

**CENTER FOR DRUG EVALUATION AND RESEARCH**

*APPLICATION NUMBER:*

**20-695/S-003**

**MICROBIOLOGY REVIEW**

DF  
APR 29 1998

**MICROBIOLOGY REVIEW**  
**DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS**  
**(HFD-590)**

**NDA #:** 20-695/SLR-003

**REVIEWER:** Peter A. Dionne  
**CORRESPONDENCE DATE:** 31-MAR-98  
**CDER DATE:** 01-APR-98  
**REVIEW ASSIGN DATE:** 07-APR-98  
**REVIEW COMPLETE DATE:** 14-APR-98

**SPONSOR:**

GlaxoWellcome Inc.  
Five Moore Drive  
P.O. Box 13398  
Research Triangle Park, North Carolina 27709

**CONTACT PERSON:**

Betsy J. Waldheim  
Project Director, Regulatory Affairs  
Phone Number: (919) 483-5319

**SUBMISSION REVIEWED:**

Supplement SLR-003 (request to add *Legionella pneumophila* and *L. pneumophila* to the *in vitro* list)

**DRUG CATEGORY:**

Antimicrobial: Fluoroquinolone

**INDICATIONS:**

ABECB, CAP, Gonorrhea, Nongonococcal Urethritis and Cervicitis

**DOSAGE FORM:**

Grepafloxacin hydrochloride tablets 200 mg/tablet

**DRUG PRODUCT NAME**

**PROPRIETARY:**

RAXAR™ Tablets

**NONPROPRIETARY/USAN:**

grepafloxacin hydrochloride tablets

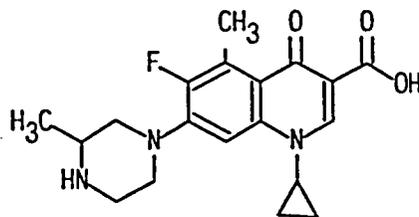
**CODE:**

OPC-17116; OPC-106

**CHEMICAL NAME:**

(±)-1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid monohydrochloride sesquihydrate

**STRUCTURAL FORMULA:**



•HCl•3/2H<sub>2</sub>O

**Molecular Formula:** C<sub>18</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>3</sub>HCl•3/2 H<sub>2</sub>O  
**Molecular Weight:** 422.88

SUPPORTING DOCUMENTS: IND 35,464—Otsuka grepafloxacin hydrochloride

BACKGROUND:

This supplement contains data from four *in vitro* studies on the activity of grepafloxacin against *Legionella pneumophila* and [redacted]. The applicant wishes to add these organisms to the *in vitro* listing in the Microbiology section of the package insert.

Study #1:

*In vitro* and *in vivo* antibacterial activity of four newly developed quinolone agents against *Legionella* Infection.

In this study from Japan, 41 reference strains (14 *L. pneumophila*) and 27 clinical isolates (25 *L. pneumophila*) of *Legionella* species were studied. The MICs were determined by the microdilution method using buffered yeast extract supplemented with [redacted] (BYE<sub>9</sub>) broth. The inoculum was 10<sup>6</sup> cfu/well and the microplates were incubated at 35°C for 48 hours. TABLE 1 shows the MIC ranges for the reference strains.

TABLE 1  
MIC Ranges (µg/mL) of 41 Reference Strains

	Grepafloxacin	Sparfloxacin	Ofloxacin	Ciprofloxacin	Erythromycin
<i>L. pneumophila</i> (n=14)	0.008-0.06	0.004-0.06	0.03-0.06	0.008-0.06	0.125-1.0
<i>Legionella</i> spp. (n=27)	0.008-0.125	0.002-0.06	0.03-0.125	0.008-0.06	0.125-2.0

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ON ORIGINAL

TABLE 2 shows the MIC<sub>50</sub> and MIC<sub>90</sub> and the range of MICs of the 27 *Legionella* clinical isolates.

