Colistin as a potentiator of anti-TB drug activity against *Mycobacterium tuberculosis*

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Received 31 March 2015; returned 28 April 2015; revised 6 June 2015; accepted 11 June 2015

Objectives: The mycobacterial cell wall is an effective permeability barrier that limits intracellular concentrations of anti-TB drugs and hampers the success of treatment. We hypothesized that colistin might enhance the efficacy of anti-TB drugs by increasing mycobacterial cell wall permeability. In this study, we investigated the additional effect of colistin on the activity of anti-TB drugs against *Mycobacterium tuberculosis* in vitro.

Methods: The concentration-dependent and time-dependent killing activity of isoniazid, rifampicin or amikacin alone or in combination with colistin against *M. tuberculosis* H37Rv was determined. Mycobacterial populations with both high and low metabolic activity were studied, and these were characterized by increasing or steady levels of ATP, respectively.

Results: With exposure to a single drug, striking differences in anti-TB drug activity were observed when the two mycobacterial populations were compared. The addition of colistin to isoniazid and amikacin resulted in sterilization of the mycobacterial load, but only in the *M. tuberculosis* population with high metabolic activity. The emergence of isoniazid and amikacin resistance was completely prevented by the addition of colistin.

Conclusions: The results of this study emphasize the importance of investigating mycobacterial populations with both high and low metabolic activity when evaluating the efficacy of anti-TB drugs in vitro. This is the first study showing that colistin potentiates the activity of isoniazid and amikacin against *M. tuberculosis* and prevents the emergence of resistance to anti-TB drugs. These results form the basis for further studies on the applicability of colistin as a potentiator of anti-TB drugs.

Introduction

TB is still a major global health problem, with 9 million people affected worldwide. It is the second most common cause of mortality related to infectious diseases, being responsible for 1.5 million deaths each year.1 Long-term treatment with multiple anti-TB agents is necessary and is often accompanied by drug toxicities, drug–drug interactions, a lack of patient compliance and the emergence of drug resistance. Powerful new treatment strategies are needed that aim to improve the potency of antimycobacterial drugs and prevent the emergence of drug resistance while minimizing drug toxicities.

The mycobacterial cell wall is known for its complex lipid architecture, forming an effective permeability barrier that contributes to the intrinsic resistance to multiple antimicrobial agents and limited efficacy of the currently used anti-TB drugs. In general, high concentrations of anti-TB drugs are needed to prevent the selection of drug-resistant mutants. In clinical practice, high dosing of anti-TB drugs is usually limited by toxic side effects. Increasing the permeability of the lipid bilayer in the mycobacterial cell wall could be a way to increase intrabacterial anti-TB drug concentrations. Different studies have shown that changes in the mycolate composition of the mycobacterial cell wall result in increased antimycobacterial drug susceptibility in both *Mycobacterium tuberculosis* (Mtbb) and *Mycobacterium avium*.2,3

Colistin, a member of the polymyxin group of drugs, is currently used in the treatment of drug-resistant Gram-negative bacterial infections. The proposed mechanism of action of colistin is through interaction with the Gram-negative bacterial membrane.4 This includes electrostatic interactions between positively charged groups on the polymyxins and negatively charged components of LPS, as well as interactions between the polymyxin fatty acid tail and the lipids of the bacterial membrane.4 Destabilization of the cytoplasmic bacterial membrane occurs, resulting in leakage of intracellular contents and apoptosis.4 Although the composition of the mycobacterial cell wall is

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different from that of Gram-negative bacteria, an interaction with colistin and colistin-like particles has been shown, resulting in increased permeability.\(^5,6\) We therefore hypothesized that colistin might enhance anti-TB drug efficacy by increasing mycobacterial cell wall permeability, allowing increased intracellular concentrations of anti-TB drugs.

In the present study, we investigated the activity of various combinations of anti-TB drugs and colistin against extracellular Mtb in vitro. We compared the activity against Mtb populations with high and low metabolic activity, as we have previously shown that the activity of anti-TB drugs depends on the metabolic activity of the mycobacteria.\(^7\) In most in vitro studies, anti-TB drug activity is determined using the MIC assay, providing endpoint data and determining only the inhibition of mycobacterial growth. In the present study, we used the time–kill kinetics assay, providing unique information on the concentration-dependent and time-dependent killing capacity of anti-TB drugs.

