

COMPARATIVE TREATMENT OF *ACNE VULGARIS* USING PALOMAR LUX APPLIQUÉ TECHNIQUE AND DIRECT INTRALESIONAL INJECTION

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Intralesional injection of triamcinolone (TMC) preparations is an effective therapy for cystic acne lesions. However, invasive delivery techniques limit the use of this modality to a relatively narrow class of cases. Skin permeability can be enhanced through creating a lattice of micro-zones (islets) of light-induced limited thermal damage in the upper layers of epidermis. In this paper, we directly compared safety and efficacy of delivering TMC acetone with this novel technique versus conventional intralesional injection for treatment of inflammatory acne lesions. A combination of an intense pulsed light system and a specially designed appliqué with a pattern of absorbing centers has been used to create the lattice of islets of damage (LID). Quantitative analysis has included estimation of the following parameters: redness, diameter, and height of acne lesions. Clinical photography has been used to document dynamics of lesion development at successive visits (two hours, 24 hours and one week post-treatment). Seven subjects have participated in the study. No difference in lesion dynamics between the treatment and control groups was observed at two-hours follow-up. At 24-hours/one-week follow-ups, TMC-injected and TMC-LID-delivered groups have demonstrated 82%/93% and 80%/89% improvement in height of lesions in comparison to control (60%/68%). Delivery of TMC with the newly proposed LID technique is at least as effective as intralesional injection for treating inflammatory acne lesions. Enhancement of skin permeability using LID approach is a promising technique for accelerating delivery of various compounds to their target areas in the skin.

Keywords: Acne vulgaris; drug delivery; skin permeability enhancement.

1. Introduction

Acne vulgaris is a skin disease affecting approximately 40 million adolescents and 25 million adults and accounts for more than 30% of all visits to the dermatologist.^{1,2} This is a follicular disorder that affects pilosebaceous follicles, primarily of the face, neck, and upper trunk, and is characterized by both noninflammatory and inflammatory lesions. *Acne vulgaris* is manifested by comedones, papules, pustules, and cysts.

Hyperkeratosis with obstruction of the follicular opening, increased production of sebum (lipids secreted by the androgen-sensitive sebaceous glands), and proliferation of bacteria, plays the main role in the development of the disease. The microbiology of the pilosebaceous unit involves three coexisting groups of microorganisms: gram-positive, coagulase-negative cocci (staphylococci and micrococci); anaerobic diphtheroids (*Propionibacterium acnes* and *Propionibacterium granulosum*); and lipophilic yeasts (*Pityrosporum* species).³ The excessive accumulation of sebum and bacteria in the pilosebaceous unit leads to formation of pustules, inflammatory papules and cysts. Severe acne may leave permanent scarring.⁴

Acne vulgaris often represents as a therapeutic problem. In mild forms of the disease, the local therapy is advantageous, mostly reaching a very good therapeutic effect.⁵ Severe forms of acne (large *acne papulopustulosa*, *acne nodularis*, *acne conglobata*, *acne fulminans*, *acne tetradia*) need the application of systemic treatment. Complex therapy of acne involves the application of oral antibiotics, hormones, isotretinoin and bacterial vaccines.^{3,5,6}

Intralesional injection of corticosteroids is effective for relief of severe nodulocystic lesions.^{3,7-9} Triamcinolone (TMC) acetonide is the preparation most commonly used in dermatological practice.⁷ TMC preparations are applied when topical or oral therapy is not completely effective or when a more rapid response is necessary. Intralesional steroid injections may produce dramatic flattening of most of the acne nodules in 48–72 hours.⁸ In the study conducted by Mahajan and Gard,⁸ intralesional injections of lincomycin hydrochloride (75 mg/ml) with TMC acetonide (2.5 mg/ml) resulted in the healing of all nodulocystic lesions of acne within 48 hours to one week. As TMC acetonide is a long acting steroid, the drug remained *in situ* long enough to produce a substantial anti-inflammatory effect.⁸ Mahajan and Gard did not believe that

any improvement was due to the systemic effects of TMC since a total dose of 5 mg/ml in any patient was not exceeded. In an earlier study, Levine and Rasmussen⁹ have shown that TMC acetonide at a concentration of 0.63 mg/ml was as efficacious as that at a higher concentration of 2.5 mg/ml.

