Med. Mycol. J.
Vol. 52, 145－152, 2011
ISSN 2185－6486

Original Articles

Therapeutic Effects of Cinnamaldehyde and Potentiation of its Efficacy in Combination with Methylcellulose on Murine Oral Candidiasis

Yuuki Taguchi[1], Kazumi Hayama[2], Masashi Okada[2], Takehito Sagawa[1], Ryo Arai[1] and Shigeru Abe[2]

[1]*Research and Product Development Division, S&B Foods Inc.*

[2]*Teikyo University Institute of Medical Mycology*

ABSTRACT

We examined the therapeutic effects of cinnamaldehyde and the potentiation of those effects with cassia and cinnamaldehyde when combined with the food additive methylcellulose against murine oral candidiasis. When 19.5 mg/ml of cinnamaldehyde was administered in the oral cavity of *Candida* infected mice, the oral symptoms were improved. Furthermore, when either a cassia or a cinnamaldehyde preparation in combination with methylcellulose was administered to oral candidiasis-inflicted mice, the therapeutic effects of cassia or cinnamaldehyde potentiated. Methylcellulose itself did not affect the oral symptoms or the viable number of *C. albicans* cells. GC/MS analysis showed that the dose of cinnamaldehyde remaining in the tongue tissue of mice treated with the cinnamaldehyde-methylcellulose mixture was higher than that in mice administered cinnamaldehyde alone, and also showed that cinnamaldehyde was not detected in the blood of any of the tested mice. These findings suggested that the combination of cassia or cinnamaldehyde and methylcellulose may be a useful prophylactic or therapeutic tool against oral candidiasis.

Key words : *Candida albicans*, murine oral candidiasis, cassia, cinnamaldehyde, methylcellulose

Introduction

The overgrowth of *Candida albicans*, which is one of the members of the oral microbial flora in a healthy human, causes pathogenic symptoms such as oral candidiasis[1,2]. Oral candidiasis accompanied with severe inflammation aggravates the quality of life of immunosuppressed individuals and elderly people[3,4]. As spices and herbs have been widely used as anti-microbiological and anti-infectious tools in daily life, we and our colleagues investigated their anti-*Candida* activity to develop new therapies for oral candidiasis[5－7]. In the preceding paper, we reported that a cassia (*Cinnamomum cassia*, synonym *C. aromaticum*) preparation exerted a therapeutic effect on a murine oral candidiasis model and that *in vitro* experiments and GC/MS analysis suggested that cinnamaldehyde, which is the major component of the essential oil of cassia, was responsible for the inhibitory activity in the cassia preparation[7]. In this study, we have checked the therapeutic efficacy of cinnamaldehyde on murine oral candidiasis. Furthermore, we attempted to increase the efficacy by its combined use with a food additive[8]. Here we report the therapeutic effects of cinnamaldehyde and the potentiation of therapeutic effects of cassia and cinnamaldehyde by the food additive methylcellulose against this condition[7,9].

Materials and Methods

C. albicans strain

The *C. albicans* strain of TIMM 1768 was isolated clinically and maintained at Teikyo University

Address for correspondence : Yuuki Taguchi
Research and Product Development Division, S&B Foods Inc., 38-8 Miyamoto-cho, Itabashiku, Tokyo 174-8651, Japan
Received : 14, October 2010. Accepted : 3, February 2011

Institute of Medical Mycology; this strain, which was shown to induce oral candidiasis in a murine model[10−12], has been used for animal experiments. Cultures were stored in our laboratory at −80℃ in Sabouraud dextrose broth (Becton Dickinson, MD, USA) containing 0.5% yeast extract (Becton Dickinson), and 10% glycerol (v/v, final concentration) until use. Strain TIMM 1768 was cultured on a Sabouraud dextrose agar plate for 18 h at 37℃. The cells were harvested with a microspatula and suspended in RPMI 1640 medium containing 2.5% fetal calf serum (RP medium). The culture was used for *in vivo Candida* oral inoculation.

