Indocyanine green-laser thermolysis of *acne vulgaris*

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ABSTRACT

The near-infrared (NIR) laser radiation due to its high penetration depth is widely used in phototherapy and photothermolysis. 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 photothermolysis in combination with topical application of ICG suggested for treatment of *acne vulgaris*.

Two volunteers with back-located acne were enrolled. Skin sites of subjects were stained by ICG and irradiated by NIR laser-diode light (803 or 809 nm). The individual acne lesions were photothermally treated at 18 W/cm^2 (803 nm, 0.5 sec) without skin surface cooling or at 200 W/cm^2 (809 nm, 0.5 sec) with cooling.

The results of the observations during a month after the treatment have shown that ICG stained acne inflammatory elements were destructed for light exposures of 0.5 sec.

Keywords: NIR laser irradiation, dye, selective laser thermolysis

1. 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 noninflammatory and inflammatory lesions. Hyperkeratosis 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.

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

(I) Suppression of *Propionibacterium acnes* by limiting its growth or by increasing the kill rate of the bacteria.
(II) Reduction of sebum excretion rate by decreasing the proliferation, or by increasing the rate of sebocyte apoptosis or/and necrosis.
(III) Reduction or arrest of follicle ductal hypercornification.
(IV) Reduction or complete resolution of the inflammatory response in the dermis and epidermis surrounding the follicles affected by acne.

Despite the many effective treatments currently available for acne, there remain many patients who have problematic side-effects[3-8]. Minocycline carries the uncommon risks of benign intracranial hypertension, lupus erythematosus-like syndromes and hepatitis[3,4]. Oral isotretinoin frequently produces significant muco-cutaneous symptoms and, less frequently, systemic symptoms as myalgia, head-aches, occasionally depression and other[1,5-8]. Topical retinoids are associated with irritant side effects, producing local erythema, dryness, peeling, burning, itching, and causing increased sun sensitivity in the skin[9-11]. Furthermore, bacterial resistance is an increasing problem[12,13]. Therefore, various new therapeutic possibilities using light irradiation were intensively studied[14-23].

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To enhance photodynamic bacteria killing and to provide effective modification of SG apparatus exogenous or inductive-exogenous, like Aminolevulinic acid (ALA), were used[17,20,21]. 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[24-26]. ICG is tricarbocyanine dye with strong absorption bands between 600 and 900 nm[27,28]. Recently application of ICG and diode laser irradiation for acne treatment was described as a new approach based on selective photothermolysis of the sebaceous glands[23].

The photodynamic properties of ICG have been investigated *in vitro*[29-31]. Based on these studies and accounting that local hyperthermia may induce cell apoptosis[32] we may hypothesize that 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[23,33,34].

Lasers have been used to correct a wide variety of congenital vascular disorders under the skin surface[21,35,36]. 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[20,21,23,33,34,37-41]. 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 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 photothermal action[29-31]. Therefore, ICG application for acne treatment is preferable.

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

2. MATERIALS AND METHODS

The present study is based on a few biophysical phenomena: 1) the selective targeting of skin appendages by dyes incorporated in a solution, 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 necrosis (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 included the skin site cleaning by ethanol. 1 mg/ml - solution of ICG in mixture with ethanol, glycerol, propylene glycol, and distillated water was applied for 15 min 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.

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. The original ASAH 430P diode-laser system (809 nm) with hand-piece of 4 mm in diameter was also explored for lesion coagulation. 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/cm^2

during 0.5 sec; and 2) with skin surface cooling by irradiating the skin site by the laser beam (809 nm) at 200 W/cm^2 during 0.5 sec. The individual acne lesions were treated.

Two males with severe *acne vulgaris* on there backs were enrolled. The age of volunteers was 20 and 23 years. Subjects were excluded if they had used any topical acne treatment, systemic antibiotics in the past two weeks, or systemic retinoids in the past year. People who plan to have excessive sun exposure, or with history of keloid or photosensitivity disorder were also excluded. All patients gave their informed consent for participation and the study protocol.

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. A linear polarizing filter (Crystal Optics, Japan) was placed on the lens to reduce specular reflection from the skin surface.

3. RESULTS AND DISCUSSION

The absorption spectra of ICG in various solvents with concentration of 1 mg/ml are presented in Fig. 1. The glycerol-ethanol-propylene-glycol-water solution as the most efficient staining lotion for diode laser thermolysis was used. The maximum of absorption coefficient of the solution is at the wavelength 789 nm. In tissues and cells the IR ICG absorption peak moves to longer wavelengths, 805-810 nm, due to binding with cell proteins[33,34], that makes light-tissue interaction mediated by ICG more efficient for diode lasers with 809 nm.

