Pharmacodynamic properties of HMR 3004, a novel ketolide, on respiratory pathogens, enterococci and *Bacteroides fragilis* demonstrated by studies of time kill kinetics and postantibiotic effect

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Objective: The pharmacodynamic properties of the novel ketolide (a new class of macrolide) antibiotic, HMR 3004, were investigated by studying time-kill kinetics and postantibiotic effect.

Methods: The time-kill kinetics were studied at two inocula against three strains each of *Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis, Enterococcus faecium* and *Bacteroides fragilis*. The postantibiotic effects of HMR 3004 were also investigated on these organisms at concentrations equivalent to 1, 4 and 10 × MIC.

Results: The time kill-kinetic data demonstrated that HMR 3004 is inoculum dependent and predominantly bacteriostatic being only slowly bactericidal at higher concentrations. HMR 3004 exhibited a significant postantibiotic effect with all strains studied, ranging from 1.9–6.2 h at 10 × MIC.

Conclusions: The bacteriostatic activity and significant postantibiotic effect demonstrated by HMR 3004 are similar to those previously obtained with other macrolides.

Key words: Ketolide, HMR 3004, time-kill kinetics, postantibiotic effect.

INTRODUCTION

The ketolide HMR 3004 belongs to a new 14-membered ring macrolide class of antibiotics which are characterized by a 3-keto group on the erythronolide A ring instead of the α-L-cladinose moiety [1]. The ketolides possess the antibacterial spectrum of erythromycin A [2], and HMR 3004 has demonstrated outstanding activity against multiresistant pneumococci and activity against *Haemophilus influenzae*, *Streptococcus pneumoniae* and the enterococci. Against strains susceptible to macrolides, the activity of HMR 3004 is one to two orders of magnitude higher than that of clarithromycin and erythromycin [3].

Pharmacodynamic parameters have become increasingly important for the determination of optimal dosing schedules of antibiotics. The in vitro pharmacodynamic parameters of an antimicrobial include time kill kinetics and postantibiotic effect (PAE). Time kill curves are pharmacodynamic examples of bactericidal activity expressed as rate of killing by a fixed concentration of antimicrobial and constitute one of the most reliable methods of determining tolerance [4]. PAE is the term used to describe the continued suppression of the growth of an organism after a short exposure to an antimicrobial agent [5]. Clinically, the importance of PAE is in antimicrobial agent dosing regimens and it is particularly relevant to short half-life compounds. An infection caused by an organism susceptible to an agent with a significant PAE may require less frequent dosing than an infection susceptible to agents that do not demonstrate a PAE, other factors being equal.

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We investigated the rate of killing and PAE of HMR 3004 on aerobic bacteria implicated in upper and lower respiratory tract infections, and, in addition, enterococci and the anaerobe *Bacteroides fragilis*. The effect of two inocula on the rate of kill was studied. Previous pharmacodynamic studies have shown that erythromycin A is predominantly bacteriostatic, although at higher concentrations it is slowly bactericidal [6]. Macrolides have demonstrated significant PAEs of >3 h with staphylococci and streptococci [5].

MATERIALS AND METHODS

Bacterial strains and culture conditions

Two recent clinical isolates and a type culture of each of the following organisms were studied: *Staphylococcus aureus* and ATCC 29213, *H. influenzae* and NCTC 10479, *Moraxella catarrhalis* and NCTC 3622, *Streptococcus pneumoniae* and NCTC 7465, *Enterococcus faecalis* and NCTC 12201, *E. faecium* and NCTC 12204 and *B. fragilis* and NCTC 9343. Three recent clinical isolates of *Streptococcus pyogenes* were also included. Iso-Sensitest agar and broth (Unipath, Basingstoke, UK) supplemented with 20 mg/L nicotinamide adenine dinucleotide (NAD) (Sigma, Poole, UK) and 5% horse blood (E and O Laboratories Ltd, Bonnybridge, UK) were used for the aerobic bacteria. Wilkins–Chalgren agar and broth (Unipath) were used throughout for the anaerobic investigations. All the anaerobic work was performed in an anaerobic cabinet (Don Whitley Scientific Ltd, Skipton, UK) containing an atmosphere of 80% nitrogen, 10% carbon dioxide and 10% hydrogen. To ensure anaerobiosis, broths and diluents were incubated in the cabinet for at least 48 h before use and a reasazurin indicator (BDH, Poole, UK) was employed.