Preparation of cassia, cinnamaldehyde, and a cassia−, cinnamaldehyde−methycellulose mixture

Cassia was collected and imported by S&B Foods Inc. (Tokyo, Japan)[7]. Food additives such as guar gum, xanthan gum, carboxymethylcellulose, and gelatin were also provided by S&B Food Inc. Cinnamaldehyde and methylcellulose 25 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Cassia or the cassia−methylcellulose mixture was suspended in hot water at 90℃ and bathed in a water bath of the same temperature for 5 min. The suspensions were collected and designated as 100% cassia or 100% cassia + methylcellulose preparation, respectively[7]. Cinnamaldehyde and the cinnamaldehyde−methylcellulose mixture were suspended with water which included a 1.0% Tween 80 solution[13].

Animals for oral candidiasis model

All animal experiments were performed in accordance with the guidelines for the care and use of animals approved by Teikyo University. The experimental procedure of the murine oral candidiasis model was described previously[6,7]. Six−week−old female ICR mice (Charles River Japan, Inc., Yokohama, Japan) were used for all animal experiments. The mice were randomized, kept in cages housing 3 to 4 individuals, and were given food and water ad libitum. During the experiments, the photoperiods were adjusted to 12 h of light and 12 h of darkness daily, and the environmental temperature was maintained at 21℃ all day. Animals were separated into groups for the oral candidasis model and for the measurement of cinnamaldehyde in the tongue and blood. To induce an orally immunosuppressed condition, 100 mg/kg of prednisolone (Mitaka Pharmaceutical Co., Tokyo, Japan) was injected subcutaneously to mice 20 to 24 hours before oral infection. Following this injection, 0.08% of tetracycline hydrochloride (Takeda Shering Purau Animal Health Co., Tokyo, Japan) was administered in drinking water. On the day of infection, animals were anesthetized by intramuscular injection with 14.4 mg/kg of chlorpromazine chloride in the femur, and were orally infected with about 2.0×10^8cells/ml of viable cells of *C. albicans* TIMM 2640 in RP medium. Oral infection was performed by means of rubbing and rolling a cotton swab (baby cotton buds; Johnson & Johnson Co., Tokyo, Japan) inside all parts of the mouth. The number of *Candida* cells inoculated in the oral cavity was calculated to be about 1×10^6 cells/mouse based on the difference in viable cell number adhering to the cotton swabs before and just after oral inoculation.

Oral administration of cassia, cinnamaldehyde and cassia−, cinnamaldehyde−methylcellulose mixture

Since the animals did not take the cassia preparation, cinnamaldehyde preparation, cassia−methylcellulose mixture, or cinnamaldehyde−methylcellulose mixture ad libitum via drinking water, 50 μl of the samples was administered in the oral cavity of the *Candida* infected mice three times: at 3 hour, 21 hour, and 27 hour after *C. albicans* inoculation, using a top−rounded needle, and was spread over all parts of the mouth.

Scoring the severity of oral infection

The procedure of scoring the severity of oral infection was performed as described previously[6,7]. On the third day after inoculation mice were sacrificed and the severity of the lesion of the tongue was evaluated by scoring the fur on each tongue and the squamous disorder as follows: 0, normal; 1, fur on less than 20%; 2, fur on more than 21% but less than 90%; 3, fur on more than 91% and on the squamous layer; 4, thick fur on more than 91% and on the squamous layer.

Evaluation of the number of viable *Candida* cells on tongue of a mouse

Cheek, tongue, and soft palate of the mice were swabbed using a cotton swab on the third day after inoculation for microbiological evaluation. After swabbing, the cotton end was cut off and placed in 3 ml of sterile saline. *Candida* cells were resuspended by mixing on a vortex mixer and diluted by a series of 20−fold and 100−fold dilutions

of sterile saline. Fifty μl of each dilution was incubated on a *Candida* GS plate for 20 h at 37℃. The CFU of *Candida* cells were counted and then the total numbers per swab were calculated.

