

In Vitro Activity of Torezolid (TR-700) versus Linezolid against *Chlamydia* species

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Abstract

Background
Torezolid (TR-700) and Linezolid (LZD) belong to the oxazolidinone class of antibiotics. TR-700 has shown increased potency against selected pathogens, including some LZD-resistant isolates. LZD is approved for the treatment of Gram-positive infections, but has not been shown to be very effective against Gram-negative bacteria.

Chlamydia are Gram-negative, obligate intracellular pathogens. They are medically important because they cause respiratory, ocular and urogenital infections, which can lead to adverse sequelae like blindness and female infertility. They have also been implicated in chronic diseases like coronary heart disease and adult onset asthma. *C. psittaci* (CPs) is endemic among birds, but can cause zoonotic disease in humans (respiratory psittacosis) which can become a life-threatening pneumonia. It is classified as a category B select agent.

Methods
Human epithelial cell lines HEp2 and HeLa 229 were infected with *Chlamydia pneumoniae* (CPn) and *Chlamydia trachomatis serovar D* (CTD) respectively, followed by addition of serial dilutions of TR-700 or LZD (0 - 64 mg/ml). The effect on *Chlamydia* development was observed after 40 - 48 hours by IF staining of inclusions. Cells from parallel experiments were collected and titred on HEp2 or HeLa cells to enumerate inclusion forming units (IFUs). Parallel experiments also included CTD infected cells that were treated with drugs for 40 hours, followed by removal of drugs and incubation for additional 26 hours before IF staining to determine inclusion recovery (size and numbers).

Results
TR-700 displayed better activity (MIC = 4 mg/ml for CTD and 1 mg/ml for CPn) than LZD (MIC = 32 mg/ml for CTD and 4 mg/ml for CPn). Neither drug demonstrated bactericidal activity since we were able to recover CTD inclusions after drug removal from both TR-700 (up to 16 mg/ml) and LZD (up to 32 mg/ml) treated wells.

Conclusions
TR-700 demonstrated 4-8 fold greater activity than LZD against CTD and CPn infections. We are presently also testing the activity of these compounds against CPs infections.

Introduction

Torezolid (TR-700) and Linezolid (LZD) belong to the oxazolidinone class of antibiotics, which inhibit protein synthesis by binding to the 50S subunit of the prokaryotic ribosome. In several studies, TR-700 has shown greater activity than LZD against selected pathogens, including some LZD-resistant isolates. LZD is FDA approved for treatment of Gram-positive bacteria, but has not been shown to be very effective against Gram-negatives.

Chlamydia are Gram-negative obligate intracellular bacteria. They are medically important because they cause respiratory, ocular and urogenital infections leading to adverse sequelae like blindness and female infertility. *C. pneumoniae* is implicated in chronic diseases including coronary heart disease and adult onset asthma. *C. psittaci* is a category B select agent, which can cause respiratory psittacosis in humans that can become a life-threatening pneumonia. Development of effective new antibiotics is essential to maintain an effective repertoire of chemotherapeutic agents against *Chlamydia*.

Methods

Organisms: Three *Chlamydia* species, *Chlamydia trachomatis serovar D* (CTD), *Chlamydia psittaci* 6BC (CPs) and *Chlamydia pneumoniae* AR39 (CPn) were used in these studies. We maintain and routinely use these strains in our laboratory.

Antibiotic agents: TR-700 (provided by Trius Therapeutics, San Diego, CA) was tested in comparison to Linezolid (LZD). Freshly prepared stock solutions were made in DMSO, followed by serial dilutions in cell culture media. Doxycycline (Sigma) was used for MIC comparison in some experiments.

Antimicrobial susceptibility testing. 48-well microtiter plates containing confluent human epithelial cell lines HEp2 and HeLa, and mouse fibroblast cell line L Cells were infected with CPs, CTD and CPn respectively. This was immediately followed by the addition of serial dilutions of TR-700 or LZD (0 - 64 µg/ml). Triplicate wells were set up for each dilution of antibiotic compound. Cultures were incubated for 40 - 48 hours, and the effect on *Chlamydia* development (number and size of inclusions) was microscopically observed by immunofluorescence (IF) staining, and images were saved. Cells from parallel experiments were collected in sucrose phosphate glutamate (SPG) buffer and stored at -80°C. These cultures were later titred to enumerate 'inclusion forming units' (IFU) of viable *Chlamydia* present after drug treatment. Parallel experiments also included drug treatment for 40 hours, followed by removal of drugs and incubation for additional 24-30 hours to determine if antibiotic treatment was cidal or static (reactivation). IF staining and titration also were carried out on the 'reactivation experiments' samples as described.

Results

- MIF studies revealed that TR-700 displayed better activity (MIC = 4 µg/ml for CPs and 2 µg/ml for CTD) than LZD (MIC = 32 µg/ml for both CPs and CTD). Activity against CPn was the same for both TR-700 and LZD (MIC = 1 µg/ml). Inhibitory drug concentrations prevented inclusion development (Figure 1 a-f).
- Presence of viable *Chlamydia* was estimated by titration of cells collected from drug treated cultures. For both TR-700 and LZD-treated cultures, the titer (IFU/ml) decreased in proportion to drug concentration (Table 1/ Figure 2 a-c). TR-700 had greater effect on all species of *Chlamydia* tested compared with LZD, since the titers of cells collected from cultures treated with TR-700 were orders of magnitude less than those from cells collected after LZD treatment at similar drug concentrations.
- The titration data are consistent with the MIF images of inclusion development.
- Removal of TR-700 and LZD after treatment resulted in recovery of viable *Chlamydia* from cultures (Table 1/ Figures 1 and 2), thus both TR-700 and LZD result in stasis rather than bactericidal activity.
- Control experiments using 0.5 and 5.0 µg/ml Doxycycline resulted in bactericidal activity in CPn infected cultures, indicating that our methods for measuring bactericidal activity were effective.

