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REVIEW ARTICLE

A review of the mechanism of action of lasers and photodynamic therapy for onychomycosis

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Abstract Onychomycosis is one of the most common diseases in the field of dermatology. It refers to the fungal infection of the nail plate or nail bed with high incidence in the general population. The available treatment options for onychomycosis have limited use due to side effects, drug interactions, and contraindications, which necessitates the application of an alternative treatment for onychomycosis. In the recent years, lasers and photodynamic therapy (PDT) have been recognized as alternative treatment options. Most of the previous studies have found them to be safe and effective treatment modalities in this indication; however, the results varied greatly and the in vitro and in vivo outcomes are contradictory. In the present review, studies related to the mechanism of action of lasers and PDT for the treatment of onychomycosis will be discussed, with a focus on to find explanation to the contradictory results.

Keywords Laser therapy · Photodynamic therapy · Onychomycosis

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Introduction

Onychomycosis is a fungal infection of the nail bed accounting for 30% of skin and skin structure fungal infections [1–3]. Sigurgeirsson et al. [4] and others reported that *Trichophyton rubrum* (*T. rubrum*) is the most common pathogen of onychomycosis,;the clinical isolation rate is up to 80% [5]. Onychomycosis not only affects the appearance of the nail, but can also lead to secondary infections [4]. Onychomycosis has always been difficult to treat; all traditional methods of treatment, for instance, topical antifungals, oral antifungal, and surgical or chemical removal of the nail have some limitations [6]. Due to the dense structure of the nail, topical antifungals could hardly penetrate into the lesion, thus greatly reducing the effect of drugs; furthermore, the treatment time is longer, and to some extent, the patient's compliance is also decreased. Although oral antifungals have good effect on onychomycosis, they could have serious side effects, such as liver toxicity, and they should not be applied in patients who have kidney disease, in children, and in the elderly. Surgical or chemical removals of the nail seriously affect the appearance of the nail; therefore, the quality of life of the patients, and on the other hand, onychomycosis can easily recur.

Due to the lack of resounding success in the treatment of onychomycosis with the abovementioned conventional methods, researchers and clinicians have been looking for a more effective, more convenient, and safe treatment modality. In the recent years, laser and PDT treatments of onychomycosis have become more and more popular due to their effectiveness without serious side effects, especially among the elderly, immunocompromised patients, and patients who have liver and/or kidney dysfunction [7–9]. Currently, the most commonly applied and US Food and Drug Administration (FDA)-approved lasers for the treatment of onychomycosis in the clinical practice are long-pulsed

neodymium-doped yttrium aluminum garnet (Nd: YAG) 1064-nm laser, Q-switched Nd:YAG 1064- and 532-nm Nd:YAG lasers, carbon-dioxide (CO_2) laser, and near-infrared diode laser [7]. The present review will cover all currently available articles discussing the mechanism of action of photodynamic therapy and of these lasers.

Mechanism of action of Nd: YAG lasers (long-pulsed 1064 nm, Q-switched 1064 nm, 532 nm)

Currently, long-pulsed 1064-nm Nd:YAG laser is the most commonly used among laser devices for the treatment of onychomycosis [7]. It has good therapeutic effect on different types of onychomycosis. The treatment is safe, effective, and simple, without serious adverse reactions. It provides a new choice of treatment for the elderly, patients with low immunity, or liver and kidney dysfunction [7]. This leads to unique therapeutic advantages for these types of patients with onychomycosis. There is also an ongoing trial investigating the effect of 1064-nm Nd:YAG laser in patients with diabetes at risk for foot complications [10]. Most of the studies have reported good therapeutic efficacy, with clearance rates ranging between 51 and 100% [11–18]. However, there were randomized controlled trials, which could not find significant therapeutic effect, compared to the untreated group [19]. In these studies, the treatment parameters (pulse duration, energy fluence), number of sessions, and treatment intervals varied greatly, which could explain the different results. The other point can be the different strains of fungi, causing onchomycosis. It has been shown that nails infected by *T. rubrum* had the best treatment response [20].

