Activity of JNJ-Q2 against *Staphylococcus aureus* Isolated from Patients with Acute Bacterial Skin and Skin-Structure Infection Obtained During a Phase II Clinical Trial

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Amended Abstract

Objective: To determine the activity of JNJ-Q2 against *S. aureus* isolated from patients with clinically diagnosed acute bacterial skin and skin-structure infection (ABSSSI) in the United States (USA) during a Phase II clinical trial and to determine the mechanisms of fluoroquinolone (FQ) resistance (R) in FQ-R strains. JNJ-Q2 is a broad-spectrum bactericidal 4-fluoroquinolone with potent activity against Gram-positive and -negative pathogens.

Methods: During the Phase II clinical trial, a total of 111 baseline *S. aureus* isolates were obtained from 111 patients, diagnosed with ABSSSI by strict criteria (including 37.8% methicillin-susceptible [MSSA] and 62.2% methicillin-resistant [MRSA]). Susceptibility testing was performed by the CLSI broth microdilution. Type II topoisomerase quinolone-resistant determinant regions (QRDR) were amplified by PCR and sequenced for FQ-R strains.

Results: JNJ-Q2 demonstrated good activity against all *S. aureus* and was very active against both MSSA ($MIC_{50/90}$, 0.008/0.12 mg/L) and MRSA ($MIC_{50/90}$, 0.12/0.12 mg/L). 51 strains (45.9%) had moxifloxacin MIC values of ≥1 mg/L (non-susceptible) and all of these strains carried at least one *par*C mutation (S80Y). Additionally, 43 of 51 had *gyr*A S84L mutations and 3 strains also had *par*C E84G (1 strain), E84I (1), or *par*E T461I (1). Furthermore, 49 of 51 strains had a JNJ-Q2 MIC of 0.12 mg/L (range for 51, 0.12-0.25 mg/L). All isolates were susceptible to linezolid (LZD) and vancomycin (VAN). JNJ-Q2 was the most active agent tested with a MIC_{90} 16-, 64- 16-, and eight-fold lower than MOX, levofloxacin (LEV), LZD and VAN, respectively.

Conclusions: JNJ-Q2 demonstrated very potent activity against contemporary *S. aureus* isolated from patients in the USA with clinically diagnosed and microbiologically confirmed ABSSSIs. JNJ-Q2 exhibited greater activity compared to LEV and MOX, including strains R to currently utilized FQs. These encouraging results support the further clinical development of JNJ-Q2 for ABSSSIs.

Introduction

Quinolone resistance in *Staphylococcus aureus* results from either mutations in the quinolone-resistance determinant regions (QRDRs) of the target enzymes, most commonly DNA gyrase and topoisomerase IV, or by drug efflux and/or decreased uptake. JNJ-Q2, a novel fluorinated 4-quinolone, has been shown to have balanced potency against both DNA gyrase and topoisomerase IV, excellent *in vitro* activity against both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA), and is in clinical development for the treatment of acute bacterial skin and skin-structure infection (ABSSSI).

The aims of this study were to determine comparative *in vitro* activity for JNJ-Q2 tested against *S. aureus* obtained from a recent (2010) randomized, controlled, double-blind, double-dummy, multicenter Phase II study for JNJ-Q2 compared with linezolid for the treatment of ABSSSI (trial #NCT01128530).

Materials and Methods

Bacterial Strain Collection. During the Phase II clinical trial, a total of 111 baseline *S. aureus* isolates were obtained from 111 patients, diagnosed with ABSSSI by strict criteria, in 15 medical centers throughout the United States (USA). Species identifications were performed by the submitting laboratories with confirmation performed by the central reference laboratory (JMI Laboratories, North Liberty, Iowa, USA).

Susceptibility Test Methods. All isolates were tested for susceptibility by reference broth microdilution methods using the Clinical and Laboratory Standards Institute recommendations (CLSI; M07-A9, 2012) in validated panels manufactured by ThermoFisher Scientific Inc, formerly TREK Diagnostics Systems (Cleveland, Ohio, USA). Categorical interpretation of comparator MIC values was performed according to CLSI (M100-S22, 2012) and EUCAST (2012) criteria. The quality assurance of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S22, 2012) control strains, including *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. The inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event. All QC results were within established ranges.

