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The soy isoflavone daidzein improves the capacity of tamoxifen to prevent mammary tumours☆

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Abstract

The aim of this study was to determine how the efficacy of tamoxifen is affected when combined with soy isoflavones. To address this, female Sprague–Dawley rats were placed on diets supplemented with tamoxifen, genistein, daidzein, or a combination of each isoflavone with tamoxifen; a week later mammary tumours were induced by 7,12 dimethylbenzanthracene. The most effective diet was the tamoxifen/daidzein combination. It reduced tumour multiplicity by 76%, tumour incidence by 35%, tumour burden by over 95%, and increased tumour latency by 62% compared with positive controls. The tamoxifen/daidzein combination diet was in all aspects more effective while the tamoxifen/genistein combination was less effective than the tamoxifen diet. The tamoxifen/daidzein diet significantly decreased 8-oxo-deoxyguanosine levels (an indicator of oxidative DNA damage) in the mammary glands. This study conclusively shows for the first time the combination of daidzein with tamoxifen produces increased protection against mammary carcinogenesis, while the combination of genistein with tamoxifen produces an opposing effect when compared with tamoxifen alone.

Keywords: Breast cancer; Chemoprevention; Daidzein; DNA damage; DMBA; Equol; Genistein; Isoflavones; Soy; Tamoxifen

1. Introduction

In 1998, the National Surgical Adjuvant Breast and Bowel Project (NSABP) demonstrated that tamoxifen treatment reduced the incidence of both invasive and non-invasive breast cancer in women at high risk for the disease [1]. Following the results of this study, women at high risk for developing breast cancer are prescribed tamoxifen to prevent primary breast cancer; tamoxifen is also prescribed to those women with oestrogen receptor (ER)-positive breast cancer to prevent secondary tumours. In recent years, soy phyto-oestrogens (mainly the isoflavones genistein and daidzein) are being increasingly used as dietary supplements among women of western societies for their apparent benefits against breast cancer, cardiovascular disease, and postmenopausal symptoms. Consequently, some women who are prescribed tamoxifen may also consume soy products or take a mixture of isoflavones, composed mainly of genistein and daidzein, as dietary supplements.

Most previous studies have focused on genistein, due to its relatively strong binding (in comparison to daidz-

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ein) to ERβ and ERα and its oestrogenic/anti-oestrogenic activities which are stronger than those of other isoflavones [2–4]. At relatively high concentrations and *in vitro*, genistein is known to inhibit enzymatic activities crucial for tumour cell proliferation [5], such as the protein tyrosine kinases [6] and topoisomerase II [7–9]. Genistein has also been found to be effective in preventing dimethylbenzanthracene (DMBA)-induced mammary tumorigenesis in female rats, but only when administered to neonatal or prepubescent rats younger than 35 days old [10,11]. However, another study found genistein to have no protective effect on DMBA-induced mammary tumours in mice and even suggested a potentially adverse effect on tumour development when high levels of genistein are consumed [12]. Two studies reported no significant differences in the effects of isoflavone-containing or -depleted soy protein isolate in DMBA-induced mammary carcinogenesis [13,14]. The effects (adverse or beneficial) of soy and genistein on breast cancer risk reduction have been reviewed [15,16].

Daidzein has over 60-fold weaker affinity for ERβ than genistein, and it does not inhibit protein tyrosine kinases or topoisomerases. Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman], a metabolite of daidzein, had been reported initially to have a strong affinity for both ERα and ERβ, but in recent studies it failed to bind to recombinant ERα and therefore it is considered to be selective for ERβ [17]. The affinity of equol for ERβ is similar to that of genistein, displaying an affinity approximately 200-fold less than 17β-oestradiol [17]. Equol was determined to induce transcription more effectively than any other isoflavone [18]. Equol is exclusively a product of intestinal bacteria. Due to their large caecum and abundance of microflora, rats produce large quantities of equol. However, only approximately 53% of humans are ''equol-producers'' [19].

