Research Article

Anti-Inflammatory Effects of the Nicotinergic Peptides SLURP-1 and SLURP-2 on Human Intestinal Epithelial Cells and Immunocytes

Alex I. Chernyavsky,1 Valentin Galitovskiy,1 Igor B. Shchepotin,2 and Sergei A. Grando1,3,4

1 Department of Dermatology, University of California, 134 Sprague Hall, Irvine, CA 92697, USA
2 National Cancer Institute, Kiev 03022, Ukraine
3 Department of Biological Chemistry, University of California, 134 Sprague Hall, Irvine, CA 92697, USA
4 Institute for Immunology, University of California, 134 Sprague Hall, Irvine, CA 92697, USA

Correspondence should be addressed to Sergei A. Grando; sgrando@uci.edu

Received 18 March 2014; Accepted 17 April 2014; Published 4 May 2014

A search for novel and more efficient therapeutic modalities of inflammatory bowel disease (IBD) is one of the most important tasks of contemporary medicine. The anti-inflammatory action of nicotine in IBD might be therapeutic, but its toxicity due to off-target and nonreceptor effects limited its use and prompted a search for nontoxic nicotinergic drugs. We tested the hypothesis that SLURP-1 and -2—the physiological nicotinergic substances produced by the human intestinal epithelial cells (IEC) and immunocytes—can mimic the anti-inflammatory effects of nicotine. We used human CCL-241 enterocytes, CCL-248 colonocytes, CCRF-CEM T-cells, and U937 macrophages. SLURP-1 diminished the TLR9-dependent secretion of IL-8 by CCL-241, and IFN-γ-induced upregulation of ICAM-1 in both IEC types. rSLURP-2 inhibited IL-1β-induced secretion of IL-6 and TLR4- and TLR9-dependent induction of CXCL10 and IL-8, respectively, in CCL-241. rSLURP-1 decreased production of TNF-α by T-cells, downregulated IL-1β and IL-6 secretion by macrophages, and moderately upregulated IL-10 production by both types of immunocytes. SLURP-2 downregulated TNFα and IFNγR in T-cells and reduced IL-6 production by macrophages. Combining both SLURPs amplified their anti-inflammatory effects. Learning the pharmacology of SLURP-1 and -2 actions on enterocytes, colonocytes, T cells, and macrophages may help develop novel effective treatments of IBD.

1. Introduction

A search for novel and more efficient therapeutic modalities of inflammatory bowel disease (IBD) is one of the most important tasks of contemporary clinical and experimental medicine. Both ulcerative colitis (UC) and Crohn’s disease (CD) are epidemiologically related to smoking [1–4]. Most patients with UC are nonsmokers, and patients with a history of smoking usually acquire their disease after they have stopped smoking [5–7]. Upon cessation of smoking, patients with UC experience more severe disease progression that can be ameliorated by returning to smoking [8–10]. In contrast, patients with CD experience severe disease when smoking, requiring an immediate and complete cessation of any tobacco usage [3, 11]. Nicotine administration in transdermal patches or enema inhibits inflammation associated with UC [8, 12–16]. Nicotine also exhibits a local therapeutic effect in CD [17], despite the fact that smoking worsens this disease. It is believed that the therapeutic effects of nicotine in IBD are mediated by the nicotinic acetylcholine (ACh) receptors (nAChRs) of gut immune cells that inhibit production of inflammatory mediators and correct specific alterations in cell cycle responses [18–20]. We have previously demonstrated that nicotinic agonists abrogate PHA-dependent upregulation of TNFα and IFNγ receptors (IFNγR) in the human leukemic T-cell line CCRF-CEM.
therefore help develop novel effective treatments of UC and on enterocytes, colonocytes, T-cells, and macrophages may facilitate healing of intestinal ulcers. The results demonstrated that SLURPs can abolish expression of the IBD-related protein-(SLURP-)1 and impaired T-cell activity [43]. SLURP-2 expression was also discovered in the skin [44]. While various subtypes of nAChRs can be involved in the physiological regulation of cell functions by SLURPs, the biological effects of SLURP-1 are predominantly mediated by α7 nAChR and those of SLURP-2 by non-α7 nAChRs [45]. Cell function and gene expression studies [46, 47] suggested that SLURPs may play important roles in regulating both epithelial cells and immunocytes. Since nicotine has been shown to alter expression of SLURP-1 in IEC [48], we hypothesized that auto/paracrine action of SLURPs on IEC may, in part, mediate the anti-inflammatory activities of nicotine in IBD.

