Pharmacokinetics/Pharmacodynamics of Colistin and Imipenem on Mucoid and Nonmucoid \textit{Pseudomonas aeruginosa} Biofilms\(^\dagger\)†

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The time course of activity of colistin and imipenem against mucoid and nonmucoid \textit{Pseudomonas aeruginosa} growing in a biofilm showed that compared with those for planktonic bacteria, the kinetics of colistin and imipenem retained the concentration- and time-dependent killing, respectively, but higher doses of antibiotics and longer dosing periods were required for biofilm eradication. Biofilms of mucoid \textit{P. aeruginosa} were more difficult to eradicate than nonmucoid biofilms.

\textit{Pseudomonas aeruginosa} growing as biofilm is the main cause of chronic lung infection in cystic fibrosis (CF) patients (17). Several \textit{in vitro} studies have shown that bacteria growing in a biofilm could become 10 to 1,000 times more resistant than planktonic bacteria of the same strains (8, 24, 25).

Dosage optimization is important to maximize the effect of antibiotics on the infection outcome and to prevent further development of antimicrobial resistance (36). The pharmacokinetics (PK) and pharmacodynamics (PD) of antimicrobial agents can be used reliably to choose dosing regimens and to achieve the maximum bactericidal effect and minimize development of antimicrobial resistance (32). Previous studies of PK and PD were related mainly to planktonic cells and not to biofilm cells. Killing curves of antibiotics on biofilms have not been reported previously.

The present study was performed to explore the susceptibilities of nonmucoid and mucoid \textit{P. aeruginosa} growing in a biofilm, to compare the killing curves of colistin and imipenem on planktonic and biofilm-growing nonmucoid and mucoid \textit{P. aeruginosa}, and to optimize PD targets for the treatment of biofilm-associated infections.

The nonmucoid wild-type \textit{P. aeruginosa} strain PAO1, its isogenic mucoid variant Alg\(^+\) PAOmicA22 (PDO300) (33), and the nonmucoid strain PAO579 were used in this study.

Biofilm assays were performed by a modified Calgary biofilm device method (8, 25). To establish the killing curves of antibiotics on biofilm, peg lids were placed in flat-bottom microtiter plates containing colistin or imipenem (0 to 512 µg/ml) and incubated for 0 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h at 37°C. A standard susceptibility assay and determination of killing curves of antibiotics on planktonic cells were performed as previously described (2).

A mouse model of lung biofilm infection (30, 38) was used for the pharmacokinetic studies of colistin and imipenem. Female 10-week-old NMRI mice were used. Experiments were performed with authorization from the National Animal Ethics Committee of Denmark. The concentrations of colistin and imipenem in serum were measured by a biological method with the indicator bacteria \textit{Streptococcus} sp. EB68 (6) and \textit{ Bordetella bronchiseptica} ATCC 4617 (21). PK parameters, including peak concentration (\(C_{\text{max}}\)), time required to reach maximum concentration (\(T_{\text{max}}\)), area under the concentration-time curve from zero time to the last sampling time (AUC\(_{\text{last}}\)), and half-life of elimination (\(T_{1/2}\)), are shown in Table 1. The details of methods and a part of the results can be found in the supplemental material.

Bacterial cells growing in a biofilm showed sharply increased resistance to imipenem compared to that of planktonic cells, and the resistance levels increased in mature (3- and 7-day-old) biofilms over that in young (1-day-old) biofilms (Fig. 1). This is probably due to development of biomass on day 3 biofilm with the increased proportion of biofilm cells in stationary phase (10, 15) and/or the accumulation of \(\beta\)-lactamase in mature biofilms compared to findings for young biofilms (5–7, 18).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result (mean ± SD) for single intrapertitoneal dose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Colistin</td>
</tr>
<tr>
<td>(C_{\text{max}}) (mg/liter)</td>
<td>15.43 ± 1.78</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>25 ± 7.75</td>
</tr>
<tr>
<td>(T_{1/2}) (min)</td>
<td>31.72 ± 2.95</td>
</tr>
<tr>
<td>AUC(_{\text{last}}) (mg · min/liter)</td>
<td>1,073.65 ± 43.19</td>
</tr>
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\(\dagger\) Results were measured following intraperitoneal administration of single doses in mice (\(n = 18\)) with \textit{P. aeruginosa} biofilm lung infection: 16 mg/kg (colistin) or 64 mg/kg (imipenem).