Antimicrobial agent

HMR 3004 was obtained from Roussel UCLAF, Paris, France. It was stored and prepared throughout these studies using the manufacturer's guidelines.

MIC determinations

MICs were determined for all organisms studied by a broth dilution method [7] with an inoculum of approximately 5×10^5 CFU/mL. The MIC was defined as the lowest concentration at which there was no visible growth after 18–24 h (48 h for anaerobes) of incubation at 35–37°C.

Time kill kinetics

HMR 3004 stock solutions were added to logarithmic-phase broth cultures of approximately 10^5 and 10^7 CFU/mL, to give concentrations equivalent to 2 and 10×MIC. An antibiotic-free growth control was included at both inocula. Viable counts were performed (three replicates) at 0, 2, 4, 6 and 24 h after antibiotic addition, by the Miles and Misra technique [8], on agar plates following serial dilution in phosphate-buffered saline, pH 7.3 (Unipath). The bacteria were enumerated after 48 h of incubation at 35–37°C and the time kill kinetics were plotted as $\log_{10}$ CFU/mL against time. Bactericidal activity is defined as a 3 $\log_{10}$ decrease in CFU/mL (99.9% kill) [4]. The lower limit of bacterial enumeration using this method was 2.7 $\log_{10}$ CFU/mL. Bacteriostatic activity was defined as a <2 $\log_{10}$ decrease in CFU/mL. Antibiotic carryover was not deemed to be a problem in our determinations as this occurs at higher concentrations of >16×MIC [4] and serial dilution was employed, which minimizes any carryover effects.

Postantibiotic effect

HMR 3004 stock solutions were added to logarithmic-phase broth cultures of approximately 10^5 CFU/mL, to give concentrations equivalent to 1, 4 and 10×MIC, and a ketolide-free culture was included as a growth control. The cultures were incubated at 35–37°C for 1 h. The HMR 3004 concentrations were then reduced by 1000-fold dilution into prewarmed broth and the cultures were incubated at 35–37°C for 24 h. The control cultures were treated in exactly the same way. Viable counts were performed on antibiotic-free agar plates prior to exposure, hourly for 6 h and at 24 h after neutralization by dilution. Viable counts were determined with a spiral plater (Don Whitley Scientific Ltd) following appropriate dilution in phosphate-buffered saline, pH 7.3. The bacteria were enumerated after 24 h of incubation at 35–37°C. The counts of $\log_{10}$ CFU/mL were plotted against time, and the PAE was defined as PAE = $T - C$, where T is the time required for the count in the test culture to increase 1 $\log_{10}$ above the count observed immediately after dilution, and C is the time required for the count in the control to increase 1 $\log_{10}$ above the count observed immediately after dilution [5]. A significant PAE is defined as >0.5 h.

RESULTS

MICs

Table 1 shows that HMR 3004 has activity against respiratory pathogens, enterococci and *B. fragilis*.

Time kill kinetics

Figure 1 depicts the time kill curves of HMR 3004 against *Staphylococcus aureus* ATCC 29213, *E. faecalis* T398 and *B. fragilis* B1 studied at an inoculum of

Table 1 Bactericidal rates (change in $\log_{10}$ CFU/mL) of HMR 3004 at concentrations equivalent to 2 and 10 × MIC

