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J. Pharm. Pharmacol. 1988, 40: 509–511
Communicated January 4, 1988

Sustained anti-adherence activity of taurolidine (Taurolin) and noxythiolin (Noxyflex S) solutions

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Abstract—Taurolidine (2% w/v) and noxythiolin (1% w/v and 2·5% w/v) solutions inhibit the adherence in-vitro of *Escherichia coli* and *Staphylococcus aureus* to human epithelial and fibroblast cells. This effect, demonstrable after 30 min exposure of cells to test drugs, persists after removal of the active compound. Significantly reduced adherence of bacteria is apparent for 5 h after taurolidine treatment and for 6 h after treatment with 2·5% noxythiolin. Anti-adherence activity of taurolidine and noxythiolin may contribute to the observed clinical efficacy of these agents.

Taurolidine (Taurolin, bis-(1,1-dioxoperhydro-1,2,4-methylene thiadiazinyl-4) methane) is a novel antimicrobial for local or parenteral use. It has a unique spectrum of antimicrobial activity including Gram-positive and Gram-negative bacteria and fungi (Reeves & Schweitzer 1974; Browne et al 1977). Clinical experience suggests that the compound has useful activity in-vivo when administered by the intravenous (Nitsche et al 1985) or intraperitoneal (Browne et al 1978) routes. Of particular interest are observations of significant neutralizing activity against endotoxin in-vitro (Thomas et al 1985; Blenkharn 1987b) and against endotoxaemia in animals (Pfirrmann & Leslie 1979) and man (Nitsche et al 1985).

Recent reports have shown that taurolidine (Gorman et al 1987), and the related compound noxythiolin (Gorman et al 1986), exhibit marked anti-adherence properties in-vitro. The clinical significance of these observations remains uncertain. However, the ability to interfere with adherence of pathogenic micro-organisms to host cells may contribute significantly to the clinical efficacy of these agents. With both compounds, pretreatment of urinary or buccal epithelia, or test organisms (*Staphylococcus saprophyticus*, *Escherichia coli* and *Candida albicans*), significantly inhibits organism/cell adherence when compared with water or 0·015% *N*-methylthiourea. The extent of this apparent anti-adhesive activity, particularly with regard to the treatment intervals for these drugs, has not previously been defined and is the purpose of this report.

Materials and methods

Cell harvest and preparation. Exfoliate epithelial cells were harvested from the pooled 12 h bile drainage of two patients having external biliary decompression following surgery for choledocholithiasis. Urine from three healthy adult females was similarly pooled for cell harvest. Samples of bile (1700 mL) and urine (2400 mL) were centrifuged at 700 *g* for 60 min. The resultant cell pellets were washed six times in RPMI 1640 medium.

Cells were trypsinized by the addition of 2 mL 0·05% trypsin and 0·02% disodium EDTA in Hanks' balanced salts solution. Suspensions were shaken gently and after 15 min incubation at 37 °C diluted with an excess of RPMI 1640. After centrifugation at 500 *g* for 10 min, cell pellets were washed three times with, and finally suspended in, Eagle's minimal essential medium (MEM) containing 10% newborn calf serum.

The established HEp2 line of epithelial cells, derived from a human laryngeal carcinoma, and the MRC 5 line of human embryonic lung fibroblasts were also examined. HEp2 cells were grown in Earle's MEM supplemented with Hanks' balanced salts solution (BSS), 15% calf serum and 2 mM glutamate. For MRC 5 cells, monolayers were grown in Eagle's MEM supplemented with Eagle's BSS, 10% foetal bovine serum, 2 mM glutamate and 1% non-essential amino acids. Monolayers of

Table 1. The anti-adherence effect of taurolidine and noxythiolin solutions for biliary and urinary epithelia. (Standard deviations in parentheses.)

	Mean adherent bacteria per cell after 30 min treatment (10 replicates)							
	Saline control		2·0% taurolidine		1·0% noxythiolin		2·5% noxythiolin	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Cell source								
Bile	41·6 (2·6)	12·5 (1·1)	6·7 (0·3)	4·8 (0·6)	26·9 (3·0)	7·9 (0·8)	6·9 (0·3)	6·9 (1·5)
Urine	35·1 (2·9)	17·4 (2·5)	2·9 (0·5)	3·4 (0·7)	19·4 (6·0)	9·0 (1·8)	5·1 (0·3)	5·1 (1·2)
			$P<0·005$	$P<0·01$	$P<0·05$	$P<0·05$	$P<0·02$	$P<0·05$

approximately 25 cm^2 were trypsinized as above and washed 3 times with Eagle's MEM containing 10% newborn calf serum. Each test cell preparation was assessed for viability, using trypan blue exclusion, and sterility and adjusted to contain approximately 1×10^5 cells mL^{-1}.

Test organisms. An isolate of *E. coli* from the urine of a female with recurrent urinary tract infection and a strain of *Staphylococcus aureus* from an infected surgical wound were used together with the corresponding reference strains *E. coli* NCTC 10418 and *S. aureus* NCTC 6571.

For adherence assay, overnight nutrient broth cultures (Oxoid Limited, Basingstoke, UK) were subcultured to fresh prewarmed broth and incubated at 37 °C for 2 h in a shaking water bath. Inoculum suspensions were adjusted to contain approximately 5×10^5 colony forming units mL^{-1} (cfu mL^{-1}).

Taurolidine: Taurolidine, batch 40499/6, was prepared as a 2·0% w/v solution in 0·85% saline and used 60 min after reconstitution. Biliary and urinary epithelial cells, and HEp2 and MRC 5 cell suspensions, were treated by addition of 2 mL test solution to pellets of 2×10^5 cells. These were immediately mixed and incubated, with gentle agitation, at 37 °C for 30 min. After treatment, cells were deposited by brief centrifugation, washed three times and re-suspended in pre-warmed Eagle's MEM supplemented with 10% newborn calf serum.

