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Synergistic Effect of the Combination Triclosan with 2-Phenylphenol against *Pseudomonas aeruginosa* and Fungi

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Abstract

Triclosan is active against Gram-positive bacteria and against Gram-negative bacteria but less so against *Pseudomonas aeruginosa*. 2-phenylphenol is considered as effective against fungi, however, its antifungal action is more important than its antibacterial activity. The aim of this study is to evaluate the bactericidal and fungicidal activities of triclosan and 2-phenylphenol alone, and in combination against standard strains of bacteria and fungi. The antibacterial activity of the combination triclosan-2-phenylphenol was significantly enhanced over that of each agent used alone against *Pseudomonas aeruginosa*. Synergistic effect was also observed against all tested strains of fungi. This combination can be considered to enhance the antimicrobial activity of the two agents.

Key words: Triclosan, 2-phenylphenol, Antibacterial, Antifungal

Introduction

Triclosan, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, is a broad-spectrum antimicrobial agent, with low activity against *Pseudomonas aeruginosa*. Its efficacy against gram-negative bacteria and yeast can be enhanced by formulation (McDonnell and Russell, 1999). Triclosan is used in many contemporary consumer and professional health care products. These include medicated soaps, surgical scrubs, deodorant products, hand lotion and creams, toothpastes, mouthwashes, and other dermatological formulations (Arweiler *et al.*, 2001; Gaffar *et al.*, 1994; Herbert, 2001; Marchetti *et al.*, 2003). The concentration of triclosan used in most preparations ranges from 0.5-2% (Bending, 1990; Larson, 1995; Webster, 1992). Many recent studies, showed triclosan acts on a defined bacterial target in the bacterial fatty acid biosynthetic pathway, NADH-dependent enoyl-[acyl carrier protein] reductase (FabI) (Heath *et al.*, 1999; Josephine and Martin, 2002; Levy *et al.*, 1999; Ward *et al.*, 1999). The fatty acid biosynthetic (Fab) pathway is an excellent target for antibacterial agents. It plays a pivotal role in providing metabolic precursors for several important cellular functions, including cell wall biogenesis (phospholipids, lipopolysaccharides and lipoproteins) and the synthesis of acylated homoserine lactones required for virulence factor gene expression (Chuanchuen *et al.*, 2001; McMurry *et al.*, 1998). The antimicrobial action of 2-phenylphenol, like that of most phenol derivatives, has a broad-spectrum, is widely used, and has become one of the most important phenolic biocides for application in hospital-type disinfectants, cosmetics, and in industrial preservation. However, its

antifungal action is more important than its antibacterial action (Seymour, 2001). 2-phenylphenol nonspecifically denatures microbial cell wall component and inhibits various enzyme systems, including NADH-oxidase (Lueck, 1980).
In this study, the antibacterial and antifungal activities of the combination of triclosan and 2-phenylphenol were evaluated in accordance with the French Standards (NF) from the Association Française de Normalisation –AFNOR (Hernandez *et al.*, 2000).