Statistical analysis

The data of scores were compared by the non-parametric Mann-Whitney U test. Statistical analysis of the $\log_{10}$ CFU of *C. albicans* isolated from each mouse part was compared using a Student's t test. P values of < 0.05 were considered statistically significant. All mean values given in the text include the standard deviation of the mean.

Histological analysis

The histological analysis was performed as described previously[7,11]. Specimens of tongue were taken from sacrificed animals, fixed in 10% formalin solution and embedded in paraffin. Specimens 4-μm thick were sectioned and stained with periodic acid-Schiff (PAS) stain for histological observation.

Measurement of cinnamaldehyde in the tongue and blood of mice

One hour before oral administration of the samples, the animals were injected with 14.4 mg/kg of chlorpromazine chloride in the femur. Fifty μl of 9.75 mg/ml of cinnamaldehyde or 9.75 mg/ml cinnamaldehyde + 1.0% methylcellulose mixture was administered in the oral cavity of the mice. After 30 and 120 minutes of oral administration, the mice were anesthetized with diethyl ether (Wako Pure Chemical Industries, Ltd.) and their tail cut off. The blood from the tail was placed in a plastic tube with saline including 1,000 units of heparin sodium injection (Mitsubishi Tanabe Pharma Co., Osaka, Japan). The mice were sacrificed and each tongue kept in a plastic tube with 1.5 ml of saline. Cinnamaldehyde was extracted with dichloromethane (Kanto Chemical Co., Inc, Tokyo, Japan) and subjected to gas chromatography/mass spectrometry (GC/MS) analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis

The contents of cinnamaldehyde in the tongues and blood of the mice were analyzed by GC/MS using a Shimadzu model GCMS-QP2010 Plus equipped with an Rtx-5MS column (RESTEK, 30 m × 0.25 mm, 0.25 μm film thickness)[7,14,15].

Results

The effects of oral administration of cassia and methylcellulose mixture on candidiasis

To potentiate the therapeutic effects of the cassia preparation against oral candidiasis following its prolonged retention in the oral cavity, we examined the effects of modified oral application methods using food thickeners. In preliminary *in vivo* experiments, we examined several food thickeners such as methylcellulose, guar gum, xanthan gum, carboxymethylcellulose (polysaccharides) and gelatin (a mixture of proteins), which were chosen from among the materials legally authorized as food or pharmaceutical additives, and found that methylcellulose might be a good candidate[16].

The efficacy of administering the cassia and methylcellulose mixture was evaluated using the murine oral candidiasis model with TIMM 1768. In this model the pathological symptoms of oral candidiasis on the lingual mucosa of the animals can be displayed as a symptom score. In this experiment, considering the physical properties of methylcellulose and the laws concerning food additives as they pertain to the concentration of methylcellulose, 1.0% or 2.0% was used. As shown in Fig. 1, in the group of mice administered 50% of cassia preparation alone, symptom scores of infected tongues tended to be lower than those of the control group, but were not statistically different. When the cassia preparation at a dose of 50% with 1.0% and 2.0% methylcellulose preparation was administered, the symptom scores were lower than those of the control group. In the group of mice administered methylcellulose alone, there was no significant difference in either scores or viable cell number of *Candida* cells from the mice in the control group. These results suggest that application of the cassia preparation with methylcellulose improved the effectiveness of cassia, however, viable cell numbers of *Candida albicans* did not decrease in any of the tested groups.

