S2 - 3

Anticarcinogenic Effects of Trypsin Inhibitors, Lectins, Lunasin and Isoflavones in Soybean

Jong-Sang Kim

Department of Animal Science & Biotechnology, Kyungpook National University, Daegu 702-701, Korea. E-mail: vision@knu.ac.kr

INTRODUCTION

Cancer is one of the leading causes of death worldwide, generally exceeded only by cardiovascular disease in the developed world. The number of people diagnosed with cancer within the next few decades is expected to double. There will therefore be increased demand for novel diagnostic and medical therapies that use new non-traditional sources. Soy components have been proven to have cancer protective effect in many studies. Many lines of evidence also suggest that soy consumption may decrease risk of not only cancer but several chronic diseases such as coronary heart disease, renal disease, osteoporosis and post-menopausal complications. The U.S. Food and Drug Administration approved the health claim that eating 25 g/d of soy protein reduces the risk of heart disease on the basis of numerous studies demonstrating that soy protein reduces serum cholesterol levels (Norman et al., 2003).

The components responsible for cancer preventive activity have been extensively searched and narrowed down to isoflavones and some proteinous compounds including trypsin inhibitors, lectins, and lunasin.

This paper will give an overview of the most recent evidence on the possible chemopreventive effects of some protein components and isoflavones in soy.

Anticancer Effect of Trypsin Inhibitors

Traditionally protease inhibitors have been considered as anti-nutritional factors until 1980 when Dr. Walter Troll of the New York University Medical Center discovered that they inhibit cancer in animals. He reported that protease inhibitors inhibited breast cancer by 50%, and also skin and bladder cancers. Since then many scientists have reported the anti-carcinogenic effect of protease inhibitors on colon, lung, pancreas, mouth, and esophagus (Messina et al., 1994).

The soybean-derived protease inhibitor Bowman-Birk inhibitor (BBI) drew most attention due to its heat stability as well as a potent chemopreventive activity in both in vivo and in vitro carcinogenesis assay systems (Kennedy, 1998). BBI is a protein of a molecular weight of 8,000 with a well-characterized ability to inhibit trypsin and chymotrypsin. As an anticarcinogenic agent, BBI has been studied as purified BBI as well as in the form of a soybean extract in which BBI has been concentrated, termed BBI concentrate (BBIC); purified BBI works as well as BBIC as an anticarcinogenic agent over a range of doses in both in vitro transformation systems and in vivo carcinogenesis assay systems (St Clair et al., 1990; Kennedy et al., 1993). Because the use of purified BBI in a human trial would be prohibitive in cost, BBIC was developed for use in large-scale human cancer-prevention trials. BBIC has achieved Investigational New Drug Status (IND No. 34671; sponsor, Ann R Kennedy) from the US Food and Drug Administration (FDA), and trials to evaluate BBIC as an anticarcinogenic agent in human populations began in 1992 (Kennedy, 1998). In BBIC, the active anticarcinogenic activity has been shown to be chymotrypsin inhibitor activity, which is present only in BBI.

It is thought that the strength of BBI as a cancer preventive agent lies in its ability to reverse the initiation of cells. The ability to reverse initiation was observed first in in vitro studies (Yavelow et al., 1985). Similar results suggesting the reversal of the initiated state were observed in in vivo carcinogenesis studies (Kennedy et al., 1993). Both purified BBI and BBIC are effective as anticarcinogenic agents even when given long after carcinogen exposure, both in in vivo and in vitro studies. In animal studies in both oral and colon carcinogenesis, BBI and BBIC treatment can begin 3 months after carcinogen exposure in a 6-mo assay period and still suppress carcinogenesis. In systems such as these, premalignant lesions can be observed at 3 months. The yields of tumors and premalignant lesions being markedly reduced in BBI-treated animals suggest that BBI treatment destroys premalignant lesions.

