20-695

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-695

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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW

PHARMACOLOGY / TOXICOLOGY REVIEW AND EVALUATION

NDA#: 20-695
Type: New Correspondence (Toxicology Information)
Date of Submission: 11/16/00

Review Division: Special Pathogen and Immunologic Drug Products
HFD-590
Reviewer: Stephen G. Hundley, Ph.D., Pharmacologist

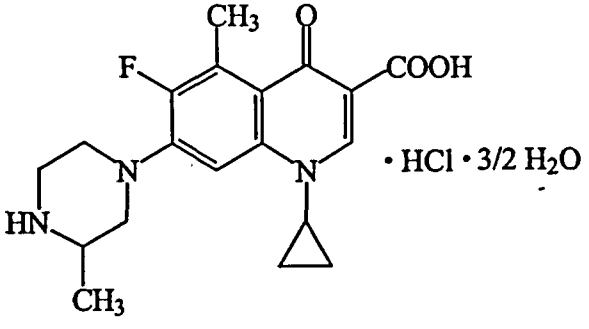
Review Completion Date: 6/8/01

Sponsor: Otsuka American Pharmaceutical Inc.
Maryland Office of Clinical Research
2440 Research Boulevard
Rockville, MD 20850
Phone: 301-902-3792

Drug Information

Name: Grepafloxacin
Drug Name: RAXAR®
Chemical Name: (±)-1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid monohydrochloride sesquihydrate
CAS#: 161967-81-3
Molecular Formula: $C_{19}H_{22}FN_3O_3 \cdot HCl \cdot 3/2\ H_2O$
Molecular Weight: 422.88 (as the monochloride sesquihydrate)

Molecular Structure:



Drug Category: Antimicrobial – Fluoroquinolone

Related Submissions: IND's 35,464 & 51,295

Current Indications: Acute exacerbation of chronic bronchitis, community-acquired pneumonia, uncomplicated gonorrhea, and nongonococcal urethritis and cervicitis.

BACKGROUND

Grepafloxacin is a fluoroquinolone approved for use as a broad-spectrum antibacterial agent. This submission contains nonclinical studies that assess the electrophysiological effects of grepafloxacin in *in vitro* systems and cardiovascular effects *in vivo*. These studies were submitted in response to a letter from the Division of Special Pathogen and Immunologic Drug Products (dated 6/9/00) requesting data regarding QTc prolongation *in vivo* and *in vitro* studies from ventricular monocytes assessing effects on action potential duration or from cells transfected with the HERG gene assessing effects on the IKr channel.

The current submission contains an electrophysiological study with dog Purkinje fibers, an *in vitro* evaluation of the IKr (potassium channel) with Chinese Hamster Ovary cells transfected with HERG cDNA, an electrophysiological study with guinea pig myocytes, and studies of cardiovascular effects in anesthetized rabbits and dogs.

NONCLINICAL SAFETY PHARMACOLOGY STUDIES

General Pharmacology of OPC-17116: Effects of New Quinolones on Blood Pressure Heart Rate and Electrocardiograms in Anesthetized Rabbits (Report no. 009120). [Study was reviewed in prior submission.]

Effect of Grepafloxacin and the Comparator Substances Ciprofloxacin, Moxifloxacin, and Sparfloxacin on Action Potential Parameters in Dog Isolated Purkinje Fibers (Report no. 013730).

General Phamacology of OPC-17116: Effects of OPC-17116 on Respiration and Cardiovascular Systems in Anesthetized Rabbits (Report no. 004845). [Study was reviewed in prior submission.]

General Pharmacology of OPC-17116: Effects of OPC-17116 on Respiratory and Cardiovascular Systems in Anesthetized Mongrel Dogs (Report no. 004700). [Study was reviewed in prior submission.]

[redacted](Grepafloxacin, Quinolone Antibiotic) Safety Pharmacology: Effects on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres (Glaxo Wellcome [redacted] Otsuka Report no. 013434).

The Electrophysiological Effects of Grepafloxacin and Comparator Compounds on Guinea-Pig Isolated, Paced Ventricular Myocytes (Glaxo Wellcome – [redacted] Otsuka Report no. 012751).

