parts of chest and back). For each score, at least two sites (ICG-light treated and untreated) were photographed and analyzed. The comparison of the

baseline, the single-treatment, and multiple-treatment groups using photographs was also done.

For objective testing of recovery process, measurements of status of microflora using fluorescence technique and sebum excretion rate using sebum-absorbent tape were provided. For testing of microflora, fluorescence images of skin excretion on a glass plate ($2.5 \times 7.5 \text{ cm}^2$) were captured before and after acne photo-treatment. For sampling, a glass plate was pressed to the skin surface for a few seconds. The orange-red fluorescence (above 590 nm) was detected by the luminescence microscope Lumam RPO-11 (LOMO, St. Petersburg, Russia) with optical filters at excitation on 400–420 nm.

Sebum-absorbent tape (Sebutape ViewPro-Kit S111, CuDerm, Dallas, TX) was used for non-invasive and easy evaluation of patient's sebum output before and after the treatment. The subject's skin within the site of treatment was cleaned thoroughly with cotton pads soaked in 90% ethanol. When the skin was completely dry, the adhesive patch Sebutape was adhered to this site for an hour. After removal from the skin, the tape was placed on a clear card for the photometer analysis. During the contact with the surface of the skin, the micro-pores of the patch are filled up with sebum. Oiliness is revealed as transparent spots on the white (scattering) background. We measured the optical transmittance of the patches with the photocolormeter FK-120 (Russia). As a reference the transmittance of a clear card was used.

Subject Selection

Twenty-two subjects of both sexes with light to severe *acne vulgaris* enrolled between February and June 2001, October and November 2001, and March–April 2002 were separated in four groups: I—light (grades 0.25–0.75), II—moderate (1.0–1.5), III—moderate–severe (2.0–3.0), and IV—severe (4.0–8.0) graded in accordance with classification of Burke and Cunliffe (1984) [2]. For group I—open and closed comedones ($\sim 1 \text{ mm}$ in diameter), single small papules and pustules (or pimples, white heads) (1–2 mm) with 1–2 active elements within the area of $4 \times 5 \text{ cm}^2$ were seen. Open (black heads) and closed comedones ($\sim 1 \text{ mm}$), a number of papules and pustules (2–5 mm), macules, post-inflammatory pigmentation spots, and small scars (increased tissue formation or loss of tissue) with 2–3 active elements within the area of $4 \times 5 \text{ cm}^2$ were typical for group II. Numerous, partly integrated papules and pustules (5–10 mm), macules, post-inflammatory pigmentation spots, and scars with 2–3 active elements within the area of $4 \times 5 \text{ cm}^2$ were found for group III. Quite large papules and pustules (10–20 mm), sinuses, cysts, macules, post-inflammatory pigmentation spots, and scars; highly inflammatory acne covering the most of the face, neck, chest, and back, with 4–5 active elements within the area of $4 \times 5 \text{ cm}^2$ were characteristic for group IV.

People were excluded if they had used any topical acne treatment, systemic antibiotics in the past 2 weeks, or systemic retinoids in the past year. People who plan to have excessive sun exposure, or with history of keloid or photosensitivity disorder, pregnant and lactating

women, and mentally incompetent subjects were also excluded.

Subjects were randomly divided into single-treatment and multiple-treatment groups. Each patient's affected area was typically divided into two $4 \times 5 \text{ cm}^2$ (or $3 \times 4 \text{ cm}^2$) areas—one for combined ICG and NIR laser treatment and another for control. Sites were marked with a marker to precisely relocate each test area. At baseline clinical evaluation was performed. When the area with acne lesions was rather large (typically patient's back), it was divided into four $4 \times 5 \text{ cm}^2$ (or $3 \times 4 \text{ cm}^2$) areas for providing more objective control: areas for combined ICG and NIR light (treatment), for ICG only (control), for laser light only (control), and free of any action (control).

Usually, in the multiple-treatment group subjects were treated twice per week for four consecutive weeks. In both groups, clinical evaluation of treatment was carried out weekly for a month or 2 months after the last treatment.

RESULTS

Soft Acne Treatment With Low Light Intensity

The absorption spectra of staining lotions at ICG concentration of 1 mg/ml are presented in Figure 2. The glycerol–ethanol–propylene–glycol–water solution as the most efficient staining lotion for diode laser therapy was used in this work [30]. In tissues and cells, the IR ICG absorption peak moves to longer wavelengths, 805–810 nm, due to binding with cell proteins [25,47–50], that makes light-tissue interaction mediated by ICG more efficient for diode lasers with 809 nm.

Preliminary control studies were performed for group of six patients with *acne vulgaris* of different severity of the disease: light acne—2 patients (1 male and 1 female); moderate acne—1 patient (female); moderate-severe acne—2 patients (1 male, 1 female); severe acne—1 patient (male). The treatment was carried out separately by application of ICG lotion or laser irradiation at 803 nm. The

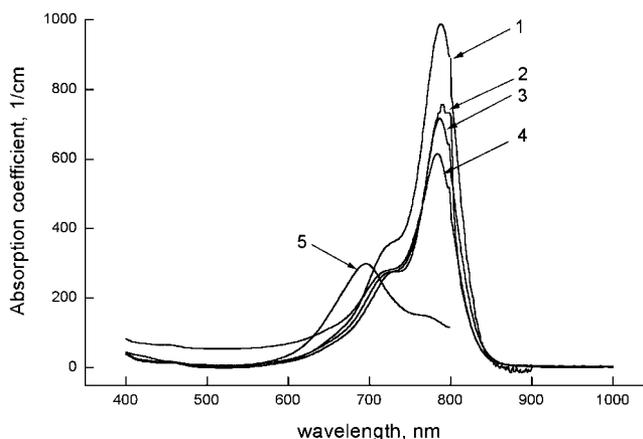


Fig. 2. Absorption spectra of indocyanine green (ICG) in various solvents at concentration of 1 mg/ml (1, ICG in glycerol–ethanol–propylene–glycol–water solution; 2, ICG in glycerol; 3, ICG in propylene glycol; 4, ICG in ethanol; 5, ICG in water).

time of staining was 5 minutes. The time of irradiation varied from 5 to 10 minutes, and power density of irradiation varied from 10 to 50 mW/cm². Total number of treatments varied from 1 to 3. Results of the observations have shown that at the separate application of dye or laser radiation no noticeable improvement of the skin lesions was seen for all patients.

