British Journal of Nutrition (2002), **88**, 439–441
DOI: 10.1079/BJN2002711

Invited commentary

Mechanistic explanations for the chemopreventive action of soyabean isoflavones: reducing the possibilities

There is increasing evidence in favour of a link between diets high in soyabeans, a rich source of the isoflavones genistein and daidzein, and reduced incidence of colon cancer and hormone-dependent cancers, such as breast and prostate cancer (for review, see Bingham *et al.* 1998). A number of studies in animal models demonstrate a chemopreventive effect of soyabean and/or genistein. For example, dietary soyabean and genistein inhibited the growth of transplantable tumours in rats (Schleicher *et al.* 1999; Zhou *et al.* 1999) and dietary isoflavones were shown to reduce the induction of tumours in rats by chemical carcinogens (Onozawa *et al.* 1999).

The isoflavones are polyphenolic compounds with structural similarity to mammalian oestradiol and belong, therefore, to a family of compounds referred to as phytoestrogens. The reported effects of genistein on cells are wide-ranging and numerous. Actions potentially involved in the chemopreventive effect of genistein include effects mediated through binding to oestrogen receptors (ER) (Kuiper *et al.* 1997), inhibition of DNA topoisomerase (Markovits *et al.* 1989), antioxidant action (Wei *et al.* 1993; Suzuki *et al.* 2002) and effects on cell signalling pathways. The latter include inhibition of tyrosine-specific protein kinases (Akiyama *et al.* 1987), inhibition of epidermal growth factor-induced phosphatidylinositol turnover (Imoto *et al.* 1988), inhibition of nuclear factor-κ B activation (Davis *et al.* 1999) and stimulation of transforming growth factor β synthesis and/or release (Kim *et al.* 2001). Induction of apoptosis, well established as a response of cancer cells to genistein (Balabhadrapathruni *et al.* 2000; Salti *et al.* 2000), is a potential link between a number of these cellular effects and anti-cancer action. Oestrogen promotes breast-cell proliferation, possibly through reduced apoptosis, and is considered a risk factor for breast cancer (Nenci *et al.* 1988). Therefore, genistein-induced apoptosis in breast-cancer cells may be the result of antagonism of oestrogen effects at ER. Po *et al.* (2002) address this possibility in a study reported in the present issue of the *British Journal of Nutrition*.

Po *et al.* (2002) show that genistein bound to the ER-α expressed heterologously in (ER-negative) HepG2 cells to increase expression of a reporter gene under the control of the oestrogen response element. They also demonstrate that genistein failed to antagonise stimulation by 17β-oestradiol of reporter gene expression. In other words, genistein is reported to show oestrogenic rather then anti-oestrogenic activity. In agreement with these observations, the paper reports induction by genistein in MCF-7 breast cancer cells of pS2 and Bcl-2 mRNA. Both are transcripts of genes well established as oestrogen-responsive (Ciocca & Elledge, 2000). Po *et al.* (2002) also demonstrate that, in MCF-7 cells, genistein increased expression of Bak, an apoptosis-promoting factor (Chittenden *et al.* 1995), and reduced expression of Bcl-xL, a factor that inhibits apoptosis (Cheng *et al.* 1996). All effects reported occurred at concentrations of genistein that were shown to induce apoptosis in MCF-7 cells. The evidence presented, therefore, does not support an anti-oestrogenic effect of genistein at ER-α in driving apoptosis. Commensurate with this new evidence is the finding that human breast-cancer cell lines did not differ in their apoptotic response to genistein, irrespective of whether or not they expressed ER (Shao *et al.* 1998).

It has been established that variants of MCF-7 cells differ in, among other features, their ER status. Some variants express no ER whilst others express ER-α or ER-α and -β (Burow *et al.* 2000; Jacobs *et al.* 2000). Po *et al.* (2002) do not confirm the ER status of the MCF-7 cells used in their study. The apoptotic response to genistein of the MCF-7 cells might, therefore, be ER-β-mediated. The results of reporter gene experiments like those presented by Po *et al.* (2002), but using ER-negative cells transfected with ER-β rather than ER-α, will establish unequivocally whether genistein antagonises the action of 17β-oestradiol at ER-β. Similarly, the study by Shao *et al.* (1998) reporting that ER-positive and -negative breast-cancer cell lines did not differ in their anti-proliferative response to genistein did not distinguish between expression of ER-α and/or -β. Studies comparing the effects of genistein in cell lines of precisely defined ER status will be of value in establishing the relative importance of genistein interaction with the respective receptor subtypes in chemopreventive action.

