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Original Contribution

Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage

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Abstract

Procyanidins can exert cytoprotective, anti-inflammatory, and anticarcinogenic actions in the gastrointestinal tract. Previous evidence has shown that procyanidins can interact with synthetic membranes and protect them from oxidation and disruption. Thus, in this study we investigated the capacity of a hexameric procyanidin fraction (Hex) isolated from cocoa to protect Caco-2 cells from deoxycholic (DOC)-induced cytotoxicity, cell oxidant increase, and loss of monolayer integrity. Hex interacted with the cell membranes without affecting their integrity, as evidenced by a Hex-mediated increase in the transepithelial electrical resistance, and inhibition of DOC-induced cytotoxicity. DOC induced an increase in cell oxidants, alterations in the paracellular transport, and redistribution of the protein ZO-1 from cell-cell contacts into the cytoplasm. Hex partially inhibited all these events at concentrations ranging from 2.5 to 20 μM. Similarly, Hex (5–10 μM) inhibited the increase in cell oxidants, and the loss of integrity of polarized Caco-2 cell monolayers induced by a lipophilic oxidant (2,2′-azobis (2,4-dimethylvaleronitrile). Results show that the assayed procyanidin fraction can interact with cell membranes and protect Caco-2 cells from DOC-induced cytotoxicity, oxidant generation, and loss of monolayer integrity. At the gastrointestinal tract, large procyanidins may exert beneficial effects in pathologies such us inflammatory diseases, alterations in intestinal barrier permeability, and cancer.

Keywords: Flavonoid; Flavanol; Polyphenol; Intestinal barrier permeability; Bile acids; Membrane interactions; Gastrointestinal tract; Cocoa

Introduction

Flavonoids are found in a wide variety of vegetables and fruits, and numerous studies have associated flavonoid consumption with health benefits. A specific class of flavonoids known as procyanidins are oligomers of flavan-3-ol subunits, i.e., (+)-catechin and (−)-epicatechin (EC), which are present in high concentrations in cocoa, red wine, cranberries, and apples [1,2]. Procyanidins in cocoa are primarily composed of several units of EC linked by 4β→8 bonds (Fig. 1).

While there is no conclusive information regarding the bioavailability and biotransformation of procyanidins, a study in humans demonstrated that cocoa procyanidins are not degraded in the stomach and can reach the intestine [3]. Of the procyanidins that reach the intestine, only dimers have been shown to be absorbed into the circulation [4,5]; higher molecular weight procyanidins are poorly absorbed in the intestinal lumen [6,7]. The human colonic microflora is capable of degrading procyanidins to aromatic acids [8], but this degradation decreases with the degree of procyanidin oligomerization [9]. In addition, procyanidins with more than 3 subunits are not transported across Caco-2 cell monolayers,

Abbreviations: AMVN, 2,2′-azobis (2,4-dimethylvaleronitrile); DCDHF, 5-(and-6)-carboxy-2′7′-dichlorodihydrofluorescein diacetate; DOC, deoxycholate; DPI, diphenyleneiodonium chloride; EC, (−)-epicatechin; FSA, fluorescein sulfonic acid; Hex, hexameric procyanidin fraction; LDH, lactate dehydrogenase; TEER, transepithelial electrical resistance.

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and can remain in part adsorbed to the apical surface of cells [8].

The above evidence supports the concept that relevant amounts of procyanidins can reach the intestine and exert anti-inflammatory, anticarcinogenic, or other beneficial effects within the gastrointestinal tract [10,11]. These effects could be relevant in diseases in which alterations in the permeability of the intestinal barrier are associated with gastrointestinal dysfunctions, such as Crohn's disease [12], celiac disease, alcoholic liver disease, fatty acid liver disease, food allergies, and acute pancreatitis (reviewed in [13]). Furthermore, the integrity of the apical plasma membrane and the conservation of intercellular tight junctions of the enterocyte are essential for maintaining the intestinal epithelial barrier, which is necessary for preventing the penetration of external toxic agents and microorganisms [14,15].

Although bile acids have relevant physiological functions in the intestinal lumen, they are also involved in the pathogenesis of several disorders affecting the intestine. Deoxycholic acid (DOC) can promote intestinal tumorogenesis [16,17] that may be associated with bile acid-mediated activation of several cell signals [18,19]. DOC, a hydrophobic bile acid, was recently shown to perturb the cell membrane, affecting its lipid distribution and physical properties. It was proposed that the observed alterations in cell signaling are induced by the effects of DOC at the level of membrane microdomains [19].