Though effective, intralesional injection of corticosteroids is an invasive delivery technique and limits the use of this modality to a relatively narrow class of cases. A less traumatic delivery system may expand the usability of the drug and benefit a number of cystic acne sufferers. However, slow diffusion of drugs through a human skin barrier makes practical application of the method difficult.

Low-intensity light phototherapy is a developing therapeutic technique for acne patients. It has been shown that irradiation of the affected skin with UV (390 nm), violet (405 nm), blue (415 and 470 nm), white (full spectrum), and red (660 nm) light leads to an improvement in the state of the skin, with different degrees of effectiveness depending on the wavelength of light and irradiation dose.¹⁰⁻¹³ Violet and blue lights are theoretically the most effective visible wavelengths for photoactivation of the major endogenous porphyrin component of *P. acnes*, but they have poor penetration depth into the skin.^{10,12} Green light is also capable of activating porphyrins and may be more efficient because of a higher penetration into the skin.¹¹ Red light is less effective at photoactivating porphyrins, but it penetrates more deeply into tissue.^{12,13} In addition, red light may have wound-healing properties by influencing the release of proinflammatory cytokines from macrophages, which stimulate fibroblast proliferation and the production of growth factors.¹² In this case the mixed low-intensity blue–red light phototherapy is an effective treatment for mild-to-moderate *acne vulgaris*.¹²

To enhance photodynamic bacteria killing and to provide effective modification of sebaceous gland apparatus, the exogenous or inductive-exogenous photosensitizers have been used. Topically applied aminolevulinic acid (ALA) converts into a potent photosensitizer, protoporphyrin IX, in human hair follicles and sebaceous glands. Combination of ALA with violet light is effective for photoactivation of protoporphyrin IX.¹⁴ Photosensitizers like methylene blue (MB), indocyanine green (ICG), activated by red and near-infrared (NIR) laser irradiation, respectively, are preferred due to high penetration depth of light within tissue.¹⁵⁻¹⁷

High-intensity NIR light sources (e.g., diode laser operating at the wavelength of 800–805 nm) in combination with ICG have been successfully used for treatment of acne.¹⁷ The precise mechanism of action is not completely understood, but the underlying physical process is photothermal, with bulk absorption of optical energy by tissue water.

In this paper, light is employed as a means to facilitate delivery of a topical medication through the *stratum corneum* without seriously compromising skin barrier function. Recently, a method of accelerating penetration of medicaments due to enhancement of epidermal permeability by creating a lattice of microzones (islets) of limited photothermal damage or lattice of islets of damage (LID) in the *stratum corneum* was proposed.^{17,18} LIDs are created as a result of the absorption of a sufficient amount of optical energy by the lattice of microzones. The absorption leads to temperature elevation in the localized zones of interaction. Since the zones of interaction contact the skin surface, some of the thermal energy transfers to the *stratum corneum*.¹⁸ As the *stratum corneum* is not viable tissue, the long-term effect of such damage is only the transient deterioration of the function of the skin barrier. That leads to the local increase of agent penetration.¹⁸

The goal of this paper is the investigation of the effectiveness of the LID method for enhancement of *stratum corneum* permeability for TMC acetonide. We directly compared the safety and efficacy of TMC acetonide delivered with this novel technique versus conventional intralesional injection for treatment of inflammatory acne lesions.

2. Materials and Methods

2.1. Selection of subjects

This study was an open clinical trial performed in Family Doctor Clinics, Ltd. (Saratov, Russia) from February to April 2006. Seven patients, one female and six males, with Fitzpatrick's skin type I–III and mild to severe acne lesions involving the back and/or the chest participated in this study. The average age was 19 years (female, 18 years; male, 19 years, range 18–20 years). Mild to severe acne was determined in accordance with the classification of Burke and Cunliffe (1984).⁴ To be included, patients had to have more than five inflammatory lesions. They were healthy volunteers, and free of any systemic or dermatological diseases other than *acne vulgaris*.

