E-160

In Vitro Activity of Garenoxacin (BMS-284756) Against 465 of Clinical Anaerobes Isolated From Intra-abdominal Infections

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Revised Abstract

Background Garenoxacin (BMS-284756) is a novel fluoroquinolone in development that has shown high activity against many anaerobes. Anaerobes were isolated from intra-abdominal infections as part of a multi-national clinical study. As this source yielded numerous species of anaerobes, the data will be limited to *Bacteroides*, *Prevotella*, *Fusobacterium, Clostridium, Eubacterium*, and *Peptostreptococcus* species. **Methods** Each isolate was identified using selective growth media and various biochemical profiles. The activity of garenoxacin was compared to other relevant compounds used for anaerobic therapy. Susceptibility testing was performed using the concentration gradient agar diffusion method (Etest) and interpreted following manufacturers instructions and NCCLS guidelines. **Results** The susceptibility results of the 465 isolates as a group are listed in Table 1. Garenoxacin MIC_{90}s for all species reported ranged from 0.5 to 16 mcg/mL with a mean of 1 mcg/mL. Garenoxacin anaerobic activity was equivalent to that of ampicillin-sulbactam, amoxicillin-clavulanic acid and piperacillin-tazobactam in all species except *Eubacterium*.

Table 1.

	Bacteroides spp (n=266)		*Prevotella spp* (n=42)		*Fusobacterium spp* (n=16)		*Clostridium spp* (n=67)		*Eubacterium spp* (n=25)		*Peptostrep spp* (n=49)	
Antibiotic	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Garenox	0.25	1	0.5	2	0.5	16	0.25	0.5	0.03	1	0.25	4
Amox/clav	0.5	2	0.06	0.5	0.12	1	0.06	0.5	0.03	0.5	0.12	1
Amp/sulb	1	4	0.06	0.5	0.06	0.5	0.06	2	0.03	0.5	0.12	2
Clinda	2	> 256	2	4	0.25	> 256	0.06	0.5	0.06	4	≤ 0.015	> 256
Metro	0.5	16	2	64	0.25	8	0.06	> 256	0.12	> 256	0.5	4
Pip/tazo	2	16	0.12	4	0.25	32	0.03	> 256	0.03	8	0.03	4

Conclusion Garenoxacin, a novel fluoroquinolone, demonstrates in vitro anaerobic activity comparable to β-lactam/β-lactamase inhibitor compounds for most anaerobes tested.

Introduction

Anaerobes play a significant role in abdominal infections as well as dental, diabetic, gynecological and obstetric infections with mixed aerobic and anaerobic bacteria. Anaerobes, particularly *Bacteroides* spp and some *Prevotella* spp and *Fusobacterium* spp, are becoming increasingly resistant to beta-lactams through beta-lactamase production and other mechanisms [1]. Resistance to typical anaerobic drugs like clindamycin and metronidazole are also on the rise, especially in some gram-positive anaerobic species.

Older quinolones such as ciprofloxacin, lomefloxacin, enoxacin and ofloxacin are mostly inactive against anaerobes. Newer quinolones, especially those with enhanced gram-positive activity, have better anaerobic activity and may be good choices for therapy against anaerobes [2]. Garenoxacin a new des-fluoro-6 quinolone has demonstrated significant in vitro gram-negative activity with enhanced gram-positive and anaerobic activity [3] [4]. The high activity and broad-spectrum of garenoxacin would make it a good candidate for mono-drug therapy in the treatment of anaerobic infections. The purpose of this study was to report the in vitro activity of garenoxacin and comparative agents against relevant anaerobic pathogens from a phase III clinical trial limited to intra-abdominal infections in a multi-center international study.

During the course of a phase III clinical trial in which garenoxacin was evaluated, 465 clinically relevant anaerobic pathogens were collected for evaluation during the 2001-2002 calendar years. There were 67 multi-national centers involved in this study of which 55 contributed at least one anaerobic isolate to this study.

Materials and Methods

- Clinical isolates were collected in 55 laboratories from 11 countries during 2001-2002.
- Identifications were performed by using the RapID ANA II System (Remel Inc. Lenexa, KS) and additional PRAS biochemicals (Anaerobe Systems, Morgan Hill, CA.) as needed.
- A total of 502 anaerobes were isolated in this study. This report is limited to the 465 anaerobes of the most common species isolated in the clinical setting: *Bacteroides spp, Clostridium spp, Eubacterium spp, Fusobacterium spp, Peptostreptococcus spp and Prevotella spp*.

Antimicrobial Susceptibility Testing

- Antibiotics tested were garenoxacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, clindamycin, metronidazole and piperacillin/tazobactam.