Examination of the Effects of 4 Compounds on HERG and KCNQ1 / KCNE1 Mediated Potassium Channels (Otsuka Report no. 013660).

General Pharmacology of OPC-17116: Re-evaluation of the Effects of OPC-17116 and Its Reference Drugs on ECG in Anesthestized Dogs (Report no. 011794).

Submitted but not Reviewed:

General Pharmacology of OPC-17116: Effects of Sparfloxacin on the Respiratory and Cardiovascular Systems in Anesthetized Dogs.

NONCLINICAL SAFETY PHARMACOLOGY STUDIES REVIEWS

Effect of Grepafloxacin and the Comparator Substances Ciprofloxacin, Moxifloxacin, and Sparfloxacin on Action Potential Parameters in Dog Isolated Purkinje Fibers (Report no. 013730).

——— (Grepafloxacin, Quinolone Antibiotic) Safety Pharmacology: Effects on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres (Glaxo Wellcome ———. Otsuka Report no. 013434).

The studies were conducted by ——— under contract from Otsuka Pharmaceutical Co., Ltd., in accordance with GLP requirements and audited by a Quality Assurance group. The compounds that were evaluated included grepafloxacin, sparfloxacin, moxifloxacin, and ciprofloxacin. The positive control selected for the dog purkinje fiber assay was *dl*-sotalol.

Ventricular Purkinje fibers were isolated from the hearts of beagle dogs. Four fibers were used for each compound and were evaluated in an *in vitro* perfusion chamber. The individual fibers were secured to the Sylgard base of the perfusion chamber with entomology pins; silver bipolar stimulating electrodes were positioned at one end of the fiber to enable electrical stimulation. Electrophysiological measurements were made with glass microelectrodes inserted into the cells of the Purkinje fiber. Perfusion media was circulated over each fiber for approximately 60 minutes to achieve stable waveform. Exposures to each of the fluoroquinolones were initiated at the lowest fluoroquinolone concentration at 1 Hz electrical stimulation. The electrophysiological measurements were made over 30 minutes and the process was repeated at 0.5 Hz stimulation. The same procedure was followed at each succeeding fluoroquinolone concentration. The terminal exposure was to 50 μM *dl*-Sotalol to confirm the responsiveness of each Purkinje fiber.

The electrophysiological measurements included action potential duration at 60 or 90 percent repolarization ($APD_{90 \text{ or } 60}$), maximum rate of depolarization (MRD), upstroke amplitude (UA), and resting membrane potential (RMP). The sponsor expressed the

perfusion concentrations for each fluoroquinolone in terms of μg/ml; 3, 10, 30, and 100 μg/ml for each fluoroquinolone. The following chart lists the μM perfusion concentration for each fluoroquinolone based upon their respective molecular weights.

	μM Perfusion Concentration			
Perfusion Level	Grepafloxacin	Sparfloxacin	Moxifloxacin	Ciprofloxacin
3 μg/ml	7.1	7.7	6.8	7.8
10 μg/ml	23.7	25.5	22.8	25.9
30 μg/ml	71	76.5	68.4	77.7
100 μg/ml	237	255	228	259

Results from the series of incubations with each fluoroquinolone (four Purkinje fiber incubations for each fluoroquinolone) indicated no compound-related effects at any concentration upon MRD, UA, and RMP. Statistically significant prolongations of APD_{90} and APD_{60} values were observed for each fluoroquinolone. The APD_{90} values, for example, were significantly increased by sparfloxacin and moxifloxacin at all perfusion concentrations (3 to 100 μg/ml) at electrical stimulations of 0.5 and 1 Hz. The APD_{90} and APD_{60} values were least affected by ciprofloxacin where statistical significance was consistently observed only at the highest perfusion concentration (100 μg/ml). Grepafloxacin unequivocally prolonged the APD_{90} and APD_{60} values at perfusion concentrations of 10 μg/ml and above (0.5 and 1 Hz stimulation). The following chart lists the average percent increases in the APD_{90} values for all of the fluoroquinolones tested in this study (results were similar for APD_{60} values).

