The Influence of Factors which Inhibit or Stimulate Intracellular Protein Aggregation on Antibiotic Tolerance in Bacteria

Daria Leszczyńska

Persisters are antibiotic-tolerant cells which usually constitute a small part of bacterial populations. Metabolism of persisters is limited, the cells don't divide or divide very slowly. In contrast to antibiotic - resistant cells, persisters are not mutants, but variants of wild type cells which become susceptible to antibiotics upon regrowth. The formation of persister cells can be induced by various stress factors, e.g. starvation, oxidative stress or thermal shock and activation of toxin-antitoxin modules. Particularly high levels of persisters can be found in biofilms and during the late stationary phase of growth. Apart from high levels of persisters, protein aggregation is another hallmark of aging bacterial cultures. I planned to check whether there exists a link between the level of antibiotic - tolerant cells and protein aggregation in stationary *E. coli* cultures. I found that the inhibition of protein aggregation by osmotically active compounds (trehalose, betaine, glucose, glycerol) or 40 mM MOPS pH 7,4 reduced the level of persisters tolerant to ampicillin, kanamycin and ofloxacin. On the other hand, protein aggregation induced by sodium acetate and higher concentrations of osmolytes was correlated with increased persister levels. I found that the number of antibiotic tolerant cells was independent of the extent of protein oxidation, the concentration of ATP and the level of VBNC (*viable but not culturable*) or dead cells. It has appeared that MOPS and osmotically active compounds were able to sensitize persister cells to kanamycin and ampicillin. The strongest effect was observed in the presence of trehalose - almost 80 % of persisters became susceptible to kanamycin. I confirmed that trehalose influenced persister formation by investigating the effect of the Δ*otsA* mutation. The lack of trehalose in Δ*otsA* cells caused a significant increase in persister level in the early stationary phase. Moreover, the induction of persister cells after heat and cold shock was more effective in the Δ*otsA* culture than in the wild type strain.

One of the main problem concerning persisters is the lack of effective method to isolate high number of persisters without antibiotic treatment. I found that using Percoll density - gradient centrifugation a subpopulation enriched in persisters can be obtained from *E. coli* cultures submitted to heat shock.

Further experiments were performed with clinical isolates of uropathogenic *E. coli* and *Pseudomonas aeruginosa* PAO1-L WT and *4* strains. The Δ*4* strain is devoid of four operons

coding for toxin - antitoxin systems, and therefore produces low persisters levels. I demonstrated that trehalose inhibited the generation of persisters tolerant to ampicillin in one of two examined uropathogenic *E. coli* strains. I found also that trehalose and other osmolytes diminished the frequency of persisters in *P. aeruginosa* WT strain, but did not cause any further decrease in persisters level in *P. aeruginosa* Δ*4* culture. This result may suggest that osmotically active compounds inhibit directly or indirectly the activation of toxin - antitoxin modules and thereby reduce persisters level.