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Studies of the Efficacy of a New Fluoroquinolone, JNJ-Q2, in Skin, Respiratory, and Systemic Murine Models of *Staphylococcus aureus* and *Streptococcus pneumoniae* Infection

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Abstract

The in vivo efficacy of JNJ-Q2, a new broad-spectrum fluoroquinolone (FQ), was evaluated in a murine septicemia model with methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus,* and in a *S. pneumoniae* lower respiratory tract infection model. JNJ-Q2 and comparators were also evaluated in an acute murine skin infection model against a community-acquired MRSA strain, and in an established skin infection (ESI) model against a hospital-acquired strain, for which the selection of resistant mutants was also determined. JNJ-Q2 demonstrated activity in the MSSA septicemia model that was comparable to moxifloxacin (JNJ-Q2 ED_{50} = 0.2 mg/kg, SC; 2 mg/kg, PO) and activity in the MRSA septicemia model that was superior to vancomycin (JNJ-Q2 ED_{50} = 1.6 mg/kg, SC). In a *S. pneumoniae* lower respiratory tract infection model, JNJ-Q2 displayed activity (ED_{50} = 1.9 mg/kg, SC; 7.4 mg/kg, PO) which was comparable to gemifloxacin and superior to moxifloxacin. In both MRSA skin infection models, treatment with JNJ-Q2 resulted in dose-dependent reductions in bacterial titers in the skin, with the response to JNJ-Q2 at each dose exceeding that of the comparators ciprofloxacin, moxifloxacin, linezolid or vancomycin. Additionally, in the ESI model, JNJ-Q2 showed a low or non-detectable propensity for ciprofloxacin-resistance selection, in contrast to the selection of ciprofloxacin-resistant mutants observed for both ciprofloxacin and moxifloxacin. JNJ-Q2 demonstrated comparable or improved activity compared to fluoroquinolone or anti-staphylococcal comparators in several local and systemic skin infection models with both *S. aureus* and *S. pneumoniae* and is currently being evaluated in Phase II human clinical trials.

Introduction

Successive improvements in the spectrum and antimicrobial potency of agents within the fluoroquinolone class have resulted in widespread clinical utility of these agents, and the activities of levofloxacin and moxifloxacin against Gram-positive pathogens, particularly *Streptococcus pneumoniae*, have contributed to the adoption of these agents for the empiric treatment of respiratory tract infections in the community setting. Although fluoroquinolone resistance in *S. pneumoniae* remains low, with levofloxacin resistance in U.S. isolates typically reported at less than 1% (13), fluoroquinolone resistance has, in selected populations or geographic regions, been reported to be greater than 10% (1). In association with the introduction of the seven-valent pneumococcal vaccine (PCV7), an increased prevalence in non-PCV7 serotypes has been observed (12, 13), including several predominant fluroquinolone-resistant and multi-drug resistant clones (4, 10). Several of the marketed fluoroquinolone agents also display in vitro activity against *Staphylococcus aureus* isolates and have been used successfully to treat staphylococcal infections (34), although none of these marketed agents is approved for the treatment of methicillin-resistant *S. aureus* (MRSA) infections. MRSA has become an increasingly important pathogen in community infections (21), and the increased incidence of infection is associated with elevated resistance, with levofloxacin resistance observed in 70% of recent U.S. clinical MRSA isolates (20). Community staphylococcal isolates typically express elevated levels of several virulence determinants, which are associated with increased virulence in murine models of bacteremia and skin abscess infection (22). Efficacy in murine models of MRSA infection is a key attribute for new antibacterial agents targeted for the treatment of staphylococcal infections, including MRSA infections in the community setting. Several investigational fluoroquinolones active against MRSA (2, 5, 17, 19, 35) are reported to be the subject of ongoing clinical studies investigating their efficacy in the treatment of acute

bacterial skin and skin structure infections caused by MRSA. The development of new fluoroquinolone agents retaining activity against multi-drug resistant *S. pneumoniae* isolates and displaying potent anti-staphylococcal activities may prove valuable as a therapeutic option for the treatment of respiratory and skin infections.