Etest® susceptibility testing:

- MIC's were determined by using the concentration gradient agar diffusion method (Etest, AB Biodisk, Sweden). Testing was performed according to NCCLS guidelines [5] and manufacturer's instructions.
- Organism suspension was inoculated into Brain Heart Infusion broth equivalent to a 1 McFarland standard.
- PRAS Brucella agar w/ Vitamin K and Hemin (Anaerobe Systems, CA) was used as growth medium for testing.
- The determination of endpoints was determined according to Etest guidelines [6].
- Quality Control of antibiotic Etest strips and media was performed using *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotamicrons* ATCC 29741, *Eubacterium lentum* ATCC 43055 [7].

Results

The results are presented in the Tables 2 - 3 below.

The 465 anaerobic isolates in this study belonged to 48 species collected during a phase III clinical trials evaluating garenoxacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, clindamycin, metronidazole and piperacillin/tazobactam in intra-abdominal infections. The most frequent species encountered was *B. fragilis* (n=113) comprising 24.3% of all isolates.

Table 2. List of 465 Anaerobic Isolates and 48 species

Organism	N	% Total
Bacteroides caccae	7	1.5
Bacteroides capillosus	2	0.4
Bacteroides distasonis	21	4.5
Bacteroides fragilis	113	24.3
Bacteroides gracilis	7	1.5
Bacteroides ovatus	6	1.3
Bacteroides stercoris	4	0.9
Bacteroides thetaiotaomicron	52	11.2
Bacteroides uniformis	28	6
Bacteroides ureolyticus	2	0.4
Bacteroides vulgatus	24	5.2
Clostridium bifermentans	1	0.2
Clostridium butyricum	5	1.1
Clostridium clostridiiforme	12	2.6
Clostridium difficile	2	0.4
Clostridium hastiforme	5	1.1
Clostridium histolyticum	1	0.2
Clostridium innocuum	3	0.6
Clostridium limosum	1	0.2
Clostridium perfringens	19	4.1
Clostridium ramosum	10	2.2
Clostridium sordellii	1	0.2
Clostridium subterminale	6	1.3
Clostridium tertium	1	0.2
Eubacterium aerofaciens	2	0.4
Eubacterium lentum	20	4.3
Eubacterium limosum	3	0.6
Fusobacterium mortiferum	2	0.4
Fusobacterium necrophorum	5	1.1
Fusobacterium nucleatum	6	1.3
Fusobacterium spp	2	0.4
Fusobacterium varium	1	0.2
Peptostreptococcus anaerobius	7	1.5
Peptostreptococcus asaccharolyticus	1	0.2
Peptostreptococcus indolicus	1	0.2
Peptostreptococcus magnus	1	0.2
Peptostreptococcus micros	25	5.4
Peptostreptococcus prevotii	11	2.4
Peptostreptococcus productus	2	0.4
Peptostreptococcus tetradius	1	0.2
Prevotella bivia	2	0.4
Prevotella buccae	7	1.5
Prevotella denticola	5	1.1
Prevotella intermedia	9	1.9
Prevotella loescheii	3	0.6
Prevotella melaninogenica	10	2.2
Prevotella oralis	3	0.6
Prevotella oris	3	0.6
Total	48	465 100

Table 3. In Vitro Activity of Garenoxacin and Comparators against 465 strains of 6 Anaerobic Species