At present, there is no clear understanding how individual soy isoflavones affect the risk of developing breast cancer when combined with tamoxifen. Previous studies raised the possibility that genistein may compete with tamoxifen for the oestrogen receptors and, consequently, the consumption of soy products could reduce the efficacy of tamoxifen [20,21]. In a recent study, genistein negated the inhibitory effect of tamoxifen on the growth of oestrogen-dependent MCF-7 cells implanted in athymic mice [22], raising more concerns about the combined use of tamoxifen and soy products. Based on these results, medical practitioners in the United States suggest women who have been prescribed tamoxifen avoid consuming soy products or taking soy isoflavone supplements that contain genistein, the presumed active component of soybeans.

However, experimental evidence is beginning to support the possibility that a soy component (or combination of components) may work together with tamoxifen to provide a stronger antitumour effect than tamoxifen alone. For example, miso (containing soy isoflavones) in combination with tamoxifen was more effective than tamoxifen alone in preventing *N*-nitroso-*N*-methylurea-induced rat mammary cancers [23]. We have also found that tamoxifen combined with soy protein isolate provides more protection against DMBA-induced mammary carcinogenesis in rats than it does by itself [24]. These findings support the premise that one or more soy component(s) work in the same direction as tamoxifen to provide protection against mammary carcinogenesis.

Our first goal was to identify the components of soybeans working together with tamoxifen to prevent mammary tumours in female Sprague–Dawley rats. Our second goal was to gain insight into possible mechanisms of action.

2. Materials and methods

2.1. Chemicals and tissue culture

All chemicals, unless otherwise specified, were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Genistein and daidzein were purchased from the Indofine Chemical Co. (Somerville, NJ, USA). All tissue culture components were obtained from Invitrogen Corp. (Carlsbad, CA, USA) unless otherwise stated.

2.2. Chemoprevention study

The diets were formulated by Harlan–Teklad (Madison, WI, USA) to contain approximately the same amounts of protein, fat, and carbohydrates. The basal diet AIN-76A is free of soy products. The diets were also adjusted to contain similar amounts of methionine, cystine, and choline because these nutrients have been reported to affect mammary carcinogenesis. Daidzein and genistein were added in the basal diets as necessary (Table 1), at 140 and 105 mg/kg of diet, respectively. The selected isoflavone concentrations were based on the amounts present in the well tolerated 16% (w/w) SPI diet [13]. The tamoxifen dose of 0.125 mg/kg diet was selected based on a previous study [24]. Additional information relating to the other components of the diets has been reported previously in [13].

Virgin female Sprague–Dawley rats at 35 days of age were randomised by weight into five groups (20 rats per group) and fed the basal AIN-76A diet. At 43 days of age, all but the control groups received basal diet supplemented with the appropriate test agents. After an additional week, the animals received a single dose of DMBA intragastrically (12.5 mg) in sesame oil.

During the experimental period, the animals were weighed weekly. Palpation of mammary tumours began 4 weeks after the animals received DMBA and contin-

Table 1
Composition of diets and their effects on final tumour incidence, incidence rate, multiplicity, survival, and body weight

Group	Added diet component (mg/kg diet)	Final tumour incidence[a] (%)	Incidence rate (*P*-value)[b]	Multiplicity (*P*-value)[c]	Latency (*P*-value)[d]	Survival (%)	Mean body weight (% of control)
+Control (DMBA)	None	95	–	–	–	90	100.0
TAM	0.125	73*	0.015	0.012	0.013	90	94.4
GEN	140.0	85	0.408	0.596	0.326	95	100.7
DAI	105.0	85	0.366	0.049	0.322	95	99.2
TAM/GEN	0.125/140.0	79	0.823	0.029	0.280	89	96.3
TAM/DAI	0.125/105.0	60**	0.005	0.001	0.002	100	94.4

Abbreviations: DMBA, dimethylbenzanthracene; TAM, tamoxifen; GEN, genistein; DAI, daidzein.
All Statistical comparisons were made for experimental groups ($n = 20$) versus control (DMBA) group.
[a] Comparisons were made by Fisher's exact test. Statistical significance is as follows: *, $P < 0.05$; **, $P < 0.01$.
[b] Comparison of data shown in Fig. 1(a) with log-rank analysis [26].
[c] Comparison of data shown in Fig. 1(b) with one-tailed test for trends in proportions.
[d] Comparisons made by unpaired *t*-test.