Studies on the biological action of genistein using cell line models offer advantages in terms of precise dosing and ease of molecular analysis. A limitation of such studies, however, is that they focus on the biological actions of the parent compound. Genistein is extensively metabolised *in vivo* to glucuronides (Adlercreutz *et al.* 1995) and by cytochrome P450 to hydroxylated derivatives (Roberts-Kirchoff *et al.* 1999; Kulling *et al.* 2001). Furthermore, halogenated and nitrated derivatives of genistein can be formed as a result of reaction with oxidants produced by inflammatory cells (Boersma *et al.* 2001). It

has been proposed that the anti-cancer effect of soyabean isoflavones may be attributable partly to competitive inhibition of carcinogen-activating enzymes (Setchell *et al.* 1984). Whilst metabolism of genistein by enzymes endogenous to breast-cancer cell lines has been reported (Peterson *et al.* 1996), systemic metabolism and metabolism at the target site that requires enzymes not expressed in cell line models is likely to modulate the biological effects.

There remain many possible mechanisms through which soyabean isoflavones may effect chemopreventive action. The activity of isoflavone metabolites requires elucidation and, through polymorphism in the relevant enzymes, may have implications with regard to inter-individual variability in chemopreventive response to dietary isoflavones. In addition to potential effects mediated through binding to ER-α, relevant actions may be ER-β-mediated or mediated through non-oestrogenic pathways and may be cell-type specific. The paper presented by Po *et al.* (2002) makes an important contribution to this field of research by adding to accumulating evidence (e.g. Shao *et al.* 1998) that, at least in breast-cancer cell lines, effects through binding to ER may not be of fundamental importance.

Dianne Ford

School of Cell and Molecular Biosciences
University of Newcastle
Kings Road
Newcastle UponTyne
NE1 7RU
UK

References

Adlercreutz H, van der Wildt J, Kinzel J, Attalla H, Wahala K, Makela T, Hase T & Fotsis T (1995) Lignan and isoflavonoid conjugates in human urine. *Journal of Steroid Biochemistry and Molecular Biology* **52**, 97–103.

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M & Fukami Y (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *Journal of Biological Chemistry* **262**, 5592–5595.

Balabhadrapathruni S, Thomas TJ, Yurkow EJ, Amenta PS & Thomas T (2000) Effects of genistein and structurally related phytoestrogens on cell cycle kinetics and apoptosis in MDA-MB-468 human breast cancer cells. *Oncology Reports* **7**, 3–12.

Bingham SA, Atkinson C, Liggins J, Bluck L & Coward A (1998) Phyto-oestrogens: where are we now? *British Journal of Nutrition* **79**, 393–406.

Boersma BJ, Barnes S, Kirk M, Wang C-C, Smith M, Kim H, Xu J, Patel R & Darley-Usmar VM (2001) Soy isoflavonoids and cancer – metabolism at the target site. *Mutation Research* **480–481**, 121–127.

Burow ME, Weldon CB, Chiang TC, Tang Y, Collins-Burow BM, Rolfe K, Li S, McLachlan JA & Beckman BS (2000) Differences in protein kinase C and estrogen receptor alpha, beta expression and signalling correlate with apoptotic sensitivity of MCF-7 breast cancer cell variants. *International Journal of Oncology* **16**, 1179–1187.

Cheng EH, Levine B, Boise LH, Thompson CB & Hardwick JM (1996) Bax-independent inhibition of apoptosis by Bcl-XL. *Nature* **379**, 554–556.

Chittenden T, Harrington EA, O'Connor R, Flemington C, Lutz RJ, Evan GI & Guild BC (1995) Induction of apoptosis by the Bcl-2 homologue Bak. *Nature* **374**, 733–736.

Ciocca DR & Elledge R (2000) Molecular markers for predicting response to tamoxifen in breast cancer patients. *Endocrine* **13**, 1–10.

Davis NJ, Kucuk O & Sarkar FH (1999) Genistein inhibits NF-kappa B activation in prostate cancer cells. *Nutrition and Cancer* **35**, 167–174.

