ABT492 and levofloxacin: comparison of their pharmacodynamics and their abilities to prevent the selection of resistant Staphylococcus aureus in an in vitro dynamic model

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Objective: To compare the kinetics of killing/regrowth of differentially susceptible clinical isolates of Staphylococcus aureus exposed to ABT492 and levofloxacin and to explore their relative abilities to prevent the selection of resistant mutants.

Methods: Three clinical isolates of S. aureus—including two ciprofloxacin-susceptible S. aureus, 201 and 480—and a ciprofloxacin-resistant S. aureus 866, were exposed to clinically achievable ratios of area under the curve (AUC) to MIC in a dynamic model that simulated human pharmacokinetics of ABT492 (400 mg) and levofloxacin (500 mg) as a single dose. In addition, S. aureus 201 was exposed to single and multiple doses of ABT492 and levofloxacin (both once daily for 3 days) over wide ranges of 24 h AUC/MIC (AUC24/MIC) including clinically achievable AUC24/MIC ratios.

Results: With each isolate, ABT492 at clinically achievable AUC/MICs produced greater anti-staphylococcal effects than levofloxacin. Areas between the control growth and the time–kill curves (ABBC in single dose simulations and the sum of ABBCs determined after the first, second and third dosing in multiple dose simulations—ABBC 1+2+3) were higher with ABT492 than levofloxacin. Moreover, at comparable AUC/MICs and AUC24/MICs, the maximal reductions in the starting inoculum of ABT492-exposed S. aureus were more pronounced than with levofloxacin. Loss in susceptibility of S. aureus 201 exposed to ABT492 or levofloxacin depended on the simulated AUC24/MIC. Although the maximal increase in MIC (MICfinal) related to its initial value (MICinitial) was seen at a higher AUC24/MIC ratio of ABT492 (120 h) than levofloxacin (50 h), similar AUC24/MICs (240 and 200 h, respectively) were protective against the selection of resistant S. aureus. These threshold values are readily achievable with 400 mg ABT492 (AUC24/MIC 870 h) but not with 500 mg levofloxacin (AUC24/MIC 70 h).

Conclusion: Overall, these findings predict greater efficacy of clinically achievable AUC/MIC (or AUC24/MIC) of ABT492 both in terms of the anti-staphylococcal effect and prevention of the selection of resistant mutants.

Keywords: S. aureus, resistance, in vitro models

Introduction

A novel 1-(6-amino-3,5-difluoropyridin-2-yl)-8-chloroquinolone antibiotic, ABT492, has been shown to be more active than other fluoroquinolones against Streptococcus pneumoniae, Staphylococcus aureus and Enterococcus faecalis.1 The present study examines the comparative pharmacodynamics of ABT492 and levofloxacin with differentially susceptible strains of S. aureus in an in vitro dynamic model that simulates human pharmacokinetics. Also, the relative ability of ABT492 and levofloxacin to prevent the selection of resistant S. aureus was compared using a recently described method.2

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Materials and methods

Antimicrobial agents and bacterial strains

ABT492 and levofloxacin (kindly provided by Abbott Laboratories, North Chicago, IL, USA and Ortho-McNeill Pharmaceuticals, Raritan, NJ, USA, respectively) were used in the study. Three differentially susceptible clinical isolates of S. aureus including two ciprofloxacin-susceptible strains, S. aureus 201 and 480 (MICs of ciprofloxacin 0.8 and 2 mg/L, respectively) and a ciprofloxacin-resistant strain 866 (MIC of ciprofloxacin 4 mg/L) were selected for the study. S. aureus 201 was kindly provided by Dr E. Gugutsidze from Moscow Clinical Hospital and S. aureus 480 and 866 were kindly provided by Dr M. Edelstein from the Institute of Antimicrobial Chemotherapy of Smolensk State Medical Academy (Russia).

