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Abstract of Thesis/Dissertation

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Title : Studies on metabolism and functional role of dietary phospholipids in the digestive sysyem

(食餌性リン脂質の消化器官における代謝並びに機能に関する研究)

Abstract

Introduction: Ethanolamine glycerophospholipid (EtnGpl) is a major subclass of glycerophospholipid in biological membranes and exists in three forms (alkenyl, alkyl, and acyl). The alkenyl form is referred to as ethanolamine plasmalogen (PlsEtn) and has a unique property of a vinyl ether linkage at the *sn*-1 position of the glycerol moiety. PlsEtn are critical for human health and have established roles such as reservoirs of second messengers, involved in membrane fusion, ion transport, cholesterol efflux, neuronal development, and as endogenous antioxidants. Dysregulation of PlsEtn or low levels have been associated with several diseases such as Zellweger syndrome spectrum disorders, Alzheimer's disease, cardiovascular disease, and certain forms of cancer. In the recent past many researcher are interested in the health benefits of PlsEtn; however, despite of the above-mentioned functional roles, biological functions and the underlying mechanistic bases of dietary PlsEtn in colonic health are not well defined. Currently, the incidence if inflammatory bowel disease (IBD) and colon cancer are on the rise, globally. It is generally recognized that patients with IBD have significant increased risk of developing colon cancer primarily a result of chronic intestinal inflammation. Therefore, the main objective of my PhD study was to elucidate the functional role and metabolism of dietary PlsEtn during intestinal inflammation-related disorders using suitable *in vivo* and *in vitro* experimental models.

In the first study (Chapter 2), I used 1,2-dimethyhydrazine (DMH) carcinogen to induce aberrant crypt foci (ACF) formation in the colon of mice. In this study, I hypothesized that dietary PlsEtn may suppress colon inflammatory stress and subsequent formation of colon precancerous lesions (aberrant crypt foci – ACF) due to the abundance of vinyl ether linkages at the *sn*-1 position. Therefore, I investigated the effect of diets containing 0.1% purified EtnGpl from ascidian muscle with high PlsEtn (87.3 mol%) and from porcine liver with low PlsEtn (7.2 mol%) levels and consisting of relatively same n-3/n-6 ratio by supplementing with 1% fish oil on the formation of ACF using DMH-induced colon carcinogenesis mice model. **Results and conclusion**: Dietary EtnGpl suppressed DMH-induced aberrant crypt with one foci (AC1) and total ACF formation ($P < 0.05$). ACF suppression by dietary ascidian muscle EtnGpl was higher compared with dietary porcine liver EtnGpl. Additionally, dietary EtnGpl decreased DMH-induced oxidative damage, overproduction of TNF-α, and expression of apoptosis-related proteins in the colon mucosa. The effect of dietary ascidian muscle EtnGpl showed superiority compared with dietary porcine liver EtnGpl. Our results demonstrate the mechanisms by which dietary PlsEtn suppresses ACF formation and apoptosis. Dietary PlsEtn attained this suppression by reducing colon inflammation and oxidative stress hence a reduction in DMH-induced intestinal impairment. These findings provide new insights about the functional role of dietary PlsEtn during colon carcinogenesis.

In the second study (Chapter 3), I used lipopolysaccharide (LPS) to induced cell injury in differentiated Caco-2 cells. I hypothesized that extrinsic PlsEtn may inhibit apoptosis in human intestinal tract cells under LPS-induced inflammatory stress. Here, I clarified that PlsEtn with abundant vinyl ether linkages has the potential to reduce the degree of LPS-induced apoptotic cells using differentiated Caco-2 cells as an *in vitro* model. **Results and conclusion**: Lipopolysaccharide (LPS) induced apoptosis of differentiated Caco-2 cells, which was suppressed by EtnGpl in a dose-dependent manner. Cells treated with ascidian muscle EtnGpl containing high levels of PlsEtn demonstrated a lower degree of apoptosis, and downregulated TNF-α and apoptosis-related proteins compared to those treated with porcine liver EtnGpl containing low PlsEtn. This indicates PlsEtn exerted the observed effects, which provided protection against induced inflammatory stress. Overall, our results suggest PlsEtn with abundant vinyl ether linkages is potentially beneficial in preventing the initiation of inflammatory bowel disease (IBD) and colon cancer.

In the third study, (Chapter 4), I used 1.5% dextran sulfate sodium (DSS) to induced colitis in mice colon. I hypothesized that dietary PlsEtn may suppress colon inflammatory mediators and histological damage due to the abundance of vinyl ether linkages at the *sn*-1 position. In this study, I investigated the effect of diets containing 0.1% purified EtnGpl from ascidian muscle with high

PlsEtn (86.2 mol%) and from porcine liver with low PlsEtn (7.7 mol%) levels and consisting of relatively similar n-3/n-6 ratio by supplementing with 1% fish oil on the impairment of colon lining using dextran sulfate sodium (DSS)-induced colitis in mice. **Results and conclusion**: Two groups of mice received AIN-93G diet with 1% fish oil (blank and control group) and another two groups of mice receive AIN-93G diet with 1% fish oil and either high PlsEtn level (ascidian muscle group) or low PlsEtn level (porcine liver group). After 38 days, DSS treatment shortened the colon length, decreased the body weight, and increased spleen weight compared to the blank group, which were improved by dietary EtnGpl, with ascidian muscle group showing superior effects. After 16 days (early/middle stage of inflammation), DSS treatment elevated MPO activity, TBARS, pro-inflammatory cytokines and proapoptosis-related proteins levels in the colon mucosa compared to the blank, which were lowered by dietary EtnGpl with ascidian muscle group showing higher suppression. Furthermore, ascidian muscle compared to porcine liver group led to higher levels of plasmalogens in the colon mucosa and plasma. Taken together, these results indicate that diets with abundant PlsEtn exert more anti-colitis effects by modulating apoptosis and inflammatory mediators in the colon mucosa.

Taken together, the three studies strongly correlate the functional role of dietary PlsEtn with inhibition of chronic colon inflammation, hence the suppression of the associated IBD and colon cancer initiation. Moreover, the abundance of vinyl ether linkage showed enhanced suppression. Therefore, the findings of these studies indicate the potential of utilizing food sources rich in PlsEtn as dietary therapies for colon inflammation-related disorders such as IBD and colon cancer.