ued until termination of the study. The date of appearance and location of every palpable tumour were recorded. Rats were observed daily to assess their general health. Rats were sacrificed by CO_2 asphyxiation. Mammary tumours were coded by location, removed from the rat, and weighed. Sections of normal mammary tissue were also taken and either flash-frozen in liquid nitrogen or formalin-fixed for paraffin-embedding. The experiment and protocol was approved and performed in compliance with relevant laws and institutional guidelines according to the Animal Care Committee (01-142) and Animal Welfare Assurance (A3460.01) at the University of Illinois at Chicago.

2.3. DNA hydrolysis

DNA was extracted from normal mammary tissue from six different rats from each group and dissolved in water at approximately 2 mg/ml. Aliquots (50-μl) containing approximately 100 μg of DNA were mixed with 50 μl of buffer (50 mM ammonium acetate, 0.2 mM $ZnCl_2$, pH 5.3), 10 μl of nuclease P1 (0.4 unit/μl), and 8 μl of alkaline phosphatase (1 unit/μl in 10 mM Tris pH 7.4) and incubated at 37 °C for 30 min. For DNA oxidation detection, a 60-μl aliquot of the DNA digest and 10 μl of internal standard mixture (containing 3.0 ppb [$^{13}C_{10}$, $^{15}N_5$]-8-oxo-dG and 1.5 ppb [$^{13}C_{10}$, $^{15}N_5$]-8-oxo-dA) were combined in a 30,000 molecular weight cut-off ultrafiltration centrifuge tube (Amicon; Bedford, MA, USA). The solution was centrifuged for 15 min at 12 000 rotations per minute (rpm) and 4 °C just before LC–UV–MS–MS analysis.

2.4. LC–UV–MS–MS quantification of oxidised nucleosides

The high-performance liquid chromatography (HPLC) system consisted of a ThermoFinnigan (San Jose, California USA) Surveyor MS pump coupled with an autosampler, a PDA detector and a YMC (Wilmington, NC, USA) ODS-AQ C_{18} column (2.0 × 250 mm, 5 μm), and guard column (4.0 × 20 mm). Absorbance detection at 240–290 nm was used for on-line HPLC quantification of dG and dC in the DNA hydrolysate, and a standard curve was prepared using standard solutions of dG and dC in the initial mobile phase. The flow rate was 200 μl/min, and the injection volume was 20 μl. For DNA oxidation, the solvent system consisted of water/methanol 90:10 (v/v) at the start point and a linear gradient from 10 to 25% (v/v) methanol over 0–25 min. Then the methanol was increased to 90% (v/v) in 30 s. After that, the mobile phase was held at 90% (v/v) methanol for 7.5 min.

Selected reaction monitoring (SRM) was carried out using a TSQ Quantum triple quadruple mass spectrometer equipped with negative ion (for DNA oxidation) electrospray ionisation. Nitrogen was used as the sheath gas and auxiliary gas at 25 and 10 arbitrary units, respectively. The spray voltage was 30 eV. Collision-induced dissociation (CID) was carried out using argon at 1.0–1.5 mTorr with the collision energy of 10–20 V. The span-time per ion channel was 0.5 s during SRM. For 8-oxo-deoxyguanosine (8-oxo-dG), m/z 282 > 192 was selected as SRM (m/z 297 > 204 for the isotopically labelled internal standard). For 5-MedC, m/z 242 > 126 was selected as SRM (m/z 245 > 129 for the isotopically labelled internal standard).

