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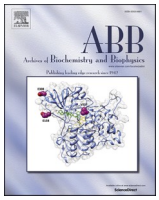

Genistein and coumestrol reduce MCF-7 breast cancer cell viability and inhibit markers of preferential metastasis, bone matrix attachment and tumor-induced osteoclastogenesis

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ABSTRACT

The propensity of breast cancer to preferentially metastasize to the skeleton is well known. Once established in bone metastatic breast cancers have a poor prognosis due to their ability to promote extensive bone loss which augments tumor burden. Unfortunately, current anti-resorptive therapies for skeletal metastasis are typically prescribed after secondary tumors have formed and are palliative in nature. One group of compounds with the potential to reduce both tumor burden and osteolysis are phytoestrogens (PE), but the mechanisms mediating a beneficial effect are unclear. Therefore, the current study examined the effect of genistein and coumestrol alone or in combination on breast cancer cell number, expression of mediators of preferential skeletal metastasis, bone matrix attachment and tumor-induced osteoclast formation. Results showed that genistein and coumestrol significantly reduced viable cell number in an estrogen receptor dependent manner ($p < 0.05$), whereas combinations of PE had no effect. In addition, genistein and coumestrol significantly reduced expression of genes driving epithelial to mesenchymal transition (snail), bone attachment (CXCR4 and integrin αV) and osteolysis (PTHrP and TNF-α). In keeping with this genistein and coumestrol significantly suppressed attachment of breast cancer cells to bone matrix and inhibited tumor and RANKL-induced osteoclast formation. Our data suggests that phytoestrogens not only decrease breast cancer cell viability but also antagonize essential tumor bone interactions that establish and drive the progression of skeletal metastasis.

1. Introduction

The skeleton is a site of metastasis in 70% of patients with late-stage breast cancer and is associated with bone pain, elevated fracture risk and reduced overall survival [1,2]. Metastasis involves malignant cells developing an ability to leave the primary tumor, enter and survive in the vasculature, extravasate into distant tissues and if the local environment is conducive form secondary cancers. In the case of breast cancer, metastatic cells are preferentially targeted to the skeleton due to an aberrant expression of chemokine receptors such as CXCR4 that promote migration to bone marrow [3]. Once in bone high levels of tumor integrin expression enables bone matrix attachment and facilitates the formation of a tumorigenic osteolytic niche. Central to niche formation is the release of PTHrP and members of the TNF superfamily which promote osteoclastogenesis and bone matrix breakdown. The subsequent release of pro-tumorigenic growth factors from bone matrix generates a self-perpetuating cycle that accelerates osteolysis and disease progression [4,5]. Controlling this viscous cycle is a key clinical goal as it rapidly leads to hypercalcemia of malignancy and death.

Current treatments for skeletal metastasis, such as anti-resorptive bisphosphonates, are typically prescribed after metastasis is established and have little effect on bone tumor incidence or overall survival [6]. Consequently, to improve patient outcomes there is a need for therapeutic agents that can be proactively administered to limit preferential metastasis and bone resorption. One group of compounds with such a potential are dietary phytoestrogens (PE), which have an anti-tumorigenic and anti-osteoclastic action [7–12]. Genistein an isoflavone of the aglycone group and coumestrol a coumestan possess a diverse range of biological activities. These include, due to their estrogen like structure, a well-established interaction with steroid (ER) and G-protein coupled estrogen receptors [13]. Coumestrol also inhibits aromatase and 17β-HSD activity to suppress estrogen and androgen levels [14]. Differential binding to ER subtypes has been noted with coumestrol having similar affinities for ER α and ER β, while genistein

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may have a higher affinity for ER β than coumestrol [15]. In addition to ER mediated actions, genistein possesses antioxidant and tyrosine kinase modifying activity leading to decreases in prostaglandin production, promotion of p21 mediated cell cycle arrest and induction of apoptosis through modification of Bax/Bcl levels and AKT signalling [16]. Inhibition of topoisomerase and DNA methyltransferases has also been implicated in genistein's epigenetic action and like genistein coumestrol induces apoptosis, inhibits MAPK and AKT signalling, and modifies reactive oxygen specifies and inflammatory mediator production [17,18].