During the two weeks prior to the study, no medication was administered.

Possible subjects were excluded if they had a history of keloid formation, or have active herpes simplex in the treatment areas. Pregnant and lactating women, mentally handicapped persons, and persons who had type V or VI skin were also excluded.

The aims of the study were explained to the patients, and they gave informed consent.

2.2. Flash-lamp appliqué system

A combination of an intense pulsed light system (EsteLux, Palomar Medical Technologies, Inc., Burlington, MA, USA) and a specially designed appliqué with a pattern of absorbing centers has been used to create LID (see Fig. 1). The absorbing centers (dots) of inert and biocompatible carbon powder were printed out on transparent film, ensuring local high absorption of light energy. When a pulse of light illuminated the appliqué, the dots absorbed the light energy, which resulted in rapid local temperature elevation. Since the dots contacted the skin surface, some of the thermal energy was conducted to the *stratum corneum*, creating the corresponding pattern of lattice-wise *stratum corneum* ablation providing enhanced permeability in skin. Thus, penetration of the topical composition into the skin was accelerated, enabling faster effect of the medication. Parameters of the light/appliqué system were selected in such a way that no irreversible damage was caused to the *stratum corneum*, so that integrity of the skin barrier was restored in a short time.

The StarLux Rs handpiece with wavelength range 650–1200 nm was applied in stamping mode

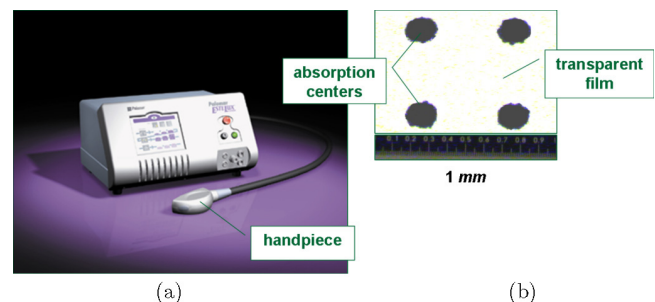


Fig. 1. Flash-lamp appliqué system: (a) flash lamp (EsteLux System, Palomar Medical Products Inc., Burlington, MA, USA) with a handpiece StarLux Rs, and (b) a specially designed appliqué on the basis of a transparent film with absorption centers.

with the following settings: fluence 20 J/cm², 20 ms pulse width, two pulses. Settings were modified at investigator's discretion to ensure subject's comfort and minimize side effects (erythema). Both the operator and subject wore protective eyewear during the treatment to exclude eye-lesion by NIR irradiation.

The pulsed-light system used in this study is FDA approved for general dermatological use. Clinical trials adhered to the International Standard GCP Procedures as per the "ICH Topic H6: Good Clinical Practice."

2.3. Study design

For each person four acne lesions were identified to receive a single treatment and one lesion served as a control. Lesions selected for treatment were divided into two groups: the first group (two lesions) was intralesional TMC-injected and the second group (two lesions) was given the light-appliqué treatment followed by topical application of the medication.

Prior to the treatment the target area was gently cleaned with a mild cleanser to remove any creams or agents from the skin surface. The skin overlying the cyst was surgically prepared with 90% ethanol solution.

For the first group of lesions, the following procedure was carried out: cyst was stabilized with the left hand and pierced at the most dependent point with a 26-gauge needle attached to a syringe. Direct intralesional injection was performed. As acne cysts vary in size, the volume of injected material was selected to individual lesions. A cyst measuring 1 cm² in size was injected with 0.1 ml of the drug. The concentration of TMC acetonide was 2.5 mg/ml (made by diluting 10 mg/ml TMC acetonide with water).

The second group of the lesions was given thermal treatment by flash-lamp-appliqué system before the drug application. The appliqué that consisted of a transparent film with a 2D-array of carbon black microdots was placed on the lesion with rough (dot) side down. A small drop of the Lux lotion was used on the rough side of the appliqué to ensure good thermal contact with the skin. Care was taken to provide uniform contact between the appliqué and the skin.