Therapeutic effects of mixture of cinnamaldehyde and cinnamaldehyde-methylcellulose on murine oral candidiasis

We have already reported that the active

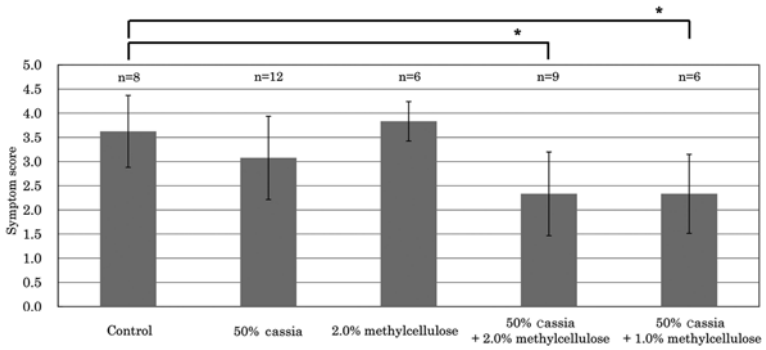

Fig. 1. The effects of cassia and cassia-methylcellulose mixture preparation by oral administration against oral candidiasis mice.
Cassia (4 g) was extracted by hot water (20 ml), and (designated to 100%) the original preparation. The preparations of 50% cassia, 2.0% methylcellulose, 50% cassia + 2.0% methylcellulose, and 50% cassia + 1.0% methylcellulose were administered into the oral cavity of oral candidiasis mice. Scores of the tongue disorder were determined as described in Materials and Methods. Viable *Candida* cell number (log_{10}CFU) in each group of mice was 5.44 ± 0.44 (Control), 5.36 ± 0.35 (50% cassia), 5.23 ± 0.25 (2.0% methylcellulose), 5.43 ± 0.25 (50% cassia + 2.0% methylcellulose), 5.41 ± 0.42 (50% cassia + 1.0% methylcellulose), respectively. P values of < 0.05 were considered as significant. (* : p values of < 0.05)

Table 1. The effects of cinnamaldehyde by oral administration against oral candidiasis mice

Mice orally treated with sample	dose (%)	Number of mice	Symptom Score	Number of mice	Viable *Candida* cells (log_{10}CFU)
Control		6	4.00 ± 0.00	6	5.42 ± 0.16
Cinnamaldehyde	19.5 mg/ml	6	2.83 ± 0.75*	6	5.53 ± 0.09

The preparation of cinnamaldehyde, prepared in water with 1.0% Tween 80, was administered into oral cavity of oral candidiasis mice. Scores of the tongue disorder were determined as described in Materials and Methods.
Viable *Candida* cells in each mouse were evaluated by CFU counting on *Candida* GS agar plate. P values of < 0.05 were considered as significant. (* : *p* values of < 0.05)

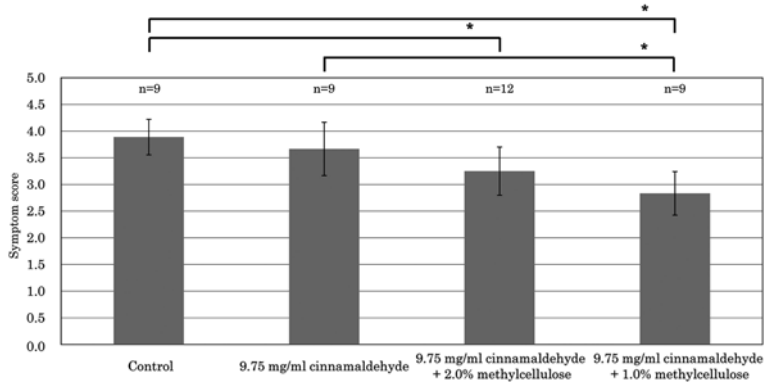

Fig. 2. The effects of cinnamaldehyde and cinnamaldehyde-methylcellulose preparation by oral administration against oral candidiasis mice. The preparations of cinnamaldehyde and cinnamaldehyde-methylcellulose were administered in the oral cavity of oral candidiasis mice, respectively. Scores of the tongue disorder were determined as described in Materials and Methods. Viable *Candida* cell number (log_{10} CFU) in each group of mice was 5.52 ± 0.26 (Control), 5.55 ± 0.23 (9.75 mg/ml cinnamaldehyde), 5.43 ± 0.20 (9.75 mg/ml cinnamaldehyde + 2.0% methylcellulose), 5.41 ± 0.15 (9.75 mg/ml cinnamaldehyde + 1.0% methylcellulose), respectively. P values of < 0.05 were considered as significant. (* : p values of < 0.05)