Combined ICG-laser treatment at 803 nm was performed for 12 subjects of both sexes with different severity of *acne vulgaris*. The ages of volunteers were from 17 to 27 years. The skin lesions were localized on subjects' faces or backs. The staining time was 5 minutes. The exposure time and power density of irradiation were varied.

Group I. Localization of acne elements was on the forehead or back. Total number of treatments varied from 1 to 4, power density of laser radiation was 10 mW/cm², time of exposure was 5 minutes. Observation of the patients and repeated treatments were carried out weekly.

It was found that treatment of young patients having light form of *acne vulgaris* is positive, but not very effective. In a week after the treatment a light recovery was watched: initial erythema (inflammation) around acne lesions decreased, elements flattened, and new elements did not appear. In general, healing was demonstrated for used light dosage, but effect was rather light and in 2 weeks new elements and erythema reappeared.

Group II. Facile localized elements were treated. Total number of treatments varied from 1 to 4, power density of laser radiation varied from 10 to 50 mW/cm², time of exposure was in the range 5–10 minutes. Observation of the patients and repeated treatments were carried out weekly.

In a week after the single treatment initial erythema (inflammation) decreased, elements flattened and new elements did not appear. On the sites of old elements macules remained. In 2 weeks, new elements appeared and erythema (inflammation) reappeared. After repeated treatments, new elements did not appear in 2–3 and more weeks in dependence on the number of treatments and power density. The macules and post-inflammatory pigmentation lightened and disappeared. In a month after 4th treatment, elements appeared but the state of lesions was lighter than the initial ones.

Group III. Localization of elements was on the face, neck, back, and chest. Total number of treatments varied from 2 to 9, power density of laser radiation varied from 10 to 50 mW/cm², time of exposure was 5–10 minutes. Observation of the patients and repeated treatments were carried out weekly. After 4th treatment for one patient, number of treatments was increased to twice per week.

In 1 week after the single treatment initial erythema (inflammation) decreased, elements flattened and new elements did not appear. On the sites of old elements macules remained. For patient of 23 years, who was available for the further studies, in 2 weeks new elements appeared and erythema (inflammation) reappeared. After four weekly treatments, new elements did not appear during 1 month, the macules and post-inflammatory pigmentation lightened and disappeared, then elements appeared again but lesions was much lighter than the initial ones.

For each treated group, the bigger light dose caused more pronounced healing of lesions. For used light exposures and power densities no threshold effect was found. The multiple photo-treatments showed significantly more improvement than the single one. Positive effect retained in about a month after the finishing of procedure. After the single photo-treatment, the positive effect retained typically only for 1 week to ten days. No significant differences between the multiple and single-treatment groups were observed in the other tested sites (control).

Figure 3a–d presents images of two regions of the back of the subject from group III: treated area and untreated one, photographed before the treatment (a, c) and in a week post-treatment (b, d), respectively. The photos were made in non-polarized light. In Figure 3a, the big papule (left-mid) and pustule (right-up) are seen. During a week after treatment they are lightened and flattened, the pustule was transformed to the macule (Fig. 3b). Control (Fig. 3c, d) has shown continuation of inflammatory process and appearance of two new papules.

There was obvious and statistically significant improvement in acne at all follow-up visits after multiple photo-treatment. Series of photos in Figure 4 presents the images of four sites on the back of the subject from IV group. They were done for the parallel polarizer and analyzer. Each image corresponds to control and different treatments of the skin site. In Figure 4a, b, the site treated by ICG and IR light is shown: a—baseline (before treatment); b—2 weeks later, during that time four procedures were done. On the right side of the photo the cyst, which consists of three to four inflammatory elements is well seen. Two weeks later no inflammatory elements are seen, erythema decreased significantly in area and intensity. The crossed-polarized images allows for more precise estimation of inflammation areas (Fig. 5a, b). These images are formed mostly by the back-reflected light from the deeper layers of the skin and showing the skin blood supply. The boundaries and intensity of inflammation of skin lesions are clearly seen, about 50% decreasing of inflammation spots area was achieved due to phototherapy, new acne elements did not appear.

Figures 4c, d and 5c, d present images of the untreated control site photographed in parallel and crossed polarizers, respectively. During 2 weeks, the observed papules has been transformed into the pustules (not shown), and macules (shown) in the course of disease. In general, the improvement of the state of this skin site was not observed.

Figures 4e, f and 5e, f show the site treated with IR light only. Initially the state of this skin site was rather satisfactory, i.e., inflammatory process was not observed. During 2 weeks, a few pustules (mostly on the top of the image f) were appeared. Larger redness area of the skin site is seen in 2 weeks.

Within the area treated by ICG only (Figs. 4g, h and 5g, h) we also can see transformation of papules into pustules, no ICG action is found. Thus, only the combined dye and laser light treatment gives a well-recognized improvement in acne lesions within 2 weeks. The other three skin sites (untreated, light only, and ICG only) watched during 2 weeks showed either transfer to worse status of acne