TABLE 2  
 MICs of Eight Agents Against 27 Clinical Isolates of *Legionella*

AGENTS	RANGE (µg/ml)	MIC <sub>50</sub> (µg/ml)	MIC <sub>90</sub> (µg/ml)
Temafloxacin	0.004-0.03	0.008	0.008
Grepafloxacin	0.008-0.06	0.008	0.03
Sparfloxacin	0.002-0.06	0.004	0.03
Y-26611	0.015-0.125	0.06	0.06
Ofloxacin	0.03-0.06	0.06	0.06
Ciprofloxacin	0.004-0.06	0.03	0.06
Norfloxacin	0.03-0.5	0.25	0.25
Erythromycin	0.125-1.0	0.125	0.5

\* 25 strains of *L. pneumophila* and one each of *L. dumoffii* and *L. micdadei*

The study did not separate the *Legionella pneumophila* isolates from the two other species, but since 25/27 isolates were *L. pneumophila* it is probably safe to assume that the MIC<sub>90</sub> value given represents the MIC<sub>90</sub> value for *L. pneumophila*. This study shows that most fluoroquinolones had a MIC<sub>90</sub> value for *L. pneumophila* of 0.03-0.06 µg/mL. Norfloxacin had a higher value and temafloxacin a lower value.

This study also determined the intracellular to extracellular concentration ratio (I/E ratio) in human polymorphonuclear leukocytes (PMN). The PMN suspension was incubated for 30 minutes with each agent at a final concentration of 50 µg/mL. After incubation the PMNs were separated from the extracellular solution using a silicone oil velocity gradient centrifugation method. The I/E ratio for grepafloxacin was 14.99 ± 0.72; for sparfloxacin it was 13.70 ± 2.20; for temafloxacin it was 12.69 ± 2.06; and for ofloxacin it was 9.07 ± 0.76. This shows that all fluoroquinolones are concentrated intracellularly in PMNs.

Another experiment demonstrated the activity of the agents on *L. pneumophila* grown in guinea pig peritoneal macrophages. A macrophage suspension was mixed with *L. pneumophila* at a concentration of  $2-4 \times 10^6$  cfu/mL. After incubation, extracellular organisms were washed out and the macrophage suspension was incubated with twice the MIC of each agent. At 0, 12, 24, and 36 hours the extracellular organisms were washed out. Macrophages were disrupted with \_\_\_\_\_, and the number of viable organisms were counted. None of the agents allowed *Legionella pneumophila* to multiply in guinea pig peritoneal macrophages. With most agents, including grepafloxacin, the count decreased 1.5 to 2  $\log_{10}$  in 24 hours and continued to decrease to a 2.5  $\log_{10}$  reduction in 36 hours.

The therapeutic effect of the agents on experimental *Legionella pneumonia* in guinea pigs was also studied. Animals were infected with  $2.5 \times 10^8$  cfu/mL. Treatment was started 24 hours after infection and was administered twice a day for 7 days. The daily dose was 10 mg/kg for the quinolones and 20 mg/kg for erythromycin. The therapeutic effect of each agent was followed for 14 days and evaluated by survival rate and mean survival dates after inoculation. The survival rates up to 14 days after infection were 62.5%, 50%, 75%, 50%, and 12.5%, respectively, in the groups treated with Y-26611, grepafloxacin, ofloxacin, ciprofloxacin, or erythromycin, compared to 0% in the untreated controls. The mean survival time was 10.4 days for Y-26611, 8.4 days for grepafloxacin, 11.3 days for ofloxacin, 8.1 days for ciprofloxacin, 4.4 days for erythromycin, and 3.1 days without treatment. This demonstrates that grepafloxacin is better than erythromycin or no treatment and about as good as ciprofloxacin in this animal model of *Legionella pneumonia*. Ofloxacin was slightly better.

