Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1867–1870, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Experimental observations on the bacteriological controls of antibiotics — II. Inactivation of the antimicrobial activity of membranes employed for the filtration of antibiotics

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Keywords: *Membrane filtration; bacteriological controls; antibiotics inactivation.*

Introduction

Previous research in the authors' laboratories demonstrated that the membranes employed for the filtration of antibiotic posses antibacterial activity and can interfere with the growth of micro-organisms. Washing with 300 ml of peptone water does not generally eliminate antibacterial activity. In this work the effects of two techniques that can be used for the inactivation of antibiotic activity have been investigated: washing the membranes with different volumes of peptone water; treatment of the membranes with inactivating substances.

Experimental

Antibiotics

The following antibiotics were filtered on 0.22-μm membrane filters with a hydrophobic surface: oxacillin (1 g), amoxycillin (875 mg) + clavulanic acid (125 mg), ceftriaxone (1 g), rolitetracycline (275 mg), chloramphenicol (250 mg), tobramycin (100 mg), erythromycin (500 mg), vancomycin (500 mg), rifamycin SV (90 mg), aztreonam (1 g) and ofloxacin (300 mg).

Washing fluid

Various volumes of peptone water were filtered through the membranes: 300, 500, 750 and 1000 ml.

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.

Inactivating substances

The efficacy of enzymes able to neutralize beta-lactam antibiotics has been examined. Beta-lactamase from *Bacillus cereus* was inoculated on the membrane and after storage at 37°C for 30 min solutions were filtered. The membranes were then washed with 50 ml of peptone water.

Evaluation of the antibacterial activity of the membranes

The antibacterial activity of the membranes used for antibiotic filtration, treated with various volumes of peptone water or beta-lactamase, was determined by the following techniques: (a) Transfer of the membrane to the surface of Mueller Hinton agar plates inoculated with sensitive micro-organisms (*Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922). After incubation at 37°C for 16–18 h, the inhibition zones were measured. (b) Filtration of 1 ml of micro-organism suspension (about 100 cfu ml^{-1}) and transfer of the membrane to the surface of Tryptic soy agar plates. The bacterial count was effected after incubation at 37°C for 3 days and compared with the bacterial count of untreated membranes.

Results

The results obtained with the washing technique are reported in Tables 1 and 2; those for the enzymatic inactivation method are shown in Table 3.

The results demonstrate that washing with large volumes of peptone water reduces but never eliminates the antibacterial activity of the membranes. The reduction in the inhibition zones depends upon the antibiotics tested (Table 1).

Interference with microbial growth is 81–100% with 300 ml of washing fluid, 74–100% with 500 ml, 55–100% with 750 ml and 20–100% with 1000 ml of fluid (Table 2).

Enzymatic inactivation of the beta-lactam antibiotics results in complete disappearance of the inhibition zones and eliminates the negative influence on microbial growth subsequent to filtration (Table 3).

Conclusions

The washing of membranes used for antibiotic filtration reduces their antimicrobial

Table 1
Influence of various volumes of washing fluids on the inhibition zones (mm) induced by membranes employed for antibiotics filtration

No.	Antibiotic	*Staph. aureus* 300 ml	500 ml	750 ml	1000 ml	*E. coli* 300 ml	500 ml	750 ml	1000 ml
1	Oxacillin	13.5	10	7	3				
2	Amoxycillin + clavulanic acid	2	2	1.5	2				
3	Ceftriaxone	2	2	1	0				
4	Rolitetracycline	7.5	6	4	3	6	5	2.5	1
5	Chloramphenicol					9.5	8	4.5	2
6	Tobramycin					5.5	5.5	5	4
7	Erythromycin	13.5	10.5	6	2				
8	Vancomycin	16	9	4	1.5				
9	Rifamycin SV	9.5	9.5	8	7				
10	Aztreonam					14	9	5	2
11	Ofloxacin	10.5	10	9.5	8				

Table 2
Influence of various volumes of washing fluids on growth inhibition in membranes employed for antibiotics filtration

No.	Antibiotic	*Staph. aureus.* cfu on Tryptic Soy Agar					*E. coli* cfu on Tryptic Soy Agar				
		Suspension cfu	300 ml	500 ml	750 ml	1000 ml	Suspension cfu	300 ml	500 ml	750 ml	1000 ml
1	Oxacillin	105	3 (−97%)	5 (−95%)	36 (−66%)	84 (−20%)					
2	Amoxycillin + clavulanic acid	102	0 (−100%)	0 (−100%)	4 (−96%)	11 (−89%)					
3	Ceftriaxone	110	21 (−81%)	28 (−74%)	49 (−55%)	85 (−23%)	86	0 (−100%)	3 (−96%)	13 (−85%)	20 (−77%)
4	Rolitetracycline						114	0 (−100%)	0 (−100%)	1 (−99%)	3 (−97%)
5	Chloramphenicol						98	0 (−100%)	1 (−99%)	6 (−94%)	10 (−88%)
6	Tobramycin										
7	Erythromycin	107	0 (−100%)	0 (−100%)	0 (−100%)	0 (−100%)					
8	Vancomycin	116	0 (−100%)	2 (−98%)	30 (−74%)	48 (−59%)					
9	Rifamycin SV	95	0 (−100%)	0 (−100%)	2 (−98%)	2 (−98%)	104	1 (−99%)	5 (−95%)	13 (−87%)	28 (−73%)
10	Aztreonam										
11	Ofloxacin	102	0 (−100%)	0 (−100%)	6 (−94%)	8 (−92%)					

Table 3
Influence of the beta-lactamase treatment on the membranes used for antibiotics filtration on the appearance of inhibition zones and on the growth of *Staph. aureus*

No.	Antibiotic	Inhibition zones (mm) on Mueller Hinton agar inoculated with *Staph. aureus*	Suspension cfu	cfu on Tryptic Soy Agar
1	Oxycillin	—	105	110 (+5%)
2	Amoxycillin + clavulanic acid	—	102	98 (−4%)
3	Ceftriaxone	—	110	107 (−3%)

activity; this reduction is greater as the volume of fluid is increased. Total removal of the antibiotic activity is not possible. The residual antimicrobial activity can interfere with the growth of micro-organisms. The washing technique with large volumes of fluid is slow and difficult in the case of some antibiotics.

The beta-lactam antibiotics can be submitted to filtration, if this technique is accompanied by treatment of the membranes with beta-lactamase. The enzymatic treatment results in the disappearance of the inhibition zones and does not inhibit bacterial growth.

Complete elimination of the antibacterial activity of the other groups of antibiotics presents many difficulties; only a few substances are able to neutralize the biological activity of antibiotics; these substances must preserve the viability of the micro-organisms. Thus it is necessary to find other inactivating substances; the need is important and urgent. An alternative is the development of new filtering materials that do not retain antibiotic molecules.

[Received for review 16 May 1989; revised manuscript received 20 July 1989]