**Materials and methods**

**Bacterial strain and culture**

The Mtb strain used was Mtb H37Rv (ATCC 27294), a clinical isolate and reference strain commonly used in TB studies. Mtb suspensions were cultured in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI, USA), supplemented with 10% OADC (Baltimore Biological Laboratories, Baltimore, MD, USA), 0.5% glycerol (Scharlau Chemie SA, Sentmenat, Spain) and 0.02% Tween 20 (Sigma Chemical Co., St Louis, MO, USA) under shaking conditions at 96 rpm at 37°C. Vials with Mtb suspensions were stored at −80°C. Cultures on solid medium were grown on Middlebrook 7H10 agar (Difco), supplemented with 10% OADC and 0.5% glycerol for 21 days at 37°C with 5% CO\(_2\). The susceptibility of the Mtb strain in terms of MICs, determined at the Dutch National Reference Laboratory according to the CLSI guidelines,\(^8\) was 0.125 mg/L for isoniazid, 0.125 mg/L for rifampicin, 2 mg/L for amikacin and >1024 mg/L for colistin.

**Anti-TB drugs and colistin**

Isoniazid was purchased from Sigma Chemical Co., rifampicin was purchased from Aventis Pharma BV (Hoevelaken, the Netherlands), amikacin was purchased from Hospira Benelux BVBA (Brussels, Belgium) and colistin sulphate was purchased from Spruyt Hillen (IJsselstein, The Netherlands; 20400 IU/mg).

**Time–kill kinetics assay**

The concentration-dependent and time-dependent killing capacity of the different anti-TB drugs alone or in combination with colistin was determined as previously described.\(^7\) Mtb populations with high and low metabolic activity were prepared for testing the activity of the anti-TB drugs. The metabolic activity of the Mtb cultures was assessed in a previous study by measuring the ATP level using the firefly luciferase bioluminescence assay.\(^7\) The Mtb population with high metabolic activity, starting at a density of \(7.2 \times 10^5\) cfu/mL (range \(5.2–10^5\)), showed a 6-fold increase in ATP concentration per viable Mtb after 3 days of incubation. Mtb cultures with low metabolic activity (LA-Mtb) were prepared by culturing Mtb at a density of \(7.2 \times 10^5\) cfu/mL in Middlebrook 7H9 broth under anaerobic conditions with 5% CO\(_2\).

\[ \text{INH} 0.031 \, \text{R} = 100\% \]
\[ \text{INH} 0.125 \, \text{R} = 100\% \]
\[ \text{INH} 0.5 \, \text{R} = 100\% \]
\[ \text{INH} 2 \, \text{R} = 100\% \]
\[ \text{INH} 8 \, \text{R} = 100\% \]

\[ \text{INH} 0 \, \text{R} = 0\% \]
\[ \text{INH} 0.031 \, \text{R} = 0\% \]
\[ \text{INH} 0.125 \, \text{R} = 0\% \]
\[ \text{INH} 0.5 \, \text{R} = 0\% \]
\[ \text{INH} 2 \, \text{R} = 0\% \]

**Figure 1.** Concentration-dependent and time-dependent bactericidal activity of isoniazid against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb). Mtb cultures were exposed to 2-fold increasing isoniazid concentrations for 6 days at 37°C under shaking conditions. On days 1, 2, 3 and 6, samples were collected, centrifuged and subcultured onto antibiotic-free and isoniazid-containing solid media and incubated for 21 days at 37°C with 5% CO\(_2\) to determine the cfu count. INH, isoniazid; R, isoniazid-resistant mutants.
metabolic activity obtained after 4 days of incubation started at a density of $1.8 \times 10^7$ cfu/mL (range $0.8 - 3.4 \times 10^7$) and showed steady ATP levels. Mtb cultures with high and low metabolic activity were exposed to anti-TB drugs and/or colistin for 6 days at 37°C under shaking conditions at 96 rpm. On days 1, 2, 3 and 6 during exposure, samples were collected, centrifuged at 14,000 g to avoid drug carry-over and subcultured onto solid medium. The plates were incubated for 21 days at 37°C with 5% CO2 to determine the number of cfu representing viable bacteria. The lower limit of detection was 5 cfu/mL (log 0.7).

