Proceedings of the Nutrition Society (2012), **71 (OCE1)**, E9 doi:10.1017/S0029665112000377

Scottish Section Meeting, 26–27 March 2012, Future food and health

Physiological levels of soy isoflavones reduce proliferation and promote apoptosis in the ERβ-positive breast cancer cell line MDA-MB-231

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Dietary soy, particularly the high levels consumed as part of traditional Eastern-Asian diets, may be protective against certain subtypes of breast cancer[1]. This effect is partly related its high isoflavone phytoestrogen content (genistein and daidzein). However much *in vitro* evidence suggests a contradictory, growth promoting impact of physiological (serum) levels of isoflavones in oestrogen receptor alpha positive (ERα+) breast cancer cell lines[2]. This has led to the contraindication of isoflavone supplement use in post menopausal breast cancer survivors. The inconsistency between epidemiological and *in vitro* evidence may relate to the relative expression levels of ERα and its counterpart ERβ. Unlike in cell lines predominantly expressing ERα, the proliferation of ERβ-dominant cell lines can be inhibited by concentrations of genistein as low as 1 nM[3,4]. This information is relevant as the ERα−/β+ subgroup represent approximately 18% of all breast tumours[5] but the beta-receptor is not routinely tested for upon diagnosis.

We assessed the impact of physiologically relevant concentrations (serum levels achievable through diet: 0.01 nM to 31.6 μM) of the soy isoflavones genistein and daidzein on proliferation (MTT assay) and apoptosis with the Annexin V-Cy3™ Apoptosis Detection Kit and DAPI staining/Nuclear Area Factor (NAF) calculation in the ERα−/β+ breast cancer cell line MDA-MB-231.

Low doses of genistein or daidzein (≥ 0.01 nM) inhibited MDA-MB-231 proliferation by around 20%, although this frequently failed to achieve statistical significance. The highest concentrations used (31.6 μM) resulted in a dramatic decrease in percent proliferation compared with a vehicle only control (mean ± SD : 48.5 ± 12.6, $p = 0.004$ and 48.4 ± 13.1, $p < 0.001$ for genistein and daidzein respectively; $n = 3$). 17β-oestradiol (E2; the major circulating oestrogen) also slightly reduced proliferation at its pre- and post-menopausal serum concentrations (1 nM and 1 pM respectively). This reduction in proliferation was associated with an observed increase in cell death at 10 and 31.6 μM genistein and daidzein, which was at least partly explained by an increase in the percentage of apoptotic cells. While the combination of E2 and isoflavones failed to show any synergistic growth inhibitory, or pro-apoptotic effects, some inhibition of proliferation was still documented. This implies that the growth inhibitory effects of genistein, daidzein and E2 may not be regulated by the same mechanisms. However, more importantly, the apoptotic cell death observed with soy isoflavone treatment was not abrogated by the addition of physiological E2 concentrations.

Overall, our data suggests that at concentrations achievable through the diet (≤ 10 μM) soy isoflavones may be potentially beneficial as chemotherapeutic agents against ERα−/β+ breast cancers, through their ability to reduce proliferation and promote apoptosis, even in the presence of physiological E2 levels. Routine determination of the expression levels of ERβ in addition to ERα in breast cancer would be an invaluable biomarker to indicate the potential efficacy of this cheap and readily available treatment, and indicates a possible pharmacological target.

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