In this study, we analyzed the roles of SLURP-1 and -2 in the physiological regulation of the key elements of the pathobiology of IBD controlling intestinal inflammation and facilitating healing of intestinal ulcers. The results demonstrated that SLURPs can abolish expression of the IBD-related mediators of inflammation in both IEC and immunocytes. Learning the pharmacology of the SLURP-1 and -2 actions on enterocytes, colonocytes, T-cells, and macrophages may therefore help develop novel effective treatments of UC and CD.

2. Materials and Methods

2.1. Cells and Reagents. Human IEC: the small intestine enterocyte cell line CCL-241 and the colonocyte cell line CCL-248, human lymphoblastoid T-cell line CEM, and human monoblastoid tumor cell line U937 were purchased from ATCC (Manassas, VA) and grown in the respective ATCC complete growth media at 37°C in a humid, 5% CO₂ incubator. To differentiate into macrophages, the U937 cells were treated with 200 nM PMA (Sigma-Aldrich Corporation, St. Louis, MO) and allowed to adhere to tissue culture plate for 3 days [49]. The full length recombinant (r)SLURP-1 and rSLURP-2 were manufactured at Viruses Corporation (Sykesville, MD), as detailed elsewhere [50]. The previously characterized anti-SLURP-1 and -2 monoclonal antibodies 336H12-1A3 and 341F10-1F12, respectively [46, 47], were from Research and Diagnostic Antibodies (North Las Vegas, NV). Normal mouse IgG (NlgG) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Primary mouse antibodies to human ICAM, IL-1β, IL-6, IL-10, TNFα, and IFNγ receptor (IFNγR) and ELISA kits for measuring human IL-6 and CXCL10 were purchased from R&D Systems (Minneapolis, MN). The IL-8 ELISA kit was from BD Biosciences (San Jose, CA). Both recombinant IL-1β and INFγ were from R&D Systems and both E. coli DNA and LPS from E. coli K12 strain (LPS-EK) were purchased from InvivoGen (San Diego, CA).

2.2. Quantitative Immunocytochemical Assay (QIA). The QIA (a.k.a. in-cell western), a high throughput quantitative assay of cellular proteins, was performed in situ, as described in detail elsewhere [46], using the reagents and equipment from LI-COR Biotechnology (Lincoln, NE). The CCL-241, CCL-248, CEM, or U937 cells, 1 × 10⁶/well of a 96-well plate, were incubated in respective growth media with or without rSLURPs for 16 h, fixed in situ, washed, permeabilized with Triton solution, incubated with the LI-COR Odyssey Blocking Buffer for 1.5 h, and then treated overnight at 4°C with a primary antibody. The cells were then washed and stained for 1 h at room temperature with a secondary antibody, and expression of the protein of interest was quantitated using the LI-COR Odyssey Imaging System. Sapphire700 (1:1000) was used to normalize for cell number/well.

2.3. Statistical Analysis. Results were expressed as mean ± SD, and statistical significance was determined by ANOVA with Dunnett’s posttest using the GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA). The differences were deemed significant when the calculated P value was <0.05.

3. Results

3.1. Anti-Inflammatory Effects of rSLURP-1 and -2 on IEC. In in vitro experiments utilizing cultured human enterocytes and colonocytes, CCL-241 and CCL-248, respectively, we recreated an aspect of IBD pathophysiology involving the proinflammatory action of IL-1β, IFNγ, and Toll-like receptor 4- (TLR4-) and TLR9-ligands (i.e., LPS-EK and E. coli DNA, resp.) on intestinal epithelium [51-53]. TLR4 and TLR9 regulate cytokine secretion, cell survival, and intestinal barrier function, and their expression on IEC is upregulated in IBD [52-57]. We hypothesized that, in response to these mediators, CCL-241 and CCL-248 cells would express proinflammatory molecules eliciting mucosal homing of T-cells and recruiting other types of inflammatory cells. Exposed
IEC indeed showed upregulated expression of IL-6, IL-8, CXCL10, and ICAM-1 (Figure 1).