Materials and Methods

Microorganisms: Antibacterial activity of the two compounds and combination were tested against: *Staphylococcus aureus* CIP 53.154, *Enterococcus hirae* CIP 5.855. *Pseudomonas aeruginosa* CIP A22 and *Escherichia coli* CIP 54.127. Antifungal activity of the two compounds and combination were tested against: *Candida albicans* IP 1180.79, *Aspergillus versicolor* IP 1187.79, *Penicillium verrucosum var. cyclopium* IP 1231.80, and *Absidia corymbifera* IP 1129.75. All were obtained from the culture collection of the Pasteur Institute, Paris, France.
Chemical agents: Stock solutions were prepared from pure substances of both chemical compounds, triclosan was obtained from (Ciba-Geigy, Greensboro, NC, USA) and 2-phenylphenol was obtained from (Aldrich Chemical Company, Inc, Milwaukee, USA). Both agents were solubilized in 95% ethanol, at double concentrations, and then diluted to give a final concentration of 1% in the reaction mixture for each agent tested alone and 0.5% of each agent in the combination. All solutions were used within 2 h of preparation.
Biocidal activity: The bactericidal and fungicidal activities were assessed by means of AFNOR guidelines. Preliminary test was carried out to validate the method and prove the efficacy of the neutralizing solution (3% polysorbate 80 v/v, 0.4% w/v sodium lauryl sulfate, and 0.3% lecithin w/v).
Bactericidal activity: For testing chemical disinfectants against vegetative bacteria the quantitative suspension test involving dilution-neutralization was used (NF T 72-150). Homogeneous suspensions of $3x10^8$ cfu/ml of each test strain were prepared. The suspension (1ml) was pipetted into a tube containing 4 ml distilled water and after 5 min at 23°C; 5 ml disinfectant prepared at double concentration was added. After 5 min contact at 23°C, 1 ml of the test mixture was pipetted into a tube containing 9 ml of neutralizer. After 10 min neutralization at 23°C, two samples of 1ml of the mixture were transferred into separate Petri dishes and 15ml of melted medium was added (2.5 g/L yeast extract, 1 g/L glucose, 5 g/L tryptic peptone of casein and 15 g/L agar).
Fungicidal activity: For testing chemical disinfectants against fungi the quantitative suspension test involving dilution-neutralization was used (NF T 72-200). The procedure was as described for bactericidal activity except for a contact time of 15 min at 23°C. The recovery medium was 5 g/L of yeast extract, 20 g/L of glucose and 15 g/L of agar.

Results

Preliminary tests were directed towards a search for an effective neutralizer, which would protect the microorganisms exposed to the disinfectant. The neutralizer had to be harmless to the bacteria. Once the preliminary test conditions were met, and when

neutralizer was effective in the inhibition of antimicrobial activity of the 1% of two compounds and combination in each test carried out for all microorganisms tested. The actual tests consisted of placing the microbial suspension in contact with the disinfectant and determining n (number of surviving cfu/ml after contact with the disinfectant, 5 min for bacteria and 15 min for fungi), and then to be compared with N (number of cfu/ml in an inoculum dilution), as described in AFNOR guidelines.
The results shown in Table 1 indicate that 1% triclosan had bactericidal activity after 5 min exposure against *S. aureus* ≤1.5 µg/ml, *E. hirae* 7 µg/ml, *Peudomonas aeruginosa* 500 µg/ml and *E. coli* 3 µg/ml. When tested using NF T 72-150 (99.999%) reduction.

Table 1. Antimicrobial activity of triclosan (1%).

Microorganism	$\frac{N}{10}$ 10-30	Concentration of triclosan (% w/v) (n)							
		0.00015	*0.0003*	*0.0007*	*0.0015*	*0.003*	*0.006*	*0.0125*	*0.025*
As a bactericidal:									
S. aureus CIP 53.154	20	2	0	0	0	0	0	0	0
E. hirae CIP 5.855	23	+	6	0	0	0	0	0	0
Ps. aeruginosa CIP A22	22	+	+	+	+	+	+	+	(500 µg/ml)
E. coli CIP 54.127	24	19	0	0	0	0	0	0	0
As a fungicidal:									
C. albicans IP 1180.79	23	+	+	+	15	0	0	0	0
A. versicolor IP 1187.79	21	+	+	+	11	0	0	0	0
P. verrucosum var. cyclopium IP 1231.80	23	+	+	+	+	9	0	0	0
A. corymbifera IP 1129.75	20	+	+	+	13	0	0	0	0

+: Presence of more than 300 colonies.
0: Absence of colonies.
n: Number of surviving cfu/ml after contact with the disinfectant (5 min for bacteria, 15 min for fungi).
N: Number of cfu/ml in an inoculum dilution.