Immediately after the treatment with the appliqué, gauze soaked with the TMC acetonide solution was applied to the lesion site. Then the

site was covered with an occlusive bondage, which was worn for at least two hours.

Immediately after the treatment, each treatment site was evaluated for adverse effects, including pigmentary changes, erythema, and edema.

The control group of lesions was not treated.

2.4. Analysis of the results

The following evaluation measurements were performed at baseline, two hours, 24 hours and one week after the beginning of the trial: (1) digital photography, (2) clinical evaluation using acne severity grading, and (3) self-evaluation questionnaire.

Standard clinical digital photography system (Nikon D-80, Japan) was deployed at the clinical site and used by personnels involved in the study. Photographs were taken at each study visit.

The following parameters were graded by experienced dermatologist for each targeted lesion using the following scales indicated in Table 1: redness, diameter, and height associated with the lesion. For the best comparison of the results, we tried to choose the lesions with similar initial state.

Subjects completed a final self-assessment questionnaire based on the overall product effectiveness. Subjects were asked to compare the appearance and feel of their skin now as compared to the time prior to the treatment (two mirrors or computer-connected digital camera were used). The scoring was based on a 1-to-5 scale (1 = disagree completely and 5 = agree completely).

This study was a single-blind, randomized study. Measurements and scores obtained from all treated sides were compared to the corresponding pre-treatment measurements and control sites. Differences in acne severity were determined by comparing the treated lesion values to those of the baseline values before treatment and to those of the untreated control site. The comparisons were made at each follow-up interval.

Table 1. Grading scale of parameters used for evaluation of skin state.

Redness	Diameter	Height
0 = None	0 = 0 mm	0 = Completely flat
1 = Slight	1 < 2.5 mm	1 = Slightly raised
2 = Mild	2 = 2.5–3.0 mm	2 = Mildly raised
3 = Moderate	3 = 3.1–4.0 mm	3 = Moderately raised
4 = Severe	4 > 4 mm	4 = Severely raised/swelled

3. Results and Discussion

Figure 2 shows the dynamics of the state of lesions from three groups. The row number in the figure corresponds to the number of groups: group 1 includes Figs. (a) to (d) — they present the images of the lesion, which was directly injected by TMC acetamide; group 2 includes Figs. (e) to (h) — they present the images of the lesion, which was treated by light-appliqué before topical application of the TMC acetamide; and group 3 includes Figs. (i) to (l) — they present the images of the control lesion.

Four columns correspond to the time of observation: Figs. (a), (e) and (i) are the images of the lesions in initial state; Figs. (b), (f) and (j) are the images of the lesions in two hours; Figs. (c), (g) and (k) are the images of the lesions at 24-hours

follow-up and Figs. (d), (h) and (l) are the images of the lesions in a week post-treatment.

In the images it is seen that the lesions had similar initial state. Intralesional steroid injection produced dramatic drying of acne lesion in two hours and whole healing of the lesion in a week. Both parameters “redness” and “diameter” significantly fell in 24 hours. In a week after the treatment the lesion from the first group became completely flat.

After the treatment the inflammation of the lesions in group 2 gradually decreased. In a week we could see light trace of the lesion. The active element in the form of pustule had been transformed into a macule. The control lesion in this case did not show decreasing of the parameters. The diameter of the lesion even increased. The active element

