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Antimicrobial efficacy of potassium salts of four parabens

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SUMMARY

The antimicrobial activity of the soluble potassium salts of methyl, ethyl, propyl, and butyl parabens were evaluated to determine whether they would be more effective than their respective parabens (esters of *p*-hydroxybenzoic acids). The potassium salts of the methyl and ethyl parabens as well as methyl and ethyl parabens were microbiocidal against the fungus *Aspergillus niger* and five bacteria, whereas the potassium salts of propyl and butyl parabens and their respective parabens were not microbiocidal against all the test organisms. In the presence of several ingredients frequently used in pharmaceutical and cosmetic formulations, ethylenediaminetetraacetate (EDTA) and magnesium hydroxide did not interfere with the antimicrobial activity of the potassium salts of parabens and appeared to be microbiocidal against three of four test organisms. Simethicone and Tween 80 interfered with the antimicrobial activity of the preservatives. At pH 4–6, the potassium salt of butyl paraben, the only preservative tested, was active against more organisms than at pH 7–8. Overall, the highly soluble potassium salts of parabens showed microbiocidal activity against more of the test organisms than the less soluble parabens.

INTRODUCTION

This study was initiated to determine whether the more water-soluble potassium salts of the methyl, ethyl, propyl and butyl parabens (KMP, KEP, KPP and KBP, respectively) might have greater microbiocidal activity than the less soluble methyl, ethyl, propyl, and butyl parabens (MP, EP, PP and BP, respectively), which are widely used as preservatives in pharmaceutical preparations and cosmetics.

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There were four points of interest concerning parabens. First, the parabens are sufficiently active against fungi and gram-positive bacteria such as the staphylococci but not very active against the gram-negative bacteria such as the *Enterobacter* and *Pseudomonas* species whose presence in pharmaceuticals and cosmetics are undesirable [7,10,13]. Second, the antimicrobial activity of the parabens has been reported [16] to be dependent upon the amount that is soluble in water. Third, Gottfried [9] reported that activity increases with an increase in the alkyl chain length, with maximum activity obtained with butyl paraben. Fourth, Furia [6] reported more effective antimicrobial action by high-

er molecular weight parabens at concentrations exceeding substrate solubility.

Several ingredients used in pharmaceutical products and cosmetics were tested to determine whether they would interfere with the microbiocidal activity of the parabens and potassium salts of the parabens. Finally, the effect of pH on the microbiocidal activity of KBP, a representative potassium salt of a paraben in glucose nutrient salts medium, was determined.

EXPERIMENTAL

Minimum microbiocidal concentration

Initially, an attempt was made to determine the minimum inhibitory concentrations (MICs) as well as the minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs). However, due to the turbidity in some of the media after the addition of the parabens and potassium salts of the parabens, the MICs could not be accurately determined.

The microorganisms used in the tests were *Aspergillus niger* ATCC 1022, *Enterobacter cloacae* RM 211, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* RM 18, *Pseudomonas aeruginosa* RM 92 and *Staphylococcus aureus* ATCC 6539. The RM strains were isolated from raw materials or products. The bacteria used as inoculum were grown at 32°C for 24 h on Trypticase Soy Agar (TSA) in Roux bottles; cells were harvested, washed, and suspended in sterile 0.85% saline. *A. niger* was grown on Sabouraud Dextrose Agar (SDA) at 28°C for 10 days and the spores were also harvested, washed, and suspended in sterile saline. The test medium consisted of 0.05 M NH_4Cl, 0.05 M $MgCl_2 \cdot 6H_2O$, 0.005 M Na_2SO_4, 0.05 M $NaHPO_4$, 0.05 M NaH_2PO_4, 0.05 M KH_2PO_4 and 0.0056 M glucose [16] adjusted to pH 7.0. The MBC/MFC tests were carried out for 28 days with weekly sampling.

The potassium salts of the parabens are highly soluble when compared to the parabens and were obtained from NIPA Laboratories (Wilmington, DE). The solubilities of the potassium salts of the parabens and parabens in deionized water, w/w at 20°C, are KMP = 33%, KEP = 50%, KPP = 50%, KBP = 50%, MP = 0.23%, EP = 0.075%, PP = 0.03%, and BP = 0.01% as reported by NIPA Laboratories and the Merck Index [14].