Study #2:

*In vitro* activity, post antibiotic effect and human monocyte activity of grepafloxacin against *Legionella* species

This study was performed in Quebec, Canada. The *in vitro* activity of grepafloxacin was compared with sparfloxacin, ciprofloxacin, clarithromycin, erythromycin, and rifampin against 214 *Legionella* isolates, 181 of which were *Legionella pneumophila*. MICs were determined by agar dilution using buffered yeast extract (BYE) agar. The inoculum was  $10^4$  cfu/spot and plates were incubated at 35°C for 48 hours. Reference strains were used for quality control. TABLE 3 shows the results of testing against *Legionella pneumophila*.

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**TABLE 3**  
**Susceptibility of *Legionella pneumophila***

Microorganism	Antibiotic	MIC (µg/mL)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Legionella pneumophila</i> serogroup 1 (71 isolates)	Grepafloxacin	≤0.004-0.06	0.015	0.015
	Sparfloxacin	≤0.004-0.06	≤0.004	≤0.004
	Ciprofloxacin	0.01-0.25	0.03	0.03
	Clarithromycin	≤0.004-0.12	0.06	0.06
	Erythromycin	0.06-1.0	0.25	1.0
	Rifampin	≤0.004-0.008	≤0.004	0.008
	<i>Legionella pneumophila</i> serogroup 2 (17 isolates)	Grepafloxacin	≤0.004-0.03	0.008
Sparfloxacin		≤0.004	≤0.004	≤0.004
Ciprofloxacin		≤0.004-0.03	0.015	0.015
Clarithromycin		≤0.004-0.06	0.03	0.06
Erythromycin		0.008-0.5	0.25	0.25
Rifampin		≤0.004	≤0.004	≤0.004
<i>Legionella pneumophila</i> serogroup 3 (14 isolates)		Grepafloxacin	≤0.004-0.015	0.008
	Sparfloxacin	≤0.004-0.008	≤0.004	≤0.004
	Ciprofloxacin	≤0.004-0.03	0.03	0.03
	Clarithromycin	0.03-0.06	0.03	0.06
	Erythromycin	0.12-0.5	0.25	0.5
	Rifampin	≤0.004	≤0.004	≤0.004
	<i>Legionella pneumophila</i> serogroup 4 (21 isolates)	Grepafloxacin	≤0.004-0.015	0.008
Sparfloxacin		≤0.004-0.008	≤0.004	≤0.004
Ciprofloxacin		0.03-0.12	0.03	0.06
Clarithromycin		0.03-0.06	0.06	0.06
Erythromycin		0.06-0.5	0.5	0.5
Rifampin		≤0.004-0.008	≤0.004	≤0.004
<i>Legionella pneumophila</i> serogroup 5 (15 isolates)		Grepafloxacin	≤0.004-0.06	0.015
	Sparfloxacin	≤0.004-0.06	≤0.004	0.015
	Ciprofloxacin	0.015-0.06	0.03	0.03
	Clarithromycin	0.015-0.06	0.03	0.06
	Erythromycin	0.06-1.0	0.25	0.5
	Rifampin	≤0.004	≤0.004	≤0.004
	<i>Legionella pneumophila</i> serogroup 6 (39 isolates)	Grepafloxacin	≤0.004-0.015	0.008
Sparfloxacin		≤0.004-0.008	≤0.004	≤0.004
Ciprofloxacin		≤0.004-0.03	0.03	0.03
Clarithromycin		≤0.004-0.06	0.015	0.06
Erythromycin		0.008-0.25	0.12	0.25
Rifampin		≤0.004-0.008	≤0.004	≤0.004
<i>Legionella pneumophila</i> serogroup 7 (2 isolates)		Grepafloxacin	≤0.004-0.008	≤0.004
	Sparfloxacin	≤0.004	≤0.004	≤0.004
	Ciprofloxacin	0.03	0.03	0.03
	Clarithromycin	0.015-0.06	0.016	0.06
	Erythromycin	0.12-0.5	0.12	0.5
	Rifampin	≤0.004	≤0.004	≤0.004
	<i>Legionella pneumophila</i> serogroup 8 (2 isolates)	Grepafloxacin	0.008	0.008
Sparfloxacin		≤0.004	≤0.004	≤0.004
Ciprofloxacin		0.03	0.03	0.03
Clarithromycin		0.06	0.06	0.06
Erythromycin		0.25	0.25	0.25
Rifampin		≤0.004	≤0.004	≤0.004

