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IN VITRO ACTIVITY OF DELAFLOXACIN AND MICROBIOLOGICAL RESPONSE AGAINST FLUOROQUINOLONE SUSCEPTIBLE AND NON-SUSCEPTIBLE *S. AUREUS* ISOLATES FROM TWO PHASE 3 STUDIES OF ACUTE BACTERIAL SKIN AND SKIN STRUCTURE INFECTIONS (ABSSSI)

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ABSTRACT

Background: Delafloxacin (DLX) is an investigational anionic fluoroquinolone antibiotic with broad-spectrum activity, including Gram-positive organisms, Gram-negative organisms, atypical organisms, and anaerobes. The *in vitro* activity of DLX and percent microbiological response in subjects infected with fluoroquinolone non-susceptible *S. aureus* isolates was determined from 2 global Phase 3 studies of DLX vs vancomycin plus aztreonam (VAN/AZ) in ABSSSI.

Methods: Patients were enrolled in 23 countries predominately in the US but also Eastern Europe, South America, and Asia. The microbiological intent-to-treat population (MITT) included 1042 patients from which 1716 isolates were submitted for identification and susceptibility testing per CLSI guidelines at a central laboratory (JMI Laboratories, N. Liberty, IA). Comparator fluoroquinolone antibiotics included levofloxacin and ciprofloxacin. Non-susceptibility to these antibiotics was determined using CLSI breakpoints.

Results: *S. aureus* isolates were 33.8% levofloxacin non-susceptible (FQ-NS). DLX activity and percent microbiological response are presented in the following table.

Baseline Organisms (MITT; both treatment arms)	Number of Isolates FQ-S/FQ-NS	DLX FQ-S $MIC_{50/90}$ (µg/mL)	DLX FQ-S Range (µg/mL)	DLX FQ-NS $MIC_{50/90}$ (µg/mL)	DLX FQ-NS Range (µg/mL)	Number of Subjects with FQ-S/FQ-NS Isolates (ME Inv-Assessed Endpoint at Follow-Up)	Percent Micro Response for Subjects with FQ-NS Isolates
S. aureus[1]	455/232	0.008/0.008	0.002-0.12	0.25/0.25	0.004-4	168/81	80/81 (98.8%)
MRSA	101/195	0.008/0.008	0.002-0.12	0.25/0.25	0.004-4	37/71	70/71 (98.6%)
MSSA	358/39	0.008/0.008	0.002-0.12	0.12/0.25	0.004-0.5	132/10	10/10 (100%)

[1]Patients with MRSA and MSSA were counted only once in *S. aureus*.

Conclusion: DLX demonstrated high rates of microbiological response against FQ-NS isolates. The data suggest that DLX could be a good treatment option for *S. aureus* ABSSSI isolates where high rates of ciprofloxacin and levofloxacin non-susceptibility are observed, including MRSA.

INTRODUCTION

DLX is a novel investigational anionic fluoroquinolone antibiotic with broad-spectrum activity, including Gram-positive organisms, Gram-negative organisms, atypical organisms, and anaerobes. DLX can be administered by either an intravenous (IV) infusion route or orally as a tablet for the treatment ABSSSI. Two global Phase 3 studies of DLX vs VAN/AZ in ABSSSI have been completed (RX-3341-302 and RX-3341-303).

DLX is more active than levofloxacin against most Gram-positive pathogens. Notably, the MIC_{50} is at least 32-fold lower for DLX compared to levofloxacin and ciprofloxacin for methicillin-resistant *Staphylococcus aureus* (MRSA).[1] The increased potency of DLX relative to other fluoroquinolones against Gram-positive bacteria and the enhanced activity of DLX at acidic pH are largely due to its structure-activity relationships profile. The collaboration between a large N1 substitution and a weakly-polar group at C8 results in increased potency against quinolone-resistant Gram-positive bacteria, a phenotype common among MRSA. The basicity at C7 leads to increased potency at acidic pH.[2] DLX is equally effective at stabilizing cleavable complexes by binding either gyrase or topoisomerase IV in both *S. aureus* and *E. coli* and, as such, is considered dual-targeting fluoroquinolone.