2.5. LC–MS analysis of isoflavone levels

Serum isoflavone levels of animals on the diets for 4 weeks without DMBA treatment were analysed. Blood was collected by orbital bleeding. Serum (400 μl) from each sample was transferred into a 2-ml Eppendorf tube. To each tube, 100 μl of 1 M (pH 5.0) buffer was added followed by vortex mixing, then 40 μl sulphatase (50 mg/ml, Sigma # S-3009) enzyme solution was added. After shaking, the mixture was incubated overnight in a 37 °C water bath (>17 h). The enzymatic hydrolysis was stopped by adding 1 ml ice-cold

ethanol/acetonitrile (1:1; v/v), mixed and centrifuged at 4 °C for 15 min (10 000 rpm). The supernatant was removed and evaporated to dryness under vacuum. The resulting residue was reconstituted with 150 μl internal standard solution, and three aliquots (15 μl each) were analysed using LC–MS (HP 1100 LC–MSD) over an Xterra RP-18 column (2.1 × 100 mm, 3.5 μm). The mobile phase was 0.1% (v/v) formic acid followed by acetonitrile (containing 0.1% (v/v) formic acid). Elution was at 200 μl/min. The MSD was operated in negative electrospray ionisation mode with selected ion monitoring of *m/z* 253 for daidzein and internal standard (chrysin), *m/z* 269 for genistein, and *m/z* 241 for equol. Three to four samples from each group were analysed and resulting values are means ± standard deviations (SD).

2.6. Statistical analysis

A significant inhibition of tumour induction achieved by administration of an inhibitor was defined as a statistically significant decrease (at 5% level) in tumour incidence, multiplicity, or latency period. Statistical comparison of tumour latency between groups was by unpaired *t*-test. The statistical significance of differences between mean tumour multiplicities was assessed using analysis of variance (ANOVA), and the Armitage's test for trends in proportions [25]. Tumour incidence rates were generated by the life-table method, and compared by (one tailed) log-rank analysis [25].

3. Results

3.1. Effects of diets on tumour incidence, multiplicity, latency and burden

The effects of the experimental diets on tumour incidence and multiplicity over the length of the study (114 days) are shown in Fig. 1. A decrease in the tumour incidence rate was evident in all groups fed the experimental diets in comparison to the group fed the basal AIN76A diet and DMBA (+control). The effect of genistein- or daidzein-containing diets on the tumour incidence rate was statistically non-significant ($P = 0.408$, $P = 0.366$, respectively, Fig. 1(a) and Table 1). Tamoxifen produced a significant reduction ($P = 0.015$) and the tamoxifen/daidzein combination diet was the most effective in reducing incidence rate ($P = 0.005$). Daidzein improved the effect of tamoxifen with respect to tumour incidence, whereas genistein weakened it, and the tamoxifen/genistein combination failed to produce a significant reduction in the tumour incidence rate in comparison to the control ($P = 0.823$). All diets containing tamoxifen produced a small effect on body weight, but it remained within 94% of the control value (Table 1).

