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# Cellular uptake of clindamycin and lincomycin

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**Summary.** Neither clindamycin nor lincomycin killed intracellular *Staphylococcus aureus* over a 4-h period. Bio-assay of neutrophil sonicates after exposure to antibiotic showed the presence of active clindamycin at approximately 20 times the extracellular concentration. Clindamycin and lincomycin kill staphylococci relatively slowly, particularly in cell culture medium and balanced salt solution. This might account for their failure to kill intracellular staphylococci despite intracellular accumulation. The neutrophil experiments could not be extended to a time period (20 h) over which the antibiotics would kill *S. aureus*, as the presence of these bacteria within neutrophils for this length of time resulted in considerable cell lysis.

Keywords: clindamycin, lincomycin, cellular uptake, antibiotics

Pathogenic bacteria that can survive within phagocytic cells are not only protected against most other host defence mechanisms, but also against many antibiotics. The ability of antibiotics to enter phagocytic cells and kill ingested bacteria would, therefore, be a significant advantage in the therapy of such infections.

Johnson *et al.* (1980) investigated the uptake of a range of radiolabelled antibiotics by rabbit alveolar macrophages. Although clindamycin was very rapidly concentrated within cells to 40 times the extracellular level, there was no evidence of any effect on intracellular bactericidal activity. Interestingly the closely related antibiotic lincomycin was taken up relatively poorly.

These observations with radiolabelled clindamycin were confirmed in human neutrophils by Klempner & Styrt (1981). They did, however, find useful intracellular bactericidal activity over a 24-h period.

In this paper we have examined the effect of unlabelled lincomycin and clindamycin on the survival of *S. aureus* within human peripheral blood neutrophils and have looked for evidence of biologically active clindamycin in neutrophil sonicates after incubation with clindamycin.

## Materials and methods

*Bacteria. S. aureus* NCTC 6571 was stored in liquid nitrogen and maintained on Columbia Agar.

*Antibiotics used.* Lincomycin and clindamycin (Upjohn), rifampicin (Lepetit) and benzylpenicillin (Glaxo).

*Minimum inhibitory and bactericidal concentrations (MIC/MBC).* These were determined by a standard broth dilution technique using Hanks' balanced salt solution, tissue culture medium 199 (Gibco) and antibiotic sensitivity broth (Isosensitest). The inoculum was $10^5$ colony-forming units (c.f.u.) of an overnight culture of *S. aureus*. After overnight incubation at 37° tubes showing no visible growth were subcultured on antibiotic-free agar (Columbia Agar-Lab M).

*Bacterial killing curves.* An overnight culture of *S. aureus* 6571 was washed and suspended at a concentration of approximately $10^6$ c.f.u. in medium 199, Hanks' balanced salt solution (Gibco) or either without (control) or with varying concentrations of antibiotic. At intervals of 2, 4, 6 and 20 h, 0.1 ml aliquots were taken, diluted 10-fold in ice-cold saline and surface viable counts performed.

*Effect of antibiotics on intracellular survival of S. aureus. S. aureus* suspended in medium 199 was opsonized with 10% pooled human serum for 15 min at 37°, washed and resuspended at a concentration of $5 \times 10^6$ c.f.u./10 ml. Human neutrophils were separated from heparinized venous blood by dextran sedimentation for 45 min at room temperature. The leucocyte-rich plasma layer was harvested and residual red cells lysed by a 10-min exposure to 0.83% Tris-buffered ammonium chloride. After two washes the cell suspension (>85% neutrophils) was adjusted to a concentration of $2 \times 10^6$ neutrophils/ml medium 199.

Aliquots (0.5 ml) of neutrophil and bacterial suspensions were mixed on a roller for 15 min at 37° to allow bacterial ingestion. Extracellular and cell-adherent *S. aureus* were removed by treatment with 5 μg lysostaphin (Becton Dickinson). After further washing an aliquot of the neutrophil suspension containing ingested *S. aureus* was added to 0.9 ml cold distilled water. The neutrophils were disrupted by sonication and viable counts performed on 10-fold dilutions. This was the $T_0$ count. The remaining suspension was incubated with or without antibiotic for varying periods of time and further viable counts performed as described above. The percentage intracellular survival of *S. aureus* was calculated from these viable counts.