Susceptibility testing was performed in triplicate using broth microdilution techniques at 24 h post-exposure, with the organism grown in Ca\(^{2+}\) (20–25 mg/L)– and Mg\(^{2+}\) (10–12.5 mg/L)-supplemented Mueller-Hinton broth (MHB, BBL, Becton Dickinson and Company, Sparks, MD, USA) at an inoculum size of 10\(^6\) cfu/mL. To establish precise values, MICs were determined using doubling dilutions, with starting concentrations of 3, 4 and 5 mg/L, as described previously. The MICs of ABT492 for S. aureus 201, 480 and 866 were 0.02, 0.06 and 0.12 mg/L, respectively. The respective MICs of levofloxacin were 0.6 and 1 and 3 mg/L.

The mutant prevention concentrations (MPCs) of ABT492 and levofloxacin for S. aureus 201 were determined as described elsewhere. Briefly, the tested microorganisms were cultured in MHB and incubated for 24 h. Then, the suspension was centrifuged (4000 \(g\) for 10 min) and re-suspended in MHB to yield a concentration of 10\(^8\) cfu/mL. A series of agar plates containing known fluoroquinolone concentrations was then inoculated with \(~10^{10}\) cfu of S. aureus 201. The inoculated plates were incubated for 48 h at 37°C and logarithms of bacterial numbers were plotted against fluoroquinolone concentrations. MPC was taken as the point where the plot intersected the x-axis, i.e. the lowest fluoroquinolone concentration that completely inhibited growth. The MPCs of ABT492 and levofloxacin were estimated at 0.07 and 1.75 mg/L, respectively.

Simulated pharmacokinetic profiles

Reported concentration–time data obtained in humans after a single 400 mg dose of ABT492 and after daily dosing for 5 days were fitted by a three-exponential equation with an absorption half-life of 0.2 h, a distribution half-life of 1.7 h and an elimination half-life (terminal half-life) of 25 h (Figure 1, upper panel). Best fit estimates were obtained by a non-linear regression analysis using TOPFIT software (V. 1.1 — Gödecke, Schering, Thomae, 1991). Then, the same fitting procedure was applied to combined data obtained after ABT492 administration at doses 100, 200 and 400 mg.

Time-courses of the geometric mean ABT492 concentration (\(C\)) were normalized by the dose (\(D\)). As seen in the bottom panel of Figure 1, there is no dose-dependent shift in C/D-time curves after the first and fifth doses of ABT492. This allows fitting the data with a linear model. The three-exponential equation fits the dose-normalized data with an absorption half-life of 0.2 h, a distribution half-life (\(t_{1/2}\)) of 1.9 h and an elimination half-life (\(t_{1/2}\)) of 23 h. This parameter set was used in in vitro simulations of ABT492 pharmacokinetics.

Because of the relatively minor impact of the absorption phase on the observed pharmacokinetics (the partial area that reflects the contribution of the absorption phase was estimated at only 9% of the total area under the curve (AUC)), a bi-exponential concentration decay of ABT492 was simulated with \(t_{1/2}\) of 2 h and \(t_{1/2}\) of 23 h. Two quasi-linear portions of each concentration-time curve (Figure 2, left panel) approximated this bi-exponential profile. With levofloxacin, a series of mono-exponential profiles were simulated with \(t_{1/2}\) of 6.8 h (Figure 2, right panel) that represent weighted means of the values reported in humans: 6.0–7.4 h.
In single dose simulations, *S. aureus* 201 was exposed to eight-fold ranging AUC/MIC of both ABT492 and levofloxacin, i.e. from 60–480 h (Figure 2, upper panel). In addition, three therapeutically achievable AUC/MICs that correspond to a 400 mg dose of ABT492 and a 500 mg dose of levofloxacin were simulated with each of the three *S. aureus* strains (Figure 2, bottom panel). In multiple dose simulations with *S. aureus* 201, when daily dosing of ABT492 and levofloxacin was mimicked for three consecutive days, 24 h AUC (AUC24) to MIC ratios varied from 60–480 h and from 15–200 h, respectively (Figure 3). In addition, the clinically achievable AUC24/MICs of 870 h (ABT492) and 70 h (levofloxacin) were simulated.

