Ex vivo laser confocal microscopy findings of cultured Acanthamoeba trophozoites

Natsuko Yamazaki¹
Akira Kobayashi¹
Hideaki Yokogawa¹
Yasuishi Ishibashi²
Yosaburo Oikawa³
Masaharu Tokoro⁴
Kazuhisa Sugiyama¹

¹Department of Ophthalmology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; ²Department of Ophthalmology, East Washinomiya Hospital, Kuki, Japan; ³Department of Medical Zoology, Kanazawa Medical University, Kahoku, Japan; ⁴Department of Parasitology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

Purpose: The purpose of the current study was to investigate ex vivo laser confocal microscopic findings of cultured Acanthamoeba trophozoites obtained from Acanthamoeba keratitis patients.

Methods: Eight cultured samples of Acanthamoeba trophozoites from eight eyes of seven patients (mean age, 26.9 years; age range, 18–52 years) were used. Seven samples were from corneal scrapings of Acanthamoeba keratitis patients and one sample was from the solution in a soft contact lens case. Ex vivo laser confocal microscopy was performed to qualitatively evaluate the shape and degree of light reflection of the living Acanthamoeba trophozoites.

Results: Ex vivo laser confocal microscopy demonstrated highly reflective, high-contrast Acanthamoeba trophozoites with no walls (mean size, 25.4 µm; range, 17.1–58.5 µm). The shapes of the trophozoites were highly pleomorphic, and some showed characteristic acanthopodia by laser confocal microscopy.

Conclusion: Ex vivo laser confocal microscopy was effective in demonstrating cultured Acanthamoeba trophozoites of various shapes and sizes. The observations of the current study may be helpful when similar structures are identified under in vivo conditions.

Keywords: Acanthamoeba, trophozoite, laser confocal microscopy

Introduction

Acanthamoeba is a ubiquitous, free-living amoeba found in water (eg, swimming pools, hot tubs, tap water, and contact lens solution), air, and soil, but Acanthamoeba keratitis is a relatively new entity.¹ The first case of Acanthamoeba keratitis was reported in 1974,¹ and the first case in Japan was reported in 1988.² As the use of soft contact lenses increased in the early 1980s, the incidence of reported Acanthamoeba keratitis increased dramatically. Acanthamoeba keratitis is relatively uncommon, but is a potentially blinding corneal infection. Clinical diagnosis is very difficult, especially in the early phase of the disease, and it is often misdiagnosed and treated as a herpes simplex infection.³ A definitive diagnosis is made by confirmation of Acanthamoeba in corneal lesions by direct examination, by corneal biopsy, or by culture. However, these methods are invasive and time-consuming, and are not always routinely available. The invasive methods are often postponed until there is a high index of suspicion for the disease and when there has been no response to treatments for bacterial, viral, and/or fungal keratitis.⁴ Unfortunately, delayed diagnosis of Acanthamoeba keratitis often results in poorer patient outcomes.

In vivo confocal microscopy has been used as a noninvasive technique for the observation of normal and pathological corneal microstructures. The usefulness of
this device in diagnosis and monitoring the improvement of Acanthamoeba keratitis has been reported.\textsuperscript{4,11} Previously, we\textsuperscript{12} and others\textsuperscript{13} reported ex vivo confocal microscopic images of cultured Acanthamoeba cysts. As a result, Acanthamoeba cysts were observed as highly reflective round- or stellate-shaped high-contrast particles (10–20 microns in diameter).\textsuperscript{12,13} In this study, we investigated ex vivo laser confocal microscopic findings of cultured Acanthamoeba trophozoites.

Patients and methods
The present study was approved by the Ethical Committee of the Kanazawa University Graduate School of Medical Science and followed the tenets of the Declaration of Helsinki.

