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Original research

Application of combination bubble liposomal amphotericin B and sonication has the dramatic effect on oral candidiasis

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ABSTRACT

Objective: We previously reported that ultrasound-mediated destruction of microbubbles might be an innovative non-invasive drug delivery system. AmBisome is a single bilayer liposomal drug delivery system consisting of unilamellar bilayer liposomes with amphotericin B, an antifungal drug, intercalated within the membrane. In the present study, we developed a novel fungicidal drug delivery system using ultrasound and bubble liposomal amphotericin B prepared by enclosing perfluoropropane gas within AmBisome.
Methods: Candida albicans growth inhibition was determined by measuring optical density (OD), counting the number of colony forming units (CFU), and measuring the colony diameters.
Result: Treatment with bubble liposomal amphotericin B and ultrasound significantly reduced the OD, CFU and diameter of colonies of *C. albicans* compared with ultrasound alone, liposomal amphotericin B alone, bubble liposomal amphotericin B alone, liposomal amphotericin B with ultrasound.
Conclusion: These results indicate that treatment with bubble liposomal amphotericin B with ultrasound efficiently inhibited the growth of *C. albicans*, suggesting this novel approach may have a beneficial effect on the treatment of oral candidal disease.

1. Introduction

With the rapid aging of our society, the incidence of fungal oral infections has increased recently because of the growing number of high-risk patients [1]. Most oral, oropharynx, and esophagus candidal diseases are caused by a single infectious agent, *Candida albicans*, or by mixed infection with other bacteria [2]. *C. albicans* is present on the skin and mucosal surfaces and is therefore a human microbial flora. *C. albicans* causes opportunistic infections such as oral candidiasis, oesophageal candidiasis, and vaginal candidiasis. It is known that oral and oesophageal candidiasis cause oral cavity pain, tongue pain, taste disturbance, odynophagia or dysphagia.

Oral candidiasis often develops as a severe oral fungal infection. Often acute oral candidiasis of patients without systemic disease, awareness or objective symptoms disappear following treatment with an antifungal drug administered short term. Holbrook et al. reported that ninety-five patients received anticandidal therapy, and 56% showed clinical improvement within 1 month; however, 13% of the treated patients with chronic hypertrophic candidiasis or endocrine disease required treatment for more than 1 year [3]. Therefore, novel and fast-acting antifungal treatments against these unmanageable infections are required.

Intravenous administration of Amphotericin B (AMB) is the main therapy for severe, fatal fungal infections because of its high efficiency and potency against many fungi. AMB acts by binding to the sterol component, ergosterol, of the cell membrane of susceptible fungi. It forms transmembrane channels leading to alterations in cell permeability through which monovalent ions leak out of the cell, resulting in cell death. While AMB has a higher affinity for the ergosterol component of the fungal cell membrane, it can also bind to cholesterol membrane components of mammalian cells, leading to nephrotoxicity and hypokalemia [4].

To reduce these side effects while maintaining the effectiveness of AMB, Liposomal AMB (AmBisome) was developed [5].

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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AmBisome is a unilamellar bilayer liposomal drug delivery system (<100 nm in diameter) with AMB intercalated within the membrane. The biochemical properties of the liposomal preparation allow it to access infected tissues when blood vessel permeability is enhanced [6]. After the attachment of AmBisome to the cell wall, it becomes disrupted and the enclosed AMB penetrates through the cell wall and damages the fungal cell membrane. However, a detailed mechanism of this system has not been reported to date. Because its structure does not become disrupted until it adheres to the cell wall of the fungus, its potential cytotoxity to animal cells is minimized. Therefore the toxicity of AmBisome to human cells is lower compared with AMB. However, other side effects such as vasodilatation, dorsal pain, chest pain have been reported [7] and careful administration is still necessary.

A previous report has shown that antibiotic treatment of *Pseudomonas aeruginosa* or *Escherichia coli* coupled with ultrasound irradiation enhanced the bactericidal activity [8]. In addition, Ikeda-Dantsuji et al. reported that combined ultrasound with bubble liposomes and antibiotic treatment was effective against Chlamydia-infected cells [9]. However, it is not clear whether the combined ultrasound and antibiotic treatment are effective against fungi, e.g. *C. albicans*. If *C. albicans* infection could be efficiently eradicated from an infected person, chronic antibiotic treatment and potential side effects might be avoided.