In this study 181 isolates were tested. The highest MIC<sub>90</sub> value was 0.03 µg/mL for serogroup 5. All other serogroups had MIC<sub>90</sub> values of 0.008 or 0.015 µg/mL. Grepafloxacin was more active than erythromycin (MIC<sub>90</sub> 0.25-1.0 µg/mL) and clarithromycin (MIC<sub>90</sub> 0.06 µg/mL) against all of the strains tested, whereas, rifampin was slightly more active (MIC<sub>90</sub> ≤ 0.008 µg/mL) than grepafloxacin. Sparfloxacin was slightly more active than grepafloxacin against most serogroups. Grepafloxacin was usually more active than ciprofloxacin with the exception of serogroups 2 and 5, where MIC<sub>90</sub> values for both drugs were equivalent. In general, *Legionella pneumophila* serogroups 6-8 were more susceptible to grepafloxacin than serogroups 1-5. Overall, all of the agents tested showed good activity against *Legionella pneumophila*.

The MIC<sub>90</sub>s of the test drugs against other \_\_\_\_\_ are shown in TABLE 4.

TABLE 4  
Susceptibility of \_\_\_\_\_

Microorganism	Antibiotic	MIC (µg/mL)		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>

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In this study the post-antibiotic effect (PAE) was determined using a broth technique. Colonies of *Legionella* were grown in buffered yeast extract (BYE) broth. A final concentration of  $10^6$ - $10^7$  cfu/mL was used. Tubes containing the drug at four times the MIC and the test culture were incubated in a shaking water bath at 37°C for 1-2 hours. Drug was then removed by centrifugation. Bacterial counts were performed on all colonies at time zero, before and after washing and every hour until turbidity developed. Counts were mapped on a graph and PAE calculated. In this experiment the PAE of grepafloxacin (2.62 hours) against erythromycin-susceptible *L. pneumophila* was longer than for the other drugs tested. The PAE of grepafloxacin (4.18 hours) against erythromycin-resistant *L. pneumophila* was also longer than for the other drugs tested.

PAE is dependent on the species tested, the concentration of drug used, and the time the culture is exposed to drug. A longer PAE may indicate that the drug may be given less frequently, but the clinical significance of a long PAE is not known.

This study also demonstrated that grepafloxacin, sparfloxacin, ciprofloxacin, and clarithromycin all inhibited the growth of *Legionella pneumophila* in human monocytes. Only grepafloxacin and ciprofloxacin, however, prevented regrowth of *Legionella pneumophila* after the removal of extracellular antibiotic.

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ON ORIGINAL**

Study #3:  
*In vitro* activity of grepafloxacin against : \_\_\_\_\_  
\_\_\_\_\_ and *Legionella* spp.

This study was performed in London. \_\_\_\_\_

\_\_\_\_\_ . These data are not relevant to the labeling change under consideration, so they are not reviewed here. The activity of grepafloxacin was compared to ofloxacin, clarithromycin, and rifampin against *Legionella*. MICs for *Legionella* species were determined using an agar dilution method in supplemented buffered yeast extract (BYE) medium containing 5% lysed horse blood. The inoculum was  $10^4$  cfu/spot. Incubation was at 37°C for 48-72 hours. Ninety-eight isolates of *Legionella pneumophila* were tested, \_\_\_\_\_ were also tested. TABLE 5 shows the results of this study.