Strain	MIC (mg/L)	Change in $\log_{10}$ CFU/mL from 0 to 24 h			
		10^7 CFU/mL 2 × MIC	10^7 CFU/mL 10 × MIC	10^5 CFU/mL 2 × MIC	10^5 CFU/mL 10 × MIC
Staphylococcus aureus ATCC 29213	0.03	2.33	0.93	0.65	>−3.28
Staphylococcus aureus F551	0.03	2.15	2.42	3.98	1.30
Staphylococcus aureus F104	0.03	2.22	−1.19	1.25	>−3.11
Streptococcus pneumoniae NCTC 7465	0.008	ND	ND	ND	ND
Streptococcus pneumoniae P591	0.008	ND	ND	ND	ND
Streptococcus pneumoniae P416	0.008	ND	ND	ND	ND
H. influenzae NCTC 10479	1	1.61	−3.03	1.16	−3.25
H. influenzae A40	0.5	1.59	0.93	0.32	−3.23
H. influenzae A330	1	1.52	1.18	−0.03	−3.28
M. catarrhalis NCTC 3622	0.06	−1.14	−1.23	>−2.28	>−3.18
M. catarrhalis R79	0.06	−1.18	−1.21	−2.08	>−1.84
M. catarrhalis R65	0.06	−1.04	−1.41	>−3.15	>−3.18
Streptococcus pyogenes P44	0.008	0.80	1.05	3.53	>−3.18
Streptococcus pyogenes P199	0.008	1.27	−0.37	2.00	>−2.15
Streptococcus pyogenes P404	0.008	−0.70	−1.70	1.65	>−2.48
E. faecalis NCTC 12201	1	1.60	1.38	3.49	2.22
E. faecalis T392	0.03	1.84	1.58	3.30	1.98
E. faecalis T398	0.03	1.60	1.39	3.30	2.08
E. faecium NCTC 12204	0.5	2.01	1.67	3.48	−1.10
E. faecium T388	0.015	1.66	1.43	3.66	3.62
E. faecium T445	0.015	1.64	1.65	3.39	0.10
B. fragilis NCTC 9343	1	>−5.20	>−5.20	>−3.28	>−3.28
B. fragilis B1	0.25	>−5.36	>−5.08	>−3.11	>−3.08
B. fragilis B2	1	−1.95	>−5.18	>−3.15	>−3.00

ND, not determinable due to autolysis.

approximately 10^5 CFU/mL. Table 1 summarizes the bactericidal rates (change in $\log_{10}$ CFU/mL from 0 to 24 h) of HMR 3004 on all strains investigated in this study. Bactericidal activity is defined as a 3 $\log_{10}$ decrease in CFU/mL; however, in some instances when studying the 10^5 CFU/mL inoculum a 3 $\log_{10}$ decrease could not be determined (due to lower limit of detection) but a decrease of >2 $\log_{10}$ was observed. In these cases, if no regrowth was observed for the duration of the experiment, the effect was termed bactericidal. The time kill kinetic data demonstrated that HMR 3004 is predominantly bacteriostatic and only slowly bactericidal at higher concentrations. The 10 × MIC concentration of HMR 3004 was bactericidal (99.9% kill) at 24 h with all strains except *Staphylococcus aureus* F551, the enterococci and *Streptococcus pneumoniae* at inocula of 10^5 CFU/mL. The 10 × MIC concentration of HMR 3004 was bactericidal at 4–6 h with the *Streptococcus pneumoniae* strains at inocula of 10^5 CFU/mL, except for strain P416, on which HMR 3004 was bacteriostatic. The 10 × MIC concentration of HMR 3004 was bactericidal at 24 h and at an inoculum of 10^7 CFU/mL only for one strain of *H. influenzae* and *B. fragilis* strains. The 2 × MIC concentration of HMR 3004 was bactericidal at 24 h only with all strains of *B. fragilis* and *M. catarrhalis* at inocula of 10^5 CFU/mL.

Postantibiotic effect

Figure 2 depicts the PAE of HMR 3004 on *Staphylococcus aureus* ATCC 29213, *E. faecalis* T398 and *B. fragilis* B1. Table 2 summarizes the PAE (h) of HMR 3004 for all the strains investigated in this study. HMR 3004 exhibited a significant PAE with all strains studied at 4 and 10 × MIC. The PAEs at 1, 4 and 10 × MIC of HMR 3004 were 0–3.5, 1.1–5.5 and 1.9–6.2 h, respectively.