Selection of drug-resistant Mtb

In order to assess the selection of drug resistance after 6 days of drug exposure, subcultures were also performed on solid media containing anti-TB drugs. The drug concentrations in the subculture plates were 4-fold the critical concentrations, i.e. 0.8 mg/L isoniazid, 4 mg/L rifampicin and 20 mg/L amikacin.

Endpoints

The two endpoints of this study were: (i) synergy between the anti-TB drug and colistin; and (ii) the prevention of emergence of anti-TB drug resistance. Synergistic activity of a drug combination was defined as $\geq 99\%$ killing compared with the killing obtained with the most active single drug or when the drug combination achieved sterilization of Mtb after 6 days of drug exposure that was not achieved during single-drug exposure.

Results

Concentration- and time-dependent bactericidal activity of anti-TB drugs and colistin at single-drug exposure in relation to the metabolic activity of the mycobacteria

As shown in Figures 1–4 (all tested concentrations are depicted in Figures S1–S4, available as Supplementary data at JAC online), the anti-TB drugs and colistin differed with respect to their concentration-dependent and time-dependent killing capacity. In the absence of anti-TB drugs or colistin, the Mtb population with high metabolic activity showed an average increase from $7.7 \times 10^5$ cfu/mL to $6.1 \times 10^7$ cfu/mL within 6 days of incubation. The Mtb population with low metabolic activity showed only a modest increase (average from $2.1 \times 10^7$ to $7.2 \times 10^7$ cfu/mL) within 6 days.

Isoniazid showed a concentration-dependent effect on the Mtb populations with high and low metabolic activity (Figure 1 and Figure S1), and $\geq 99\%$ killing was rapidly achieved at low concentrations in the Mtb population with high metabolic activity. Sterilization was observed only at extremely high concentrations ($\geq 128$ and $\geq 256$ mg/L, respectively). Both Mtb populations became 100% isoniazid resistant at concentrations ranging from 0.031 to 64 mg/L and 0.125 to 128 mg/L, respectively.

Rifampicin showed a concentration-dependent effect, particularly in the Mtb population with high metabolic activity (Figure 2 and Figure S2). In both Mtb populations, mycobacterial killing was less rapid than with isoniazid. Sterilization was achieved at...
Figure 3. Concentration-dependent and time-dependent bactericidal activity of amikacin against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb). Mtb cultures were exposed to 2-fold increasing amikacin concentrations for 6 days at 37°C under shaking conditions. On days 1, 2, 3 and 6, samples were collected, centrifuged and subcultured onto antibiotic-free and amikacin-containing solid media and incubated for 21 days at 37°C with 5% CO₂ to determine the cfu count. AMK, amikacin; R, amikacin-resistant mutants.

Figure 4. Concentration-dependent and time-dependent bactericidal activity of colistin against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb). Mtb cultures were exposed to 2-fold increasing colistin concentrations for 6 days at 37°C under shaking conditions. On days 1, 2, 3 and 6, samples were collected, centrifuged and subcultured onto antibiotic-free solid medium and incubated for 21 days at 37°C with 5% CO₂ to determine the cfu count. CST, colistin sulphate.
occurred. It was achieved only at high concentrations and sterilization never
was prevented by the addition of colistin to the Mtb population showing high metabolic activity and at 1024 mg/L in the Mtb population with low metabolic activity. In the Mtb population with high metabolic activity, a synergistic effect was observed when lower concentrations of amikacin (0.125 and 0.25 mg/L) were combined with low concentrations of isoniazid (0.125 mg/L) and rifampicin (0.031 mg/L), resulting in sterilization (Figures 5 and 7). In the presence of colistin, isoniazid and amikacin achieved sterilization at lower concentrations (0.125 and 0.25 mg/L, respectively) than isoniazid and amikacin at single-drug exposure (128 and 1 mg/L, respectively). Synergy was not observed when isoniazid was combined with rifampicin (Figure 6). In the Mtb population with low metabolic activity, there was no synergistic effect of colistin and anti-TB drugs (Figures 5–7).

