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Laser delivery and spectral study of a modern chlorine-containing drug for the treatment of onychomycosis at laser radiation with a wavelength of 450 nm

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The possibility of active laser delivery of a modern chlorine-containing photosensitizing drug Chloderm (Chloderm, Russia) under the nail plate by laser radiation with a wavelength of 450 nm for the purpose of photodynamic therapy of onychomycosis is studied. In an in vitro experiment, sequential laser microporation of the nail plate and active delivery of the drug under the nail plate by this laser radiation with an intensity of up to 200 W/cm^2 were investigated. The results of studying the absorption spectra of an aqueous solution of Chloderm in the range of 400−900 nm before and after exposure to 450 nm laser radiation are presented. It is demonstrated that sequential laser microporation and active laser drug delivery under the nail plate is possible at laser radiation intensity greater than 178 W/cm^2. It is shown that the maximum rate and efficiency of nail plate ablation by 450 nm laser radiation is achieved at an intensity of 200 W/cm^2 and is 2750 ± 30 μm/s and 1.47 ± 0.05 μm/mJ, respectively. The delivery rate of Chloderm under the nail plate is 1.15 ± 0.1 mg/s. It is shown that exposure to 450 nm laser radiation at the intensity of 200 W/cm^2 for a time sufficient to deliver the drug under the nail plate does not change the extinction coefficient of the drug at the laser wavelength and slightly changes the conformational state of Chloderm.

Keywords: laser delivery, ablation, wavelength, absorption coefficient, chlorine-containing photosensitizing drug, laser radiation.

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Introduction

Onychomycosis is one of the most prevalent nail diseases, which occurs due to overgrowth of fungus in the nail bed, leading to hyperkeratinization [1]. Onychomycosis strikes 10−40% of population [2,3]. The available treatment options for onychomycosis are facing significant barriers in successfully eradicating the disease. This is because topical medications cannot penetrate via nail plate to reach nail bed and systemic antifungal agents possess certain adverse effects on long-term use and also have some contraindications. Conventional surgical treatments of onychomycosis are quite painful, require professional assistance and have long rehabilitation period.

In recent years, photodynamic therapy (PDT) is suggested for onychomycosis treatment [1], which is a promising method of therapy in many fields of medicine, including oncology, urology, ophthalmology, otorhinolaryngology, dentistry and others [4–8]. Prospects of photodynamic therapy for onychomycosis treatment are discussed in [9–14], where, among other things, attention is drawn to the necessity to improve the efficiency of known photodynamic drugs delivery to the nail bed and search for new photodynamic drugs and light sources.

The method of photodynamic therapy consists in that a photosensitizer drug penetrates into the tissue followed by irradiation with visible light of a wavelength that is absorbed by the photosensitizer drug. Upon absorption of a photon, the photosensitizer undergoes one or more energy transitions and usually emerges in its excited triplet state. The triplet can participate in a one-electron oxidation–reduction reaction (type I photochemistry) with a neighboring molecule, producing free radical intermediates that can react with oxygen to produce peroxyradicals and various reactive oxygen species. Alternatively, the triplet-state photosensitizer can transfer energy to ground state oxygen (type II photochemistry), generating singlet molecular oxygen, a highly reactive form of oxygen that reacts with many biological molecules, including lipids, proteins, and nucleic acids [15].

The selection of photosensitizer (PS) plays a decisive role in PDT treatment [16]. Currently it is considered that optimum chlorine-containing photosensitizers are those of the second generation based on chlorine e6 (Ce6) [17–19]. A considerable number of experimental studies are known on the investigation of spectral and luminescent properties of chlorine e6 (Ce6) in terms of aggregation and

its subsequent effect on photophysical properties of the photosensitizer drug, which is of critical importance for the PDT. The absorbance spectrum of Ce6 is characterized by presence of the following bands: the most intensive is B-band (Soret band) having its peak at a wavelength of about 401 nm, which corresponds to two close Bx- and By-transitions, Qx 00- and Qx 01-bands (near 505-510 nm), as well as Qy 00-band having its peak at a wavelength of about 664 nm. B-band is quite wide and extends from 320 to 480 nm by its base. It is known that Ce6 in the ethanol solution and in the alkaline aqueous solution is predominantly in a monomer form [20,21]. Photodynamic therapy mainly uses light sources with a wavelength falling into the peak of Qy 00-band, which is related to the fact that light with this wavelength penetrates deep enough into biological tissues. However, this radiation is not absorbed by photosensitizer as effectively as the radiation falling into the peak of B-band. And the use of sources with wavelengths within the B-band is limited by the shallow penetration depth of their radiation into biological tissue. In this context the use of radiation with a wavelength falling into the B-band but not corresponding to its absorbance peak should allow selecting the conditions for the light to penetrate deeper into the biological tissue, but at the same time the absorption of light by the photosensitizer should be higher or at the same level as under the exposure to the light of the Qy 00-band peak. The wavelength of 450 nm meets the above-listed criteria.