We have recently shown that different flavonoids can interact with synthetic membranes (liposomes), protecting the bilayer from both the disruption induced by a detergent and free radical-mediated lipid oxidation [20,21]. Procyanidins were particularly active in these protective actions and their effects consistently depended on the degree of oligomerization [22]. Here we hypothesize that procyanidins, through their interaction with cell membranes, could participate in the protection of the gastrointestinal tract from the deleterious effects of bile acids. To test this hypothesis, we used Caco-2 cells, a well-established model of intestinal epithelium [23], to investigate the capacity of a hexameric procyanidin fraction (Hex) to interact with and protect cell membranes from DOC-induced oxidant production and membrane damage. The effects of DOC were compared to those of a lipophilic oxidant, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). We observed that Hex interacts with Caco-2 cells, protecting them from DOC-induced: (a) cytotoxicity, (b) increase in cell oxidants, (c) redistribution of tight junction proteins (ZO-1), and (d) monolayer permeabilization. Accordingly, Hex inhibited an AMVN-mediated increase in cell oxidants and the loss of monolayer integrity.

Materials and methods

Materials

Procyanidins were purified [2,24,25] and supplied by Mars Incorporated (Hackettstown, NJ). The Hex fraction was composed of 76% hexamers, 4.5% monomers, 2.2% dimers, 1.0% trimers, < 1.0% tetramers, 11.5% pentamers, and 4.1% procyanidins larger than hexamers. Caco-2 cells were from the

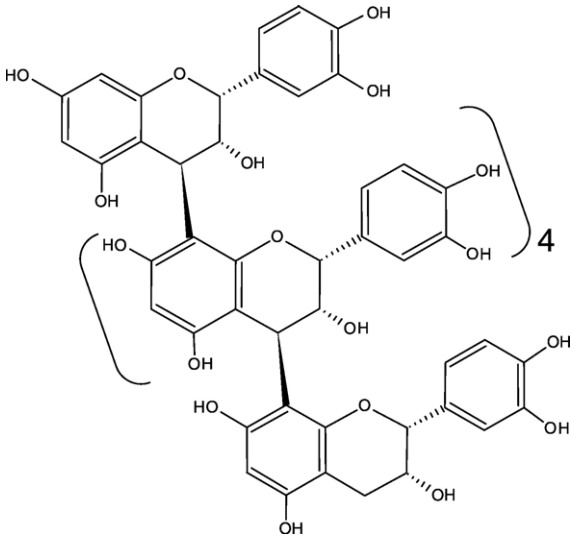

Fig. 1. Chemical structure of B-type procyanidins. N is 4 for hexameric procyanidins.

American Type Culture Collection (Rockville, MA). Cell culture media and reagents were from InVitrogen Life Technologies (Carlsbad, CA). ZO-1 antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). Cell culture transwell permeable supports were from Corning Incorporated (Corning, NY). The CellTiter-Glo Luminiscent Cell Viability assay was from Promega (Madison, WI). Lactate dehydrogenase (LDH) activity assay kit was from Wiener Lab, Rosario, Argentina. 5-(and-6)-carboxy-2′7′-dichlorodihydrofluorescein diacetate (DCDHF), fluorescein sulfonic acid (FSA), and propidium iodide were from Molecular Probes (Eugene, OR). AMVN was from Wako Pure Chemical Ind. (Osaka, Japan). DOC, EC, apocynin, and diphenyleneiodonium chloride (DPI) were from Sigma Chem. Co. (St. Louis, MO).

Cell culture and incubations

Caco-2 cells were cultured at 37°C in a humidified atmosphere of CO_2/air (5/95) in EMEM medium supplemented with 10% (v/v) fetal bovine serum and antibiotics (50 U/ml penicillin, and 50 μg/ml streptomycin). For the experiments we used both unpolarized (at confluence after 7–10 days in culture) and polarized cells. Polarized cells were obtained by seeding the Caco-2 cells on 0.4-μm-pore polyester membranes in transwell inserts (6- or 12-well plates) for 17–20 days until the formation of a cell monolayer. During this culture period the media in the apical and basolateral chambers were changed every 3 days.

For the treatments, unpolarized or polarized cells were washed, and the media replaced by fetal bovine serum-free EMEM. Cells were then preincubated for 30 min in the presence of EC or procyanidins at the concentrations described for each experiment, after which 0.2 mM DOC or 10 mM AMVN was added to the cell culture. Depending on the experiment, EC and procyanidins remained or were removed from the cell culture medium during the subsequent incubation. After the corresponding incubation, the medium was collected and/or the cells