JNJ-Q2 is a new broad-spectrum fluoroquinolone that displays potent in vitro activity against *S. pneumoniae*, including levofloxacin-resistant and multi-drug resistant isolates, and *S. aureus* including MRSA and ciprofloxacin-resistant MRSA isolates (14, 26) and is currently being evaluated in clinical studies for the treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). JNJ-Q2 displayed lower MIC values than comparator fluoroquinolones against *S. pneumoniae* and *S. aureus* isolates, including MRSA (26) and also displayed a lower propensity for resistance selection against these Gram-positive pathogens (26). Herein we report the in vivo activities of JNJ-Q2 in murine models of septicemia with MSSA and CA-MRSA strains, *S. pneumoniae* lower respiratory tract infections, and MRSA acute and established skin infections. The selection of MRSA mutants with reduced susceptibility to test agents in the established skin infection model is also reported. In an accompanying article, the in vitro activities of JNJ-Q2 are presented, highlighting the inhibitory activity against purified target enzymes and in vitro biofilms, and in vitro resistance development in MRSA. (Morrow, et al., Antimicrob. Agents Chemother., submitted in parallel)

Portions of this work were previously presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy 50th Annual Meeting, 2010 [Fernandez et al., (F1-2093)](15)

Materials and Methods

Antimicrobial Agents

JNJ-Q2 was synthesized at Johnson & Johnson Pharmaceutical Research & Development, L.L.C. Moxifloxacin was obtained from Bayer AG, gemifloxacin from CB Research and Development, Inc. (New Castle, DE), and linezolid from Organix, Inc. (Wolburn, MA). Ciprofloxacin hydrochloride was obtained from Pentax-Bayer (Kankakee, IL). Vancomycin hydrochloride was purchased from MP Bio (Irvine, CA).

Microorganisms

S. pneumoniae (ATCC 6301), *S. aureus* Smith (MSSA, ATCC 13709), and methicillin-resistant *S. aureus* MRSA (ATCC 43300) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). MRSA OC 8525, a community-acquired MRSA strain was obtained from Dr. Barry Kreiswirth of the Public Health Research Institute, Newark, NJ.

Animals

Female CF-1 mice (20-22 g) and female Crl:SKH1-hrBr hairless, immunocompetent mice (20-25 g) were purchased from Charles River Laboratories (Wilmington, MA) and female Swiss-Webster mice (20-22 g) were obtained from Taconic Farms, Inc. (Hudson, NY). Animals were allowed free access to food and water and were maintained on a 12 hour light/dark cycle. Mice were allowed to acclimate for five days after receipt from the vendor. All animal studies were reviewed and approved by the Johnson & Johnson Pharmaceutical Research & Development Institutional Animal Care and Use Committee. Animal numbers were justified by a power

117 analysis of the treatment group sample size necessary to detect a statistically significant
118 decrease in bacterial CFU or mortality by use of Dunnett's multiple comparison test (36).

119 **Inoculum Preparation**

120 For respiratory studies, *S. pneumoniae* was grown overnight on tryptic soy agar (TSA)
121 containing 5% sheep blood at 35°C, 5% CO_2. Isolated colonies were inoculated into trypticase
122 soy broth with 5% heat-inactivated goat serum (Rockland Immunochemicals, Inc., Gilbertsville,
123 PA) and incubated at 35°C until at mid-log growth phase, centrifuged, then concentrated to
124 approximately 1 x 10^8 CFU/animal for inoculation. For septicemia studies, overnight cultures of
125 *S. aureus* Smith ATCC 13709 (MSSA) or *S. aureus* OC 8525 (CA-MRSA) were inoculated from
126 frozen glycerol stocks into brain-heart infusion (BHI) media and shaken for 18 h at 37°C,
127 centrifuged, diluted in 7.0% hog gastric mucin (Sigma-Aldrich Chemical Company, St Louis, MO)
128 in saline to approximately 3 x 10^5 CFU/animal (MSSA) or 1 x 10^7 CFU/animal (CA-MRSA). In
129 skin studies, overnight cultures of *S. aureus* OC 8525 (CA-MRSA) or MRSA ATCC 43300 were
130 centrifuged as described above and diluted in BHI media with 131-220 µm Cytodex®
131 microcarrier dextrin beads (Sigma-Aldrich Chemical Company, St Louis, MO) added to a final
132 concentration of 0.1% (v/v) to the inoculating dose (7 x 10^6 CFU/mouse, CA-MRSA, acute skin
133 infection model or 9 x 10^6 CFU/mouse, MRSA ATCC 43300, established skin infection model).

134 **In Vitro Susceptibility**

135 Minimal Inhibitory Concentrations (MICs) were determined by microbroth dilution methods
136 according to CLSI guidelines (9).