450 nm laser is used for medical purposes, including surgery, because it effectively vaporizes and coagulates soft tissues without significant thermal impact on the surrounding biological tissues [22,23]. This wavelength is promising for antimicrobial photodynamic therapy [24]. In dermatology the 450 nm laser is successfully used in the treatment of port wine stains [25]. In dentistry the 450 nm laser with a power of 1500 mW in combination with low concentrations of hydrogen peroxide is used for the teeth bleaching [26]. Not long ago, lasers with this wavelength started to be widely used for intravascular laser blood irradiation (ILBI) [27]. Also, it is known that the phototherapy by radiation with a wavelength of 450 ± 25 nm is an effective method of treatment of newborn children with conjugated jaundice [28,29]. Thus, 450 nm laser is already used and has a great potential for creation of new biomedical technologies, including PDT.

To improve the efficiency of topical drugs delivery, including photosensitizers, various methods are used aimed at destruction of the nail plate: full or partial chemical ablation of the nail plate, ionophoresis, sonophoresis, low-frequency ultrasonic therapy, mechanical and laser microporation [30]. Laser microporation differs from other methods of drug delivery efficiency improvement by its minimum invasiveness, painlessness, better pharmacokinetics of the drug and high localness [30]. The most promising for microporation of nail plate are Er- and CO_2-lasers due to their highly efficient ablation effect on superficial layers of biological tissue [31–34].

Drug delivery through a laser-microporated nail plate to the infected nail bed can be passive or active, i.e. occur without or as a result of external action [33,35]. However, in the case of passive delivery, water-based drugs do not penetrate into the micropored nail plate because of high surface tension coefficient [36]. At the same time, it is known that in the case of active delivery laser-induced hydrodynamic processes can increase the drug penetration rate into biological tissues [37,38] as a result of pressure waves generation.

An important issue is retention of drug properties after its active laser delivery. It is known that laser radiation causes increase in adrenoblocking properties of propranolol, weakening of the cardiotonic activity of adrenaline and dobutamine, increase in the antiarhythmic activity of verapamil and lidocaine [39]. This may be related to the laser radiation effect on the drug structure, their hydrate shell and, as a consequence, change in the interaction of substances with the receptors [39]. Laser radiation can affect the optical properties of drugs, including as a result of change in their conformational state [40,41]. Photodynamic efficiency of a photosensitizer drug also can be related to its conformational state and evaluated by investigating the absorbance spectrum [42,43].

In the process of the laser delivery and photodynamic therapy under exposure to light with a wavelength corresponding to the peak of drug absorption (resonance impact), as a result of the effective photodestruction of the photosensitizer, as well as due to the fact that wavelength of the chlorine-containing photosensitizer drug absorption peak is shifted in this circumstances, the absorption of the photosensitizer drops dramatically during the irradiation and the photodynamic effect is significantly decreased [43,44]. Exposure of the photosensitizer drug to the radiation with a wavelength out of the absorption band peak, but within the absorption band (non-resonance exposure out of the absorption peak, for example at a wavelength of 450 nm) can weaken both the above-listed effects and result in a positive consequences in terms of retention of photodynamic activity of the drug. In this context the investigation of behavior of the photosensitizer drug absorbance spectrum under such a non-resonance exposure is a relevant task. The non-resonance laser impact should result in a lower degree of photosensitizer drug absorption (as compared to the resonance impact) at the excitation wavelength within the exposure time, because in this case processes of photodestruction at the excitation wavelength are either weak or completely absent, and the destruction of the photosensitizer takes place not only under the action of light, but also under the action of other factors (temperature, acidity, etc.). In this case in the process of treatment, for example, of a fungoid disease in dermatology, to compensate for the loss of photodynamic activity of the drug, lower intensities of the radiation should be needed as compared with the resonance impact, which should allow

avoiding unnecessary injury of the biological tissue around the place of impact, while keeping high efficiency of the photodynamic action, i.e. it should improve safety of the treatment.

One of promising modern photosensitizer drugs based on chlorine e6 is Chloderm (Chloderm, Russia). The dynamics of absorption spectra of this drug under photodynamic impact by LED radiation with a wavelength of 656 ± 10 nm was investigated in [44]. Processes of microporation of nail, delivery of Chloderm to the nail bed and PDT under exposure to laser radiation with a wavelength of 450 nm have not been studied so far, which, along with the above-listed issues, define the relevance of investigation of these processes.

The purpose of this work was to obtain new scientific knowledge on the laser delivery of modern photosensitizer drugs and photodynamic therapy of onychomycosis using laser radiation with a wavelength of 450 nm. In this context a successive microporation of the nail plate and active laser delivery of modern chlorine-containing photosensitizer drug (Chloderm) using 450 nm laser under the nail plate was studied *in vitro*. Also, a extinction spectra of this photosensitizer drug were investigated before and after the exposure to the laser radiation with an intensity sufficient for microporation of the nail plate.

Materials and methods

In the *in vitro* study we used fragments of healthy human nail plates of one volunteer extracted as the nail was growing. A total of 20 fragments were studied. Before the experiments, fragments were mechanically cleaned and washed with distilled water. Average thickness of fragments was $370 \pm 20\,\mu$m.