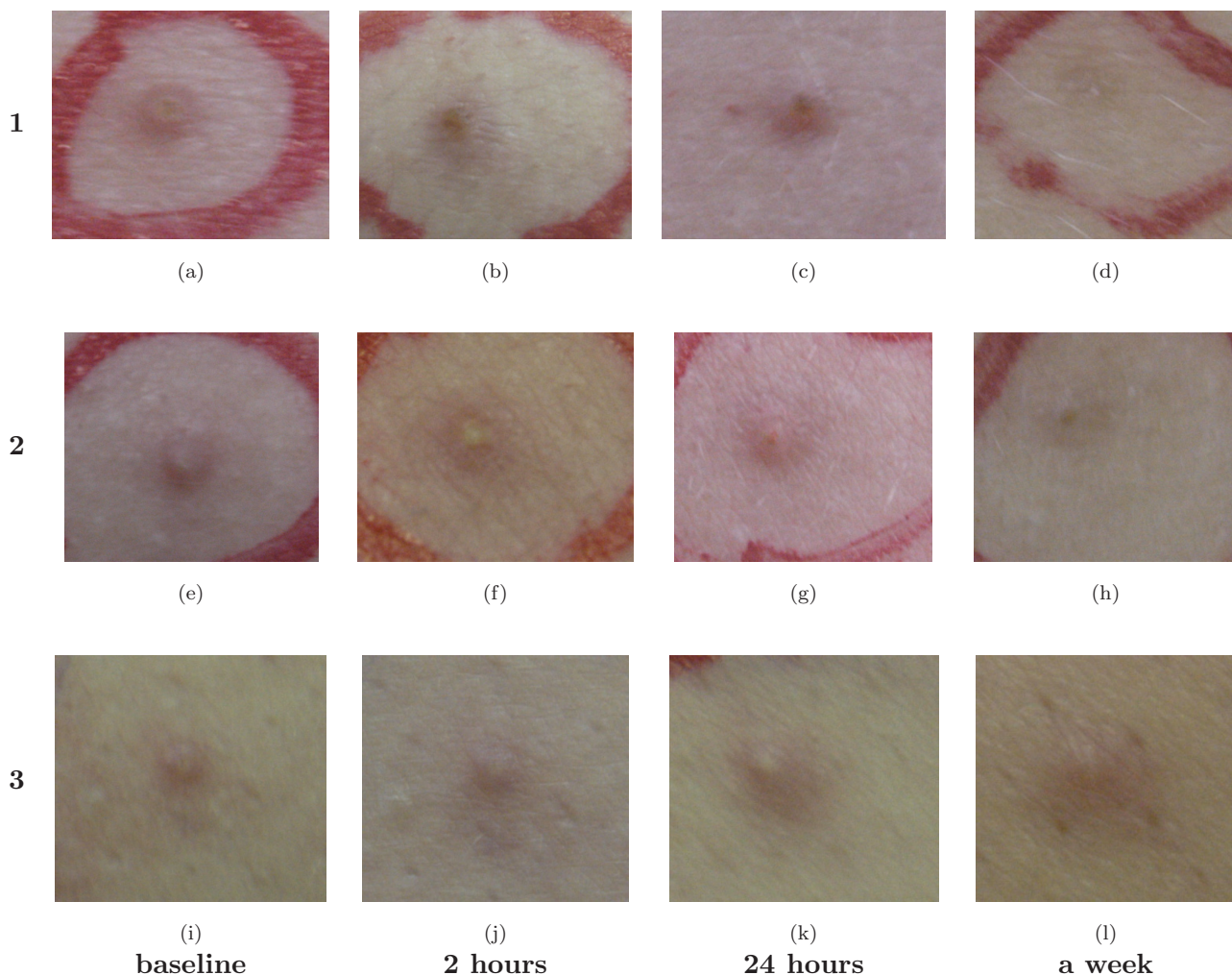


Fig. 2. The images of the lesions under scoring. The numbers of the rows correspond to the lesions from the respective groups: 1 — the lesion was directly TMC-injected; 2 — the lesion was treated by light-appliqué before topical application of TMC; 3 — the control lesion. Columns correspond to the interval of evaluation measurements.

in the form of papule had been transformed into a large pustule.

Quantitative analysis of the lesions is presented in diagrams. Figures 3(a), 3(b) and 3(c) show the temporal change of investigated parameters: redness, diameter, and height, respectively. In the figures, red columns correspond to the first group of the lesions; green columns correspond to the second group of the lesions; and blue columns correspond to the control group of the lesions.

The vertical axis reflects the severity of the lesions according to the grading scale of parameters presented in Table 1. The height of both red and green columns equal to the values of the parameters averaged from 14 lesions; the height of blue column was obtained by taking average of the values from seven lesions.

