Rifampin Protects Human Lung Epithelial Cells Against Cytotoxicity Induced by Clinical Multi and Pandrug-resistant Acinetobacter baumannii

Younes Smani, Juan Domínguez-Herrera, and Jerónimo Pachón
Service of Infectious Diseases, Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío/CSIC/University of Seville, Spain

Objective. Recently, it has become apparent that rifampin can act on eukaryotic cells modulating production of host mediators. We aimed to study the cytoprotective effect of rifampin against multidrug- and pandrug-resistant Acinetobacter baumannii-induced cell death using human lung epithelial cells.

Methods. We pretreated A549 cells with rifampin and infected them with 3 different A. baumannii strains (susceptible, multidrug-resistant, and pandrug-resistant) that induce cell death. Cellular viability, apoptosis and host mediators, free radicals, and proinflammatory cytokines associated with A. baumannii pathogenesis were studied. Moreover, bacterial concentrations in A549 cells culture were determined.

Results. Rifampin-pretreated A549 cells demonstrated decreases in apoptosis and cell death induced by A. baumannii. The oxidative stress and proinflammatory responses to A. baumannii were reduced in rifampin-pretreated A549 cells, as shown by decreased superoxide anion, tumor necrosis factor-α, and interleukin-6. Furthermore, bacterial count performed in A549 cell culture medium showed that rifampin did not reduce significantly the bacterial concentrations.

Conclusion. These data demonstrate that rifampin is able to attenuate the cellular damage induced by multidrug- and pandrug-resistant A. baumannii clinical isolates without being relevantly bactericidal. Indeed, the cytoprotective effect of rifampin was observed on the decrease of dead cells induced by A. baumannii by reducing oxidative stress and proinflammatory cytokines release.

In recent years, substantial increases have occurred in the prevalence of resistance to the major antimicrobial treatment options for Acinetobacter baumannii: fluoroquinolones, β-lactams including carbapenems, and aminoglycosides. These changes in the A. baumannii resistance rates have led clinicians to adopt other therapeutic options, such as colistin, which has returned to clinical use. Moreover, different combinations of antimicrobials have been evaluated in vitro and in experimental infections [1], such as rifampin plus imipenem or sulbactam [2], and rifampin plus colistin [2, 3]. The combination of colistin plus rifampin has been also evaluated clinically, mostly in patients with pneumonia and bacteremia [4–6].

Rifampin is a macrocyclic antimicrobial that is highly effective in tuberculosis treatment [7]. Its antimicrobial activity is mediated by bacterial RNA polymerase inhibition. Several researchers have shown that, in addition to its antimicrobial activity, rifampin regulates various immune responses, influencing such functions as lymphocyte migration, cytokine and nitric oxide production, antigen presentation, and phagocytosis [8–13].

In addition to these immunologic effects, neuroprotective effects of rifampin have been observed in cerebral ischemia and in neurodegenerative disorders, such as Alzheimer and Parkinson diseases. It has been reported that rifampin reduces brain injury after cerebral ischemia [14] and inhibits beta-amyloid toxicity.
Moreover, cytoprotective effects of rifampin have been studied in different works. Rifampin has been shown to bind to and to activate human glucocorticoid receptor (GR) in hepatocellular carcinoma cell line [20], inhibit prostaglandin E2 (PGE2) and arachidonic acid (AA) releases in human epithelial cells [21], and down-regulate tumor necrosis factor (TNF)-α on macrophages [9]. GR activation and PGE2, AA, and TNF-α inhibitions resulted in inhibition of cell death [22–24]. Moreover, it was demonstrated that rifampin inhibits CD95-induced apoptosis in human cells by modulating the expression of various molecules regulating apoptosis [25, 26]. These rifampin cytoprotective effects have also been demonstrated in tuberculosis and meningitis infections. It was shown that rifampin exerts immunosuppressive effects on macrophages by means of decrease of TNF-α production as well as inhibition of apoptosis induced by Mycobacterium tuberculosis [8]. Moreover, rifampin reduced early mortality, reduced inflammation by decreasing cerebrospinal fluid PGE2 and free radicals production, and decreased neuronal cell death in mouse and rabbit experimental models of Streptococcus pneumoniae meningitis [27–30].

Thus, accumulating evidence from different studies indicates that rifampin may play a major role in the protection of induced cell death in neurodegenerative disorders and in infectious diseases.