An aqueous solution of Chloderm photosensitizer drug (Chloderm, Russia) was used. Weight concentration (C) of Chloderm in water was $C = 0.65\%$.

When studying the rate and efficiency of laser ablation of nail, as well as the rate of active laser delivery of Chloderm through a single microhole, laser radiation with a wavelength of 450 nm was used. Average power of the radiation P was 1.24, 1.69 and 1.90 W and was limited by parameters of laser diode used. Diameter of the laser beam on the nail plate surface was 1.1 ± 0.1 mm. Thus, the intensity of laser radiation on the nail surface in the experiment was 131, 178 and 200 W/cm^2 for the above-listed average power values, respectively. The time of exposure to laser radiation (t) corresponded to 0.04, 0.06, 0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 and 1.5 s. The scheme of experimental setup was similar to that described in [41,45] and shown in Fig. 1.

On the outer surface of the specimen a microcuvette was formed from glued layers of Scotch tape, thickness of the microcuvette was $h = 100 \pm 10\,\mu$m, its length and width were ~ 1.5 mm. A specimen of nail plate, in turn, was placed of a paper substrate, and the substrate was placed on the surface of a glass slide.

At the first stage (Fig. 1, *a*) the microcuvette was not filled with the photosensitizer drug, the laser radiation was focused on the dorsal surface of the nail plate. As a result of laser impact with a wavelength of 450 nm a microhole was made in the nail plate. The process of nail plate microporation was monitored using a „DTX 50“ digital USB-microscope (Levenhuk, Inc., USA), connected to a computer and located from the rear side of the paper substrate. The moment of microporation corresponded to the moment of defect emerging on the rear side of the paper substrate.

At the second stage (Fig. 1, *b*) the microcuvette was filled with the photosensitizer drug. Immediately after that its active delivery was performed as a result of laser impact with a wavelength of 450 nm. The moment of photosensitizer drug delivery corresponded to the moment when the paper substrate with the specimen placed on it changed its color to that of the drug, and this moment was recorded by the digital USB-microscope. Appearance of the nail plate dorsal surface and the side of paper substrate facing the microscope, where the nail plate specimen was placed at the moment of Chloderm aqueous solution ($C = 0.65\%$) delivery is shown in Fig. 2.

In the experiment the rate and efficiency of laser ablation of the nail plate, the time needed to create a single through microhole in the nail plate, and the time required for active delivery of photosensitizer drug were determined depending on the power of the laser radiation. Also, the mass ($PM_{Chloderm}$) of photosensitizer drug penetrated (delivered) through the single hole at the moment of its delivery under the nail plate and the rate ($V_{Chloderm}$) of drug delivery through the single microhole were determined. The rate of ablation was determined as a ratio between the crater depth in the nail plate and the time of laser radiation action. The efficiency of ablation was determined as a ratio between the crater depth in the nail plate and the energy required to create the crater. To estimate the crater depth, the nail plate fragment with crater was ground by an abrasive tool along the crater axis and a longitudinal thin section of the crater was formed passing through the crater axis. The longitudinal thin section of the crater was photographed using a „DTX 50“ USB-microscope (Levenhuk, Inc., USA), the obtained image was analyzed in CorelDRAW Graphics Suite 2021 software package (Corel Corporation, Canada) to determine the crater depth.

The microporation rate was determined as a ratio between the nail plate thickness and the time required to create a single through microhole in the nail plate. The microporation efficiency was determined as a ratio between the nail plate thickness and the energy required to create a single through microhole in the nail plate. The mass of $PM_{Chloderm}$ was calculated on the basis of results of paper substrate weighing before and after the active laser delivery of the photosensitizer drug and determined as the difference between paper substrate masses before and

