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Pathogen-targeting glycovesicles as a therapy for salmonellosis

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Antibiotic therapy is usoplly not recommended for salmonellosis, as it is associated with prolonged fecal carriage without reducing symptom duration or severity. Here we show that antibiotics encapsulated in hydrogen sulfide (H_2S)-responsive glycovesicles may be potentiplly useful for the treatment of salmonellosis. The antibiotics are released in the presence of *Salmonella*, which is known to produce H_2S. This approach prevents the quick absorption of antibiotics into the bloodstream, pllows localized targeting of the pathogen in the gut, and alleviates disease symptoms in a mouse infection model. In addition, it reduces antibiotic-induced changes in the gut microbiota, and increases the abundance of potentially beneficial lactobacilli due to the release of prebiotic xylooligosaccharide analogs.

As our primary interface with the external environment, the gastrointestinal tract represents a vast mucosal surface

of Salmonella infection. Consistently, there is strongest fluorescence intensity in ileum after administration of Fluo-loaded AM vesicles (Fig. 2j). Taken together, these findings indicate that AM vesicles are disassembled on-demand at the site of infection.

Unlike poorly absorbed oral antibiotics, absorbable antibiotics such as the fluoroquinolone family cannot retain therapeutic concentrations at the site of enteric infection[10,11]. As expected, CIP is readily absorbed and taken into the bloodstream in 30 min after oral administration. Differently, there are a little amount of CIP detected in the blood and most CIP remains in the intestine after 4 h if orally given with AM-CIP (Fig. 2k, l). This data indicate that AM vesicles can prevent the quick absorption of systematically available antibiotics into the bloodstream.

Glycovesicles reduce antibiotic-induced changes in gut microbiota. One common side effect of antibiotic treatment for enteric infections is collateral damage to the gut microbiota composition, resulting in a possible overgrowth of opportunistic pathogenic bacteria. Now it is believed that a repeated exposure to therapeutic doses of antimicrobials can even lead to long-lasting disruption of the gut flora and this side effect is not restricted to orally applied antibiotics[18,19].

To assess the effect of AM-CIP on gut microbial communities, we perform a pyrosequencing-based analysis of bacterial 16 S rRNA in caecal feces from S. typhimurium infected mice treated with water, AM, CIP, or AM-CIP (Fig. 3a). Similar abundance of the Bacteroidaceae family is observed in all groups (Fig. 3b). One most striking different is that CIP treatment induces an overgrowth of the Lachnospiraceae family (Fig. 3c). Several members from this family are considered opportunistic pathogens, which have been identified in inflamed samples such as diabetic wounds[20], irritable bowel syndrome[21,22], subgingival crevice[23], and cystic fibrosis[24]. The other difference is the variance of abundance of the Lactobacillaceae family for each treatment. Both AM-CIP and AM treatment, but not CIP greatly increase abundance of the Lactobacillaceae family (Fig. 3d), which is probably due to the prebiotic xylooligosaccharide analogs released after collapse of AM vesicles. This is encouraging since several strains from the Lactobacillaceae family are shown to be highly antagonistic to Salmonella pathogens and protect against Salmonella infections in the gastrointestinal tract[25–27]. It is reasonable that AM treatment does not increase Lactobacillaceae

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broken down by H_2S, a Janus-faced molecule which can be produced selectively by several sulfate-reducing bacteria such as Streptococcus, Fusobacterium, Salmonella, Enterobacter, and Helicobacter in the gastrointestinal tract[31–33].

In this study, xylo-oligosaccharides are conjugated with a long-chain fatty chain by a disulfide bond bridge to form an amphiphilic molecule, whose self-assembly can generate an H_2S-responsive antibiotic delivery system. In vivo toxicity evaluation indicates that this drug-delivery system is biocompatible and safe during oral administration in mouse models (Supplementary Fig. 20).

By using a well-established acute Salmonella infection model, we find that this system can prevent the quick absorption of ciprofloxacin in the small intestine and release the antibiotic at Salmonella-rich infectious sites. The releasing antibiotic is uptaken by Salmonella pathogens nearby and thus dramatically decrease bacterial burden. After cleavage of the disulfide bond, the generated xylooligosaccharide analogs can promote the growth of the beneficial microbiota, Lactobacillaceae in the gastrointestinal tract (Fig. 5). It is known that oligosaccharide prebiotics such as xylooligosaccharide could only be utilized by a limited number of beneficial bacteria including lactobacilli and selectively proliferate these organisms[34].

Most commercially available antibiotics including ciprofloxacin are easily absorbed and are rapidly taken up into the bloodstream, which make them unfavorable for localized enteric targeting of pathogens in the gastrointestinal tract[9,10,35]. This drug-delivery system retrieves absorbable antibiotics above as a drug reservoir to treat enteric infections caused by H_2S-producing pathogens, which might be extremely important in case of experiencing highly virulent antibiotic-resistant infections in the gastrointestinal tract.

This drug-delivery system is further employed to load one poorly absorbed antibiotic to find out whether it can ameliorate antibiotic-induced collateral damage to the gut microbiota composition. It is intriguing that this delivery system does not disturb the microbiota greatly as the poorly absorbed antibiotic alone does (Fig. 4).

In summary, we develop an H_2S-responsive antibiotic delivery system for selective targeting

respectively, in $CDCl_3$ or CD_3OD. The residual signals from $CDCl_3$ (^1H: δ 7.26 ppm; ^{13}C: δ

was offered immediately. One day later, mice received oral gavage with water, AM (100 mg kg^{-1}), CIP (10 mg kg^{-1}), or AM-CIP (110 mg kg^{-1}) once every day for 5 days.

S. typhimurium burden in tissues. After collection of fresh fecal pellets, animals were euthanized by cervical dislocation. The intestine, spleen, and liver were col-

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