	Average Percent Increase in APD_{90}			
	1 Hz Electrical Stimulation			
	3 μg/ml	10 μg/ml	30 μg/ml	100 μg/ml
Grepafloxain	3.1	**16.5**	**36.3**	**74**
Ciprofloxacin	No Effect	5.1	7.5	**19**
Moxifloxacin	**7.1**	**15**	**33**	**74**
Sparfloxacin	**12**	**32**	**65**	**105**
	0.5 Hz Electrical Stimulation			
Grepafloxacin	7.9	**23.4**	**51.5**	**92.8**
Ciprofloxacin	No Effect	6.7	7.8	**20**
Moxifloxacin	**11.6**	**18.3**	**43.7**	**103**
Sparfloxacin	**14.7**	**42.2**	**79.2**	**151**

The **bold type** values were derived from APD_{90} msec values that were statistically greater than values from the zero-level incubation concentrations.

Sparfloxacin caused the greatest percent increase in APD_{90} values regardless of whether the perfusion concentration data were expressed as μg/ml or μM. The effects of grepafloxacin and moxifloxacin upon APD_{90} were similar regardless the way perfusion concentrations were expressed. Ciprofloxacin exhibited the least effect upon the prolongation of APD_{90} values with an approximate 20 percent increase at 100 μg/ml (259 μM).

The 50 μM concentration of *dl*-sotalol resulted in an approximate 45% increase (130 msec) in the $APD_{60 \text{ and } 90}$ values at 1 Hz stimulation and an approximate 64% increase (210 msec increase) at the 0.5 Hz stimulation rate. The *dl*-sotalol results confirmed the viability of each of the Purkinje fibers used in this study.

The sponsor also conducted a preliminary study with only grepafloxacin in accordance with the procedures previously described. The perfusion concentrations examined were 1, 3, 5, and 10 μg/ml (equivalent to 2.4, 7.1, 11.9, and 23.7 μM). APD_{90} values were prolonged by approximately 13% at the 10 μg/ml perfusion concentration at the 1 Hz electrical stimulation rate. Non-significant (statistically) increases in the APD_{90} values were observed at the 3 and 5 μg/ml perfusion concentrations. These results were similar to those observed for grepafloxacin in the definitive study.

The Electrophysiological Effects of Grepafloxacin and Comparator Compounds on Guinea-Pig Isolated, Paced Ventricular Myocytes (Glaxo Wellcome - ——— Otsuka Report no. 012751).

Ventricular myocytes were isolated from guinea pig hearts and maintained in cell culture for electrophysiological measurements. Cells were mounted in a glass coverslip base of a perspex flow chamber; a perfusion solution flowed over the myocytes. Action potentials were electrically stimulated at a frequency of 1 Hz. Action potential durations were measured with an intracellular glass microelectrode. The perfusion concentrations of grepafloxacin were 1, 3, 5, and 10 μg/ml. Comparator compounds included sparfloxacin (3, 10, and 30 μg/ml), trovafloxacin (1 and 10 μg/ml), ofloxacin (3 and 30 μg/ml), erythromycin (10 and 100 μg/ml), and *dl*-sotalol (3, 10, and 30 μg/ml). This study was not conducted in accordance with GLP requirements and was not audited.

Trovafloxacin and ofloxacin did not effect the APD_{90} values at the perfusion concentrations that were examined. Grepafloxacin prolonged the APD_{90} values by approximately 10 and 16 percent at the 5 and 10 μg/ml perfusion concentrations, respectively. Sparfloxacin prolonged the APD_{90} values by 9, 15, and 27 percent at perfusion concentrations of 3, 10, and 30 μg/ml, respectively. The effect of *dl*-sotalol was similar to sparfloxacin with 16 and 22 percent prolongation of APD_{90} values at the 10 and 30 μg/ml concentrations. Erythromycin produced minimal effects upon APD_{90} values with an approximate 9 percent increase at the 100 μg/ml perfusion concentration.

The following table lists the highest perfusion concentration for each compound as μg/ml, and μM, and the percent prolongation of the APD_{90} values.

