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Interaction of phagocytic cells with antibiotics: uptake and intracellular killing activity

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Antibiotics can affect the interaction of phagocytes with microbes in several ways.

I Phagocytes may provide a protected environment for some microbes, in which the phagocyte cannot kill the organisms, and antimicrobial agents are either unable to reach the microbes or are inactivated in the phagosome.

II Antibiotics may affect phagocytes directly, either potentiating or inhibiting their migratory, phagocytic and microbidical functions.

III Finally antibiotics may affect the microbes in such a way that their phagocytosis and killing by neutrophils and monocytes is altered.

Intracellular survival of infectious agents within human phagocytic cells is best exemplified by patients with chronic granulomatous disease (CGD). Due to a metabolic defect CGD phagocytes lack endogeneous intracellular microbial killing and therefore represent a unique opportunity to study the intracellular bactericidal activity of exogeneous agents such as antibiotics [10].

In order to be effective in CGD--Patients, antimicrobial agents have to fulfill three requirements:

I The must be able to penetrate the outer cell membrane and then the phagosomal membrane, in order to reach invaders surviving inside the phagosomes.

Lipophilic antibiotics or drugs taken up by active transport systems would have an advantage over hydrophilic antibiotics.

II They must be able to exert antimicrobial killing activity also inside the phagosomes. Bactericidal antibiotics would have an advantage over bacteriostatic antibiotics.

III Finally they must possess an antimicrobial spectrum directed against catalase-positive invaders. Antibiotics directed at staphylococci and enterobacteriaceae would be most successful.

This review will summarize data on uptake and intracellular killing activity of 4 Antibiotics:

1. *Rifampin;* 2. *Trimethoprim;* 3. *Clindamycin;* 4. *Fosfomycin*

From these data recommendations of antimicrobial prophylaxis and treatment for CGD-patients are derived.

Methods

Uptake of antibiotics by phagocytes can only be maesured by a system clearly separating the intracellular or cell-associated antibiotics from the extracellular antibiotics. One suitable way is to first incubate phagocytes with radiolabelled antibiotics followed by rapid separation of cells from medium by centrifugation through a layer of waterimpermeable silicone oil [11]. The ratio of cell-associated to extracellular antibiotic concentration (C/E) is then determined.

A C/E-value of 1 means equal distribution in the two compartments, a C/E < 1 exclusion from the cells and a C/E > 1 concentration within the cells. Such experiments cannot localize the antimicrobial agents inside the cell, nor can they tell whether intracellularly accumulated drugs are still bioactive.

The antibiotic effect on intracellular microbes can only be assessed by a bacterial killing test removing the non-ingested bacteria almost completely. In neutrophils one successful approach is the use of multiple washes. [11].

In detail, phagocytes are first added to fresh normal serum and Staph. aureus in logarithmic growth phase to allow opsonization and phagocytosis. After 30 min. the mixture is centrifuged and the cell pellet washed vigorously 3 times which removes most of the extracellular bacteria. Only then is medium with or without antibiotics added.

At 0,60 and 120 min. an aliquot of the reaction mixture is lysed for quantitation of viable phagocyte-associated microorganisms. While CGD neutrophils on their own show only slight killing, in the presence of intracellularly active antibiotics killing will be nearly normalized.

Results

1. *Rifampin*

Both form s of rifampin the quinone and the hydroquinone accumulate equally well in normal neutrophils with a maximal cellular to extracellular concentration of 9 after 5 – 10 min. incubation, and in CGD neutrophils with a C/E max. of 14.

Uptake is not influenced by metabolic inhibitors and even occurs within dead neutrophilis fixed by glutaraldehyde [5]. At first shown by Ezer and Soothill [2] rifampin significantly reduces the number of staphylococci surviving inside CGD cells at extracellular concentrations ranging from $0.06 - 5\ \mu g/ml$ easily achievable in vivo, thus compensating for the bactericidal defect inherent with this disease.

Though these in vitro results look very promising, rifampin alone cannot be used for long term prophylaxis of infections in CGD due to the selection of resistant mutants, but it has proven to be effective in the treatment of overt staphylococcal infections if combined with a second antibiotic.