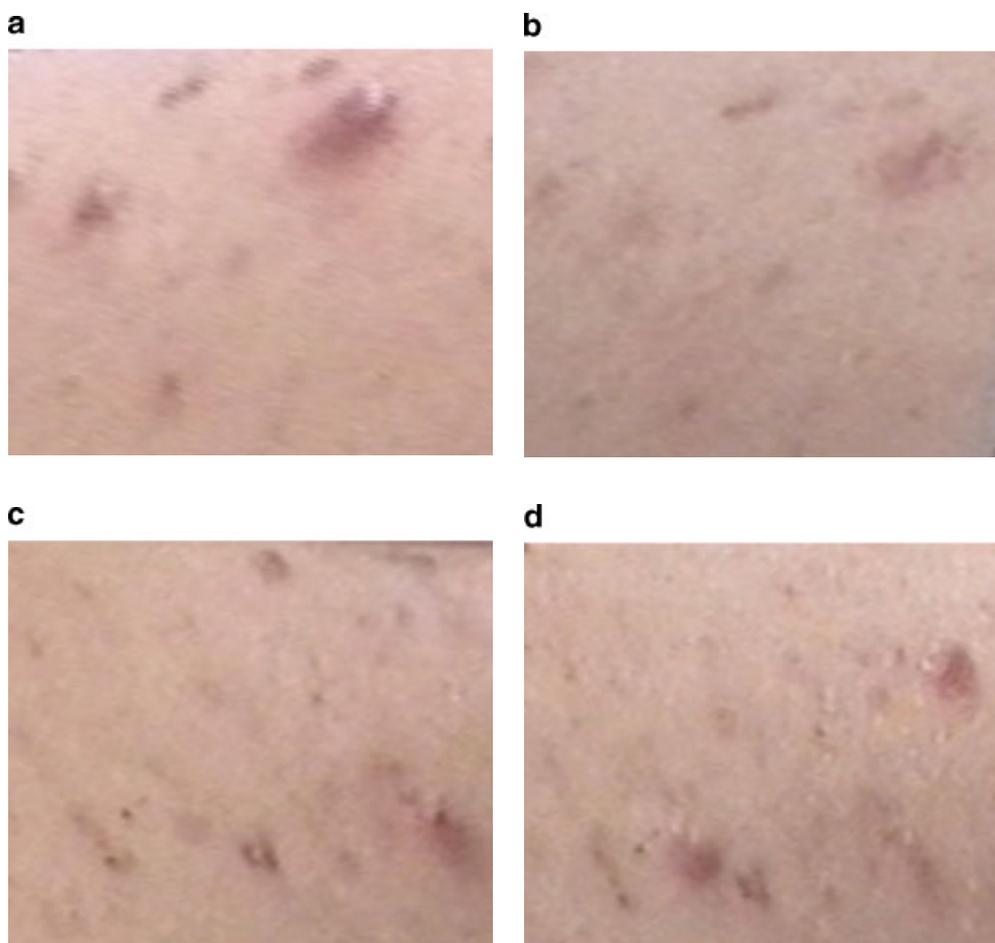


Fig. 3. Transient acneiform improvement after a single treatment (ICG and IR light). **a:** Baseline of the treated site; **(b)** 1 week post-treatment; **(c)** baseline of the untreated control site; **(d)** 1 week later. Skin site area of $3 \times 4 \text{ cm}^2$. [Figure can be viewed in color online via www.interscience.wiley.com.]

lesions or were not significantly different from the baseline for all visits of the patients.

Figure 6 illustrates the possibility of objective monitoring of results of acne treatment by detection of fluorescence of skin excretion samples before and after treatment. Orange-red fluorescence allows for recognition of *P. acnes* colonies taken from the skin site at excitation of bacteria produced porphyrins. It is well seen that after treatment much low intensity and smaller areas of fluorescence exhibit on the samples of skin excretion. Therefore, low-intensity acne treatment is connected with photodynamic bacteria suppression.

The sebum excretion rate of the treated skin sites determined by using of sebum-absorbent tape was not significantly different from the baseline.

Results of the treatment were more pronounced for moderate to severe groups of patients, having large inflammatory elements. Only slight improvement of the state of patients from group I, having mainly open and closed comedones, was observed. This is just associated with inhibition of *P. acnes* inducing inflammation.

Soft Acne Treatment With Medium Light Intensity

For the medium-intensity treatment, the same procedures for skin sites cleaning and staining as for the low-intensity treatment were used, but to increase the efficiency of follicular tissue, staining exposure to ICG-lotion was prolonged to 15 minutes. To increase the efficiency of bacteria killing and to provide additional mechanisms of acne treatment, much higher power densities of $150\text{--}190 \text{ mW/cm}^2$, more precise overlapping of bound ICG molecular band (810 nm) [50] and laser wavelength (809 nm), and more prolonged light exposure of 15 minutes were utilized. Universal infrared diode-laser system with Fresnel's lens, presented in Figure 1, was used.

In the study, four volunteers with different severity of the disease were treated: two females (22 and 29 years) with moderate acne, one male (16 years) with moderate-severe acne, and one male (23 years) with severe acne (see, Table 1). For two first patients (I and II) facile localized acne elements were treated. Elements were in the form of open