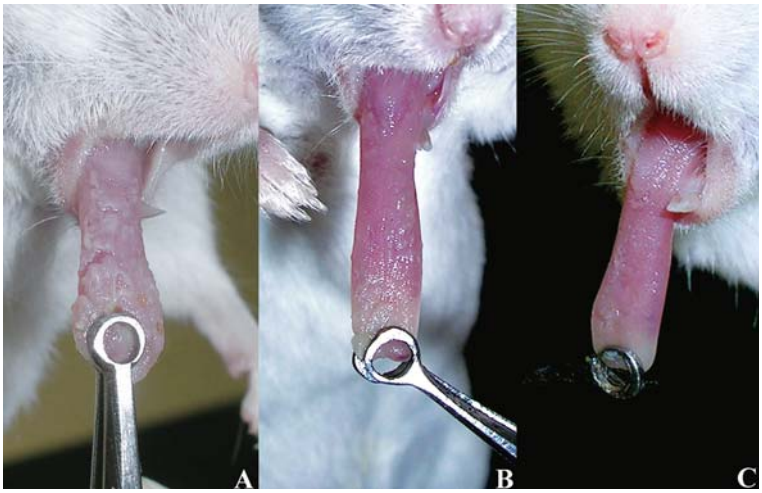

Fig. 3. Macroscopic observation of improvement on tongue of oral candidiasis mice by administration of the 9.75 mg/ml cinnamaldehyde + 2.0% methylcellulose mixture. A: oral candidiasis (control) (white patches spread out on the tongue.), B: treated with cassia preparation, C: healthy

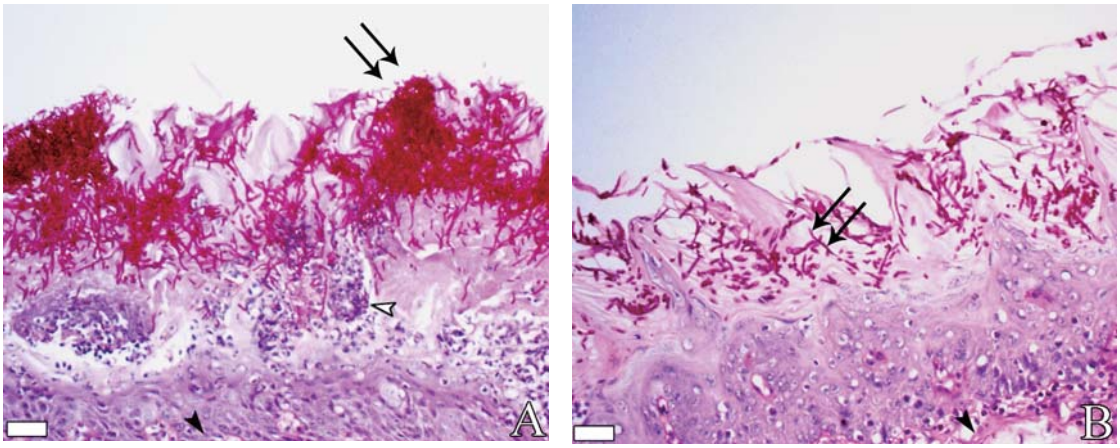

Fig. 4. The effect on lingual mucosa of a *Candida*-infected mouse administered 9.75 mg/ml cinnamaldehyde + 2.0% methylcellulose preparation. Samples were stained with PAS stain. White bars indicate 20 μm. Black arrows indicate *Candida* cells. White and black arrow-heads indicate inflammatory cells and lamina propria, respectively. A: oral candidiasis without any treatment (control), B: oral candidiasis treated with 9.75 mg/ml cinnamaldehyde + 2.0% methylcellulose mixture.