2. *Trimethoprim*

The lipid-soluble TMP is concentrated- by normal and CGD neutrophils 2-fold and sulfamethoxazol (SMX) 3.5-fold, which is less than rifampin, but much better that penicillin G [4].

If TMP is combined with SMX at extracellular concentrations still achievable in vivo (5 and 100 $\mu g/ml$ TMP/SMX) normalization of bacterial killing by CGD neutrophils is seen [11] (Figure 1).

These in vitro data have been confirmed by Jacobs and Wilson [6, 7].

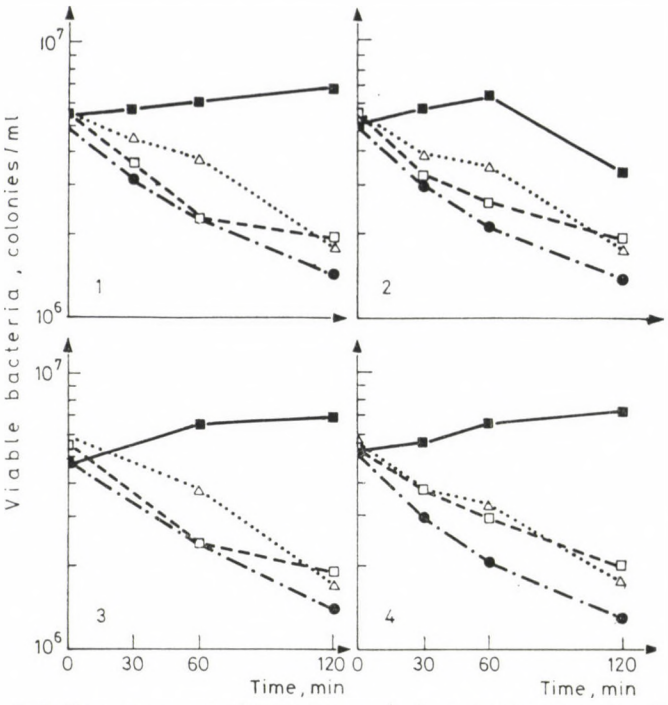

FIG. 1. Effect of Sulfamethoxazol (SMX 100 μg/ml) and trimethoprim (TMP 5 μg/ml) on the killing of S. aureus by neutrophils from four patients with CGD. The number of total viable bacteria in the reaction mixture is plotted as a function of time at which the mixture is sampled. (11) ■——■ CGD cells without SMX/TMP, □——□ CGD cells with SMX/TMP (105 μg/ml), ●——● normal cells without antibiotic, △——△ antibiotics without cells for comparison

In their study TMP/SMX at 4 and 80 μg/ml as well as TMP/rifampin at 4 and 1 μg/ml were both able to reverse the bactericidal defect of CGD neutrophils.

Such data indicate that TMP/SMX and-in case of sulfonamide allergy — TMP/rifampin may be suitable candidates for continuous therapy in CGD patients.

The mode of action of the intracellular killing activity of TMP/SMX is probably a purely antibiotic effect [3].

The improvement of deficient microbicidal killing in CGD by TMP/SMX is not due to: 1) direct generation of H_2O_2; 2) inhibition of bacterial catalase; 3) improvement of neutrophil oxygen metabolism; 4) synergism with non-oxygen dependent killing mechanisms.

Such negative data do suggest that the reduction of intracellular bacteria by TMP/SMX is simply due to a direct antibacterial action.

3. *Clindamycin*

Clindamycin, a hydrophilic antibiotic, is nevertheless concentrated 6.5-fold by neutrophils of normal

controls and CGD patients. Accumulation of clindamycin is an active energy-requiring process dependent at least in part upon glycolysis [9].

Being a weak base, clindamycin is transported from areas of higher pH e.g. the extracellular medium, to areas of lower pH, probably the lysosomal and phagosomal compartments.

At least four studies, [1, 6, 7, 13], such as the one by Jacobs and Wilson [6, 7], have now shown that the addition of clindamycin to a suspension of CGD neutrophils following the ingestion of Staph. aureus increased intracellular killing at extracellular concentrations achievable in vivo.

There are as yet no clinical studies on the use of clindamycin for treatment of infections in CGD. If the emergence of resistant staphylococcal strains occurs during the usual TMP/SMX prophylaxis, they should be eradicated with other drugs, and high dose clindamycin therapy could be one useful adjunct.