		MICs (mg/mL)			Susceptibilities (%)*		
Species	Drug	MIC Range	MIC_{50}	MIC_{90}	Sus	Int	Res
All species (n=465)	Garenox	≤ 0.002 - > 32	0.25	1	95.9	0.4	3.7
	Amox/Clav	≤ 0.015 - > 256	0.25	2	97.0	1.7	1.3
	Amp/Sulb	≤ 0.015 - > 256	0.5	4	97.4	1.7	0.9
	Clinda	≤ 0.015 - > 256	1	> 256	71.2	10.3	18.5
	Metro	≤ 0.015 - > 256	0.5	32	87.5	1.7	10.8
	Pip/Tazo	≤ 0.015 - > 256	1	16	95.9	1.5	2.6
Bacteroides spp (n=266)	Garenox	≤ 0.002 - > 32	0.25	1	96.6	0.0	3.4
	Amox/Clav	≤ 0.015 - > 256	0.5	2	96.2	2.7	1.1
	Amp/Sulb	≤ 0.015 - > 256	1	4	96.6	2.3	1.1
	Clinda	≤ 0.015 - > 256	2	> 256	63.5	11.7	24.8
	Metro	≤ 0.015 - > 256	0.5	16	88.7	1.9	9.4
	Pip/Tazo	≤ 0.015 - > 256	2	16	95.1	2.3	2.6
Clostridium spp (n=67)	Garenox	≤ 0.002 - > 32	0.5	2	97.0	0.0	3.0
	Amox/Clav	≤ 0.015 - 8	0.06	0.5	98.5	1.5	0.0
	Amp/Sulb	≤ 0.015 - 8	0.06	0.5	100.0	0.0	0.0
	Clinda	≤ 0.015 - > 256	2	4	74.6	17.9	7.5
	Metro	≤ 0.015 - > 256	2	64	82.1	1.5	16.4
	Pip/Tazo	0.0230 - > 256	0.12	4	98.5	0.0	1.5
Eubacterium spp (n=25)	Garenox	≤ 0.002 - > 32	0.5	16	88.0	0.0	12.0
	Amox/Clav	≤ 0.015 - 2	0.12	1	100.0	0.0	0.0
	Amp/Sulb	≤ 0.015 - 1	0.06	0.5	100.0	0.0	0.0
	Clinda	≤ 0.015 - > 256	0.25	> 256	84.0	0.0	16.0
	Metro	≤ 0.015 - > 256	0.25	8	92.0	0.0	8.0
	Pip/Tazo	≤ 0.015 - 32	0.25	48	100.0	0.0	0.0
Fusobacterium spp (n=16)	Garenox	≤ 0.002 - 0.5	0.25	0.5	100.0	0.0	0.0
	Amox/Clav	≤ 0.015 - 64	0.06	0.5	93.7	0.0	6.3
	Amp/Sulb	≤ 0.015 - 64	0.06	2	93.7	0.0	6.3
	Clinda	≤ 0.015 - 128	0.06	0.5	93.7	0.0	6.3
	Metro	≤ 0.015 - > 256	0.06	> 256	87.5	0.0	12.5
	Pip/Tazo	≤ 0.015 - > 256	0.03	> 256	81.3	0.0	18.7
Peptostreptococcus spp (n=49)	Garenox	≤ 0.002 - > 32	0.03	1	98.0	0.0	2.0
	Amox/Clav	≤ 0.015 - 2	0.03	0.5	100.0	0.0	0.0
	Amp/Sulb	≤ 0.015 - 4	0.03	0.5	100.0	0.0	0.0
	Clinda	≤ 0.015 - 16	0.06	4	89.8	6.1	4.1
	Metro	≤ 0.015 - > 256	0.12	> 256	81.6	0.0	18.4
	Pip/Tazo	≤ 0.015 - 32	0.03	8	100.0	0.0	0.0
Prevotella spp (n=42)	Garenox	≤ 0.002 - > 32	0.25	4	90.5	4.7	4.8
	Amox/Clav	≤ 0.015 - > 256	0.12	1	95.2	0.0	4.8
	Amp/Sulb	≤ 0.015 - 16	0.12	2	95.2	4.8	0.0
	Clinda	≤ 0.015 - > 256	≤ 0.015	> 256	76.2	4.8	19.0
	Metro	≤ 0.015 - > 256	0.5	4	92.9	4.8	2.4
	Pip/Tazo	≤ 0.015 - > 256	0.03	4	95.2	2.4	2.4

Garenox: garenoxacin; Amox/Clav: amoxicillin/clavulanic acid; Amp/Sulb: ampicillin/sulbactam; Clinda: clindamycin; Metro: metronidazole; Piperacillin/Tazo: piperacillin/tazobactam
* Breakpoints defined by NCCLS interpretive criteria, 2003; document M100-S13. Garenoxacin tentative breakpoints (mg/mL) defined as: Sus ≤ 4; Int = 8; Res ≥ 16.

Figure 1. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

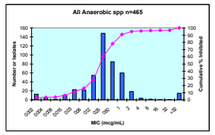


Figure 2. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

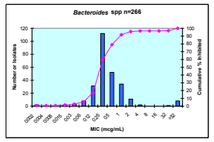


Figure 3. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

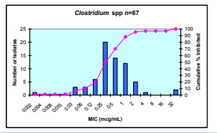


Figure 4. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

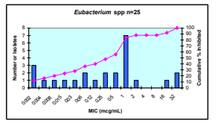


Figure 5. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

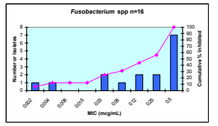


Figure 6. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited

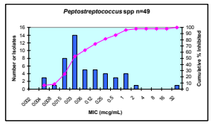
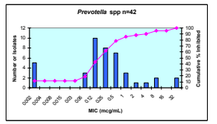


Figure 7. Garenoxacin MIC Frequency Distribution and Cumulative Percent Inhibited



Conclusions

- Garenoxacin demonstrates in vitro activity comparable to the beta-lactam/beta-lactam inhibitor compounds against *Bacteroides* spp, *Clostridium* spp and *Peptostreptococcus* spp; and better activity against *Fusobacterium* spp; and slightly less activity against *Eubacterium* spp.
- Garenoxacin demonstrated better activity than clindamycin and metronidazole against all anaerobic isolates except *Eubacterium* spp.
- The beta-lactamase/beta-lactamase inhibitors, amoxicillin/clavulanic acid, ampicillin/sulbactam and piperacillin/tazobactam inhibited 100% of all *Eubacterium* spp.
- Garenoxacin's MIC_{90} of 1 mcg/mL against *Bacteroides* spp. and all species combined was the lowest of all study drugs.
- Garenoxacin appears to be a promising agent for the treatment of infections caused by anaerobic pathogens.

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