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Compared with those of planktonic bacteria (3, 13, 14, 34, 35), the kinetics of colistin and imipenem retained concentration-dependent and time-dependent killing, respectively, on both mucoid and nonmucoid bacteria growing as biofilm. However, for treatment of *P. aeruginosa* growing in a biofilm, higher doses of antibiotics and longer treatment times were required than for planktonic cells (Fig. 2 and 3). The error bars of Fig. 2 and 3 show means ± standard deviations (SD). The maximum bactericidal effects of colistin and imipenem were attained at 1 to 8 h and 4 to 8 h for planktonic *P. aeruginosa* and at 2 to 12 h and 6 to 24 h for *P. aeruginosa* growing as biofilm, respectively. Mature biofilm (day 3) was more resistant than young biofilm (day 1). The maximum killing effects of colistin and imipenem occurred at 4 and 8 h, respectively, for young biofilm of PAO1 but at 8 and 24 h, respectively, for mature biofilms (Fig. 3). Mucoid PDO300 growing in a biofilm was more resistant to colistin and imipenem than nonmucoid PAO1. To reach the maximum killing effect of imipenem, treatment was required for 6 h and at least 64 μg/ml for young biofilm (day 1) of PAO1 but for at least 8 h and at 512 μg/ml for young biofilm of PDO300; as for colistin, it was required for at least 4 h and at least 32 μg/ml for young biofilm of PAO1 and for 12 h and at least 64 μg/ml for young biofilm of PDO300 (Fig. 3).

Traditionally, the MIC and the minimal bactericidal concentration (MBC) as a single static parameter *in vitro* are used for the selection of antimicrobial agents to treat planktonic bacterial infections (4, 31). Similarly, minimal biofilm inhibitory concentration (MBIC) and minimal biofilm eradication concentration (MBEC) measurements provide only a snapshot of the effects of antibiotic agents on the basis of a single concentration. Killing-curve approaches provide more-appropriate information about the interaction between bacteria and antimicrobial agents, since they depict the interaction in a multidimensional way via a dynamic integration of concentration and time (26–28). Thus, while in Fig. 1 the MBEC of imipenem reached 1,024 μg/ml, the concentrations in both mucoid and nonmucoid strains were similar, and the killing curve (Fig. 3) showed that treatment was required for a longer period of time to kill mucoid PDO300 than was the case for the nonmucoid strain PAO1.

When most planktonic cells are killed by antibiotics at concentrations of MBC, the planktonic residue can be eradicated by the immune system *in vivo*. However, a large part of biofilm cells might be able to escape from the attack of both antibiotic and immune systems (11, 19). The biofilm will be restored quickly from residual biofilm cells that survived the antibiotic treatment (9). Therefore, the clinical target of antibiotic treatment for biofilm infections is different from that for infections caused by planktonic cells (22). It was difficult to reach the MBEC of colistin and imipenem *in vivo* due to the side effects and toxicity of the antibiotics (Fig. 4 and Table 2). Therefore, biofilm eradication seems impossible using antibiotic monotherapy via systemic administration. Inhalation and other topical administrations are obvious options for treatment of biofilm infections due to the possibility of achieving higher concentrations of antibiotic in biofilm, avoiding side effects and toxicity (12).
FIG. 2. Kill curves of planktonic *Pseudomonas aeruginosa* nonmucoid PAO1 and mucoid PDO300 with imipenem and colistin. (a) PAO1, planktonic, imipenem; (b) PDO300, planktonic, imipenem; (c) PAO1, planktonic, colistin; (d) PDO300, planktonic, colistin.
However, inhalation of antibiotics cannot reach all foci of infection in the airways. Long-term and high-dose antibiotic therapies have been recommended for biofilm infection (12). However, the side effects and toxicity of antibiotics can impair the therapeutic effects (29, 39, 40) and result in failure of antibiotic therapy in some biofilm infections.

In conclusion, the ideal concentration of antibiotics for eradication of mature *P. aeruginosa* biofilm is difficult to obtain *in vivo*. Aggressive and early treatment with the combination of antibiotics is highly recommended for young-biofilm eradication (Fig. 4). In addition, combination therapy with quorum-sensing inhibitors, enzymes, biofilm disassembling agents, and efflux pump inhibitor (1, 10, 16, 20, 23, 37) can be expected in the future. The kinetics of colistin and imipenem showed concentration-dependent and time-dependent killing, respectively, for *P. aeruginosa* growing in a biofilm, and $C_{\text{max}}$/MBIC, $C_{\text{max}}$/MBEC, AUC/MBIC, and AUC/MBEC are probably the targets for dosing with polymyxin peptide, with the times during...
FIG. 4. Pharmacokinetics in mouse serum of colistin and imipenem versus MIC, MBIC, MBIC, and MBEC of P. aeruginosa PAO165307.  ■, 16 mg/kg of colistin;  ●, 64 mg/kg of imipenem with one-dose intraperitoneal administration.

which the drug concentration is greater than the MBIC and MBEC (T>MBIC and T>MBEC) being the targets for dosing with beta-lactam antibiotics. However, additional in vivo experiments are required to validate the PK-PD simulation in P. aeruginosa growing in a biofilm. PK-PD targets specific for biofilm-associated infections and toxicity evaluation will contribute to the increased efficacy of antibiotic therapy for biofilm-associated infections.

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REFERENCES

TABLE 2. Pharmacokinetic/pharmacodynamic modeling in P. aeruginosa biofilm lung infection model in mice

<table>
<thead>
<tr>
<th>Parameter&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>7.72</td>
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<sup>a</sup> n = 18 mice.
<sup>b</sup> T>MIC, time during which the drug concentration is greater than the MIC.

Other variables are defined in the text.