*Bactericidal activity of neutrophil sonicates.* Neutrophils were incubated with varying concentrations of antibiotic. The cells were centrifuged rapidly (12000 *g* for 1 min) through silicone fluid, which acted as a water excluding layer. The silicone fluid (Dow Corning) was a 4:7 mixture of the 0.94-g/ml and 1.07-g/ml fluids. The neutrophil pellet was disrupted by sonication and the bactericidal activity of the sonicate bioassayed using an agar plate diffusion technique with *S. aureus* as the indicator organism.

Results shown are from individual experi-

**Table 1.** Effect of clindamycin and lincomycin on intracellular staphylococcal survival

| Extracellular antibiotic concentration (mg/l) | Viable count (c.f.u. × $10^5$) (±SD) | | Staphylococcal survival (%) |
|---|---|---|---|
| | $T_0$ | $T_4$ | |
| Control | 6.2 (0.2) | 3.0 (0.2) | 48 |
| Clindamycin 1 | 5.0 (0.8) | 2.6 (0.1) | 52 |
| Clindamycin 5 | 5.4 (0.2) | 3.0 (0.1) | 56 |
| Clindamycin 20 | 5.4 (0.4) | 2.6 (0.1) | 48 |
| Penicillin 1 | 5.2 (0.2) | 3.1 (0.1) | 60 |
| Rifampicin 1 | 5.2 (0.3) | 0.71 (0.07) | 14 |
| Control | 9.1 (0.4) | 4.2 (0.3) | 46 |
| Lincomycin 1 | 10.0 (0.3) | 4.7 (0.5) | 47 |
| Lincomycin 5 | 9.2 (0.1) | 4.4 (0.1) | 48 |
| Lincomycin 20 | 8.5 (0.2) | 4.0 (0.1) | 47 |
| Penicillin 1 | 9.4 (0.4) | 5.5 (0.3) | 58 |
| Rifampicin 1 | 8.3 (0.4) | 0.76 (0.07) | 9 |

**Table 2.** Effect of clindamycin added before the initiation of phagocytosis on intracellular staphylococcal survival

| Extracellular antibiotic (mg/l) | Viable count (c.f.u. $\times 10^5$) | | Staphylococcal survival (%) |
|---|---|---|---|
| | $T_0$ | $T_{2.5}$ | |
| None | 1.04 | 0.32 | 30.8 |
| Clindamycin 1 | 0.99 | 0.29 | 29.3 |
| Clindamycin 10 | 0.85 | 0.32 | 37.6 |

ments but all were repeated a minimum of five times with similar results.

*Determination of intracellular volume.* The total volume of the pellet was found by incubating cells with [$^3$H]-water, specific activity 1 mCi/ml (Amersham), for 5 min at 37°C. The extracellular volume was similarly determined with [$^3$H]-inulin, specific activity 1 $\mu$Ci/ml (Amersham). The intracellular volume of the PMN in the pellet was then calculated by subtracting the extracellular from the total pellet volume. This was 1.00 $\mu$l/4 $\times$ 10$^6$ PMN.

## Results

Table 1 shows the effect of varying extracellular concentrations of clindamycin and lincomycin on the survival of *S. aureus* over a 4-h period. Even at 20 mg/l neither agent had any detectable effect on ingested staphylococci. This was in contrast to the effect of extracellular rifampicin which was used as a positive control. Even the addition of antibiotics to the medium before phagocytosis began had no effect on subsequent intracellular survival (Table 2).

The lack of effect of clindamycin was surprising, given that the minimum bactericidal concentration for the test *S. aureus* was only 1.0 mg/l and the report by Klempner & Styrt (1981) of a $\times$40 concentration of clindamycin in human neutrophils.