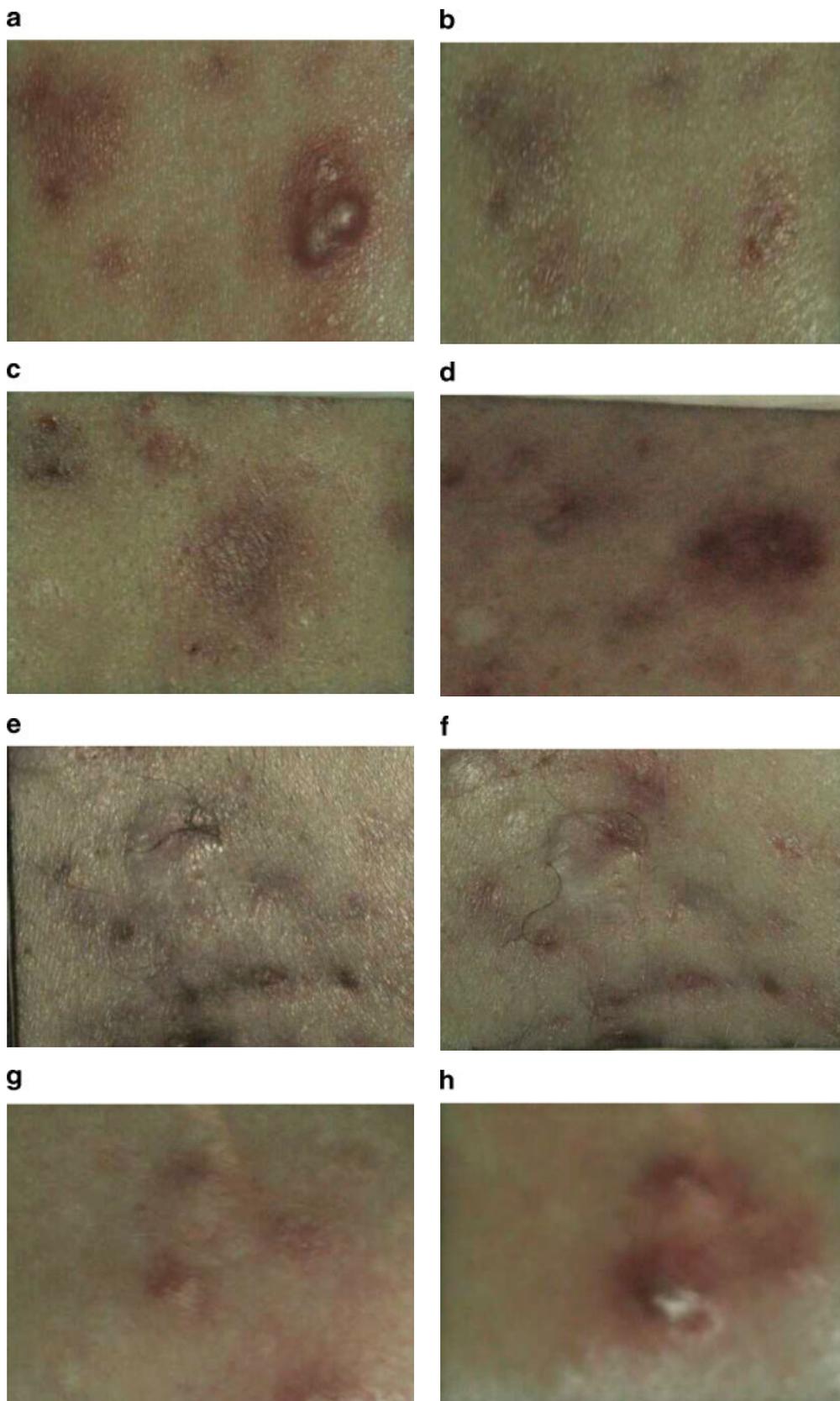


Fig. 4.

and closed comedones, papules, and pustules. There were macules and post-inflammatory pigmentation. For the patient III facile elements were in the form of multiple pustules and papules. For the patient IV back-localized elements were treated. Elements were in the form of quite large pustules (1–2 cm in diameter), sinuses, and cysts. Highly inflammatory acne covered most of the face, neck, chest, and back. There were macules and post-inflammatory pigmentation, and scars.

For each patient total number of treatments were 8–9, twice per week. The same as for the low-intensity protocol, only the combined dye and laser light treatment gave a well-recognized improvement in acne lesions. Observation of the patients was carried out once per 2 weeks during 1–2 months after completion of multiple treatment. For all these patients treatment gave more pronounced, stable, and prolonged effect than for low-intensity protocol. Images of patient's skin before and after treatment illustrate the efficiency of the designed laser-ICG technique of acne recovery. More objective representation of results of treatment was done by counting of the number of active acne elements within the control and treated skin site areas of $4 \times 5 \text{ cm}^2$.

Figure 7 shows the dynamics of results of medium-intensity soft acne treatment in a week and in a month after completion of multiple treatment. For all four patients positive effect of treatment was achieved. For three of them, effect in comparison to control skin sites was rather significant to the 7th day after completion of multiple treatment. Important to note that control (and initial) skin sites have their own dynamics of active elements formation, therefore sometimes, when acne is a developing process, phototreatment could not overcome acne elements development for a short period after multiple treatment (see, diagrams for patient I, Fig. 7a). Nevertheless, in a month after treatment active elements were suppressed for this patient as well. For two patients (III and IV), which were available for two months after completion of treatment, approximately the same positive results during the next month were seen as at a month after treatment (see, Fig. 7c,d).

The objective monitoring of acne treatment by detection of orange-red fluorescence of skin excretion samples before and after treatment and/or for control and treated sites also allows us to consider photodynamic *P. acnes* bacteria suppression as a leading mechanism of acne treatment. In contrast to low-intensity protocol, the sebum excretion rate of the treated skin sites determined by using of sebum-absorbent tape was significantly different from the baseline. Less optical transmittance of the patch indicates less volume of skin surface lipids (sebum excretion). Typical values for different treated skin sites at moderate intensity protocol are collected in Tables 1 and 2.

Results summarized in Table 2 were received for patient III, when sebum was collected twice per day during 1 week before treatment and after multiple treatment, which was done during a week (one procedure per day). Corresponding images of patient's skin site before and after treatment are presented in Figure 8. A recovery process is well seen. Some discrepancy of the absolute values of sebum samples optical transmittance for this patient presented in Table 1 and Table 2 is explained by rather prolonged time difference between two sets of measurements, more than 3 months, corresponding to different whether seasons. The first set of measurements (Table 1) was done in November 2001, and the second one in March–April 2002. It is remarkable that for the patients with lighter severity of disease sebum excretion rate did not change or did change (reduced) only slightly (about 10%) due to treatment, but for moderate-severe and severe acne reduction of sebum excretion rate is much higher, up to 40%. This means that more serious abnormalities of functioning of pilosebaceous apparatus at severe acne are corrected more noticeably than for lighter acne with a slightly destroyed apparatus.

Laser Thermotherapy (Photothermolysis) of Acne

A few skin acne lesions (about 30) of one patient of 23 years with severe acne were treated by photothermolysis. To provide selectivity of photothermolysis, skin was stained by ICG-lotion for 15 minutes using technology applied for soft acne treatment. Two protocols were used: (1) without skin surface cooling by irradiating the skin site by the laser beam (803 nm) at 18 W/cm^2 for 0.5 seconds; and (2) with skin surface cooling by irradiating the skin site by the laser beam (809 nm) at 200 W/cm^2 for 0.5 seconds.

Using the first protocol, 20 acne elements were treated. Elements were in the form of pustules. Observations of the patient were carried out twice per week. Results of the observations have shown that in 3 days after the treatment, the top of the pustule dried up. Erythema (inflammation) decreased. In a week after the treatment erythema decreased greatly. The element flatted and disappeared. In 10 days after the treatment erythema disappeared totally. Pigmented spot did not arise.

Using the second protocol 10 acne elements were treated. Elements were in the form of pustules. Observations of the patient were carried out twice per week. Results of the observations were identical to results received using protocol I, but after treatment slightly visible scars were found (see, Fig. 9).

DISCUSSION AND CONCLUSION

To summarize results on the soft low-intensity acne treatment, we may state that only the combined ICG and

Fig. 4. State of patient's skin after a multiple treatment. Images are captured in parallel polarizers for four sites on the back of the patient: ICG and IR light (a, baseline (before treatment); b, 2 weeks later); untreated control place (c, baseline; d, 2 weeks later); IR light only (e, baseline; f, 2 weeks later); ICG only (g, baseline; h, 2 weeks later). Skin site area of $3 \times 4 \text{ cm}^2$. [Figure can be viewed in color online via www.interscience.wiley.com.]

