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The Activity of Apo-transferrin on the Growth of *Staphylococcus pseudintermedius*

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Abstract : Apo-transferrin is an iron-binding protein that has been reported to have an antimicrobial effect. It is considered a major component of the host defense mechanism as it limits microbial access to iron. This study was performed to investigate whether bovine apo-transferrin would have an inhibitory effect on the growth of *S. pseudintermedius*, which is one of the most isolated bacteria from dogs, and to compare the antimicrobial efficacy with bovine holo-transferrin. *S. pseudintermedius* were grown at 37°C in 96-well culture plates using Muller Hinton broth containing bovine apo-transferrin or bovine holo-transferrin at concentrations ranging from 0.5 or 2.5 to 5.0 mg/ml. The optical densities of the wells were then measured at 570 nm. In this study, the apo-transferrin showed dose-dependent antimicrobial effect against *S. pseudintermedius* while holo-transferrin did not inhibit the growth of *S. pseudintermedius* effectively. The results suggest that iron deprivation is an important pathway for inhibiting bacterial growth and bovine apo-transferrin has great antimicrobial effects against *S. pseudintermedius.*

Key words **:** apo-transferrin, iron, dog, *S. pseudintermedius.*

Introduction

Iron is an essential nutrient for pathologic microorganisms and their hosts (12,14). Several studies demonstrated that many pathogens compete with their host for iron, and within hours of infection in humans and other vertebrates, concentrations of iron in extracellular fluid and plasma decrease dramatically (7,9,12,16).

Transferrin, widely known as an iron-binding protein, is found in physiological fluids such as serum, bile, milk, and cerebrospinal fluid (3). Synthesized predominantly by hepatocytes, transferrin consists of a single polypeptide chain folded into two globular domains, with each domain having a single iron-binding site (6,15). Unsaturated transferrin, called apo-transferrin, has no irons; studies demonstrate that apo-transferrin has the ability to sequester free iron as a means of reducing bacteria or yeast infections and limiting access of microorganisms to the skin surface (2,4,8,17,19). In addition, bacterial adhesion has been shown to be inhibited only by apo-transferrin and not by holo-transferrin, the saturated form of transferrin (4,8).

Staphylococcus pseudintermedius is the most common pathogens isolated from skin infection in dogs (5,23). Although this microorganism is also cultured in healthy dogs, the bacteria can overwhelm and induce skin disease in dogs suffering cutaneous, metabolic or immunologic abnormalities (20,21, 23). Previous studies have shown that iron-binding proteins (IBPs) can have bacteriostatic or bactericidal effects against *Staphylococcus* species (1,19,22,25). However, the effects of IBPs on the growth of *S. pseudintermedius* have not yet been reported.

The purpose of this study was to investigate the effects of apo-transferrin on the growth of *S. pseudintermedius* and compare its efficacy with holo-transferrin *in vitro*.

Materials and Methods

Microorganism

S. pseudintermedius was isolated from a pustular lesion from a dog with recurrent superficial pyoderma. The isolated microorganism was identified and confirmed to be *S. pseudintermedius* on the basis of gross appearance, pigment, hemolytic pattern, the results of Gram stain, catalase and coagulase tests, and biotyping using the API STAPH-IDENT system (bioMerieux, Lyon, France) and Biolog Microstation System (Biolog Inc, Hayward, CA, USA). The microorganism was cultured on sheep blood agar plates (Asan Pharmaceutical, Hwasung, Korea) at 37°C for two days prior to use. The cells were incubated for 24 hours in Muller Hinton Broth (MHB; Difco, Detroit, MI, USA) at 37°C. Then, the cell suspension was centrifuged at 1200 g for 15 minutes and washed twice with phosphate-buffered solution (PBS, pH 7.4; Sigma, Poole, UK). The resulting supernatant was discarded. The bacteria inoculum was made by suspending *S. pseudintermedius* cells in sterile PBS, at a concentration of about 5×10^6 colony-forming units/ml.

Preparation of test materials

Bovine apo-transferrin (T-1428) and bovine holo-transfer-

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rin (T-1283) were purchased from Sigma (St Louis, MO, USA). All test materials were dissolved in sterile PBS. Triplicate wells in sterile 96-well tissue culture plates (Becton-Dickinson, Oxnard, CA) were inoculated with 100 µl of MHB, 25 µl of the *S. pseudintermedius* suspension, and 100 µl of either PBS containing each of the test materials or PBS alone. The final concentrations of bovine apo- or holo- transferrin in the wells were 0.5 or 2.5 to 5.0 mg/ml.

Assessment of bacterial growth

Bacterial growth was assessed by measuring optical density (OD) using a plate reader (Spectramax 250 Microplate reader, Molecular Devices, Sunnyvale, CA, USA). The optical density of *S. pseudintermedius* was measured at 570 nm. The OD values in the wells were obtained after 0-60 hours of incubation.