Noxythiolin. 1·0% w/v and 2·5% (w/v) noxythiolin solutions (Noxyflex S, *N*-methyl-*N'*-hydroxymethylthiourea), batch 800637, were prepared in 0·85% NaCl and used 60 min after reconstitution. Cell suspensions were treated for 30 min with solutions of either concentration and washed to remove test drug as described for taurolidine.

Adherence assay. To evaluate the duration of anti-adherence effect of taurolidine and noxythiolin, treated cells in prewarmed drug-free Eagle's MEM containing 10% newborn calf serum were held at 37 °C for up to 6 h. At hourly intervals commencing immediately after taurolidine or noxythiolin treatment, aliquots were withdrawn for adherence assay.

Organism/cell adherence was evaluated by addition of equal volumes of organism suspension to taurolidine- or noxythiolin-treated cell suspensions. Control cell preparations were treated with 0·85% NaCl. Blank cell preparations, not challenged with test organisms, were also examined. Test, control and blank preparations were run in duplicate. Organism/cell suspensions were incubated at 37 °C for 1 h, with gentle agitation, after which approximately 0·1 mL volumes were transferred to glass slides. After air-drying and methanol fixation, slides were stained using Wright-Giemsa stain. Slides were examined for adherent bacteria at × 1200 magnification. Bacteria associated with cells in ten randomly selected high power fields were counted, the results being expressed as number of adherent bacteria per cell.

Results and discussion

Background counts of adherent bacteria to untreated biliary and urinary epithelia averaged < 1·0 organism per cell. Mean numbers of *E. coli* adherent to saline-treated biliary epithelium was 41·6 (s.d. 2·6) bacteria per cell and to urinary epithelium 35·1 (s.d. 2·9) per cell (Table 1). 30 min exposure to 2·0% taurolidine before immediate challenge significantly reduced the adherent bacteria per cell (Mann Whitney U test, $P<0·005$). No differences were observed between wild strain *E. coli* and the corresponding NCTC strain. Total numbers of adherent *E. coli* NCTC 10418 cells were reduced by approximately 50% compared with the wild strain. *S. aureus* adherence to exfoliate epithelia was less than with *E. coli*. However, both agents achieved significant reduction of adherent staphylococci compared with controls. The NCTC strain of *S. aureus* showed vastly reduced adherence to all cell types in this study compared with the clinical isolate or with *E. coli* and the number per untreated cells was insufficient for statistical analysis. Inhibition of adherence to HEp2 (epithelial) and MRC 5 (fibroblast) cells appears similar to that for exfoliate epithelia. However, after 30 min drug exposure excessive cell death, by trypan blue exclusion testing, prevented further study. This is in concordance with the previously reported selective toxicity of these agents toward cells exhibiting degrees of neoplasticity (Blenkharn 1987a). No such adverse effect was observed with either taurolidine or noxythiolin treatment of exfoliate biliary or urinary epithelia.

The duration of inhibition of adherence was evaluated by treatment of cells with test drugs followed by a period of up to 6 h in drug-free medium. With *E. coli* as indicator, significant reduction of adherent bacteria ($P<0·05$) was observed for up to 4 h with 2·0% taurolidine and 2·5% noxythiolin and 2 h with 1·0% noxythiolin. No differences were observed between biliary or urinary epithelia. Using staphylococci, the duration of anti-adherence effect was 5 h for taurolidine, 4 h with 1·0% noxythiolin and 6 h with 2·5% noxythiolin. Evaluation of taurolidine and noxythiolin anti-adherence activity using HEp2 and MRC 5 cells appears comparable with exfoliate epithelia but excess cell death prevented detailed analysis using these cells.

Adherence to cell surfaces may have a fundamental role in the pathogenesis of many bacterial infections. The capacity for adherence in-vitro has been shown with Gram positive and Gram negative species (Almeida & Jorgensen 1984; Svanborg-Eden et al 1977). In-vivo, adherence is difficult to demonstrate but appears related to the severity of infection (Svanborg-Eden et al 1976). Results of the present study confirm the anti-adherence capacity of taurolidine and noxythiolin. Additionally, delayed challenge after removal of test drug highlights a sustained effect which, for noxythiolin, may be dose-dependent.

Noxythiolin may be instilled, and retained for 30–60 min, in the bladder for treatment of infection (British National Formulary 1987). Additionally, it is used for intermittent peritoneal

irrigation as an adjunct to surgical treatment of bacterial peritonitis (Pickard 1972). Taurolidine is administered intravenously for treatment of systemic sepsis (Pfirrmann & Leslie 1979) but may be substituted for noxythiolin in peritonitis where it offers enhanced local and systemic antibacterial and anti-endotoxin activity (Krawzak & Stremmel 1987).

The anti-adherence activity of both agents may contribute to their observed clinical efficacy. The effect is apparent with epithelia of different origins and may be valid for fibroblasts also. The duration of effect at full dosage suggests an apparently sustained activity. The effect of interaction of either agent with blood and other body fluids, and the effect of dilution, have not been separately examined. However, the results suggest that, in-vitro, the anti-adherence properties of taurolidine and noxythiolin may be more extensive than previously recognized (Gorman et al 1986, 1987). It is possible that this activity may be manifest in-vivo also, for significant periods throughout the treatment interval, and may account for the high clinical efficacy observed in situations where theoretical considerations suggest that antibacterial activity alone would be unlikely to afford significant protection (Khan et al 1987).

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