Tables 3 and 4 summarize the effect of colistin on the bactericidal activity of the anti-TB drugs. Selection of drug resistance after exposure to anti-TB drugs combined with colistin

The selection of isoniazid resistance observed after single-drug exposure was completely prevented by the addition of high-dose colistin in the Mtb population showing high metabolic activity and was reduced in the population showing low metabolic activity (Tables 3 and 4). There was no amikacin resistance after single-drug exposure in the Mtb population with high metabolic activity. In the population with low metabolic activity, the addition of colistin to amikacin completely prevented the selection of amikacin resistance (Table 4). Rifampicin resistance did not occur after single-drug exposure in the Mtb population with high metabolic activity. In the Mtb population with low metabolic activity, colistin was not able to prevent the selection of rifampicin resistance (Table 4).

### Table 1. Concentration-dependent bactericidal activity (≥99% killing) against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb) during 6 days of exposure to anti-TB drugs or colistin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Best MIC (mg/L)</th>
<th>MIC (mg/L)</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>1</td>
<td>1</td>
<td>0.125</td>
<td>0.031</td>
<td>0.063</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>32</td>
<td>128</td>
<td>2</td>
<td>0.31</td>
<td>0.25</td>
</tr>
<tr>
<td>Amikacin</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>256</td>
<td>64</td>
<td>512</td>
</tr>
</tbody>
</table>

### Table 2. Concentration-dependent sterilizing activity against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb) after 6 days of exposure to anti-TB drugs or colistin

<table>
<thead>
<tr>
<th>Drug</th>
<th>Lowest concentration (mg/L) resulting in ≥99% killing of Mtb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>128</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>8</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1</td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>

Sterilization was defined as ≤5 cfu/mL as this was the lower limit of detection of our time–kill kinetics assay.

≥8 mg/L in the Mtb population with high metabolic activity and at 1024 mg/L in the Mtb population with low metabolic activity. Rifampicin-resistant mutants were selected only in the Mtb population with low metabolic activity, and resistance increased slowly over concentrations ranging from 0.25 to 512 mg/L.

Amikacin showed a strong concentration–dependent effect on the Mtb population with high metabolic activity (Figure 3 and Figure S3). In both Mtb populations, ≥99% killing was rapidly achieved. Sterilization was observed at ≥1 mg/L in the Mtb population with high metabolic activity and at 1024 mg/L in the Mtb population with low metabolic activity. Amikacin-resistant mutants were selected only in the Mtb population with low metabolic activity, and resistance increased slowly over concentrations ranging from 1 to 512 mg/L.

The effect of colistin on Mtb was modest in both Mtb populations (Figure 4 and Figure S4). In the two populations, ≥99% killing was achieved only at high concentrations and sterilization never occurred.

Tables 1 and 2 summarize the comparative bactericidal activity of the anti-TB drugs and colistin at single-drug exposure.

### Effect of colistin on the concentration- and time-dependent bactericidal activity of anti-TB drugs in relation to the metabolic activity of the mycobacteria

The colistin concentrations used in the combination experiments were 8 and 32 mg/L, based on the activity against Mtb at single-drug exposure. Colistin at 8 mg/L showed no activity against Mtb and colistin at 32 mg/L showed only a modest, inhibitory effect on mycobacterial growth (Figure 4). The anti-TB drug concentrations in the combination experiments studying the Mtb population with high metabolic activity were based on the MIC values of the anti-TB drugs. Because sterilization was achieved at the MIC of amikacin, a lower amikacin concentration was used (1/8×MIC). In the combination experiments studying the Mtb populations with low metabolic activity, we also used higher concentrations of anti-TB drugs, which were associated with the selection of drug resistance after single-drug exposure (isoniazid and amikacin, 4×MIC; rifampicin, 8×MIC).

The concentration- and time-dependent killing capacity of isoniazid, rifampicin and amikacin combined with colistin are depicted in Figures 5–7.