137

138 **Mouse Septicemia Model**

To study the comparative efficacy of JNJ-Q2, ciprofloxacin, moxifloxacin, and gemifloxacin in the mouse septicemia model (16, 18), Swiss Webster mice were infected intraperitoneally (IP) with *S. aureus* Smith (MSSA) ATCC 13709 (≈ 3 x 10^5 colony forming units (CFU)/mouse ,10-100X the LD_{50}) or with CA-MRSA OC 8525 (≈ 1 x 10^7 CFU/mouse, 10-100X the LD_{50}), then treated either subcutaneously or orally 1 hour (JNJ-Q2), or 1 and 3 h (comparators), post infection and mortality was monitored for 3 days. The dosing regimen (QD vs. BID) was determined from pilot studies conducted in the mouse septicemia model (data not shown). The 50% effective dose (ED_{50}) and the 90% effective dose (ED_{90}), the dose where 50 and 90% survival was observed, respectively, and 95% fiducial limits were calculated from the survival curves using a logistical regression. All statistical analyses were carried out in SAS version 5 or 9.1.3 (SAS Institute, Cary, NC).

Lower Respiratory Tract Infection Model

To study the comparative efficacy of JNJ-Q2, moxifloxacin, and gemifloxacin in a murine lower respiratory tract infection model (16, 18), female CF-1 mice were briefly anesthetized with isoflurane and infected by placing 50 μL of *S. pneumoniae* ATCC 6301 (≈ 1 x 10^8 CFU/mouse) at the tip of the nares and allowing the mouse to inhale the inoculum. Mice were then treated subcutaneously or orally, once, 24 hour post-infection, and mortality was monitored over 2 days. The dose that provided 50 or 90% survival (ED_{50} or ED_{90}) was calculated from the survival curves using a logistic regression.

Acute Skin Infection Model

To study the comparative efficacy of JNJ-Q2, linezolid, and vancomycin against *S. aureus* in an acute skin infection model (15, 16, 18), female SKH1 mice were anesthetized with isoflurane and given a 0.2 mL subcutaneous (SC) injection of a Cytodex® bead inoculum containing ≈ 7 x

10^6 CFU/mouse flank *S. aureus* (CA-MRSA OC 8525) on the left and right flanks. Animals received JNJ-Q2, linezolid, or vancomycin 1, 3, 25, and 27 hours post infection at a dose of 1.6, 6.2, 25, or 100 mg/kg/day. All drugs were delivered orally except for vancomycin, which was given subcutaneously. Animals were euthanized by CO_2 asphyxiation 48 hours after infection, and the lesions on each flank were measured. A lesion volume score was calculated from the following equation; $LV=(\pi/6)(LxW^2)$, where LV = lesion volume, L = length of the lesion in mm, and W = width of the lesion in mm (7). For determination of CFU/g skin, the skin from the infected areas was disinfected with Nolvasan® (chlorhexidine diacetate, Fort Dodge Animal Health, Fort Dodge, IA), excised, weighed, and homogenized in 1 mL of saline (4°C, 35,000 rpm for 0.5 minutes [Omni Prep Tissue Homogenizer and Omni Tip™ Disposable Rotor Stator Generator Probes, Omni International, Marietta, GA]). Serial 100-fold dilutions in saline (0.85% Saline, Remel, Lenexa, KS) were plated (Autoplate® 4000, Spiral Biotech, Inc., Norwood, MA) on TSA plates. The plates were incubated for 18 h at 37°C and the CFUs counted (Q-Count, Spiral Biotech, Inc. Norwood, MA).

Established Skin Infection Model

To study the comparative efficacy of JNJ-Q2, ciprofloxacin and moxifloxacin against *S. aureus* in an established skin infection model(16), mice were anesthetized with isoflurane and given a 0.2 mL subcutaneous injection of the Cytodex® bead inoculum containing ≈ 9 x 10^6 CFU/mouse flank *S. aureus* (MRSA ATCC 43300) on the left and right flanks. Then 3 days post-infection, twice-daily treatment with JNJ-Q2, ciprofloxacin, or moxifloxacin (25-200 mg/kg/day) was initiated, and administered for 3 days. All drugs were delivered orally except for ciprofloxacin, which was given intraperitoneally. Twenty-four hours following the last dose (to minimize any drug carryover effect), the animals were euthanized, and skin tissue was processed and analyzed as described previously for the acute skin infection model.

To assess in vivo resistance selection, an undiluted skin sample was spiral plated on TSA plates containing 2 μg/mL of ciprofloxacin. The plates were incubated for 48 hours at 37°C and the number of colony forming units was determined. Resistance was defined as any colony that grew on a TSA plate containing 2 μg/mL of ciprofloxacin (4x the MIC) within 48 h.

Statistical Methodology

Preliminary evaluation using descriptive summary statistics suggested mean treatment differences between JNJ-Q2 and comparative treatment groups. Evaluation to determine whether these differences were significant was then performed using logistic regression (septicemia, lower respiratory tract infection, and resistance selection) (32) or linear mixed effects modeling (acute and established skin infection models) (23) to explain the response ratios as a function of log transformed dose and treatment groups. The modeling was adjusted to account for over-dispersion as appropriate.