Grepafloxacin	10 μg/ml	28 μM	16 %
Sparfloxacin	30 μg/ml	77 μM	27 %
Trovafloxacin	10 μg/ml	24 μM	No Change
Ofloxacin	30 μg/ml	83 μM	No Change
Erythromycin	100 μg/ml	136 μM	8.5 %
dl-Sotalol	30 μg/ml	110 μM	22 %

Comments:

The electrophysiological study with ventricular myocytes from guinea pigs was a probe or pilot project that was limited in scope. The only electrophysiological measurement was the action potential duration at 90 percent repolarization (APD_{90}). There was also no rationale given for the perfusion concentrations used for grepafloxacin and the comparator fluoroquinolones. The highest grepafloxacin concentration examined was 10 μg/ml, whereas sparfloxacin and ofloxacin were evaluated at 30 μg/ml. The maximum prolongation of APD_{90} was approximately 27 % at the 30 μg/ml sparfloxacin concentration indicating that all of the fluoroquinolones could have been examined at perfusion concentrations up to 100 μg/ml.

Examination of the Effects of 4 Compounds on HERG and KCNQ1 / KCNE1 Mediated Potassium Channels (Otsuka Report no. 013660).

This study was contracted by Otsuka Pharmaceutical to [redacted] and was not conducted in accordance with GLP requirements. Chinese hamster ovary (CHO) cells were transfected with human genes that encoded for the HERG and KCNQ1/KCNE1 potassium channels. The transfected CHO cell cultures expressed the respective potassium channel genes and electrophysiological measures of the potassium channel activity were made by the patch-clamp technique under defined experimental conditions. Cell culture concentrations of grepafloxacin, ciprofloxacin, sparfloxacin, and moxifloxacin were 10, 30, and 100 μg/ml while sparfloxacin was also evaluated at 3 μg/ml. These fluoroquinoline concentrations were used to evaluate the effect upon the HERG potassium channel. The effect of these fluoroquinolones upon the KCNQ1/KCNE1 potassium channels was evaluated at a single concentration of 30 μg/ml for grepafloxacin and moxifloxacin, 100 μg/ml for ciprofloxacin, and 10 μg/ml for sparfloxacin. No postive control was used in the assays.

The conditions under which peak tail currents for the HERG potassium channel were measured included a –80 mV holding potential that was depolarized to +40 mV in 1 sec., followed by repolarization in increments of 20 mV at a stimulation frequency of 0.1 Hz. The reduction in peak tail currents was measured at each fluoroquinolone concentration. An IC_{50} value was determined for each fluoroquinolone and are listed as μg/ml and μM (based upon molecular weights).

	IC_{50} Values	
Grepafloxacin	37 μg/ml	87 μM
Ciprofloxacin	---	---
Sparfloxacin	13 μg/ml	33 μM
Moxifloxacin	35 μg/ml	80 μM

Ciprofloxacin did not reduce the peak tail current at the highest incubation concentration of 100 μg/ml ($\approx$260 μM).

CHO cells transfected with the KCNQ1/KCNE1 gene were also maintained at a –80 mV holding potential then depolarized to +60 mV in 2 sec., at a stimulation frequency of 0.1 Hz. The cells were then depolarized to +40 mV in 1 sec. The sustained current amplitude was measured. Both grepafloxacin and moxifloxacin at 30 μg/ml (≈70 μM) reduced the current amplitude by 30 percent. Sparfloxacin reduced the current amplitude by 12 percent at 10 μg/ml (25 μM) while no effect was observed with ciprofloxacin at the cell culture concentration of 100 μg/ml (≈260 μM).

Comments:

No positive control was used in these assays. A positive control is desirable in order to make relative comparisons in IC_{50} values between the selected fluoroquinolones and an agent that has potent cardiac activity. The effect of grepafloxacin and the selected fluoroquinolone comparators on the KCNQ1/KCNE1 potassium channels needed to be evaluated at three or more cell culture concentrations of each fluoroquinolone in order to establish approximate IC_{50} values.

General Pharmacology of OPC-17116: Effects of New Quinolones on Blood Pressure Heart Rate and Electrocardiograms in Anesthetized Rabbits (Report no. 009120). [Study was reviewed in prior submission.]