Next, we sought to determine if rSLURP-1 or -2 can inhibit production of these proinflammatory molecules. rSLURP-1 significantly \((P < 0.05)\) diminished the TLR9-dependent secretion of IL-8 by CCL-241, but not CCL-248, and the IFN\(\gamma\)-induced upregulation of ICAM-1 in both types of IEC (Figure 1). rSLURP-2 inhibited the IL-1\(\beta\)-induced secretion of IL-6 and TLR4- and TLR9-dependent induction of CXCL10 and IL-8, respectively, in CCL-241. The specificity of these effects was demonstrated by ability of anti-SLURP antibodies to abolish the inhibitory activity of corresponding rSLURP. A mixture of both nicotinergic peptides almost completely inhibited upregulated expression of all tested inflammatory molecules in both types of IEC (Figure 1), which is in keeping with the synergistic mechanisms of their biological action \([58, 59]\).

3.2. Anti-Inflammatory Effects of rSLURP-1 and -2 on Immunocytes. rSLURP-1 significantly \((P < 0.05)\) decreased production of TNF\(\alpha\) by CEM, downregulated IL-1\(\beta\) and IL-6 secretion by U937 cells, and moderately upregulated IL-10 production by both types of immunocytes (Figure 2). rSLURP-2 significantly \((P < 0.05)\) downregulated TNF\(\alpha\) and IFN\(\gamma\)R in CEM and reduced IL-6 production by U937 cells (Figure 2). Combining both rSLURPs amplified their anti-inflammatory effects.
that could be activated by rSLURP-2. Activation of inhibits immunoreactivity [72, 73].

of immunocytes [21, 22] and IEC. By RT-PCR, CCL-241 cells subtypes expressed on the cell membrane of different kinds uniquely express α3, whereas CCL-248, α2 and α5, and both cells also express α7 and α9 nAChRs (data not shown), which is different from the colonic cell line HT29 that carries α4-made nAChR [38]. The variations of the nAChR profiles among distinct IEC types help explain regional variations of intestinal responses to smoking/nicotine [4, 70, 74–76].

Previous studies indicated that SLURP-1 can potentiate the ACh action at α7 nAChR leading to modifications in functions of cutaneous epithelial cells [77] and immunocytes [78]. Since both IEC and immune cells express this nAChR subtype, the anti-inflammatory effects of SLURP-1 in the gut may result from its action on both cells types simultaneously. Additionally, since SLURP-1 has been shown to upregulate production of ACh by immunocytes [78], this endogenously produced and secreted agonist may further potentiate the α7-mediated anti-inflammatory effect of SLURP-1.

5. Conclusions

Both rSLURP-1 and -2 inhibit production of inflammatory mediators in human enterocytes, colonocytes, T-cells, and macrophages. Combining both rSLURP proteins amplifies the anti-inflammatory effects. The anti-inflammatory effects of nonotoxic nAChR ligands such as SLURPs may therefore ameliorate disease in CD and UC patients. Identification of the predominant types of nAChRs mediating anti-inflammatory effects of each SLURP protein on IEC and immunocytes should help elucidate the intracellular signaling pathways.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported, in part, by internal funds from University of California-Irvine School of Medicine.
References

[1] R. J. Motley, J. Rhodes, S. Kay, and T. J. Morris, “Late presentation of ulcerative colitis in ex-smokers,” International Journal of Colorectal Disease, vol. 3, no. 3, pp. 171–175, 1988.

[2] I. Koutroubakis, O. N. Manousos, S. G. M. Meuwissen, and A. S. Pen, “Environmental risk factors in inflammatory bowel disease,” Hepato-Gastroenterology, vol. 43, no. 8, pp. 381–393, 1996.