DISCUSSION

Our MIC data confirm the preliminary study by Agouridas et al [3], in that the ketolides have activity against respiratory pathogens and enterococci. The time kill kinetic data demonstrate that HMR 3004 exhibits concentration- and inoculum-dependent bacteriostatic activity on all strains studied. Slow time-dependent bactericidal activity (99.9% kill at 24 h) was only seen at the higher concentration studied, but not

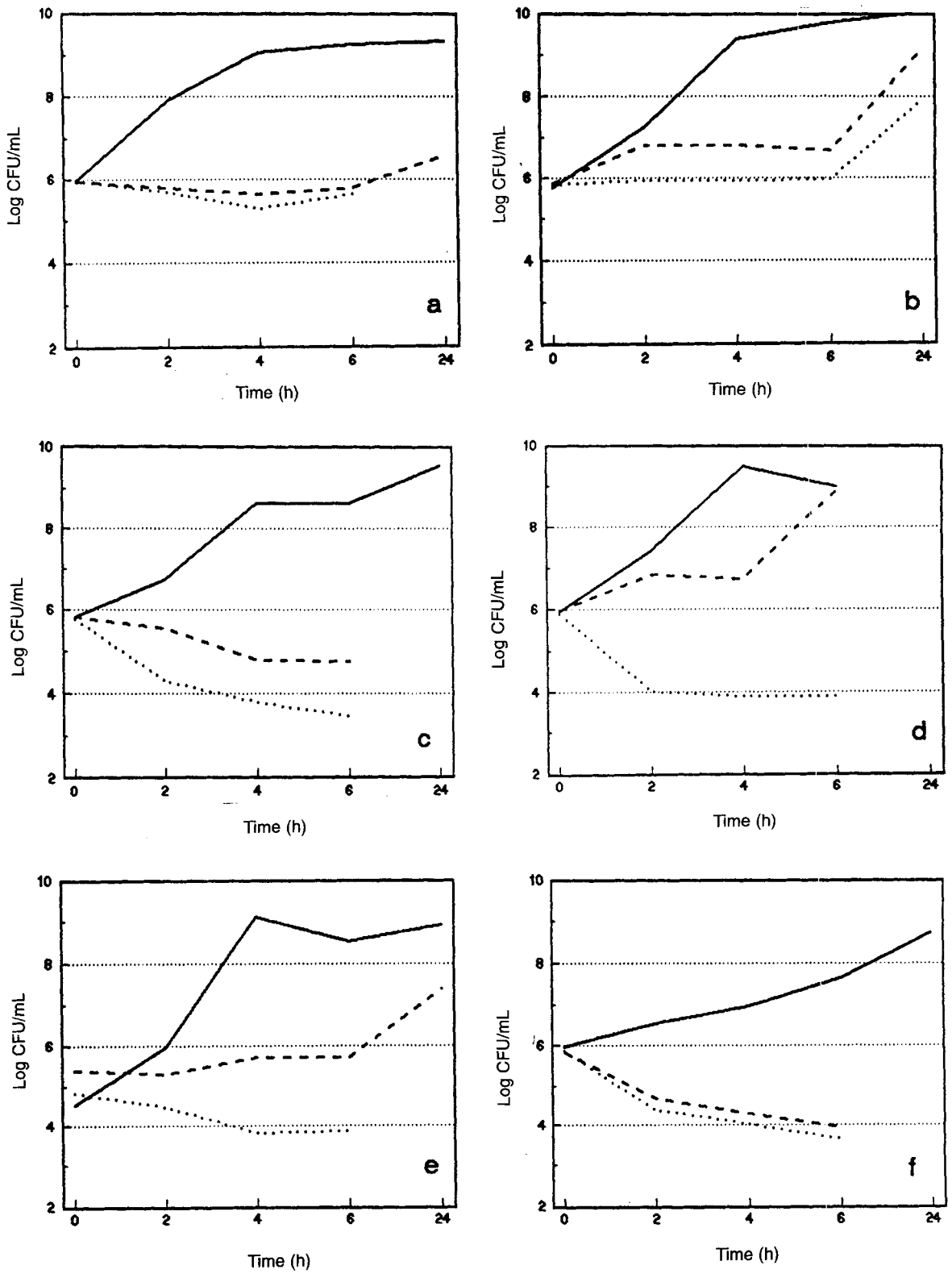