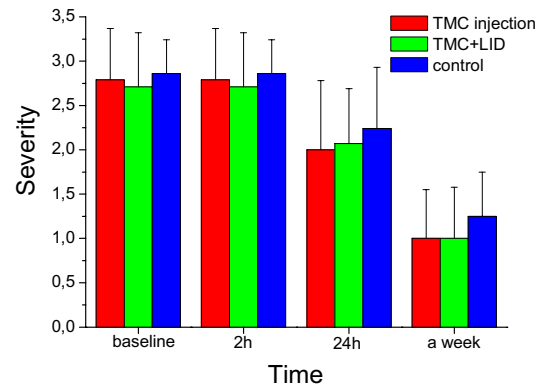
The horizontal axis is the time scale. The first group was measured before the treatments and the following groups were measured at the follow-ups of two hours, 24 hours and one week. The bars reflect standard deviations from the mean value.

Absolute values of the parameters are presented in Fig. 3. For both the treatment and the control groups no difference in lesion dynamics was observed at the follow-up of two hours. During the observation period it is clearly seen that values of all parameters decreased gradually with time. However the rate of decrease of parameters was different.

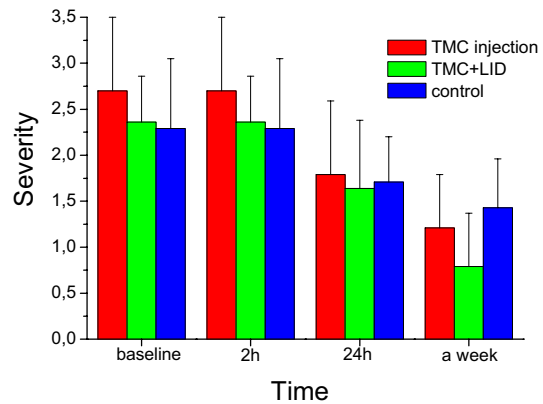
Figure 4 illustrates the dynamics of normalized parameters during a week. Symbols correspond to values of the parameters. The bars reflect confidence interval. Values of significance level $p < 0.05$ were accepted as statistically significant. In the figure it is well seen that the values of parameters of the lesions from both the first and the second groups were lower than that of the lesions from the control group during a week post-treatment.

Improvement of the skin state has been estimated with the help of the following expression: $(\bar{P}_{\text{baseline}} - \bar{P}_{24\text{-hours/week}})/\bar{P}_{\text{baseline}}$, where $\bar{P}_{\text{baseline}}$ is the average value of the studied parameter at the baseline follow-up, $\bar{P}_{24\text{-hours/week}}$ is the average value of the studied parameter at 24 hours' or a week's follow-ups. At 24-hours/week follow-ups, improvement of the "redness" was 75%/87%, 72%/89% and 73%/86% for groups 1, 2, and 3, respectively.

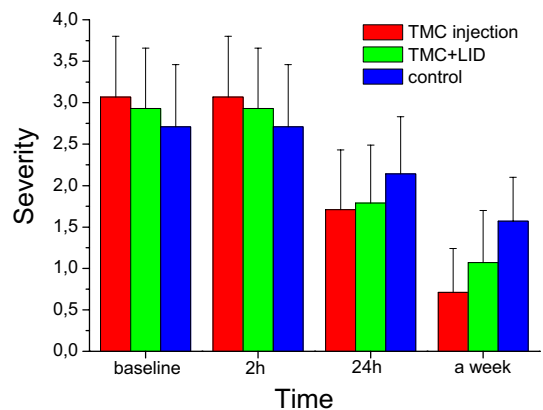
Improvement of the "diameter" of the lesions in both groups 1 and 2 at 24-hours/week follow-ups has very close values, namely 56%/80% and 56%/78%, respectively. The decrease in lesion diameter in the control group was 60%/68%.



