The effect of phospholipids and fatty acids on tight-junction permeability and bacterial translocation

Abstract The activity of phospholipase A₂ (PLA₂) is elevated in the intestinal epithelia of patients with inflammatory bowel disease (IBD). We recently reported that PLA₂ mediates hydrolysis of phosphatidylcholine (PC) to lysophosphatidylcholine (L-PC) when both are applied to the apical surface of cultured EC monolayers, resulting in increased bacterial translocation (BT) and decreased transepithelial electrical resistance (TEER). Free fatty acids (FFA) are the other products of this reaction, however, their effect on Caco-2 cell permeability has not been reported. In addition to PC, other luminal phospholipids are present at the surface of the enterocyte. PLA₂ may also mediate the hydrolysis of luminal phospholipids other than PC. The aim of this study was to examine the effects of phospholipids other than PC and common FFA on intestinal epithelial permeability and BT. Human Caco-2 enterocytes were grown to confluence on porous filters in the apical chamber of a two-chamber cell-culture system. Monolayer integrity and tight-junction permeability were measured as TEER. First, common FFA released by PC hydrolysis were determined using thin-layer chromatography (TLC). In separate experiments, monolayers were treated with phosphatidylethanolamine (PE), lysophosphatidylethanolamine (L-PE), or palmitoleic acid, oleic acids, linoleic acids, and arachidonic acid solubilized in solution with PC. The magnitude of BT was determined 2 h after treatment by adding Escherichia coli C25 to the apical chamber followed by quantitatively culturing basal-chamber samples. Statistical analysis was by the Kruskal-Wallis test. TLC of PC samples incubated with PLA₂ on the apical surface of Caco-2 monolayers demonstrated the production of palmitoleic acid, oleic acids, linoleic acids, and arachidonic acid. L-PE significantly decreased TEER compared to controls, but to a lesser degree than L-PC alone. L-PE had no effects on BT. Palmitoleic acid and oleic acid likewise significantly decreased TEER compared to controls, however, less than L-PC. All FFA tested had no effect on BT. Phospholipids applied to the apical surface of enterocytes, such as those found in vivo in mucus, can be hydrolyzed by the enzyme PLA₂ resulting in lysophospholipid and FFA species that can alter enterocyte monolayer permeability. However, FFA and L-PL, other than L-PC, appear to have no effect to stimulate BT. This observation may have clinical implications in the pathogenesis and treatment strategies for IBD patients in whom enterocyte PLA₂ activity has been shown to be elevated.

Keywords Phospholipase A₂ · Phosphatidylethanolamine · Lysophosphatidylethanolamine · Free fatty acids · Bacterial translocation · Transepithelial electrical resistance · Inflammatory bowel disease

Introduction Traditionally viewed as an organ of nutritional absorption, the gut also has complex defense mechanisms to prevent bacteria from entering the systemic circulation. The mucous layer is a component of the intestinal barrier, segregating potentially harmful luminal bacteria from the mucosal epithelial surface. We have previously reported that intestinal mucous phospholipid concentrations and composition are altered in neonatal, compared to adult, rabbits [13]. Phosphatidylcholine (PC), lysophosphatidylcholine (L-PC), phosphatidylethanolamine (PE), and lysophosphatidylethanolamine (L-PE) are major components of mucus phospholipids. We have also recently reported that the addition of L-PC into the apical surface promotes bacterial translocation (BT) in an enterocyte cell-culture model.
Several human studies have documented that the activity of secretory phospholipase A₂ (PLA₂) is elevated in the intestinal mucosa and serum of patients with Crohn’s disease or ulcerative colitis [1, 7–9, 4]. PLA₂ comprises a family of lipolytic enzymes that catalyze the hydrolysis of the fatty acyl ester bond at the sn-2 position of glycerol-3-phospholipids to produce free fatty acids (FFA) and lysophospholipids such as L-PC. PLA₂ within the lumen of the intestine is derived from the pancreas or from mucosal Paneth-cell secretion (secretory PLA₂). Pancreatic PLA₂ functions in dietary and biliary phospholipid digestion, whereas secretory PLA₂ functions within or on the exofacial surface of intestinal mucosal epithelia. We have previously demonstrated that PLA₂ mediates hydrolysis of PC to L-PC in an enteroctye cell-culture system. When both PLA₂ and PC are applied to the apical surface of cultured EC monolayers, the results are increased BT and decreased transepithelial electrical resistance (TEER) [17].

