EFFECTS OF NITROGEN LASER IRRADIATION AND PSORALEN PHOTOTOXICITY ON THE GROWTH OF CANDIDA ALBICANS

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A nitrogen laser operating at 337 nm, with an energy density of 2.9 mJ/cm²/pulse, and pulse rate of 2-3 pulses/s, was used to irradiate the fungal suspensions. Irradiation for 2, 5 and 10 min produced moderate inhibition of the growth of Candida albicans (C. albicans), and the number of colonies formed decreased with increasing exposure time. Such irradiation in the presence of methoxsalen caused a lethal effect on the fungus, but methoxsalen application alone did no harm to it. Exposure of the fungal suspensions for 20 min resulted in a lethal effect even in the absence of methoxsalen. The findings suggest possible application of the ultraviolet (UV) laser in photochemotherapy.

KEY WORDS
Nitrogen laser Near-uv Photosensitization Pulse Photochemotherapy Candida albicans Fungal suspensions

Introduction

Nitrogen lasers have a pulsed output in the near-uv at 337 nm. It has not yet been determined whether the lasers have potential clinical applications. Although it is well known that psoralen ultraviolet A (PUVA) can be used to treat a wide variety of skin diseases, little has been published covering the effects of coherent ultraviolet A (UVA) in combination with psoralen. The aim of the present study was to examine in vitro the effects of nitrogen laser irradiation on the growth of C. albicans in the presence or absence of methoxsalen.

Materials and Methods

Preparation of the Fungal Suspensions

A strain of C. albicans from our laboratory culture collection was growth on Sabouraud glucose agar for 72 h at 25°C. A fungal suspension was prepared by suspending a loopful of the culture in normal saline. The suspended yeast cells were counted and diluted to a density of 4000 yeast cells/ml.

One ml of the fungal suspension was added to each of three test tubes. Then 0.25 ml of 1% methoxsalen in a solvent of ethanol, acetone, and propylene glycol was added to the first tube, making a concentration of 0.2% methoxsalen. Normal saline (0.25 ml) was added to the second tube and 0.25 ml of the vehicle to the third tube. Each of these mixtures contained 32 yeast cells/0.01 ml.

Light Source

A pulsed nitrogen laser operation at a wavelength of 337 nm was used for irradiating the fungal suspensions. The energy density was 2.9 mJ/cm² per pulse and the pulse rate varied from 2 to 3 pulses per second.

Experimental Procedures

Prior to irradiation, 0.01 ml of the fungal suspension containing methoxsalen was taken from the first tube and inoculated onto Sabouraud glucose agar in a small flask as a control. The remaining fungal suspension in the tube was then directly and evenly exposed to pulsed, near-uv laser light offered by the nitrogen laser at a distance of 10 cm. At the end of 2, 5, 10, and 20 min, 0.01 ml of the irradiated fungal suspension was transferred with a new sterile pipette to separate flasks of Sabouraud glucose agar.

For comparison, the fungal suspension containing the vehicle and the suspension in normal saline were similarly irradiated. Parallel cultures were made from each of them before and 2, 5, 10, and 20 min after exposure to laser irradiation. All the inoculated flasks of Sabouraud glucose agar were incubated at 25°C and observed daily for 1 week for fungal growth, which was quantitated on a − to +3 basis.
Results

The results of the treatment of different fungal suspensions with nitrogen laser irradiation are summarized in Table 1 and three photographs (Figures 1–3).

All the fungal suspensions showed excellent growth in the cultures made before exposure to laser irradiation even in the presence of methoxsalen. Following in vitro irradiation with the nitrogen laser for 2, 5, and 10 min, the fungal suspension in normal saline showed slight, moderate, and marked inhibition of fungal growth respectively as compared with the control. Similar effects were observed in the vehicle treated fungal suspension after exposure for the same periods. In both cases the number of colonies formed decreased with increasing exposure time. On the other hand, such irradiation in the presence of methoxsalen resulted in complete inhibition as evidenced by the total absence of colony formation. Exposure of the fungal suspensions for 20 min also resulted in complete inhibition of fungal growth even in the absence of methoxsalen.

Discussion

We have demonstrated that exposure of suspensions of C. albicans in vitro to near-uv (337 nm) laser irradiation results in a dose-dependent inhibition of fungal growth. There was a distinct correlation between the decrease in the colony-forming ability and the duration of the irradiation. The longer the exposure time, the more marked was the response. Fungal suspensions irradiated for 20 min all ended in complete inhibition. These data agree with those of Kagawa. However, the effects were not solely dependent upon the duration of irradiation. Previous studies in our laboratory indicated that in vitro irradiation at lower energy densities (less than 0.6 mJ/sq cm/pulse) were usually not effective in producing inhibition of fungal growth.2

Addition of methoxsalen to the fungal suspension followed by nitrogen laser irradiation for 2 to 10 min resulted in complete inhibition of fungal growth. The effect cannot be explained on the basis of laser irradiation alone, for such irradiation in the absence of methoxsalen only caused slight to marked inhibition. Treatment with methoxsalen alone did no harm to the fungus. Thus the effect on the fungal cells was a photosensitization and was similar to the fungicidal effect of PUVA on suspensions of T.

Table 1. Growth of C. albicans from different fungal suspensions before and after nitrogen laser irradiation (2.9 mJ/sq cm/pulse)

<table>
<thead>
<tr>
<th>Fungal suspension</th>
<th>Control</th>
<th>Exposure time (min)</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline treated</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle treated</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxsalen treated</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-: No growth; +: less than 10 colonies; ++: 11-25 colonies; +++: more than 25 colonies.

Figure 1. Saline treated exposure for 2 to 10 min: slight to marked inhibition; exposure for 20 min: complete inhibition.
mentagrophytes as demonstrated by Horio et al.\textsuperscript{2} We previously reported that fungal growth was not inhibited when the fungal suspensions were exposed to a nitrogen laser for up to 90 min at an energy density of 0.2 mJ/sq cm/pulse and pulse rate of 6 pulses/s. In contrast, such irradiation for 30 min or longer in the presence of methoxsalen resulted in complete inhibition.\textsuperscript{2} Near-uv (337 nm) irradiation offered by nitrogen lasers seems to be much more harmful to the fungus than ordinary UVA irradiation. Horio et al. demonstrated that UVA irradiation to suspensions...
of *T. mentagrophytes* caused no lethal or inhibitory effect on the fungus. The mechanism by which the near-uv laser light interacts with the fungus appears to be predominantly photochemical rather than photothermal. If severe, irreversible or lethal damage occurs, cell death follows. The above observation that methoxsalen plus the 337 nm beam was capable of producing psoralen phototoxicity suggests possible clinical application of nitrogen laser in PUVA therapy. Cripps demonstrated that the range of photosensitivity for methoxsalen was 310–380 nm with peak sensitivity at 330 nm. Nitrogen lasers at 337 nm could be an attractive alternative because it has its own unique properties of influencing a living cell. It might be possible to enhance the therapeutic effect and/or decrease the risk of adverse effects by using nitrogen lasers as the light source. Studies are now under way to try to explore the utility of nitrogen lasers for localized PUVA therapy.

**References**