Due to enhanced potency of DLX against MRSA, it was of interest to further investigate the *in vitro* activity of DLX and the percent microbiological response in Phase 3 clinical trial subjects infected with fluoroquinolone non-susceptible *S. aureus* isolates. Further, microbiological responses were examined for clinical trial *S. aureus* isolates characterized for mutations in the quinolone resistance determining region (QRDR).

MATERIALS AND METHODS

RX-3341-302 and **RX-3341-303 Study Design**:

Pooled data from the 2 pivotal, global, Phase 3 ABSSSI studies (RX-3341-302 and RX-3341-303) are presented.

- Stratified, randomized, double-blind, phase 3, multicenter studies of DLX vs VAN/AZ for the treatment of ABSSSIs
- Patients had wounds, burns, major abscesses, or cellulitis with lesions of ≥75 cm^2 in size; ≥2 systemic signs of infections; and met study entry criteria
- Patients randomly assigned in 1:1 ratio to either DLX 300 mg IV/450 mg oral q12h, or VAN 15 mg/kg IV (actual body weight) with AZ. Total treatment duration was 5-14 days at investigators' discretion
- Patients evaluated at screening, daily on therapy, at Follow-up (FU, Day 14 ± 1) and Late Follow-up (LFU, Day 21-28)
- Efficacy evaluated through assessments of signs and symptoms of infection; measurement of lesion size by digital planimetry; and culture and susceptibility testing of bacterial isolates

Microbiological Analysis Populations

- **Microbiological ITT (MITT) Analysis Set**
  - All patients in the ITT analysis set who had a baseline bacterial pathogen identified by the sponsor that was known to cause ABSSSI.
  - The MITT population for the 2 global phase 3 studies consisted of 1042 subjects (N=518 DLX arm; 524 VAN/AZ arm).
- **Microbiologically Evaluable (ME) Analysis Sets**
  - All patients in the MITT analysis set who also met the criteria for the corresponding clinically evaluable analysis sets.
  - The ME at follow-up population for the two global phase 3 studies consisted of 806 subjects (N=410 DLX arm; 396 VAN/AZ arm)

Microbiology Outcomes

Microbiological response for patients in the ME and MITT analysis sets were based on results of baseline and post-baseline cultures (FU and LFU) and susceptibility testing, together with the clinical response assigned by investigators. The definitions of documented eradicated, presumed eradicated and documented persisted were:

- **Documented eradicated** - The baseline pathogen was absent in cultures of the original site of infection at the post-baseline visit. Investigator-assessed response was not considered a determining factor for this microbiologic response definition.
- **Presumed eradicated** - There was no material available for culture or no culture was done and the patient had an investigator-assessed response of success.
- **Documented persisted** - The baseline pathogen was present in cultures of the original site of infection at the visit. Investigator-assessed response was not considered a determining factor for this microbiologic response definition.
- **Presumed persisted** - There was no material available for culture or no culture was done and the patient had an investigator-assessed response of failure.

Microbiology Methods

- Isolates (1716 *S. aureus* isolates) were submitted for identification and susceptibility testing per CLSI guidelines at a central laboratory (JMI Laboratories, N. Liberty, IA).[3]
- Comparator fluoroquinolone antibiotics included levofloxacin and ciprofloxacin. Non-susceptibility to these antibiotics was determined using CLSI breakpoints.[4]
- For analysis tables using subject outcome and isolates microbiological data, fluoroquinolone susceptibility/non-susceptibility was based upon levofloxacin data.
- For QRDR analysis, testing was performed on fluoroquinolone (ciprofloxacin or levofloxacin) resistant isolates. Molecular characterization of the QRDR was performed by PCR amplification of the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC*/*grlA* and *parE*/*grlB*) genes, followed by sequencing of the amplicons. Protein amino acid sequences of selected isolates were compared to wild type sequences of *S. aureus* NCTC 8325 strain.