**In vitro dynamic model**

A previously described dynamic model11 was used in the study. Briefly, the model consists of two connected flasks, one containing fresh MHB and the other a magnetic stirrer; and a central unit, with the same broth containing either a bacterial culture alone (control growth experiments) or a bacterial culture plus antimicrobial (killing/regrowth experiments). Peristaltic pumps circulate fresh nutrient medium to the flasks: from the central 70 mL unit at a flow rate of 22.4 mL/h during first 16 h after dosing of ABT492 and then at a flow rate of 2.1 mL/h. With levofloxacin, the central unit volume was 60 mL and the flow rate 6.1 mL/h. The reliability of fluoroquinolone pharmacokinetic simulations and the high reproducibility of the time–kill curves provided by the model have been reported elsewhere.11

The system was filled with sterile MHB and placed in an incubator at 37°C. The central unit was inoculated with an 18 h culture of *S. aureus*. After the bacteria had been incubated for 2 h, the resulting exponentially growing cultures reached ∼10^6 cfu/mL in single-dose simulations, or 10^8 cfu/mL (6 × 10^9–7 × 10^9 per 60–70 mL central compartment) in multiple dose simulations, at which time ABT492 or levofloxacin was injected into the central unit.

**Quantification of the time–kill curves and antimicrobial effect**

In each experiment, multiple sampling of bacteria-containing media from the central compartment was performed throughout the observation period. One hundred microlitre samples were serially diluted as appropriate, and 100 µL was plated onto agar plates. The duration of the experiments was defined in each case as the time—after a single or the final dose—when antibiotic-exposed bacteria reached the maximum numbers observed in the absence of antibiotic (>10^8 cfu/mL). In multiple dose studies, the minimum duration was 72 h if no regrowth occurred. The lower limit of accurate detection was 2 × 10^2 cfu/mL. A level of 10 cfu/mL was considered to be the theoretical limit of detection.
Based on time–kill data obtained in single-dose simulations, the area between the normal growth curve and the curve of bacteria exposed to antibiotic (ABBC) was calculated from time zero to 24 h. In multiple dose simulations, a cumulative antimicrobial effect was expressed as the sum of ABBC determined within the first, second and third dosing interval (ABBC1, ABBC2 and ABBC3, respectively): ABBC1 + ABBC2 + ABBC3 = ABBC1+2+3. The upper limit of bacterial numbers, i.e. the cutoff level on the regrowth and control growth curves used to determine ABBC and ABBC1+2+3 was 10^9 cfu/mL. The computation of ABBC, ABBC1, ABBC2 and ABBC3 is depicted graphically in Figure 4.

ABBC versus AUC/MIC relationships were fitted by the Boltzmann function.
Results

Single dose simulations

Time courses of killing and regrowth of *S. aureus* 201 exposed to bi-exponentially decreasing concentrations of ABT492 and monoeXponential concentration decay of levofloxacin at AUC/MIC of 60–480 h are shown in the upper panel of Figure 5. The time–kill curves observed with both quinolones yielded similar patterns: regrowth followed a prompt reduction in bacterial numbers. At a given AUC/MIC ratio, the maximal reductions in the starting inoculum of ABT492-exposed *S. aureus* were greater than those with levofloxacin, but it showed longer times to regrowth than ABT492. At AUC/MIC of 60, 120, 240 and 480 h, the minimal numbers \( N_{\text{min}} \) of surviving *S. aureus* exposed to ABT492 were lower than with levofloxacin: *1.8 x 10^8* versus *1.5 x 10^9*, *1.3 x 10^8* versus *3.5 x 10^7*, *7 x 10^5* versus *1 x 10^4* and *1.5 x 10^5* versus *1 x 10^4* cfu/mL, respectively. The respective times to regrowth were 9 versus 16 h, 11 versus 22 h, 11 versus 26 h and 15 versus 30 h. However, at AUC/MIC ratios provided by the clinical dose of ABT492 (400 mg), regrowth of all three strains of *S. aureus—S. aureus* 201, 480 and 866—was observed much later than in simulations of the clinically achievable AUC/MIC of levofloxacin (500 mg) (Figure 5, bottom panels). Again, the maximal reductions in viable counts after ABT492 exposure were greater than with levofloxacin: \( N_{\text{min}} \) of *1.5 x 10^9* versus *1.1 x 10^8* cfu/mL (*S. aureus* 201), *6.5 x 10^8* versus *9.3 x 10^7* cfu/mL (*S. aureus* 480) and *7 x 10^8* versus *1.5 x 10^7* cfu/mL (*S. aureus* 866), respectively.