It was previously believed that very small amounts of the soybean derived protease inhibitors would be taken up into the bloodstream and distributed to organs outside the gastrointestinal tract after dietary ingestion. However, several studies supported that a sufficient amount of BBI was taken up from the gastrointestinal tract and into the bloodstream to result in a cancer-preventive effect in most organ systems.

Anticancer Effects of Lectins and Lunasin

Recently, there has been increased interest in the potential health benefits of bioactive polypeptides and proteins from soybeans, including lunasin and lectins. Lectins are glycoproteins that selectively bind carbohydrates; lectins are used in medicine in a variety of new applications (de Mejia et al., 2003). Lunasin is a polypeptide that arrests cell division and induces apoptosis in malignant cells. Additional research, including clinical trials, should continue to examine and elucidate the therapeutic effects, nutritional benefits, and toxic consequences of commonly ingested soybean lectins and lunasin.

Lectins are a significant group of bioactive proteins found in almost all organisms, including plants, bacteria, and viruses. The ability of lectins to agglutinate cells is a well-recognized physiologic effect that is dependent on their specific, high-affinity binding to particular carbohydrate moieties on the cell surface. Lectins also combine with sugar residues of polysaccharides, glycoproteins, or glycolipids, which can be either independent or an integral part of cell membranes. Some lectins are composed of subunits with different binding sites. For instance, soybean seed lectin is a tetrameric 120-kD glycoprotein that accumulates during embryogenesis, normally constitutes 1 to 2% of the seed protein mass, and recognizes terminal α-linked 2-acetamido-2-deoxy-D-galactosyl or α- or β-D-galactosyl sugar residues. Because subunits have very different specificities for cell surface receptors, each combination is considered to have a different function. The accurateness of the different binding sites suggests that there are endogenous saccharide receptors in the tissues from which they are derived or on other cells with which the lectin is specialized to interact. These structure sites include metal binding sites, hydrophobic sites, glycosylation sites, and carbohydrate binding sites. Characterization of the primary structure, isoforms, and molecular modeling of lectins is a very active field of research. Dietary lectins have been shown to enter the systemic circulation intact. Although concentrations in blood are low, the overall effect of the uptake of dietary lectins might affect cell adhesion and proliferation of certain cells. Despite the beneficial qualities of soy protein in nutrition and health, adverse nutritional and other effects following consumption of raw soybean meal have been attributed to endogenous inhibitors of digestive enzymes, poor digestibility, and the presence of lectins. Soybean cultivars contain 6.5 g of lectin per kg of defatted meal. The ingestion of pure lectins in the diet of animals has several biochemical, physiologic, and nutritional implications. Pure soybean lectins can release cholecystokinin, thus stimulating pancreatic enzyme secretion in anesthetized rats. Once ingested, lectin activity largely persists during

passage through the gastrointestinal tract. Soybean lectins that bind to motifs containing N-acetyl-D-galactosamine tend to stimulate intestinal cells, and thus can interfere with intestinal absorption of nutrients. For these reasons, soybean-derived lectins have traditionally been recognized as having anti-nutritional properties. However, soybean lectins and endogenous inhibitors are largely eliminated by fractionation during food processing or inactivated by heat treatment. The hemagglutinating activity of lectins, for example, can be abolished within 5 minutes of heat treatment at 92°C on most soybean cultivars. Such treatment significantly improves the nutritional quality of soy foods. For example, soaked microwave-heated soybeans have a higher protein efficiency ratio compared with dry microwave-heated soybeans. The stability and resistance to degradation of soybean lectins depend on its tertiary and quaternary structures (de Mejia et al., 2003).

There is evidence that lectins may be involved in the recognition between cells or between cells and various carbohydrate-containing molecules involved in the regulation of physiologic functions. Lectins have been used as diagnostic reagents for stomach cancer, in cancer treatment combined with anticancer drugs, and in the removal of tumor cells from bone marrow, as well as in targeted drug delivery treatments. They are also involved in a variety of biomedical applications, including fractionation of bone marrow cells, the treatment of ulcerative colitis, and other biologic uses, such as cancer diagnosis and treatment.