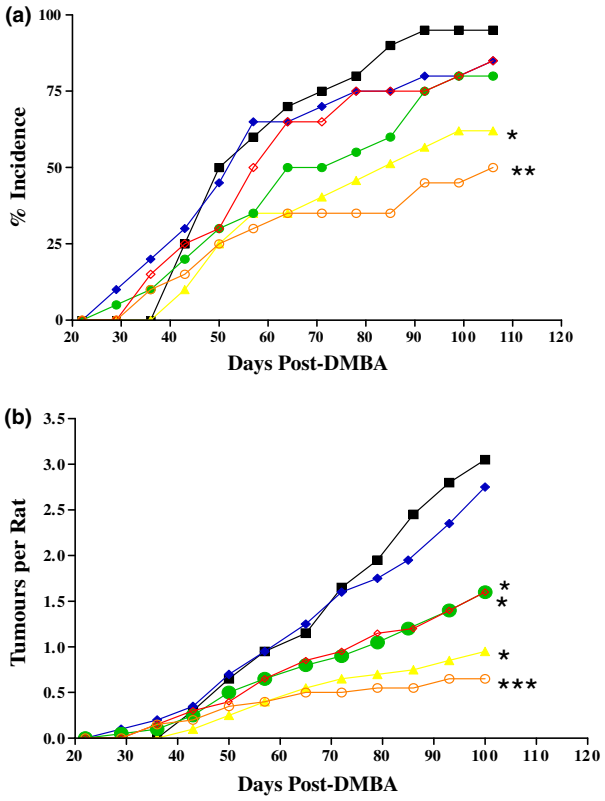

Fig. 1. The effect of experimental diets, on tumour incidence (a) and mean number of tumours (b) in female Sprague–Dawley rats exposed to dimethylbenzanthracene (DMBA). Rats ($n = 20$) were started on basal AIN-76A diet (black) or diets containing tamoxifen (TAM) (yellow), genistein (GEN) (blue), TAM/GEN (green), daidzein (DAI) (red), or TAM/DAI (orange) 1 week prior to administration of DMBA. Rats were maintained on the diet throughout the experiment. Tumours were detected by palpation during the study. *, $P < 0.05$ vs.+control. **, $P < 0.01$ vs.+control. ***, $P = 0.001$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Tumour multiplicity was significantly reduced in the groups fed tamoxifen ($P = 0.012$), daidzein ($P = 0.049$), tamoxifen/daidzein ($P = 0.001$) and tamoxifen/genistein ($P = 0.029$). Thus, daidzein improved and genistein reduced the effect of tamoxifen with respect of tumour multiplicity (Fig. 1(b) and Table 1).

Tumour latency increased in all experimental groups in comparison to the control group (Fig. 2(a) and Table 1). However, the increase was statistically significant only in the tamoxifen ($P = 0.013$), and tamoxifen/daidzein ($P = 0.002$) groups (Table 1). The increase over the control value in the tamoxifen group was 46% compared with 62% in the tamoxifen/daidzein group and 18% in the tamoxifen/genistein group. Therefore, daidzein improved the effect of tamoxifen on tumour latency and genistein curtailed it.

A similar effect was evident in tumour burden (Fig. 2(b)), defined as the average tumour weight per group. The mean tumour weight in the control group was

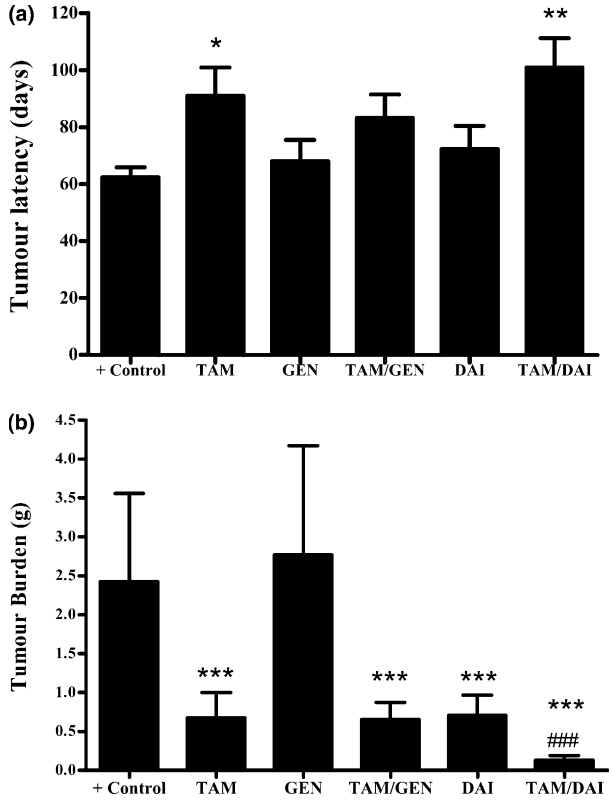

Fig. 2. The effect of experimental diets on mean latency (a) and tumour burden (b) of mammary tumours in female Sprague–Dawley rats exposed to dimethylbenzanthracene. Latency was determined by the first appearance of palpable tumours in the rats. Data are means with standard deviations (SD) shown as T-bars ($n = 20$). Statistical comparison of tumour latency between groups was by the unpaired t-test. Abbreviations: TAM, tamoxifen; GEN, genistein; DAI, daidzein. Statistical significance is as follows: *, $P < 0.05$ vs.+control group. **, $P < 0.01$ vs.+control group. ***, $P < 0.001$ vs.+control group. ### , $P < 0.001$ TAM/DAI group vs. TAM group.