In the present study, we evaluate the cytoprotective effect of rifampin on multidrug- and pandrug-resistant A. baumannii-induced cell death using human lung epithelial cells. The data presented here indicate that rifampin inhibits the cell death induced by A. baumannii, without eradicating bacteria, through the reduction of free radicals and proinflammatory cytokines releases.

**MATERIALS AND METHODS**

**Bacterial Strains**

A. baumannii ATCC 19606 strain, susceptible to all antimicrobials; clinical multidrug-resistant A. baumannii isolate 1327M strain (a mutant strain obtained from an original multidrug-resistant 1327 with minimal inhibitory concentration [MIC] to rifampin of 4 µg/mL); and clinical pandrug-resistant A. baumannii isolate 113-16 strain were used. Original multidrug-resistant 1327 and pandrug-resistant 113-16 strains were isolated in University Hospitals Virgen del Rocío. All strains were grown in a Mueller Hinton broth (MHB) at 37°C for 20-24 h. Cultured strains were washed with phosphate-buffered saline (PBS) and resuspended in Dulbecco’s modified Eagle’s medium (DMEM) before use in eukaryotic cell culture experiments.

**In Vitro Susceptibility Testing and Time-kill Experiments**

Rifampin MICs against ATCC 19606, 1327M, and 113-16 strains were determined by microdilution in 2 independent experiments, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [31]. Escherichia coli ATCC 25922 and Pseudomonas aeruginosa 27853 were used as control strains. Rifampin activity was tested using 2 different inocula: 5 x 10^5 colony-forming units (cfu)/mL (standard inoculum) [32] and 1 x 10^8 cfu/mL (high inoculum). Time-kill kinetic assays were conducted on MHB at rifampin concentrations of 1x, 2x, and 4x MIC with initial log-phase inocula of 5 x 10^5 cfu/mL (standard inoculum) [33] and 1 x 10^8 cfu/mL (high inoculum). Rifampin-free broth was evaluated in parallel as a control, and cultures were incubated at 37°C. Viable counts were determined by serial dilution at 0, 2, 4, 8, and 24 h after adding the rifampin, and plating 100 µL of control, test cultures, or dilutions at the indicated times onto sheep blood agar plates (Blood-Agar Columbia, Becton Dickinson). Plates were incubated for 24 h, and after colony counts, the log_{10} of viable cells (cfu/mL) was determined. Rifampin was considered to be bactericidal when a ≥3 log_{10} decrease in cfu/mL was reached relative to the initial inoculum.

**Human Cell Culture and Infection**

Type II pneumocyte cell line A549 derived from a human lung carcinoma were a gift from Felipe Fernandez-Cuenca, MD, and were grown in DMEM supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) (Gibco), vancomycin (50 µg/mL), gentamicin (20 µg/mL), amphotericin B (0.25 µg/mL; Gibco), and 1% 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid in a humidified 5% CO₂ at 37°C. A549 cells were passaged every 3-4 days. A549 cells were seeded 24 h in 96-well plates (for 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT] assay) and in 24-well plates (for the rest of experiments) before infection with A. baumannii at a multiplicity of infection of 100. Immediately before infection, A549 cells were washed thrice with prewarmed PBS and further incubated in DMEM without FBS and the antibiotics previously mentioned.

**Cellular Viability and Apoptosis**

A549 cells were infected with 10^8 cfu/mL of ATCC 19606, 1327M, and 113-16 strains for 24 h in the presence or absence of rifampin at concentrations of 0.5x, 1x, and 2x MIC, corresponding to each strain. Before the evaluation of A. baumannii cytotoxicity, we removed viable A. baumannii strains from A549 cell cultures, and we washed A549 cells 5 times with prewarmed PBS. Then, A. baumannii cytotoxicity was initially assessed quantitatively by monitoring the mitochondrial reduction activity using the MTT assay (Sigma), as described elsewhere [34]. Simultaneous determination of live and dead cells was achieved with the LIVE/DEAD kit, in accordance with the manufacturer’s instructions (Invitrogen).
To monitor apoptosis, cell nuclei were visualized using 4′,6-diamidino-2-phenylindole (DAPI). A549 cells, grown on glass coverslips in 24-well plates, were washed with cold PBS, fixed in methanol for 8 min at -20°C, incubated for 10 min at room temperature with DAPI (0.5 μg/mL), washed with PBS, mounted with SlowFade Gold antiFade reagent (Invitrogen), and visualized using a Leica fluorescence microscope (DM-6000).