Further comparison of the effects of J&J-Q2 with comparative treatments was assessed by using a linear contrast argument within this model. Additionally, modeling diagnostics were provided for assessing goodness-of-fit. Differences were considered significant at the 0.05 level.

Results

In Vitro Susceptibility

The MICs for JNJ-Q2 and all comparators against *S. pneumoniae* ATCC 6301, *S. aureus* Smith (MSSA, ATCC 13709), and methicillin-resistant *S. aureus* (MRSA, OC 8525 and ATCC 43300) are shown in Table 1. Against *S. pneumoniae*, JNJ-Q2 was 2-, 16-, 16-, 32- and 64-fold more

potent than gemifloxacin, ciprofloxacin, moxifloxacin, vancomycin and linezolid, respectively. Against MSSA, JNJ-Q2 and gemifloxacin were equipotent, and both were 8-, 32-, 128-, and 512-times more potent than moxifloxacin, ciprofloxacin, vancomycin and linezolid, respectively. Against MRSA, JNJ-Q2 was 4- to 128-fold more potent than the fluoroquinolone comparators and 64 to 512-fold more potent than the anti-MRSA comparators linezolid and vancomycin.

Mouse Septicemia Model

The activities (ED_{50}s and ED_{90}s) of JNJ-Q2, moxifloxacin, and gemifloxacin are summarized in Table 2. In the systemic infection model with *S. aureus* Smith, JNJ-Q2 was 6- and 9-times more potent than ciprofloxacin by the oral ($p<0.0021$) and subcutaneous ($p<0.0001$) routes of administration, respectively. JNJ-Q2 displayed ED_{50} values that were similar to moxifloxacin and gemifloxacin by the subcutaneous route of administration. However, JNJ-Q2 was more potent ($p<0.0001$) than gemifloxacin when the slopes of the dose-response profiles were compared. When administered orally, ED_{50} values of JNJ-Q2 were similar to moxifloxacin ($p>0.85$), but slightly less potent than gemifloxacin ($p>0.14$).

JNJ-Q2 was also compared to the anti-MRSA comparators linezolid and vancomycin against a CA-MRSA (OC 8525) strain in the murine septicemia model (Table 2). Oral activity (ED_{50}s) of JNJ-Q2 was less than that of linezolid ($p<0.0087$) in this model; however, JNJ-Q2 was 2- and 8-fold more active than linezolid ($p<0.0004$) and vancomycin ($p<0.0001$), respectively, when administered subcutaneously.

Lower Respiratory Tract Infection Model

The efficacies (ED_{50}s and ED_{90}s) of JNJ-Q2, moxifloxacin, and gemifloxacin in the mouse *S. pneumoniae* lower respiratory tract infection model are detailed in Table 3. JNJ-Q2 was 2- to 10-fold more active than moxifloxacin by the oral ($p<0.0086$) and subcutaneous ($p<0.0001$) routes of administration, respectively. JNJ-Q2 displayed similar activity to gemifloxacin when administered either subcutaneously or orally in this model.

Acute Skin Infection Model

The effect of JNJ-Q2, linezolid, and vancomycin against acute skin infections mediated by a CA-MRSA strain (OC 8525) are shown in Figure 1. In untreated control animals, the starting inoculum of ≈ 6.8 $\log_{10}$ CFU increased to 7.5 $\log_{10}$ CFU (8 $\log_{10}$ CFU/g skin tissue) during the 48 hour testing period. At every dose tested (1.6 to 100 mg/kg/day), JNJ-Q2 displayed greater reductions in bacterial burden in the skin of mice than linezolid ($p<0.0001$) or vancomycin ($p<0.0045$). At the highest dose tested (100 mg/kg/day), JNJ-Q2 reduced the bacterial burden in the skin by 2.5 and 1.3 $\log_{10}$ CFU/g more than linezolid and vancomycin, respectively, and reduced the bacterial titer by nearly 3 $\log_{10}$ CFU/g from the starting inoculum.

The skin lesion volumes resulting from infection with the CA-MRSA strain are shown in Figure 2. The reductions in lesion volume were concordant with the reductions in CFU (Figure 1). Animals treated with JNJ-Q2 had the smallest lesion volumes at every dose when compared to those of linezolid ($p<0.0001$) and vancomycin ($p<0.0001$).