The inherent difference in ABT492 and levofloxacin pharmacodynamics is visualized by the AUC/MIC relationships of ABBC (Figure 6, left panel). A specific strain-independent relationship was inherent for each quinolone, and equation 1 fits the data. Although more pronounced effects of levofloxacin were seen for most AUC/MIC ranges (60–480 h), the clinically achievable AUC/MIC ratios of ABT492 were more efficient than the respective AUC/MICs of levofloxacin. For example, with *S. aureus* 201, an 870 h AUC/MIC produced 1.7 times higher ABBC than a 70 h AUC/MIC of levofloxacin: 140 versus 83 (log cfu/mL) x h. With two other organisms, the clinically achievable AUC/MICs of ABT492 (290 h for *S. aureus* 480 and 145 h for *S. aureus* 866) also produced greater effects than the respective AUC/MICs of levofloxacin (55 h for *S. aureus* 480 and 18 h for *S. aureus* 866): 1.5-fold difference in the ABBCs with *S. aureus* 480 and four-fold difference with *S. aureus* 866 (Figure 7).

Multiple dose simulations

Time–kill dynamics. Time courses of ABT492- and levofloxacin-exposed *S. aureus* 201 are shown in Figure 8. At most studied AUC/MIC ratios, regrowth followed a prompt reduction in bacterial numbers after each dosing. However, at the clinically achievable AUC/MIC ratio of ABT492 (870 h) but not levofloxacin (70 h), no bacterial regrowth occurred. AUC/MIC-dependent relationships of *ABBC_1+2+3* (Figure 6, right panel) were similar to the AUC/MIC relationships of ABBC delineated in single-dose simulations. Because of fewer points with ABT492, these data could not be accurately approximated by equation 1. Therefore, quasi-linear portions of both ABT492 and levofloxacin plots were fitted by equation 2. Like single-dose simulations, with ABT492 the 870 h clinical value of the AUC/MIC ratio produced a 1.7 times greater antimicrobial effect than a 70 h value for levofloxacin: *ABBC_1+2+3* of 505 versus 290 (log cfu/mL) x h.

Bacterial resistance

Significant increases in MIC of ABT492 were observed at AUC/MIC of *60 and 120 h*, and with levofloxacin at AUC/MIC of *25–100 h* after 3 days exposure of *S. aureus* 201 (Figure 9). With both quinolones, these increases were most pronounced after the third quinolone dose. Serial passages of resistant isolates onto antibiotic-free plates revealed minimal or no changes in the elevated MICs, showing stable resistance after five passages (data not shown). At the higher AUC/MIC (≥240 h with ABT492 and 200 h with levofloxacin), no loss in the susceptibility of *S. aureus* was documented. Therefore, these threshold values may be considered to be protective against the selection of resistant mutants.

Plotting the ratio of the elevated MIC (MIC_{final}) to the starting values (MIC_{initial}) against the simulated AUC/MIC ratio resulted in two different relationships that appeared to be quinolone-specific (Figure 10, upper panel). As seen in the figure, the ABT492 curve is shifted towards the higher AUC/MICs compared with the levo
Figure 8. Killing/regrowth kinetics of *S. aureus* 201 exposed to the quinolones. The simulated AUC$_{24}$/MIC (h) is indicated by the number at each curve. The arrows reflect quinolone dosing.
ABT492 pharmacodynamics and staphylococcal resistance

floxacin curve. The clinically achievable AUC$_{24}$/MIC ratio of ABT492 (870 h) is far in excess of the 240 h protective value. Unlike ABT492, the clinically achievable AUC$_{24}$/MIC ratio of levofloxacin (70 h) is less than its protective value (200 h).