principle of cassia preparation was shown to be cinnamaldehyde by *in vitro* experiments and GC/MS analysis. Results of the analysis appeared to show that the cinnamaldehyde content in 100% cassia preparation was 19.5 mg/ml[7]. Table 1 shows that oral treatment with 19.5 mg/ml of cinnamaldehyde improved the oral symptoms of the *Candida* infected mice, but did not reduce the viable cell number of *Candida albicans*.

The additive effects of methylcellulose on therapeutic efficacy were examined using a suboptimal dose of cinnamaldehyde of 9.75 mg/ml, as shown in Fig. 2. In the group of animals administered this dose, scores tended to be lower than those of the control group, but the difference was not statistically significant. When the cinnamaldehyde preparation was administered with 1.0% and 2.0% methylcellulose, the symptom scores were significantly improved. Especially when 1% of methylcellulose was added, the scores of the animals administered 9.75 mg/ml of cinnamaldehyde were significantly lower than

Table 2. Contents of cinnamaldehyde in the tongue and blood obtained from the animals after sample administration

Dose of cinnamaldehyde (mg/ml)	Dose of methylcellulose (%)	[a] Contents of cinnamaldehyde (μg) per 1 g of tongue tissue		[b] Contents of cinnamaldehyde (μg) per 1 g of the blood	
		30 minutes after administration	120 minutes after administration	30 minutes after administration	60 mimutes after administration
9.75	1.0	76.5 ± 25.1** (n=6)	55.6 ± 30.3* (n=6)	N.D.	N.D.
9.75	0	18.3 ± 11.7 (n=6)	26.8 ± 8.0 (n=6)	N.D.	N.D.

9.75 mg/ml of cinnamaldehyde and 9.75 mg/ml cinnamaldehyde + 1.0% methylcelullose mixture were prepared for oral inoculation.
Tongue and blood samples were collected from the animals after 30 minutes and 120 minutes of oral administration.
[a), b)] Each content was determined by GC/MS analysis.
P values of < 0.05 were considered as significant. (** : *p* values of < 0.01, * : *p* values of < 0.05)
Less than 0.2 μg of cinnamaldehyde in 1 g of tissue or blood sample was regarded as not detected (N.D.).

when 9.75 mg/ml of cinnamaldehyde alone was used. Viable *Candida* cell numbers of all groups of mice did not statistically differ.

A typical tongue of the control group, the group administered 9.75 mg/ml of cinnamaldehyde and 2.0% of methylcellulose mixture, and the group uninfected with *C. albicans* is shown in Fig. 3A, 3B, and 3C, respectively; the tongue treated with the combination appeared normal and partially healthy.

In histological studies, PAS-positive fungi were observed on severe lesions near the oral epithelium of tongues of the control mice as shown in Fig. 4A. Figure 4B shows that there were fewer PAS-positive hyphae and the inflammatory cell infiltration was relatively mild on the tongues treated with 9.75 mg/ml of cinnamaldehyde and 2.0% of the methylcellulose mixture.

Quantification of the amount of cinnamaldehyde in the oral cavity and blood in the mice

We measured the content of cinnamaldehyde in the murine tongue tissue and blood after oral application of cinnamaldehyde alone or in combination with methylcellulose. As shown in Table 2, a significant amount of cinnamaldehyde was detected from tongue tissues, but not from blood. Table 2 also shows that at both 30 minutes and 120 minutes after administration, tongues obtained from the animals treated with the cinnamaldehyde-methylcellulose mixture contained 2-4 times more of the cinnamaldehyde than those of mice treated with the cinnamaldehyde alone. This suggests that methylcellulose supports the retention of cinnamaldehyde in the tongue.