[3] D. T. Rubin and S. B. Hanauer, “Smoking and inflammatory bowel disease,” European Journal of Gastroenterology and Hepatology, vol. 12, no. 8, pp. 855–862, 2000.

[4] R. Eliakim, F. Karmeli, P. Cohen, S. N. Heyman, and D. Rachmilewitz, “Dual effect of chronic nicotine administration: augmentation of jejunitis and amelioration of colitis induced by iodoaceticamide in rats,” International Journal of Colorectal Disease, vol. 16, no. 1, pp. 14–21, 2001.

[5] A. D. Harries, A. Baird, and J. Rhodes, “Non-smoking: a feature of ulcerative colitis,” British Medical Journal, vol. 284, no. 6317, p. 706, 1982.

[6] R. F. Logan, M. Edmond, K. Somerville, and M. J. S. Lanman, “Smoking and ulcerative colitis,” British Medical Journal, vol. 288, no. 6419, pp. 751–753, 1984.

[7] R. J. Motley, J. Rhodes, and G. A. Ford, “Time relationships between cessation of smoking and onset of ulcerative colitis,” Digestion, vol. 37, no. 2, pp. 125–127, 1987.

[8] H. de Castella, “Non-smoking: a feature of ulcerative colitis,” British Medical Journal, vol. 284, no. 6330, p. 1706, 1982.

[9] J. Birtwistle and K. Hall, “Does nicotine have beneficial effects in the treatment of certain diseases?” British Journal of Nursing, vol. 5, no. 19, pp. 1195–1202, 1996.

[10] J. M. Wolf and B. A. Lashner, “Inflammatory bowel disease: sorting out the treatment options,” Cleveland Clinic Journal of Medicine, vol. 69, no. 8, pp. 621–631, 2002.

[11] R. J. Hilsden, D. C. Hodgins, A. Timmer, and L. R. Sutherland, “Helping patients with Crohn’s disease quit smoking,” American Journal of Gastroenterology, vol. 95, no. 2, pp. 352–358, 2000.

[12] G. A. Thomas, J. Rhodes, and J. R. Ingram, “Mechanisms of disease: nicotine—a review of its actions in the context of gastrointestinal disease,” Nature Clinical Practice Gastroenterology & Hepatology, vol. 2, no. 11, pp. 536–544, 2005.

[13] B. Coulie, M. Camilleri, A. E. Bharucha, W. J. Sandborn, and D. Burton, “Colonic motility in chronic ulcerative proctosigmoiditis and the effects of nicotine on colonic motility in patients and healthy subjects,” Alimentary Pharmacology and Therapeutics, vol. 15, no. 5, pp. 653–663, 2001.

[14] J. McGrath, J. W. McDonald, and J. K. Macdonald, “Transdermal nicotine for induction of remission in ulcerative colitis,” Cochrane Database of Systematic Reviews, no. 4, Article ID CD004722, 2004.

[15] R. D. Pullan, J. Rhodes, S. Ganesh et al., “Transdermal nicotine for active ulcerative colitis,” New England Journal of Medicine, vol. 330, no. 12, pp. 811–815, 1994.

[16] J. T. Green, G. A. O. Thomas, J. Rhodes et al., “Pharmacokinetics of nicotinic carboxam enemas: a new treatment modality for ulcerative colitis,” Clinical Pharmacology and Therapeutics, vol. 61, no. 3, pp. 340–348, 1997.

[17] J. R. Ingram, J. Rhodes, B. K. Evans, and G. A. Thomas, “Nicotine enemas for active Crohn’s colitis: an open pilot study,” Gastroenterology Research and Practice, vol. 2008, Article ID 237185, 6 pages, 2008.

[18] A. Bai, Y. Guo, and N. Lu, “The effect of the cholinergic anti-inflammatory pathway on experimental colitis,” Scandinavian Journal of Immunology, vol. 66, no. 5, pp. 538–545, 2007.

[19] M. C. Aldhous, R. J. Prescott, S. Roberts, K. Samuel, M. Waterfall, and J. Satsangi, “Does nicotine influence cytokine profile and subsequent cell cycling/apoptotic responses in inflammatory bowel disease?” Inflammatory Bowel Diseases, vol. 14, no. 11, pp. 1469–1482, 2008.