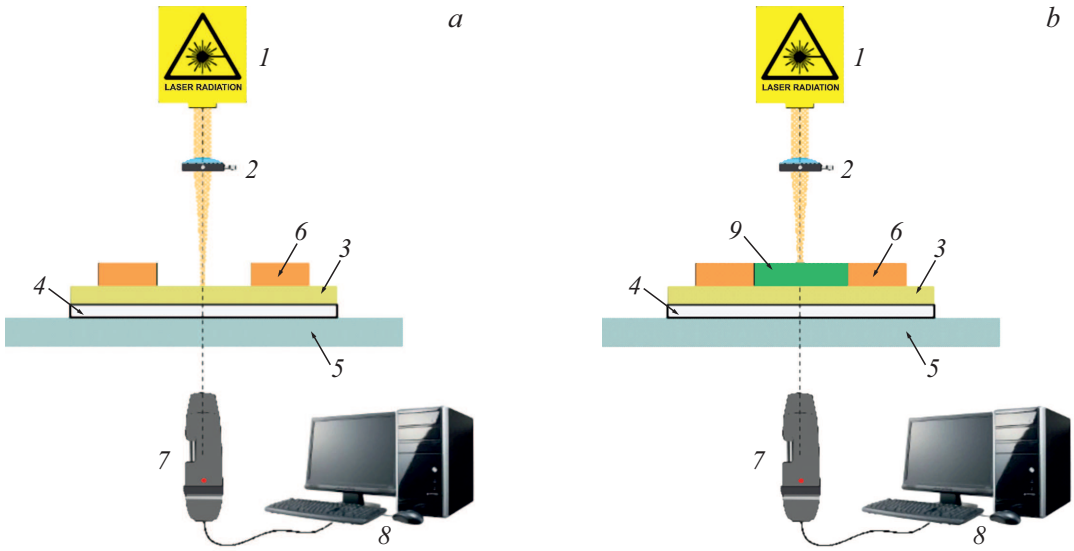

Figure 1. Scheme of the experimental setup for successive microporation (*a*) and active laser delivery (*b*) of Chloderm (Russia) under the nail plate: *1* — laser ($\lambda = 450$ nm); *2* — lens ($F = 50$ mm); *3* — nail plate fragment; *4* — paper substrate; *5* — glass slide; *6* — microcuvette; *7* — DTX 50 digital USB-microscope (Levenhuk, Inc., USA); *8* — computer; *9* — aqueous solution of Chloderm ($C = 0.65\%$).

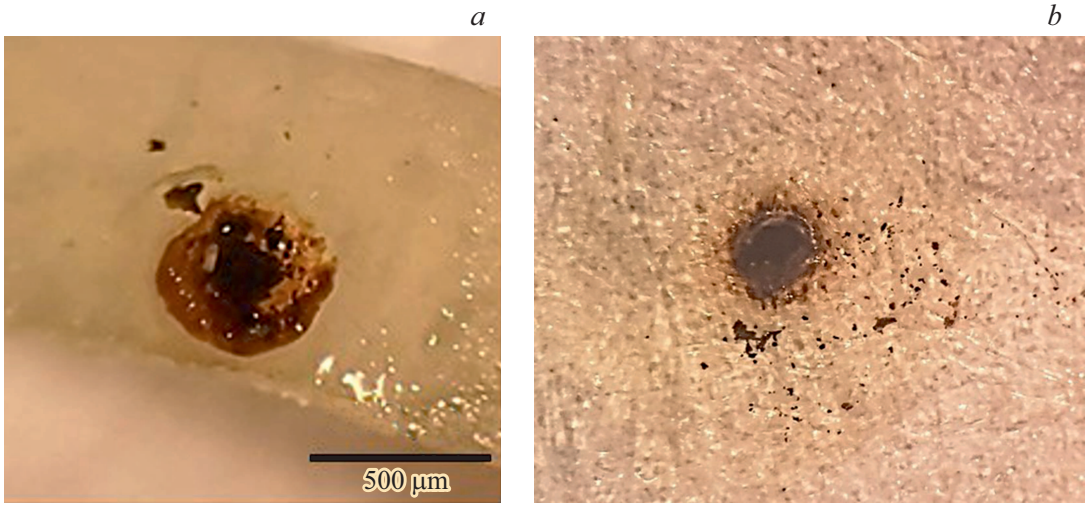

Figure 2. Typical photo of the nail plate dorsal surface (*a*) and the side of paper substrate facing the microscope, where the nail plate specimen was placed (*b*) at the moment of Chloderm aqueous solution ($C = 0.65\%$) delivery by 450 nm laser ($P = 1.90$ W, $t = 0.6$ s).

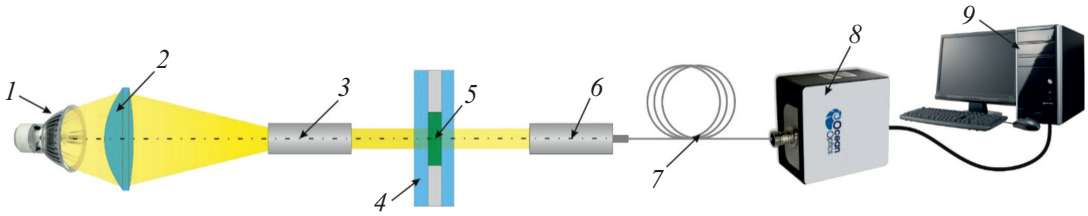

Figure 3. Scheme of the setup for investigation of extinction spectra of Chloderm aqueous solution: *1* — halogen lamp (50 W); *2* — focusing lens ($F = 80$ mm); *3* — collimator; *4* — cuvette; *5* — Chloderm aqueous solution; *6* — receiving collimator; *7* — spectrometer fiber; *8* — SD2000 spectrometer (Ocean Optics Inc., USA); *9* — computer.

after the delivery. The weighing was performed in 5–10 s after the delivery. At the same time, the decrease in the paper substrate weight related to the microhole formed in it after the microporation of the nail plate was taken into account. Weight of paper substrate material removed by the microporation was determined as the difference between weight of the paper substrate before and immediately after the microporation. In the experiments, weight of the paper substrate material removed by the microporation was from 0.04 to 0.08 mg, depending on the laser radiation power and exposure time. The rate of V_{Chloderm} delivery was determined as a ratio between the $\text{PM}_{\text{Chloderm}}$ mass and the time required for the active laser delivery of the photosensitizer drug.