Molecular Methods. QRDR mutations were detected by PCR amplification and sequencing of *gyr*A, *gyr*B, *par*C and *par*E as previously described (Horii, et al., 2003). Sequences were aligned against *S. aureus* ATCC 25923 using MegAlign (DNAStar, Lasergene, Madison, Wisconsin, USA).

Results

- Of the 111 *S. aureus* isolated, 37.8% were methicillin-susceptible (MSSA) and 62.2% methicillin-resistant (MRSA). JNJ-Q2 demonstrated good activity against all isolates and was very active against both MSSA ($MIC_{50/90}$, 0.008/0.12 mg/L) and MRSA ($MIC_{50/90}$, 0.12/0.12 mg/L) with all isolates inhibited at JNJ-Q2 MIC values of ≤0.25 mg/L (Table 1).
- Levofloxacin and moxifloxicin resistance rates were high at 46.9 and 31.5%, respectively (Table 2). Levofloxacin/ moxifloxicin resistance rates were much higher in MRSA (59.4/40.6%) compared to MSSA (26.2/16.7%), respectively. JNJ-Q2 was the most active agent tested with a MIC_{90} 16-, 32-, 16-, and eight-fold lower than moxifloxacin, levofloxacin, linezolid and vancomycin, respectively.
- 51 strains (45.9%) had moxifloxacin MIC values of ≥1 mg/L (non-susceptible) and all of these strains carried at least one *par*C mutation (S80Y). Additionally, 43 of 51 had *gyr*A S84L mutations and 3 strains also had *par*C E84G (1 strain), E84I (1), or *par*E T461I (1). Furthermore, 49 of 51 strains had a JNJ-Q2 MIC of 0.12 mg/L (range for 51, 0.12-0.25 mg/L).
- Overall erythromycin resistance was also elevated at 64.9%. Most isolates were susceptible to clindamycin (97.3%) and trimethoprim-sulfamethoxazole (94.6%). All isolates were susceptible to linezolid and vancomycin.

Table 1. MIC frequency and cumulative percent inhibited distributions of JNJ-Q2 tested against 111 *S. aureus*[a].

Organism (no. tested)	No. (cum. %) of isolates inhibited at JNJ-Q2 MIC (mg/L)						
	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25
S. aureus (111)	27 (24.3)	25 (46.9)	7 (53.2)	1 (54.1)	0 (54.1)	49 (98.2)	2 (100.0)
MSSA (42)	12 (28.6)	2 (57.2)	6 (71.4)	1 (73.8)	0 (83.0)	10 (97.6)	1 (100.0)
MRSA (69)	15 (21.7)	13 (40.6)	1 (42.0)	0 (42.0)	0 (42.0)	39 (98.6)	1 (100.0)

a. Isolates from a Phase II clinical trial.

Table 2. Antimicrobial activity of JNJ-Q2 and comparator antimicrobials against 111 baseline *S. aureus* obtained during a Phase II ABSSSI clinical trial.

Organism (no. tested)/ Antimicrobial agent[a]	MIC in mg/L			CLSI[b] %S / %R
	MIC_{50}	MIC_{90}	Range	
S. aureus – all isolates (111)				
JNJ-Q2	0.015	0.12	≤0.004 – 0.25	-[c] / -
Moxifloxacin	0.25	2	≤0.06 – 8	54.1 / 31.5
Levofloxacin	2	4	0.12 – >8	47.8 / 46.9
Oxacillin	>2	>2	≤0.25 – >2	37.8 / 62.2
Erythromycin	>8	>8	0.25 – >8	35.1 / 64.9
Clindamycin	≤0.12	≤0.12	≤0.12 – >8	97.3 / 2.7
Linezolid	1	2	1 – 2	100.0 / 0.0
TMP/SMX[d]	≤0.5	≤0.5	≤0.5 – >4	94.6 / 5.4
Daptomycin	≤0.25	0.5	≤0.25 – 0.5	100.0 / -
Vancomycin	1	1	0.5 – 2	100.0 / 0.0
MRSA (69)				
JNJ-Q2	0.12	0.12	≤0.004 – 0.25	- / -
Moxifloxacin	1	2	≤0.06 – 8	42.0 / 40.6
Levofloxacin	4	8	0.12 – >8	40.6 / 59.4
Erythromycin	>8	>8	0.25 – >8	15.9 / 84.1
Clindamycin	≤0.12	≤0.12	≤0.12 – >8	95.7 / 4.3
Linezolid	1	2	1 – 2	100.0 / 0.0
TMP/SMX[d]	≤0.5	≤0.5	≤0.5 – 1	100.0 / 0.0
Daptomycin	≤0.25	0.5	≤0.25 – 0.5	100.0 / -
Vancomycin	1	1	0.5 – 2	100.0 / 0.0
MSSA (42)				
JNJ-Q2	0.008	0.12	≤0.004 – 0.25	- / -
Moxifloxacin	≤0.06	2	≤0.06 – 8	73.8 / 16.7
Levofloxacin	0.25	4	0.12 – >8	59.5 / 26.2
Erythromycin	0.5	>8	0.25 – >8	66.7 / 33.3
Clindamycin	≤0.12	≤0.12	≤0.12 – 0.25	100.0 / 0.0
Linezolid	1	2	1 – 2	100.0 / 0.0
TMP/SMX[d]	≤0.5	>4	≤0.5 – >4	85.7 / 14.3
Daptomycin	≤0.25	≤0.25	≤0.25 – 0.5	100.0 / -
Vancomycin	1	1	0.5 – 2	100.0 / 0.0