As well, the fungicidal activity of 1% Triclosan was determined after 15 min exposure against *C. albicans, A. versicolor* and *A. corymbifera,* 30 µg/ml, and *P. verrucosum var. cyclopium* 60 µg/ml,

Table 2 indicates the bactericidal activity of 1% 2-pheylphenol after 5 min exposure against *S. aureus*, *E. coli* at 30 µg/ml, *E. hirae* 60 µg/ml, and *Pseudomonas aeruginosa* 250 µg/ml

And also shows the fungicidal activity of 1% 2-phenylphenol after 15 min exposure against *C. albicans* 7 µg/ml, *A. versicolor, A. corymbifera*, 15 µg/ml and *P. verrucosum var. cyclopium* 30 µg/ml.

Table 3 shows the bactericidal activity of the combination of 0.5% triclosan and 0.5% 2-phenyphenol. Considerable Synergistic effect was observed against *Ps. aeruginosa* 15 µg/ml, 33-fold of triclosan and 17-fold of 2-phenylphenol when the two agents used alone against this microorganism, at concentrations tested.

Synergistic effect of the combination was also observed after 15 min exposure against *C. albicans*, *A. versicolor, A. corymbifera* ≤ 1,5 µg/ml, and *P. verrucosum var. cyclopium* at 3µg/ml.

Table 2. Antimicrobial activity of 2-phenylphenol (1%).

Microorganism	$\frac{N}{10}$ 10-30	Concentration of 2-phenylphenol (% w/v) (n)							
		0.00015	*0.0003*	*0.0007*	*0.0015*	*0.003*	*0.006*	*0.0125*	*0.025*
As a bactericidal:									
S. aureus CIP 53.154	22	+	+	+	12	0	0	0	0
E. hirae CIP 5.855	24	+	+	+	+	8	0	0	0
Ps. aeruginosa CIP A22	21	+	+	+	+	+	+	+	0
E. coli CIP 54.127	20	+	+	+	14	0	0	0	0
As a fungicidal:									
C. albicans IP 1180.79	19	+	+	0	0	0	0	0	0
A. versicolor IP 1187.79	22	+	+	+	0	0	0	0	0
P. verrucosum var. *cyclopium* IP1231.80	21	+	+	+	+	0	0	0	0
A. corymbifera IP 1129.75	23	+	+	+	0	0	0	0	0

Discussion

In the current study, the two agents were selected because both of them are widely used in many preparations. Triclosan is a broad-spectrum against all bacteria except *Pseudomonas aeruginosa* that requires higher concentration of 100-1000 µg/ml (Russell *et al.,* 1999). Because of its favorable safety profile, triclosan was incorporated into a variety of many antimicrobial products alone or in combination with other agents such as zinc citrate, sodium fluoride, ethylenediamine tetra-acetic acid (EDTA), chlorhexidine, pyrophosphate, and povidone-iodine (Bruhn *et al.,* 2002; Faogali *et al.,* 1995; Healy *et al.,* 2000; Nogueira-Filho *et al.,* 2000; Webster, 1992). On the other hand, 2-phenylphenol is active against fungi rather than bacteria, it may be formulated alone or in combination with alkyl, halogenated phenolic derivatives and other agents (Seymour, 2001). The two agents were also selected because they exert different mechanisms of action: 2-phenylphenol denatures microbial cell wall, causes enzymes whose normal role is to synthesize the cell wall to reverse their role in some way and effect its disruption; whereas triclosan causes disorganization of the cytoplasmic membrane, resulting in leakage of a group of characteristic chemical species, such as amino acids, purines, and pyrimidines that are essential for microbial survival (Hugo and Russell, 1998). According to those mechanisms and to the synergism of their combination, 2-phenylphenol may render *Pseudomonas aeruginosa* species more sensitive, to the action of triclosan, possibly by altering the permeability of the outer envelope.