Discussion

In this study we have obtained two major findings: 1) the therapeutic efficacy of cinnamaldehyde on murine oral candidiasis and 2) potentiation of the therapeutic effects of cinnamaldehyde by methylcellulose. The oral symptoms of *Candida* infected mice administered 19.5 mg/ml of cinnamaldehyde improved significantly. Previously we demonstrated that 100% of cassia preparation, which contained 19.5 mg/ml of cinnamaldehyde, improved lesions on the lingual surface of the mouse tongue in a murine oral candidiasis model, and that it also inhibited *Candida* mycelia growth in *in vitro* assay and GC/MS analysis[7]. From these results, we clearly demonstrated that cinnamaldehyde is responsible for the therapeutic effect of cassia. This is the first report, as far as we know, to show that aldehyde obtained from food exerts a therapeutic effect against oral candidiasis[7,17].

As an even more important finding, we have shown that we obtained the protective action of cassia or cinnamaldehyde against oral candidiasis by combined treatment with a food additive such as methylcellulose[8]. Cassia and cinnamaldehyde have significant therapeutic effects against oral candidiasis, but they also have a pungent taste[18]. We therefore attempted to decrease the oral doses of cassia and cinnamaldehyde needed to exert a therapeutic effect so that they can be combined with other food agents and used safely[16,19]. Methylcellulose is one of the food agents providing stability, thickness, and viscosity and is authorized as a safe additive in laws concerning foods[16]. In this study, we demon-

strated that methylcellulose potentiated the therapeutic effect of cinnamaldehyde when given at a legal dose. (Methylcellulose and other food thickeners are permitted by law to be used in an amount less than 2.0% of the total food weight[16].)

To examine how methylcellulose potentiated the therapeutic effects of cassia and cinnamaldehyde, we also measured the content of the latter in the tongue tissue and blood treated with cinnamaldehyde or cinnamaldehyde-methylcellulose mixture. GC/MS analysis showed that methylcellulose assisted in the retention of cinnamaldehyde in tongue tissue and that it was not detected in the blood. We therefore concluded that the potentiating effect of methylcellulose was not systemic but local[8].

This is also the first report, as far as we know, to show the potentiation of the therapeutic effects of cassia and cinnamaldehyde by addition of a food thickener, methylcellulose. These results suggested that cassia and cinnamaldehyde in combination with methylcellulose is a useful therapeutics tool against oral candidiasis. Further work will be needed to establish details of their therapeutic usage with methylcellulose for oral candidiasis: the conditions of preparation, optimal dosage, period of administration, other food additive candidates, and the influence of such additives on the intestinal microbiota[15,20,21,22]. It is our wish that our studies will facilitate development of the application of spices and herbal foods in conventional use to prevent and improve the instances of oral candidiasis in elderly people and immunocompromised patients.

