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Soy Saponin Suppresses Colon Carcinogenesis through Diverse Mechanisms

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According to the national statistics, deaths from colon cancer had increased 75.6% between 1990 and 1999 (2000). Colon cancer is considered as the cancer site most closely related to diet (Doll & Peto, 1981). A highly positive correlation has been found between colon cancer incidence and the consumption of beef, animal proteins, and total calories. On the other hand, evidences suggest colon cancer risk may be reduced by high intake of dietary fiber or other components found in plant foods. Plant foods contain a wide variety of micronutrients and non-nutritive compounds with anticarcinogenic properties.

Like other legumes, soybeans are an excellent source of plant protein. Epidemiological studies showed a negative relationship between soy food consumption and the risk of cancer development (Jacobsen et al., 1998; Goodman et al., 1997, Kim et al., 2002). However, lack of a consistent relationship (Wu et al., 2000; Dai et al., 2001) has indicated that the role of individual anticarcinogens present in soybeans as well as other dietary factors should be carefully evaluated before making any conclusion.

Saponins are glycosidic compounds consisted of a non-polar aglycon and sugars attached to it. Due to their chemical nature, saponins possess surface-active property. The hemolytic activity of saponin is one of the most commonly known biological effects. It is generally accepted that saponins bind to cell membrane components and bring about changes in cell membrane permeability, which induce osmotic cell lysis. However, depending on their chemical structures, saponins are known to possess different membranolytic activity.

Previous studies reported that saponins from a few non-edible plant sources had direct cytotoxic effects in different cancer cell lines (Sati et al., 1985; Ravikumar et al., 1979; Konoshima and Lee, 1986). Reverse transformation of melanoma cells was observed when cells were treated with ginsenosides (Rh2), a triterpenoid saponin extracted from the roots of *Panax ginseng*, or glycyrrhizin, a glycoside extracted from the roots of *Glycyrrhizae globa* (Ota et al., 1987; Abe et al., 1987). Saponins extracted from Quillaja bark had significant immune-stimulatory effects (Chavali et al., 1987; Chavali and Campbell, 1987). Also, the ability of saponins to bind bile acids (Oakenfull and Sidhu, 1983), known promoters of abnormal cell proliferation in the colonic epithelium (Wargovich et al., 1984), is another possible anticancer property of saponins. However, as indicated, most of these studies have used saponins from non-dietary sources, and possible biological effects mediated through dietary saponins have been overlooked.

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The major sources of dietary saponins are legumes, especially soybeans. Countries such as Japan and Korea consume 28 and 31 g/day/person legumes, respectively, which is about 10 times more than the amount consumed by their western counterparts (OECD, 1988; The Ministry of Health & Welfare of Korea, 2000). Saponin content of major soyfoods are listed in Table 1. However, few studies have been conducted to evaluate chemopreventive effects and metabolic fate of soybean saponins. An animal study indicated soy saponins were hydrolyzed by cecal microflora to sapogenins and sugars. Therefore, it is likely that saponins or sapogenins reach the colon and interact with free or membrane bound sterols in the colon. The interaction of saponins with intestinal mucosa was also demonstrated by Alvarez and Torres-Pinedo (1982). Mucosal uptake of radiolabelled glycinin wa significantly increased when mucosal samples were incubated in the presence of soybean saponins indicating changes in the permeability.

Previous reports showed soybean saponins suppress the growth of colon tumor cells in vitro (Sung et al., 1995a), and a 2% crude soybean saponin diet inhibited chemically-induced colonic aberrant crypt formation in CF1 mice (Koratkar and Rao, 1997). However, mechanisms involved in their anticarcinogenicity are not well defined. We conducted studies to elucidate some of their modes of actions and the results are summarized in this review.

Table 1. Saponin Contents in soybeans

Product	Saponin
	g/100 g dry matter
Soymilk	0.26～0.39
Natto	0.41
Miso	0.10
Tofu	0.30～0.33
Okara	0.15
Yuba	0.25
Soybeans	0.50～5.6*

(Anderson & Wolf, 1995; *Okenfull, 1981)

Effect of soybean saponins on aflatoxin B1-induced DNA adduct formation

Genotoxic compounds and free radicals can cause DNA damages by forming DNAadducts, which can initiate cancer process. Previous studies (Gestetner, 1963; Sung, 1995b) indicated that saponins bind membrane lipid components affecting cell membrane permeability. Saponins from Quillaja bark have high affinity to cholesterol, while soybean saponins show a significant binding activity towards sphingomyelin (Sung, 1995b). When these saponins were incubated with red blood cells, soybean saponins were less hemolytic, and this difference may be related to their binding capacity to different membrane lipids. Saponin- membrane lipid complexes possibly change the permeability and/or fluidity of the membrane, which may affect the transport of genotoxins.

