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Title of the Thesis: Therapeutic intervention of *Shigella flexneri* host pathogen interaction

ABSTRACT

Antibiotics are the only options for treatment of *Shigella flexneri* infection; while incidences of antibiotic resistance in the causal organism are remarkably rising worldwide. In this context, newer approaches are needed to reduce *S. flexneri* burden. This study largely focuses on the role of a herbal compound capsaicin (Caps) in inhibiting intracellular *S flexneri* growth and evaluating the role of autophagy behind bacterial clearance. In addition, this work aims to characterize new drug targets to intervene *S. flexneri* host pathogen interaction.

In this study, Caps induced *S. flexneri* clearance was assessed by intracellular invasion assay in intestinal cells and macrophages. Autophagy was monitored using electron and confocal microscopy. Autophagic gene regulation by Caps in *S. flexneri* infected and uninfected cells were analysed by PCR array. The impact of autophagy on bacterial growth was evaluated in overexpression and knockdown studies by lipofectamine mediated transfection. Immunoblotting and quantitative PCR were used to assess the underlying mechanism behind Capsaicin induced autophagy. Promoter binding ability of TFEB was checked by TFEB-TF and chromatin immunoprecipitation assays. Interaction between bacterial effector protein IpaH9.8 and host protein ZKSCAN3 was observed using co-immunoprecipitation. *in vivo* studies were performed to validate *in vitro* results.

Caps reduced intracellular bacterial burden both in pre- and post-treated condition. From broth dilution assay, it has been observed that it is not directly killing the invaded bacteria. Rather, capsaicin inhibited *S. flexneri* growth by augmenting autophagy. It induced autophagy by targeting a transcription factor TFEB, which triggers autophagosomal lysosomal genes. Eventually, augmentation of autophagy by Caps resulted in killing of intracellular *S. flexneri*.

Our findings revealed that TFEB knockdown retained intracellular survival of *S. flexneri* hence Caps induced TFEB nuclear translocation is one of the key factors responsible for bacterial clearance. Mechanistic details showed that capsaicin induced TFEB activation is a cumulative effect of a series of phosphorylation and dephosphorylation events. AMPK phosphorylation and subsequently Akt and mTOR dephosphorylation contributed to TFEB activation. Moreover, the efficacy of Caps in reducing *S. flexneri* growth via autophagy is confirmed in animal model. Autophagic targets like TFEB has been well characterised and another transcription factor ZKSCAN3 was also established as a target involved in pathogenesis during infection. ZKSCAN3, a repressor of autophagy is induced due to *S flexneri* infection in intestinal cells.Further, we observed that bacterial virulence factor IpaH9.8 interacts with host protein ZKSCAN3.Hence, mechanistic details behind autophagic modulation are unearthed during intracellular *S flexneri* infection. Moreover,the synergistic effect of capsaicin along with antibiotics treatment against multidrug resistant *S. flexneri* clinical isolates was monitored in *in vitro* as well as *in vivo* conditions. This study showed for the first time that *S. flexneri* infection can be intervened by inducing autophagy. Our observations suggest that capsaicin activates TFEB to induce autophagy and thereby combat *S. flexneri* infection. Capsaicin and antibiotics combination may be synergistically used as a potent therapeutic alternative against antibiotic resistant *Shigella flexneri* infected condition in near future.

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