In the Mtb population with high metabolic activity, a synergistic effect was observed when high colistin concentrations (32 mg/L) were combined with low concentrations of isoniazid (0.125 mg/L) or amikacin (0.25 mg/L), resulting in sterilization (Figures 5 and 7). In the presence of colistin, isoniazid and amikacin achieved sterilization at lower concentrations (0.125 and 0.25 mg/L, respectively) than isoniazid and amikacin at single-drug exposure (128 and 1 mg/L, respectively). Synergy was not observed when colistin was combined with rifampicin (Figure 6). In the Mtb population with low metabolic activity, there was no synergistic effect of colistin and anti-TB drugs (Figures 5–7).

Tables 3 and 4 summarize the effect of colistin on the bactericidal activity of the anti-TB drugs.

### Selection of drug resistance after exposure to anti-TB drugs combined with colistin

The selection of isoniazid resistance observed after single-drug exposure was completely prevented by the addition of high-dose colistin in the Mtb population showing high metabolic activity and was reduced in the population showing low metabolic activity (Tables 3 and 4). There was no amikacin resistance after single-drug exposure in the Mtb population with high metabolic activity. In the population with low metabolic activity, the addition of colistin to amikacin completely prevented the selection of amikacin resistance (Table 4). Rifampicin resistance did not occur after single-drug exposure in the Mtb population with high metabolic activity. In the Mtb population with low metabolic activity, colistin was not able to prevent the selection of rifampicin resistance (Table 4).
Figure 5. Concentration-dependent and time-dependent bactericidal activity of isoniazid combined with colistin against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb). Mtb cultures were exposed to isoniazid or colistin alone or in combination for 6 days at 37°C under shaking conditions. On days 1, 2, 3 and 6, samples were collected, centrifuged and subcultured onto antibiotic-free and isoniazid-containing solid media and incubated for 21 days at 37°C with 5% CO2 to determine the cfu count. INH, isoniazid; CST, colistin sulphate; R, isoniazid-resistant mutants.

Figure 6. Concentration-dependent and time-dependent bactericidal activity of rifampicin combined with colistin against the Mtb population with high metabolic activity (HA-Mtb) and the Mtb population with low metabolic activity (LA-Mtb). Mtb cultures were exposed to rifampicin or colistin alone or in combination for 6 days at 37°C under shaking conditions. On days 1, 2, 3 and 6, samples were collected, centrifuged and subcultured onto antibiotic-free and rifampicin-containing solid media and incubated for 21 days at 37°C with 5% CO2 to determine the cfu count. RIF, rifampicin; CST, colistin sulphate; R, rifampicin-resistant mutants.
In this study, we assessed the concentration-dependent and time-dependent activity of anti-TB drugs in combination with colistin against Mtb. We identified two new potent anti-TB drug combinations that induced a strong and rapid killing of Mtb and prevented the emergence of drug resistance. To our knowledge, this is the first study to provide evidence that colistin can potentiate the activity of isoniazid or amikacin against Mtb in vitro. The addition of colistin to low concentrations of isoniazid and amikacin resulted in sterilization of the mycobacterial load, which is one of the main goals of TB treatment. Interestingly, synergy was only achieved in the Mtb population with high metabolic activity. In addition, in the presence of colistin, the emergence of isoniazid resistance was completely prevented in the Mtb population with high metabolic activity, but not in the population with low metabolic activity. Colistin combined with amikacin completely prevented the emergence of amikacin resistance in the Mtb population with low metabolic activity. The results of our study emphasize the importance of investigating Mtb populations

Table 3. Synergistic activity and prevention of selection of drug resistance in the Mtb population with high metabolic activity (HA-Mtb)

<table>
<thead>
<tr>
<th></th>
<th>CST 8</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>no CST, day 6</td>
<td></td>
<td>day 1 synergy</td>
<td>day 2 synergy</td>
</tr>
<tr>
<td>resistance (%)</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>INH 0.125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIF 0.125</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AMK 0.25</td>
<td>0</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

CST, colistin sulphate; INH, isoniazid; RIF, rifampicin; AMK, amikacin; +, synergy; S, sterilization.