Imoto M, Yamashita T, Sawa T, Kurasawa S, Naganawa H, Takeuchi T, Bao-quan Z & Umezawa K (1988) Inhibition of cellular phosphatidylinositol turnover by psi-tectorigenin. *FEBS Letters* **230**, 43–46.

Jacobs E, Bulpitt PC, Coutts IG & Robertson JF (2000) New calmodulin antagonists inhibit in vitro growth of human breast cancer cell lines independent of their estrogen receptor status. *Anticancer Drugs* **11**, 63–68.

Kim H, Xu J, Su Y, Xia H, Li L, Peterson G, Murphy-Ullrich J & Barnes S (2001) Actions of the soy phytoestrogen genistein in models of human chronic disease: potential involvement of transforming growth factor beta. *Biochemical Society Transactions* **29**, 216–222.

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S & Gustafsson JA (1997) Comparison of the ligand binding specificity and transcript tissue distribution of the estrogen receptors alpha and beta. *Endocrinology* **138**, 863–870.

Kulling SE, Honig DM & Metzler M (2001) Oxidative metabolism of the soy isoflavones daidzein and genistein in humans in vitro and in vivo. *Journal of Agriculture and Food Chemistry* **49**, 3024–3033.

Markovits J, Linassier C, Fosse P, Couprie J, Pierre J, Jacquemin-Sablon A, Saucier JM, Le Pecq JB & Larsen AK (1989) Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian topoisomerase II. *Cancer Research* **49**, 5111–5117.

Nenci I, Marchetti E & Querzoli P (1988) Commentary on human mammary pre-neoplasia: the estrogen receptor hypothesis. *Journal of Steroid Biochemistry and Molecular Biology* **30**, 105–106.

Onozawa M, Kawamori T, Baba M, Fukuda K, Toda T, Sato H, Ohtani M, Akaza H, Sugimura T & Wakabayashi K (1999) Effects of a soybean isoflavone mixture on carcinogenesis in prostate and seminal vesicles of F344 rats. *Japanese Journal of Cancer Research* **90**, 393–398.

Peterson TG, Coward L, Kirk M, Falany CN & Barnes S (1996) The role of metabolism in mammary epithelial cell growth inhibition by the isoflavones genistein and biochanin A. *Carcinogenesis* **17**, 1861–1869.

Po LS, Wang TT, Chen Z-Y & Leung LK (2002) Genistein-induced apoptosis in cell lines involves changes in Bak and Bcl-x without evidence of anti-oestrogenic effects. *British Journal of Nutrition* **88**, 463–469.

Roberts-Kirchoff ES, Crowley JR, Hollenberg PF & Kim H (1999) Metabolism of genistein by rat and human cytochrome P450s. *Chemical Research in Toxicology* **12**, 610–616.

Salti GI, Grewal S, Mehta RR, Das Gupta TK, Boddie AW Jr & Constantinou AI (2000) Genistein induced apoptosis and topoisomerase II-mediated DNA breakage in colon cancer cells. *European Journal of Cancer* **36**, 796–802.

Schleicher RL, Lamartiniere CA, Zheng M & Zhang M (1999) The inhibitory effect of genistein on the growth and metastasis of a transplantable rat accessory gland carcinoma. *Cancer Letters* **136**, 195–201.

Setchell KDR, Borriello SP, Humle P, Kirk DN & Axelson M (1984) Non-steroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *American Journal of Clinical Nutrition* **40**, 569–578.

Shao Z-M, Alpaugh ML, Fontana JA & Barsky SH (1998) Genistein inhibits proliferation similarly in estrogen receptor-positive and negative cell lines characterised by P21WAF1/CIP1 induction, G2/M arrest and apoptosis. *Journal of Cellular Biochemistry* **69**, 44–54.

Suzuki K, Koike H, Matsui H, Ono Y, Hasumi M, Nakazato H, Okugi H, Sekine Y, Oki K, Yamamoto T, Fukabori Y, Kurokawa K & Yamanaka H (2002) Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. *International Journal of Cancer* **99**, 846–852.

Wei H, Wei L, Frenkel K, Bowen R & Barnes S (1993) Inhibition of tumour promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. *Nutrition and Cancer* **20**, 1–12.

Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL & Clinton SK (1999) Soybean phytochemicals inhibit the growth of transplantable prostate carcinoma and tumour angiogenesis in mice. *Journal of Nutrition* **129**, 1628–1635.