Focusing on the energy, it was found by Ghavam et al. [21] that low-power laser systems modified colonies, but did not have any inhibitory effects on the fungal colonies, while in the high power laser category, the Q-switched Nd: YAG 532 nm in 8 J/cm2, Q-switched Nd: YAG 1064-nm laser at 4 to 8 J/cm2, and pulsed dye laser (PDL) in 8 J/cm 2595 nm to 14 J/cm2 can significantly inhibit the growth of *T. rubrum* [21] and could significantly change the microstructure of *T. rubrum* [5]. Henrik et al. [22] reported that this effect could be due to unspecific tissue heating with a subsequent increase in circulation due to vasodilatation and stimulation of the immunological processes. Kozarev et al. [15] and others consider that 1064-nm long-pulsed Nd: YAG laser is mainly dependent on the thermal effect of the laser. Moreover, Galvan Garcia et al. [16] believe that Nd: YAG Q-switched laser generates high-energy peaks with many repetitions, which do not warm the tissue (it is painless); thus, it produces impact energy that mechanically damages only the fungi. The authors suggest that laser therapy acts via "selective photothermolysis" that works according to the type of pigment, type of light, and pulse frequency (light + heat + impact power). Vural et al. [23] reported that the inhibition of fungal colonies by Q-switched Nd: YAG laser is most likely to be due to nonspecific thermal damage. Another study [24] has reported that 532-nm Q-switched Nd:YAG laser can suppress *T. rubrum* due to the large number of xanthomegnin that it contains; therefore, *T. rubrum* can be sensitive to the 532-nm wavelength of light. In contrast, that Q-switched Nd:YAG laser at 1064-nm wavelength is also beyond the absorption spectra of xanthomegnin, and it has similar inhibitory effect on *T. rubrum*. The great amount of melanin in the cell wall of *Trichophyton* species could be an explanation of this inhibitory effect. The absorption spectrum of a hair color group is also 1064 nm [24]. In view of the foregoing, long-pulsed Nd:YAG 1064-nm laser is based on the principle of selective photothermolysis. The laser acts on the chromophore of the fungal tissue. Chromophores absorb enough heat, which is shifted to the fungal tissue, resulting in the elevation of the tissue temperature, leading to the damage of the fungi. Nd:YAG laser can effectively reach the depth of the nail bed, the site of the fungus colony. The surface temperature is controlled on 43~51 °C, which kills the fungus completely, resulting in the cure of onychomycosis [25]. However, Paasch et al. [26] found, while irradiating fungal pathogens cultured in liquid media, that complete clearance was achieved only when the temperature exceeded 50 °C. When the temperatures were not high enough, they observed stimulation in the growth of fungi, especially in case of *Microsporum gypseum* and *T. rubrum*. They stated that for long-pulsed near-infrared lasers, heat is the most likely mechanism of action [26].

Other studies have indicated that UV, heat stress, H_2O_2, and other external pressure, mainly through the induction of mitochondrial apoptotic pathway lead to the apoptosis of fungi [9]. Reactive oxygen species (ROS) produced by the mitochondria, are major fungal molecules, which mediate apoptosis. ROS include hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and superoxide anion (O_2^-). The main way via ROS that induce the apoptosis of fungus is the ROS-mediated oxidation–reduction reaction, which is through the combination of electronic O_2 of fungal mitochondrial respiratory chain, leading to the production of excessive amount of ROS that induce fungal cell apoptosis or necrosis [27]. At the same time, ROS, which may be oxidized by certain fungi in vivo, change the mitochondrial membrane potential, resulting in the release of cytochrome C (Cyt c), and activation of caspases (metacaspases), causing cell mitochondria apoptosis and cell damage. According to Yu Ri Kim et al.'s [28] hypothesis, Nd:YAG laser irradiation has effect on cell metabolism, such as reducing membrane permeability and generating ROS, thereby inducing the destruction of fungal cells. Another possibility is that laser irradiation of the nail matrix accelerates the nail's growth rate. Nevertheless, this explanation has not been supported by objective results. Carney et al. [29] reported that the main point in the mechanism of action is the temperature

increase, induced by energy absorption by the abundant lipids and moisture within the fungus and host cells, with the heat shock response, affecting transcription and translation, which processes lead to death by the induced cell imbalance. *T. rubrum* death occurred after 15 min of exposure on 50 °C [29], and the expression of heat shock protein 70 was increased after the irradiation [30]. Another hypothesis is that xanthomegnin and melanin produced by *T. rubrum* act as chromophores. These pigments are known to protect fungi from host immune responses and reactive oxygen species. It is therefore reasonable to assume that the destruction of these pigments could lead to an antifungal effect [31], as it was mentioned previously.

