Summary

In bacteria, topoisomerase IV (topo IV) catalyses the decatenation of DNA-daughter chromosomes after replication and drives replication and recombination by relaxation of positive DNA-supercoils. Topo IV and the structurally similar topoisomerase II (gyrase) are both lethal targets of fluoroquinolone antibiotics. By using genetically defined mutants of *E. coli* carrying mutations rendering either gyrase or topo IV to the primary target of quinolones, this study aimed at determining the cellular effects, e.g. induction of the SOS response and loss of viability, triggered by the selective inhibition of either type II topoisomerase with various inhibitors including fluoroquinolones. In addition, the study focused on the elucidation of the mechanisms involved in the transcriptional regulation of the *parC* and *parE* genes encoding the two subunits of topo IV, on possible implications for the target concentration, and thus, the cellular susceptibility to inhibitors of topo IV.

In exponentially growing cultures, fluoroquinolones caused loss of viability and induction of the SOS response, characterized by filamentation of the cells and induction of *recA* transcription, irrespective of gyrase or topo IV being the primary target. After inhibition of topo IV with norfloxacin both the induction of the SOS response and the loss of viability were less pronounced than after inhibition with ciprofloxacin, or inhibition of gyrase with both norfloxacin and ciprofloxacin. Loss of viability was also modulated by the rate of cell division as well as growth phase and composition of the growth medium. Resting cultures were not killed by fluoroquinolones, but those with higher affinity to gyrase and topo IV (e.g., moxifloxacin) were more effective in inhibiting the re-growth of such cultures especially if gyrase was the primary target.

Investigation of other inhibitors showed that etoposide, an inhibitor of eucaryotic topoisomerase II, primarily targeted gyrase while rutin primarily targeted topo IV. Both inhibitors induced the SOS response, indicated by filamentation of growing cells, but only etoposide also caused cell death. Furthermore, an in vitro-generated quinolone-resistant *E. coli* strain ($GyrA^{S83W}$) was demonstrated to be hypersensitive to etoposide while the quinolone-sensitive wild-type strain was resistant, thereby confirming former *in vitro* data.

Thus, targeting of topo IV with quinolones or other inhibitors not only differed from that of gyrase with respect to drug interaction, but also to the extent of cellular effects caused by inhibition. The Investigations show, that both topo IV and gyrase are promising antibacterial targets for approved and new drug classes even in the face of the growing resistance against fluoroquinolones.

The transcriptional regulation of *parC* and *parE* expression was examined under different physiological conditions with real-time RT-PCR and a newly established fluorescence-based method to map transcription start sites. The latter method revealed for the first time experimently different start sites dependent upon exponential or stationary growth of a batch culture. DNA sequence alignments of the regions upstream from the transcription start sites of both *parC* and *parE* revealed putative σ^{70}-dependent promoters and putative binding sites for H-NS pointing to a negative transcriptional regulation by a decrease in DNA supercoiling. Such

dependence was confirmed for *parC* and to a lesser extent for *parE*. Thus, a temporary relaxation of DNA by cold shock or by inhibition of gyrase with novobiocin caused a significant reduction of *parC* expression. This proof of DNA supercoiling-dependent transcription of *parC* underlines the role of topo IV in maintaining a global degree of negative DNA-supercoiling.

By targeting either gyrase or topo IV, fluoroquinolones did not influence the transcription of *gyrA* or *parE* but in contrast reduced *parC* expression after inhibition of topo IV only.