Sample collection and culturing of Acanthamoeba trophozoites
Eight cultured samples of trophozoites from eight eyes of seven patients (mean age, 26.9 years; age range, 18–52 years) were used. Seven samples were from corneal scrapings of Acanthamoeba keratitis patients and one sample was from the solution from a soft contact lens case (Case 3). All patients were seen and treated at the Department of Ophthalmology, Kanazawa University Graduate School of Medical Science between August 2006 and November 2008. The demographic data and treatments are shown in Table 1. The culture medium was an amoeba saline containing 0.012% NaCl, 0.00035% KCl, 0.0003% CaCl\textsubscript{2}, and 0.0004% MgCl\textsubscript{2}·7 H\textsubscript{2}O in 0.05 mM Tris-HCl with a pH of 6.8, supplemented with Escherichia coli.\textsuperscript{14} The detailed culture method was described previously.\textsuperscript{14}

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Eye</th>
<th>Stage</th>
<th>CL</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>F</td>
<td>B</td>
<td>Early</td>
<td>2WSCL</td>
<td>Topical 0.1% micafungin, topical 0.02% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
<td>R</td>
<td>Early</td>
<td>2WSCL</td>
<td>Topical 0.1% micafungin, topical 0.1% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>M</td>
<td>L</td>
<td>Early</td>
<td>2WSCL</td>
<td>Topical 0.1% micafungin, topical 0.1% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>M</td>
<td>R</td>
<td>Advanced</td>
<td>HCL</td>
<td>Topical 0.05% micafungin, topical 0.05% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>M</td>
<td>R</td>
<td>Early</td>
<td>2WSCL</td>
<td>Topical 1% voriconazole, topical 0.02% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>F</td>
<td>L</td>
<td>Early</td>
<td>1MSCL</td>
<td>Topical 0.05% micafungin, topical 0.05% chlorhexidine, oral itraconazole</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>M</td>
<td>L</td>
<td>Early</td>
<td>2WSCL</td>
<td>Topical 0.05% micafungin, topical 0.05% chlorhexidine, oral itraconazole</td>
</tr>
</tbody>
</table>

Abbreviations: M, male; F, female; R, right eye; L, left eye; B, bilateral eyes; 2WSCL, two-week disposable soft contact lens; 1MSCL, one-month disposable soft contact lens; HCL, hard contact lens.

Ex vivo laser confocal microscopy
After applying a large drop of contact gel (Comfort Gel ophthalmic ointment\textsuperscript{15}; Bosch and Lomb, GmbH, Berlin, Germany) on the front surface of the microscope lens and ensuring no air bubbles had formed, a Tomo-Cap\textsuperscript{16} (Heidelberg Engineering GmbH, Dossenheim, Germany) was mounted on the holder to cover the microscope lens. A suspension of cultured Acanthamoeba trophozoites was then dropped into a small space created in front of the Tomo-Cap using Scotch\textsuperscript{17} tape. Ex vivo laser confocal microscopy (Heidelberg Retina Tomograph 2-Rostock Cornea Module, HRT 2-RCM; Heidelberg Engineering GmbH) was performed to qualitatively evaluate the shape and degree of light reflection of the living Acanthamoeba trophozoites. The HRT 2-RCM uses a 60x water-immersion objective lens (Olympus Europa GmbH, Hamburg, Germany) and utilizes a 670-nm diode laser as the light source, with a 400-μm\textsuperscript{2} area of observation.

Results
Light microscopic and ex vivo laser confocal microscopic observation
Light microscopic observation of cultured Acanthamoeba trophozoites showed amorphous Acanthamoeba trophozoites (Figure 1A). In all culture samples, ex vivo laser confocal microscopy demonstrated highly reflective, high-contrast Acanthamoeba trophozoites with no walls (mean size, 25.4 μm; range, 17.1–58.5 μm) (Figure 1B–D). The shapes of the trophozoites were highly pleomorphic and some showed characteristic acanthopodia by HRT2-RCM (Figure 1E). In some culture samples, clusters of Acanthamoeba cysts were sporadically observed as highly reflective round- or stellate-shaped high-contrast particles (10–20 microns in diameter).
trophozoites obtained herein may this allows a result, we consistently observed highly reflective, high-contrast amorphous structures. No walls were observed around the trophozoites (bar = 100 μm). (C) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 2 (bar = 100 μm). (D) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 6 (bar = 100 μm). (E) Acanthopodia (arrowhead), which are characteristic of Acanthamoeba trophozoites, were observed in some of the trophozoite images (Case 6) (bar = 100 μm). (F) Clusters of Acanthamoeba cysts were also observed as highly reflective, high-contrast stellate-shaped particles 10–20 μm in diameter (Case 2) (bar = 100 μm).