Lunasin was discovered from mid-maturation soybean seeds in 1997 (Galvez et al., 1999; Galvez et al., 2001). This unique 43-amino acid soybean peptide, whose carboxyl end contains nine Asp (D) residues, an Arg-Gly-Asp (RGD) cell adhesion motif, and a helix with structural homology to a conserved region of chromatin-binding proteins, is now known as lunasin. Commercial soy products contain reasonable amounts of lunasin, ranging from 5.48 mg of lunasin/g of protein (defatted soy flour) to 16.52 mg of lunasin/g of protein (soy concentrate). Synthetic lunasin is heat stable, surviving temperatures up to 100°C for 10 minutes. Animal studies indicate that lunasin resists digestion, gets absorbed, and enters target tissues. Lunasin is now known to be a major component of the Bowman-Birk protease inhibitor (BBIC), a cancer-preventing component from soybeans Investigators believe that BBIC may protect lunasin from being digested in the gastrointestinal tract. Removal of lunasin from BBIC by antibody affinity column and resin treatment significantly reduced the ability of BBIC to inhibit cell transformation. In 1999, Galvez and coworkers (1999) discovered that injecting the lunasin gene into cancer cells arrested cell division and induced apoptosis. The antimitotic effect is thought to be related to lunasin's preferential binding to hypoacetylated chromatin, leading to the displacement of kinetochore proteins. Lunasin does not appear to affect kinetochore assembly as the cells go through normal mitosis. It rather inhibits the transformation of normal embryo fibroblast cells into cancerous cells by preventing chromatin acetylation and oncogene activation in cells with mutated tumor suppressor gene. Furthermore, lunasin competitively binds to membrane integrins required by metastatic cells for attachment to the extracellular matrix and/or proliferation. Lunasin appears to be alternative novel cancer chemopreventive agent from soybeans although further human bioavailability and toxicity studies must be performed prior to clinical use.

Anticancer Mechanism of Soy Isoflavones

Some early studies reported the conflicting results regarding tumor-promoting effect of soy proteins. After 1990, the situation was reversed and soy protein was emerged as potential chemopreventive agents. In particular, isoflavones in soy protein got most attention as preventive agents against several kinds of cancer. The estrogenic action of isoflavones was viewed as a negative property. However, during the past 10~15 y, beneficial effects have been observed in humans and some animals. Although confusion remains as to what isoflavones really are,

the phytoestrogens are widely accepted as chemopreventive agents. At least, prepubertal exposure to genistein was clearly confirmed to suppress carcinogen-derived tumorigenesis in rodent model.

Treatment of rats given a combination of 17β-estradiol and progesterone just before puberty led to a 90% reduction in mammary tumors induced by the carcinogen N-methyl-N-nitrosourea (Grubbs et al., 1985). Similarly, Lamartiniere's group (Lamartiniere et al., 2002; Fritz et al., 1998) showed that rats exposed to dietary levels of genistein during puberty have a lower incidence of mammary tumors when challenged by the carcinogen 7,12-dimethylbenz[a]anthracene (Barnes, 2004).

To search for possible anti-carcinogenic mechanism of isoflavones we examined the effect of isoflavones on protein expression profile in cell culture model using proteomic approach. Genistein appeared to regulate the expression and/or posttranslational modification of several heat shock proteins and glycolytic enzymes. Interestingly, genistein inhibited 2-deoxyglucose uptake in cancerous cells in dose-dependent manner, possibly providing another anticancer mechanism of isoflavones.