In addition to hydrolyzing PC to L-PC, PLA₂ in the lumen and possibly at the apical enterocyte surface may also hydrolyze other phospholipids such as PE, resulting in L-PE as well as FFA. Oleic acid and linoleic acid have been shown to induce increases in mucosal permeability in developing piglet intestine [20, 21]. It is reported that arachidonic acid (AA), in particular, plays a role in endothelial permeability [15]. Thus, PLA₂ hydrolysis products other than L-PC on the apical surface of epithelial cells could theoretically affect mucosal permeability and BT. The purpose of this study was to test whether PLA₂ hydrolysis products other than L-PC alter intestinal epithelial permeability and BT.

**Materials and methods**

Human colonic carcinoma (Caco-2) cells were obtained from the American Type Culture Collection No. HTB 37 (Manassas, VA). *Escherichia coli* C25 was provided by Dr. Henri R. Ford (Pittsburgh, PA). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, non-essential amino acid (NEAA) solution, sodium pyruvate, penicillin G, streptomycin, and trypsin-EDTA were purchased from Gibco (Grand Island, NY). A two-chamber cell-culture system (pore size 3.0 μm, diameter 6.5 mm), chloroform, methanol, CuSO₄ pentahydrate, concentrated H₂PO₄, and MacConkey’s agar were obtained from Fischer (Pittsburgh, PA). Rat-tail type I collagen, Dextran blue, PC, and L-PC were purchased from Sigma (St Louis, MO). Brain-heart infusion medium was obtained from BBL (Cockeysville, MD). Precoated silica gel plates (TLC plates, Silica gel 60) were purchased from EM SCIENCE (Gibbstown, NJ).

Caco-2 cells are transformed human colon carcinoma cells that display many features of differentiated small-intestinal enterocytes. They spontaneously form polarized monolayers with tight junctions, and the apical surfaces of the cells have well-developed microvilli that contain disaccharidases and peptidases typical of normal small-intestinal villous cells. Moreover, Caco-2 cell line has been used extensively to study enterocyte interactions with bacteria such as *E. coli*. Cell passages 26–37 were grown on 100-mm dishes in DMEM supplemented with 10% fetal bovine serum, 1% NEAA solution, 1% sodium pyruvate, penicillin G (100 IU/ml), and streptomycin (100 μg/ml) in a 5% CO₂ atmosphere at 37 °C. After reaching 60%–70% confluence, cells were harvested by trypsinization with trypsin-EDTA, washed, resuspended in DMEM and then seeded at a density of 1 × 10⁴ per well (0.33 cm²) onto collagen-coated porous filters in the apical chamber of a two-chamber cell-culture system. Collagen coating of the plates was accomplished by incubation in 30 μl 1.0 mg/ml rat-tail type I collagen. The cells were grown for 14 days in DMEM to reach confluence and fully differentiate. Media were changed every 2nd day.

The tested FFA were not able to solubilize in PBS because they are hydrophobic. Although PE and L-PE are amphipathic, PE or L-PE alone were unable to form micelles. PC, which is present in mucus, was added to the FFA, PE, and L-PE in order to solubilize

![Fig. 1 Confirmation of phospholipase A₂ (PLA₂) mediated hydrolysis of phosphatidylcholine (PC) to free fatty acids: thin-layer chromatography of representative samples taken from apical Caco-2 monolayer surface treated with PLA₂ + PC (2 mM) (lane 1); standard free fatty acids; palmitoleic acids (lane 2); oleic acid (lane 3); linoleic acid (lane 4); and arachidonic acid (lane 5) (Image 304x219 to 547x716)](image)