Aminoglycoside Effects on *Chlamydia Trachomatis* Bacteria

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Introduction

Aminoglycoside antibiotics inhibit protein synthesis and are commonly believed to be ineffective against intracellular pathogens (1). Previous studies reveal differing results on whether aminoglycosides can affect the growth of *Chlamydia trachomatis* (Ct), an intracellular bacterium (2-5). My project focused on testing several aminoglycosides on *Chlamydia* to see if they affected chlamydial inclusion formation and recovery of infectious progeny.

Chlamydia has a biphasic developmental life cycle consisting of the infectious extracellular elementary bodies (EBs) and metabolically active intracellular reticulate bodies (RBs). *Chlamydia* enters the host cell by the naked EBs attaching to the cell membrane by bacterial ligands and host receptors. It then injects pre-packaged effectors inside the host cytosol which enables invasion. During internalization, the EBs form a vacuole called an inclusion, which is what we quantify to determine the effect of aminoglycosides on these chlamydia infected cells (4-5).

Aminoglycoside antibiotics inhibit protein synthesis by binding to the A-site of the bacterial ribosome, misreading mRNA and causing a disruption in protein synthesis. Aminoglycosides disperse through porin channels in the outer membrane of susceptible organisms. Once inside the bacterial cell, streptomycin binds to 30S ribosomes, but other aminoglycosides bind to additional sites on 50S subunit (1).

The four aminoglycosides used throughout my experiments were kanamycin, geneticin (G418), streptomycin and hygromycin B. The first part of the project consisted of testing different concentrations of aminoglycosides on *Chlamydia* infected cells. *Chlamydia* can be grouped into serovars that infect three distinct sites: the eyes, genital tract, or macrophages within lymph nodes of the rectum. I worked with the serovar termed CT/L2 which infects macrophages. **We hypothesized that aminoglycoside antibiotics would affect chlamydial inclusion formation.** Our results showed that the four aminoglycosides tested had no effect on primary inclusion formation. However, when we measured the recovery of infectious units (IFU), we found that aminoglycoside treatment reduced recovery of infectious units (IFU)**.**

This led us to conclude that aminoglycosides penetrate the host cell, but not the chlamydial inclusion during primary infection. **However, when infected cells are disrupted to measure Chlamydia replication by IFU, aminoglycosides gain access to chlamydial EBs, decreasing their infectivity.** Our studies confirm early reports in the *Chlamydia* literature indicating bacterial sensitivity to some aminoglycosides, such as kanamycin and geneticin (G418) (2-3).