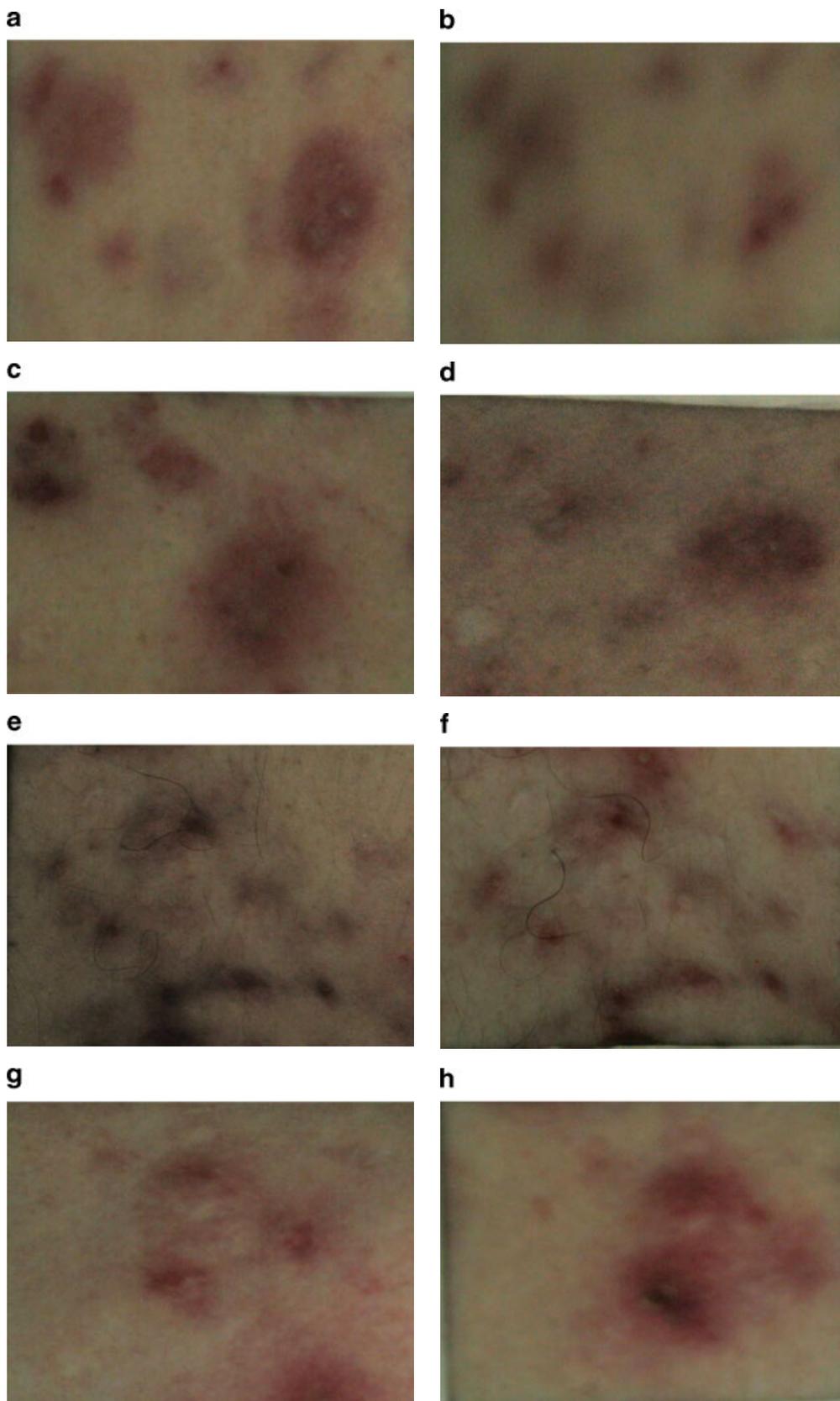


Fig. 5.

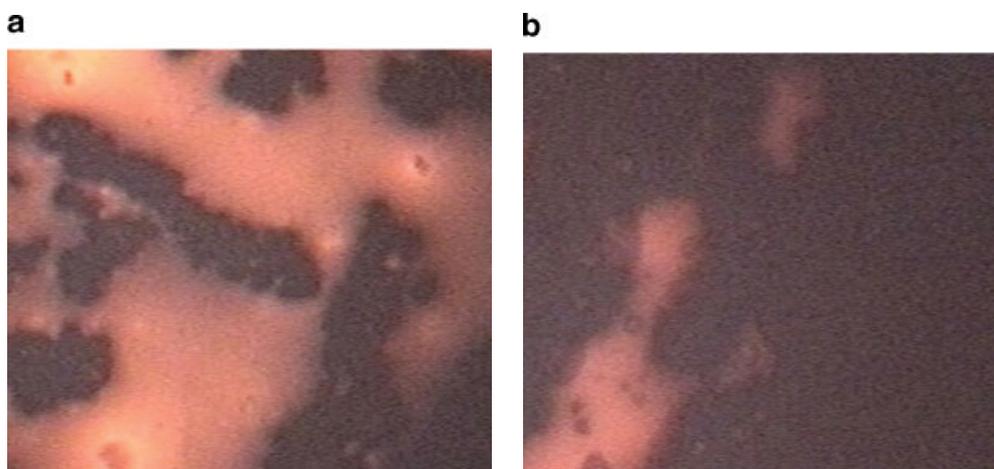


Fig. 6. Two fluorescence images of facial skin excretion at glass plate of the patient with *acne vulgaris* before (a) and after (b) soft acne treatment by ICG-lotion and laser irradiation at 803 nm. The orange-red fluorescence (above 590 nm) at excitation on 400–420 nm. [Figure can be viewed in color online via www.interscience.wiley.com.]

laser light treatment gives a well-recognized improvement in acne lesions. The other skin sites (untreated, light only, and ICG only) showed either transfer to worse status of acne lesions or were not significantly different from the baseline for all visits of the patients. For each treated group and for used light exposures and power densities no threshold effect was found; the major light dose gave more significant therapeutic effect. In general, results of observations have shown that photo-treatment induced decrease of erythema, flattening and predrying of elements (see, Figs. 3–5). For single treatment during 1 week to 10 days, post-treatment new elements as well as papules and pustules did not appear in most cases. If the elements appeared, their size was smaller than initial ones. Furthermore, only mild inflammation was observed. Approximately the same clinical results can be received at short course of antibiotic therapy.

The treatment was more effective for moderate to severe groups of patients, having large inflammatory elements. The multiple photo-treatments showed significantly more improvement than the single one. Positive effect retained for about a month after the finishing of procedure. In a month, new elements appeared but the state of lesions was lighter than initial ones.

