

Caenorhabditis elegans as a simple in vivo model for combinatorial therapy with thioridazine and dicloxacillin against infections with methicillin-resistant Staphylococcus aureus

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Abstract

The shortage of drugs active against methicillin-resistant Staphylococcus aureus (MRSA) is a growing clinical problem. In vitro studies indicate that the phenothiazine thioridazine (TZ) might enhance the activity of the β -lactam antibiotic dicloxacillin (DCX) to a level where MRSA is killed, but positive in vivo studies have yet to be performed. We have introduced Caenorhabditis elegans infected by MRSA as an in vivo model to test the effect of TZ as a helper drug in combination with DCX. Because TZ is an anthelmintic, initial experiments were carried out to define the thresholds of toxicity, determined by larval development, and induction of stress-response markers. No measurable stress effects were observed at the concentrations below 64 mg/L TZ. Of seven tested MRSA strains the most pathogenic strain (ATCC 33591) was chosen for treatment analysis. Full-grown C. elegans were exposed to the test strain for three days and subsequently treated with 8 mg/L DCX and 8 mg/L TZ for two days. This resulted in a 14-fold reduction in the intestinal MRSA load as compared with untreated controls. Each drug alone resulted in a two to threefold reduction in MRSA load. In conclusion, we have proved C. elegans as a simple model for testing synergy between TZ and DCX against MRSA. Moreover, we have shown that TZ enhances the activity of DCX in a simple in vivo host model as C. elegans leading to a decrease of bacterial load of MRSA in the nematode gut and intestine.

Treatment with methicillin-resistant Staphylococcus aureus (MRSA) is increasingly becoming a global problem. Many species are drug-resistant and there is a lack of effective drugs due to the slow progress in developing new antibiotics (Fischbach & Walsh, 2009). Therefore, there is an urgent need for new MRSA management strategies.

MRSA is resistant to dicloxacillin (DCX) and another penicillins are resistant to linase, which is the drug of choice for the treatment of methicillin-susceptible S. aureus (MSSA). We have previously shown that neuroleptic drug thioridazine (TZ) works in conjunction with DCX to the point that it reverses the tendency of MRSA to DCX and increases the tendency of MSSA to DCX in vitro (Klitgaard et al., 2008; Poulsen et al., 2013). In addition, we analyzed molecular mechanisms that work between the interactions between TZ and DCX (Bonde et al., 2011; Klitgaard et al., 2008; Thorsing et al., 2013). Most importantly, TZ has been shown to affect the cell wall pathway of biosynthesis by disrupting the construction of the pentaglycine bridge of peptidoglycan precursors, leading to improved sensitivity to DCX (Thorsing et al., 2013). From a clinical point of view, the combination of TZ and DCX looks promising. Before this combination can be tested in a clinical setting, however, animal testing is required.

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