**Bacterial Count in Human Cell Culture**

After A549 cells infection with 10⁸ cfu/mL of ATCC 19606, 1327_M, and 113-16 strains in the presence or absence of rifampin at concentrations of 0.5x, 1x, and 2x MIC, corresponding to each strain, A549 cells culture medium was removed and serially diluted. Enumeration of developed colonies was determined as described above.

**Superoxide Anion Assay**

Superoxide anion (O₂⁻) production was assayed by spectrophotometric measurement of ferricytochrome c reduction, as described elsewhere [35]. After infection of A549 cells with 10⁸ cfu/mL of ATCC 19606, 1327_M, and 113-16 strains in the presence or absence of rifampin at concentrations of 0.5x, 1x, and 2x MIC, corresponding to each strain, A549 cells were washed thrice with prewarmed PBS and incubated with 0.5 mL of reaction mixture consisting of 150 mM oxidized (Fe³⁺) cytochrome c in EDTA (100 μM) and sodium phosphate buffer (50 mM; pH 7.5) at 37°C for 1 h. Then, the supernatants were collected and used to quantify the amount of reduced cytochrome c by absorbance at 550 nm. O₂⁻ release was calculated using the ferricytochrome c extinction coefficient (21.1 mM.cm⁻¹).

**Cytokine Assay**

After A549 cells infection with 10⁸ cfu/mL of ATCC 19606, 1327_M, and 113-16 strains in the presence or absence of rifampin at concentrations of 0.5x, 1x, and 2x MIC, corresponding to each strain, A549 cells were washed thrice with prewarmed PBS and incubated with 0.5 mL of reaction mixture consisting of 150 mM oxidized (Fe³⁺) cytochrome c in EDTA (100 μM) and sodium phosphate buffer (50 mM; pH 7.5) at 37°C for 1 h. Then, the supernatants were collected and used to quantify the amount of reduced cytochrome c by absorbance at 550 nm. O₂⁻ release was calculated using the ferricytochrome c extinction coefficient (21.1 mM.cm⁻¹).

**Statistical Analysis**

Group data are presented as mean ± standard deviation (SD). The Student t test was used to determine differences between means. Difference was considered significant at P < .05. SPSS (version 15.0) statistical package was used (SPSS).

**RESULTS**

**In Vitro Susceptibility of A. baumannii to Rifampin**

According to the broth-microdilution method with standard inoculum, rifampin MIC against ATCC 19606, 1327_M, and 113-16 strains were 4, 128, and 1024 μg/mL, respectively (Table 1).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 19606</td>
<td>4</td>
</tr>
<tr>
<td>1327_M</td>
<td>128</td>
</tr>
<tr>
<td>113-16</td>
<td>1024</td>
</tr>
</tbody>
</table>

**Time-Kill Curves of A. baumannii at Standard Inoculum with Rifampin**

Time-kill curves are represented in Figure 1. For the ATCC 19606 strain, rifampin was bactericidal at 4x MIC at 24 h. For the 1327_M strain, rifampin was bactericidal at 1x, 2x, and 4x MIC at 8 h. At 24 h, we observed a regrowth of the 1327_M strain at 1x MIC. Meanwhile, for the 113-16 strain, rifampin was bactericidal at 1x, 2x, and 4x MIC at 24 h.

**Rifampin Effect on Cell Death Induced by A. baumannii**

The assessment of cell survival by the MTT reduction assay showed that A549 cells incubation with ATCC 19606, 1327_M, and 113-16 strains at 10⁸ cfu/mL reduced significantly the cell viability to 97.05% ± 15.06%, 75.82% ± 18.97%, and 75.68% ± 6.9%, respectively. Pretreatment with rifampin produced dose-dependent improvement in cell survival in the presence of ATCC 19606 and 1327_M strains. With rifampin at 0.5x, 1x, and 2x MIC, corresponding to each strain, increasing cell viability reached to 83.81% ± 6.63%, 87.12% ± 6.58%, and 97.27% ± 9.22%, for 1327_M strain (Figure 2A).

In the case of exposure to the 113-16 strain, pretreatment with rifampin improved cell survival only with rifampin at 0.5x MIC, increasing cell viability to 100.22% ± 4.11%. Rifampin at 1x and 2x MIC produced nonsignificant increase in cell survival: 83.45% ± 17.74% and 85.65% ± 19.11%, respectively. Consequent to these results, we studied whether rifampin at high concentration affects the cell viability of A549 cells. Rifampin concentrations of both 1x and 2x MIC (1024 and 2048 μg/mL), when incubated alone with A549 cells, induced decrease in cell viability to 80.81% ± 5.08% and 76.25% ± 24.79%, respectively; meanwhile, rifampin at 0.5x MIC (512 μg/mL) did not reduce the cell viability (98.97% ± 15.45%) (Figure 2B).