Resistance of quinolone-exposed *S. aureus* 201 also correlated with the time above MPC ($T_{>\text{MPC}}$). As seen in the bottom panel of Figure 10, longer $T_{\text{MPC}}$s were associated with less pronounced increases in MIC, although a specific $T_{\text{MPC}}$ relationship of MIC$_{\text{final}}$/MIC$_{\text{initial}}$ was inherent for each quinolone. To protect from the selection of resistant *S. aureus*, the shorter $T_{\text{MPC}}$s were needed with ABT492 (<10 h) than levofloxacin (18 h).

Discussion

Single and multiple dose simulations at comparable AUC/MIC or AUC$_{24}$/MIC ratios (from 50–60 to 100–120 h) showed more pronounced killing ($N_{\text{final}}$) of *S. aureus* 201 with ABT492 than levofloxacin, although the antimicrobial effects (ABBC) were smaller. Despite lower bacterial counts after ABT492 exposure, the times to regrowth were shorter than with levofloxacin. This earlier regrowth occurred because of the relatively rapid decline in ABT492 concentrations to the MIC level. Unlike levofloxacin, most events reflected by the time–kill curves at ABT492 AUC/MICs of 60–480 h were associated with the distribution phase ($t_{1/2\alpha}$ 1.9 h) rather than the elimination phase ($t_{1/2\beta}$ 23 h) of the simulated concentration–time curves (Figure 2, upper panel).

It is fair to say that $t_{1/2\beta}$ of 23 h simulated in this study differs from values reported in human studies with ABT492: 4.2–8.5$^{14}$ and 8.3–9 h. This difference is due to the fact that the reported $t_{1/2\beta}$s ignore the terminal phase of the concentration-time curves (from 12–48 h) that reflects enterohepatic recycling of ABT492. In this light, $t_{1/2\beta}$ simulated in the present study may be considered as an apparent half-life that describes ABT492 pharmacokinetics as observed in humans, regardless of the underlying mechanisms.

Despite the relatively smaller anti-staphylococcal effects of ABT492 compared with levofloxacin at a given AUC/MIC or AUC$_{24}$/MIC ratio, the clinically achievable AUC/MIC or AUC$_{24}$/MIC ratios, i.e. those provided by a 400 mg dose of ABT492, produced much greater ABBCs with *S. aureus* 201 (Figure 6), 480 and 866 (Figure 7) than did clinically achievable AUC/MICs or AUC$_{24}$/MICs of 500 mg levofloxacin. Moreover, similar 1.7-fold differences in the antimicrobial effect in terms of ABBC or ABBC$_{1+2+3}$ were observed in single- and multiple-dose simulations. Concordant estimates of relative anti-staphylococcal efficacy have been reported with moxi-

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**Figure 9.** Changes in the susceptibility of *S. aureus* 201 exposed to ABT492 and levofloxacin at different AUC$_{24}$/MICs.

**Figure 10.** AUC$_{24}$/MIC and $T_{\text{MPC}}$ relationships of *S. aureus* 201 resistance to ABT492 and levofloxacin.
floxacin and levofloxacin in previous single-dose and 3 day dosing in vitro studies.\textsuperscript{16}

Like our study with four fluoroquinolones,\textsuperscript{2} loss in the susceptibility of ABT492- and levofloxacin-exposed \textit{S. aureus} \textsuperscript{201} (MIC\textsubscript{final}/MIC\textsubscript{initial}) depended on the simulated AUC\textsubscript{24}/MIC (Figure 10, upper panel). However, with ABT492, the maximal increase in MIC\textsubscript{final} was seen at a higher AUC\textsubscript{24}/MIC ratio (120 h) than with levofloxacin (50 h). Resistance of quinolone-exposed \textit{S. aureus} \textsuperscript{201} was also related to \(T_{\text{MPC}}\): the longer the \(T_{\text{MPC}}\), the less pronounced the loss in susceptibility. Like AUC\textsubscript{24}/MIC relationships, a specific \(T_{\text{MPC}}\) relationship with MIC\textsubscript{final}/MIC\textsubscript{initial} was inherent in each drug (Figure 10, bottom panel). To protect against the selection of resistant \textit{S. aureus}, shorter \(T_{\text{MPC}}\)s were needed with ABT492 (<10 h) than levofloxacin (18 h). In contrast to data reported with more slowly eliminated quinolones,\textsuperscript{3} increases in ABT492 MICs did not correlate with the time when ABT492 concentrations were within the mutant selection window (MSW), i.e. between MPC and MIC (\(T_{\text{MSW}}\)). With four of five simulated regimens (including AUC\textsubscript{24}/MIC of 60 and 120 h) where loss in susceptibility was observed and AUC\textsubscript{24}/MIC of 240 and 480 h without such a loss, \(T_{\text{MSW}}\) were similar (~17% of the dosing interval). At the same time, no increases in MIC were associated with AUC\textsubscript{24}/MIC of 870 h when \(T_{\text{MSW}}\) was 38% of the dosing interval.