2.5 g, but ranged greatly from 0.01 to 14 g. Daidzein reduced tumour burden as effectively as tamoxifen, while genistein proved ineffective. The tamoxifen/daidzein and tamoxifen/genistein combinations also reduced tumour burden significantly in comparison to the control value. The reduction in tumour burden by the tamoxifen/daidzein group was statistically significant even in comparison to tamoxifen ($P < 0.001$). Therefore, the combination of genistein with tamoxifen neither improved nor attenuated the effectiveness of tamoxifen on average tumour burden, whereas a substantial improvement was evident in the group fed the tamoxifen/daidzein combination diet.

3.2. Effect of diets on oxidative DNA damage and serum isoflavone levels

LC–UV–MS–MS was used to determine oxidative DNA damage in the mammary glands, a method known to be highly selective and sensitive. This is the most suitable method for detecting the oxidation product 8-oxo-deoxyguanosine (8-oxo-dG), considered to be one of the most critical lesions leading to carcinogenesis. The effect of the experimental diets on the ratio of 8-oxo-dG/dG was compared with that of the control diets, and the results are shown in Fig. 3. As expected from an earlier report [26], the 8-oxo-dG levels in the DMBA group (+control) were higher than the group not exposed to DMBA (−control). Tamoxifen, genistein, and tamoxifen/genistein diets were ineffective in suppressing these DMBA-induced DNA alterations. However, daidzein significantly ($P < 0.05$) suppressed the levels of 8-oxo-dG equal to those of the group never exposed to DMBA (−control). The tamoxifen/daidzein combination markedly lowered the damage with respect to the +control ($P < 0.001$). More importantly, the tamoxifen/daidzein diet lowered oxidative DNA damage to the levels below the −control group ($P < 0.05$). This finding suggests the tamoxifen/daidzein combination not only blocks DMBA initiated oxidative DNA damage, but it also reduces endogenous oxidative DNA damage. Thus, both diets containing daidzein are effective against oxidative DNA damage, as determined by measuring the 8-oxo-dG levels.

The genistein, daidzein, and equol isoflavone levels were measured in the serum of 3–4 animals from groups on the same diets for 4 weeks and were never exposed to DMBA. As expected, the control (basal diet) and tamoxifen groups had very low levels of genistein and daidzein. The genistein and tamoxifen/genistein groups showed very low levels of daidzein; the daidzein and tamoxifen/daidzein groups had very low levels of genistein. Equol, the end product of the metabolic pathway of daidzein [19], was also measured in the serum of the

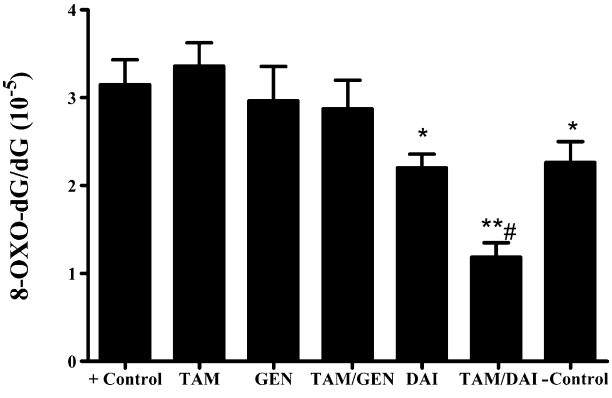

Fig. 3. LC–MS–MS analysis of 8-oxo-deoxyguanosine (8-oxo-dG) from the normal mammary glands of female Sprague–Dawley rats fed control or experimental diets. All groups were exposed to dimethylbenzanthracene (DMBA) except −control. Data representing the ratio of 8-oxo-dG to deoxyguanosine (dG) are means of triplicate samples with SD shown as T-bars ($n = 6$). Statistical significance is as follows: *, $P < 0.05$ vs.+control group. **, $P < 0.01$ vs.+control group; #, $P < 0.05$ TAM/DAI group vs. −control. Abbreviations: TAM, tamoxifen; GEN, genistein; DAI, daidzein.