Here, we investigated the synergistic use of ultrasound and antifungals to kill *C. albicans*. This report presents the results of the first step to examine the *in vitro* response of *C. albicans* to a combination of ultrasound and bubble liposomal AMB.

2. Materials and methods

2.1. Fungus strain and mediums

C. albicans ATCC (American Type Culture Collection) 18804 was used in this study and cultured in YEAST MOLD (YM) broth (Becton, Dickinson, Franklin Lakes, NJ, USA) and YM agar (Becton, Dickinson, Franklin Lakes, NJ, USA) at 30 °C.

2.2. Bubble liposomal amphotericin B

Bubble liposomal AMB was prepared from liposomal AMB and perfluoropropane gas (Takachiho Chemical Inc., Co., Ltd., Tokyo, Japan). First, 5 mL sterilized vials containing 2 mL of liposomal AMB suspension (lipid concentration: 0.4 mg/mL) were filled with perfluoropropane gas, capped, and then pressurized with 7.5 mL of perfluoropropane gas. The vials were placed in a bath-type sonicator (42 kHz, 100 W, Bransonic 2510J-DTH, Branson Ultrasonics Co., Danbury, CT, USA) for 30 s to form bubble liposomal AMB.

2.3. Growth inhibition in planktonic C. albicans

After the solution of AmBisome (0.5 μg/mL) was added to the wells, the fungus liquid was exposed to ultrasound for 60 s at room temperature using a ultrasonication transducer (Sonitron 2000 V, Rich Mar Inc., Inola, OK, USA) at 1 MHz, with an output intensity of 2.0 W/cm^2 and a 10% duty cycle for the delivery of AMB. For the latter, the head of the transducer was directly immersed into the fungus liquid. When possible, the ultrasonication probe and well plate were fixed to a stand to avoid dislocation during ultrasound exposure (Fig. 1A). The growth inhibition of *C. albicans* was

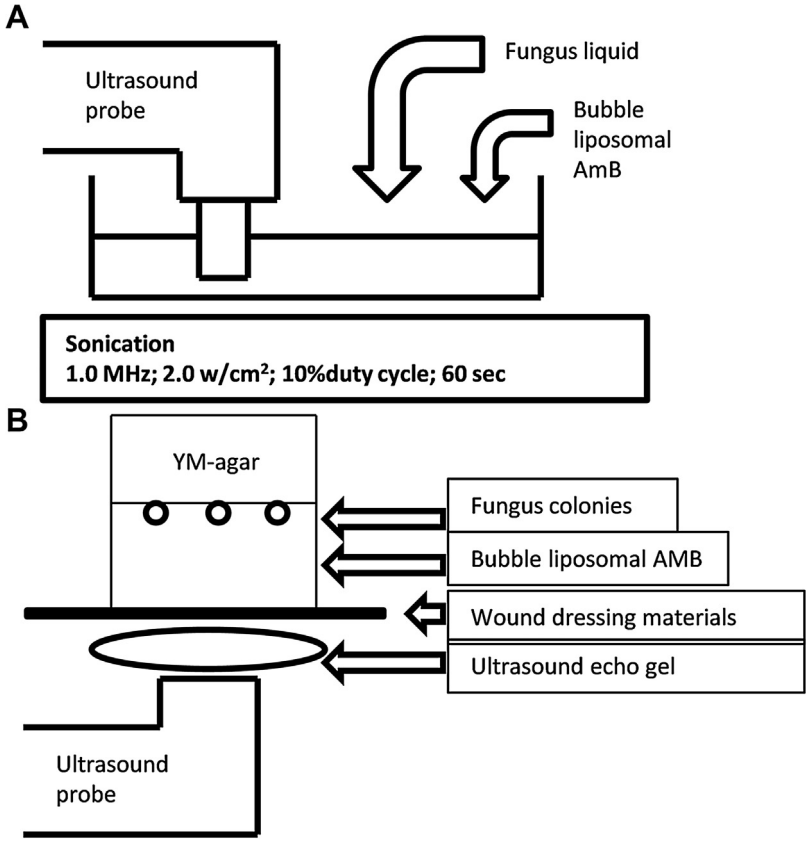

Fig. 1. Schematics of the study in planktonic fungus (A) and fungus colonies (B).

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