This study was previously reviewed under the original NDA 20-695 submission (submission received 11/8/96; Pharmacology and Toxicology review completed 2/10/97). Male New Zealand white rabbits were anesthetized and administered grepafloxacin and selected comparator fluoroquinolones by *iv* infusion at infusion rates of 30 mg/kg/min. Doses of each fluoroquinolone were administered in ascending order with a 30 minute interval between the termination of one dose level and the initiation of the next dose level. Grepafloxacin was administered at dose levels of 10, 30, and 100 mg/kg. Ciprofloxacin and lomefloxacin were administered at dose levels of 10, 30, 100, and 300 mg/kg. Electrocardiograms, heart rate, and blood pressure were determined at each dose level while the rabbits were maintained under anesthesia.

Arrhythmia was induced by grepafloxacin at all dose levels whereas ciprofloxacin and lomefloxacin induced arrhythmias at the 100 mg/kg dose level and not at the lower dose levels. The following table lists the arrhythmia rate and fatal arrhythmias.

	Incidence of Arrhythmias			
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Grepafloxacin	1/4	2/4	4/4 (2)	---
Ciprofloxacin	0/4	0/4	1/4	4/4 (4)
Lomefloxacin	0/4	0/4	2/4	4/4 (4)

Values in parentheses indicate the number of fatal arrhythmias.

Grepafloxacin also increased the heart rate and decreased the blood pressure at each dose level. The maximum blood pressure decrease in surviving animals was 54 % at the 100

mg/kg dose level. Ciprofloxacin dosing resulted in a decreased heart rate and a maximum increase in blood pressure of 10 % at the 100 mg/kg dose level. Lomefloxacin also caused a decreased heart rate and decreased blood pressure (6 % and 11 % at 30 and 100 mg/kg, respectively). These data indicated that grepafloxacin was more potent in inducing cardiac arrhythmia than ciprofloxacin and lomefloxacin in the anesthetized rabbit model.

General Pharmacology of OPC-17116: Effects of OPC-17116 on Respiratory and Cardiovascular Systems in Anesthetized Mongrel Dogs (Report no. 004700). [Study was reviewed in prior submission.]

General Pharmacology of OPC-17116: Re-evaluation of the Effects of OPC-17116 and Its Reference Drugs on ECG in Anesthestized Dogs (Report no. 011794).

This study was previously reviewed under the original NDA 20-695 submission (submission received 11/8/96; Pharmacology and Toxicology review completed 2/10/97). Anesthetized mongrel dogs (two males and two females for each compound) received *iv* infusions of grepafloxacin and selected comparator fluoroquinolones. The dose levels for grepafloxacin and ofloxacin were 1, 3, 10, and 30 mg/kg. Ciprofloxacin dose levels were 0.3, 1, 3, and 10 mg/kg and norfloxacin was administered at dose levels of 0.1, 0.3, 1, and 3 mg/kg. The *iv*-infusion rate was 10 mg/kg/min for all dose levels and doses were administered in ascending order. Blood pressure, heart rate, and respiration rate were monitored at sequential time intervals following each dose. Electrocardiograms (ECG's) were also generated following each *iv* infusion.

The 30 mg/kg *iv* dose of ofloxacin resulted in the death of one dog approximately 50 minutes following the dose. Two dogs died approximately 10 minutes following the 3 mg/kg dose of norfloxacin. There were no clinical signs reported by the sponsor nor any indication as to cause of death. There were no mortalities as a result of dosing with grepafloxacin and ciprofloxacin. Heart rates were reported to have increased after dosing with ciprofloxacin and grepafloxacin, although no quantitative data were presented. Heart rates decreased after dosing with ofloxacin and were unaltered by norfloxacin. Summarized quantitative data were presented for blood pressure effects and are listed in the following table.