TABLE 5

The MICs of Grepafloxacin and Comparators against *Legionella pneumophila* (98 isolates)

ANTIBIOTIC	MIC <sub>50</sub>	MIC <sub>90</sub>	RANGE
Grepafloxacin	0.015	0.015	0.008-0.03
Ofloxacin	0.015	0.03	0.008-0.03
Clarithromycin	0.03	0.03	0.015-0.06
Rifampin	0.008	0.008	0.004-0.03

[  
These data demonstrate that grepafloxacin has good activity against *Legionella pneumophila*. Its activity is about equal to that of ofloxacin and clarithromycin and slightly less than that of rifampin. \_\_\_\_\_  
\_\_\_\_\_

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ON ORIGINAL

Study #4:  
*In vitro* antimicrobial activity of grepafloxacin and its therapeutic efficacy on  
respiratory infections

This study was performed in Japan. The *in vitro* activity of grepafloxacin was compared with ofloxacin, tosufloxacin, ciprofloxacin, sparfloxacin, and erythromycin against ~~25 clinical strains of Legionella pneumophila~~ and ~~25 clinical strains of Legionella pneumophila~~ 25 clinical strains of *Legionella pneumophila* and ~~25 clinical strains of Legionella pneumophila~~. The results against *Legionella* are shown in TABLE 6.

TABLE 6  
MICs of Grepafloxacin and Comparators against *Legionella* (Study #4)

Organism	Antimicrobial	MIC range ( $\mu\text{g}/\text{mL}$ )	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>L. pneumophila</i> (25 isolates)	Grepafloxacin	0.008-0.06	0.008	0.015
	Ofloxacin	0.03-0.06	0.06	0.06
	Tosufloxacin	0.004-0.03	0.008	0.015
	Ciprofloxacin	0.015-0.06	0.03	0.06
	Sparfloxacin	0.002-0.03	0.004	0.008
	Erythromycin	0.015-0.25	0.06	0.125

The data in the above table demonstrate that the MIC<sub>90</sub> of grepafloxacin for clinical isolates of *Legionella pneumophila* was equal to that of tosufloxacin, one dilution higher than that of sparfloxacin and superior to that of ofloxacin, ciprofloxacin, and erythromycin. All the fluoroquinolones showed good activity and were better than erythromycin.

SUMMARY

Four studies have been included in this submission. TABLE 7 summarizes the *in vitro* activity of grepafloxacin against *Legionella pneumophila* clinical strains. Only clinical isolates have been included since the Points to Consider document states that data must be from recent clinical isolates.

TABLE 7  
 Summary of Grepafloxacin *in vitro* Activity Against *Legionella pneumophila*

Species	# Tested	MIC Range (µg/mL)	MIC <sub>90</sub> (µg/mL)	Reference
<i>L. pneumophila</i>	25 <sup>1</sup>	0.008-0.06	0.03	1—Japan
<i>L. pneumophila</i> (serogroup 1)	71	≤0.004-0.06	0.015	2—Canada
<i>L. pneumophila</i> (serogroup 2)	17	≤0.004-0.03	0.015	2
<i>L. pneumophila</i> (serogroup 3)	14	≤0.004-0.016	0.015	2
<i>L. pneumophila</i> (serogroup 4)	21	≤0.004-0.016	0.015	2
<i>L. pneumophila</i> (serogroup 5)	15	≤0.004-0.06	0.03	2
<i>L. pneumophila</i> (serogroup 6)	39	≤0.004-0.016	0.008	2
<i>L. pneumophila</i> (serogroup 7)	2	≤0.004-0.008	0.008	2
<i>L. pneumophila</i> (serogroup 8)	2	0.008	0.008	2
<i>L. pneumophila</i>	98	0.008-0.03	0.015	3—England
<i>L. pneumophila</i>	25	0.008-0.06	0.015	4—Japan
TOTAL	329	≤0.004-0.03	0.008-0.03	

1) *Legionella pneumophila* was not separated from two other species of *Legionella* in this study. The MIC<sub>90</sub> and range given is for all 27 *Legionella* species, but 25 of the 27 isolates were *Legionella pneumophila*.