To find an explanation for our observation, we therefore looked more closely at the killing of *S. aureus* by clindamycin and lincomycin. Conventional MBC tests did in fact give differing results when bacteriological media, tissue culture media or Hanks'

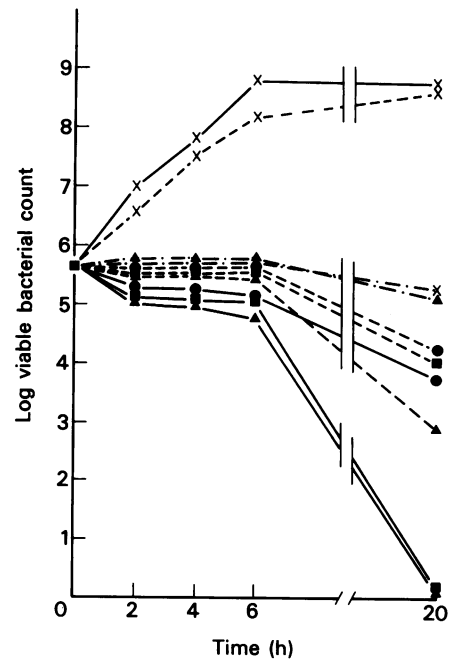


**Fig. 1.** Effect of clindamycin on survival of *S. aureus* in broth, Hank's balanced salt solution and Medium 199 at 37°. ×——×, no antibiotic, broth; ×– – –×, no antibiotic, 199; ×–·–×, no antibiotic, HBSS; ▲–·–▲, clindamycin 20 mg/l, HBSS; ▲– – –▲, clindamycin 20 mg/l, 199; ▲——▲, clindamycin 20 mg/l, broth, ■– – –■, clindamycin 5 mg/l, 199; ■——■, clindamycin 5 mg/l, broth, ●– – –●, clindamycin 1 mg/l, 199; ●——●, clindamycin 1 mg/l, broth.

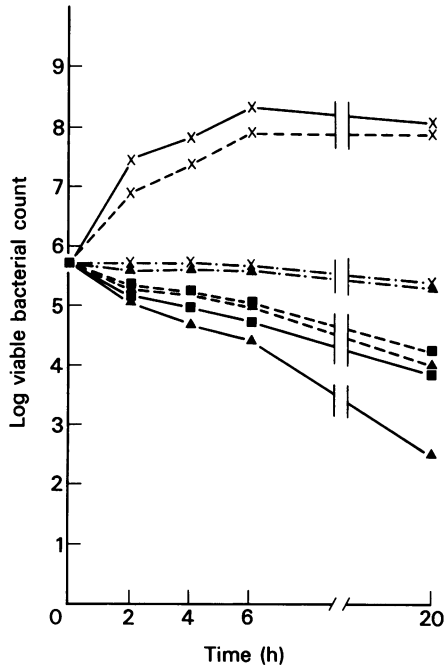


**Fig. 2.** Effect of lincomycin on survival of *S. aureus* in broth, Hanks' balanced salt solution and Medium 199 at 37°. ×——×, control, broth; ×– – –×, control, 199; ×–·–×, control, HBSS; ▲–·–▲, lincomycin 20 mg/l, HBSS; ▲– – –▲, lincomycin 20 mg/l, 199; ▲——▲, lincomycin 20 mg/l, broth; ■– – –■, lincomycin 5 mg/l, 199; ■——■, lincomycin 5 mg/l, broth.

balanced salt solution (HBSS) were used. The MBC of lincomycin was 2.0 mg/l in Todd Hewitt broth, 5 mg/l in 199 medium and >20 mg/l in balanced salt solution. The corresponding figures for clindamycin were 1 mg/l, 1 mg/l and >20 mg/l.

Of more immediate relevance to our experiments with phagocytic cells (than conventional MBCs) were the quantitative killing curves for the two antibiotics. These are shown in Figs 1 and 2. Two points stand out. First, the influence of the medium on bacterial killing. No killing was seen in HBSS, maximum killing occurred in standard bacteriological medium, while tissue culture medium occupied an intermediate position. Second, that killing by both clindamycin and lincomycin was at best a relatively slow process, little taking place in the first 3–6 h. This might account for the minimal effect of both agents on intracellular staphylococcal survival over a 4-h period.

