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Interaction between cell wall synthesis inhibitors and human granulocytes and monocytes

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Human monocytes enhance the antibacterial activity of penicillins against ingested and non-ingested Staphylococcus aureus (S. aureus) [1, 3, 4]. The increased activity against extracellular bacteria has been ascribed to a factor secreted by the monocytes [1, 2]. This factor is probably a peptidoglycan-degrading enzyme differing from lysozyme and N-acetyl-β-D-glucosaminidase [2, 5]. We performed the present study to find out whether the antibacterial activity of vancomycin against non-phagocytosed S. aureus is enhanced by the presence of monocytes and whether the presence of granulocytes influences the antibacterial activity of penicillin G against non-phagocytosed S. aureus.

Assessment of the influence of the presence of phagocytes on the antibacterial activity of antibiotics

A mixture of equal volumes of a suspension of 1×10^7 non-opsonized S. aureus/ml and 1×10^7 granulocytes or monocytes/ml was incubated with penicillin G or vancomycin under rotation (4 rpm) at 37 °C. At 0, 60, 120, and 180 min, samples were taken and brought into ice-cold gelatin-HBSS. The total number of viable bacteria in the samples was determined microbiologically [1].

Results

The antibacterial activity of the antibiotics against S. aureus in the presence of granulocytes or monocytes was determined in the absence of serum, which meant that ingestion and intracellular killing of the bacteria by the phagocytes could not occur [1]. The antibacterial activity of penicillin G against S. aureus was greater in the presence than in the absence of granulocytes or monocytes (Table I). The antibacterial effect of vancomycin on S. aureus was also enhanced by the presence of monocytes (Table I).

To find out whether the oxygen-dependent killing mechanism of the phagocytes is involved in the enhancement of the antibacterial effect of

TABLE I

Antibacterial effect of vancomycin and penicillin G in the presence of normal and PMA-treated phagocytes*

	Antibacterial effect** at 180 min of incubation	
	penicillin G (0.100 μg/ml)	vancomycin (0.500 μg/ml)
Absence of phagocytes	0.70	0.82
Granulocytes		
normal	1.19	N.D.
PMA-treated	0.77	N.D.
Monocytes		
normal	1.78	1.28
PMA-treated	1.75	0.91

* Results of a representative experiment.
** Results expressed as antibacterial effect (E):

$$E = \log Na_t - \log Nb_t.$$

For the calculation of E in the absence of phagocytes, Na is the total number of viable bacteria in gelatin-HBSS and Nb that in gelatin-HBSS containing antibiotic. For calculation of the antibacterial effect in the presence of phagocytes, Na is the total number of viable bacteria in gelatin-HBSS with phagocytes and Nb that in gelatin-HBSS containing phagocytes and antibiotic.

the antibiotics, we treated the granulocytes and monocytes with 100 ng/ml phorbol myristate acete (PMA) for 5 min before they were used in the assay. Exposure of the cells to this high dose of PMA abolishes the production of hydrogen peroxide and oxygen radicals by the oxygen-dependent killing mechanism [6]. After PMA treatment the granulocytes did not increase the antibacterial effect of penicillin G or the monocytes that of vancomycin (Table I). The enhancement of the antibacterial effect of penicillin G by monocytes was not abolished by PMA treatment.

DISCUSSION

The present findings show that the presence of either granulocytes or monocytes increased the antibacterial activity of penicillin G against S. aureus. Monocytes increased the effect of vancomycin. Since no serum was present during incubation, this enhancement could not have been the result of phagocytosis and intracellular killing of S. aureus by the phagocytes. The oxygen-dependent killing mechanism seems to play a role in the enhancement of antibacterial activity of penicillin G by granulocytes and of vancomycin by monocytes, since pretreatment of the cells with PMA, which inhibits this killing system, abolished the enhancement of the activity. The enhancement of the effect of penicillin G by monocytes was not prevented by pre-treatment of the monocytes with PMA, which indicates that this enhancement was due to some other mechanism. This finding supports that the earlier described monocyte factor which enhances the antibacterial effect of penicillin G on S. aureus, is responsible for the greater antibacterial effect of penicillin G in the presence of monocytes [1]. This factor probably plays a role in the non-oxidative killing mechanism of the monocytes.

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