Figure 1 Light and confocal microscopic observation of cultured Acanthamoeba trophozoites. (A) Light microscopic observation of cultured Acanthamoeba trophozoites (arrows) (one scale = 10 μm). Note that the shape of the Acanthamoeba trophozoites was amorphous. (B) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 1. The Acanthamoeba trophozoites were observed as highly reflective, high-contrast amorphous structures. No walls were observed around the trophozoites (bar = 100 μm). (C) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 2 (bar = 100 μm). (D) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 6 (bar = 100 μm). (E) Acanthopodia (arrowhead), which are characteristic of Acanthamoeba trophozoites, were observed in some of the trophozoite images (Case 6) (bar = 100 μm). (F) Clusters of Acanthamoeba cysts were also observed as highly reflective, high-contrast stellate-shaped particles 10–20 μm in diameter (Case 2) (bar = 100 μm).

Figure 1 Light and confocal microscopic observation of cultured Acanthamoeba trophozoites. (A) Light microscopic observation of cultured Acanthamoeba trophozoites (arrows) (one scale = 10 μm). Note that the shape of the Acanthamoeba trophozoites was amorphous. (B) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 1. The Acanthamoeba trophozoites were observed as highly reflective, high-contrast amorphous structures. No walls were observed around the trophozoites (bar = 100 μm). (C) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 2 (bar = 100 μm). (D) Ex vivo laser confocal microscopic observation of cultured Acanthamoeba trophozoites from Case 6 (bar = 100 μm). (E) Acanthopodia (arrowhead), which are characteristic of Acanthamoeba trophozoites, were observed in some of the trophozoite images (Case 6) (bar = 100 μm). (F) Clusters of Acanthamoeba cysts were also observed as highly reflective, high-contrast stellate-shaped particles 10–20 μm in diameter (Case 2) (bar = 100 μm).

stellate-shaped particles that were 10–20 μm in diameter (Figure 1F).

Discussion

In this study, we have reported the ex vivo laser confocal microscopic findings of cultured Acanthamoeba trophozoites obtained from Acanthamoeba keratitis patients. As a result, we consistently observed highly reflective, high-contrast Acanthamoeba trophozoites with no walls (mean size, 25.4 μm; range, 17.1–58.5 μm). This is consistent with the previously published trophozoite sizes evaluated by in vivo confocal microscopy (25–50 μm in diameter). The shapes of the trophozoites were highly pleomorphic not only by light microscopy, but also by confocal microscopy. Acanthamoeba trophozoites were characterized by the presence of acanthopodia, which was also visible by ex vivo laser confocal microscopy.

In clinical settings, direct scraping is still the “gold standard” for definitive diagnosis of Acanthamoeba keratitis. Recently, HRT2-RCM was shown to provide high-resolution images of Acanthamoeba cysts with round or ovoid-shaped structures from 10–20 μm in diameter; this allows for the rapid and non-invasive diagnosis of early-stage Acanthamoeba keratitis. However, it was quite difficult to identify Acanthamoeba trophozoites in keratitis patients. Shiraishi et al reported putative Acanthamoeba trophozoites in stromal images obtained by HRT2-RCM in only one case out of nine keratitis patients. They found that the Acanthamoeba trophozoites showed acanthopodia, nucleus, and karyosomes. We could not detect definitive Acanthamoeba trophozoites in the corneal epithelium and/or stroma, possibly because they cannot be distinguished from other highly reflective pathological structures.

The limitation of this study is that the size and shape of the Acanthamoeba trophozoites obtained herein may not be readily applicable to images of in vivo conditions in the corneal tissue. However, considering the difficulty of visualizing Acanthamoeba trophozoites in corneal tissue by confocal microscopy, the ex vivo morphological data may be a first step to better recognizing Acanthamoeba trophozoite images in vivo by confocal microscopy.

In conclusion, ex vivo laser confocal microscopy was effective in demonstrating cultured Acanthamoeba trophozoites with various shapes and sizes. The observations of the current study may be helpful when similar structures are identified under in vivo conditions.

Disclosure

The authors report no conflicts of interest. They received no financial support for this study.

References