To detect possible structural changes in Chloderm in the process of active laser delivery under 450 nm laser impact, the process was physically modelled and extinction coefficients of the photosensitizer drug were determined before and after the laser impact. Extinction spectra of the Chloderm aqueous solution were recorded using a SD2000 spectrometer (Ocean Optics Inc., USA). Measurements were performed in relation to two glass slides identical to cuvette walls and transparent for the light. The scheme of experimental setup was similar to that described in [41,46] and shown in Fig. 3.

The Chloderm aqueous solution ($C = 0.65\%$) filled the entire inner cavity of the quartz cuvette, which was sized identical to parameters of the inner cavity of the microcuvette formed on the nail surface to investigate the active laser delivery of Chloderm (see above). The transmittance spectrum of Chloderm aqueous solution before the laser impact was recorded. The Chloderm aqueous solution was irradiated by scanning of the laser radiation with a wavelength of 450 nm along the surface of the photodynamic drug. This was performed with maximum intensity of the laser radiation equal to 200 W/cm^2. The scanning was performed in a stepwise manner from point to point with a step equal to the laser beam diameter, until the whole surface of the drug was irradiated. Time of the laser impact on one point was 0.3, 0.4, 0.7 and 0.8 s. Then the irradiated Chloderm aqueous solution inside the cuvette was thoroughly agitated (for 10 ± 1 s) with following recording of its transmittance spectrum after the laser impact. The spectrum recording took 10–15 s.

Based on the obtained transmittance spectra, in accordance with Beer–Lambert–Bouguer law the extinction spectrum of the Chloderm aqueous solution before and after the laser impact was calculated. In the experiments, two absorption bands were investigated: with peak at a wavelength of 664 ± 3 nm (Qy 00-band of drug absorption) and with peak at a wavelength of 710 ± 3 nm, which correspond to conformation states of chlorine e6 (Ce6) in the form of monomer and tetramer, respectively [21]. Also, in this study we evaluated the extinction coefficient at a wavelength of 405 ± 5 nm that corresponds to the peak of B-band of the drug absorption, at a wavelength of 450 ± 2 nm that corresponds to the wavelength of laser radiation, and at a wavelength of 507 ± 5 nm that corresponds to the peak of Qx 00-band of the drug absorption. In this study we evaluated the coefficient of spectral transformation (k_t) equal to the ratio between the extinction coefficient of Chloderm (μ_t) at a wavelength corresponding to the resonance absorption of monomers and the extinction coefficient of Chloderm at a wavelength corresponding to the resonance absorption of tetramers. Since the experimentally obtained spectra were of complex nature, with overlapped bands, to obtain the true value of k_t, we decomposed the spectra into individual lines of the Gaussian profile and determined their peaks from the experimentally obtained spectra as corresponding to wavelengths of 664 ± 5 nm (monomers) and 705 ± 5 nm (tetramers). The spectra were processed by built-in functions of the „Origin Pro 2021“ applied software package (OriginLab Corporation, USA). If $k_t = 1$, tetramers present in the solution equally to monomers, if $k_t > 1$ — monomers prevail in the solution, not tetramers, and if $k_t < 1$ — tetramers prevail over monomers. We investigated the change in k_t as a function of the laser impact time t.

The statistical processing of the experimentally obtained data consisted in determining mean values and standard deviation of measured quantities and performed in the STATGRAPHICS Plus 5.0 software package (Statistical Graphics Corp., USA).

Results and discussion

Dependencies of rate and efficiency of nail plate ablation on the time of exposure to the laser impact with a wavelength of 450 nm at different power of the laser radiation are shown in Fig. 4, *a* and 4, *b*, respectively.

It can be seen, that the rate and efficiency of ablation decrease with growth of the laser impact time. It may be related to the gradual increase in the distance between the crater bottom and the plane of laser beam constriction during the period of laser impact, as well as to the accumulation of laser destruction products inside the crater that absorb the radiation. In both cases the energy reaching the crater bottom decreases. Also, it should be noted that the rate and efficiency of nail plate ablation increase with growth of laser radiation power, which is evidently caused by the increase in the volume of the nail plate where laser radiation intensity exceeds the ablation threshold.

Maximum ablation rate of the nail plate by radiation with a wavelength of 450 nm was $2750 \pm 30\,\mu$m/s, while nail plate ablation efficiency was $1.47 \pm 0.05\,\mu$m/mJ at a laser radiation power of $P = 1.90$ W.