The rifampin effect on cell survival was also investigated using a 2-color fluorescence cell viability assay (Figure 2B). In contrast to intense and uniform green fluorescence based on intracellular esterase activity displayed by control A549 cells, cells exposed for 24 h to ATCC 19606, 1327_M, and 113-16 strains were stained mainly with ethidium homodimer-1, producing a bright red fluorescence indicative of dead cells (Figure 2C). Pretreatment
with rifampin at 0.5x, 1x, and 2x MIC in A549 cells infected with ATCC 19606 and 1327\textsubscript{M} strains reduced dose-dependently the number of dead cells (Figure 2C). In contrast, as we showed with the MTT assay, pretreatment with rifampin at 0.5x MIC in A549 cells infected with 113-16 strain reduced the number of dead cells to zero. These last reappeared with rifampin at 1x and 2x MIC (Figure 2C).

Taken together, these data indicate that rifampin reduces the cell death induced by \textit{A. baumannii}.

**Effect of Rifampin on \textit{A. baumannii}-Induced Cell Apoptosis**

The nature of cell death induced by \textit{A. baumannii} was examined by DAPI staining. Morphological examination of cellular nuclei stained with DAPI showed that A549 cells exposed to ATCC 19606, 1327\textsubscript{M}, and 113-16 strains presented typical apoptotic morphology, with condensation of chromatin and fragmentation of the nuclei (Figure 2D). Pretreatment with rifampin significantly reduced cell apoptosis in a dose-dependent manner in the presence of ATCC 19606 and 1327\textsubscript{M} strains, with concentrations of 0.5x, 1x, and 2x MIC, corresponding to each strain (Figure 2D). In the case of 113-16 strain, rifampin at 0.5x MIC nearly mediated complete abolition of apoptotic cell death, whereas with rifampin at 1x and 2x MIC some apoptotic cells were present (Figure 2D). Taken together, these results indicate that rifampin protects A549 cells from death by reducing cell apoptosis induced by \textit{A. baumannii}.

**Effect of Rifampin on \textit{A. baumannii} Clearance from A549 Culture Medium**

We next sought to determine whether the rifampin cytoprotective effect is associated with its bactericidal effect against \textit{A. baumannii}, as shown in time-kill curves. Bacterial counts of ATCC 19606 strain found in the A549 cells culture medium in the presence of rifampin at 0.5x, 1x, and 2x MIC showed a decrease of \textit{A. baumannii} concentrations after 24 h (Figure 3). Surprisingly, bacterial counts of 1327\textsubscript{M} and 113-16 strains found in the A549 cells culture medium in the presence of rifampin at 0.5x, 1x, and 2x MIC showed that rifampin had no significant effect on the bacterial concentration decrease after 24 h (Figure 3).

**Time-kill Curves of \textit{A. baumannii} at High Inoculum with Rifampin**

A possible reason that rifampin did not play its bactericidal role in A549 cells culture medium, as observed above with time-kill curves with standard inoculum, might depend on the inoculum-size effect, as demonstrated with other bacteria and other antimicrobials [36,37].

For ATCC 19606, 1327\textsubscript{M}, and 113-16 strains at high inoculum, rifampin was not bactericidal at 1x, 2x, and 4x MIC during 24 h (Figure 4). The reduction in cfu/mL for these strains was \( <3 \log_{10} \). Moreover, increased rifampin MIC value was observed for ATCC 19606, 1327\textsubscript{M}, and 113-16 strains from 4 to 1024, 128 to 2048, and 1024 to \( >4096 \) \( \mu g/mL \), respectively (Table 1).
Effect of Rifampin on Oxidative Stress Induced by A. baumannii

In another study, we observed that A. baumannii induces an oxidative stress that was involved in cell death (Y. Smani et al, unpublished data, 2009). Here, we found that exposure of A549 cells to ATCC 19606 and 1327M strains for 6 and 24 h induced a significant increase in the O$_2^-$ liberation, whereas 113-16 strain induced a significant increase in the O$_2^-$ liberation only after 24 h (Figure 5). A549 cells pretreatment with rifampin at 1x and 2x MIC reduced significantly the O$_2^-$ liberation induced by the ATCC 19606 strain after 6 and 24 h. A549 cells pretreatment with rifampin at 1x and 2x MIC reduced significantly the O$_2^-$ liberation induced by the 1327M strain after 24 h, whereas at rifampin concentration of 2x MIC, the O$_2^-$ liberation was significantly reduced after 6 and 24 h (Figure 5). For A549 cells infected with the 113-16 strain, pretreatment with rifampin at 1x and 2x MIC prevents the increase of O$_2^-$ liberation induced by this strain after 24 h (Figure 5). According to these results, rifampin was able to reduce the free radicals production induced by A. baumannii.