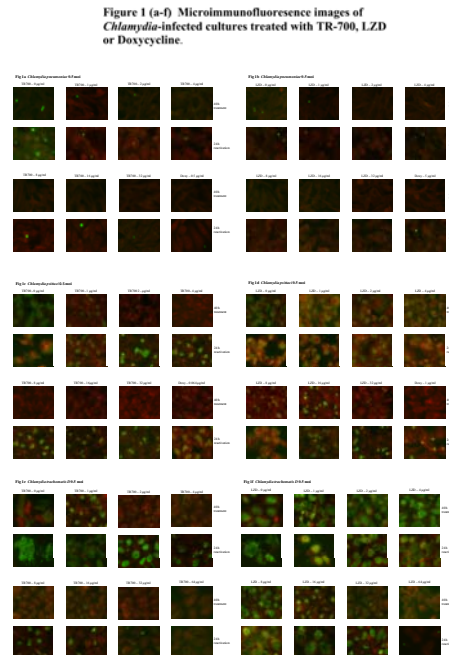
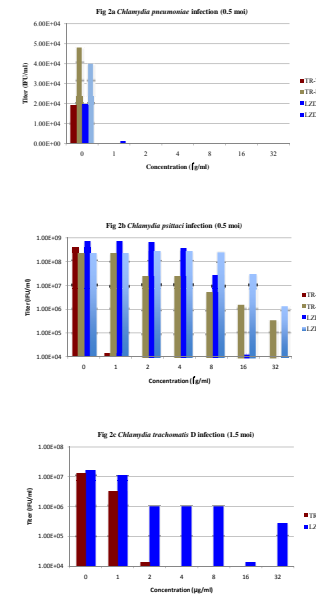


Figure 2 (a-c) Effect of TR-700 and LZD on *in vitro* chlamydial infections



TR-T = TR-700 40h treatment; TR-R = TR-700 Reactivation
LZD-T = Linezolid 40h treatment; LZD-R = Linezolid Reactivation

Table 1. Comparison of Titration data from TR-700, LZD and Doxycycline treated *Chlamydia*-infected cultures

| Drug compound | Concentration µg/ml | CPn titer IFU/ml (0.5 ml infection) | | CPs titer IFU/ml (0.5 ml infection) | | CTD titer IFU/ml (1.5 ml infection) | |
|---------------|---------------------|-------------------------------------|-----------------------|-------------------------------------|-----------------------|-------------------------------------|------------------|
| | | 40h Treatment | Reactivation 40h | 40h Treatment | Reactivation 40h | 40h Treatment | Reactivation 40h |
| TR-700 | 0 | 1.2 x 10 ⁸ | 4.0 x 10 ⁷ | 1.0 x 10 ⁸ | 2.1 x 10 ⁷ | 1.2 x 10 ⁸ | - |
| | 1 | 0 | 0 | 1.4 x 10 ⁷ | 2.3 x 10 ⁶ | 1.1 x 10 ⁷ | - |
| | 2 | 0 | 0 | 0 | 2.3 x 10 ⁵ | 1.1 x 10 ⁶ | - |
| | 4 | 0 | 0 | 0 | 2.2 x 10 ⁴ | 0 | - |
| | 8 | 0 | 0 | 0 | 5.9 x 10 ³ | 0 | - |
| | 16 | 0 | 0 | 0 | 1.6 x 10 ³ | 0 | - |
| LZD | 0 | 2.0 x 10 ⁸ | 3.9 x 10 ⁷ | 7.4 x 10 ⁷ | 2.1 x 10 ⁷ | 1.6 x 10 ⁷ | - |
| | 1 | 0 | 1.2 x 10 ⁷ | 0.1 x 10 ⁷ | 2.3 x 10 ⁶ | 1.1 x 10 ⁶ | - |
| | 2 | 0 | 0 | 4.9 x 10 ⁶ | 0.6 x 10 ⁶ | 1.5 x 10 ⁶ | - |
| | 4 | 0 | 0 | 1.3 x 10 ⁶ | 2.8 x 10 ⁵ | 1.0 x 10 ⁶ | - |
| | 8 | 0 | 0 | 1.0 x 10 ⁵ | 7.9 x 10 ⁴ | 1.0 x 10 ⁵ | - |
| | 16 | 0 | 0 | 1.4 x 10 ⁴ | 1.0 x 10 ⁴ | 2.6 x 10 ⁴ | - |
| Doxycycline | 0.004 | - | 0 | 1.1 x 10 ⁷ | - | - | - |
| | 0.5 | - | 0 | 4.0 x 10 ⁶ | - | - | - |
| | 5 | - | 0 | - | - | - | - |
| | 0 | - | - | - | - | - | - |
| | 0 | - | - | - | - | - | - |
| | 0 | - | - | - | - | - | - |

Conclusions

- TR-700 demonstrated 8 to 16 fold greater activity than LZD against CPs and CTD.
- Results from reactivation studies revealed that TR-700 and LZD did not cause bactericidal activity against CPs and CTD infections at the concentrations and for the length of time tested in these studies. Removal of drug compounds after treatment resulted in resumed growth of bacteria
- CPn infection was susceptible to both TR-700 and LZD treatment, but we could not reactivate CPn from TR-700 treated cultures, suggesting that this species may be more amenable to oxazolidinone antibiotics