Established Skin Infection Model

The efficacies of JNJ-Q2, ciprofloxacin, and moxifloxacin in mice with established skin infections (3 days) due to MRSA ATCC 43300 are shown in Figure 3. Bacterial burdens in untreated control mice increased 1.4 $\log_{10}$ CFU to 7.7 $\log_{10}$ CFU (8.2 $\log_{10}$ CFU/g skin tissue) over the six-

day testing period. Oral treatment with JNJ-Q2 at 25, 50, 100, and 200 mg/kg/day resulted in dose-dependent reductions of 0.7, 1.1, 2.4, and 3.0 $\log_{10}$ CFU/g skin tissue. In contrast, treatment with either ciprofloxacin ($p<0.0007$) or moxifloxacin ($p<0.0379$) did not result in reductions in CFU below the initial infecting inocula (6.8 $\log_{10}$ CFU). At the highest dose tested, 200 mg/kg/day, JNJ-Q2 had 2.8- and 1.9-fold greater reductions of MRSA in the skin of mice than ciprofloxacin and moxifloxacin, respectively.

Skin lesion volumes for mice infected with MRSA ATCC 43300 and treated with JNJ-Q2, ciprofloxacin, or moxifloxacin are shown in Figure 4. Control mice had a mean lesion volume of 611 mm^3. Treatment with JNJ-Q2 resulted in lesion volumes that were 46 to 63% smaller than those in control mice, in comparison with reductions in lesion volume of 19 to 46% and 19 to 28% for ciprofloxacin ($p<0.0092$) and moxifloxacin ($p<0.0001$), respectively.

The propensities for JNJ-Q2, ciprofloxacin and moxifloxacin to select for ciprofloxacin resistance in the established mouse skin infection model are summarized in Table 4. No resistant colonies were detected in any of the samples from 50 to 200 mg/kg/day of JNJ-Q2. Samples from animals treated with 25 mg/kg/day of JNJ-Q2 contained low levels of resistant colonies which grew in the presence of 2 μg/mL ciprofloxacin in 3/16 skin samples; however, counts were below the limit of reliable detection. In contrast, resistant colonies were recovered from each dose group of animals receiving ciprofloxacin. The density of resistant bacterial cells averaged approximately 3 $\log_{10}$ CFU/g skin tissue in each ciprofloxacin dose group. Ciprofloxacin-resistant colonies were also cultured from samples taken from moxifloxacin-treated animals (50 to 200 mg/kg/day), with resistant bacterial densities ranging from 2.6 to 3.2 $\log_{10}$ CFU/g skin tissue. The selection of resistance in infected animals treated with ciprofloxacin or moxifloxacin was statistically significant ($p<0.001$) when compared to the incidence of resistance following treatment of animals with JNJ-Q2.

Discussion

The activity of the new fluoroquinolone JNJ-Q2 compared to other fluoroquinolones and anti-MRSA comparators in murine models of systemic, respiratory, and localized skin infections was assessed. These activities included the efficacy of JNJ-Q2 in treating murine systemic and skin infections caused by MRSA and an established skin infection model with a virulent CA-MRSA isolate.

The fluoroquinolone moxifloxacin has demonstrated increased utility in treating respiratory tract infections, as it is associated with high rates of microbiological success in the clinic (3). In vitro, moxifloxacin displays activity against *S. pneumoniae*, including some ciprofloxacin-resistant isolates carrying QRDR mutations, however, the MICs of JNJ-Q2 were 32-fold lower than those of moxifloxacin against these *S. pneumoniae* isolates (27). The greater in vitro potency of JNJ-Q2 was reflected in the relative activities of these agents in the murine lower respiratory tract infection model, in which JNJ-Q2 displayed lower ED_{50} and ED_{90} values than moxifloxacin.

The increased in vitro activity of JNJ-Q2, in comparison with other fluoroquinolone agents, against *S. aureus*, including MRSA and ciprofloxacin-resistant MRSA (27), was likewise reflected in the murine septicemia model with *S. aureus* MSSA and MRSA strains. Against MSSA, peroral ED_{50} values for JNJ-Q2 were comparable to moxifloxacin, although JNJ-Q2 was more active by the subcutaneous route of administration. The moxifloxacin ED_{50} values for *S. aureus* Smith in our study closely matched those published previously for a systemic infection model (29). In the septicemia model with MRSA, JNJ-Q2 was more active than either of the anti-MRSA agents linezolid or vancomycin.