RESULTS

- *S. aureus* isolates from subjects in the MITT analysis population (both treatment arms, pooled data) were 33.7% levofloxacin non-susceptible (Table 1).
- DLX demonstrated potent activity against both *S. aureus* and MRSA.
- DLX demonstrated potent activity against levofloxacin non-susceptible isolates. The DLX MIC_{90} against levofloxacin non-susceptible *S. aureus*, MRSA and MSSA was 0.25 µg/mL (Table 1).
- DLX demonstrated high rates of microbiological response against levofloxacin-non-susceptible isolates (Table 2).
  - Based on the ME population (pooled data, DLX treatment arm), *S. aureus* was eradicated or presumed eradicated in 98.4% (245/249) in DLX-treated patients (Table 2).

TABLE 1: DELAFLOXACIN *IN VITRO* ACTIVITY AGAINST BASELINE *S. AUREUS* BASED ON LEVOFLOXACIN SUSCEPTIBILITY (MITT-DELAFLOXACIN AND COMPARATOR TREATMENT ARMS, POOLED DATA)

	Levofloxacin Susceptible			Levofloxacin Non-susceptible			Overall (N=1042)			
Organism	n	Range µg/mL	$MIC_{50/90}$ µg/mL	n	Range µg/mL	$MIC_{50/90}$ µg/mL	n	Range µg/mL	$MIC_{50/90}$ µg/mL	% LVX-NS
S. aureus	455	0.002-0.12	0.008/0.008	232	0.004-0.25	0.25/0.25	685	0.002-4	0.008/0.25	33.7
MRSA	101	0.002-0.12	0.008/0.008	195	0.004-0.25	0.25/0.25	294	0.002-4	0.12/0.25	66.0
MSSA	358	0.002-0.12	0.008/0.008	39	0.004-0.5	0.12/0.25	395	0.002-0.5	0.008/0.03	9.6

Minimum inhibitory concentration (MIC) results are in µg/mL. MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*; LVX: levofloxacin; NA: not applicable; N: total number of patients; n: total number of MIC values from isolates cultured at baseline from the primary infection site or blood. Overall includes subjects from Asia, Latin America, North America, and Europe.

TABLE 3: ANALYSIS OF QRDR MUTATIONS FROM BASELINE DELAFLOXACIN ARM GRAM-POSITIVE CLINICAL STUDY ISOLATES RESISTANT TO LEVOFLOXACIN AND CORRESPONDING MICROBIOLOGICAL RESPONSE (MEFUI)

		QRDR Mutation Profile				Microbiological Response ME at Follow-up	Delafloxacin		Levofloxacin		Ciprofloxacin	
Baseline Organism	n	*gyrA*	*gyrB*	*parC*	*parE*	(% Eradicated[a])	MIC range	$MIC_{50/90}$	MIC range	$MIC_{50/90}$	MIC range	$MIC_{50/90}$
S. aureus	1	E88K S84L	WT	E84G S80Y	WT	1/1 (100%)	4	–/–	> 8	–/–	> 8	–/–
	80	S84L	WT	S80Y	WT	80/80 (100%)	0.12-0.25	0.12/0.25	4-8	4/8	8-> 8	8/> 8
	5	S84L	WT	S80F	WT	4/5 (80%)	0.25-0.5	–/–	8-> 8	–/–	> 8	–/–
	3	S84L	WT	S80Y	P451S	3/3 (100%)	0.25-0.5	–/–	> 8	–/–	> 8	–/–
	1	S84L S85P	WT	S80F	D432N	1/1 (100%)	0.5	–/–	> 8	–/–	> 8	–/–
	3	S85P	WT	S80F	WT	3/3 (100%)	0.03	–/–	2	–/–	8	–/–
	1	S84L	WT	S80F S80Y	WT	0/1 (0%)	0.25	–/–	8	–/–	> 8	–/–
	1	WT	WT	S80F	WT	1/1 (100%)	0.015	–/–	1	–/–	4	–/–
	1	S84L	WT	E84Q S80Y	WT	0/1 (0%)	0.5	–/–	> 8	–/–	> 8	–/–