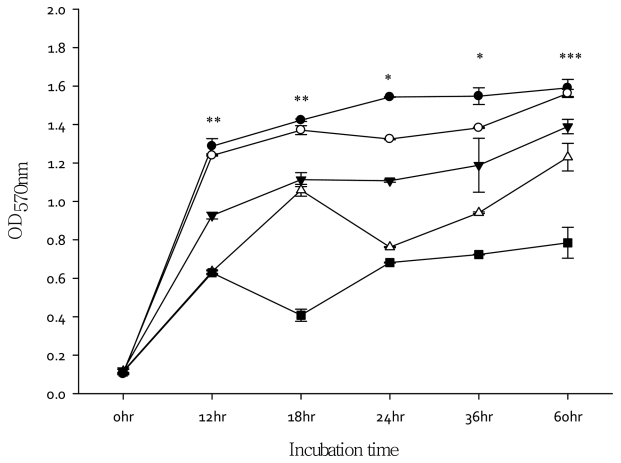

Fig 1. The growth of *S. pseudintermedius* in MHB with or without apo-transferrin. Symbols designated MBH supplemented with: control, medium alone (●); 0.5 mg/ml apo-transferrin (○); 1.0 mg/ml apo-transferrin (▼); 2.5 mg/ml apo-transferrin (△); 5.0 mg/ml apo-transferrin (■). Values presented are an average of at least three optical density measurements at 570 nm.

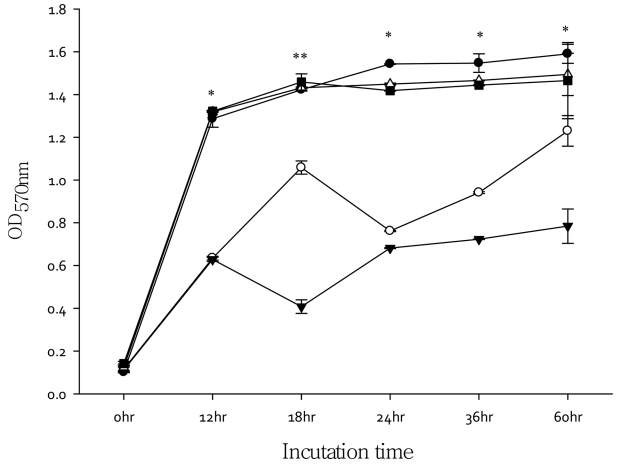

Fig 2. Comparison of efficacies between apo-transferrin and holo-transferrin on the growth of *S. pseudintermedius*. Symbols designate MBH supplemented with: control, medium alone (●); 2.5 mg/ml apo-transferrin (○); 5.0 mg/ml apo-transferrin (▼); 2.5 mg/ml holo-transferrin (△); 5.0 mg/ml holo-transferrin (■). Values presented are an average of at least three optical density measurements at 570 nm.

Results

Bovine apo-transferrin inhibited the growth of *S. pseudintermedius* in a dose-dependent manner. After at least 12 hours of incubation, bovine apo-transferrin inhibited the growth of *S. pseudintermedius* at all concentrations except 0.5 mg/ml in dose-dependent manner (Fig 1). Bovine apo-transferrin and holo-transferrin had different inhibitory efficacies on the growth of *S. pseudintermedius*. Bovine apo-transferrin decreased bacterial growth after incubation. However, bovine holo-transferrin didn't inhibit the growth effectively at all testing times (Fig 2).

Discussion

In the present study, bovine apo-transferrin, at concentrations of 0.5 to 5.0 mg/ml, inhibited the growth of *S. pseudintermedius* in a dose dependent manner. In comparison, when bovine holo-transferrin was used at the same concentrations, did not inhibit bacterial growth. Compared to previous studies, the degree of antimicrobial activity may be different depending upon the microorganism or species of transferrin (8,19,25). Bond *et al* demonstrated that the growth of *Malassezia pachydermatis* is inhibited by bovine apo-transferrin as well as bovine holo-transferrin (8). Furthermore, compared to our study, similar concentration of transferrin showed more intense bacteriostatic effects (8).

Iron is an important nutrient to microorganisms, and high levels of free iron promote the growth of pathogens (10,12, 18). Many previous studies demonstrate the bacteriostatic or bactericidal effects of IBPs, such as transferrin or lactoferrin against several pathogens (1,2,4,8,11,17,19,22,25). *S. aureus* is one of the most prevalent infectious pathogen in humans and the growth of this bacterium is inhibited under iron-restricted conditions by IBDs (1,23,24). *S. aureus* has an iron-regulated surface determinant protein, IsdA and this protein plays important roles in growth and adhesion to host cells. Although our knowledge, not all of surface proteins of *S. pseudintermedius* have been identified, *S. pseudintermedius* expresses surface proteins resembling those from *S. aureus* and is believed to express a homologue of IsdA (13).

Since transferrin has been found on skin of other species, it possibly exist on canine skin. In this study, we evaluated the efficacy of bovine transferrin not transferrin of canine. Further studies based on the use of transferrin of canine should be performed, and *in vivo* tests are needed to determine whether transferrin inhibits bacterial adherence to canine corneocytes.

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