Rate and efficiency of healthy nail plate ablation by radiation of CO_2 ($\lambda = 10.6\,\mu$m) laser were investigated in [47,48], ablation by radiation of Er:YAG ($\lambda = 2.94\,\mu$m), Ho:YSGG ($\lambda = 2.08\,\mu$m), femtosecond Ti:Sapphire ($\lambda = 1.053\,\mu$m), XeCl ($\lambda = 0.308\,\mu$m) lasers were investigated in [46], and ablation by radiation of Er:YLF ($\lambda = 2.81\,\mu$m) laser was investigated in [31]. In [49] authors investigated the rate and efficiency of

ablation of a nail plate affected by *T. rubrum* or *T. mentagrophytes* using Er:YAG ($\lambda = 2.94\,\mu$m) laser and it was shown that the rate and efficiency of ablation of the affected nail plate by laser radiation are comparable with those for healthy nail plate. In our case the efficiency of healthy nail plate ablation under laser impact with a wavelength of 450 nm is higher than the efficiency of Ho:YSGG ($0.05\,\mu$m/mJ) and XeCl ($0.03\,\mu$m/mJ) lasers, comparable with the efficiency of CO_2 ($0.55\,\mu$m/mJ), Er:YAG ($0.2-0.4\,\mu$m/mJ) and Ti:Sapphire ($1\,\mu$m/mJ) lasers, but inferior to the ablation efficiency of Er:YLF ($4.6\,\mu$m/mJ) laser, that can be explained by the difference in both the absorption ability of the nail plate and spatial-time characteristics of the laser radiation.

The time of laser impact required for microporation through the nail plate in our case was 0.4, 1.0 and 1.5 s for laser radiation power of 1.90, 1.69 and 1.24 W, respectively.

The dependence of mass $PM_{Chloderm}$ of the Chloderm aqueous solution penetrated through a single hole in the nail plate at the moment of its delivery under the nail plate on the time of laser impact at different power of the laser radiation is shown in Fig. 5, *a*. The dependence of rate ($V_{Chloderm}$) of active laser delivery of Chloderm aqueous solution on the time of laser impact at different power of the laser radiation is shown in Fig. 5, *b*.

Dependencies of $PM_{Chloderm}$ and $V_{Chloderm}$ are shown only for $P = 1.69$ W and $P = 1.90$ W, because at $P \leq 1.69$ W there was no delivery. It can be seen that $PM_{Chloderm}$ and $V_{Chloderm}$ increase with growth of the laser impact time, as well as with increase in radiation power. This is evidently related to the growth of the laser radiation energy exposure.

Maximum mass $PM_{Chloderm} = 0.92 \pm 0.09$ mg and delivery rate $V_{Chloderm} = 1.15 \pm 0.10$ mg/s were recorded at $P = 1.90$ W and $t = 0.8$ s. Minimum time required for the active laser delivery was observed at $P = 1.90$ W and was equal to 0.3 s. In [37] authors investigated the rate of laser delivery of methylene blue (MB) aqueous solution and mass of the drug delivered under the nail plate by the radiation of Er:YLF ($\lambda = 2.81\,\mu$m) laser. It was shown that mass of the delivered drug is $PM_{MB} = 1.04 \pm 0.27$ mg, while delivery rate is $V_{MB} = 0.26 \pm 0.03$ mg/pulse, that, at a laser pulse length of $t_p = 270 \pm 10\,\mu$s, corresponds to $V_{MB} = 963 \pm 0.03$ mg/s. It is evident that in our case the mass of delivered drug and the rate of delivery are considerably lower, which may be related to both the difference in viscosity of the delivered drugs and the significant differences in the ability of drugs to absorb radiation of these lasers and difference in the pulse power of the laser sources affecting the intensity of the running hydrodynamic processes.

Typical extinction spectra of Chloderm aqueous solution ($C = 0.65\%$) before and after the laser impact with a wavelength of 450 nm and a power of $P = 1.90$ W (intensity of 200 W/cm^2) are shown in Fig. 6, *a*. The dependence of transformation coefficient k_t on the time of laser impact is shown in Fig. 6, *b*.

Before the laser impact the extinction coefficient μ_t of Chloderm aqueous solution at a wavelength of 405 ± 2 nm was 0.28 cm^{-1}, at a wavelength of 450 ± 5 nm it was 0.59 cm^{-1}, at a wavelength of 507 ± 5 nm it was 0.69 cm^{-1}, at a wavelength of 664 ± 5 nm it was 0.84 cm^{-1}, and at a wavelength of 710 ± 3 nm it was 0.47 cm^{-1}. The fact that the extinction coefficient at a wavelength of 664 ± 3 nm is greater than the coefficient μ_t at a wavelength of 710 ± 3 nm indicates that in the initial Chloderm aqueous solution predominant are monomers of chlorine e6, and not its tetramers.

When the gel is exposed to laser radiation with a wavelength of 450 nm, with a power of 1.90 W (Fig. 6, *a*) within a period of time equal to 0.3, 0.4, 0.7 and 0.8 s, the extinction coefficient μ_t at a wavelength of 405 ± 2 nm remains unchanged; at a wavelength of 450 ± 5 nm it remains unchanged at an exposure time of 0.3 s and increases to 0.60, 0.60 and 0.64 cm^{-1} at other times of the laser impact; at a wavelength of 507 ± 5 nm it increases to 0.72, 0.74, 0.79 and 1.03 cm^{-1}; at a wavelength of 664 ± 3 nm it increases to 0.89 cm^{-1}, 0.88 cm^{-1}, 0.94 cm^{-1} and 1.25 cm^{-1}, at a wavelength of 710 ± 3 nm it increases to 0.51, 0.56, 0.66 and 1.05 cm^{-1}, respectively. The increase may be related to an increase in concentration of photosensitizer (chlorine e6) in the drug due to the water evaporation under the laser impact.