References

1) Odds FC: A Review and Bibliography. *Candida* and candidosis: 4-129, Bailliere Tindale, London, 1988.
2) Dongari-Bagtzoglou A, Dwivedi P, Ioannidou E, Shaqman M, Hull D, Burleson J: Oral *Candida* infection and colonization in solid organ transplant recipients. Oral Microbiol Immunol **24**: 249-254, 2009.
3) Bodey GP: Pathogenesis, Diagnosis and Treatment. Candidiasis 2nd edition: Raven Press, New York, 1993.
4) Sullivan D, Coleman D: *Candida dubliniensis*: characteristics and identification. J Clin Microbiol **36**: 329-334, 1998.
5) Taguchi Y, Komiya M, Ishibashi H, Inoue S, Yamaguchi H, Arai R, Abe S: Inhibition of mycelial growth of *Candida albicans* by spices and herbs *in vitro* and effects of their oral administration on intestinal colonization of *Candida albicans* in mice. Pharmacometrics **64**: 53-57, 2003.
6) Taguchi Y, Ishibashi H, Takizawa T, Inoue S, Yamaguchi H, Abe S: Protection of oral candidiasis in mice by oral or intragastric administration of herbal food, clove (*Syzygium aromaticum*). Jpn J Med Mycol **46**: 27-33, 2005.
7) Taguchi Y, Takizawa T, Ishibashi H, Sagawa T, Arai R, Inoue S, Yamaguchi H, Abe S: Therapeutic effects on murine oral candidiasis by oral administration of Cassia (*Cinnamomum cassia*) preparation. Jpn J Med Mycol **51**: 13-21, 2010.
8) Dobaria NB, Mashru RC, Badhan AC, Thakkar AR: A novel intravaginal delivery system for itraconazole: *in vitro* and *in vivo* evaluation. Curr Drug Deliv **6**: 151-8, 2009.
9) Abe S: Development of murine experimental model for candidiasis and its application. Jpn J Med Mycol **45**: 227-231, 2004.
10) Takakura N, Wakabayashi H, Ishibashi H, Teraguchi S, Tamura Y, Yamaguchi H, Abe S: Oral lactoferrin treatment of experimental oral candidiasis in mice. Antimicrob Agents Chemother **47**: 2619-2623, 2003.
11) Takakura N, Sato Y, Ishibashi H, Oshima H, Uchida K, Yamaguchi H, Abe S: A novel murine model of oral candidiasis with local symptoms characteristic of oral thrush. Microbiol Immunol **47**: 321-326, 2003.
12) Hayama K, Ishibashi H, Kitadate K, Yamazaki M, Abe S: Therapeutic effect of oligonol, a low-molecular polyphenol formulation derived from lychee fruits on murine oral candidiasis. Jpn J Med Mycol **51**: 137-142, 2010.
13) Nozaki A, Takahashi E, Okamoto K, Ito H, Hatano T: Antifungal activity of essential oil and their constituents against *Candida* spp. and their effects on activity of Amphotericin B. Yakugaku Zasshi **130**: 895-902, 2010.
14) Abe S, Sato Y, Inoue S, Ishibashi H: Anti-*Candida albicans* activity of essential oil including lemongrass (*Cymbopogon citratus*) oil and its component, citral. Jpn J Med Mycol **44**: 285-291, 2003.
15) Giordani R, Regli P, Kaloustian J, Portugal H: Potentiation of antifungal activity of amphotericin B by essential oil from *Cinnamomum cassia*. Phytother Res **20**: 58-61, 2006.
16) Ministry of Health Labour and Welfare: Japan's specifications and standards for food additives, 7th edition: 2000.
17) Ooi LS, Li Y, Kam SL, Wang H, Wong EY, Ooi VE: Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am J Chin Med **34**: 511-522, 2006.
18) Ministry of Health Labour and Welfare: The Japanese Pharmacopeia, 15th edition: 1272-1273, 2006.
19) Valencia-Chamorro SA, Perez-Gago MB, Del Río MA, Palou L: Curative and preventive activity of hydroxypropyl methylcellulose-lipid edible com-

posite coatings containing antifungal food additives to control citrus postharvest green and blue molds. J Agric Food Chem **57**: 2770–2777, 2009.
20) Swidsinski A, Ung V, Sydora BC, Loening-Baucke V, Doerffel Y, Verstraelen H, Fedorak RN: Bacterial overgrowth and inflammation of small intestine after carboxymethylcellulose ingestion in genetically susceptible mice. Inflamm Bowel Dis **15**: 359–364, 2009.
21) Hammer KA, Carson CF, Riley TV: Antimicrobial activity of essential oils and other plant extracts. J App Microbiol **86**: 985–990, 1999.
22) Rauha JP, Remes S, Heinonen M, Hopia A, Kahkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P: Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol **56**: 3–12, 2000.