Over 300 clinical isolates (>100 isolates are usually required for inclusion in the label) were tested in more than one laboratory. Although no studies were conducted with United States isolates, one major study which tested over 180 clinical isolates was conducted in North America (Canada). All studies demonstrated that grepafloxacin's MICs against *Legionella pneumophila* were ≤0.03 µg/mL. *Legionella pneumophila* may be placed into the *in vitro* activity section of the package insert.

Table 8 summarizes the *in vitro* activity of grepafloxacin against other  
Here again, only clinical isolates are included.

TABLE 8



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ON ORIGINAL





**MICROBIOLOGY REVIEW**  
**DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS**  
**(HFD-590)**

**NDA #:** 20-695/SLR-003/BL      **REVIEWER:** Peter A. Dionne  
**CORRESPONDENCE DATE:** 22-SEP-98  
**CDER DATE:** 23-SEP-98  
**REVIEW ASSIGN DATE:** 11-DEC-98  
**REVIEW COMPLETE DATE:** 14-DEC-98

**SPONSOR:** GlaxoWellcome Inc.  
 Five Moore Drive  
 P.O. Box 13398  
 Research Triangle Park, North Carolina 27709

**CONTACT PERSON:** Betsy J. Waldheim  
 Project Director, Regulatory Affairs  
 Phone Number: (919) 483-5319

**SUBMISSION REVIEWED:** Supplement SLR-003 (request to add *Legionella pneumophila* and \_\_\_\_\_ to the *in vitro* list)  
 (BL) Submission is revised package insert with only *Legionella pneumophila* added.

**DRUG CATEGORY:** Antimicrobial: Fluoroquinolone

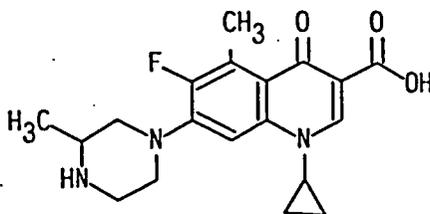
**INDICATIONS:** ABECB, CAP, Gonorrhea, Nongonococcal Urethritis and Cervicitis

**DOSAGE FORM:** Grepafloxacin hydrochloride tablets 200 mg/tablet

**DRUG PRODUCT NAME**

**PROPRIETARY:** RAXAR™ Tablets  
**NONPROPRIETARY/USAN:** grepafloxacin hydrochloride tablets  
**CODE:** OPC-17116; OPC-106  
**CHEMICAL NAME:** (±)-1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid monohydrochloride sesquihydrate

**STRUCTURAL FORMULA:**



•HCl•3/2H<sub>2</sub>O

**Molecular Formula:** C<sub>19</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>3</sub>HCl•3/2 H<sub>2</sub>O  
**Molecular Weight:** 422.88

SUPPORTING DOCUMENTS: IND 35,464—Otsuka grepafloxacin hydrochloride

BACKGROUND:

This submission revises the package insert. Only *Legionella pneumophila* is included. \_\_\_\_\_ has been removed as requested in the Microbiology Review of supplement SLR-003 that is dated April 14, 1998.

CONCLUSIONS & RECOMMENDATIONS:

The label is satisfactory from the microbiological viewpoint.

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ON ORIGINAL

/S/

Peter A. Dionne  
Microbiologist HFD-590

CONCURRENCES:

HFD-590/Div Dir  
HFD-590/TLMicro

/S/

Signature 2/18/99 Date  
Signature 1/7/99 Date

CC:

HFD-590/Original NDA # 20695/SLR-003/BL  
HFD-590/Division File  
HFD-590/Micro/PDionne  
HFD-590/MO/AMeyerhoff  
HFD-520/Pharm/TPeters  
HFD-590/Chem/GHolbert  
HFD-590/CSO/RAnderson