Discussion

In this study, we assessed the concentration-dependent and time-dependent activity of anti-TB drugs in combination with colistin against Mtb. We identified two new potent anti-TB drug combinations that induced a strong and rapid killing of Mtb and prevented the emergence of drug resistance. To our knowledge, this is the first study to provide evidence that colistin can potentiate the activity of isoniazid or amikacin against Mtb in vitro. The addition of colistin to low concentrations of isoniazid and amikacin resulted in sterilization of the mycobacterial load, which is one of the main goals of TB treatment. Interestingly, synergy was only achieved in the Mtb population with high metabolic activity. In addition, in the presence of colistin, the emergence of isoniazid resistance was completely prevented in the Mtb population with high metabolic activity, but not in the population with low metabolic activity. Colistin combined with amikacin completely prevented the emergence of amikacin resistance in the Mtb population with low metabolic activity. The results of our study emphasize the importance of investigating Mtb populations
with both high and low metabolic activity when evaluating anti-TB drug efficacy in vitro as these drugs can behave differently depending on the metabolic activity of the mycobacteria. This is supported by striking differences in the anti-TB drug activities of isoniazid, rifampicin and amikacin at single-drug exposure as well as differences in the emergence of anti-TB drug resistance between the two Mtb populations. The observation that the potentiating effect of colistin on isoniazid and amikacin activity was only observed in the Mtb population with high metabolic activity might be related to the fact that higher drug concentrations were needed to achieve sterilization of the Mtb population with low metabolic activity. It can be speculated that these concentrations cannot be achieved even with the addition of colistin, explaining the lack of synergy when studying the Mtb population with low metabolic activity. Distinguishing between the two different Mtb populations might be clinically relevant as the Mtb population with high metabolic activity is supposed to be responsible for spreading TB, whereas the Mtb population with low metabolic activity. It can be speculated that these concentrations were needed to achieve sterilization of the Mtb population with low metabolic activity. It can be speculated that these concentrations were needed to achieve sterilization of the Mtb population with low metabolic activity.

It is surprising that, in the present study, the addition of colistin did not result in enhanced efficacy of rifampicin; neither did it prevent the emergence of rifampicin resistance. An explanation might be that the influence of colistin on anti-TB drug activity depends on the lipophilicity of the anti-TB drug. It has been suggested that the lipophilicity of antimycobacterial agents is correlated with their ability to invade the mycobacterial cell wall and therefore with antimycobacterial drug activity. Rifampicin is known for its lipophilic properties, whereas isoniazid and amikacin are hydrophilic agents. It has been shown that the activity of isoniazid against M. avium can be improved by using a more lipophilic isoniazid derivate, strengthening the hypothesis that a lipophilic nature is associated with increased efficacy. The pronounced ability of rifampicin, compared with isoniazid and amikacin, to cross the mycobacterial lipid bilayer might explain the fact that the increasing mycobacterial cell wall permeability brought about by colistin was not beneficial when it was combined with rifampicin. In addition, it has been proposed that cationic peptides, such as colistin, form micelle-like aggregates spanning the cytoplasmic membrane and providing water channels for the movement of ions and hydrophilic molecules across the bacterial membrane. This mechanism might also contribute to the synergistic effect observed between colistin and hydrophilic compounds such as isoniazid and amikacin as opposed to the lipophilic rifampicin. However, our results are in contrast to the findings of Korycka-Machala et al., who showed increased susceptibility to rifampicin in Mycobacterium vaccae treated with polymyxin B nonapeptide (PMBN), which was linked to changes in the lipid composition of the mycobacterial cell wall. Since the cell wall of M. vaccae is known to be different from that of most other mycobacterial species, it is unclear whether similar results would have been obtained with Mtb or whether structural differences would have been observed between PMBN and colistin account for these differences. The study by Yuan et al. also showed that changes in the cell wall lipid composition of a mutant Mtb strain were associated with increased rifampicin susceptibility, whereas susceptibility to isoniazid remained unchanged. Although colistin has been shown to increase the permeability of the mycobacterial cell wall, a role for other mechanisms in colistin’s potentiation of anti-TB drug activity cannot be excluded. For instance, colistin has recently been shown to induce hydroxyl radical production. The hypothesis that colistin causes increased intramyocobacterial drug concentrations needs to be confirmed by measuring intramyocobacterial anti-TB drug concentrations.

