REVIEW

Recent Studies on Oral Candidiasis using a Murine Model

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Abstract: In order to develop new treatments for oral candidiasis, Takakura et al. recently established a new murine model of this condition showing local symptoms. The present article reviews studies using this murine model to ascertain the clinical application of essential oils, and antimicrobial agents found in saliva. Applying tea tree oil to the mouth of mice with C. albicans reduced the viable cell count and improved inflammatory symptoms. When mice were given tap water containing bovine lactoferrin, they were similarly also protected from oral candidiasis, but protection was clearly observed only from 6 days after infection. Furthermore, we were able to identify the existence of a factor which detached C. albicans that had attached to oral tissue by forming biofilms. When human saliva was administered to the mice orally inoculated with C. albicans, viable cell counts and inflammatory symptoms declined significantly. We expect this murine model of oral candidiasis to contribute to clarification of the host defense mechanisms against the disease, and to aid in the development of new therapeutic agents.

Introduction

Oral candidiasis is an infectious disease of the oral cavity caused by Candida spp., in particular C. albicans, which is a member of the normal microbial flora of the mouth. While Candida spp. rarely cause candidiasis in healthy adults, it can cause opportunistic infections in vulnerable hosts, such as the elderly, immunocompromised patients, or patients with malignant tumors. Oral candidiasis may also result from decreased salivary secretion or long-term use of an antimicrobial agent or immunosuppressant.

New research on treatment for oral candidiasis requires an appropriate animal model. Although numerous animal models for this condition have been developed, researchers have lacked a convenient and reproducible technique for preparing adequate numbers of animals for statistical analysis. In recent years, Takakura et al. developed a murine model of oral candidiasis showing local symptoms, which makes it possible to clarify the effects of various antimicrobial agents in vivo, not just in vitro. While azole antifungal agents are commonly used to treat oral candidiasis, intractable cases of candidiasis and recurrent candidiasis remain problematic in clinical settings. Satisfactory treatment of candidiasis will require the development of a new treatment. The present article summarizes studies using the murine model of oral candidiasis by Takakura et al. to ascertain the effects of essential oils and salivary antimicrobial agents.

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Preparing the Murine Model of Oral Candidiasis

Mice were selected as a model animal because numbers adequate for statistical analysis are easily prepared. Five-week-old ICR mice were used for the present study. To establish oral infection of *C. albicans* in these animals, the host defense mechanism was suppressed by several methods. Prednisolone, an immunosuppressant, and chlorpromazine, a tranquilizer, were administered to lower the immune functions of the mice. The chlorpromazine tranquilizer that lowers leukocyte functions also suppresses saliva secretion and facilitates the colonization of *C. albicans* perhaps due to prolonged sedative periods. Prednisolone was injected into the mice subcutaneously at the same time tetracycline hydrochloride was provided in drinking water. Twenty-four hours after initiation of tetracycline administration, chlorpromazine was injected intramuscularly. A small cotton pad was soaked in a *C. albicans* solution and placed in the oral cavity of each mouse. For two days after the inoculation, the inflammatory responses of the oral cavity were observed, the tongue primarily. The inflammation peaked five days after the inoculation, then subsided thereafter. The number of viable *C. albicans* cells peaked three days after inoculation. In mice with oral candidiasis, curd-like patches (in which numerous mycelia of *C. albicans* cells grow) surrounded by redness were widely visible on the tongue surface. A histopathological study using PAS staining showed that *C. albicans* with hyphae was attached in layers to the oral epithelia on the dorsum of the tongue. These observations suggested that this murine model exhibited symptoms quite similar to human oral candidiasis. Fluconazole, an antifungal agent, was given to the mice orally infected with *C. albicans* in the animals’ drinking water. No inflammatory symptoms were observed in the mice, and virtually no *C. albicans* cells were detected, indicating that the curd-like patches observed on the tongue in the murine oral cavity were caused by the inoculation of *C. albicans* (Fig. 1). These findings indicate that the murine model prepared by the procedure described above is a suitable animal model for oral candidiasis.

Developing New Agents for Treatment of Oral Candidiasis using the Murine Model

1. Pharmacological effects of essential oils

As mentioned above,azole antifungal agents are widely used to treat candidiasis. However, due to the high incidence of recurrence and new emergence of *Candida* strains resistant to these drugs, new treatments are urgently needed. Essential oils are known to sterilize and kill various microbes, however, few studies have investigated the therapeutic effects of essential oils in cases of oral candidiasis caused by *C. albicans*. Komiyama, *et al.* used the present murine model to ascertain the pharmacological effects of tea tree oil, an essential oil. *C. albicans* was inoculated into the mouth of mice using a cotton pad. 3 hours and 24 hours later, 4% tea tree oil was applied to the mouth. Forty eight hours after inoculation, viable cell counts and inflammation were assessed. The results showed lower viable cell counts and reduced inflammation. This finding indicates that the tea tree oil was also effective against *C. albicans* strains resistant to azole agents and this suggests that tea tree oil may be used to treat oral candidiasis unsusceptible to chemotherapies. Other studies in recent years have suggested that a herbal preparation clove extract may protect mice from oral candidiasis as effectively as tea tree oil.

