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ORIGINAL RESEARCH

Substance P Mediates Proinflammatory Cytokine Release From Mesenteric Adipocytes in Inflammatory Bowel Disease Patients

Aristea Sideri,[1,2] Kyriaki Bakirtzi,[1] David Q. Shih,[3] Hon Wai Koon,[1] Phillip Fleshner,[3] Razvan Arsenescu,[4] Violeta Arsenescu,[5] Jerrold R. Turner,[6,7] Iordanes Karagiannides,[1] and Charalabos Pothoulakis[1]

[1]*Inflammatory Bowel Disease Center, and Neuroendocrine Assay Core, Division of Digestive Diseases, David Geffen School of Medicine, University of California, Los Angeles, California;* [2]*Postgraduate Program in Molecular Medicine, Medical School, University of Crete, Heraklion, Crete, Greece;* [3]*F. Widjaja Foundation, Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, California;* [4]*Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition, and* [5]*Inflammatory Bowel Diseases Center, Mucosal Immunology Laboratory, Division of Gastroenterology, Wexner Medical Center, Ohio State University, Columbus, Ohio;* [6]*Department of Pathology and* [7]*Department of Medicine, University of Chicago, Chicago, Illinois*

SUMMARY

Preadipocytes in inflammatory bowel disease (IBD) have acquired, differential disease-dependent characteristics that lead to changes in the release of inflammation-associated mediators after substance P (SP) treatment; interleukin 17 (IL-17) is the most consistently regulated mediator in isolated human mesenteric preadipocytes.

BACKGROUND & AIMS: Substance P (SP) neurokinin-1 receptors (NK-1Rs) are expressed in mesenteric preadipocytes, and SP binding activates proinflammatory signaling in these cells. We evaluated the expression levels of SP (Tac-1), NK-1R (Tacr-1), and NK-2R (Tacr-2) mRNA in preadipocytes isolated from patients with inflammatory bowel disease (IBD) and examined their responsiveness to SP compared with control human mesenteric preadipocytes. We investigated the effect of the neuropeptide SP on cytokine expression in preadipocytes of IBD versus control patients and evaluated the potential effects of these cells on IBD pathophysiology via SP-NK-R interactions.

METHODS: Mesenteric fat was collected from control, ulcerative colitis (UC) and Crohn's disease patients (n = 10–11 per group). Preadipocytes were isolated, expanded in culture, and exposed to substance P. Colon biopsy samples were obtained from control and IBD patients.

RESULTS: Tacr-1 and -2 mRNA were increased in IBD preadipocytes compared with controls, but Tac-1 mRNA was increased only in UC preadipocytes. SP differentially regulated the expression of inflammatory mediators in IBD preadipocytes compared with controls. Disease-dependent responses to SP were also observed between Crohn's disease and UC preadipocytes. Interleukin 17A (IL-17A) mRNA expression and release increased after SP treatment in both Crohn's disease and UC preadipocytes; IL-17RA mRNA increased in colon biopsies samples from IBD patients.

CONCLUSIONS: Preadipocyte SP-NK-1R interactions during IBD may participate in IBD pathophysiology. The ability of human preadipocytes to release IL-17A in response to SP together with increased IL-17A receptors in the IBD colon suggests that a fat-colonic mucosa inflammatory loop may be active during IBD. (*Cell Mol Gastroenterol Hepatol 2015;1:420–432; http://dx.doi.org/10.1016/j.jcmgh.2015.03.003*)

Keywords: Cytokines; Interleukin-17; Preadipocytes; Substance P.

Substance P (SP) is an endecapeptide[1] member of the tachykinin family of peptides and a product of the preprotachykinin-A (Tac1) gene.[2] SP signals via binding to three G-protein–coupled neurokinin receptors (NK-1R-2R-3R), with highest affinity for NK-1R.[2] SP is expressed in numerous tissues and organs, including the gastrointestinal tract.[2,3] SP is also expressed in cells of the immune system, and it functions both as a neurotransmitter and an immune modulator in many disease states, including several intestinal diseases with an inflammatory phenotype.[2,4]

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease, comprises complex diseases of unknown etiology. The pathophysiology of these diseases involves complex interactions between genetic, microbial, and immune factors.[5] Our group and others have shown that SP and NK-1R have a role in the pathophysiology of intestinal inflammation, including IBD.[6–8] NK-1R expression is increased in the intestinal mucosa of mice with

Abbreviations used in this paper: **b-FGF, basic fibroblast growth factor; BMI, body mass index; CSF-2, colony-stimulating factor 2; CXCL, chemokine (C–X–C motif) ligand; IBD, inflammatory bowel disease; IFNγ, interferon γ; IL, interleukin; IL-17RA, interleukin 17 receptor A; LTB, leukotriene B; MCP-1, monocyte chemotactic protein 1; MIP, macrophage inflammatory protein; NK-1R, neurokinin-1 receptor; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T-cell expressed and secreted; SP, substance P; Tac1, preprotachykinin-A; TNFα, tumor necrosis factor α; UC, ulcerative colitis; VEGFA, vascular endothelial growth factor A.**