The monitoring of bacterial porphyrins fluorescence has indicated that low-intensity acne treatment is connected with photodynamic bacteria suppression. The fact that sebum excretion rate of the treated skin sites determined by using sebum-absorbent tape was not significantly different from the baseline also supports the hypothesis that low-intensity acne therapy has a bacterial suppression nature and does not influence seriously on the sebum excretion

rate and follicle ductal hypercornification, because of insufficient light intensities. Therefore, healing was not prolonged and multiple treatment was needed.

Photoactivation of ICG by irradiation with a diode laser (805 nm) effectively kills human keratinocytes due to formation of reactive oxygen species [27]. Intracellular uptake is highly cumulative (up to 40-fold at 1 hour of incubation). Thus, intracellular ICG accumulates by transport against a concentration gradient [25]. To provide effective cell damage intracellular concentration of ICG, about 5,000 μM is needed [27], accounting that cumulative effect for 5–15 minutes of cell incubation with ICG gives at least of three to tenfolds increase in cellular uptake, applied ICG concentration of 500–1,700 μM (0.5–1.8 mg/ml) should be good. Therefore, in this paper and in References [51,52] for acne treatment, lotions with ICG concentration of 1 mg/ml and staining (incubation) time of 5–15 minutes were used.

At the chosen ICG concentration, doses from 50 to 170 J/cm^2 should be sufficient to damage keratinocytes and sebocytes [38]. It is expected that light doses to damage bacteria should be lower than for damage of keratinocytes. Therefore, in this study soft acne treatment at low ICG intracellular concentration (staining time of 5 minutes) and lower irradiation density (up to 50 mW/cm^2), and irradiation time (5–10 minutes) were effective mostly for bacteria killing. At 5 minutes of staining at concentration of 1 mg/ml (925 μM), intracellular (intrabacterial) concentration should be of 2,800 μM . The irradiation dose in the range from 15 to 30 J/cm^2 should be sufficient to kill bacteria, but not enough to damage keratinocytes and other tissue components including sebocytes.

Fig. 5. State of patient's skin after a multiple treatments. Images are captured in crossed polarizers for the same four sites on the back of the patient as presented in Figure 4: ICG and IR light (a, baseline (before treatment); b, 2 weeks later);

untreated control site (c, baseline; d, 2 weeks later); IR light only (e, baseline; f, 2 weeks later); ICG only (g, baseline; h, 2 weeks later). Skin site area of $3 \times 4 \text{ cm}^2$. [Figure can be viewed in color online via www.interscience.wiley.com.]

TABLE 1. Sebum Output Estimation for Acne Patients Treated at Moderate Intensity Protocol Upto 1 Month After Multiple Treatments

Number	M/F	Age (years)	Severity of acne [2]	Acne sites	Transmittance	
					Control	Treatment
I	F	22	1.0–1.5	Cheeks (right (control) and left (treated))	3.50 ± 0.10	3.50 ± 0.10
II	F	29	1.0–1.5	Cheeks (right (control) and left (treated))	2.80 ± 0.08	2.50 ± 0.08
III	M	16	2–3	Cheeks (right (control) and left (treated))	2.10 ± 0.06	1.30 ± 0.04
IV	M	23	4–8	Upper symmetric parts of the back	5.80 ± 0.17	3.20 ± 0.10

“SEBUTAPE” technology was used; patch optical transmittance at 700 nm is presented (less transmittance means less sebum excretion).