KMP, KEP, KPP and KBP were dissolved in deionized water, sterilized by passing the solutions through 0.22 μm pore size filters and added to 15 ml of sterile glucose nutrient salts medium in tubes. MP, EP, PP, and BP were dissolved in small amounts of ethanol, sterilized through a 0.22 μm pore size filter and added to 15 ml of sterile glucose nutrient salts medium in tubes. All media in tubes were heated at 80°C for 20 min before use. Duplicate tubes for each level of the test preservatives were inoculated with suspensions containing $\approx 10^6$ colony-forming units (CFUs) per ml of the test organism and incubated at room temperature (23–25°C). One milliliter of inoculated medium was diluted in 5.0 ml of neutralizer solution containing 10% Tween and 3% Asolectin in 10% Trypticase Soy Broth (TSB) [22] and the entire mixture was cultured in 40 ml of TSA or SDA containing 1% Tween 80 and 0.3% Asolectin in 15 × 150 mm pour plates after 7, 14, 21 and 28 days. MBC and MFC endpoints were recorded as no CFU after 48 h incubation at 32°C for the bacteria and after 4 days of incubation at room temperature for *A. niger*. Concentrations of parabens beyond their soluble points were tested, but concentrations of the potassium salts of parabens higher than 3.4 mg/ml were not tested because crystals formed in the media (crystals formed at concentrations of 0.3 mg/ml for KBP, 0.5 mg/ml for KPP, and 1.6 mg/ml for KEP and KMP).

Effect of ingredients on preservatives

Several ingredients used in formulations (aluminum hydroxide, ethylenediaminetetraacetate, magnesium hydroxide, propylene glycol, simethicone, sorbitol, and Tween 80) were tested in the glucose nutrient salts medium to determine their effect on the activity of 1.6 mg/ml of KMP, KEP, KPP and KBP and 1.0 mg/ml of MP, EP, PP, and BP. The preservatives were active at these levels against the sensitive organisms. The concentrations of the ingredients simulated levels in products

(Table 2). No attempt was made to adjust the pH of the media which were altered as a result of the presence of raw materials. The tubes containing glucose nutrient salts medium, ingredients used in formulation, and preservative were inoculated with suspensions containing $\approx 10^6$ CFU/ml of *A. niger, E. cloacae, P. aeruginosa* or *S. aureus* and incubated at room temperature for 28 days. One milliliter of medium was cultured weekly in TSA or SDA using the procedures as described for MBC and MFC determinations. Due to the turbidity of some of the ingredients, 0.01% triphenyl tetrazolium chloride was added to the growth media in order to detect the presence of viable organisms. Results were recorded as + (growth of organism) or − (no growth). The tube containing the highest level of preservatives and showing no growth upon sub-culture was considered to have reached the MBC or MFC endpoint.

Effect of pH

The effect of pH on the efficacy of the potassium salt of butyl paraben was determined by transferring a suspension containing $\approx 10^6$ CFU/ml of *A. niger, Candida albicans, S. aureus* or *P. aeruginosa* into glucose nutrient salts medium which had been adjusted to pH 4, 5, 6, 7 or 8 with 0.1 N NaOH or 0.1 N HCl. Potassium butyl paraben was used as the test preservative at levels of 0.03–3.4 mg/ml. In a preliminary study it was determined that several of the test organisms did not survive in the medium at pH 9, and therefore antimicrobial efficacy at >pH 8 was not tested. The tubes were incubated at room temperature, and 1 ml of the medium was cultured on days 7, 14, 21 and 28 in TSA or SDA containing neutralizer as described earlier in the determinations of MBCs and MFCs.

RESULTS AND DISCUSSION

Minimum bactericidal concentration and minimum fungicidal concentration

The MBCs and MFCs of the test organisms are shown in Table 1. Endpoints were obtained for the six test organisms more often by the potassium salts of the parabens than by the parabens.

When antimicrobial activity in terms of endpoints against the six test organisms is considered, KMP, KEP, MP and EP are the most effective preservatives. Therefore, Gottfried's report [9] of the increase in the activity of parabens with an increase in the alkyl chain length could not be confirmed in our pH 7 nutrient salts medium. However, Aalto's claim [1] that methyl and ethyl parabens were more effective than propyl and butyl parabens was substantiated. Methyl and ethyl parabens are more water-soluble than propyl and butyl parabens [16], which may influence their antimicrobial activity.

