Inactivation of Carnosine by Staphylococci

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The ability of staphylococci to inactivate natural dipeptide carnosine was revealed and a method for evaluating bacterial anticarnosine activity is described. The prevalence and expression of this sign are different in bacteria isolated from carriers and patients with pyoseptic diseases. Anticarnosine activity of staphylococci is regarded as a possible mechanism of their persistence.

Key Words: *carnosine; staphylococci; mechanisms of persistence*

Natural dipeptide L-carnosine (β-Ala-His) possesses numerous protective effects: antistress [5], immunomodulating [7], radioprotector [8], membrane protector [2], antiallergic [1], antioxidant [6], and antibacterial.

Carnosine is an endogenous component of fast skeletal muscles. However, the highest content of carnosine was found in the olfactory epithelium of the anterior nasal passage mucosa [9], the natural epitope for microorganisms (specifically staphylococci) in carriers. It is logical to presume that microorganisms persisting for a long time in epithelial cells of the nasal mucosa inactivate carnosine, a natural antiseptic of the host in this epitope. Detection of anticarnosine activity (ACA) of staphylococci may promote the detection of a new characteristic of bacterial pathogens during their persistence in the host.

MATERIALS AND METHODS

Sixty staphylococcal strains were used in the study: *S. aureus* (n=30) and *S. epidermidis* (n=30) isolated from the nasal mucosa of healthy carriers and from pathological material of patients with pyoseptic diseases (PSD) of staphylococcal etiology (sinusitis, furuncles, carbuncles, panaritia, and postinjection abscesses). Isolated strains were identified using a Staphytest system (Lachema). Carnosine from Sigma was used.

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The method for evaluating of bacterial ACA is based on the delayed antagonism principle [10]. Test culture of *M. luteus* (ATCC 15307) highly sensitive to bactericidal effect of carnosine was selected in experiments on determination of the minimal bactericidal concentration of carnosine for various microorganisms.

The bacterial culture was grown on a nutrient medium, a 10^9 suspension was prepared, 0.1 ml of this suspension was mixed with 1.7 ml meat-peptone broth (MPB), and 0.2 ml carnosine was added to a final concentration of 1 mg/ml.

The controls were as follows: 1) mixture of carnosine (0.2 ml), normal saline (0.1 ml), and MPB (1.7 ml); 2) mixture of normal saline (0.2 ml), 10^9 suspension of test culture (0.1 ml), and MPB (1.7 ml); and 3) 0.85% NaCl (0.3 ml) and MPB (1.7 ml).

After 24-h incubation at 37°C, 0.1 ml chloroform was added to experimental and control samples and after 40 min all samples were centrifuged (3000 rpm for 15 min). The supernatants (0.6 ml) were mixed with 1.1 ml of MPB and 0.1 ml micrococcal suspension (10^9 suspension was prepared from 16-18-h agar culture and diluted 1:10), incubated for 24 h at 37°C, and optical density measured on a KFK-2MP. Inactivation of carnosine by staphylococci was calculated by the formula:

$$C\text{-}C\times\left(\frac{OP_1\text{-}OP_2}{OP_3\text{-}OP_4}\right),$$

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where C is the initial concentration of carnosine, OP_1 is optical density of micrococcal suspension after co-culturing with the supernatant of test cultures, OP_2 is optical density of micrococcal suspension grown in MPB with the supernatant of test culture and carnosine, OP_3 is optical density of micrococcal suspension grown in MPB, and OP_4 is optical density of micrococcal suspension grown in MPB with carnosine.

RESULTS

Staphylococci isolated from patients with PSD and healthy carriers inactivated carnosine. Comparative analysis of ACA of strains isolated from carriers showed that carnosine was inactivated by 82% *S. epidermidis* and 78% *S. aureus*. Among the strains isolated from PSD patients, 70% *S. epidermidis* and 86% *S. aureus* possessed ACA.

Evaluation of the level of carnosine inactivation by various staphylococcus strains showed essential differences between the strains isolated from patients with different forms of infection (Fig. 1): *S. epidermidis* and *S. aureus* isolated from carriers possessed far higher activity than the strains isolated from patients with PSD.

Our experiments revealed a new characteristic of staphylococci: ACA towards host cells. It is probable that ACA of microorganisms is a mechanism of staphylococcal persistence, because the highest activity was observed in strains isolated from carriers, but not from PSD patients. These findings agree with the data on antilysozyme activity of pathogens. High prevalence of ACA in staphylococci isolated from carnosine-rich epitopes should be regarded as a realization of the principle of ecological determination of the persistence factors [3], which can be used in practical hygienic measures for microbiological monitoring of the environment [4].

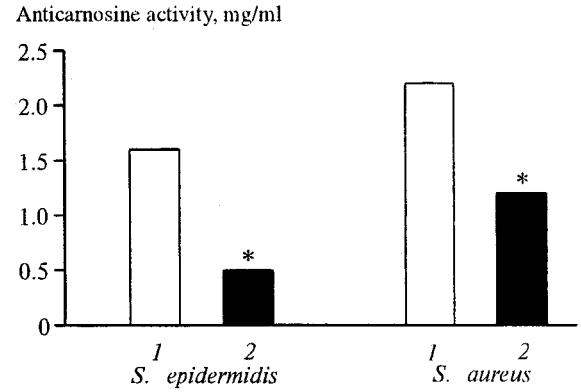

Fig. 1. Anticarnosine activity of staphylococci isolated from healthy carriers (*1*) and patients with pyoseptic diseases (*2*). *$p<0.05$ in comparison with carriers.

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