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Chlamydial Life Cycle

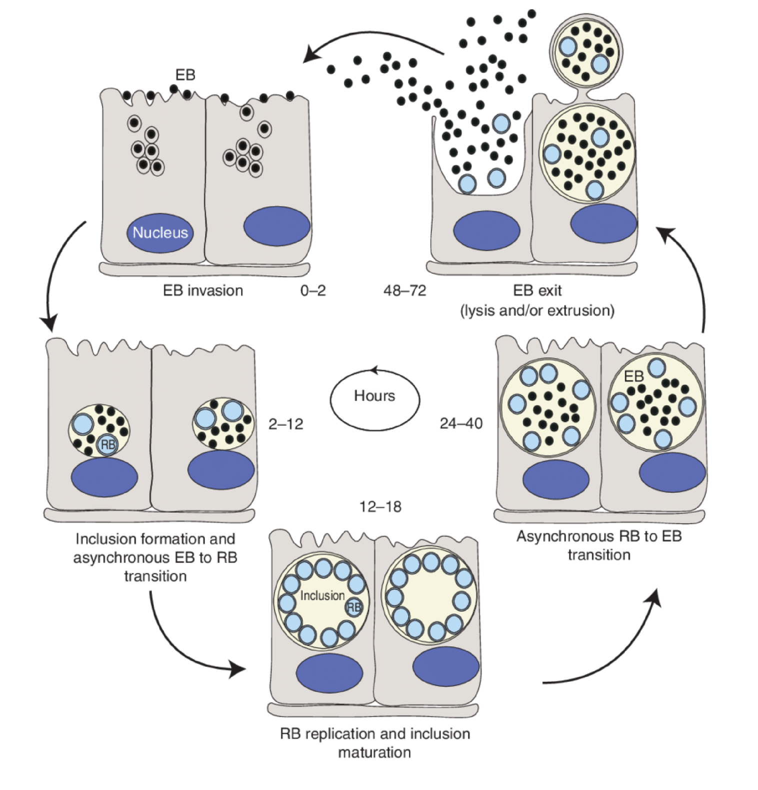
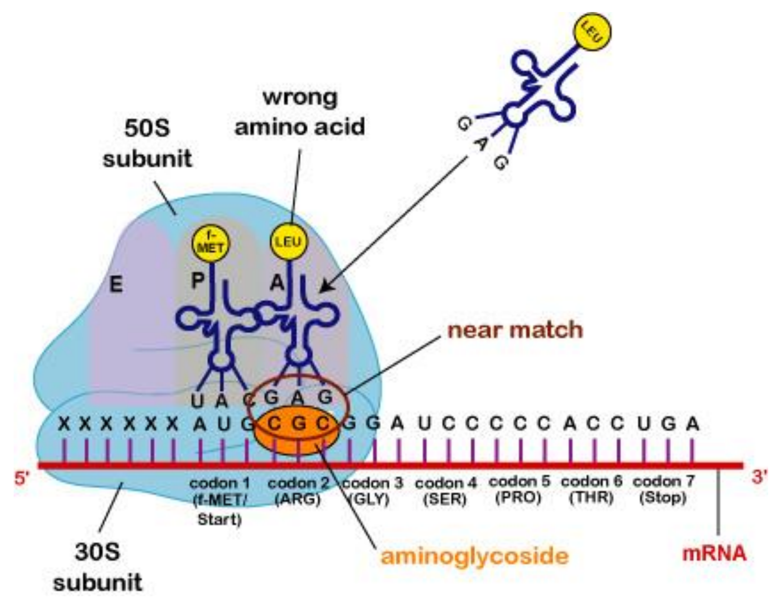


Figure 1. Biphasic Life cycle of *Chlamydia*. There are five steps to the life cycle: the infectious extracellular elementary bodies (EBs) invade the host cell and form a vacuole called an inclusion. EB's transition to metabolically active reticulate bodies (RB). RBs replicate and mature before transitioning back into EBs, at which point the EBs can exit the host through by lysis or extrusion.

Mechanism of Aminoglycosides

Figure 2. Aminoglycosides bind to the 30S ribosomal subunit and interfere with initiation of protein synthesis by fixing the 30S-50S ribosomal complex at the start codon of mRNA, preventing translation