Table 2
Mean serum levels (ng/ml) ±SEM[a] of soy isoflavones in female rats fed the control and experimental diets for 4 weeks

Diet	GEN	DAI	Equol
Basal (control)	1.2 ± 0.1	0.2 ± 0.1	0.0
TAM	0.8 ± 0.1	0.2 ± 0.0	0.0
GEN	116.3 ± 11.3	0.8 ± 0.2	0.0
TAM/GEN	119.7 ± 2.6	1.0 ± 0.1	0.0
DAI	1.2 ± 4.7	3.6 ± 0.9	115.0 ± 20.6
TAM/DAI	3.9 ± 1.0	3.6 ± 0.4	148.0 ± 16.5

Abbreviations: TAM, tamoxifen; GEN, genistein; DAI, daidzein; SEM, standard error of the mean.

[a] Serum was obtained from 3–4 rats from each group and (LC–MS) analysis was performed in triplicate for each serum sample. The MSD was operated in negative electrospray ionisation mode with selected ion monitoring of *m/z* 253 for DAI and *m/z* 241 for equol, as described in the Section 2.

rats not exposed to DMBA. The equol levels in the control, tamoxifen, genistein, and tamoxifen/genistein groups were not detectable, although these groups contained small quantities of genistein and daidzein (Table 2). Equol was present at very high concentrations in the daidzein and tamoxifen/daidzein groups, although daidzein itself was present at much lower concentrations (Table 1), suggesting the vast majority of daidzein had been metabolised to equol.

4. Discussion

In the present study, the effects of genistein against DMBA-induced rat mammary carcinogenesis are marginal. The rats were treated with carcinogen at 50 days of age at a time when the mammary gland is almost fully developed and less susceptible to differentiation. Genistein marginally reduced the tumour incidence and multiplicity and was ineffective in reducing tumour burden or increasing tumour latency (Fig. 2). Therefore, the reported efficacy of soy protein isolate against mammary carcinogenesis in the same animal model [27], cannot be attributed to genistein. Daidzein, contrary to genistein, effectively reduced tumour multiplicity and burden (Figs. 1(b) and 2(b)). Therefore, daidzein seems to account, at least partially, for the chemopreventive effects of soy protein isolate against rat mammary carcinogenesis.

Dietary genistein has been studied extensively in models of carcinogenesis with mixed results. Genistein has been determined to be effective against mammary carcinogenesis when given at relatively large doses and early in life (neonatally and prepubertally) [11]. However, it has been found to be ineffective or marginally effective in several animal models including mammary [12,14] and colon [28,29] cancers. Genistein has been reported to modulate a large number of activities and to have several biological targets, and has been determined to produce promitotic effects in ERα-positive cultured tumour cells at physiologically relevant concentrations (in the range of 0.5–2 μM) [30]. As a topoisomerase II poison, [8,31,32], genistein could produce chromosomal aberrations that can give rise to infantile acute leukaemia and secondary leukaemia [33]. On the contrary, other activities of genistein can retard carcinogenesis; these include the anti-oestrogenic effects, the induction of tumour cell differentiation, and the inhibition of protein tyrosine kinases. Therefore, the dominant activities in a particular system determine if the isoflavone will act as a promoter or inhibitor of carcinogenesis.