Effect of Rifampin on Proinflammatory Cytokines Release Induced by A. baumannii

We further tested the hypothesis that rifampin can reduce the release of proinflammatory cytokines TNF-α and IL-6 induced by A. baumannii. TNF-α assay showed that A549 cells pretreatment with rifampin at 0.5x, 1x, and 2x MIC reduced significantly the TNF-α liberation induced by ATCC 19606 strain after 6 h. A549 cells pretreatment with rifampin at 0.5x and 2x MIC reduced significantly the TNF-α liberation induced by 1327M strain after 6 h (Figure 6A). For A549 cells infected with 113-16 strain, pretreatment with rifampin at 0.5x and 1x MIC prevented the increase of TNF-α liberation induced by this strain after 6 h (Figure 6A).
significantly the IL-6 liberation induced by ATCC 19606 and 1327M strains after 6 and 24 h, and 24 h, respectively (Figure 6B). For A549 cells infected with 113-16 strain, pretreatment with rifampin at 0.5x and 1x MIC reduced significantly the IL-6 liberation after 6 and 24 h, whereas rifampin at 2x MIC reduced significantly only the IL-6 liberation at 24 h (Figure 6B). All together, these data indicate that rifampin reduces the proinflammatory cytokines release induced by A. baumannii.

**DISCUSSION**

In this study, we demonstrate that rifampin is able to attenuate the cytotoxicity effect induced by multidrug- and pandrug-resistant A. baumannii independently of its bactericidal effect. Indeed, the cytoprotective effect of rifampin was observed by the decrease of dead cells induced by A. baumannii and by the reduction of oxidative stress and proinflammatory cytokines release.

Moreover, we showed that the drug-susceptible strain induces more cell death, compared with the cell death induced by the multidrug- and pandrug-resistant strains. Recent results from our laboratory showed that the acquisition of drug resistance by A. baumannii decreases its ability to kill A549 cells. Briefly, we demonstrated that incubation of A549 cells with outer membrane proteins (OMP), isolated from ATCC 19606 or 113-16 strains, induce a dose-dependent decrease in the cell viability. This cell viability was lower with ATCC 19606 than 113-16 OMP at 50 mg/L of proteins. Relative expression of genes coding for
ompA, omp33-36 kDa, carO, and oprD, porins involved in the acquisition of carbapenems resistance and the interaction of pathogen-host cells, by 113-16 OMP was decreased, especially for CarO and OprD [38]. For A549 cells, pretreatment with rifampin and infection with A. baumannii were associated with a significant reduced rate of cell death. In other mammalian cells also, it was demonstrated that rifampin reduced cell death [14, 15, 17].

Figure 5. Rifampin effect on oxidative stress induced by Acinetobacter baumannii. Superoxide anion (O$_2^-$) concentration was determined in A549 cells pretreated or not with rifampin at 0.5x, 1x, and 2x minimum inhibitory concentration (MIC), corresponding to each strain, and infected with 10$^8$ colony-forming units (cfu/mL) of A. baumannii ATCC 19606, 1327m, and 113-16 for 6 and 24 h. Data are the means ± SD of 3 different experiments. $P < .05$: * between control and treated groups at 6 h, † between control and treated groups at 24 h, ‡ between treated groups at 6 h, $*: between treated groups at 24 h.