Figure 1 HMR 3004 time kill curves of (a) *Staphylococcus aureus* ATCC 29213, (b) *E. faecalis* T398, (c) *B. fragilis* B1, (d) *Streptococcus pneumoniae* P591, (e) *Streptococcus pyogenes* P199, and (f) *M. catarrhalis* R65. —, control; ---, 2 × MIC; ..., 10 × MIC.

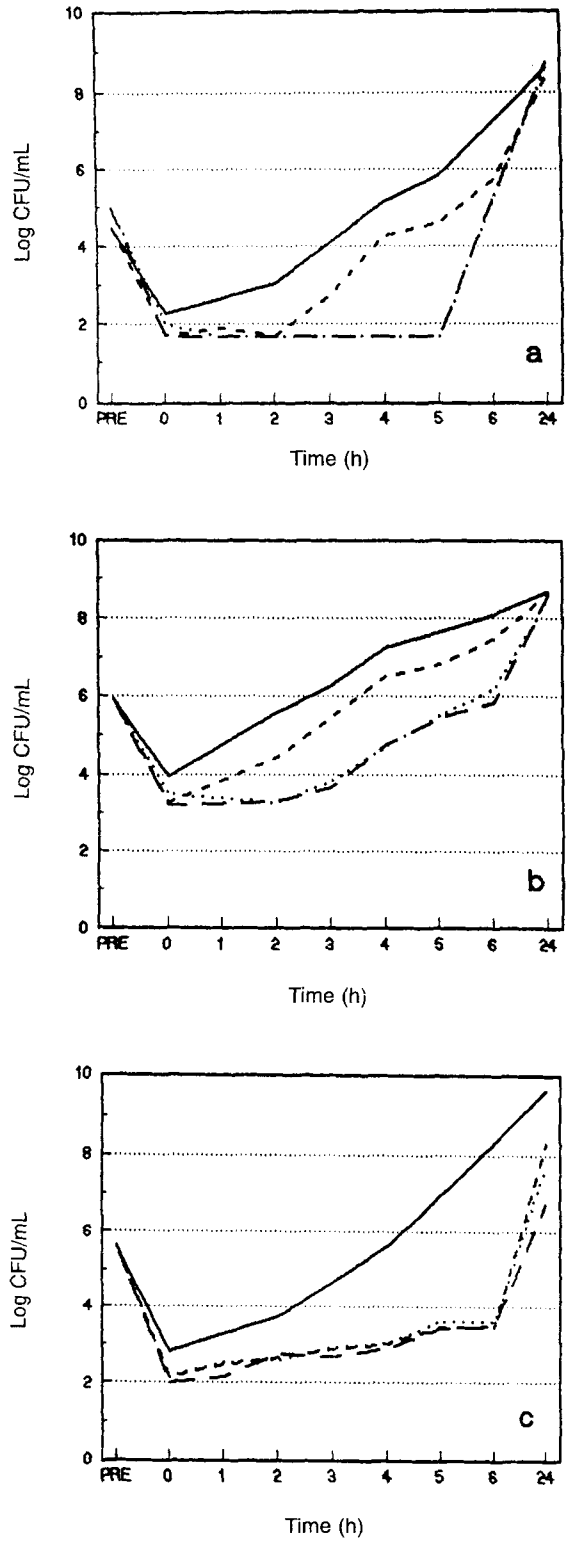

Figure 2 HMR 3004 postantibiotic effect on (a) *Staphylococcus aureus* ATCC 29213, (b) *E. faecalis* T398, (c) *B. fragilis* B1. —, control; ---, 1 × MIC; ..., 4 × MIC; –, 10 × MIC.

