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Three lysophosphatidic acids with a distinct long chain moiety differently affect cell differentiation of human colon epithelial cells to goblet cells

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ABSTRACT

Aim: The intestinal mucus layer helps maintain intestinal homeostasis. In this study, we investigated the effects of lysophosphatidic acids (LPA) on differentiation of human colon carcinoma cell line, HT-29, to goblet cells with and without sodium butyrate, a known differentiation factor for intestinal cells.

Main methods: Number and average size of cells with goblet-like morphology in five photographs per dish were measured for assessment of differentiation of HT-29 cells to goblet cells as well as their relative portion of surface of to whole surface area of the photograph.

Key findings: Our results revealed that 18:1 LPA enhanced butyrate-induced differentiation of HT-29 cells. Because increased mRNA expression of LPA_5 and decreased mRNA expression of LPA_6 were observed in HT-29 cells after treatment with butyrate, we explored the effects of alkyl LPA and 20:4 LPA, which show preferentially higher affinities to LPA_5 and LPA_6, respectively. As a result, the cell differentiation to goblet cell was increased by alkyl LPA but decreased by 20:4 LPA. Further, alkyl LPA and 18:1 LPA, but not 20:4 LPA, were found to reduce the numbers of cells surviving after incubation in a standard culture medium containing 10% fetal calf serum.

Significance: We suggest that the three LPAs positively and negatively affect the differentiation of HT-29 cells to goblet cells, which may be associated with their reduced survival through the activation of distinct LPA receptor (s).

1. Introduction

The intestinal mucus layer is located at the interface between the intestinal epithelium and the bacterial flora. To maintain the dynamics of the mucus layer, mucus is continuously secreted into the intestinal lumen by goblet cells (GC) and then flows down the digestive tract [1]. The mucus layer on the colon plays two purposes. It acts firstly as a lubricant and secondly as a protective physical barrier between the mucosal surface and luminal contents. The physiological processes for homeostasis of mucus layer are regulated by various stimuli. Sodium butyrate (NaBt), a natural product of intestinal flora, is of particular interest as an inducer of differentiation of colon cell to GCs [2,3]. In contrast to the many reports on fatty acids that include NaBt [4], only one in vivo study of the effects of lysophosphatidic acid (LPA) as a representative glycerol-backboned lysophospholipid mediator which exerts diverse physiological actions through its six G protein-coupled receptors, on intestinal GC metaplasia in the colon, but not the small intestine, of LPA_2-deficient mice has been reported [5]. On the other hand, several works showing increased differentiation of lung and airway epithelial cells to GCs were reported for platelet-activating factor (PAF) [6,7], sphingosine 1-phophate (S1P) [8,9], and LPA [10]. Our interests in examining the potential effects of LPA on intestinal cell differentiation are two-fold. First, LPA receptors, LPA_2 [11] and LPA_5 [12], were shown to be located on the apical plasma membrane of intestinal epithelial cells, indicating that they have several physiological effects in response to LPA in foods and LPA as a metabolite of its precursor phospholipids in mammalian digestive tracts [13]. In fact, direct administration of synthetic LPA or natural LPA to the lumen side of rat stomach [14] and rat rectum [15] was found to protect against gastric and colonic ulcer induced by water-immersion restraint stress and trinitrobenzene sulfonate installation, respectively. Second, LPA generated from lysophosphatidylcholines by lysophospholipase D activity of autotaxin accesses the basolateral plasma membranes of human intestinal epithelial cells [16]. Autotaxin was originally reported to be rich in human small intestine and colon [17]. Its involvement in the generation of LPA in mouse intestinal tissues are suggested [18], and submucosal mast cells in human gastrointestinal tract highly express autotaxin [19]. Here, we examined whether which molecular species of