The combination of genistein with tamoxifen was evaluated previously by Ju and colleagues [22] in athymic mice. In the mouse study, genistein was found to negate the growth inhibitory effects of tamoxifen. However, the mouse model is not ideal for the evaluation of the efficacy of potential chemopreventive agents because it relies on the growth of existing tumour cells (MCF-7 cells); consequently, the efficacy of an agent during the transformation of a normal cell to tumour cell (cancer initiation) cannot be determined. In the rat model that we used in this study, the conversion of normal mammary epithelial cells to adenocarcinomas by DMBA is relevant to the events leading to breast cancer in women. Further, this model is favoured by the National Cancer Institute for the evaluation of breast cancer chemopreventive agents [34,35]. The tamoxifen/genistein combination diet was less effective than the tamoxifen diet in reducing tumour incidence and multiplicity. However, the tamoxifen/daidzein diet was more effective than the tamoxifen diet in reducing tumour burden, incidence, and multiplicity, as well as increasing tumour latency. Therefore, in the rat model, as in the mouse model, genistein diminished the effect of tamoxifen. In the rat model, daidzein enhanced the chemopreventive effect of tamoxifen. The combination of tamoxifen/daidzein was almost as effective as the tamoxifen/soy protein isolate combination that we reported earlier [24]. The combined chemopreventive action of tamoxifen with daidzein has not been evaluated or reported in other animal models.

The protection against oxidative DNA damage, as determined by reduced 8-oxo-dG levels, in the group of rats that were fed the combination tamoxifen/daidzein diet is striking. The tamoxifen-containing diet alone did not reduce the levels of 8-oxo-dG, whereas the daidzein-containing diet produced a significant reduction compared with the levels of the positive control (Fig. 3). The combination tamoxifen/daidzein diet produced even lower levels of DNA damage, suggesting that the daidzein acted in a synergistic manner with tamoxifen to protect the mammary gland against oxidative DNA damage. In the mammary gland, tamoxifen may act in a classical sense as an antagonist to the tumour-promoting effects of oestrogen. Equol, on the

other hand, as a polyphenol and a hydrogen/electron donor may scavenge free-radicals. Equol, has been found to have the highest antioxidant activity in three different assays [36–38]. The superior antioxidant activity of equol combined with the anti-oestrogenic/antioxidant effects of tamoxifen could explain the excellent protection against carcinogenesis of the tamoxifen/daidzein diet. It is also possible that tamoxifen upregulated the activity of antioxidant enzymes such as quinone reductase (QR) by binding to the electrophile response element in the 5′ regulatory region of the QR gene [39]. This hypothesis is consistent with the observation of Montano and Katzenellenbogen who determined that anti-oestrogens increase QR activity in MCF-7 cells [40]. Therefore, the induction of phase II enzymes by tamoxifen together with the free-radical scavenging effects of equol could explain our results regarding lower 8-oxo-dG levels and tumour risk parameters by the combination tamoxifen/daidzein diet. A third possibility is that daidzein somehow causes an increase in the serum levels of tamoxifen. This remote possibility that the effective agents, through an unknown mechanism, produce increased levels of tamoxifen cannot be excluded at present.

Health-conscious women who are at high risk for breast cancer, are generally advised by medical practitioners to avoid soy products. Presently, there are no human intervention studies or animal data evaluating the combined effects of tamoxifen and soy (on breast cancer risk); therefore, this advice is not based on data from human studies. This recommendation is rather based on the (hypothetical) competition between tamoxifen and phyto-oestrogens for the same oestrogen receptor. It is assumed that, when phyto-oestrogens occupy the ERα, tamoxifen is prevented from binding and eliciting a favourable response. Based on this hypothesis, soy products were expected to reduce the efficacy of tamoxifen in animal models of carcinogenesis. Our studies have shown clearly that, although genistein may have a deleterious effect when combined with tamoxifen, the use of soybeans in combination with tamoxifen is beneficial [24], and that daidzein, the isoflavone that had been neglected in the past, seems to be the most promising component of soy in combating cancer in combination with tamoxifen. Our study suggests the benefits of daidzein might be mediated through its oestrogenic metabolite equol. In conclusion, the present study demonstrates that tamoxifen and soy components may act together to combat breast cancer more effectively than when given individually. Furthermore, the present study makes a clear distinction between the effects of genistein and daidzein with respect to their antitumour properties.

In the future, our most important challenge is to determine whether the data obtained from animal models and cultured cells are relevant to humans, and how we might be able to use natural or synthetic oestrogen agonists/antagonists to modulate the relative expression of ERα and ERβ to reduce the risk of breast cancer.

Conflict of interest statement

None declared.

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