	Baseline	Change from Baseline (mm Hg) at each mg/kg Dose					
	mm Hg	0.1	0.3	1.0	3.0	10	30
Grepafloxacin	136			- 5	- 19	- 45	- 88
Ofloxacin	136			- 7	- 49	- 85	- 92
Norfloxacin	131	- 6	- 16	- 96	- 107		
Ciprofloxacin	136		- 15	- 82	- 102	- 96	

These data represented the transitory peak depression in blood pressure following the *iv* infusion at each dose level. The sponsor did not indicate the time following dosing for peak blood pressure depression. These data suggest that grepafloxacin had the smallest

relative effect on blood pressure at the 1, 3, and 10 mg/kg dose levels. Norfloxacin and ciprofloxacin appeared to cause the greatest relative depression in blood pressure. The sponsor also indicated that *iv* infusion with grepafloxacin did not affect respiration rate whereas the respiration rate was elevated following the 3 and 10 mg/kg doses of ciprofloxacin.

The second report was generated by reevaluating ECG data collected from the previously reviewed study. Quantitative heart rate data indicated that grepafloxacin and norfloxacin had no effect upon heart rate. Both ofloxacin and ciprofloxacin appeared to lower the heart rate at time intervals from one to three minutes following the *iv* infusion. Ofloxacin reduced the heart rate by approximately 10 % at the 10 mg/kg dose level and 28 % at the 30 mg/kg dose level. Ciprofloxacin reduced the heart rate by approximately 10 % at the 3 and 10 mg/kg dose levels. Heart rates recovered to control values by 20 minutes following *iv* infusions of ofloxacin and ciprofloxacin. The ECG's demonstrated QT interval prolongation as a result of dosing with grepafloxacin, ofloxacin, and ciprofloxacin. QTc values were calculated based upon the Bazett correction factor for altered heart rate. The following table lists the maximum change in QTc for each of the fluoroquinolones at all dose levels.

Maximum Δ QTc (msec) and % Change from Baseline

	Dose Level (mg/kg)					
	0.1	0.3	1.0	3.0	10.0	30.0
Grepafloxacin			N.C.	N.C.	+31 (8.4 %)	+59 (16 %)
Ofloxacin			N.C.	+19 (5 %)	+17 (4 %)	+25 (6.5 %)
Ciprofloxacin		N.C.	+27 (7.6 %)	+14 (3.9 %)	+26 (7.2 %)	
Norfloxacin	N.C.	N.C.	N.C.	N.C.		

No change (N.C.) from baseline.

Norfloxacin did not cause QTc prolongation at the highest dose tested (3.0 mg/kg). Ciprofloxacin and grepafloxacin caused a similar degree of QTc prolongation at the 10 mg/kg dose level. Grepafloxacin induced approximately 2-fold greater QTc prolongation than ofloxacin at the 10 and 30 mg/kg dose levels. The 1.0 and 3.0 mg/kg dose levels were common to all compounds; no alterations to QTc were observed with norfloxacin and grepafloxacin at these two dose levels. QTc prolongation resulted from ofloxacin and ciprofloxacin at the 3.0 mg/kg dose level and with ciprofloxacin at the 1 mg/kg dose level.

Comments:

Mongrel dogs were used in this study which presents problems with regard to health status, age, and animal to animal variability. The intent of this research was to compare the cardiovascular effects of structurally and pharmacologically similar compounds. In

order to best achieve this comparison the sponsor should have used a pure breed of dogs such as beagles. In addition, randomization into the different dosing routines is difficult with mongrel dogs. The sponsor also did not identify the sex of each dog in the individual animal data tables.

EVALUATION AND CONCLUSIONS

The dog Purkinje fiber studies indicated that grepafloxacin and the comparator fluoroquinolones only affected the action potential duration ($APD_{60 \text{ and } 90}$). Ciprofloxacin induced the smallest increase in $APD_{60 \text{ and } 90}$ at the cell culture concentrations examined (7.8 to 259 μM). Grepafloxacin and moxifloxacin were equivalent in prolongation of the $APD_{60 \text{ and } 90}$ values at cell concentrations ranging from 23 to 237 μM (15 to 74 % increase in APD_{90} at 1 Hz stimulation). Sparfloxacin induced the greatest degree of prolongation at similar cell culture concentrations (31 to 105 % increase in APD_{90} at 1 Hz stimulation).

