New preclinical data on adjunctive antibacterial therapies

Small-molecule compound of thiohydrazides of oxamic acids inhibit T3SS of *Salmonella* in vitro and in vivo

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**Objects.** Therapeutic strategies that target bacterial virulence rather than growth have received considerable attention. The conservation of structural components of type III secretion system (T3SS) and their importance for virulence in many bacterial pathogens make them attractive targets for inhibition with small molecules. Five hundred compounds of thiohydrazides of amides of oxamic acids were tested for toxicity to eukaryotic cells and T3SS inhibitory activity. The selected T3SS inhibitors were chemically optimized which allowed to obtain the original lead compound named CL-55, characterized by low toxicity, high levels of solubility, stability, and specific efficiency.

**Methods.** Organic synthesis, microbiological and cell culture tests, SDS-PAGE, pharmacokinetic studies, toxicity studies, infection models on BALB/c, I/St and A/Sn mice.

**Results.** In this study we describe the antimicrobial action of CL-55 on T3SS *S. enterica* serovar Typhimurium. We have found that CL-55 inhibits the secretion of the early T3SS effectors encoded by SPI-1 but does not affect salmonella growth in vitro. CL-55 inhibits replication of salmonella into mice macrophages *ex vivo* that suggests that CL-55 inhibits not only SPI-1 but also SPI-2. In the model of an acute infection intraperitoneal injection of CL-55 in a dose of 10 mg/kg for four days significantly (500-fold) decreased the numbers of salmonella in spleens and peritoneal lavages and gave twofold increase in survival rates in salmonella susceptible (BALB/c, I/St) and more resistant (A/Sn) mice. 12 days long therapy gave complete eradication of salmonella in mice. We have also confirmed the absence of reactivation of infection during the two months post infection. Toxicity studies revealed that CL-55 was not cancerogenic or mutagenic, did not increase the level of chromosomal abberations in bone marrow cells and displayed low toxicity in mice, rats and rabbits. Pharmacokinetic studies have shown that compound CL-55 very rapidly leaves the systemic blood circulation and well distributed in organs.

**Conclusion.** Our data demonstrates that the selected compound CL-55 - derivative of thiohydrazides of amides of oxamic acids affects bacterial T3SS activity in *S. typhimurium* in vitro and in vivo and hence could be used as a substance in the design of bacterial T3SS specific inhibitors for pharmaceutical intervention of bacterial virulence. The activity of CL-55 on SPI-1 and SPI-2 effectors of salmonella suggests that this compound possesses protective potential for both acute and chronic infection. Preclinical studies on pharmacokinetics showed higher relative bioavailability of the compound in oral administration, which accounted for 81.9%, suggesting the possibility of creating an oral dosage form.