Scholars have recognized that long-pulsed Nd:YAG 1064-nm laser with high energy can cause deep penetration of heat without gene mutations and is easy to operate with it. The destruction of fungus within the onychomycotic nail by long-pulsed Nd:YAG 1064-nm laser is not only related to treatment times and parameters but also related to the mechanism of action [17]. Nevertheless, further studies are needed to identify the optimal parameters in the long-pulsed Nd: YAG 1064-nm laser settings for the treatment of onychomycosis to achieve the best therapeutic effect. To promote this purpose, investigations for the best laser settings in in vitro models could be performed according to Vila et al. [32] who determined the fungicidal activity of lasers on sterile human nail fragments covered with fungal biofilms.

Mechanism of action of fractional CO_2 laser

CO_2 laser is the first laser used for the treatment of skin diseases. In the recent years, it has been applied for the treatment of onychomycosis with high cure rate and low recurrence rate [33]. Bhatta et al. [34] reported 80% cure rate with fractional CO_2 laser combined with topical antifungal cream for 3 months with three sessions of laser treatment at 4-week intervals. The mechanism of action of CO_2 laser is partially based on a photothermal effect: the fungal tissue is heated; the water within the fungal tissue is converted into steam, which causes swelling and increased pressure within the fungal body, leading to micro-explosions and, eventually, catabolism. Higher local temperatures can directly kill the fungus. On the other hand, micro-holes made by fractional CO_2 laser may improve the penetration of the topical antifungal agent into the nail bed [34]. Generally, thermal damage of fungi can occur on a specific temperature (more than 40 °C), resulting in protein denaturation and inactivation of fungi. The continuous repeated stimulation of fungi via thermal effects eventually exerts bacteriostatic or bactericidal effect [35]. Beckham et al. [36] found that within a relatively narrow temperature range, heat shock protein (HSP) expression and laser-induced thermal stress effect are positively correlated; therefore, the detection of HSP may reflect the degree of fungal cell damage. The application of photodynamic therapy combined with fractional CO_2 laser pretreatment has been also published, reporting good efficacy [33]. Summarizing, CO_2 laser treatment for onychomycosis is simple and cost-effective, with good patient compliance. Therefore, it can be considered as an alternative treatment option, especially for the elderly, in case of liver or kidney dysfunction, and in immunocompromised patients [33, 34]. Nevertheless, there is no standard protocol for energy density, depth and breadth of the wavelength. It remains to be further explored and summarized with its proper mechanism of action, too.

Mechanism of action of near-infrared diode laser

Near-infrared diode laser is a two-wavelength laser, which can emit 870- and 930-nm near-infrared light. It leads to the cure of onychomycosis through thermal effects [14]. Diode laser causes a decreased mitochondrial membrane potential and increased production of reactive oxygen species (ROS), which play a role in the killing effect on *Candida albicans*, *Trichophyton rubrum*, *Staphylococcus aureus*, and *Escherichia coli*, confirmed by in vitro experiments [37, 38].

