Influence of Culture Media on the Antistaphylococcal Activity of Fosfomycin

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Antagonism or double zones of inhibition (or both) resulted when Staphylococcus aureus was exposed to fosfomycin in Brain Heart Infusion, trypsic/papacic digest of casein/soybean, and Mueller Hinton culture media; neither occurred with a totally defined, completely synthetic medium. The extent of antagonism was quantitated by determination of the absolute minimal inhibitory concentration of fosfomycin in each medium. By using the synthetic medium and inducers, the hexose-phosphate transport system appeared to be more important than the \( \alpha \)-glycerophosphate transport system in providing ingress of fosfomycin into staphylococci.

Fosfomycin, formerly known as phosphonomycin, appears to gain access into bacterial cells by way of both the \( \alpha \)-glycerophosphate and the hexose transport systems (2, 4). The relative importance of these pathways has not been weighed although it is a matter of importance to susceptibility testing in vitro. Small quantities of glycerol and glycerophosphate, hexoses and hexosephosphates may act as inducers of these transport enzymes, facilitating the expression of fosfomycin-mediated antibiotic effect by assuring entry of the drug into the cell. However, such substances, if present in high concentrations, will also be competitive with fosfomycin for transport into the cell. Moreover, NaCl may be antagonistic to fosfomycin (4). Since inducers, competitors, and NaCl are present in most culture media, the apparent activity of fosfomycin might vary considerably depending on the culture medium employed for susceptibility testing.

MATERIALS AND METHODS

Four kinds of agar culture media were used: Brain Heart Infusion (BHI, Difco); trypsic/papacic digests of casein/soybean (TSB, BioQuest Laboratories, Baltimore, Md.); Mueller Hinton (MH, Difco); synthetic amino acid medium [SAAM, compounded as described previously (3)]. Each medium was inculated while molten (at 45°C) with Staphylococcus aureus (strain HLR82, kindly supplied by Stanley Gould, Hoffman-La Roche, Inc., Nutley, N.J.) to give a final density of \( 10^8 \) cocci per ml just before distribution.

For qualitative study, each medium was added to a quadrant of polystyrene petri dishes (82 mm diameter, built-in dividers, Falcon Plastics Division of BioQuest Laboratories, Baltimore, Md.). After gelling, wells 7 mm in diameter were cut in the center of each quadrant with a sterile cork borer. One-tenth micro-mole of fosfomycin (18.2 \( \mu \)g of disodium fosfomycin, kindly supplied by E. L. Foltz, Merck, Sharpe and Dohme, Inc., Rahway, N.J.), dissolved in sterile distilled water, was added to each well. Incubation at 37°C was continued for 24 hr.

For quantitative study, plates were prepared with seeded culture media, as above; three wells, 7 mm in diameter, were cut in each plate, leaving about 35 mm between wells. Each of the several concentrations of fosfomycin under study was applied in quintuplicate. The radii of zones of inhibition (less the radius of the well) were read after 24 hr of incubation at 37°C. The square of the average value for a given concentration was plotted against the log of that concentration to enable construction of a straight line. Extrapolation to zero zone size yielded the absolute minimal inhibitory concentration, the \( m' \) value (1).

Similarly, quantitative evaluation was made of the effect of inducers of the \( \alpha \)-glycerophosphate and hexose transport systems on the antistaphylococcal activity of fosfomycin. SAAM was used since the basal concentrations of inorganic phosphate (2 mm) and hexose (\( \beta \)-glucose, 1.4 mm) are specified. The \( m' \) values were determined for fosfomycin dissolved in water and 0.5 mm solutions of either \( \beta \)-glucose-6-phosphate (alone, with 0.5 mm \( \alpha \)-glycerophosphate, or with 0.5 mm glycerol), \( \alpha \)-glycerophosphate, or glycerol.

RESULTS

Fosfomycin appeared to be about as active in SAAM as in MH but there was a double zone of inhibition in MH. It was less active in TSB, again giving a double zone of inhibition, and it was almost without effect in BHI (Fig. 1).

The absolute minimal inhibitory concentrations were confirmatory of the qualitative quadrant plate assessment of relative activity, according to
Fig. 1. Effect of synthetic amino acid medium (SAAM), Mueller Hinton (MH), Brain Heart Infusion (BHI), and trypsin/pepsin digests of casein/soybean (TSB) on the antistaphylococcal activity of fosfomycin.

Fig. 2. Absolute minimal inhibitory concentration of fosfomycin for Staphylococcus aureus HLR 82 was determined by the method of Cooper (1963) in synthetic amino acid medium (SAAM), Mueller Hinton (MH), Brain Heart Infusion (BHI), and trypsin/pepsin digest of casein/soybean (TSB) media.

culture medium (Fig. 2). In descending order: BHI [1.95 µmole (355 µg of disodium fosfomycin) per ml]; TSB [0.26 µmole (47.3 µg of disodium fosfomycin) per ml]; SAAM [0.19 µmole (34.6 µg of disodium fosfomycin) per ml]; MH [0.18 µmole (32.8 µg of disodium fosfomycin) per ml].

The antistaphylococcal activity of fosfomycin was significantly increased in the presence of D-glucose-6-phosphate (Fig. 3). Neither α-glycerophosphate nor glycerol had significant effect when exhibited alone (curves d and e compared with f). However, α-glycerophosphate clearly augmented D-glucose-6-phosphate (curve a compared with c), whereas glycerol added nothing to the effect of D-glucose-6-phosphate (curves b and c).

DISCUSSION

Since fosfomycin displayed some activity in each of the four media that were tested, determination of the absolute minimal inhibitory concentration for S. aureus HLR 82 in each medium was of considerable value to comparative assessment. The greater activity of fosfomycin in
TABLE 1. Concentrations of inorganic phosphate and glucose in synthetic amino acid medium (SAAM), Mueller Hinton (MH), Brain Heart Infusion (BHI), and tryptic papaic digest of casein/soybean (TSB) media

| Addition       | Conc in culture medium (mM) |
|----------------|-----------------------------|
|                | SAAM | MH  | BHI | TSB |
| Inorganic phosphate | 2   | <5  | 17.2| 14.4|
| Glucose        | 1.4  | 11  | 13.9|

*Values given for MH, BHI, and TSB were calculated from the manufacturer's formulas. They must be minimal values since additional hexoses, inorganic, and organic phosphates are surely contributed by the hydrolysates of proteins and infusates used in compounding these media.

SAAM and MH could result either from enhancement in these media or from suppression of activity in the other culture media. The latter possibility appears to be most reasonable since both phosphate and glucose are present in much higher concentrations in BHI and TSB than in SAAM or MH (Table 1).

Fosfomycin is an inhibitor of cell wall synthesis (1). It is tempting, therefore, to speculate that salts might appear to antagonize fosfomycin through stabilization of cell wall-defective bacteria. However, none of the four culture media that were studied is hypertonic.

From these data, it appears that the hexose transport system is the more important, the more efficient pathway for moving fosfomycin into *S. aureus*. The α-glycerophosphate transport system does also convey fosfomycin into staphylococci for there was a significant additive effect from simultaneous activation of both transport systems.

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