JNJ-Q2 exhibited dose-dependent reductions in bacterial load in an established MRSA mouse skin infection model, with limited evidence of resistance selection. In the MRSA established skin infection model, no resistant colonies were selected by JNJ-Q2 at doses of 50 to 200 mg/kg/day. This was in contrast to the comparator fluoroquinolone agents ciprofloxacin and moxifloxacin, which both selected for ciprofloxacin-resistant colonies within infected skin at the same doses. Poor efficacy in the established skin infection model observed in ciprofloxacin-treated animals was possibly due to the selection of resistant bacteria during treatment. Resistance selection in ciprofloxacin-treated animals was extensive, with 70 to 100% of treated mice yielding resistant colonies from infected skin samples. Given the clinical experience of ciprofloxacin, against which resistance in MRSA was observed to emerge rapidly (6), the reduced potential for resistance selection is a key attribute for a new fluoroquinolone developed to treat MRSA infections. Weight loss was noted in the ciprofloxacin high dose group (data not shown), which precluded increasing the dose to further evaluate efficacy. Concordant with our efficacy results, Cagni and colleagues (8) reported only minimal activity with ciprofloxacin (administered bid for 7 days) in a 21 d established rat tissue cage infection model, but in contrast, they did not isolate any resistant colonies following therapy. The difference in resistance selection observed here in the skin infection model reflects differences in the in vitro resistance rates observed for JNJ-Q2 and ciprofloxacin with MRSA, including isolates carrying QRDR mutations and displaying elevated ciprofloxacin MICs (27). Minimal efficacy was noted with moxifloxacin, even when tested at 200 mg/kg/day. Like ciprofloxacin, resistant isolates were recovered from every dose level tested, but to a lesser extent, which may have negatively impacted the efficacy of moxifloxacin in this setting. This model of an established skin infection was fairly robust, in that the infection involved a bacterial population of 8 $\log_{10}$ CFU/g tissue (7.7 $\log_{10}$ CFU total), permitting the differentiation of agents with disparate propensities for resistance selection.

In both the acute and established skin infection models, the observed reductions in lesion volume were generally dose-dependent; however, overall smaller lesions were observed in animals in the established skin infection model. This may be reflective of staphylococcal infections being self-limiting in these murine models and could in part be due to reported enhanced ability of *S. aureus* to bind hemoglobin derived from humans as compared to other mammals including mice (31).

The increased efficacy of JNJ-Q2 in comparison to ciprofloxacin and moxifloxacin in several mouse models of infection may result from the lower MIC values for JNJ-Q2 against the infecting strains, and the pharmacokinetic exposures of JNJ-Q2 in the mouse underscore the potency of this compound. JNJ-Q2 is 67% bound to mouse plasma proteins and was 8% orally bioavailable in the mouse (S. Steller and A. Streeter, unpublished data). This compares to mouse oral bioavailablity of 38 and 78% for ciprofloxacin and moxifloxacin, respectively (24, 33). JNJ-Q2 was 65% orally bioavailable in dogs, monkeys (G. Eichenbaum and S. Stellar, unpublished data) and humans (11). In the mouse, an oral 10 mg/kg dose of JNJ-Q2 yielded an AUC of 0.13 μg*h/mL (S. Steller and A. Streeter, unpublished data), a value that is 11-fold and 6-fold lower than oral ciprofloxacin and moxifloxacin mouse exposures, respectively (25, 30). In the established skin infection model, studies with ciprofloxacin and moxifloxacin included doses of 150 and 200 mg/kg, respectively, achieving in the mouse exposures comparable to human doses of 750 and 400 mg, respectively (25, 28, 30). In the septicemia model, the lower ED_{50} value for the subcutaneous administration of JNJ-Q2 in comparison to moxifloxacin reflects the lower MIC values for JNJ-Q2, but the lower ED_{50} values for moxifloxacin following peroral administration likely resulted from its greater oral bioavailability in the mouse. In contrast, in the lower respiratory tract infection model, JNJ-Q2 displayed lower ED_{50} values than moxifloxacin by both oral and subcutaneous routes of administration, possibly resulting from increased in vitro potency and the potential for increased lung exposure for JNJ-Q2.

In conclusion, JNJ-Q2 displayed promising levels of efficacy in a variety of local and systemic mouse infection models and warrants further study.

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468
469 Table 1. In Vitro Susceptibility of JNJ-Q2 and Comparators Against Gram-Positive Pathogens
470 Used in the Mouse Infection Models

Drug	MIC (µg/mL)			
	S. pneumoniae ATCC 6301	MSSA ATCC 13709	MRSA OC 8525	MRSA ATCC 43300
JNJ-Q2	0.015	0.008	0.015	0.004
Ciprofloxacin	0.25	0.25	2	0.5
Moxifloxacin	0.25	0.06	0.25	0.03
Gemifloxacin	0.03	0.008	0.06	0.03
Linezolid	1	4	4	2
Vancomycin	0.5	1	1	1

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Table 2. In Vivo Efficacy of Single Dose JNJ-Q2 and Comparators in a Murine Septicemia Model with *S. aureus* Smith (MSSA) or OC 8525 (CA-MRSA)

