Influence of Subinhibitory Concentrations of Clindamycin on Opsonophagocytosis of Staphylococcus aureus, a Protein-A-Dependent Process

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Incubation in subinhibitory concentrations of clindamycin enhanced the uptake of protein A-rich Staphylococcus aureus strains by polymorphonuclear leukocytes. This enhancement of uptake appeared to be due, in part, to a reduction in the amount of protein A at the bacterial cell surface by clindamycin.

Exposure to antibiotics at concentrations lower than the MIC is known to induce morphological changes in certain bacteria (5, 8), and it also renders these organisms more susceptible to phagocytosis (2, 4, 6–9). Because opsonization of Staphylococcus aureus is necessary for phagocytosis, the enhanced uptake by phagocytes of these antibiotic-treated bacteria may be due to more efficient opsonization (4, 10).

The synthesis of protein A, a component of the cell wall of S. aureus known to impair opsonization (12, 13, 15), is reduced when S. aureus is grown in the presence of low concentrations of clindamycin (3). Therefore, we studied the influence of subinhibitory concentrations of clindamycin on the opsonophagocytosis of S. aureus strains with different amounts of protein A.

Bacteria. Opsonophagocytosis of S. aureus Cowan I; S. aureus Cowan I NG, a protein A-deficient mutant of S. aureus Cowan I; S. aureus Wood 46; S. aureus HSmR; and three strains isolated from patients with staphylococcal infections was studied. The strains were grown overnight at 37°C in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) containing 5 μCi of [3H]thymidine (Amer- sham International plc, Amersham, United Kingdom) per ml in the presence or absence of one-quarter or one-half of the MIC of clindamycin. For trypsin treatment, 10 μg radioactively labeled S. aureus isolates, grown overnight in MHB without clindamycin, were incubated in 5 ml of trypsin (1 mg/ml; Boehringer GmbH, Mannheim, Federal Republic of Germany) for 30 min at 37°C. A total of 5 ml of trypsin inhibitor (1 mg/ml; from lyophilized soybean; Boehringer) was used to stop the reaction (37°C for 15 min).

Antibiotics. Clindamycin hydrochloride was obtained from The Upjohn Co., Ede, The Netherlands. The MIC of clindamycin was 0.08 μg/ml for S. aureus Wood 46 and 0.16 μg/ml for the other S. aureus strains tested.

Polymorphonuclear leukocytes. Polymorphonuclear leukocytes (PMNs) were isolated from fresh blood of healthy volunteers. Briefly, after dextran sedimentation of the erythrocytes, PMNs were separated from mononuclear cells by centrifugation on Ficoll-Paque (Pharmacia, Fine Chemicals AB, Uppsala, Sweden). Residual erythrocytes were then lysed by hypotonic NH4Cl treatment. PMNs were washed and suspended in Hanks balanced salt solution containing 0.1% gelatin (1). Contamination with mononuclear cells never exceeded 2%. More than 95% of the cells were viable, as shown by trypan blue exclusion.

Serum sources. Normal human pooled serum was obtained from 10 healthy donors and stored at −70°C until use.

Phagocytosis assay. The uptake of radioactively labeled bacteria by PMNs was measured by the method of Verhoef et al. (14). Briefly, radioactively labeled bacteria were opsonized in different concentrations of serum for various periods of time. Equal amounts of opsonized staphylococci (5 × 10⁷/ml) and PMNs (5 × 10⁶/ml) were incubated in a 37°C shaking water bath. At indicated times phagocytosis was stopped by the addition of ice-cold phosphate-buffered saline. The percentage of staphylococci taken up by the PMNs at each time interval was calculated from the uptake of radioactivity by the phagocytes and the total added radioactivity. Radioactivity was determined by liquid scintillation counting (Philips, Almelo, The Netherlands).

Quantitation of cell wall protein A. Bacteria grown overnight in MHB in the presence or absence of clindamycin were incubated with phosphate-buffered saline containing 2.0 μg of lysostaphin per ml (Schwarz/Mann, Orangeburg, N.Y.) for 120 min at 37°C. The amount of protein A in the lysate was determined quantitatively by indirect hemagglutination with 200 μl of 1% sheep erythrocytes that were previously sensitized with a subagglutinating dose of immunoglobulin G antibody against sheep erythrocytes (National Institute for Public Health, Bilthoven, The Netherlands) (11).

In Fig. 1 it is shown that incubation in subinhibitory concentrations of clindamycin results in enhancement of phagocytosis for bacteria that are rich in protein A but not for bacteria that are protein A deficient, when 1% normal serum is used as the opsonic source. Results of previous studies have shown that the enhanced uptake observed is not due to increased attachment of the clindamycin-treated bacteria to the granulocytes but results from enhanced ingestion by the granulocytes (7, 10).

To further investigate the potential role of protein A in preventing opsonization, strains were treated with trypsin to remove cell surface proteins. Trypsin treatment of the strains grown in the absence of clindamycin resulted in values of protein A (Table 1) and rates of opsonophagocytosis (Fig. 1) that were comparable to those of strains treated with one-half the MIC of clindamycin.

The total amount of protein A present at the bacterial cell surface was determined quantitatively in both clindamycin-
peptidoglycan synthesis and assembly are defective or quantitatively deficient, resulting in peptidoglycan abnormalities. Therefore, studies need to be extended to other surface characteristics that interfere with phagocytosis.

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LITERATURE CITED