To test the ability of soybean saponins to suppress the formation of DNA-adduct by alflatoxin B_1, human colon epithelial cells were preincubated with saponins, and the formation of aflatoxin-DNA adduct was quantified (Jeon, 1998). Results showed soybean saponins inhibited DNA adduct formation by 50.71% at a concentration of 30 g/ml (Table 2). Apart from the changes in membrane permeability, soybean saponins may scavange aflatoxin B_1 epoxide. Recently, soybean saponins are reported to possess antioxidative activity (Kudou et al. 1994; Yoshiki and Okubo, 1995). The component DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) attached to the aglycon of soybean saponins, I, II, III, IV, and V, is known to exert their antioxidative capacity. Also, soybean saponins, at the concentration of 1 ppm, are shown to possess the superoxide quenching ability equivalent to 17.1 unit of SOD (Yoshiki and Okubo, 1995). In vitro antioxidative capacity of soybean saponin extracts were

tested in our lab, and saponins not only inhibit lipid peroxidation (Jeon and Sung, 1999a), but maintained antioxidative enzyme activities after tert-butyl hydroperoxide (t-BHP) treatment in hepatocytes (Park, 1998) implying that soybean saponins possess a strong antioxidatvie capacity. When the ability of soybean saponins to inhibit DNA-adduct formation in hepatocytes was compared to major antioxidants, soybean saponins showed significantly larger capacity to suppress the formation of DNA-adduct compared to either L-ascorbic acid or BHT (Table 3). These results indicate that the inhibitory action of soybean saponins may be drived from more than their radical scavenging capacity.

Table 2. Effect of soybean saponins on [^{3}H]AFB$_1$-DNA adduct formation in CCD-18Co normal human colon cells

Concentration (μg/ml)	AFB1-DNA adduct (fmol/μg DNA)	Injibition (%)[1)]
0	0.94±0.15	0^{c}
10	0.72±0.05	23.76±4.92^{b}
30	0.46±0.03	50.71±3.56^{a}
50	0.48±0.03	49.47±3.51^{a}

Inhibition (5)=100-[(dpm/ ug DNA test cell)/(dpm/ ug DNA control cell)×100]
Mean with different letters (a, b, c, d) within a row are significantly different from each other at $p<0.01$ as determined by Duncan's multiple range test.
(Jeon & Sung, 1999)

Growth inhibitory action mediated by PKC activity and differentiation

A number of growth factors and hormones mediate cell growth and function by binding with receptor proteins present in cell membrane. Receptor-growth factor complexes or receptor-hormone complexes exert their effects through signal transductions.

Protein kinase C (PKC) is known as the target for a number of tumor promoters. It has been implicated in the regulation of a range of important cellular processes in colonic epithelial cells including proliferation (Frawley et al., 1994), differentiation (Basson et al., 1995) and apoptosis (Medina et al., 1997). Diacylglycerol released from membrane phospholipids followed by an external stimulus activates PKC and procede intracellular signaling.

Table 3. Inhibition (%) of soybean saponins and major antioxidants on [^{3}H]AFB$_1$-DNA adduct formation in HepG2 cells

Treatment / Conc. (μg/ml)	Inhibition (%)				
	Soybeann saponi	L-ascorbic acid	α-tocopherol	BHT	All trans retinol
0			0z		
10	37.3±2.0^{ay}	22.2±4.9^{by}	34.5±4.7^{ay}	30.7±4.4^{aby}	42.0±4.2^{ay}
30	50.1±1.5^{bx}	38.4±3.4^{cdx}	44.6±2.9^{bcxy}	32.6±3.7^{dy}	59.8±2.2^{ax}
50	49.8±2.2^{x}	43.2±1.6^{x}	50.2±5.3^{x}	-	-

Mean with different letters (a, b, c, d) within a row are significantly different from each other at $p<0.01$ as determined by Duncan's multiple range test.
Means with different letters (x, y, z) within a column are significantly different from each other at $p<0.01$ as determined by Duncan's multiple range test.
(Jeon & Sung, 1999)

Also, TPA (12-O-tetradecanoylphorbol-13-acetate), a well-known tumor promotor, activates PKC by the translocation of the enzyme from the cytoplasmic compartment to the membrane fraction (Castagma et al., 1982). A previous report indicated that saponins extracted from soybeans possessed surface-active properties (Rao and Sung, 1995). Examination by transmission electron microscopy indicated soybean saponins induced deformations in plasma and nuclear membranes without abrupt membrane rupture.

