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*Short Communication*

# Research on sterility and contamination controls of chemotherapeutic agents by membrane filtration method

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## Introduction

According to the guidelines of the European Pharmacopoeia, sterility tests (for injectable drugs, eye drops and lotions) and microbial contamination tests (for non-injectable drugs) on chemotherapeutic pharmaceuticals must be conducted only after the elimination of antimicrobial activity.

The preferred method for the elimination of antimicrobial activity is membrane filtration of the drug solution followed by separate washing of the membrane three times with sterile peptone water (100 ml). Other techniques can be used, such as neutralization and dilution, but these methods are of limited application.

Unfortunately the results that are obtained with the membrane filtration method can be affected by the phenomenon of adsorption of antimicrobial substances (antibiotics) onto the membrane surface, as reported by Lagodsky [1], Desbordes [2] and Wagman *et al.* [3].

Investigations in the authors' laboratories [4] have been applied to many classes of antibiotics (amoxycillin + clavulanic acid, aztreonam, ceftriaxone, chloramphenicol, erythromycin, oxacillin, rifamycin SV, rolitetracycline, tobramycin, vancomycin); membrane filtration and washing resulted in the appearance of zones of inhibited microbial growth and in a decrease (81–100%) in colony-forming units (CFU). In addition, antibiotic was released into the culture broth, in which the membranes had been immersed, in 54.5% of the cases and there was subsequent growth inhibition of the contaminant micro-organisms present on their surface.

The aim of the present work was to verify also that chemotherapeutic agents can induce analogous undesirable effects.

## Experimental

*Chemotherapeutic agents*

The following 16 chemotherapeutic agents were subjected to membrane filtration: *p*-aminosalicylic acid (1500 mg), dapsone (100 mg), ethambutol (1200 mg), furazolidone (100 mg), ketoconazole (200 mg), isoniazid (300 mg), metronidazole (250 mg), nalidixic acid (500 mg), nitrofurantoin (50 mg), oxolinic acid (500 mg), pefloxacin (400 mg), pipemidic acid (400 mg), sulphaguanole (400 mg), sulphamethoxypyridazine (500 mg), sulphathiazole (1000 mg), and trimethoprim (160 mg) + sulphamethoxazole (800 mg).

*Membranes*

Membranes (0.22–0.45 μm) with a hydrophobic surface of various diameters were used.

*Antimicrobial activity of membranes after filtration and washing*

The determination of such activity was carried out by four methods.

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*Method 1.* The membrane was transferred onto the surface of Mueller Hinton agar plates previously inoculated with bacterial strains, as well as onto the surface of tryptic soy agar plates inoculated with fungi. In order to obtain clear and reliable results, two layers of medium, one deep and the other superficial were inoculated in the Petri dishes.

The bacterial suspension was added only in the superficial layer. After incubation of plates for 18 h at 37°C in the case of Mueller Hinton agar and at 22°C for 36 h in the case of tryptic soy agar inhibition zones were measured.

*Method 2.* The membranes previously used for the filtration and washing of the chemotherapeutic agents were inoculated with a suspension of viable and sensitive bacteria and fungi; the membrane was transferred onto the surface of tryptic soy agar plates after washing with 50 ml of sterile peptone water.

After incubation of the plates for 5 days at 32°C in the case of bacteria and at 22°C in the case of fungi, the colonies developed on the agar plates were counted.

At the same time analogous tests were conducted on agar plates inoculated with fresh membranes subjected to filtration (and subsequent washing) of an identical number of microbial cells in suspension.

