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In-vitro antibacterial activity of noxythiolin and taurolidine

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Abstract—The minimum inhibitory concentrations (MIC) of noxythiolin and taurolidine were determined for strains of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Tests were performed in broth alone and in broth plus 25% v/v serum or 25% v/v urine. Inoculum density was either 10^3, 10^5 or 10^7 colony forming units per mL^{-1}. Slight inoculum-dependent variation in the activity of both agents was observed for some, but not all, strains of *P. aeruginosa* and *S. aureus*. A more pronounced medium-dependent increase in activity was observed with both drugs, with up to 8-fold reduction of values for MIC when tested in the presence of serum or urine. These observations may help to clarify the disparity between the observed clinical efficacy of these agents and relatively poor in-vitro activity when tested using conventional methods in synthetic media.

Noxythiolin (*N*-methyl-*N'*-hydroxymethylthiourea) (Noxyflex S. Geistlich Sons Ltd, Chester), and the related compound taurolidine (bis [1,1-dioxoperhydro-1,2,4-methylene thiadiazinyl-4]methane) (Taurolin, Geistlich Sons Ltd, Chester, UK), are broad spectrum antimicrobials with activity against Gram-negative and Gram-positive bacteria and fungi (Reeves & Schweitzer 1974; Gorman et al 1985).

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The clinical efficacy of noxythiolin (Browne & Stoller 1970) and of taurolidine (Browne et al 1977) correlates poorly with the high minimum inhibitory concentrations (MIC) observed for commonly occurring bacterial pathogens (Reeves & Schweitzer 1974; Browne et al 1977; Brearley & George 1980). Conventional techniques used for the in-vitro study of antibiotic action may not fully reflect the in-vivo activity of these agents. In this report, the in-vitro activity of noxythiolin and taurolidine, determined in broth alone, is compared with the activity observed in the presence of serum or urine.

Materials and methods

Serial two-fold dilutions of noxythiolin and taurolidine were prepared over the range 16384 to 2 mg L^{-1} in nutrient broth (CM1, Oxoid Limited, Basingstoke) and dispensed in 100 μL volumes to sterile microdilution trays. Separate broth blanks containing no test drug were also prepared for each test strain.

Inocula were prepared by diluting overnight broth cultures of *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662, together with five clinical isolates of each of these species. For noxythiolin, inocula were adjusted to give final inoculum densities of 10^3, 10^5 and 10^7 colony forming units (cfu) mL^{-1}. For taurolidine the inoculum density was 10^5 cfu mL^{-1}. Samples of each inoculum suspension (100 μL) prepared in nutrient broth, were added to each well of the microdilution trays.

All tests, performed in duplicate, were incubated in air at 37°C for 18 h. The minimum inhibitory concentration (MIC) was defined as the lowest dilution showing no visible growth after this time.

To evaluate possible enhancement of antimicrobial activity in the presence of body fluids, the MIC of both agents was again determined for all test strains but with the addition of serum or urine to nutrient broth. For this, broth used for the preparation of drug dilutions and inocula was supplemented with 25% v/v pooled sterile human serum. For noxythiolin only, the determination of MIC values was repeated using broth supplemented with 25% v/v pooled sterile human urine also. All other test conditions were as described above.

Results

With a standard inoculum density of 10^5 cfu mL^{-1}, the MIC values of noxythiolin for the reference strains of *E. coli* and *P. aeruginosa* were 1024 mg L^{-1}. Values for MIC of the corresponding clinical isolates (*E. coli*, mean 1024, range 512–2048 mg L^{-1}; *P. aeruginosa*, mean 1024, range 1024–2048 mg L^{-1}) were similar to those for the reference strains. For all species, the values for minimum bactericidal concentration were within one dilution of the corresponding MIC.

The activity of taurolidine (*E. coli*, mean MIC 1024, range 1024–2048 mg L^{-1}; *P. aeruginosa*, mean MIC 2048, range 2048–4096 mg L^{-1}) was essentially comparable to that of noxythiolin for each clinical isolate and reference strain tested. With *S. aureus* NCTC 6571, the MIC values for noxythiolin and taurolidine were 256 mg L^{-1} with similar values obtained for all clinical isolates (noxythiolin and taurolidine; mean MIC 512 range 256–1024 mg L^{-1}).