Figure 6. Rifampin effect on proinflammatory cytokines release induced by Acinetobacter baumannii. Tumor necrosis factor (TNF)-α (A) and interleukin-6 (IL-6) (B) concentrations were determined in A549 cells pretreated or not with rifampin at 0.5x, 1x, and 2x minimum inhibitory concentration (MIC), corresponding to each strain, and infected with 10$^8$ colony-forming units (cfu/mL) of A. baumannii ATCC 19606, 1327m, and 113-16 for 6 and 24 h. Data are the means ± SD of 3 different experiments. $P < .05$: * between control and treated groups at 6 h, †: between control and treated groups at 24 h, ‡: between treated groups at 6 h, $*: between treated groups at 24 h.
This cytoprotection effect was not observed when A549 cells were infected with the pandrug-resistant strain and pretreated with rifampin concentration at 1x and 2x MIC. This effect would be due to the high rifampin concentration used in this experiment that is toxic for A549 cells. Reduction of cell death induced by rifampin was not associated with the significant decrease of multidrug- and pandrug-resistant A. baumannii concentrations in A549 extracellular medium. Previous studies have shown that rifampin was not bactericidal in the presence of a high inoculum of methicillin-susceptible and methicillin-resistant Staphylococcus aureus [36, 39]. In our study, we found that the rifampin efficacy against A. baumannii was greatly influenced by the inoculum size. Although rifampin has been generally considered to be an antimicrobial that is bactericidal for A. baumannii [2, 40–42], contradictory results are often found in the literature [3, 43, 44]. In our stationary-phase studies, in which the presence of a high inoculum was needed, rifampin showed a moderate killing effect, but it was not strictly bactericidal. The bactericidal activity of rifampin against stationary-phase A. baumannii has been considered the basis for its main role in the treatment of A. baumannii infections [3, 41, 42, 45]. Our finding that rifampin was not bactericidal with a high inoculum of A. baumannii indicates that inoculum size would be responsible for the non-decrease of A. baumannii concentrations in A549 extracellular medium. Thus, discordance exists regarding the beneficial impact of rifampin on eukaryotic cell viability and the non-bactericidal effect of rifampin when A. baumannii was in high inoculum. Several hypotheses can explain this fact. One of them is that rifampin could inhibit the bacterial toxin synthesis, as demonstrated with E. coli, without decreasing the number of live E. coli (E. A. Rahal et al, oral personal communication, 2010). Moreover, rifampin may act not only on the eradication of A. baumannii but also on eukaryotic cells physiology. Several lines of evidence strongly support the role of rifampin, a free radical scavenger, in the decrease of free radicals concentrations in mammalian cells [16, 19]. We have recently observed that A. baumannii induced the release of free radicals in human lung epithelial cells that may induce, therefore, the death of these cells (Y. Smani et al, unpublished data, 2009). In the present study, we demonstrated that rifampin is able to reduce the release of free radicals induced by A. baumannii. It is noteworthy that free radicals were involved in the epithelial membrane alteration [46, 47]. Interestingly, our study showed that preincubation of A549 cells with rifampin reduced cellular membrane alteration induced by A. baumannii in the cytoxicity experiments.

Moreover, our cytokines studies revealed that TNF-α and IL-6 release induced by A. baumannii decreased in the presence of rifampin. These results are in line with the results obtained by other authors, showing that macrophages pretreatment with rifampin decreased the TNF-α production induced by M. tuberculosis [8]. The reduction of TNF-α and IL-6 release by rifampin was also shown in vitro after epithelial cells infection with E. coli and in rat models of A. baumannii and P. aeruginosa sepsis [45, 48, 49]. Moreover, in S. pneumoniae meningitis, it was demonstrated that rifampin in combination with ceftriaxone reduced ceftriazone-induced release of deleterious bacterial products and attenuated inflammation [30]. This role of rifampin in reducing proinflammatory cytokines release could be most interesting if we consider that the rifampin concentration reached on the site of lung infection is similar to the range of concentrations used in our study for the multidrug- and pandrug-resistant A. baumannii strains. Thus, rifampin intracellular concentrations reached in alveolar macrophages are ~252 μg/mL, which are 16.26 times the plasmatic levels with the standard oral dose of 600 mg [50].

In summary, the present study demonstrates that rifampin attenuates the cytotoxicity induced by multidrug- and pandrug-resistant A. baumannii clinical isolates, without being relevantly bactericidal, in human lung epithelial cells. Our data reveal that this cytoprotection would be due to the effect of rifampin on the reduction of oxidative stress and proinflammatory cytokines release.

Funding

Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III – cofinanced by European Development Regional Fund “A Way to Achieve Europe” (ERDF), Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008) (to Y.S.); Consejería de Salud de la Junta de Andalucía (grant 288/2008).

Acknowledgments

We thank the members of the Infectious Diseases Service for helpful discussions and for critical reading of the manuscript.

References


9. Mlambo G, Sigola LB. Rifampicin and dexamethasone have similar effects on macrophage phagocytosis of zymosan, but differ in their effects on nitrite and TNF-x production. Int Immunopharmacol 2003; 3:513–22.