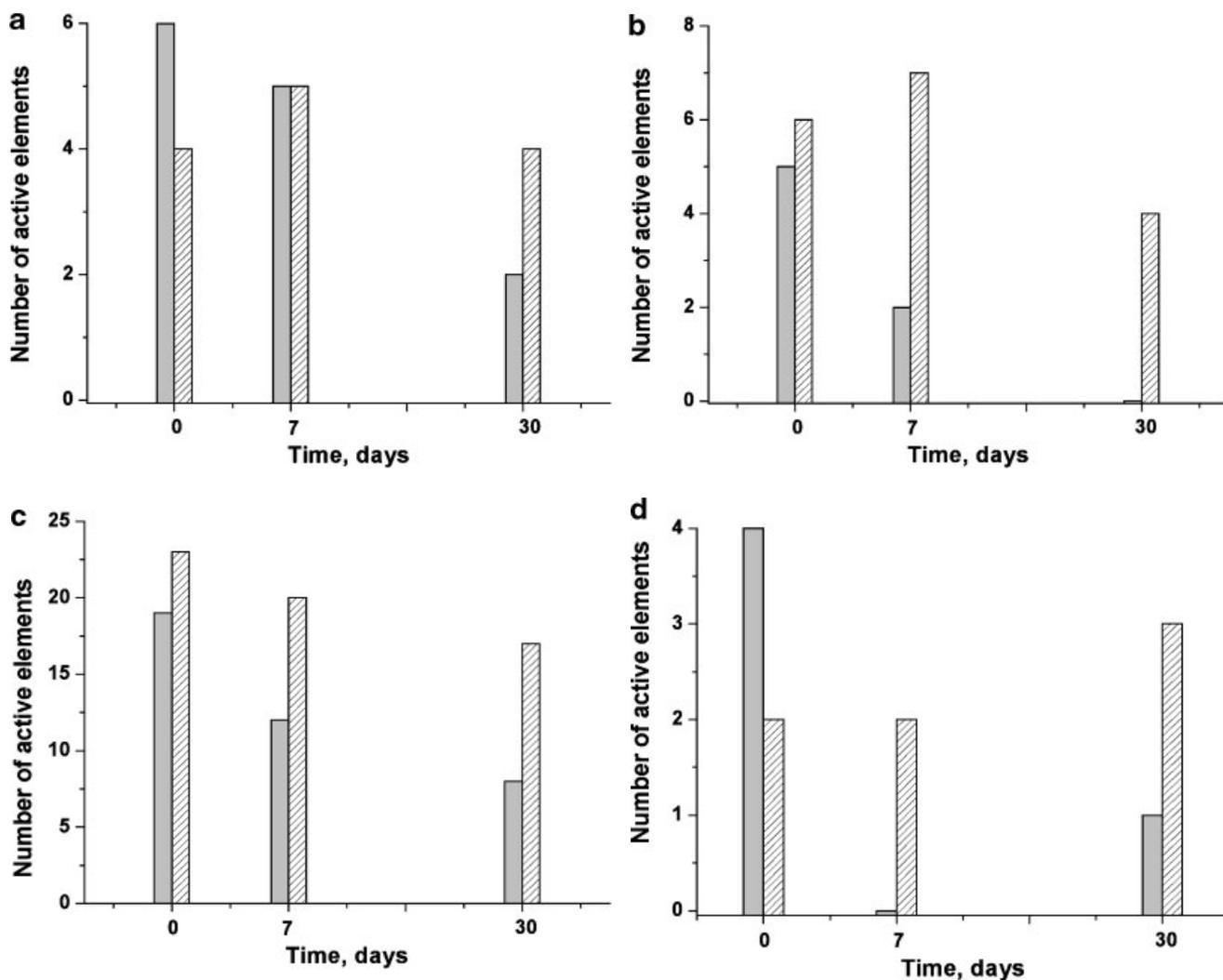


Fig. 7. Medium-intensity soft acne treatment for four volunteers with different severity of the disease and treated skin sites: two females (22 and 29 years) with moderate (facile localized) acne (**a** and **b**), one male (16 years) with moderate-severe (facile localized) acne (**c**), and one male (23 years) with severe (back localized) acne (**d**). Vertical axes is the number of active acne elements counted within the control and treated

skin sites of $4 \times 5 \text{ cm}^2$. Horizontal axes is the time scale: the first pair of columns correspond to the initial status of the skin sites (control is shown as shaded column and treated is shown as solid column); the second pair—to the status of the same skin sites in 1 week after multiple treatment during 1 month (8–9 procedures); and the third pair—in a month after the treatment.

TABLE 2. Sebum Output Estimation for Patient III Treated at Moderate Intensity Protocol During 1 Week (7 Procedures, One Per Day)

Acne sites	Transmittance			
	Before treatment		After treatment	
	Morning	Evening	Morning	Evening
Forehead	7.0 ± 4.7	5.1 ± 3.4	4.1 ± 1.7	3.9 ± 1.9
Cheeks	3.4 ± 2.2	3.5 ± 1.8	2.3 ± 1.1	2.3 ± 0.9

“SEBUTAPE” technology was used; patch optical transmittance at 700 nm is presented; data are averaged for seven measurements (mean ± SD).

Based on these results, we may hypothesize that at low-intensity treatment acne lesions healing is caused by suppression of *P. acnes* colonies and other accompany bacterial cultures characteristic for inflammation. The recent in vitro experiment for model bacteria strain *Staphylococcus* 209P have demonstrated rather high suppression efficiency (90–95%) of bacteria colonies growing at rather small light energy densities (15 J/cm²) of 810 nm light (LED) and ICG as a photosensitizer (dye concentration of 0.5 mg/ml in lotion diluted by a sugar broth) [52], and, therefore, directly supports this hypothesis.

We have also demonstrated that more prolonged ICG lotion staining time (15 minutes), irradiation by NIR light at moderate power densities (150–190 mW/cm²) during 15 minutes and more precise spectral selectivity (diode laser with 809 nm) inhibit the development of *acne vulgaris* more effectively than at low-intensities (see Figs. 7 and 8). This statement is also supported by clinical evidence of more effective treatment results than the antibiotic therapy gives.

More prolonged effect of treatment supports the hypothesis that moderate-intensity acne treatment besides bacterial suppression nature has influence on the sebum excretion rate (see Tables 1 and 2), therefore, additional photodynamic and/or photothermal mechanisms of pilosebaceous canal epithelial tissue reshaping and inactivation of sebocytes proliferation are possible.

PDT with topical application of ALA and irradiation by broadband light (550–700 nm) is more radical and has significant side effects: transient hyperpigmentation, superficial exfoliation, and crusting. It causes acute inflammation followed by partial or complete necrosis of SG [12].

In our study, we did not observe any adverse effects due to rather small power densities used in experiments, however, positive dynamics of acne treatment was shown.

Thus, we have demonstrated for the first time that topical application of ICG and followed irradiation by NIR light at a low power density inhibits the development of *acne vulgaris*. We have found using fluorescence imaging that NIR light inactivates *P. acnes* bacteria stained by ICG. The effectiveness of such soft acne treatment is due to extremely high absorption of ICG at 800–810 nm (about 1,000 cm⁻¹ at concentration of 1 mg/ml) (see, Fig. 2) and

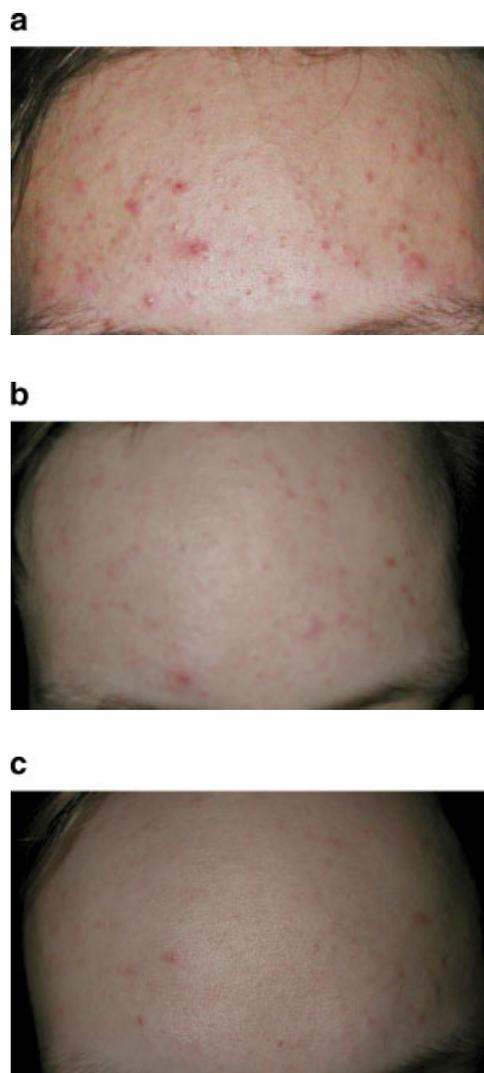


Fig. 8. Soft acne treatment with medium light intensity: images of the treated places: (a), before the treatments; (b), just after 7 daily treatments; (c), 10 days late after the end of the treatments. [Figure can be viewed in color online via www.interscience.wiley.com.]

well correspondence of laser wavelength and the absorption maximum of dye molecules bound to the target biological sites within pilosebaceous unit.