The coefficient of transformation k_t decreases from 2.73 ± 0.25 ($t = 0$ s) to 0.83 ± 0.07 ($t = 0.8$ s). This indicates that growth of t results in changes of the conformation state of the Chloderm aqueous solution, and quantity of monomers of chlorine e6 decreases, which can reduce the photodynamic activity of the Chloderm delivered to the rear side of the nail. However, up to $t = 0.7$ s the coefficient of transformation k_t after the laser impact remains greater than unit, which indicates that, as in the initial solution, monomers of chlorine e6 prevail in the Chloderm aqueous solution after the laser impact with $t \leq 0.7$ s, and not its tetramers.

The changes in the extinction spectrum of the photodynamic drug noted in this study can hardly be connected with its heating in the process of laser delivery and further cooling down at room temperature. In [50], it is noted that the heating of a chlorine-containing photodynamic drug — Revixan (Areal LLC, Russia) — can result in changes in shape of the extinction spectrum of this drug, changes in its conformation state and result in growth of monomer concentration. Also, a work is known [51], where the effect of temperature on spectral and luminescent properties of water-soluble tricarbocyanine dye is investigated and stability of this compound under heating is tested. The authors conclude that heating of this compound results in increase in monomer concentration due to decomposition of dimers in it, and absorption coefficients of the dye change. The [52] investigates the evolution of absorption spectra of an indotricarbocyanine dye resulted from the heating–cooling cycle. It is shown that the exposure to temperature results in a change of dye?s absorption spectra,

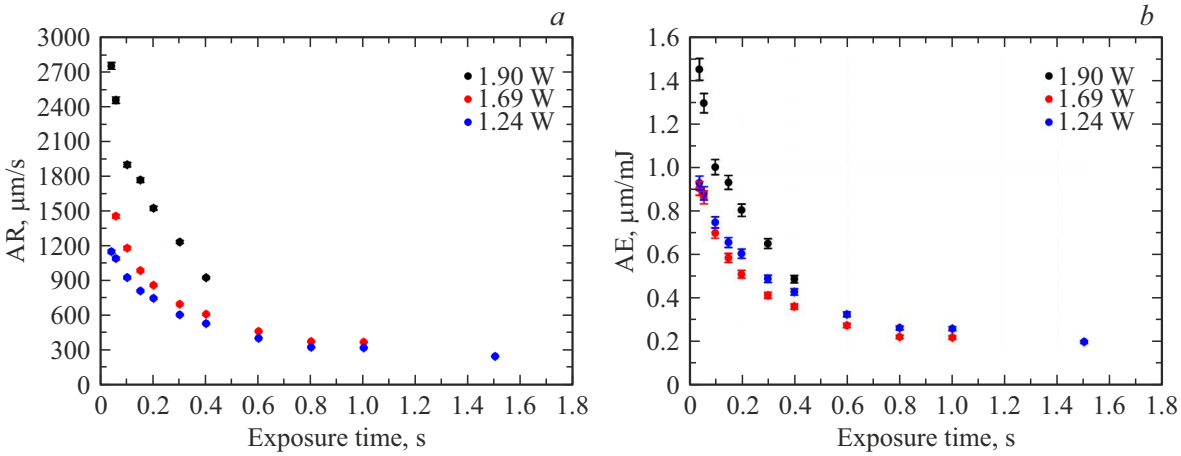

Figure 4. Dependence of rate AR (a) and efficiency AE (b) of nail plate ablation on the time of exposure to the laser impact with a wavelength of 450 nm at different average power of the laser radiation.

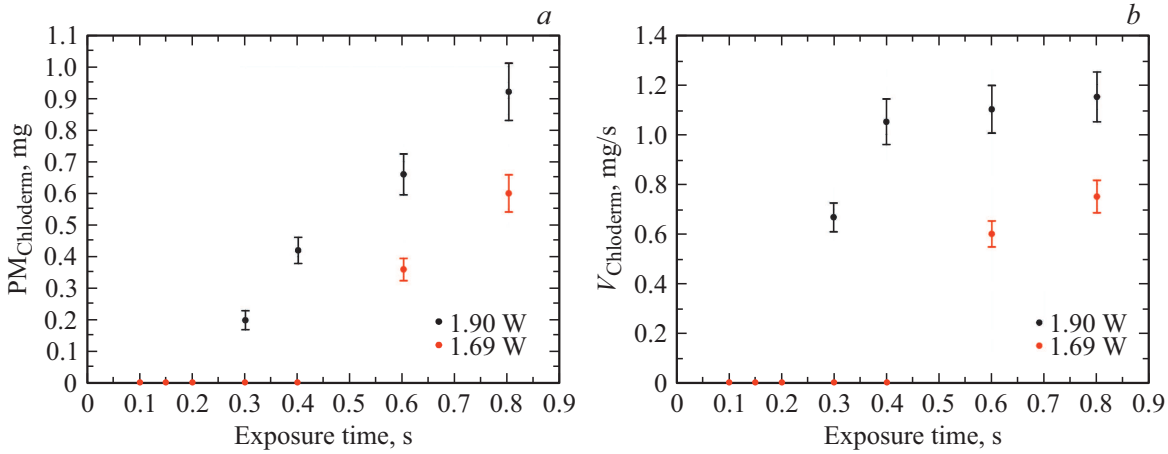

Figure 5. Dependence of mass $PM_{Chloderm}$ (a) and rate ($V_{Chloderm}$) of active laser delivery (b) of Chloderm aqueous solution ($C = 0.65\%$) on the time of laser radiation impact with a wavelength of 450 nm at different mean power of the radiation.