[20] J. Qian, V. Galitovskiy, A. I. Chernyavsky, S. Marchenko, and S. A. Grando, “Plasticity of the murine spleen T-cell cholinergic receptors and their role in vitro differentiation of nave CD4 T cells toward the Th1, Th2 and Th17 lineages,” Genes and Immunity, vol. 12, no. 3, pp. 222–230, 2011.

[21] A. I. Chernyavsky, J. Arredondo, V. Galitovskiy, J. Qian, and S. A. Grando, “Structure and function of the nicotinic arm of acetylcholine regulatory axis in human leukemic T cells,” International Journal of Immunopathology and Pharmacology, vol. 22, no. 2, pp. 461–472, 2009.

[22] A. I. Chernyavsky, J. Arredondo, M. Skok, and S. A. Grando, “Auto/paracrine control of inflammatory cytokines by acetylcholine in macrophage-like U937 cells through nicotinic receptors,” International Immunopharmacology, vol. 10, no. 3, pp. 308–315, 2010.

[23] P. Henderson, J. E. Van Limbergen, J. Schwarze, and D. C. Wilson, “Function of the intestinal epithelium and its dysregulation in inflammatory bowel disease,” Inflammatory Bowel Diseases, vol. 17, no. 1, pp. 382–395, 2011.

[24] T. W. Zimmerman and H. J. Binder, “Effect of tetrodotoxin on cholinergic agonist-mediated colonic electrolyte transport,” The American Journal of Physiology, vol. 244, no. 4, pp. G386–G391, 1983.

[25] A. Pettersson, S. Nordlander, G. Nylund, A. Khorram-Manesh, S. Nordgren, and D. S. Delbro, “Expression of the endogenous, nicotinic acetylcholine receptor ligand, SLURP-1, in human colon cancer,” Autonomic and Autacoid Pharmacology, vol. 28, no. 4, pp. 109–116, 2008.

[26] C. L. Green, W. Ho, K. A. Sharkey, and D. M. McKay, “Dextran sodium sulfate-induced colitis reveals nicotinic modulation of ion transport via iNOS-derived NO,” American Journal of Physiology-Gastrointestinal and Liver Physiology, vol. 287, no. 3, pp. G706–G714, 2004.

[27] B. Sayer, J. Lu, C. Green, J. D. Söderholm, M. Akhtar, and D. M. McKay, “Dextran sodium sulphate-induced colitis perturbs muscarinic cholinergic control of colonic epithelial ion transport,” British Journal of Pharmacology, vol. 135, no. 7, pp. 1794–1800, 2002.

[28] M. Jönsson, Ö. Norrgård, and S. Forsgren, “Presence of a marked nonneuronal cholinergic system in human colon: study of normal colon and colon in ulcerative colitis,” Inflammatory Bowel Diseases, vol. 13, no. 11, pp. 1347–1356, 2007.

[29] P. L. Wei, L. J. Kuo, M. T. Huang et al., “Nicotine enhances colon cancer cell migration by induction of fibronectin,” Annals of Surgical Oncology, vol. 18, no. 6, pp. 1782–1790, 2011.

[30] O. Lundgren, M. Jodal, M. Jansson, A. T. Ryberg, and L. Svensson, “Intestinal epithelial stem/progenitor cells are controlled by mucosal afferent nerves,” PLoS ONE, vol. 6, no. 2, Article ID e16295, 2011.

[31] J. Wei and J. Feng, “Signaling pathways associated with inflammatory bowel disease,” Recent Patents on Inflammation and Allergy Drug Discovery, vol. 4, no. 2, pp. 105–117, 2010.

[32] Y. Sun, B. Fihn, M. Jodal, and H. Sjövall, “Effects of nicotinic receptor blockade on the colonic mucosal response to luminal...
bile acids in anaesthetized rats,” Acta Physiologica Scandinavica, vol. 178, no. 3, pp. 251–260, 2003.

[33] G. M. Roomans, V. Vanthanouvong, A. Dragomir, I. Kozlova, and R. Wróblewski, “Effects of nicotine on intestinal and respiratory epithelium,” Journal of Submicroscopic Cytology and Pathology, vol. 34, no. 4, pp. 381–388, 2002.