**Table 1**
Inhibition zones (mean diameter, mm) produced by membranes after filtration of chemotherapeutic agents and washing with 300 ml of peptone water

| Chemotherapeutic agent | Inhibition zone | | |
|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Candida albicans* |
| *p*-Aminosalycilic acid | 0 | 1 | — |
| Dapsone | 0 | 0 | — |
| Ethambutol | 2 | 0 | — |
| Furazolidone | 8 | 6 | — |
| Ketoconazole | — | — | 9 |
| Isoniazid | 0 | 0 | — |
| Metronidazole | 0 | 0 | — |
| Nalidixic acid | 6 | 11.5 | — |
| Nitrofurantoin | 6 | 5.5 | — |
| Oxolinic acid | 7.5 | 11.5 | — |
| Pefloxacin | 15 | 14.5 | — |
| Pipemidic acid | 6.5 | 13 | — |
| Sulphaguanole | 0 | 0 | — |
| Sulphamethoxypyridazine | 8.5 | 8.5 | — |
| Sulphathiazole | 6 | 6.5 | — |
| Trimethoprim + sulphamethoxazole | 15 | 14 | — |

**Table 2**
Colonies forming units (CFU) and percentage variation of microbial suspensions* used to contaminate membranes washed with 300 ml of peptone water after filtration of chemotherapeutic agents

| Chemotherapeutic agent | *Staphylococcus aureus* | | *Escherichia coli* | | *Candida albicans* | |
|---|---|---|---|---|---|---|
| | CFU | % | CFU | % | CFU | % |
| *p*-Aminosalycilic acid | 0 | −100 | 3 | −96.73 | | |
| Dapsone | 140 | 1.44 | 93 | 1.08 | | |
| Ethambutol | 92 | −33.3 | 87 | −5.43 | | |
| Furazolidone | 0 | −100 | 0 | −100 | | |
| Ketoconazole | | | | | 0 | −100 |
| Isoniazid | 142 | 2.86 | 96 | 4.34 | | |
| Metronidazole | 83 | −37.1 | 90 | −2.17 | | |
| Nalidixic acid | 0 | −100 | 0 | −100 | | |
| Nitrofurantoin | 0 | −100 | 0 | −100 | | |
| Oxolinic acid | 0 | −100 | 0 | −100 | | |
| Pefloxacin | 0 | −100 | 0 | −100 | | |
| Pipemidic acid | 0 | −100 | 0 | −100 | | |
| Sulphaguanole | 0 | −100 | 0 | −100 | | |
| Sulphamethoxypyridazine | 0 | −100 | 0 | −100 | | |
| Sulphathiazole | 0 | −100 | 8 | −91.3 | | |
| Trimethoprim + sulphamethoxazole | 0 | −100 | 0 | −100 | | |

*Concentrations of microbial suspensions were: *Staphylococcus aureus* 138 CFU $ml^{-1}$, *Escherichia coli* 92 CFU $ml^{-1}$ and *Candida albicans* 100 CFU $ml^{-1}$.

The results obtained were compared with those of membranes employed for the filtration process in order to find out possible variations in the CFU number.

*Method 3.* Membranes were artificially contaminated with bacterial and fungal strains, after filtration and washing of chemotherapeutic agents, in 100 ml of fluid thioglycollate medium and 100 ml of tryptic soy broth, respectively, and the growth or absence of growth was determined after incubation for 7 days at 32°C for fluid thioglycollate medium and at 22°C for tryptic soy broth.

*Method 4.* Membranes, after chemotherapeutic substance filtration and washing with peptone water, were placed in fluid thioglycollate medium and in tryptic soy broth and, after 24 h at 32°C, the antimicrobial activity was measured by transfer of a drop of broth on the surface of agar plates.

**Table 3**
Growth inhibition* in culture broths of three microbial strains used to contaminate membranes washed with 300 ml of peptone water after filtration of chemotherapeutic compounds

| Chemotherapeutic agent | Fluid thioglycollate medium | | Tryptic soy broth |
|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Candida albicans* |
| *p*-Aminosalycilic acid | – | – | |
| Dapsone | – | – | |
| Ethambutol | – | – | |
| Furazolidone | + | – | |
| Ketoconazole | | | + |
| Isoniazid | – | – | |
| Metronidazole | – | – | |
| Nalidixic acid | + | + | |
| Nitrofurantoin | + | + | |
| Oxolinic acid | + | + | |
| Pefloxacin | + | + | |
| Pipemidic acid | + | + | |
| Sulphaguanole | – | – | |
| Sulphamethoxypyridazine | – | – | |
| Sulphathiazole | + | – | |
| Trimethoprim + sulphamethoxazole | + | + | |

*Microbial growth inhibition, +; microbial growth, –.