ME: microbiologically evaluable; MEFUI: microbiologically evaluable at follow-up for investigator-assessed response; MIC: minimum inhibitory concentration; MIC_{50}: lowest MIC that inhibits 50% of the strains (≥ 10 strains) within a single species; MIC_{90}: lowest MIC that inhibits 90% of the strains (≥ 10 strains) within a single species; MIC: minimum inhibitory concentration; QRDR: quinolone resistance-determining region; WT: wild-type strain. [a]Documented or presumed eradicated

- Similar eradication rates were observed for subjects with levofloxacin non-susceptible *S. aureus* isolates (80/81; 98.8%) (Table 2) and MRSA isolates (70/71; 98.6%) (Data not shown).
- Subjects with isolates with DLX MIC values as high as 4 µg/mL were eradicated/presumed eradicated (Table 2).
- The predominant mutation observed was Ser84-Leu in *gyrA*/Ser80-Phe in *parC*. The DLX MIC_{90} for baseline *S. aureus* isolates with this mutation was 0.25 µg/mL (n=80) whereas the MIC_{90} for levofloxacin and ciprofloxacin was 8 µg/mL and > 8 µg/mL, respectively (Table 3). Microbiological response rates for subjects with *S. aureus* isolates with this mutation were 100% (80/80).
- These data also demonstrated that DLX MIC values do not increase beyond 0.5 µg/mL until at least double mutations in both *gyrA* and *parC* are observed (Table 3).
- One baseline isolate was found with such a double mutation. The DLX MIC value for this isolate was 4 µg/mL but despite this relatively high DLX MIC value, the microbiological response for the subject with this isolate was presumed or documented eradicated (Table 3).

TABLE 2: MICROBIOLOGICAL RESPONSE AT FOLLOW-UP FOR SUBJECTS WITH *S. AUREUS* ISOLATES BY DELAFLOXACIN MIC BASED ON LEVOFLOXACIN SUSCEPTIBILITY (MICRO EVALUABLE POPULATION AT FOLLOW UP, POOLED DATA)

Micro Response in Pooled Phase 3 Delafloxacin Treated *S. aureus* Based on Levofloxacin Susceptibility				
Pathogen	**Baseline DLX MIC (µg/mL)**	**N1**	**Eradicated/Presumed Eradicated**	**Persisted/ Presumed Persisted**
Levofloxacin susceptible *S. aureus*			**165**	**3**
	0.002, n (%)	15	15 (100.0)	0
	0.004, n (%)	44	44 (100.0)	0
	0.008, n (%)	101	98 (97.0)	3 (3.0)
	0.015, n (%)	7	7 (100.0)	0
	0.06, n (%)	1	1 (100.0)	0
Levofloxacin non-susceptible *S. aureus*			**80**	**1**
	0.03, n (%)	3	3 (100.0)	0
	0.12, n (%)	38	38 (100.0)	0
	0.25, n (%)	36	35 (97.2)	1 (2.8)
	0.5, n (%)	3	3 (100.0)	0
	4, n (%)	1	1 (100.0)	0

Percentages were calculated as 100 x (n/N1). N1 was the number of subjects for each MIC value. If multiple MIC values were reported per subject per pathogen, the highest value was used.

CONCLUSION

- High rates of microbiological eradication were observed in global Phase 3 studies for ABSSSI with DLX treatment. These high eradication rates extended to include both levofloxacin non-susceptible *S. aureus* and MRSA isolates as well as *S. aureus* isolates with mutations in the QRDR.
- 100% microbiological response rates were observed for subjects with *S. aureus* isolates with mutations in Ser84-Leu in *gyrA*/Ser80-Phe in *parC,* the most commonly observed mutation in global Phase 3 studies.
- The data suggest that DLX may be considered as a treatment option for *S. aureus* ABSSSI isolates where high rates of ciprofloxacin and levofloxacin non-susceptibility are observed, including MRSA.

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