[34] I. Kozlova, A. Dragomir, V. Vanthanouvong, and G. M. Roomans, “Effects of nicotine on intestinal epithelial cells in vivo and in vitro: an X-ray microanalytical study,” Journal of Submicroscopic Cytology and Pathology, vol. 32, no. 1, pp. 97–102, 2000.

[35] I. A. Finnie, B. J. Campbell, B. A. Taylor et al., “Stimulation of colonic mucin synthesis by corticosteroids and nicotine,” Clinical Science, vol. 91, no. 3, pp. 359–364, 1996.

[36] A. Cervin, S. Lindberg, U. Mercke, and R. Uddman, “Neuropeptide Y in the rabbit maxillary sinus modulates cholinergic acceleration of mucociliary activity,” Acta Oto-Laryngologica, vol. 112, no. 5, pp. 872–881, 1992.

[37] F. J. Zijlstra, E. D. Srivastava, M. Rhodes et al., “Eicosanoids Effect of nicotine on rectal mucus and mucosal eicosanoids,” Gut, vol. 35, no. 2, pp. 247–251, 1994.

[38] A. E. Summers, C. J. Whelan, and M. E. Parsons, “Nicotinic acetylcholine receptor subunits and receptor activity in the epithelial cell line HT29,” Life Sciences, vol. 72, no. 18-19, pp. 2091–2094, 2003.

[39] M. Bencherif, P. M. Lippiello, R. Lucas, and M. B. Marrero, “Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases,” Cellular and Molecular Life Sciences, vol. 68, no. 6, pp. 931–949, 2011.

[40] J. Ghia, P. Blennnerhassett, R. T. El-Sharkawy, and S. M. Collins, “The protective effect of the vagus nerve in a murine model of chronic relapsing colitis,” American Journal of Physiology-Gastrointestinal and Liver Physiology, vol. 293, no. 4, pp. G711–G718, 2007.

[41] J. E. Ghia, P. Blennnerhassett, H. Kumar-Ondiveeran, E. F. Verdu, and S. M. Collins, “The Vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model,” Gastroenterology, vol. 131, no. 4, pp. 1122–1130, 2006.

[42] S. Nikfar, S. Ehteshami-Ashar, R. Rahimi, and M. Abdollahi, “Systematic review and meta-analysis of the efficacy and tolerance of nicotine preparations in active ulcerative colitis,” Gastroenterology, vol. 164, no. 1, pp. 47–53, 2011.

[43] H. Tsuji, K. Okamoto, Y. Matsuoka, H. Iizuka, G. Tamiya, and H. Inoko, “SLURP-2, a novel member of the human Ly-6 superfamily that is up-regulated in psoriasis vulgaris,” Genomics, vol. 81, no. 1, pp. 26–33, 2003.

[44] S. A. Grando, “Basic and clinical aspects of non-neuronal acetylcholine: biological and clinical significance of non-canonical ligands of epithelial nicotinic acetylcholine receptors,” Journal of Pharmacological Sciences, vol. 106, no. 2, pp. 174–179, 2008.

[45] J. Arredondo, A. I. Chernyavsky, R. J. Webber, and S. A. Grando, “Biological effects of SLURP-1 on human keratinocytes,” Journal of Investigative Dermatology, vol. 125, no. 6, pp. 1236–1241, 2005.

[46] J. Arredondo, A. I. Chernyavsky, D. L. Jolkovsky, R. J. Webber, and S. A. Grando, “SLURP-2: a novel cholinergic signaling peptide in human mucocutaneous epithelium,” Journal of Cellular Physiology, vol. 208, no. 1, pp. 238–245, 2006.
[63] B. H. Lee, S. H. Hwang, S. H. Choi et al., “Quercetin inhibits α3β4 nicotinic acetylcholine receptor-mediated ion currents expressed in Xenopus oocytes,” *Korean Journal of Physiology and Pharmacology*, vol. 15, no. 1, pp. 17–22, 2011.