CONCLUSION

Soybeans contain a variety of anticarcinogenic phytochemicals. Recently, there has been increased interest in the potential health benefits of bioactive polypeptides and proteins from soybeans, including trypsin inhibitor, lunasin and lectins. BBI is a protein of a molecular weight of 8000 with a well-characterized ability to inhibit trypsin and chymotrypsin. Purified BBI and its concentrate, BBIC, have comparable suppressive effects on the carcinogenic process in a variety of in vivo and in vitro systems. Lunasin is a small polypeptide that arrests cell division and induces apoptosis in malignant cells. Lectins, glycoproteins that selectively bind carbohydrates, are used in medicine in a variety of new application and there is now convincing evidence for the antitumor activity of plant lectins. In recent years, the overall effects of the isoflavones in soy on human health have been the subject of lively debate largely based on their presumed estrogenic properties. The systematic identification of the cellular and biochemical targets of isoflavones and the mechanisms that they influence remains to be clarified. Modern biological approaches and high dimensional analysis techniques offer new ways to better understand the function of how cells and integrated biological mechanisms respond to compounds such as isoflavones. Data from experiments using proteomic analysis for examining the effects of genistein in mammalian tumor cells indicate that genistein alters the expression of several proteins such as heat shock proteins and enzymes involved in carbohydrate metabolism. Additional research, including clinical trials, should continue to examine and elucidate the therapeutic effects, nutritional benefits, and toxic consequences of commonly ingested soybean components.

ACKNOWLEDGEMENTS

This study was supported by the Korea Science and Engineering Foundation (R01-2001-000-00231-0).

REFERENCES

Barnes S. 2004. Soy isoflavones--phytoestrogens and what else? *J Nutr* 134: 1225S-1228S.

de Mejia EG, Bradford T, Hasler C. 2003. The anticarcinogenic potential of soybean lectin and lunasin. *Nutr Rev* 61: 239-246.

Fritz WA, Coward L, Wang J, Lamartiniere CA. 1998. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. *Carcinogenesis* 19: 2151-2158.

Galvez A, Chen N, Macasieb J, de Lumen BO. 2001. Chemopreventive property of a soybean peptide (lunasin) that binds to deacetylated histones and inhibits acetylation. *Cancer Res* 61: 7473-7478.

Galvez AF, de Lumen BO. 1999. A soybean cDNA encoding a chromatin-binding peptide inhibits mitosis of mammalian cells. *Nat Biotech* 17: 495-500.

Grubbs CJ, Farnell DR, Hill DL, McDonough KC. 1985. Chemoprevention of N-nitroso-N-methylurea-induced mammary cancers by pretreatment with 17 beta-estradiol and progesterone. *J Natl Cancer Inst* 74: 927-931.

Kennedy AR, Billings PC, Maki PA, Newberne P. 1993. Effects of various protease inhibitor preparations on oral carcinogenesis in hamsters induced by 7,12-dimethylbenz(a)anthracene. *Nutr Cancer* 19: 191-200.

Kennedy AR. 1998. The Bowman-Birk inhibitor from soybeans as an anticarcinogenic agent. *Am J Clin Nutr* 68(6 Suppl): 1406S-1412S.

Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, Elgavish A. 2002. Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. *J Nutr* 132: 552S-558S.

Messina M, Messina V. Soyfoods and cancer prevention (Ch 8), in Soybean and Health. Paragon Press, Honesdale, PA. p 77-88.

Norman HA, Butrum RR, Feldman E, Heber D, Nixon D, Picciano MF, Rivlin R, Simopoulos A, Wargovich MJ, Weisburger EK, Zeisel SH. 2003. The role of dietary supplements during cancer therapy. *J Nutr* 133(11 Suppl 1): 3794S-3799S.

St Clair W, Billings P, Carew J, Keller-McGandy C, Newberne P, Kennedy AR. 1990. Suppression of DMH-induced carcinogenesis in mice by dietary addition of the Bowman-Birk protease inhibitor. *Cancer Res* 50: 580-586.

Yavelow J, Collins M, Birk Y, Troll W, Kennedy AR. 1985. Nanomolar concentrations of Bowman-Birk soybean protease inhibitor suppresses X-ray induced transformation in vitro. *Proc Natl Acad Sci USA* 82: 5395-5399.