Instances were observed when MBCs for EP, PP and BP against the test organism were higher than their reported solubility. This confirmed Furia's report [6] of effective antimicrobial activity by the more insoluble parabens at concentrations exceed-

Table 1

Minimum bactericidal and fungicidal concentrations of parabens and the potassium salts of parabens (mg/ml)

Preservative	*A. niger*	*E. cloacae*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
KMP	1.3	1.6	1.6	1.6	1.6	0.1
MP	0.7	0.7	0.5	0.8	0.9	0.2
KEP	1.0	1.0	0.8	0.8	1.6	0.06
EP	1.0	1.0	0.6	1.0	1.6	0.6
KPP	0.5	0.4	0.4	0.5	>3.4	0.2
PP	1.0	1.0	1.0	>1.4	>1.6	1.0
KBP	0.4	0.8	0.5	0.6	>3.4	0.2
BP	1.0	2.0	>1.4	>3.0	>3.0	1.0

ing substrate solubilities.

MBCs were higher for KMP than for MP against five of the six test organisms. The exception was *S. aureus*, in which MBCs of KMP and MP were very similar (0.1 and 0.2 mg/ml, respectively). When equivalent weights are considered, the MBCs for MP and KMP are higher than the MP/KMP ratio of 1:1.25 against the test organisms except *S. aureus*.

In the case of KEP and EP, the MBCs were similar for *A. niger, E. cloacae* and *P. aeruginosa.*

E. coli had an EP/KEP ratio of 1:1.3 and *S. aureus* had a ratio of 1:10. Therefore, the antimicrobial activities for KEP and EP as well as for KMP and MP evidently were not related to weights of preservatives.

Table 2

Efficacy of parabens (1.0 mg/ml) and potassium salts of parabens (1.6 mg/ml) in the presence of ingredients used in formulations

+ = growth; − = no growth.

Microorganisms	Antimicrobial activity after 4 weeks								Ingredients in nutrient medium (no preservative)
	KMP	MP	KEP	EP	KPP	PP	KBP	BP	
	Aluminum hydroxide (5.0 g/10 ml medium, pH 7.9)								
A. niger	+	+	−	−	−	+	−	−	+
E. cloacae	+	+	−	−	+	−	−	−	+
P. aeruginosa	−	+	+	−	+	+	−	−	+
S. aureus	−	−	−	−	−	−	−	−	+
	Ethylenediaminetetraacetate (0.05 mg/10 ml medium, pH 6.9)								
A. niger	−	−	−	−	−	−	−	−	−
E. cloacae	−	−	−	−	−	−	−	−	−
P. aeruginosa	−	−	−	−	−	−	−	−	+
S. aureus	−	−	−	−	−	−	−	−	−
	Propylene glycol (1.0 g/10 ml medium, pH 7.2)								
A. niger	−	−	+	−	−	−	−	−	+
E. cloacae	−	−	−	−	−	−	−	−	+
P, aeruginosa	−	−	−	−	−	+	+	+	+
S. aureus	−	−	−	−	−	−	−	−	+
	Simethicone (0.3 g/10 ml medium, pH 7.0)								
A. niger	−	−	+	−	−	+	−	+	+
E. cloacae	+	+	+	−	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+	+	+	+
S. aureus	+	−	−	−	+	+	+	+	+
	Sorbitol (1.0 g/10 ml medium, pH 6.9)								
A. niger	−	−	−	−	−	−	−	−	+
E. cloacae	−	−	+	−	−	−	−	+	+
P. aeruginosa	−	+	−	−	+	+	+	+	+
S. aureus	−	−	−	−	−	−	−	−	+
	Tween 80 (2.5 g/10 ml medium, pH 7.0)								
A. niger	+	+	+	+	+	+	+	+	+
E. cloacae	+	+	+	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+	+	+	+
S. aureus	−	−	+	+	−	−	−	−	+

When endpoints were obtained, the MBCs for KPP, PP, KBP and BP invariably showed that lesser amounts of the potassium salts of parabens were required than of the parabens. For the potassium salts, no endpoint was obtained only against *P. aeruginosa*, although for the parabens, no endpoint was obtained against *E. coli, K. pneumoniae* and *P. aeruginosa.*

Effect of formulation ingredients on microbiocidal activity

The effect of ingredients used in formulations on the parabens and potassium salts of parabens was variable (Table 2). All test organisms grew in the medium used in the test. EDTA has a direct effect on some microorganisms since it releases cell wall lipids by sequestering magnesium ions [4,6]. It was intrinsically microbiocidal against *A. niger, E. cloacae* and *S. aureus* but not against the strain of *P. aeruginosa* used in the test. EDTA was reported by several investigators [3,5,8,19,20,23] to induce lysis of *P. aeruginosa.* The survival of *P. aeruginosa* may have been due to strain variation, since variations in bacterial resistance have been reported by Morton [15]. Synergism between EDTA and compounds with antimicrobial properties against bacteria have been reported by MacGregor and Elliker [12] and Richards and McBride [21]. This synergistic activity of EDTA with parabens or with potassium salts of parabens resulted in the death of *P. aeruginosa*, which was not killed by EDTA alone in the growth medium.