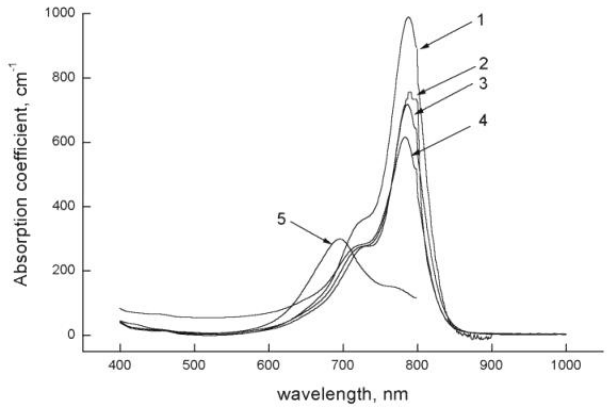


Figure 1: Absorption spectra of Indocyanine Green in various solvents at concentration of 1 mg/ml: 1 – ICG in glycerol-ethanol-propylene-glycol-water solution; 2 – ICG in glycerol; 3 – ICG in propylene glycol; 4 – ICG in ethanol; 5 – ICG in water

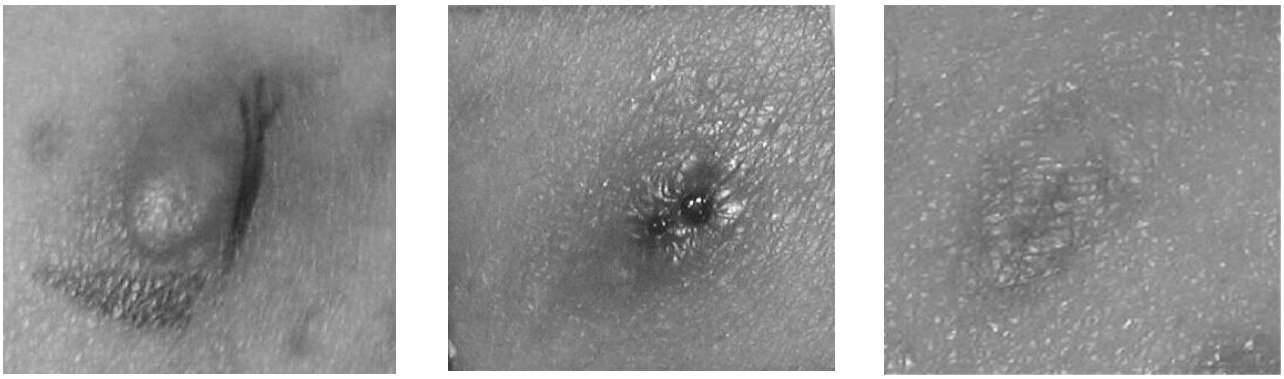
A few skin acne lesions (about 30) of two males of 20 and 23 years with severe acne were treated by photothermolysis. Two protocols were used: 1) without skin surface cooling by irradiating the skin site by the laser beam (803 nm) at 18 W/cm^2 for 0.5 sec; and 2) with skin surface cooling by irradiating the skin site by the laser beam (809 nm) at 200 W/cm^2 for 0.5 sec.

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 three days after the treatment the top of the pustule dried up. Erythema (inflammation) decreased. In a week after the treatment erythema decreased greatly. The elements became flat but the crusts appeared and remained during a month. Pigmented spot did not arise (see, Fig. 2).

a b c

Figure 2: The skin lesion on the back of the patient before (a – baseline), three days (b), and a month (c) after laser thermolysis (803 nm, 18 W, 5 mm beam size, 0.5 sec). Images were captured in parallel polarizers.

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 have shown that in a week crusts disappeared and in two weeks the elements disappeared totally. After the treatment slightly visible scars were found (see, Fig. 3). No any adverse effects were found.



a b c

Figure 3: The skin lesion on the back of the patient before (a – baseline), three days (b), and a month (c) after laser thermolysis (809 nm, 30 W, 4 mm beam size, 0.5 sec). Images were captured in parallel polarizers.

It is known that the ICG dye solution penetrates along a hair shaft to the soft nonkeratinized tissues, in particular to SG. The depth and time of dye penetration for terminal hairs are about 1 mm and 5-15 min, respectively[34]. Sebaceous follicles have very large pores, long canal free of hair, two or more sebaceous ducts, therefore, much higher staining efficiency of acne element than of normal skin.

Moreover, at acne there are additional targets and paths for ICG staining of acne lesions, like black head (an open comedon 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[42].

As it is described in literature temperatures of a few degrees above physiological one, i.e. 42-43^{0}C, can induce cell apoptosis[32]. Such temperatures are used for killing of tumor cells. Temperatures of greater than 44^{0}C (44-48^{0}C) causes prolonged necrosis, uniformly affecting all cells in tissue structure. The localized photothermolysis (momentary cell-killing temperatures, 48-100^{0}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[35,36]. A highly localized photothermal action was achieved using a combination of ICG and an 808 nm diode laser applied to murine mammary tumors[25,26.] At present ICG, as a highly selective dye having extraordinary absorption properties, is the most preferable dye for getting controllable thermal effects in tissues.

5. CONCLUSION

Thus, we have shown that staining of acne lesions by ICG allows one to reduce diode laser irradiation power density to 18 W/cm^2 to provide rather effective thermal destruction of acne inflammatory elements at exposure of 0.5 sec. The optimal power density should be in the limits from 20 to 200 W/cm^2, which at used exposure, 0.5 sec, correspond to light fluence from 10 to 100 J/cm^2. 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/cm^2, when the local temperature rise about 90^{0}C is expected[23].

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 thermal acne lesions treatment were suggested and realized.

Reduction of sebum excretion rate may be caused by decreasing the proliferation and/or by increasing the rate of sebocyte 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/cm^2 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 sec). No any adverse effects were found.

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