# Anti-*Wolbachia* drugs have no effect on the viability of extracellular *Wolbachia*.

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*Wolbachia* is an obligate symbiont of medically important filarial nematodes and is required for normal worm development, growth, embryogenesis, transmission of microfilariae and adult worm longevity. *Wolbachia* depletion with doxycycline leads to immediate and permanent sterility of adult female worms and a highly reduced adult lifespan. Other compounds in the tetracycline and rifamycin classes of antibiotics have also been shown to be active against *Wolbachia*. Interestingly, within the fluoroquinolone class some antibiotics are active whereas others have no effect on *Wolbachia* infection. More recently, candidates from the AWOL consortium were shown to have a much more rapid kill profile. The mechanism of action by which these drugs clear *Wolbachia*, and the reasons behind the differences in speed and efficacy are not yet understood. We show that anti-*Wolbachia* drugs from each class consistently increase autophagic flux in two insect cell lines and *Brugia malayi*, but not in mammalian cells. Drugs ineffective against *Wolbachia*, and sub-optimal concentrations of the anti-*Wolbachia* antibiotics, did not induce autophagy. Inhibition of autophagy, at early and late stage, reduced the effect of the drugs in C6/36 cells and *B. malayi*. Interestingly, the activation of autophagy by anti-*Wolbachia* drugs was also seen in uninfected cells, indicating its effect is independent of *Wolbachia* infection. To further investigate the role of the host cell, *Wolbachia* were purified from C6/36 cells and treated with anti-*Wolbachia* drugs. No change in bacteria viability was seen after a 7-day exposure to anti-wolbachial drugs, measured by BacLight membrane staining. This assay is based on membrane integrity, and since it is possible for the bacteria to be dead with an intact membrane, the ability of the drug exposed *Wolbachia* to re-infect was assessed. All drug exposed *Wolbachia* were able to re-infect C6/36 cells. Ultrastructural morphology of purified *Wolbachia*, naturally release *Wolbachia* and drug exposed bacteria were indistinguishable. Since *Wolbachia* are obligately intracellular, with a degenerate genome, and unable to replicate extracellularly, it could be that they are metabolically quiescent and therefore unaffected by anti-*Wolbachia* drugs, many of which are known to target protein synthesis. In order to test this, we plan on performing RNAseq on *Wolbachia* at different time points since exiting the host cell. Together, this data shows that induction of autophagic flux is required for anti-*Wolbachia* activity, that this induction is independent of *Wolbachia* infection, and that anti-*Wolbachia* drugs have no direct effect on extracellular *Wolbachia*.