Synergistic effect that was exhibited by this combination can be considered to reduce the in-use concentration of each agent used alone, that may minimize any possible side effect of the two agents. Recently many bacterial strains with resistance to triclosan have emerged (Heath and Rock, 2000; Rungtip *et al.,* 2001; Webster *et al.,* 1994; Suller and Russel, 2000). Therefore, this combination can be used to avoid bacterial resistance to one of the two agents, and also to increase the bactericidal activity against *Pseudomonas aeruginosa*. However, the two agents are not considered as antipseudomal when each agent used alone. In addition, the combination of triclosan and 2-phenylphenol can be used to enhance the fungicidal activities at lower concentrations.

Table 3. Antimicrobial activity of triclosan (0.5%) + 2-phenylphenol (0.5%)

Microorganism	$\frac{N}{10}$ 10-30	Concentration of triclosan + 2-phenylphenol (% w/v) (n)							
		0.00015	*0.0003*	*0.0007*	*0.0015*	*0.003*	*0.006*	*0.0125*	*0.025*
As a bactericidal:									
S. aureus CIP 53.154	21	1	0	0	0	0	0	0	0
E. hirae CIP 5.855	24	18	0	0	0	0	0	0	0
Ps. aeruginosa CIP A22	23	+	+	7	0	0	0	0	0
E. coli CIP 54.127	24	3	0	0	0	0	0	0	0
As a fungicidal:									
C. albicans IP 1180.79	24	2	0	0	0	0	0	0	0
A. versicolor IP 1187.79	22	1	0	0	0	0	0	0	0
P. verrucosum var. cyclopium IP1231.80	23	11	0	0	0	0	0	0	0
A. corymbifera IP 1129.75	25	1	0	0	0	0	0	0	0

References

Arweiler, N.B., Netuschil, L., Reich, E. (2001). Alcohol-free mouthrinse solutions to reduce supragingival plaque regrowth and vitality. *J. Clin. Periodontol* 28: 168-174.

Bending, J.W.A. (1990). Surgical hand disinfection: Comparison of 4% chlorhexidine detergent solution and 2% triclosan detergent solution. *Journal of Hospital Infection.* 15: 143-148.

Bruhn, G., Netuschil, L., Richter, S.T., Brecx, M., Hoffmann, T. (2002). Effect of a toothpaste containing triclosan on dental plaque, gingivitis, and bleeding on probing-an investigation in periodontitis patients over 28 weeks. *Clin. Oral. Invest.* 6: 124-127.

Chuanchuen, R., Beinlich, K., Hoang, T.T., Becher, A., Karkhoff-Schweizer, R.R., Schweizer, H.P. (2001). Cross-resistance between triclosan and antibiotics in

Pseudomonas aeruginosa is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects nfxB mutants over-expressing MexCD-OprJ. *Antimicrobial Agents and Chemotherapy*. 45: 428-432.

Faogali, J., Fong, J., Georg, N., Mahoney, P., O Rourke, V. (1995). Comparison of the immediate, residual, and cumulative antibacterial effects of Noraderm , Novascrub R, Betadine Surgical Scrub, Hibiclens, and liquid soap. *American Journal of Infection Control*. 23: 337-343.

Gaffar, A., Afflitto, J., Nabi, N., Kruger, I., Olsen, S. (1994). Recent advances in plaque, gingivitis, and tartar and caries prevention technology. *Int. Dent. J.* 44: 63-70.

Heath, J.H., Holland, D.R., Zhang, E., Snow, M.E., Rock, C.O. (1999). Mechanism of triclosan of bacterial fatty acid synthesis. *Journal of Biological Chemistry*. 274: 11110-11114.

Heath, R.J., Rock, C.O. (2000). A Triclosan-resistant bacterial enzyme. *Nature*. 406: 145.

Healy, C.M., Cruchley, A.T., Thornhill, M.H., Williams, D.M. (2000). The effect of sodium lauryl sulphate, triclosan and zinc on the permeability of normal oral mucosa. *Oral Diseases*. 6: 118-123.