[64] Y. L. Shih, H. Liu, C. Chen et al., “Combination treatment with luteolin and quercetin enhances antiproliferative effects in nicotine-treated MDA-MB-231 cells by down-regulating nicotinic acetylcholine receptors,” *Journal of Agricultural and Food Chemistry*, vol. 58, no. 1, pp. 235–241, 2010.

[65] M. Comalada, D. Camuesco, S. Sierra et al., “In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway,” *European Journal of Immunology*, vol. 35, no. 2, pp. 584–592, 2005.

[66] H. H. Kim, H. Kong, B. Choi et al., “Metabolic and pharmacological properties of rutin, a dietary quercetin glycoside, for treatment of inflammatory bowel disease,” *Pharmaceutical Research*, vol. 22, no. 9, pp. 1499–1509, 2005.

[67] J. P. Van Dijk, G. S. Madretsma, Z. J. Keuskamp, and F. J. Zijlstra, “Nicotine inhibits cytokine synthesis by mouse colonic mucosa,” *European Journal of Pharmacology*, vol. 278, no. 1, pp. R11–R12, 1995.

[68] T. Spoettl, C. Paetzel, H. Herfarth et al., “(E)-metanicotine hemigalactarate (TC-2403-12) inhibits IL-8 production in cells of the inflamed mucosa,” *International Journal of Colorectal Disease*, vol. 22, no. 3, pp. 303–312, 2007.

[69] W. J. de Jonge and L. Ulloa, “The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation,” *British Journal of Pharmacology*, vol. 151, no. 7, pp. 915–929, 2007.

[70] R. Eliakim and F. Karmeli, “Divergent effects of nicotine administration on cytokine levels in rat small bowel mucosa, colonic mucosa, and blood,” *Israel Medical Association Journal*, vol. 5, no. 3, pp. 178–180, 2003.

[71] T. Werner and D. Haller, “Intestinal epithelial cell signalling and chronic inflammation: from the proteome to specific molecular mechanisms,” *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 622, no. 1-2, pp. 42–57, 2007.

[72] K. Matsunaga, T. W. Klein, H. Friedman, and Y. Yamamoto, “Involvement of nicotinic acetylcholine receptors in suppression of antimicrobial activity and cytokine responses of alveolar macrophages to Legionella pneumophila infection by nicotine,” *Journal of Immunology*, vol. 167, no. 11, pp. 6518–6524, 2001.

[73] E. P. van der Zanden, S. A. Snoek, S. E. Heinsbroek et al., “Vagus nerve activity augments intestinal macrophage phagocytosis via nicotinic acetylcholine receptor α4β2,” *Gastroenterology*, vol. 137, no. 3, pp. 1029–1039, 2009.

[74] I. Murakami, Y. Hamada, S. Yamane, H. Fujino, S. Horie, and T. Murayama, “Nicotine-induced neurogenic relaxation in the mouse colon: changes with dextran sodium sulfate-induced colitis,” *Journal of Pharmacological Sciences*, vol. 109, no. 1, pp. 128–138, 2009.

[75] R. Eliakim, X. F. Qiu, and M. W. Babyatsky, “Chronic nicotine administration differentially alters jejunal and colonic inflammation in interleukin-10 deficient mice,” *European Journal of Gastroenterology and Hepatology*, vol. 14, no. 6, pp. 607–614, 2002.

[76] A. Karban and R. Eliakim, “Effect of smoking on inflammatory bowel disease: is it disease or organ specific?” *World Journal of Gastroenterology*, vol. 13, no. 15, pp. 2150–2152, 2007.

[77] F. Chimienti, R. C. Hogg, L. Plantard et al., “Identification of SLURP-I as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda,” *Human Molecular Genetics*, vol. 12, no. 22, pp. 3017–3024, 2003.

[78] T. Fujii, K. Horiguchi, H. Sunaga et al., “SLURP-1, an endogenous alpha7 nicotinic acetylcholine receptor allosteric ligand, is expressed in CD205(+) dendritic cells in human tonsils and potentiates lymphocytic cholinergic activity,” *Journal of Neuroimmunology*, vol. 267, no. 1-2, pp. 43–49, 2014.