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The New Oxazolidinone, TR-700 (DA-7157): Effects of pH, Inoculum, Serum and Media on Antibacterial Activity

S. CHOI, W. IM, J. RHEE

Dong-A Pharm., Yongin-Si, Republic of Korea.

Contact Information:

Sunghak Choi
Dong-A Pharm. Co., Ltd. Research Lab.
47-5 Sanggal-Dong Kiheung-Gu
Yongin-Si
Kyunggi-Do 449-905 Korea
e-mail: choish@donga.co.kr

Abstract

Background:

TR-700 (DA-7157), a parent drug of TR-701 (DA-7218), has a potent antibacterial activity against Gram-positive pathogen including MRSA and VRE. In this study, the effects of pH, inoculum size, medium and serum protein binding on TR-700 antibacterial activity were assessed.

Methods:

Using CLSI (previously NCCLS) methodology, MIC values were determined for TR-700 against three ATCC reference strains (*Staphylococcus aureus* ATCC29213 and ATCC25923, and *Enterococcus faecalis* ATCC29212) as well as clinical isolates of MRSA and VRE. Several conditions were tested including Mueller-Hinton agar adjusted to pH 6, pH 7, and pH 8, and - inocula between 10^3 and 10^5 colony forming units per spot. The activity of TR-700 was also evaluated in Mueller-Hinton agar with or without 20% human serum. MIC comparisons for TR-700 were evaluated using a range of commercially available susceptibility testing media.

Results:

The activity of TR-700 was not affected by pH, inoculum size or the addition of serum to the testing medium; all comparisons agreed within a one tube dilution. MIC values obtained in the three media tested did not vary and there was no more than a one-dilution difference between agar and broth methods.

Conclusions:

These results suggest that the activity of TR-700 is likely to be consistent under different growth conditions.

Introduction

TR-700 (DA-7157) is a new oxazolidinone being developed to satisfy needs for a new antibiotic active against a wide spectrum of Gram-positive pathogen including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and penicillin-resistant *Streptococcus pneumoniae*.

The activity of an antibiotic can be affected by a number of factors, which may be encountered during susceptibility testing and / or physiologically. Four such factors (pH, inoculum, medium and the presence of serum) were investigated for TR-700 (DA-7157), to establish whether MIC values obtained against standard Gram-positive strains and clinical isolates of MRSA and VRE are consistent under a variety of bacterial growth conditions.

Methods

Strains

Recent clinical isolates of methicillin-resistant *Staphylococcus aureus* (n=18) and vancomycin-resistant enterococci (n=17) and reference ATCC strains (*S. aureus* ATCC 29216, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212) were selected from our culture collection. Strains were grown overnight in Tryptic Soy broth (Difco). The inoculum was adjusted so that concentrations of 2 X 10^5 CFU/mL for broth and 10^4 CFU per spot for agar were obtained.

Susceptibility test

The Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method according to CLSI recommendations. Two fold MIC differences were not considered significant in these experiments.

Factors

- Overnight broth cultures were diluted to 10^3, 10^4 and 10^5 CFU per inoculum spot.
- The pH Muller Hinton agar and broth was adjusted to pH 5, 6, 7 and 8.
- Muller Hinton (MH), Tryptic Soy (TS), Brain Heart Infusion (BHI) and Nutrient agars and broths were prepared according to manufacturer's instructions.
- Human plasma was added to MHA to concentration of 20% (v/v) and compared to MHA. Both TR-700 (DA-7157) and linezolid were used in testing.

Results

- Inoculum size between 10^3 and 10^5 CFU/spot had no effect on the antibacterial activity of TR-700 (DA-7157) (Table 1, 4).
- The antibacterial activity of TR-700 (DA-7157) was unaffected by altering the acidity of the testing medium between pH 5 and 8 (Table 2, 5).
- TR-700 (DA-7157) demonstrated consistent antibacterial activity in all four types of agar and broth media examined (Table 3, 6).
- The antibacterial activities of TR-700 (DA-7157) and linezolid were unaffected by the addition of 20% human plasma in MHA - all comparisons agreed within a one tube dilution (Table 7).

Table 1. Effect of inoculum on the activity of TR-700 (DA-7157) against reference strains, expressed as MIC (μg/mL).

Strain	Inoculum density (CFU/inoculum spot)		
	10^3	10^4	10^5
S.aureus ATCC 29213	0.25	0.5	1
S.aureus ATCC 25923	0.25	0.5	1
E.facalis ATCC 29212	0.25	0.5	0.5

Table 2. Effect of pH on the activity of TR-700 (DA-7157) against reference strains, expressed as MIC (μg/mL).

Strain	pH of medium							
	pH5		pH6		pH7		pH8	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
S.aureus ATCC 29213	0.25	1	0.5	1	0.5	1	1	1
S.aureus ATCC 25923	0.25	1	0.5	1	0.5	1	1	1
E.facalis ATCC 29212	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 3. Effect of media on the activity of TR-700 (DA-7157) against reference strains, expressed as MIC (μg/mL).

Strain	Medium type							
	MH		TS		BHI		Nutrient	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
S.aureus ATCC 29213	0.5	0.5	0.25	0.25	0.5	0.25	0.5	0.5
S.aureus ATCC 25923	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5
E.facalis ATCC 29212	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 4. Effect of inoculum on the activity of TR-700 (DA-7157) against clinical isolate of MRSA and VRE.

Inoculum density (CFU/inoculum spot)	MRSA (18)			VRE (17)		
	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}
10^3	0.25-0.5	0.5	0.5	0.25-0.5	0.5	0.5
10^4	0.5	0.5	0.5	0.25-0.5	0.5	0.5
10^5	1-2	2	2	1	1	1

Table 5. Effect of pH on the activity of TR-700 (DA-7157) against clinical isolate of MRSA and VRE.

pH of medium	MRSA (18)			VRE (17)		
	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}
pH 5	0.5	0.5	0.5	0.25	0.25	0.25
pH 6	0.5	0.5	0.5	0.25-0.5	0.25	0.25
pH 7	0.5	0.5	0.5	0.5	0.5	0.5
pH 8	0.5	0.5	0.5	0.5	0.5	0.5

Table 6. Effect of media on the activity of TR-700 (DA-7157) against clinical isolate of MRSA and VRE.

Medium Type	MRSA (18)			VRE (17)		
	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}
MHA	0.5	0.5	0.5	0.5	0.5	0.5
TSA	0.5	0.5	0.5	0.5	0.5	0.5
NA	0.25-0.5	0.5	0.5	0.25-0.5	0.5	0.5

Table 7. Effect of human plasma on the activity of TR-700 (DA-7157) against clinical isolate of MRSA and VRE.

Drug	MHA containing	MRSA (18)			VRE (17)		
		MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}
TR-700 (DA-7157)	No plasma	0.5	0.5	0.5	0.25-0.5	0.5	0.5
	With 20% plasma	1	1	1	0.5	0.5	0.5
Linezolid	No plasma	2	2	2	1-2	2	2
	With 20% plasma	2-4	4	4	1-2	2	2

Conclusions

- The activity of TR-700 (DA-7157) is likely to be consistent under a variety of bacterial growth conditions, ensuring consistency of the susceptibility testing data reported by different centers.