Herbert, P. (2001). Triclosan a widely used biocide and its link to antibiotics. *FEMS Microbiology Letters*. 202: 1-7.

Hernandez, A., Martro, E., Matas, L., Martin, M., Ausine, V. (2000). Assessment of in-vitro efficacy of 1% Virkon against bacteria, fungi, viruses, and spores by means of AFNOR guidelines. *Journal of Hospital Infection*. 46: 203-209.

Hugo, W.B., Russell, A.D. (1998). *Pharmaceutical Microbiology*, 5nd edn. *Blackwell Scientific*. Oxford.

Josephine, J., Martin, C.J. (2002). The antibacterial activity of triclosan-impregnated storage boxes against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Shewanella putrefaciens in conditions simulating domestic use. *Journal of Antimicrobial Chemotherapy*. 49: 87-94.

Larson, E L. (1995). APIC guideline for handwashing and hand antisepsis in health care settings. *American Journal of Infection Control*. 23: 251-269.

Levy, C.W., Roujeinikova, A., Sedelnikova, S., Baker, P.J., Stuitje, A. R., Clayton, E. (1999). Molecular basis of triclosan activity. *Nature*. 398: 383-384.

Lueck, E. (1980). Antimicrobial Food Additives. *Springer-Verlag*. Berlin.

Marchetti, M.G., Kampf, G., Finzi, G., Salvatorelli, G. (2003). Evaluation of the bactericidal effect of five products for surgical hand disinfection according to prEN 12054 and prEN 12791. *Journal of Hospital Infection*. 54: 63-67.

McDonnell, G., Russell, A.D. (1999). Antiseptics and disinfectants: active, action and resistance. *Clinical Microbiology Reviews*. 12: 147-179.

McMurry, L.M., Oethinger, M., Levy, S.B. (1998). Triclosan targets lipid synthesis. *Nature*. 394: 531-532.

Nogueira-Filho, G.R., Toledo, S., Cury, J.A. (2000). Effect of three dentifrices containing triclosan and various additives. An experimental gingivitis study. *J. Clin. Periodontol*. 27: 494-498.

Rungtip, C., Kerry., Tung, T.H., Anna, B., Roxann, R., Karkhoff., Schweizer., Herbert, P. (2001). Cross-Resistance between triclosan and antibiotics in Pseudomonas aeruginosas is mediated by multidrug efflux pumps: Exposure of a susceptible mutant strain to triclosan selects nfxB mutants overexpressing MexCD-OprJ. *Antimicrobial Agents and Chemotherapy*. 45: 428-432.

Russell, A.D., Hugo, W.B., Aylffe, G.A.J. (1999). Disinfection, Preservation and Sterilization, 3nd edn. *Blackwell Science*. Oxford.

Seymour, S.B. (2001). Disinfectant, Sterilization, and Preservation, 5nd edn. *Lippincott Williams & Wilkins*. Philadelphia.

Suller, M.T.E., Russell, A.D. (2000). Triclosan and antibiotic resistance in Staphylococcus aureus. *Journal of Antimicrobial Chemotherapy*. 46: 11-18.

Ward, W.H.J., Holdgate, G.A., Rowsell, S., McLean, E.G., Pauptit, R. A., Clayton, E. (1999). Kinetic and structural characteristics of the inhibition of enoyl (acyl carrier protein) reductase by triclosan. *Biochemistry*. 38: 12514-12525.

Webster, J. (1992). Handwashing in neonatal intensive care nursery: product acceptability and effectiveness of chlorhexidine gluconate 4% and triclosan 1%. *Journal of Hospital Infection*. 2: 137-141.

Webster, J., Faogali, J.L., Cartwright, D. (1994). Elimination of methicillin-resistant staphylococcus aureus from a neonatal intensive care unit after hand washing with triclosan. *Journal of Pediatrics and Child Health*. 30: 59-64.

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