(a)



(b)



(c)

Fig. 3. Comparable statistical analysis of the skin state of volunteers after direct intralesional TMC-injection (red columns), light-appliqué treatment followed by topical application of TMC (green columns) and control group (blue columns) of lesions characterized by the following parameters: (a) redness, (b) diameter, and (c) height of lesions. The vertical axis is the mean severity of the lesions, the horizontal axis is the time scale: the first group of columns corresponds to the initial status of the lesions; the second group corresponds to the same lesions at two-hours follow-up; the third group is the status of the lesions at 24-hours follow-up; and the fourth group is a week after the treatment. The bars reflect standard deviations.

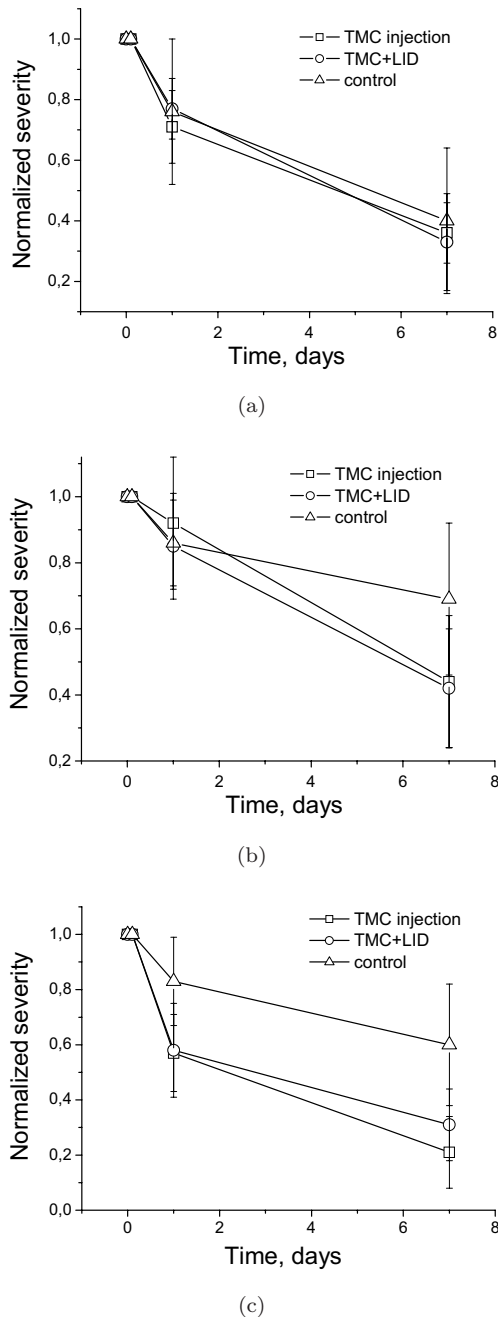


Fig. 4. Temporal dependence of the normalized values of parameters: (a) redness, (b) diameter, and (c) height of lesions. Symbols correspond to the methods of the treatment: direct intralesional TMC-injection (squares), light-appliqué treatment followed by topical application of TMC (circles), and control group of lesions (triangles). The bars reflect confidence interval.

From the diagrams it follows that the most sensitive parameter is the height of lesion. At 24-hours/week follow-ups, groups 1 and 2 have demonstrated 82%/93% and 80%/89% improvement in lesion height in comparison to control (68%/77%).

The independent *t*-test has shown that the differences in the result of the treatment with TMC-injection and TMC + LID technique were not significant at both 24-hours and a-week follow-ups for all studied parameters. The differences in the acne state between two groups TMC-injection and control were statistically significant for parameter “diameter” only at the weekly follow-ups ($p = 0.04$) and for parameter “height” at the 24-hours/week follow-ups ($p = 0.012/0.007$, respectively). The differences of the parameter “diameter” between two groups TMC + LID and control were also statistically significant at the weekly follow-up ($p = 0.04$) and for parameter “height” at the 24-hours/week follow-ups ($p = 0.027$ in both cases).

Parameter “redness” has not shown significant differences between control and experimental groups.

The results of the observations have shown that the delivery of TMC acetonide with the proposed LID technique is at least as effective as intralesional injection for treatment of inflammatory acne lesions at the evaluation of all parameters: redness, diameter and height of lesions.

