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Potential usefulness of bovine lactoferrin for adjunctive immunotherapy for mucosal *Candida* infections

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Abstract

Immunosuppressed children and adults have a higher prevalence of oropharyngeal candidiasis. In this patient population, anti-fungal therapy of this condition is often ineffective, and new approaches to treatment are needed. The use of bovine lactoferrin is considered a promising option in treating oropharyngeal candidiasis. Here we review the results of *in vitro* and *in vivo* studies that have examined the antimicrobial characteristics of bovine lactoferrin as an adjunctive therapy for oropharyngeal candidiasis.

Introduction

Oropharyngeal candidiasis (OPC) and vulvovaginal candidiasis are the major mucosal fungal infections caused by *Candida albicans* and several non-albicans *Candida* species that occur at the site where lactoferrin (Lf) usually exists at relatively high levels on the mucosal surface. It is generally accepted that Lf has a role as an integral part of the innate immune system through not only its antimicrobial activity but also its pleiotropic immunomodulatory activities which affect the function of many immune cell types and neutrophils. This suggests that Lf functions locally and systemically as a prominent component of the host defense against mucosal *Candida* infections.

Although usually associated with slight morbidity, OPC can clinically be the most significant mucosal fungal infection. Severe OPC can interfere with the administration of medications and adequate nutritional intake. Symptoms may include burning pain, altered taste sensation, and difficulty in swallowing liquids and solids. Lesions represented by white patches are most likely to be seen on the dorsum and sides of the tongue or on the buccal, palatal, and pharyngeal mucosae.

OPC occurs predominantly in patient populations who suffer from some kind of systemic or local immunosuppression. The incidence of OPC in some immunocompromised patient populations is particularly high: the disease can be present in 28% to 38% of cancer patients undergoing therapy with corticosteroids or anti-cancer agents (Yeo *et al.* 1985, Samonis *et al.* 1990) and in 92% of human immunodeficiency virus (HIV)-infected patients (McCarthy *et al.* 1991). Treatment of OPC is relatively simple, with most patients responding well to therapy with antifungal azole agents of topical use (e.g., clotrimazole) or systemic use (e.g., fluconazole). However, recurrence is not uncommon particularly among those who are severely immunosuppressed by HIV-infection or neutropenia. Another troublesome consequence of continuous or repeated therapy with fluconazole in HIV-infected patients has been the emergence of resistant disease (Fichtenbaum & Powderly 1998).

To overcome these therapeutic problems, we have been considering the potential usefulness of bovine Lf for adjunctive therapy, particularly immunotherapy, of OPC. There can be two modalities of Lf therapy: one is its use as a chemotherapeutic agent and the other as an immunotherapeutic agent.

Potential as chemotherapeutic agent

A simple way to use bovine Lf for the management of OPC is its topical application as a solution or some other formulations of bovine Lf as a kind of topical antifungal agent. Although, like human Lf, bovine Lf has substantial *in vitro* antimicrobial activity against *C. albicans* and several other *Candida* species pathogenic for humans, its reported minimum growth-inhibitory concentrations are relatively high and vary markedly from paper to paper probably due to different assay conditions (Wakabayashi *et al.* 1996a, Xu *et al.* 1999, Kuipers *et al.* 1999). Even lactoferricin B, an N-terminal peptide derived from bovine Lf, with a greater *in vitro* antimicrobial activity, is far less active against *Candida* species than most of the topical antifungal drugs currently available (Wakabayashi *et al.* 1996a, 1996b, 1998a). Considering such unreliable *in vitro* anti-*Candida* activity of bovine Lf or lactoferricin B, it could be more appropriate to use them in combination with some existing antifungal agent. Our previous studies showed that both bovine Lf and lactoferricin B synergistically enhanced the antifungal activity of several azole agents against both azole -susceptible and -resistant *C. albicans* (Wakabayashi *et al.* 1996a, 1998a, 1998b). However, it remains to be examined whether such combinations are also active *in vivo*. In this regard, there is a noteworthy paper reporting that severe, refractory OPC in HIV-infected patients was completely resolved by treatment with a mouthwash containing bovine Lf and lysozyme, in combination with the azole antifungal agent itraconazole (Masci 2000). In any case, further *in vivo* studies are needed to prove the clinical usefulness of bovine Lf and lactoferricin B as topical antifungal agents.