Compound	Organism	N[a]	Dosing Route	ED_{50}[mg/kg/day][b](95% Fiducial Limits)	ED_{90} [mg/kg/day][c] (95% Fiducial Limits	p-valve[d]
JNJ-Q2	MSSA	24	PO	2.0 (1.5 – 2.5)	4.0 (3.2 - 7.9)	NA
		16	SC	0.15 (0.05 – 0.22)	0.4 (0.3 – 0.8)	NA
	MRSA	16	PO	12 (9.1 – 20.4)	33.7 (21.1 – 108.6)	NA
		16	SC	1.6 (1.0 – 2.2)	5.4 (3.4 – 10.6)	NA
Ciprofloxacin	MSSA	16	PO	11 (6.5 – 41)	31.0 (NL)	<0.0021
		16	SC	1.4 (0.92 – 2.5)	3.3 (NL)	<0.0001
Moxifloxacin	MSSA	13	PO	1.5 (0.73 – 2.2)	3.1 (2.2 – 7.6)	>0.85
		13	SC	0.4 (0.2 – 0.8)	2.8 (1.5 - 9.7)	<0.0001
Gemifloxacin	MSSA	21	PO	1.1 (0.82 – 1.4)	2.8 (1.4 – 3036)	>0.14
		13	SC	0.1 (0.06 – 0.14)	0.2 (0.15 – 0.45)	<0.0001
Linezolid[e]	MRSA	18	PO	5.1 (3 – 8)	16.6 (11.9 – 31.5)	<0.0087
		18	SC	3.7 (2 – 6)	11.6 (8.3 – 22.8)	<0.0004
Vancomycin[e]	MRSA	32	SC	12 (10 – 14)	40.8 (29.1 – 69.4)	<0.0001

[a]Number of animals per group.
[b]ED_{50}s calculated using SAS version 5.
[c] ED_{90}s calculated using SAS version 9.1.
[d]p-value measure the significance of contrast of survival incidence of JNJ-Q2 and comparator dose response profiles.
[e] Dosed b.i.d., 1 and 3 hours post-infection.
NA – not applicable.

485

486

Table 3. In Vivo Efficacy of Single Dose JNJ-Q2 and Comparators in a Murine Lower Respiratory Tract Infection Model with *S. pneumoniae* ATCC 6301

Compound	N	Dosing Route	ED_{50} [mg/kg/day][b] (95% Fiducial Limits)	ED_{90} [mg/kg/day][c] (95% Fiducial Limits)	p-valve[a]
JNJ-Q2	16	PO	7.4 (5.3 – 10.1)	19.7 (13.8 – 38.0)	NA
	16	SC	1.9 (0.7 – 3.3)	18.3 (9.5 – 80.3)	NA
Moxifloxacin	16	PO	14 (8 – 23)	41.5 (28.3 – 84.2)	<0.0086
	16	SC	23 (NL)	NATC NATC	<0.0001
Gemifloxacin	16	PO	3.9 (2.2 – 5.8)	25.7 (16.4 – 53.2)	<0.0066
	16	SC	2.0 (1.2 – 2.9)	15.2 (8.9 – 40.6)	<0.0104

[a] p-value measure the significance of contrast of survival incidence of JNJ-Q2 and comparator dose response profiles.
[b] ED_{50}s calculated using SAS version 5.
[c] ED_{90}s calculated using SAS version 9.1.
NA – not applicable.
NL – no limits obtained.
NATC – program not able to calculate.

Table 4. Incidence of Ciprofloxacin-Resistant Bacteria arising within ATCC 43300 (MRSA) Mouse Skin Abscesses Following Treatment with JNJ-Q2 or Comparators.

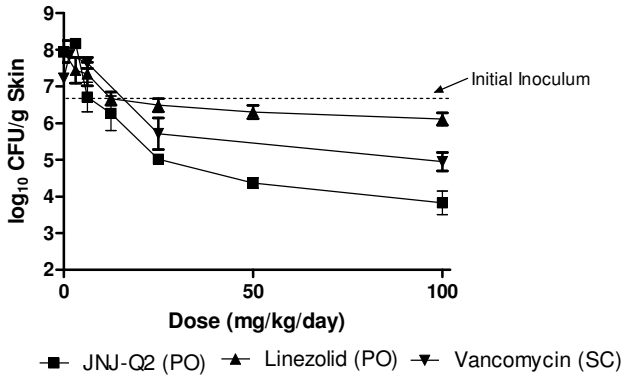
	Incidence of Resistant Colonies[a]		
Dose (mg/kg/day)	JNJ-Q2[b]	CIP	MOX
25	3/16	16/16	NT
50	0/16	16/16	5/16
100	0/14	11/16	7/16
200	0/14	11/16	2/16

NT = not tested

[a]the total number of samples that had any MRSA growth on a TSA plate containing 2 mg/L of ciprofloxacin divided by the total number of samples tested.