To examine the effect of soybean saponins, as membrane activating compounds, on protein kinase C activity, human colon cancer cells (HT-29) were treated with saponins, and then stimulated for PKC activation (Oh and Sung, 2000). The PKC activity was measured by substrate phosphorylation. The phosphorylated substrate became negatively charged and was separated on electrophoresis unit. Results showed that soybean saponin pretreatment significantly reduced the TPA stimulated total PKC activity in a dose-dependent manner (Fig. 1). This implies that saponin-membrane interaction possibly affect PKC translocation and directly interfere in the activation of the enzyme. Since there are 12 different isoforms of PKC, and physiological importance of these isoforms in carcinogenesis require further investigations.

Promoters of tumor cell growth are known to inhibit normal cellular differentiation while accelerate undifferentiated cell growth. Therefore, the degree of differentiation can be used as a marker for the diagnosis of the earliest carcinogenic changes (Kim and Boland, 1992). Tumor promoters are known to influence cell differentiation, either by inhibiting normal differentiation or by inducing inappropriate differentiation (Yamasaki, 1984). A number of antitumor compounds are known to possess differentiation-inducing properties in tumor cells (Stewart et al., 1997; Heerdt et al., 1994; Tsao et al., 1982; Shabahang et al., 1994). Induction of differentiation is associated with a restoration of normal growth control in conjunction with an upregulation of production of the differentiation- related molecules such as carcinoembryonic antigen (CEA), an intercellular adhesion glycoprotein (Reynolds et al., 1998). Also, in colon tissues, undifferentiated crypt epithelial cells not only lose their normal morphology, but also had decreased the activities of enzymes including alkaline phosphatase, sucrase-isomaltase, and dipeptidyl peptidase (Aviram et al., 1988; Wiltz et al., 1991).

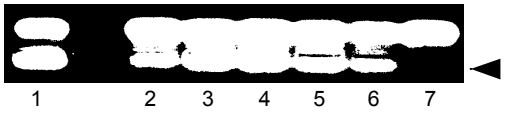

Fig. 1. Effect of soybean saponins on PKC activity induced by TPA in HT-29 colon adenocarcinoma cells. Phosphorylated band were electrophoretically separated on 0.8% agarose gels: lane 1, positive control, lane 2, no saponin (-TPA), lane 3, no saponin (+TPA), lane 4～6, TPA+saponin 150, 300, 600 ppm, lane 7, negative control. The arrow indicates the position of phosphorylated band by PKC (Oh & Sung 2001).

When soybean saponins were incubated with HT-29 cells, they stimulate the production of CEA (Fig. 2). Also, the activity of alkaline phosphatase (AP), a marker of differentiation in HT-29 cells (Herz et al., 1981) was increased (Fig. 3). AP is located on brush border membranes of intestinal cells, and its activity is significantly higher in more differentiated cells as compared to the less differentiated cells (Tsao et al, 1980). Several differentiation inducers in HT-29 cells include sodium selenite (Stewart et al., 1997), butyrate (Heerdt et al., 1994) retinoic acids (Tsao et al, 1982), and vitamin D_3 (Shabahang et al., 1994), and these are also well-known tumor suppressors. Membranopholic actions of vitamin D_3 have been suggested as critical aspects of calcium transport and an increase in intracellular calcium concentration is related to the induction of differentiation (Tanaka et al, 1989). Since soybean saponins are important membrane-activating compounds, the same hypothesis may be

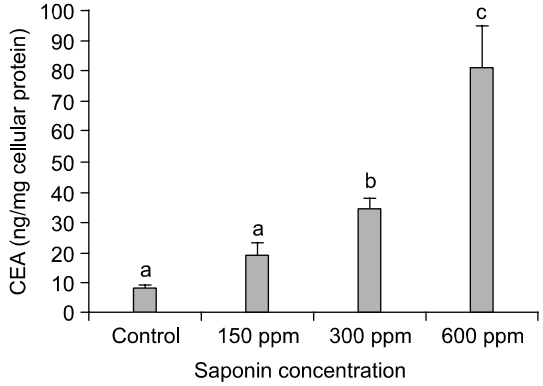

Fig. 2. Effect of soybean saponins on CEA production in HT-29 colon adenocarcinoma cells. Different concentrations of saponins were compared with two-way ANOVA. Pair-wise comparisons were made with the Duncans multiple range test. Values not sharing same letters are significantly different at $p<0.05$ (Oh & Sung 2001).

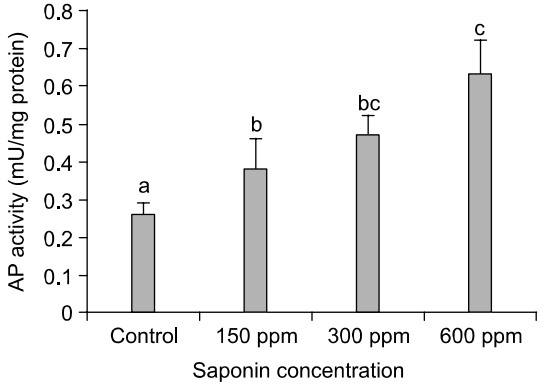

Fig. 3. Effect of soybean saponins on alkaline phosphatase activity in HT-29 colon adenocacinoma cells. Different concentrations of saponins were compared with two-way ANOVA. Pair-wise comparisons were made with the Duncans multiple range test. Values not sharing same letters are significantly different at $p<0.05$ (Oh & Sung 2001).

applied. When we measure apoptotic activity of soybean saponins in these cells, no significant effect was observed.