a. S = susceptible, R = resistant, MSSA = methicillin-susceptible *S. aureus*, MRSA = methicillin-resistant *S. aureus.*
b. Criteria as published by the CLSI (CLSI, 2012).
c. = No breakpoint has been established.
d. Trimethoprim-sulfamethoxazole.

Conclusions

- In summary, JNJ-Q2 was found to be very active against *S. aureus* isolated from patients with a definitive clinical diagnosis of ABSSSI during a 2010 Phase II clinical trial in the USA, with all 111 baseline strains inhibited at JNJ-Q2 MIC values of ≤0.25 mg/L. Additionally, JNJ-Q2 was the most active compound with activity being many-fold higher than the comparator agents tested.
- These favorable results, along with the previously demonstrated low propensity for pathogens to induce rapid mutational resistance to JNJ-Q2, support the further clinical development of JNJ-Q2 to treat ABSSSI, including MRSA.

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References

1. Blumberg HM, Rimland D, Carroll DJ, Terry P, Wachsmuth IK (1991). Rapid development of ciprofloxacin resistance in methicillin-susceptible and -resistant *Staphylococcus aureus*. *J Infect Dis* 163: 1279-1285.
2. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition.* Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement.* Wayne, PA: CLSI.
4. Covington P, Davenport JM, Andrae D, O'Riordan W, Liverman L, McIntyre G, Almenoff J (2011). Randomized, double-blind, phase II, multicenter study evaluating the safety/tolerability and efficacy of JNJ-Q2, a novel fluoroquinolone, compared with linezolid for treatment of acute bacterial skin and skin structure infection. *Antimicrob Agents Chemother* 55: 5790-5797.
5. Farrell DJ, Liverman LC, Biedenbach DJ, Flamm RK, Jones RN (2011). Surveillance of JNJ-Q2 activity tested against *Staphylococcus aureus* and beta-hemolytic streptococci as a component of the 2010 SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 71: 415-420.
6. Farrell DJ, Liverman LC, Biedenbach DJ, Jones RN (2011). JNJ-Q2: A new fluoroquinolone with potent in vitro activity against *Staphylococcus aureus*, including methicillin- and fluoroquinolone-resistant strains. *Antimicrob Agents Chemother* 55: 3631-3634.
7. Horii T, Suzuki Y, Monji A, Morita M, Muramatsu H, Kondo Y, Doi M, Takeshita A, Kanno T, Maekawa M (2003). Detection of mutations in quinolone resistance-determining regions in levofloxacin- and methicillin-resistant *Staphylococcus aureus*: Effects of the mutations on fluoroquinolone MICs. *Diagn Microbiol Infect Dis* 46: 139-145.
8. Morrow BJ, He W, Amsler KM, Foleno BD, Macielag MJ, Lynch AS, Bush K (2010). In vitro antibacterial activities of JNJ-Q2, a new broad-spectrum fluoroquinolone. *Antimicrob Agents Chemother* 54: 1955-1964.