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intestinal inflammation[9] as well as IBD patients.[7,10,11] Studies employing NK-1R knockout mice[12,13] and SP receptor antagonists[14–17] show that SP, via NK-1R, plays a dual role in the development of colitis. SP acts as a proinflammatory peptide in acute intestinal inflammation but also enhances proliferation and mucosal healing during chronic colitis[13,16,18,19] by activating distinct protective signaling pathways.[14,19,20] The mechanism involved in the proinflammatory NK-1R–associated responses includes interactions of SP with NK-1R on epithelial and inflammatory cells[10,21,22] and the release of cytokines[17,23,24] that modulate colitis and colitis-associated motility[3] primarily by activating pathways dependent on nuclear factor κB (NF-κB).[6]

A potential role for adipose tissue in IBD pathophysiology is suggested by clinical studies associating increased body mass index (BMI) with the development of active Crohn's disease and the requirement of patients for hospitalization.[25] Fat accumulation surrounding the inflamed intestine ("creeping fat") during Crohn's disease represents a hallmark of the disease.[26,27] Histologic examination of the mesenteric fat of patients with creeping fat demonstrated inflammatory changes[26] and alterations of adipokine levels in the circulation of IBD patients.[28] These data combined with the emergence of fat as an endocrine organ[29] suggest a role of intra-abdominal fat in IBD pathophysiology. Previously, we demonstrated the presence of NK-1R in human mesenteric preadipocytes[30] along with activation of inflammatory,[30] antiapoptotic,[31] and metabolic[32,33] pathways after SP treatment. We also reproduced the creeping fat phenotype in the intracolonic trinitrobenzylsulfonic acid (TNBS) mouse colitis model that was associated with increased proinflammatory cytokine expression in these depots.[30] However, modulation of expression of SP and NK-1R in adipose tissue during IBD has never been examined, and the responsiveness of IBD preadipocytes to SP has not been determined.

Here, we compared for the first time the effects of SP treatment on cytokine production in human mesenteric preadipocytes isolated from a substantial number of control, UC, and Crohn's disease patients. In these cells, we also compared the levels of expression of the Tac1 and NK-1R, NK-2R, and NK-3R genes. Initially, we demonstrate differential cytokine release from preadipocytes isolated from IBD patients compared with controls. We show that human mesenteric preadipocytes isolated from UC and Crohn's disease patients release express higher levels of NK-1R and NK-2R but not NK-3R. We also found that human mesenteric preadipocytes express Tac-1 mRNA, whose expression was elevated in UC but not Crohn's disease preadipocytes. Further, we present evidence that UC and Crohn's disease preadipocytes display differential responses after treatment with SP compared with cells from control patients. Our data also demonstrate IBD-disease dependent changes in SP-induced inflammatory stimulation of human preadipocytes, including increased interleukin 17A (IL-17A) transcription, while interleukin 17 receptor A (IL-17RA) mRNA expression is higher in colonic biopsy samples of both UC and Crohn's disease patients compared with controls.

Materials and Methods

Patients

Mesenteric fat tissues from male and female IBD (11 UC, 11 Crohn's disease) and non-IBD patients (adenocarcinoma surgery, other gastrointestinal complications, or vascular surgery, n = 10) were used. The group of control patients was either of Hispanic or (mainly) of Caucasian descent, mixed both males and females, and had an average BMI of 26.86. Their pathologies included four with adenocarcinoma, two with polyposis coli, one with Whipple disease, one with diverticulitis, one with idiopathic motility disorder, and one with tubular adenoma. The UC and Crohn's disease patients were also a mixed population of men and women with an average BMI of 27.23 and 24.12, respectively. The protocol was approved by the UCLA institutional review board for human research (11-001527-AM-00003).

All patients fasted for at least 10 hours before surgery and provided informed consent. Tissues from Cedar's Sinai were obtained after informed consent in accordance with procedures established by the Cedars-Sinai institutional review board (3358 and 23705). Tissues from Chicago were obtained in accordance with procedures established by the University of Chicago institutional review board (IRB 12960). Colon biopsy samples were collected from patients undergoing colonoscopy for colon cancer screening or IBD disease activity monitoring. The samples were obtained, immediately frozen, and used for RNA isolation.

Isolation and Cell Culture of Human Preadipocytes

We minced 2–5 g of mesenteric fat tissue from each patient into pieces. The samples were then placed in 50-mL tubes containing collagenase solution (1 mg/1 mL of phosphate-buffered saline, 3 mL solution/1 g tissue) and minced to a fine consistency. After vortexing, the tubes were placed in a 37°C shaking water bath (100 rpm) for 40 minutes. The solution was vortexed and filtered through a sterile 100-μm nylon mesh (Fisher Scientific, Hampton, NH). The homogenates were centrifuged at 1000 rpm for 10 minutes. The pellet was then resuspended in 10 mL of erythrocyte lysis buffer (cat. no. A1049201, GIBCO/Invitrogen, Grand Island, NY), placed in a 37°C shaking water bath for 5 minutes at 100 rpm, and then centrifuged at 1000 rpm for 10 minutes. The pellet was resuspended in 10 mL of plating medium (Dulbecco's modified Eagle medium, 0.1 mM penicillin, 0.06 mM streptomycin, 10% HI-fetal bovine serum, pH 7.4), vortexed, plated onto 100-mm dishes, and incubated at 37°C.

Culture of Human Preadipocytes

After 20 hours, the cells were washed three times with 10 mL of phosphate-buffered saline, and 1 mL of trypsin solution (Invitrogen, Carlsbad, CA) was added. The trypsin was inactivated with 5 mL of plating medium, and the cells were centrifuged at 1000 rpm for 10 minutes. After resuspension in plating medium, the cells were plated at 5×10^4 cells/cm^2 in plating medium and incubated at 37°C until