[b]$p<0.001$ vs. ciprofloxacin and moxifloxacin by logistic regression analysis.

506 **Figure 1. The Effect of JNJ-Q2, Linezolid and Vancomycin on Acute CA-MRSA (OC 8525)**
507 **Skin Infections**



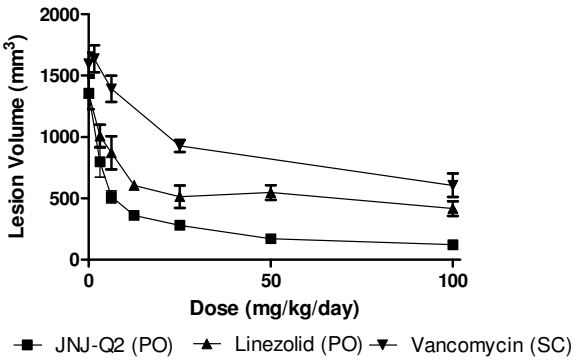
508

509 p-values were generated by comparing the slope of the dose-response curves for JNJ-Q2 vs. linezolid (p-
510 value <0.0001); JNJ-Q2 vs. vancomycin (p-value <0.0045)

511

512

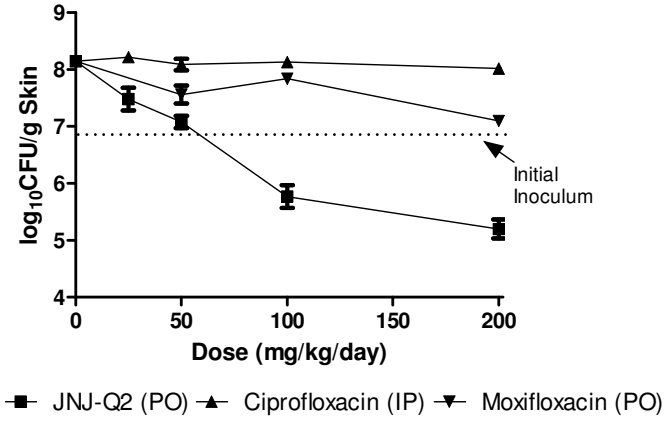
513 **Figure 2. The Effect of JNJ-Q2, Linezolid, and Vancomycin on Lesion Volume in Mice**
514 **Infected with CA-MRSA (OC 8525) in the Acute Skin Infection Model**



515

516 p-values were generated by comparing the slope of the dose-response curves JNJ-Q2 vs. linezolid (p-
517 value <0.0001); JNJ-Q2 vs. vancomycin (p-value <0.0001)[a]
518 [a]modeling contains a term for quadratic dose
519

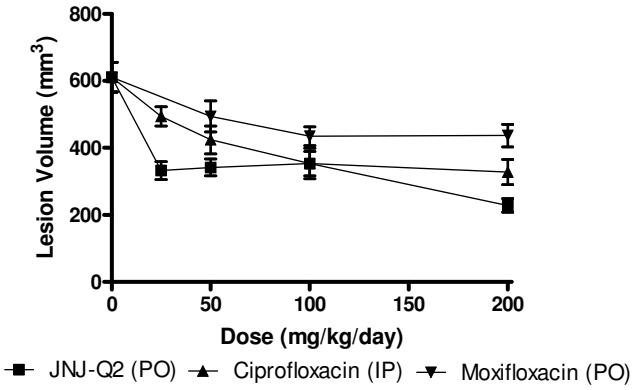
520 **Figure 3. The Effect of JNJ-Q2, Ciprofloxacin and Moxifloxacin on Established MRSA**
521 **ATCC 43300 Skin Infections**



522

523 p-values were generated by comparing the slope of the dose-response curves JNJ-Q2 vs. ciprofloxacin
524 (p-value <0.0007); JNJ-Q2 vs. moxifloxacin (p-value <0.0379)[a]
525 [a]modeling contains a term dose*treatment interaction

526 **Figure 4. The Effect of JNJ-Q2, Ciprofloxacin and Moxifloxacin on Lesion Volume in Mice**
527 **with Established MRSA ATCC 43300 Skin Infections**



528

529 p-values were generated by comparing the slope of the dose-response curves JNJ-Q2 vs. ciprofloxacin
530 (p-value <0.0092); JNJ-Q2 vs. moxifloxacin (p-value <0.0001)[a]
531 [a]modeling contains a term for quadratic dose
532