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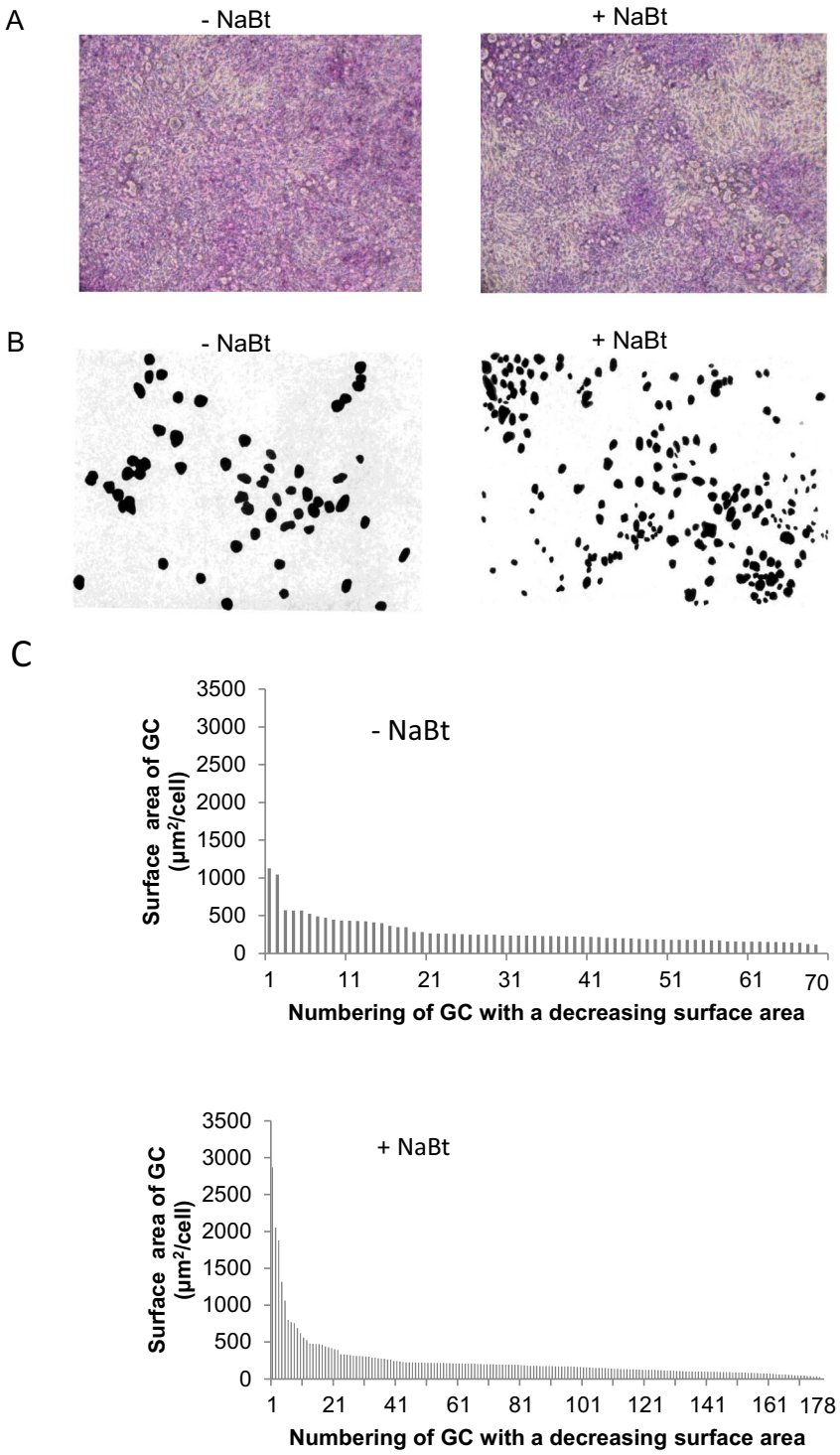

Fig. 1. Typical results of measurement of cell numbers and individual surface areas of GC cells in HT-29 cell cultures in the presence and absence of NaBt.
A: Typical microphotographs of HT-29 cells cultured with and without 3 mM NaBt for eight days.
B: The microphotographs shown in A were converted to figures with manual black-painting of GCs.
C: Histograms of surface areas/individual GCs. The GCs were numbered from No. 1 for GC with the highest surface area to No. 70 and No. 178 for GCs in the absence and presence of 3 mM NaBt, respectively, in the order of decreasing surface area.

LPA with distinct affinities to LPA receptors [20] exerts a beneficial effect on intestinal inflammation by augmenting differentiation of intestinal epithelial cells to GC, in addition to its well-known wound-healing effect. Using well-accepted human colonic tumor cell line (Caco-2), we previously examined extracellular-dependent uptake of various lysolipids including LPA [21]. The cell culture model is not ideal, however, that Caco-2 cells did not secret mucus like the natural epithelium of the intestinal tracts. In order to examine whether LPA is involved in physiological mucus synthesis and secretion, we selected HT-29 line derived from human colonic adenocarcinoma, because HT-29 cells comprise subpopulations, each of which is known to be effectively differentiated to GC and absorptive enterocytes by exposure to appropriate physiological stimuli including NaBt [2,3,22]. In addition, HT-29 cells are shown to be heterologous, and contain in a small