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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Antifibrogenic Effects of the Antimicrobial Peptide Cathelicidin in Murine Colitis-Associated Fibrosis

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SUMMARY

Administration of cathelicidin exerts effective antifibrogenic effects in colitis associated intestinal fibrosis. Cathelicidin inhibits transforming growth factor β1-induced collagen expression in fibroblasts. The antifibrogenic effect is extracellular-regulated kinase pathway dependent.

BACKGROUND & AIMS: Cathelicidin (LL-37 in human and mCRAMP in mice) represents a family of endogenous antimicrobial peptides with anti-inflammatory effects. LL-37 also suppresses collagen synthesis, an important fibrotic response, in dermal fibroblasts. Here, we determined whether exogenous cathelicidin administration modulates intestinal fibrosis in two animal models of intestinal inflammation and in human colonic fibroblasts.

METHODS: C57BL/6J mice (n = 6 per group) were administered intracolonically with a trinitrobenzene sulphonic acid (TNBS) enema to induce chronic (6–7 weeks) colitis with fibrosis. We administered mCRAMP peptide (5 mg/kg every 3 day, week 5–7) or cathelicidin gene (*Camp*)-expressing lentivirus (10^7 infectious units week 4) intracolonically or intravenously, respectively. We then infected 129Sv/J mice with *Salmonella typhimurium* orally to induce cecal inflammation with fibrosis. *Camp*-expressing lentivirus (10^7 infectious units day 11) was administered intravenously.

RESULTS: TNBS-induced chronic colitis was associated with increased colonic collagen (col1a2) mRNA expression. Intracolonic cathelicidin (mCRAMP peptide) administration or intravenous delivery of lentivirus-overexpressing cathelicidin gene significantly reduced colonic col1a2 mRNA expression in TNBS-exposed mice compared with vehicle administration. *Salmonella* infection also caused increased cecal inflammation associated with collagen (col1a2) mRNA expression that was prevented by intravenous delivery of *Camp*-expressing lentivirus. Exposure of human primary intestinal fibroblasts and human colonic CCD-18Co fibroblasts to transforming growth factor-β1 (TGF-β1) and/or insulin-like growth factor 1 induced collagen protein and mRNA expression, which was reduced by LL-37 (3–5 μM) through a MAP kinase-dependent mechanism.

CONCLUSIONS: Cathelicidin can reverse intestinal fibrosis by directly inhibiting collagen synthesis in colonic fibroblasts. *(Cell Mol Gastroenterol Hepatol 2015;1:55–74; http://dx.doi.org/10.1016/j.jcmgh.2014.08.001)*

Keywords: Antimicrobial Peptide; Collagen; Inflammatory Bowel Disease.

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis, is manifested by chronic inflammation of the gastrointestinal tract with significant morbidity and at times life-threatening complications.[1] Patients with chronic CD often develop transmural luminal narrowing and form strictures caused by excessive extracellular matrix deposition.[2,3] The mechanisms of intestinal fibrosis are complex and not well understood. Transforming growth factor-β1 (TGF-β1) levels are often higher in the mucosa overlying CD strictures compared with nonstrictured gut.[2] Therapies for fibrosis are ineffective, and surgical resection is often required.[2] Approximately 75% of CD patients eventually undergo surgery, most frequently

Abbreviations used in this paper: **CD, Crohn's disease; DSS, dextran sulfate; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HA, hemagglutinin; HEK293T, human embryonic kidney 293T cells; IBD, inflammatory bowel disease; IGF-1, insulin-like growth factor 1; LB, Lysogeny broth; LL-37, cathelicidin; LV, lentivirus; mCRAMP, cathelicidin; MEK, mitogen-activated protein kinase; PD98059, 2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one; RT-PCR, reverse-transcription polymerase chain reaction; TFA, trifluoroacetic acid; TGF-β1, transforming growth factor-β1; TNBS, trinitrobenzene sulphonic acid; TNFα, tumor necrosis factor α; U0126, 1,4-diamino-2,3-dicyano-1,4-*bis*[2-aminophenylthio]butadiene; UCLA, University of California–Los Angeles.**

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because of intestinal fibrosis.[3] Therapy with anti–tumor necrosis factor α (anti-TNFα) monoclonal antibodies can effectively reduce inflammation but may not prevent or reverse fibrosis in CD patients.[4] Previous studies also have shown that inhibition of TGF-β1 reduces colonic fibrosis in a mouse chronic colitis model.[5]

Cathelicidins (LL-37 in humans and mCRAMP in mice) represent a family of endogenous antimicrobial peptides