The four test organisms were killed in the medium containing magnesium hydroxide without preservatives as well as in the medium containing magnesium hydroxide (3.0 g/10 ml) with the preservatives (not shown in Table 2). Death of the organisms was attributed to the high alkalinity (pH 10.8) of the medium caused by the presence of magnesium hydroxide.

Simethicone and Tween 80 had an adverse effect on the activity of all of the parabens and potassium salts of parabens [26]. This was not surprising in the case of Tween 80, since it is one of the surfactants used to neutralize the antimicrobial activity of many antiseptics and preservatives. The reduction in antimicrobial activity was probably due to the solubilization of preservatives within micelles or the formation of hydrophobic complexes between preservative and surfactant [25]. Sorbitol did not appear to affect the activity of most of the parabens or potassium salts in most cases. It did have a negative effect on BP and KEP against *E. cloacae.*

Propylene glycol was reported by Rae [18] to have preservative activity at concentrations of 10–20%, while Prickett et al. [17] reported that it enhances the activity of parabens, particularly the bactericidal activity. Under our test conditions, the antimicrobial activity of parabens and potassium salts of parabens was enhanced against *E. cloacae* and *S. aureus* but not against *P. aeruginosa.* Aluminium hydroxide appeared to enhance the activity of BP and KBP but interfered with the activity of KMP, KEP, and MP against the test organisms except *S. aureus.*

The results indicate that because ingredients used in formulation can enhance, remain neutral or interfere with the parabens or potassium salts of parabens and affect specific microorganisms differently, the choice of the preservative should depend on the type of microbial contaminants which are most likely to contaminate the product. Another factor which may influence the amount and choice of the paraben or derivative is the guidelines or regulations which exist in that particular country.

Choosing a suitable preservative such as a paraben or potassium salt of the paraben for a pharmaceutical product which contains the drug in an aqueous medium with little or no additives should be a relatively straightforward method. However, choosing a preservative for a pharmaceutical or cosmetic formulation with high concentrations of additives would be a much more difficult procedure due to the potential interaction with the additives.

Effect of pH on microbiocidal activity

The parabens with longer alkyl chains, PP and BP, were reported by Gottfried [9] and Wickliffe [24] to be most active and also more stable than MP or EP at pH 8–9. Therefore the antimicrobial activity of KBP, which is much more soluble than BP,

Table 3

Effect of pH on minimum bactericidal and fungicidal concentrations of potassium butyl paraben

	Concentration (mg/ml) effecting MBC or MFC			
pH	*A. niger*	*C. albicans*	*P. aeruginosa*	*S. aureus*
4	0.4	0.2	3.0	0.3
5	0.4	0.1	2.6	0.3
6	0.4	0.1	3.4	0.5
7	0.4	0.08	>3.4	0.1
8	0.2	<0.06	>3.4	0.3

was tested at pH 4–8.

KBP was slightly more effective at pH 8 against *A. niger* and *C. albicans* (Table 3). This confirmed an earlier publication by Bandelini [2], who showed that parabens were most effective against fungi at pH > 7.0.

KBP was effective against *P. aeruginosa* at ≤pH 6 and was effective against *S. aureus* over a wide pH range. These results indicate that formulations in which KBP is to be used should be at ≤ pH 6 since it is active against both gram-positive and gram-negative bacteria and fungi, whereas at neutral or alkaline pH values it may not be effective against *P. aeruginosa*.

The results support the following conclusions:

(1) The water-soluble potassium salts of parabens showed better antimicrobial activity against more microorganisms than the less water-soluble parabens.

(2) Certain ingredients used in formulations affect the antimicrobial activity of parabens and their potassium salts by being intrinsically microbiocidal or synergistic (EDTA and magnesium hydroxide) or by interfering with the antimicrobial activity (Tween 80 and simethicone) against certain microorganisms.

(3) The antimicrobial activity of KBH was greater at ≤ pH 6.0 than at ≥ pH 7.0.

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