Aminoglycoside Activity Against *C. trachomatis*

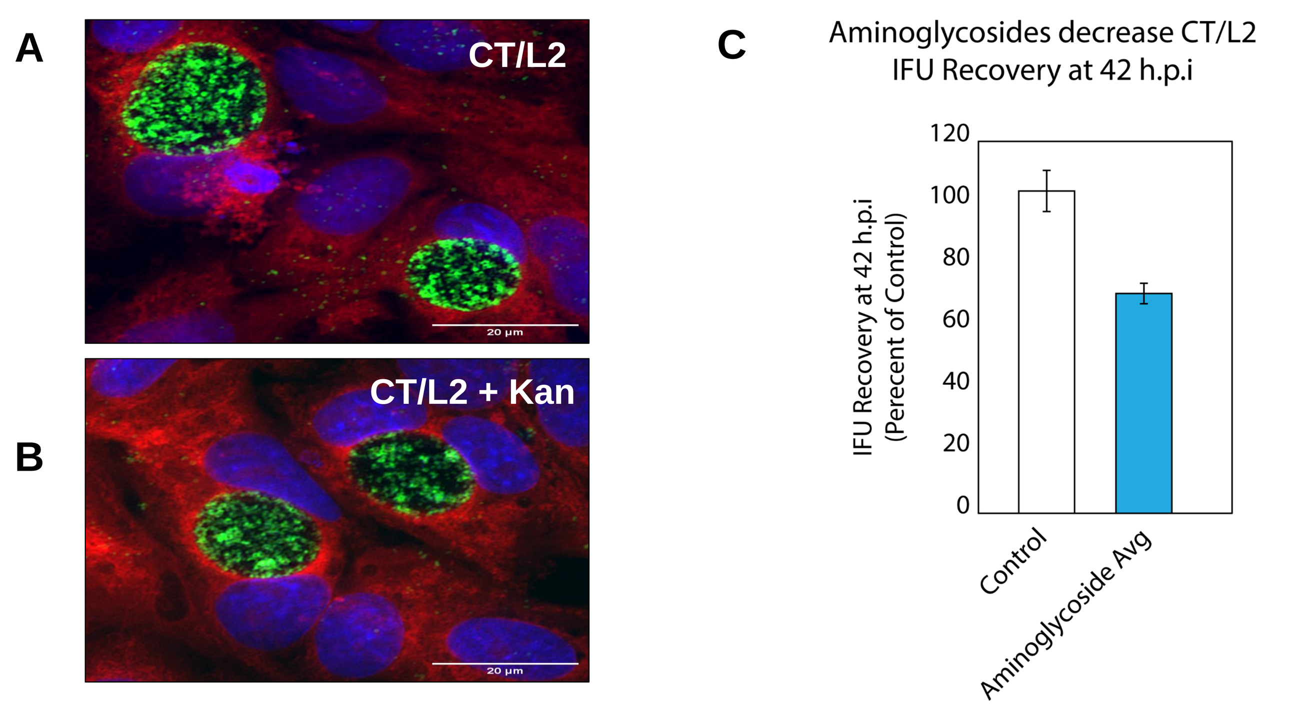
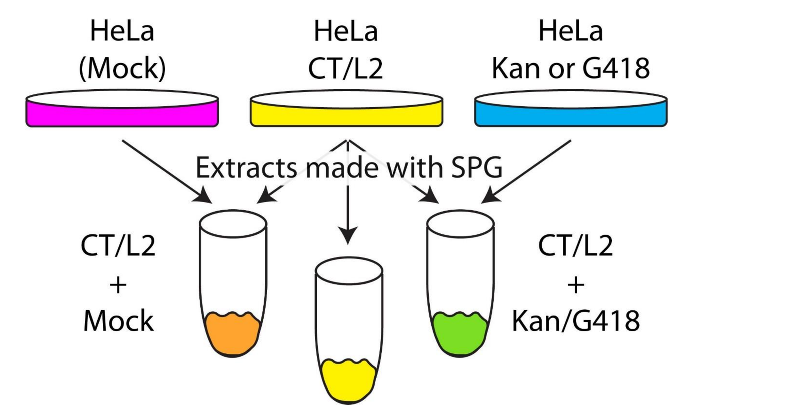


Figure 3. Representative images of **(A)** HeLa cells infected with CT/L2 or **(B)** Treated with Kanamycin at 100 µg/mL post-infection. Results similar to Kanamycin were obtained with Hygromycin B and G418. **(C)** IFU recovery from CT/L2 infections in the presence of Kanamycin/G418/Hygromycin shows ~35% decrease in chlamydial IFU recovery compared to control cells, confirming data in reference 2. Streptomycin (not shown) did not affect IFU recovery confirming data in references 2 & 3. No decrease in cell number was observed when HeLa cells were exposed to the four aminoglycosides at 100 µg/mL for 48 hours.