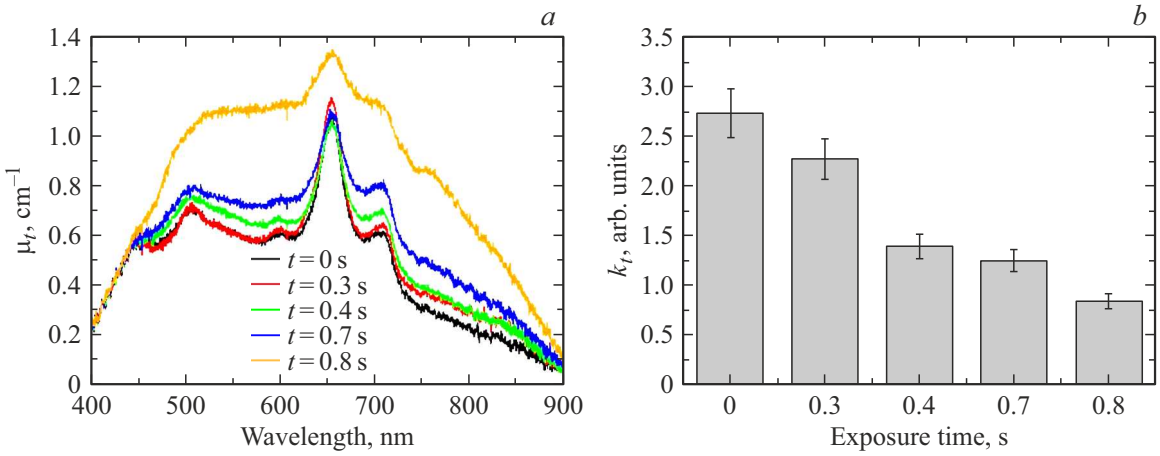

Figure 6. Typical extinction spectra of Chloderm aqueous solution ($C = 0.65\%$) before and after the laser impact at different exposure times (a) and coefficients of transformation k_t at different times of the laser impact (b) (μ_t — extinction coefficient, wavelength of the laser radiation is 450 nm, mean power is $P = 1.90$ W, intensity is 200 W/cm^2).

and an increase in temperature causes growth of monomer concentration due to decomposition of dimers. In our case the concentration of monomers decreases, which may be related in a greater degree to their photodestruction by the radiation with a wavelength of 450 nm.

The attention should be paid to the fact, that at the minimum time of the laser impact required for the active laser delivery ($t = 0.3$ s), the k_t changes by less than 20%, which allows suggesting that the laser delivery with a wavelength of 450 nm and an intensity of 200 W/cm^2 (energy exposure of 60 J/cm^2) changes insignificantly the conformation state of the Chloderm aqueous solution.

Conclusion

The possibility of microporation followed by active delivery of Chloderm (Chloderm, Russia), a modern chlorine-containing photosensitizer drug, under the nail plate by 450 nm diode laser is studied *in vitro*. The possibility of nail plate microporation by the radiation of this laser is demonstrated, and it is shown that minimum time of impact required for the nail plate microporation is 0.4 s. It is found that maximum ablation rate of nail plate by radiation with a wavelength of 450 nm is $2750 \pm 30\,\mu$m/s, while ablation efficiency is $1.47 \pm 0.05\,\mu$m/mJ. The possibility of active delivery of Chloderm, a modern chlorine-containing photosensitizer drug, under the nail plate is demonstrated. The minimum time of impact required for active delivery of Chloderm gel aqueous solution at a laser radiation intensity of 200 W/cm^2 is 0.3 s. It is shown that the rate of active laser delivery of Chloderm aqueous solution by radiation with a wavelength of 450 nm can be as high as 1.15 ± 0.10 mg/s. It is demonstrated for the first time in an *in vitro* experiment that using the laser radiation with a wavelength of 450 nm and a power of $P \geq 1.69$ W (intensity of ≥ 178 W/cm^2) it is possible to implement microporation of the nail plate with an average thickness of $350 \pm 20\,\mu$m followed by active laser delivery of the Chloderm aqueous solution ($C = 0.65\%$) with a layer thickness of $h = 100\,\mu$m.

Extinction spectra of the Chloderm aqueous solution are investigated before and after the active laser delivery. It is shown that the laser delivery with a wavelength of 450 nm does not change the extinction coefficient of the drug at the laser radiation wavelength and insignificantly changes its conformation state. The photodynamic activity of Chloderm after the 450 nm laser delivery needs further studying with the cultures of fungus that cause onychomycosis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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