During the soft acne treatment, especially when medium intensity protocol is used, a few degree temperature rise of SG tissues is expected. As it is described in literature temperatures of a few degrees above physiological one, i.e., 42–43°C, can induce cell apoptosis [28]. Such temperatures are used for killing of tumor cells. Heating to lesser temperatures appeared to be tolerated by the cells. More prolonged heating can be associated with secondary necrosis of apoptotic cells, where the cells retained some of the features of apoptosis but had superimposed features of necrosis including membrane disintegration and swelling of cytoplasmic organelles. After a few days, apoptotic cells are

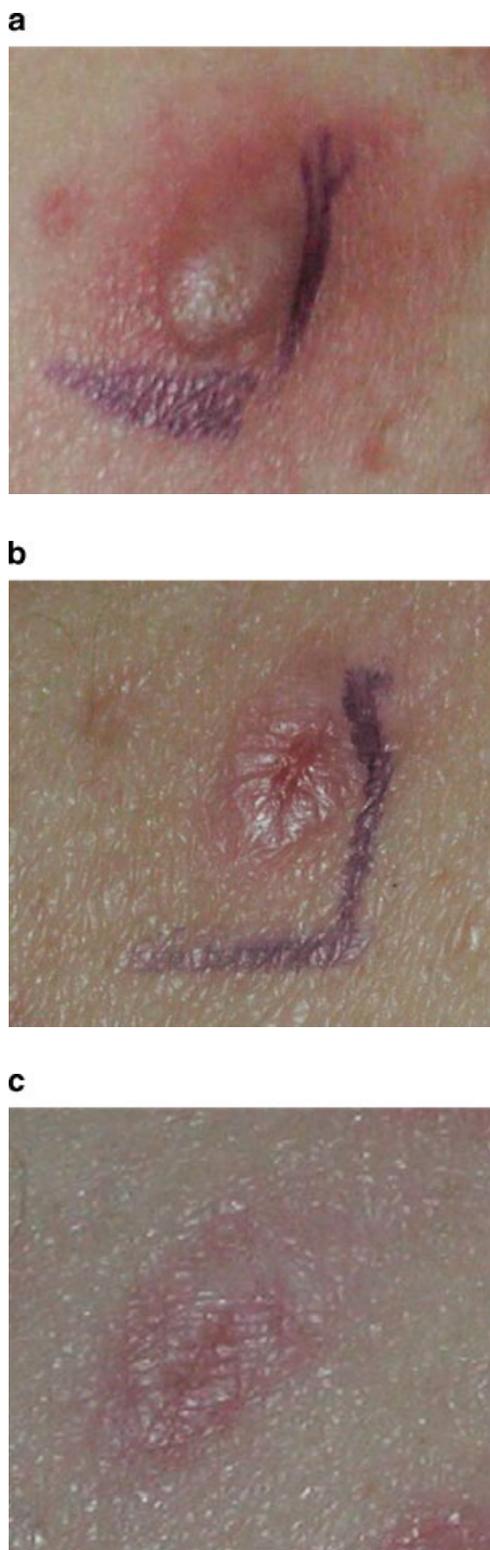


Fig. 9. The skin lesion on the back of the patient before (**a**, baseline), 2 weeks (**b**), and 1 month (**c**) after laser thermolysis (809 nm, 30 W, 4 mm beam size, 0.5 seconds). Images were captured in parallel polarizers. [Figure can be viewed in color online via www.interscience.wiley.com.]

phagocytosed by tissue mononuclear phagocytes [28]. Because apoptosis is universal mechanism of cell death, described effects should be characteristic for acne microorganisms and/or for follicular tissue, in particular for sebocytes.

It also should be noted that heating has some synergetic effect when applied together with photodynamic treatment [53], therefore, more effective bacteria killing can be provided.

Temperatures of greater than 44°C (44–48°C) causes prolonged necrosis, uniformly affecting all cells in tissue structure. The localized photothermolysis (momentary cell-killing temperatures, 48–100°C) of skin tissues can be provided at the optimal laser pulse duration lying in a microseconds or milliseconds range and at effective cooling of the skin surface [31,32]. A highly localized photothermal action was achieved using a combination of ICG and an 808 nm diode laser applied to murine mammary tumors [21,22]. At present ICG, as a highly selective dye having extraordinary absorption properties, is the most preferable dye for getting controllable thermal effects in tissues.

Thus, we have shown that staining of acne lesions by ICG allows one to reduce diode laser irradiation power density to 18 W/cm² to provide rather effective thermal destruction of acne inflammatory elements at exposure of 0.5 seconds.

The optimal power density should be in the limits from 20 to 200 W/cm², which at used exposure, 0.5 seconds, correspond to light fluence from 10 to 100 J/cm². Such estimation well correlates to experimental results on selective photodamage of ICG loaded enlarged SG at irradiation by the diode laser of 810 nm wavelength, with 50-millisecond pulse duration, and fluence of 40 J/cm², when the local temperature rise about 90°C is expected [15].

This study allows us to make some conclusions.

Based on the concept that hair follicle, especially sebaceous gland, can be intensively and selectively stained by ICG due to dye's diffusion through pilosebaceous canal and its fast uptake by living microorganisms, vital keratinocytes of epithelium of the canal and sebaceous duct and rapidly proliferating sebocytes, new technologies of soft and thermal acne lesions treatment were suggested and realized.

The hypothesis of microflora suppression as a leading mechanism of laser soft treatment is supported by *P. acnes* bacteria population suppression, detected by luminescence microscopy (see, Fig. 6), and reduction or complete resolution of the inflammatory response in the dermis and epidermis surrounding the follicles affected by acne, caused by photodynamic and/or thermal effects (see, Figs. 3–5,7,8).

The hypothesis of additional photodynamic and/or photothermal mechanism of pilosebaceous canal epithelial tissue reshaping and inactivation of sebocytes proliferation at medium-intensity soft treatment is supported by clinical evidence of more effective treatment results than the antibiotic therapy and less sebum excretion rate at the recovery stage (up to 40% less excretion was determined) (see Tables 1 and 2).

Reduction of sebum excretion rate may be caused by decreasing the proliferation and/or by increasing the rate

of sebocyte apoptosis and/or necrosis. Light induced reduction or arrest of follicle ductal hypercornification may be responsible for pilosebaceous canal epithelial tissue reshaping.

Diode laser (803 or 809 nm) power densities in the range from 18 to 200 W/cm² will provide a variety of economic and comfortable procedures of thermal destruction of ICG stained acne inflammatory elements at short light exposures (less 0.5 seconds) (see, Fig. 9).

No any adverse effects were found.

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