Activation potential duration was also evaluated in the guinea pig ventricular myocyte cell culture system at different fluoroquinolone concentrations. Grepafloxacin prolonged APD_{90} values by 16 % at a cell culture concentration of 28 μM (the highest concentration of grepafloxacin evaluated). Trovafloxacin and ofloxacin were not active at the highest cell culture concentrations that were evaluated (24 and 83 μM, respectively). Sparfloxacin prolonged APD_{90} values by 27 % at a cell culture concentration of 77 μM. This study was deficient because grepafloxacin and the comparator fluoroquinolones were not examined at the same cell culture concentrations and the highest concentration (30 μg/ml or 77 μM for sparfloxacin) was not high enough to result in at least a 50 % prolongation in APD_{90} values. The limited data from this study indicated that grepafloxacin may be as active as sparfloxacin in the guinea pig ventricular myocyte cell culture system.

IC_{50} values for grepafloxacin and comparator fluoroquinolones were determined using the HERG (potassium channel) model in transfected CHO cells. Ciprofloxacin exhibited no activity in this assay at the highest concentration evaluated (260 μM). Grepafloxacin and moxifloxacin had similar IC_{50} values of 87 and 80 μM, respectively. The IC_{50} value for sparfloxacin was 33 μM. These data suffered to some extent because the three cell culture concentrations used were inadequate to differentiate between grepafloxacin and moxifloxacin. Additionally, a non-fluoroquinolone positive control with known cardiac activity such as cisapride or terfenadine would have provided a comparison of relative potency. Grepafloxacin and moxifloxacin also reduced the current amplitude of the KCNQ1/KCNE1 potassium channel by approximately 30 % at a cell culture concentration of 70 μM while sparfloxacin reduced the current amplitude by 12 % at a concentration of 25 μM. IC_{50} values could not be determined because only one concentration was used for each fluoroquinolone.

The electrophysiological effects of grepafloxacin and comparator fluoroquinolones were assessed in anesthetized rabbits and dogs by *iv* infusion of each fluoroquinolone. In

rabbits, grepafloxacin induced cardiac arrhythmia at each dose level (10, 30, and 100 mg/kg). All four animals at the 100 mg/kg dose level exhibited pronounced arrhythmia with two of these animals suffering fatal arrhythmias. Arrhythmias were induced by ciprofloxacin in one of four rabbits at the 100 mg/kg dose but not at the lower dose levels. Lomefloxacin induced results similar to ciprofloxacin. ECG data were generated from anesthetized dogs over dose levels ranging from 1 to 30 mg/kg. Grepafloxacin prolonged the QTc interval by 8 and 16 % (31 and 59 msec) at the 10 and 30 mg/kg dose levels, respectively. Ofloxacin exhibited half the effect of grepafloxacin at the same dose levels. The percent QTc prolongation resulting from ciprofloxacin at the 10 mg/kg dose level was similar to results obtained from grepafloxacin at the same dose level but also produced QTc prolongation at dose levels of 1 and 3 mg/kg with no dose response. Ciprofloxacin was not evaluated at the 30 mg/kg dose level. Norfloxacin and ciprofloxacin caused pronounced depression in blood pressure at the 1 and 3 mg/kg dose levels. Norfloxacin was not evaluated at dose levels above 3 mg/kg due to mortality. Grepafloxacin had the least effect upon blood pressure at dose levels as high as 10 mg/kg. The anesthetized dog study, however, utilized mongrel dogs thus eliminating the effective randomization of animals into the different dose groups. A comparative study between structural analogs needs to be conducted with a pure breed of dog of similar age. In addition, the comparator fluoroquinolones in the anesthetized dog study did not include moxifloxacin which was prominently compared to grepafloxacin in the *in vitro* studies.

The data in the reports contained in the current submission indicated that grepafloxacin has potential similar to other approved fluoroquinolones to cause cardiac effects in human subjects. These observations do not impact the approved indications for grepafloxacin (RAXAR®) under NDA 20-695 but suggest the continued need for post-marketing surveillance for cardiac arrhythmia.

KEYWORDS: Fluoroquinolone, Grepafloxacin, QTc Interval, Potassium Channel, ECG, Purkinje Fibers

Stephen G. Hundley, Ph.D.

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