Mechanism of action of photodynamic therapy

Photodynamic therapy (PDT) has emerged as a new technology for the treatment of skin diseases in the recent years. It is the combination of a photosensitizer drug and a light source (red light, blue light, or even laser light). As a photosensitizer, 5-aminolevulinic acid (5-ALA) or methyl-aminolevulinic acid (MAL) are applied mostly in the clinical practice [39, 40]. Although, there are publications with hypericin, methylene blue, Photogem, and rose Bengal, reporting also good therapeutic efficacies [41–45]. After the illumination with light, ROS, including singlet oxygen are produced, leading to the selective damage of the infected tissue [46]. 5-ALA-PDT has been proven to be effective in killing bacteria, yeasts, fungi, and other microorganisms. [47–50] In an in vitro experiment, 5-ALA was added to the liquid medium of the culture of *T. rubrum. T. rubrum* converted 5-ALA to the optically active photosensitizer protoporphyrin IX (PpIX), and then underwent on a certain wavelength of laser irradiation. In the presence of oxygen, ROS were produced, attacking fungi plasma and intracellular membranes, resulting in the reduction of the number and diameter of fungal colonies, effectively inhibiting the growth of *T. rubrum* [48]. In the photochemical reactions, 5-ALA is the precursor of PpIX, which is a photoactive material, a participant in the in vivo heme biosynthetic pathway. Previous researches showed that a certain amount of PpIX is necessary to effectively destroy

C. albicans. The principle could be that the produced excited singlet oxygen destroys the fungal cell membrane and increases its permeability, resulting in the destruction of cell structure, thus accelerating cell lysis and death [38]. The mechanism of action of PDT is based on two types of physico–chemical reactions: type I and type II reactions [51, 52]. Type I reaction occurs through the generation of highly reactive free radicals (O_2^-, H_2O_2, OH^-) [53], resulting in a complex mixture of ROS, which can oxidize a variety of biomolecules [53, 54]. Type II reaction, however, is based on the generation of singlet oxygen ($^1O_2$), a highly reactive species of oxygen, which is produced by an excited-state reaction between an excited photosensitizer molecule and a vital oxygen molecule [54, 55].

Further in vitro experiments confirmed that 5-ALA-PDT leads to the production of large amount of ROS and causes nitration by photochemical reactions, effectively inhibiting the growth of *T. rubrum* and also killing it [56]. However, due to the difference between *T. rubrum* in vitro culture conditions, and the human body infected by that fungus, further studies on the exact mechanism of action of 5-ALA-PDT are needed.

Discussion

Lasers have been approved for the treatment of onychomycosis and have been vigorously promoted clinically in the recent years. It can be a suitable treatment modality for the elderly, patients with liver and or kidney dysfunction, and even for diabetic patients [7–10, 25]. The reported mycological and clinical cure rates indicate that lasers having deep red or near-infrared spectrum are the most efficient for the treatment of onychomycosis [58]. Therefore, the most favorable lasers are 1064-nm Nd:YAG, CO_2 and near-infrared diode lasers. However, in the reported studies, the treatment procedures vary greatly, resulting in different therapeutic efficacies within a wider range and also making it impossible to perform a meta-analysis of the data [7–9, 12–19]. The results of the previous in vitro and in vivo experiments are also contradictory. While most of the in vivo studies have found it to be an effective treatment modality [12–18], in in vitro experiments, it was reported that at least 45 °C is needed to effectively inhibit the growth of fungi; otherwise, increased growth can be induced [26]. Moreover, dermatophytes and yeasts could react differently to laser treatments [20, 26]. Behind the various clinical outcomes, the application of non-optimal laser systems and treatment parameters could serve as an explanation. Regarding the mechanism of action of lasers, it is based on the principle of selective photothermolysis. Xanthomegnin was recognized as a chromophore of *T. rubrum* [24, 31]. It was also reported that heat stress (thermal damage) and ROS via the induction of mitochondrial apoptotic pathway could lead to the apoptosis of fungi [9, 27, 28]. However, the exact mechanisms of action, as like the best treatment parameters, still needed to be clarified.

Photodynamic therapy is also an emerging approach to cure onychomycosis. There are several photosensitizers applied in this indication. Results of the trials confirmed that not only dermatophytes but also *C. albicans*-caused infections could be also successfully treated by this treatment modality. The main mechanism of action seems to be that ROS induced fungal cell membrane damage, leading to the lysis of the cell. However, further studies are needed to determine the proper mechanism resulting in cell death.

There are also no standard protocols available regarding the optimal parameters of the laser light and PDT [57]. Therefore, more studies are needed in order to obtain the best therapeutic effect in onychomycosis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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