Table 2 HMR 3004 postantibiotic effect (h)

	HMR 3004		
Strain	1 × MIC	4 × MIC	10 × MIC
Staphylococcus aureus ATCC 29213	0.8	5.5	5.5
Staphylococcus aureus F551	0.8	2.7	3.4
Staphylococcus aureus F104	0.3	3.1	4.3
Streptococcus pneumoniae NCTC 7465	0.8	3.1	6.2
Streptococcus pneumoniae P591	1.0	3.7	6.2
Streptococcus pneumoniae P416	0.4	1.2	3.7
H. influenzae NCTC 10479	0.0	1.6	2.3
H. influenzae A40	0.9	2.2	2.5
H. influenzae A330	0.6	1.1	3.3
M. catarrhalis NCTC 3622	1.0	2.0	3.6
M. catarrhalis R79	0.0	3.0	3.1
M. catarrhalis R65	0.5	3.5	5.0
Streptococcus pyogenes P44	0.0	1.6	6.1
Streptococcus pyogenes P199	0.2	2.0	5.0
Streptococcus pyogenes P404	1.2	3.5	4.8
E. faecalis NCTC 12201	3.5	2.9	3.7
E. faecalis T392	1.3	2.8	3.1
E. faecalis T398	0.5	2.6	2.3
E. faecium NCTC 12204	3.1	2.5	3.7
E. faecium T388	1.2	2.0	1.9
E. faecium T445	1.2	1.4	3.2
B. fragilis NCTC 9343	2.9	2.6	3.4
B. fragilis B1	2.5	2.3	2.2
B. fragilis B2	1.8	2.2	2.3

ND, not determinable.

with all strains. HMR 3004 exhibited the most rapid bactericidal activity on the *Streptococcus pneumoniae* strains. The predominant bacteriostatic activity exhibited by HMR 3004 is similar to that observed with erythromycin A [6]. Regrowth was observed during this study, and the colonies observed were morphologically identical and had MICs the same as or within one dilution step of the controls. The clinical importance of regrowth is unclear, particularly if it occurs after the usual dosing interval [4].

Bacterial growth suppression termed PAE has been observed since the advent of antibiotic therapy. The mechanism and relevance of PAE are still poorly understood, but clinically, its greater impact is on designing dosing regimens for new antimicrobials. There is inadequate clinical evidence to suggest an optimal dosage regimen for macrolides. The most common explanations for the mechanism of PAE are prolonged persistence of the antimicrobial at the cellular site of action or the true recovery period from non-lethal damage [5]. For macrolides, the duration of the PAE may be equivalent to the time required for the macrolide to detach from the ribosome. HMR 3004 produced a significant concentration-dependent PAE.

The increase in PAE with an increase in concentration was shown with all strains. For example, PAEs at 1, 4 and 10 × MIC of 0.8, 2.7 and 3.4 were seen for HMR 3004 on *Staphylococcus aureus* F551 strain. PAEs of other macrolides are similar to the PAE we observed for HMR 3004. Odenholt-Tornqvist et al [9] reported PAEs of 2.9–8 h for roxithromycin, clarithromycin and azithromycin with *Streptococcus pyogenes, Streptococcus pneumoniae* and *H. influenzae*. Scaglione et al [10] also reported a clarithromycin PAE of ≥ 4 h for *Streptococcus pyogenes, Staphylococcus aureus* and *H. influenzae*. In addition, Munckhof and Turnidge [11] recently reported similar PAEs as demonstrated here with *Staphylococus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae*. The PAE of HMR 3004 exhibited considerable strain and interspecies variation; this variation has been noted with other antimicrobials [12].

In conclusion, it would appear that HMR 3004 exhibits pharmacodynamic properties similar to those of erythromycin A, i.e. they are slowly bactericidal at high concentrations and exhibit a significant PAE.

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