We therefore extended the duration of the neutrophil experiment to 20 h. As shown in Table 3, there was apparently no intracellular staphylococcal survival after 20 h incubation in the presence of clindamycin. When the cell suspension was counted, however, few whole cells were seen. This suggested that the neutrophils had lysed over the longer incubation period exposing intracellular bacteria to antibiotics. We further investigated the problem by incubating neutrophils over 20 h either alone or with ingested staphylococci, antibiotics, or both. Neutrophil survival with or without added antibiotic was good. When the cells contained staphylococci, however, few cells remained.

**Table 3.** Survival of neutrophils incubated at 37° over a 20-h period in the presence of clindamycin and/or ingested *S. aureus*

| | PMN count | | | |
|---|---|---|---|---|
| Time | PMN | PMN + clindamycin | PMN + *S. aureus* | PMN + clindamycin + *S. aureus* |
| $T_0$ | $2.4 \times 10^6$ | $2.6 \times 10^6$ | $2.6 \times 10^6$ | $2.5 \times 10^6$ |
| $T_4$ | $2.35 \times 10^6$ | $2.7 \times 10^6$ | $2.3 \times 10^6$ | $2.2 \times 10^6$ |
| $T_{20}$ | $2.0 \times 10^6$ | $1.65 \times 10^6$ | No cells seen. Bacterial overgrowth | $2.5 \times 10^4$ |

**Table 4.** Biologically active clindamycin in neutrophil sonicates

| Extracellular clindamycin (mg/l) | Clindamycin in neutrophil sonicate (mg/l) | Intracellular concentration (× extracellular concentration) |
|---|---|---|
| 10 | 220 | 22 |
| 5 | 130 | 26 |

To measure the biological activity of cell-associated clindamycin, neutrophils were incubated with clindamycin and centrifuged through a water-excluding gradient to avoid the need for washing. The cell pellet was sonicated and the sonicate bio-assayed for activity against *S. aureus*. Table 4 shows that the sonicate had antibacterial activity, and there was in fact evidence of concentration of active clindamycin far above the extracellular level.

## Discussion

Studies with radiolabelled clindamycin in both rabbit alveolar macrophages and human peripheral blood neutrophils showed that there is a rapid intracellular accumulation of antibiotic which was dependent on an active oxidative transport system (Johnson *et al.* 1980; Klempner & Styrt 1981; Prokesch & Hand 1982). Intracellular uptake does not, however, necessarily mean useful antibacterial activity. The active agent may be broken down within the cell, it may fail to reach the phagosome or reach it in too low a concentration to be effective. Alternatively the pH or other conditions within the phagosome may be unsuitable for antibacterial activity. The ingested organism may have become resistant to certain antibiotics by virtue of reduced metabolic activity and cell wall synthesis.

Neither clindamycin nor lincomycin appeared to affect intracellular staphylococcal survival over a 4-h period. Although labelled clindamycin is taken up by human neutrophils to reach an intracellular concentration of 40 times that outside the cell within 10 min (Klempner & Styrt 1981), the relatively slow direct bactericidal action of the drug which we found against *S. aureus* might not allow significant intracellular killing in 4 h.

We were unable to extend our observations to 20 h as the presence of live or dead intracellular *S. aureus* significantly reduced neutrophil survival over this length of time. Klempner & Styrt (1981) incubated neutrophils 'overnight' with clindamycin and found a reduction in viable intracellular organism, but made no comment on neutrophil survival. Johnson *et al.* (1980) found that despite intracellular concentration, clindamycin had no effect on intracellular staphylococcal survival.

In experiments not described here we found radiolabelled clindamycin to be concentrated within human neutrophils to about 40 times the extracellular level. This was similar to that reported by Klempner & Styrt (1981).

We are now looking at the distribution of radiolabelled clindamycin within human neutrophils in order to see whether the antibiotic is concentrated within the phagocytic vacuole.

Our results illustrate the limitations of conventional MIC/MBC determinations of antibiotic activity when applied to this type of neutrophil experiment. Both the culture medium and the time of antibiotic exposure are critical and bacterial killing may be a slow event.

Any intracellular concentration of antibiotic could be of use clinically. The problem of antibiotic-associated colitis has limited the use of clindamycin to serious infection, but it

is a highly effective agent against staphylococci and anaerobes.

## Acknowledgements

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