2. Application of biological anti-*Candida* factors

Saliva contains various antimicrobial proteins. One such protein is lactoferrin, which is also found in breast milk. Hence, researchers have investigated the clinical application of lactoferrin as an antimicrobial agent. Takakura, *et al.* assessed the therapeutic effects of lactoferrin against oral candidiasis using the murine model. In their study, 0.3% bovine lactoferrin in tap water was administered to mice as drinking water. The estimated lactoferrin dosage was approximately 0.5 g/kg/day. One day after the start of lactoferrin
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administration, *C. albicans* was inoculated in the mice using a cotton pad. The results showed that the viable cell counts for mice given access to tap water containing lactoferrin fell significantly from 6 days after inoculation. Scores for tongue symptoms also decreased significantly, starting 6 days after the inoculation, confirming the efficacy of lactoferrin *in vivo*. This characteristic late expression of efficacy by lactoferrin clearly differs from results observed with conventional antifungal agents in that *Candida* growth is prohibited and the symptoms do not appear from one to two days after treatment. The therapeutic efficacy of lactoferrin was also observed even when it was administered intragastrically using a stomach tube. These findings suggest that lactoferrin does not kill *C. albicans* directly, but indirectly suppresses candidiasis, perhaps through improving the immune functions of the host\(^{(15)}\). In fact, lactoferrin has been shown to recover the function of cervical neck lymph node cells in *Candida*-infected mice. These immunomodulation activities of lactoferrin suggested that it may be effective in cases in which *C. albicans* strains are resistant to conventional antifungal agents. In AIDS patients with oral candidiasis unresponsive toazole antifungal agents, oral treatment with lactoferrin and lysozyme have improved the efficacy of azoles, supporting the results of the above study\(^{(15)}\).

3. Pharmacological effects of salivary factors for *C. albicans* detachment
Saliva contains various antimicrobial proteins,
including lactoferrin, transferrin, lysozyme, histatin, and secretory IgA\(^{5,15-16}\). We analyzed protective mechanisms of salivary fluid against oral *Candida* infection and found a factor with activity detaching *C. albicans* that had formed biofilms\(^{17}\). Colonization of *Candida* in oral tissue is the most critical step in pathogenesis of oral candidiasis. Since *C. albicans* attaches to oral epithelial cells via integrin–like proteins, detachment of colonized *C. albicans* by saliva is thought to protect the hosts from oral candidiasis\(^{19}\).

This detachment factor was shown to be different from the several salivary antimicrobial factors reported previously. To check prophylactic activity of this detachment factor against *Candida* oral infection, its effects have been assessed using the murine model of oral candidiasis\(^{19}\). Characteristics of this detachment factor are introduced and its possible role in defense mechanisms against oral candidiasis is discussed in greater details below\(^{17,19}\).

In each well of a 96-well microplate, \(3 \times 10^6\) cells of *C. albicans* were inoculated, after which the plate was incubated at 37°C for three hours to allow the formation of biofilms on the bottom of the wells. After eliminating the culture solution from each well, human saliva was added (final concentration : 30%), and the plate was incubated once again at 37°C. The results showed that *Candida* cells that had attached to the bottom of the wells by forming biofilms had been completely detached. This salivary detachment factor is a macromolecule with a molecular weight of at least 50K; its activity is lost if it is subjected to 100°C heat. Since protease treatment negates the detachment activity, the detachment factor appears to be a protein.

The level of detachment activity in saliva was analyzed in 95 subjects and the results showed that the level of activity of this factor could clearly be divided into two age groups\(^{18}\). The ratio of subjects above 60 years of age with low activity was significantly higher than that of children and adults. None of the 21 children showed low activity levels, while 12 of the 43 elderly subjects exhibited low activity\(^{18}\). The activity of the detachment factor appears to decline markedly in certain elderly subjects. This finding suggest that the reduced potency of the detachment factor in saliva may be one cause for the high incidence of oral candidiasis among the elderly.

Unlike conventional salivary factors, this proteinaceous factor detaches *C. albicans* cells that have attached to the oral tissue by forming biofilms\(^{19}\). In light of this factor’s potential as a therapeutic agent in treatment of oral candidiasis, its therapeutic activities were examined by using the murine model of oral candidiasis. *C. albicans* was inoculated into mice prepared according to the methods of Takakura, et al. (Fig. 1)\(^{17}\). Then, 0.1 ml of human saliva was administered five times to the oral cavity of the *Candida*-infected mice. The results showed that the viable cell count and tongue inflammation scores were significantly lower three days after *C. albicans* inoculation. The extent of the decline in the viable cell count was especially marked. These findings suggest successful isolation of a saliva fraction with this detachment action may result in a new therapeutic agent for treating oral candidiasis\(^{17,19}\).

We hope for further development of new treatment agents on a broader scale, based on the present murine model of oral candidiasis.

**References**