In the present study, the concentration of colistin that was needed to potentiate anti-TB drugs was relatively high, at 32 mg/L. When used intravenously, colistin is administered as the prodrug colistin methanesulphonate (CMS), which is rapidly converted into its active component in vivo. It has been reported in two studies in critically ill patients that intravenous clinical dosages of CMS resulted in suboptimal maximum plasma concentrations of active colistin of $2.21 \pm 1.08$ and $2.93 \pm 1.24$ mg/L, respectively. Colistin concentrations were undetectable in the bronchoalveolar lavage fluid. These concentrations in serum and bronchoalveolar lavage fluid are far below the colistin concentration that effected synergy in vitro in the present study. In relation to this, administration of colistin via inhalation should achieve attention. Colistin administered by

Table 4. Synergistic activity and prevention of selection of drug resistance in the Mtb population with low metabolic activity (LA-Mtb)

<table>
<thead>
<tr>
<th>No CST, day 6, resistance (%)</th>
<th>CST 8</th>
<th>CST 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH 0.125</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>INH 0.5</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>RIF 0.125</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RIF 1</td>
<td>5.7</td>
<td>4.6</td>
</tr>
<tr>
<td>AMK 2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>AMK 8</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

CST, colistin sulphate; INH, isoniazid; RIF, rifampicin; AMK, amikacin.
inhalation is already successfully used in current clinical practice for the treatment of pneumonia caused by resistant Gram-negative bacteria in patients with cystic fibrosis and patients with (ventilator-associated) pneumonia. The potential advantage of inhalation therapy is that higher local concentrations can be achieved while minimizing systemic drug concentrations and associated drug toxicities. It has recently been shown that colistin sulphate nebulization in rats resulted in concentrations in the pulmonary epithelial lining fluid that were around 1800 times higher than those achieved by intravenous colistin administration. Another recent study confirmed these findings in patients with cystic fibrosis, showing that dry powder nebulization of CMS resulted in colistin sputum concentrations of ≥128 mg/L in 38% of patients, while the remainder had levels that were <128 mg/L. Importantly, minimal systemic exposure was observed with inhalation therapy. However, whether the pulmonary colistin concentrations that can be achieved via inhalation are high enough to achieve synergy in combination with anti-TB drugs remains to be determined, especially since pulmonary surfactant has been shown to impair in vitro colistin activity.

Based on the results obtained in the present study, we suggest that inhalational colistin combined with anti-TB drugs could be a potential new strategy for the treatment of TB. In general, the concept of pulmonary delivery of anti-TB drugs is very attractive and has recently been attracting interest. In addition to achieving enhanced anti-TB drug efficacy against susceptible Mtb while minimizing systemic drug toxicity, the increase in local drug concentrations at the primary infected site could overcome drug resistance in (extensively) drug-resistant TB and perhaps decrease the contagiousness of the disease. Several anti-TB drugs have already been studied in animal models of pulmonary delivery, including the first-line agents isoniazid, rifampicin and pyrazinamide; the results have been promising. However, the translation of these experimental animal data to clinical practice remains to be determined as data on clinical efficacy and safety in humans are scarce. Further studies are needed to assess the clinical applicability of anti-TB drug inhalation and the additional value of colistin.

In conclusion, this is the first study to show that colistin potentiates the activity of isoniazid and amikacin against Mtb in vitro and prevents resistance to anti-TB drugs. These results form the basis for further studies on the applicability of colistin as a potentiator of anti-TB drug activity against intracellular Mtb hiding inside macrophages.

Funding
This work was supported by NanoNextNL, a micro and nanotechnology consortium of the Government of the Netherlands and 130 partners (project number 03D.09).

Transparency declarations
None to declare.

Supplementary data
Figures S1 to S4 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

21 Dharmadhikari AS, Mphahlele M, Venter K et al. Rapid impact of effective treatment on transmission of multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2014; **18**: 1019–25.