As a rule, concentrations of more than 5 mg/ml of TMC acetonide are not used for intralesional injections because of the risk of atrophy.^{8,19} We did not exceed a total dose of 5 mg/ml in any subject. In our study, we did not observe any adverse effects due to rather small dose used in experiments. However, positive dynamics of acne treatment was shown.

Self-evaluation of the subjects has shown that the subjects consider the TMC + LID technique having the same effectiveness as the TMC-injection and prefer the first method as it is more painless.

It is known that the penetration of agents into skin is mainly through the hair canals.²⁰ To reduce barrier function of skin epidermis, a number of different chemical and physical methods are used. Vehicles like dimethyl sulfoxide or oleic acid are used to enhance penetration.²¹ Ethanol and liposome encapsulation can also be used for penetration enhancement.^{22,23} Physical methods such as stripping,²⁴ microdermabrasion,²⁵ laser ablation of skin surface,²⁶ iontophoresis,^{27,28} ultrasound²⁸ and photomechanical waves,²⁹ and needle-free injection³⁰ were also proposed. Physical methods for transdermal-agent delivery have two advantages over formulations and chemical enhancers: (a) there is no interaction between enhancer and the agents being delivered; and (b) they reduce the risk of

additional skin irritation.²⁹ However, some methods, such as stripping and laser ablation of skin surface, can be related with the damage or ablation of large areas of *stratum corneum*. Iontophoresis results in a reduction of the resistance of the skin to diffusion of ions, but as an enhancer of the flux of uncharged molecules it is a less effective method. Ultrasound and other pressure waves can affect various skin structures and inner organs due to a large penetration depth that can be undesirable for specific tasks of drug delivery.

Enhancement of skin permeability using LID approach is a promising technique for accelerating the delivery of drugs to their target areas in the skin. In Ref. 31 it was shown that the diameters and depth of craters arising on the surface of the skin were $200 \pm 20 \mu\text{m}$ and $25 \pm 5 \mu\text{m}$, respectively.³¹ The thickness of epidermis including *stratum corneum* is $\sim 100 \mu\text{m}$.¹⁰ Thus, LID promotes better penetration of agents into tissue as it creates additional pathways for drug delivery through the rest of *stratum corneum* and living epidermis. Since the pulse width was 20 ms, therefore, the skin perforation was absolutely painless.

As we can see, the direct intralesion drug injection gives the same anti-inflammatory effect as the TMC + LID-delivery technique despite that the dose of the drug administered in the target is higher in the first case. Besides, the TMC + LID-delivery technique has the advantage of the painless procedure. Thus, we may state that noninvasive delivery of drug in the target area is practically as effective as intralesion injection of the drug.

4. Conclusion

This study was a pilot investigation of the efficacy of flashlamp-appliqué treatment procedure for mild to severe (including nodulocystic) *acne vulgaris* in comparison with direct intralesion drug injection. Results of the investigation have shown that the decrease of the lesion's inflammation parameter for the noninvasive method was as significant as that for the injection. In contrast, the control group has demonstrated worse results for every parameter. Adverse effects were not observed.

Microdamages of the skin surface reduce *stratum corneum* barrier function for some limited time and serve as microchannels for agent penetration. Thus, enhancement of skin permeability using LID approach is a promising technique for accelerating

controllable delivery of various compounds to their target areas in the skin.

Acknowledgments

The authors are grateful to Palomar Medical Technologies, Inc. (Burlington, MA, USA) for funding this work and providing the intense pulsed light system EsteLux with the StarLux Rs handpiece, and to Family Doctor Clinics Ltd. (Saratov, Russia) for providing clinical room during clinical trials, and to medical doctor I. Yu. Baratova for performing intralesional injection. The research described in this paper was also supported by Grant No. 224014 PHOTONICS4LIFE of FP7-ICT-2007-2 (2008–2013), Grant of President of RF “Supporting of Leading Scientific Schools” — NSHA-208.2008.2 (2008–2009), project Nos. 2.1.1/4989 and 2.2.1.1/2950 of PF Program on the Development of High School Potential (2009–2010), and project SSU No. 1.4.09 of Federal Agency of Education of RF (2009–2010).

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