Anti-inflammatory action

A number of clinical and epidemiological studies have clearly shown that chemopreventive effect of non-steroidal anti-inflammatory (NSAIDs) against human colon cancer (Smalley and DuBois, 1997). For the metabolic targets of the NSAIDs, prostaglandin-dependent effects via COX-2 are well defined (Duggan et al., 1980; Meade et al., 1993). Normally, the expression of COX-2 is very low, however, it is transiently induced by hormonal stimulation and tissue damages, and upon colon tumor development. In addition to NSAIDs, a number of COX-2 inhibitors including antioxidants have been elucidated. The multistage carcinogenesis models using mouse epidermis and intestinal epithelium indicated that mitogen activated protein kinases (MAPKs) pathway is involved in the expression of COX-2 followed by prostaglandin E_2 (PGE_2) and Prostaglandin F_2 (PGF_2) synthesis, and both prostaglandins induce tumor promotion. TPA, LPS, and TNF-alpha are well-known activator through which MAPK pathway is activated and decomposition of Ikappa B is increased resulting in NF-kappa B activation and COX-2 expression (Furstenberger and Mark, 2000). Nitric oxide prodution during inflammatory responses is also known to be mediated by NF-kappa B activation, and nitric oxide stimulates COX-2 expression (Swierkosz et al., 1995). Apart from the proposed role of COX-2 in tumor promotion, it has been reported that this enzyme is involved in the regulation of colon cancer cell-induced angiogenesis by modulating the generation of several angiogenic factors (Tsuji et al., 1998). Based on these findings, a number of phytochemicals have been suggested to possess an anti-inflammatory property through blocking NF-kappa B activation and/or nitric oxide production.

Since soybean saponins exertd significant antioxidative activities, we then hypothesized these saponins inhibit inflammatory responses, which are

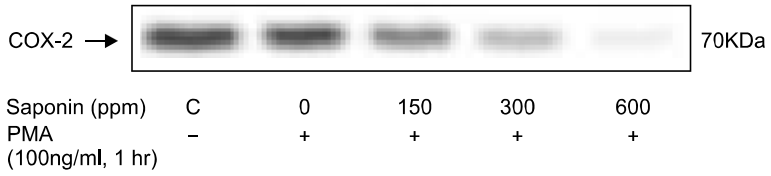

Fig. 4. Expression of PMA-induced COX-2 in HT-29 colon adenocarcinoma cells treated with saponin (Kim et al., 2002).

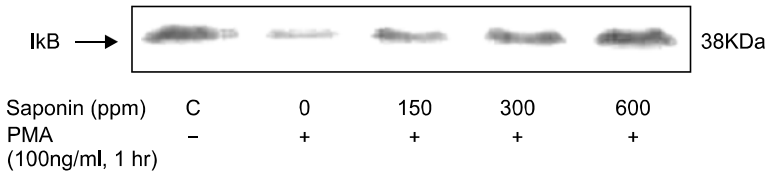

Fig. 5. Expression of IkB in HT-29 colon adenocarcinoma cells treated with saponin (Kim et al., 2002).

closely related to cancer cell growth and metastasis. Previous studies have shown that antioxidants are capable of suppressing several steps in the inflammation process (Surh, 1999; Ma and Kinneer, 2002). We examined effects of soy saponins on COX-2 expression and nitric oxide production in cell lines (Kim et al., 2002; Kang et al, 2002). When soysaponins at concentrations of 150, 300 and 600 ppm were incubated with human colon cancer cells (HT-29), PMA-induced COX-2 expression was decreased, and the decomposition of Ikappa B was reduced in a dose-dependent manner (Fig. 4, 5). When mice peritoneal macrophages were incubated with different concentrations of soy saponins, the production of pro-inflammatory molecules including PGE_2, TNF-alpha and MCP-1 was decreased in accordance with decreased COX-2 expression. In the same cell system, saponin treatment also decreased iNOS expression and nitric oxide production. These results clearly indiate that soybean saponins are effective anti-inflammatory agents and thereby may possibly suppress tumor development.

To the present, only a limited numbers of studies on chemopreventive effects of soybean saponins are executed. As presented in this review, soybean saponins may exert their anticarcinogenecity through diverse mechanisms. Further investigations on their structure-activity relationship and in vivo studies including human intervention studies are required to fully evaluate their role as anticarcinogens.

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