Gastrointestinal activity of saponins from soy and tomato

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Introduction

Saponins are a diverse family of amphipathic compounds that consist of triolepine or steroidal aglycones covalently linked to one or more sugar sapogenins and are found in a variety of edible plants such as soya, tomato, cherries and tangerine.1 Saponins are structurally similar to phytosterols (e.g., β-sitosterol). The hypolipidemic activity of phytosterols and phytostanols has resulted in their increased incorporation into food products targeted to decrease plasma cholesterol levels.2 Limited data from in vitro and animal studies have suggested that saponins also possess hypotriglyceremic activity.3

Materials and Methods

Preparation of synthetic micelles. Synthetic micelles were prepared to examine the impact of test compounds on microemulsion of cholesterol independent of food matrix. Stock solutions of phytostanols, saponins, soybean oil, oleic acid, and cholesterol were prepared in dimethylsulfoxide (DMSO). Final concentration of DMSO <0.1%. Aliquots of cholesterol containing monolayer monolayer (120 µM) were added to the microemulsion of different saponins and cholesterol were individually added to 25 µL glass vials before transfer of the solutions of saponins. After addition of reconstituted cholesterol (50 mM), each vial was dried under 100 psi gas, cholesterol-coated Teflon-lined Medium (DMEM) containing free cells (glycogen, lactobionic acid, trehalose, polyethylene glycol, sodium caseinate, and Tween 80) were added. The free cell solution was mixed with the lipids and tropolone. Vials were sonicated for 30 minutes to form micelles and then filtered (0.25 µm filter). 1H-cholestane was quantified in filtered and non-filtered aliquots of the samples to determine the extent of rehydration of cholesterol. Final ratio of cholesterol to recombine compound was 1:6.5.

Simulated digestion and micellization of cholesterol in vitro. In vitro digestion was conducted using pepsin as the food matrix as described elsewhere.2 Test compounds included saponinoside 1, saponinoside 2 (apoglycone), and saponinoside 3 (apoglycone). The impact of equimolar concentrations (90-130 µM) of test compounds on cholesterol microemulsion was determined by HPLC. Cholesterol was extracted from cholesterol-containing monolayer monolayer (120 µM) with 10% H2SO4. 1H-cholestane (100 µL, sodium carbonate 0.25 M) reaction within 15 minutes of digestion of cholesterol was compared to micellization during digestion of the matrix containing cholesterol alone. Commercial phospholipids (containing 14 µM cholesterol) served as a positive control. It is known to inhibit micellization of cholesterol. The ratio of cholesterol to saponin/phospholipid in all test samples was approximately 1:6.5. After digestion, in all samples, was centrifuged to obtain the fraction. The supernatant of cholesterol was quantified in digested and filtered aqueous phases of the samples via scintillation counting to determine the extent of micellization of cholesterol.

Cytotoxicity. Differentiated cultures of Caco-2 cells (HTB37) were exposed to a range of saponin concentrations for 24 hours. Cell viability was assessed by metabolic assay (e.g., cell proliferation). Relative cell number was estimated by cell counting.

Results

Impact of Saponins on Cholesterol Micellization using Synthetic Micelles.

α-Tomato is potent inhibitor of incorporation of cholesterol into synthetic micelles.*

Impact on Cholesterol Micellization during Simulated Digestion

α-Tomato is a potent inhibitor of cholesterol micellization.*

Impact on Cholesterol Uptake

Saponins from soy and tomato inhibit cholesterol uptake by Caco-2 cells.*

Adhesion of Salmonella Typhimurium to Caco-2 Cells

Saponins from soy reduce adhesion of Salmonella Typhimurium to Caco-2 cells. *

Cytotoxicity of saponins for Caco-2 cells is proportional to their ability to inhibit micellization of cholesterol.

Summary

Saponins, a structurally diverse family of secondary plant metabolites, possess anti-atherogenic, hypolipidemic, and immune-enhancing activities. We have initiated studies comparing the effects of saponins extracted from several soybean cultivars and tomatoes. This study investigated their role as modulators of metabolism and transport of cholesterol and on interactions between gut microbes and intestinal epithelial cells. Saponins from soybeans reduced the incorporation of cholesterol into synthetic micelles by 65%, whereas other test compounds (saponin from apricot cherries and beta-sitosterol and phytostanols) were without effect. During simulated gastric and small intestinal digestion, saponin (9 µM) from tomatoes and mixed phytosterol significantly inhibited rehydration of cholesterol from a food matrix. Saponins from soy and tomato, as well as phytostanols, also significantly impaired the gastric transfer of cholesterol into differentiated cultures of human intestinal Caco-2 cells. Additionally, soyasaponins decreased adhesion and invasion of Caco-2 cells by Salmonella enterica. These preliminary observations suggest that saponins from crops imported to Ohio’s economy may contribute to cardiovascular and gastrointestinal health.

Cytotoxicity

Cytotoxicity of saponins was highly tested to Caco-2 cells in comparison to other saponins. The greater toxicity corresponds with greater apparent affinity of α-tomatine for cholesterol in micelles and the rapid inhibitory effect on micellization during digestion.

Cholesterol Micellization

Synthetic Micelles: Saponins significantly inhibited the incorporation of cholesterol into synthetic micelles under the defined conditions, whereas the other saponins were without effect.

Simulated Digestion: Phytosterol mixture inhibited cholesterol micellization ~21%, which is similar to inhibition observed in previous studies.2

α-Tomatine was the most potent of the tested saponins for inhibition of cholesterol micellization (16%).

References


Abstract

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