Activity of Aminoglycosides Against *C. trachomatis* EBs



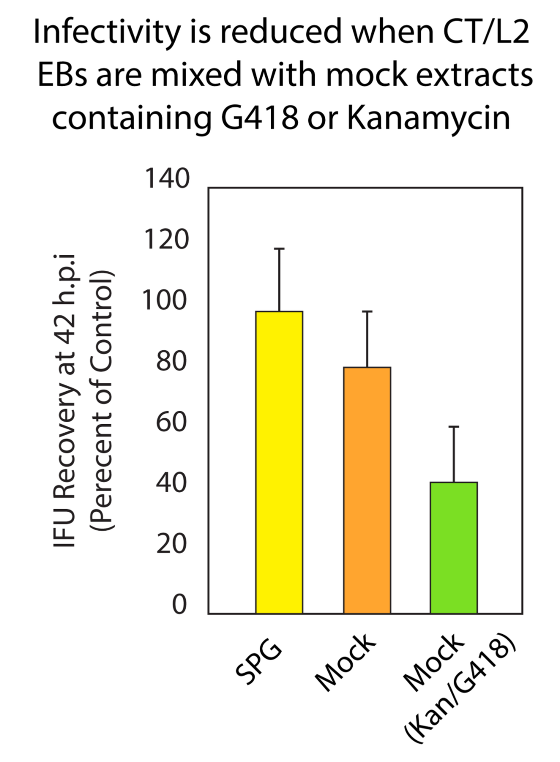
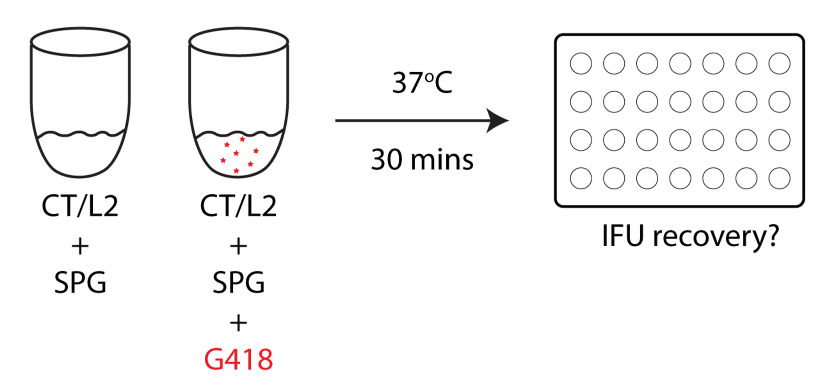


Figure 4. HeLa cells were infected with CT/L2 (yellow), mock-infected (pink) or exposed to aminoglycosides at 100 µg/mL (blue). SPG extracts made separately at 42 h.p.i and then mixed as shown, and then evaluated for IFU recovery. Kan/G418 extracts reduced IFU recovery by ~60%.



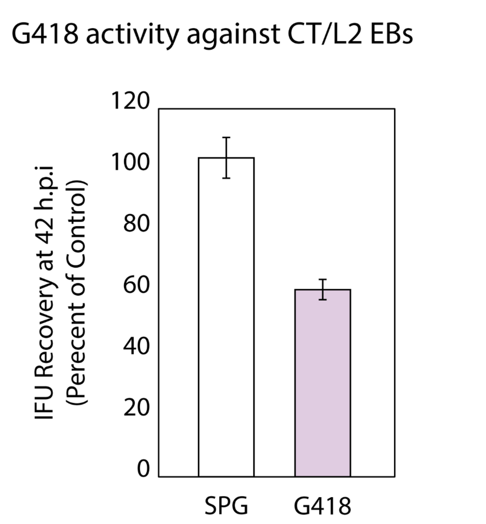


Figure 5. HeLa cell extracts containing CT/L2 EBs were incubated for 30 minutes with SPG or SPG containing G418 (100 µg/mL) . IFU recovery indicates that G418 caused ~40% reduction in CT/L2 infectivity.

Conclusions

- Results from my experiments reveal three points regarding *C. trachomatis* and aminoglycosides:

1. Aminoglycosides reduce *C. trachomatis* IFU recovery, supporting early studies indicating the trachoma agent is sensitive to several aminoglycosides (2,3).
2. G418 decreases chlamydial EB infectivity by 30-40%, suggesting that at least a portion of EBs contain actively translating ribosomes.
3. Many molecular studies with *Chlamydia* use engineered cell-lines selected with G418 or Hygromycin, which have intracellular half-lives of 4-7 days (1). This should be taken into consideration while evaluating *Chlamydia* replication in such engineered cell-lines.

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