Both AUC\textsubscript{24}/MIC and \(T_{\text{MPC}}\) relationships of the MIC\textsubscript{final}/MIC\textsubscript{initial} ratio allow prediction of threshold values that prevent the selection of resistant \textit{S. aureus}. In terms of the AUC\textsubscript{24}/MIC ratio, these threshold values are similar for ABT492 and levofloxacin (240 and 200 h, respectively), but in terms of \(T_{\text{MPC}}\) they are different: 10 versus 18 h. The clinically achievable AUC\textsubscript{24}/MIC ratio of ABT492 (870 h) is far in excess of the 240 h protective value. Unlike ABT492, the clinically achievable AUC\textsubscript{24}/MIC ratio of levofloxacin (70 h) is less than its protective value (200 h).

Based on linear relationships of AUC to the dose for ABT492 and levofloxacin\textsuperscript{13} (equation 3), the AUC\textsubscript{24}/MIC relationships of ABBC\textsubscript{1+2+3} and MIC\textsubscript{final}/MIC\textsubscript{initial} can be presented as respective dose relationships. As seen in the left upper panel of Figure 11, a 400 mg dose of ABT492 provides a 60% greater effect (ABBC\textsubscript{1+2+3}) on \textit{S. aureus} \textsuperscript{201} than a 500 mg dose of levofloxacin. Also, the clinical dose of ABT492 but not levofloxacin may be able to prevent the loss in susceptibility (Figure 11, left bottom panel).

It is fair to say that this analysis ignores protein binding and, therefore, it may overestimate the true effect of more highly bound ABT492 (84%)\textsuperscript{4} relative to levofloxacin (30%).\textsuperscript{17} If the data presented in the left panel of Figure 11 were corrected for protein binding, the described advantages of ABT492 would disappear. Unlike total concentrations, the free concentration analysis predicts similar

Figure 11. Dose-dependent ABBC\textsubscript{1+2+3} and MIC\textsubscript{final}/MIC\textsubscript{initial} for quinolone-exposed \textit{S. aureus}: impact of protein binding.
MIC_{final}/MIC_{minimal} ratios (Figure 11, right bottom panel) but lower ABBC_{1+2+3} with 400 mg ABT-492 compared with 500 mg levofloxacin (Figure 11, right upper panel). Is the free concentration analysis that is often used in studies with in vitro models more correct than that based on the total concentrations? Far from it, because this mechanistic transformation of the data does not consider the dynamic nature of the equilibrium between protein-bound and -unbound fractions. As a result, the true impact of protein binding on the anti-

MICs is underestimated. So, in terms of the dose predictions, the truth stands between those that do or do not consider protein binding. Furthermore, no protein binding effects on killing of S. aureus, S. pneumoniae or Escherichia coli were reported in a recent in vitro study with three differentially bound quinolones. Similar conclusions were drawn using a murine pneumococcal pneumonia model: both total and free concentrations of the five quinolones were equally predictive of the 50% maximal animal survival. Overall, these findings predict greater efficacy of clinically achievable AUC/MIC and AUC_{24}/MIC of ABT-492 both in terms of the anti-staphylococcal effect and prevention of the selection of resistant mutants.

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