4. *Fosfomycin*

Fosfomycin is an antibacterial agent of low molecular weight, 140 daltons, inhibiting the first step in bacterial cell wall synthesis. Its bactericidal activity in vitro includes organisms important in CGD such as staphylococci and enterobacteriaceae, e.g. E. coli, S. marcescens and salmonella.

Fosfomycin is a hydrophilic antibiotic, but is nevertheless concentrated 2-fold by neutrophils.

Fosfomycin accumulation is hindered by glycerophosphate as well as by agents interfering with energy-requiring processes.

This strongly suggests the presence of an active transport mechanism in human neutrophils, similar to that described for this drug in bacteria [4].

At extracellular concentrations achievable in vivo by iv administration (between 50 and 150 μg/ml) fosfomycin is able to kill staphylococci surviving within CGD neutrophils, thus compensating for the bactericidal deficiency in CGD [4]; (Fig. 2).

Clinical investigations are thus indicated to study whether fosfomycin can be added to the small list of antibiotics clinically useful in CGD.

Discussion

Of many antibiotics tested, only 4 have been unequivocally shown to be taken up by normal and CGD neutrophils and to reach the phagocytic vacuole in a functionally active form; TMP/SMX, rifampin, clindamycin, and fosfomycin.

The mode of uptake is either passive, due to lipid solubility, or active, due to energy requiring transport systems.

From the clinical point of view TMP/SMX or in case of sulfonamide allergy TMP/rifampin would be good candidates for longterm antibiotic prophylaxis.

Rifampin and clindamycin would be a reasonable therapeutic strategy against gram negative infections.

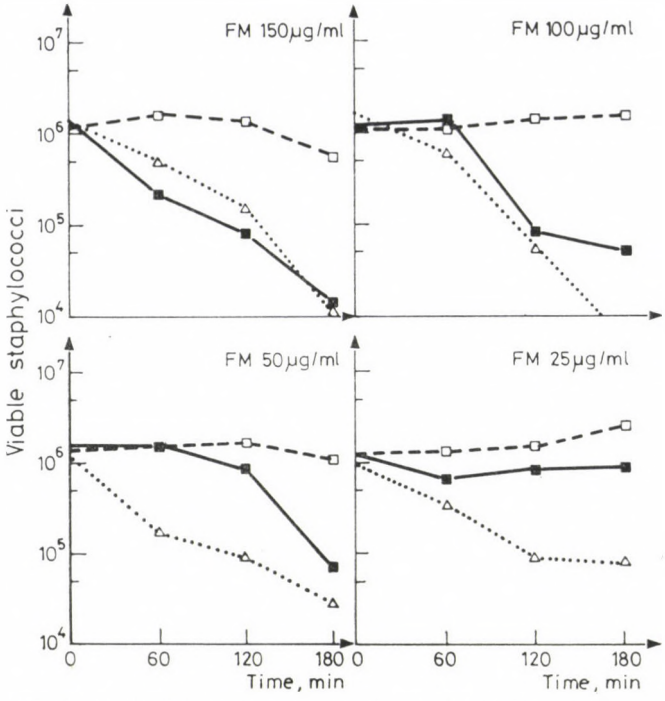

FIG. 2. Effect of fosfomycin (FM; 25—150 µg/ml) on the killing of S.aureus by neutrophils from one patient with CGD □——□ CGD cells without fosfomycin ■——■ CGD cells with fosfomycin, △——△ normal cells without antibiotic for comparison. (4)

Only TMP/SMX has been subjected to clinical trials in CGD patients, [8, 12].

The biggest retrospective study up to date has been performed by Richard Mouy and Claude Griscelli in Paris [8].

The authors found a significant reduction in the frequency and severity of bacterial infections in CGD patients under TMP/SMX-prophylaxis at 50 mg/kg/day. The mean number of infections/year was reduced fivefold from 2 to 0.4, the number of lymphadenitis episodes dropped markedly and liver abscesses and salmonella infections virtually disappeared.

If CGD patients are infected with microorganisms which develop TMP/SMX resistance additional drugs are needed.

One obvious candidate is fosfomycin and clinical trials using this drug should be considered.

It is a promising broad-spectrum antibiotic and has already shown favourable clinical results in individual CGD patients.

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