Adjustment of inoculum density to 10^3 or 10^7 cfu mL^{-1} resulted in no variation in values for MIC, which were within one dilution of values obtained with an inoculum density of 10^5 cfu mL^{-1}. However, with two of five clinical isolates of *P. aeruginosa* and one strain of *S. aureus*, modest reduction of MIC, by one to two dilutions, was observed at an inoculum density of 10^3 cfu mL^{-1}.

For all strains tested, growth in broth supplemented with 25% v/v serum or 25% v/v urine was comparable to growth in broth alone. The addition of 25% v/v serum or 25% v/v urine resulted in no significant change in values for MIC of noxythiolin when tested with inocula of 10^5 and 10^7 cfu mL^{-1}. By contrast, when tested with an inoculum of 10^3 cfu mL^{-1}, the addition of 25% v/v serum or urine resulted in 4-fold reduction of noxythiolin MIC for *E. coli* and *P. aeruginosa*, when compared with results obtained using broth alone. For *S. aureus*, up to 8-fold reduction of MIC was observed (Table 1). Similarly, the addition of serum resulted in 4-fold reduction in values for the MIC of taurolidine for all strains tested. As for noxythiolin, this effect was only apparent when tested with an inoculum density of 10^3 cfu mL^{-1}.

Table 1. Effect of serum on the activity of noxythiolin and taurolidine.

	Minimum inhibitory concentrations (mg L^{-1})	
	Broth alone*	Broth plus 25% serum*
Noxythiolin		
S. aureus	256–1024	64–256
E. coli	512–2048	128–512
P. aeruginosa	1024–2048	256–1024
Taurolidine		
S. aureus	256–1024	128
E. coli	1024–2048	256–512
P. aeruginosa	2048–4096	512–1024

* MIC range, mg L^{-1}. Inoculum 10^3 cfu mL^{-1}.

Discussion

In broth alone and irrespective of the inoculum density used, the MIC of noxythiolin for all species was at least 5 times below the minimum concentration (1% w/v) recommended for clinical use, thus confirming the potential value of this agent as a broad spectrum antimicrobial. Modest inoculum-dependent reduction of the MIC of noxythiolin was observed for some strains of *P. aeruginosa* and *S. aureus* when inocula were reduced to 10^3 cfu mL^{-1}. This is in concordance with the findings of Horsfield (1967) who reported 4-fold, or greater, reduction of MIC for strains of *Proteus morganii* and *Proteus rettgeri*, although not for *Klebsiella aerogenes*, when tested using a reduced inoculum density.

With inocula of 10^5 or 10^7 cfu mL^{-1}, the presence of serum or urine did not result in alteration of values for the MIC of noxythiolin or taurolidine. By contrast, Brearley & George (1980) suggested that the rate of bacterial killing by noxythiolin was markedly reduced in the presence of 10% blood, an observation apparently at variance with the results reported here. However, these workers observed the initial killing rate with blood was identical to that in blood-free controls. Only 1% of the original inoculum remained viable for periods not exceeding 2 h after drug addition. Beyond this time no difference in activity was observed. Thus, although the killing rate of noxythiolin in the presence of blood or serum may be slightly prolonged this would not result in alteration of values for MIC when tested using conventional techniques requiring overnight incubation.

With a reduced inoculum, the addition to broth of either serum or urine enhanced the apparent activity of noxythiolin by approximately 4- to 8-fold. Similar medium-dependent enhancement of activity was also observed with taurolidine. With neither agent were these effects apparent with inoculum densities of 10^5 or 10^7 cfu mL^{-1}.

The results obtained indicate that the apparent antibacterial activity of noxythiolin and taurolidine may be significantly enhanced in the presence of serum or urine. With some strains of *P. aeruginosa* and *S. aureus* an inoculum-dependent effect is also apparent. Although the mechanism for these effects is not known, this observation may further explain the disparity between the recognised clinical efficacy of these agents yet apparently high value for MIC when determined in broth alone.

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