Karolinska Institutet

Institutionen för Medicinsk Biokemi och Biofysik (MBB)

Role of antimicrobial peptides in combating shigellosis and in antibiotic-associated diarrhea

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Hillarp Lecture Hall, Retzius väg 8, Karolinska Institutet, Campus Solna

Fredagen den 25 maj, 2012, kl 09.00

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Stockholm 2012

ABSTRACT

Antimicrobial peptides (AMPs) constitute front-line components of innate immunity in multicellular organisms. AMPs are able to kill a wide range of pathogens and exhibit additional important functions such as chemotaxis, angiogenesis and wound healing. These peptides are constitutively expressed in immune and/or epithelial cells. However, their expression can also be induced or suppressed by different stimuli in a cell and tissue specific manner. In this thesis, the functional relevance of cathelicidins and defensins, two major families of AMPs in mammals, was studied in the context of enteric infectious diseases. Induction of these AMPs was explored in *Shigella* infection. The effect of antibiotics on the expression of AMPs in colonic epithelial cells was also investigated and the implication of this effect on *Clostridium difficile* associated diarrhea (CDAD) was assessed.

In a rabbit model of shigellosis, cathelicidin CAP-18 was downregulated in the large intestinal epithelia. Oral treatment with sodium butyrate (NaB) counteracted this downregulation and led to the conversion of the proform of CAP-18 into its active form in the stool (paper I). These findings correlated with reduced *Shigella* load in the intestinal lumen and clinical as well as histopathological recovery. Association between CAP-18 induction and reduction of bacterial load was supported by partial blocking of the antimicrobial activity in stool extract of a butyrate treated rabbit with CAP-18 specific antibody along with *in vitro* shigellacidal activity of CAP-18 peptide. In patients with shigellosis, administration of NaB enema as adjunct to antibiotic therapy led to an early improvement of rectal histopathology along with early reduction of inflammatory cells and proinflammatory cytokines in the stool compared to placebo treated patients (paper II). NaB treatment also resulted in enhanced expression of the human cathelicidin LL-37 in the rectal epithelia and sustained secretion of LL-37 in the stool. Since all patients were treated with antibiotics, attenuation of *Shigella* load in stool and clinical symptoms occurred simultaneously in both group of patients. Sodium-4-phenyl-butyrate (PB), an analogue of butyrate, was demonstrated to exhibit a similar therapeutic efficiency as NaB in the rabbit model of shigellosis (paper III). Downregulation of CAP-18 expression was also observed in the epithelia of lung and trachea. This suppression could render the respiratory tract susceptible to secondary infections during shigellosis. After oral treatment with PB or NaB, CAP-18 reappeared in the lung epithelia, which might strengthen the immunity of the respiratory tract against opportunistic respiratory pathogens.

Ciprofloxacin and clindamycin were found to suppress butyrate-induced expression of LL-37 in the colonic epithelial cell line, HT-29 (paper IV). *In vivo* inhibitory effect of ciprofloxacin was observed on CAP-18 expression in the rectal epithelia of *Shigella*- infected rabbits treated with NaB as well as of healthy rabbits. Induction of genes encoding HBD-3 and additional AMPs by NaB were also inhibited by ciprofloxacin. *In vitro* antibacterial activity of LL-37 against ciprofloxacin-resistant *C. difficile* indicates that the reduction of AMPs after antibiotic treatment may allow the overgrowth of *C. difficile* in the gut.

In conclusion, induction of AMPs is a promising therapeutic strategy against shigellosis. Suppression of AMP expression by antibiotics may contribute to CDAD, which is classically known to occur through disruption of the intestinal microbiota after antibiotic treatment.

ISBN 978-91-7457-751-8