**Table 4**
Antimicrobial properties* towards three microbial strains of culture broths inoculated with filtering membranes used for chemotherapeutic agents and subsequently washed with 300 ml of peptone water

| Chemotherapeutic agent | Fluid thioglycollate medium | | Tryptic soy broth |
|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Candida albicans* |
| *p*-Aminosalycilic acid | – | – | |
| Dapsone | – | – | |
| Ethambutol | – | – | |
| Furazolidone | – | + | |
| Ketoconazole | | | – |
| Isoniazid | – | – | |
| Metronidazole | – | – | |
| Nalidixic acid | – | – | |
| Nitrofurantoin | – | – | |
| Oxolinic acid | +++ | +++ | |
| Pefloxacin | – | – | |
| Pipemidic acid | – | – | |
| Sulphaguanole | – | – | |
| Sulphamethoxypyridazine | – | – | |
| Sulphathiazole | – | – | |
| Trimethoprim + sulphamethoxazole | ++ | ++ | |

*Absence of antimicrobial property, –; low antimicrobial property, +; moderate antimicrobial property, ++; high antimicrobial property, +++.

*Notes on methods.* The experimental conditions in method 2 are analogous to those of contamination controls whereas those in method 3 follow those of sterility controls. All experiments were run twice and the mean result was calculated.

*Micro-organisms*

The micro-organisms used in the tests were: *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922; and *Candida albicans* ATCC 10231. The concentrations of the microbial suspensions used for filtration through the membranes were: *Staphylococcus aureus* 138 CFU $ml^{-1}$, *Escherichia coli* 92 CFU $ml^{-1}$ and *Candida albicans* 100 CFU $ml^{-1}$.

## Results

The results are reported in Tables 1–4. Use of the membranes after filtration of chemotherapeutic solutions and washing resulted in the appearance of inhibition zones in 12 cases out of 16. The width of inhibition zones generally varied from 5.5 to 15.0 mm; in two cases only it was less than 3.0 mm. For dapsone, isoniazid, metronidazole and sulphaguanole there was no evidence of inhibition.

Use of membranes washed according to the European Pharmacopoeia guidelines, resulted in reduction (two preparations) or even complete elimination of CFU (11 preparations). Variations of less than 10%, frequently related to analytical variability, were not taken into account.

Membranes used for the filtration of chemotherapeutic agents caused problems, even after washing with 300 ml of peptone water; the growth of micro-organisms that were used to artificially contaminate membranes, after incubation in liquid culture media, was sometimes inhibited. Growth inhibition was observed in nine out of 16 solutions of chemotherapeutic agents.

The membranes used for the filtration of chemotherapeutic agents imparted antimicrobial properties to the culture media in which they were inoculated in the case of three substances out of 16 tested.

## Discussion

Application of membrane filtration assays to solutions of 16 chemotherapeutic agents showed that in most cases part of the substance was adsorbed onto the membranes. Consequently, the membranes possessed antimicrobial activity that was demonstrated by the presence of inhibition zones as well as by reduction or lack of growth of micro-organisms which came into contact with the membranes during filtration.

The results confirm previous observations [4] on the membrane filtration of antibiotics. Trials of methods of chemical neutralization of antimicrobial properties of membranes used for filtration of chemotherapeutic agents are in progress. If the trials are not completely successful, as in most cases of antibiotics, it will be desirable to study new approaches for solving the problem. However, it is necessary to ensure always that the production of chemotherapeutic agents is carried out according to stringent criteria of asepsis in order to avoid microbial contamination which can only be detected with difficulty.

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