Potential as immunotherapeutic agent

It is well known that patients with HIV infection or neutropenia are at high risk of developing OPC, suggesting the important role of cell-mediated immunity and neutrophils in the host defense against the disease. The stimulatory effect of Lf on neutrophil functions was reported by several groups of investigators (Oseas *et al.* 1981, Gahr *et al.* 1991, Miyauchi *et al.* 1998). Our previous *in vitro* studies demonstrated that the anti-*Candida* activity of neutrophils was substantially augmented by bovine Lf in a concentration-dependent manner. (Okutomi *et al.* 1997, Wakabayashi *et al.* 1998b). The results of these studies suggest two possible action mechanisms of bovine Lf toward neutrophils: one is enhancement of adhesion to or engulfment by phagocytes of microbial cells (Miyauchi *et al.* 1998) and the other stimulation of extracellular release of some endogenous antimicrobial molecules (Okutomi *et al.* 1997, Wakabayashi *et al.* 1998b).

In vivo anti-*Candida* activity of bovine Lf was first demonstrated in cyclophosphamide-immunosuppressed mice with systemic *C. albicans* infection (Abe *et al.* 2000). In this murine model of lethal candidiasis, oral administration of bovine Lf at a daily dosage of 50 mg for 4 consecutive days prior to the infection significantly prolonged the survival period of infected mice. The therapeutic efficacy of oral doses of bovine Lf was also shown in the guinea pig model of dermatophytosis (tinea) (Wakabayashi *et al.* 2000a, 2000b). The clinical usefulness of bovine Lf in the treatment of dermatophytosis was confirmed by a placebo-controlled double-blind clinical study in which patients with tinea pedis (athlete's foot) were treated with bovine Lf at an oral dose of 600 or 2,000 mg a day (Yamauchi *et al.* 2000a, 2000b). Moreover, immunological analyses of peripheral blood samples and spleen cells from guinea pigs immunized with heat-killed dermatophyte conidia revealed that feeding of bovine Lf augmented the functions of splenic mononuclear cells (Wakabayashi *et al.* 2002). Therefore, oral administration of bovine Lf may potentiate the antifungal host defense by modulating immune responses to fungal cells in infected or immunized hosts.

With this postulation, *in vivo* studies were carried out to examine the possible efficacy of orally administered bovine Lf against OPC (Takakura *et al.* 2003a). For this purpose, experiments were conducted using the animal model of OPC that was considered suitable for testing the therapeutic effect of antifungal or immunomodulating agents because of the development of white patches, a characteristic presentation of OPC mimicking that seen in naturally occurring infection in humans (Takakura *et al.* 2003b). The results of experiments with the murine OPC model are as follows.

Bovine Lf was administered to a group of mice by allowing the animals to ingest drinking water containing 0.3% bovine Lf *ad libitum* from one day prior to the *Candida* infection throughout the 7-day experimental period. As the usual amount of drinking water taken in a day was approx. 4 mL per mouse in aver-

age, a daily dose of bovine Lf was roughly estimated to be 500 mg/kg. In bovine Lf-untreated control mice both the severity of local symptoms and fungal burden of the oral cavity gradually increased. In bovine Lf-treated mice, although infection initially developed up to the 4th day postinfection, significant symptomatological and mycological improvement occurred thereafter. It is to be noted that a similar improvement of infection was also seen when bovine Lf at a daily dose of 500 mg/kg was administered directly into the stomach by gastric gavage.

These results suggest that bovine Lf worked by some host-mediated mechanism of action rather than by direct antimicrobial action. This possibility was further supported by our recent studies in which the effect of orally administered bovine Lf on immune responses in the same murine OPC model was investigated. It was demonstrated that the bovine Lf-treatment induced not only a significant increase in number of regional or cervical lymph node cells but also an increased production by these cells of the cytokines IFN-γ, TNF-α, and IL-12 (Takakura *et al.*, 2004). Considering this and an earlier report (Tansho *et al.* 1994) that the anti-*Candida* activity of murine neutrophils was augmented by murine TNF-α and IFN-γ, we are led to the possibility that the *in vivo* activity of orally administered bovine Lf against experimental OPC in mice is principally exerted through local and systemic immune responses.

Conclusions

The incidence of OPC has been rapidly increasing with the increase in number of immunosuppressed individuals. The current therapy for OPC with the available antifungal agents is often ineffective in the setting of immune suppression. Hence, immunotherapy is a rational approach to treatment of the disease because it is intended to enhance immune function.

We consider bovine Lf as a promising candidate for the treatment of a variety of fungal infections including OPC. Although bovine Lf has substantial *in vitro* antimicrobial activity against *C. albicans* and several other *Candida* species it appears more likely that the immunomodulating activity of the protein to enhance host defense against these pathogenic yeasts is the primary contributor to the therapeutic effect. As reviewed here, there is increasing evidence that supports the potential usefulness of bovine Lf for adjunctive immunotherapy in the treatment of OPC and some other fungal infections. The promise of bovine Lf underscores the need for interdisciplinary research to establish parameters for its use, and offers a major challenge to both scientists and clinicians.

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