Similar to cancer incidence PE levels vary, with Asian populations typically having greater PE concentrations and lower incidence of breast cancer than Westerners [19] and phytoestrogen sources correlating globally with reduced breast cancer risk [20]. PE have also been shown to reduce metastatic burden [21,22], but the cellular mechanism mediating this beneficial action is unclear. This could arise due to multiple actions including general tumor suppressive effects, inhibition of preferential metastasis, suppression of the pro-tumorigenic osteolytic response or a combination of these actions. Therefore, to identify cellular mechanisms of action the current study investigated the effect of PE known to inhibit osteoclast formation on breast cancer survival, expression of genes involved in skeletal metastasis, bone matrix adhesion and tumor-mediated osteoclast formation.

2. Materials and methods

2.1. Media and reagents

Human MCF7 breast cancer cells (ATCC, UK) were incubated in phenol red-free RPMI-1640 medium or phenol red-replete RPMI 1640 medium (Gibco Thermo Fisher Scientific, UK) supplemented with 10% charcoal stripped fetal calf serum (Autogen Bioclear, U.K.) and 2 mmol/l glutamine. Incubations were performed at 37 °C in 5% CO_2, and cultures fed every 2–3 days by replacing half of the culture volume with fresh medium. The non-selective estrogen antagonist ICI 182,780 was obtained from Tocris Biosciences (Bristol, UK). Cell permeable caspase 3 inhibitor was obtained from Calbiochem (Merck, Gillingham, Dorset, UK). All other reagents were obtained from Sigma (Poole, Dorset, UK) unless stated.

2.2. Measurement of the impact of phytoestrogens on viable cell number

Cells were transferred to 96-well plates at a density of 5×10^3 cells per well and cultured with combinations of genistein and coumestrol (10^{-5} to 10^{-8} M) for 72 h. The number of live metabolically active cells was assessed using an AQueous one cell assay (Promega UK) according to manufacturer's instructions. Initial studies determined the profile of PE concentrations able to significantly alter viability, subsequent experiments assessing synergistic or antagonistic interactions combined these effective concentrations. Experiments examining the role of estrogen receptor signaling were performed by incubating cells in the presence of the estrogen antagonist ICI 182,780 (10^{-5} M). The role of apoptosis in the response to phytoestrogens was assessed by incubating cells with phytoestrogens in the presence of Z-VAD-FMK a pan-caspase inhibitor of apoptosis (1 μM, Promega, UK). Viable cell number was then assessed as previously. Caspase activity was assessed in black 96 well plates (Nuncleon, UK) following phytoestrogen treatment using a fluorescent Apo-ONE homogenous caspase 3/7 Assay (Promega, UK) according to manufacturer's instructions. Fluorescence was measured on a BMG Labtech Fluostar Omega spectrofluorometer at 499 nM excitation and 521 nM emission. Caspase activity was expressed relative to control and all assays were performed in triplicate.

2.3. Real time quantitative PCR analysis of metastatic marker expression

Quantitative RT-PCR (qPCR) was used to detect gene expression of key mediators of preferential metastasis using the $\Delta\Delta C_T$ methodology. Cells (5×10^5 cells) were incubated in 25 cm^2 flasks for 72 h with genistein or coumestrol. Total RNA was extracted from these cultures using a Sigma genelute RNA isolation kit and reversed transcribed with M-MLV reverse transcriptase using random nonamer primers. ΔΔcT qPCR was performed on a StepOne PCR system (Applied Biosystems, UK) using the DNA-binding dye SYBR green for detection of PCR products. A total of 1 μl of cDNA was added to a final reaction volume of 12.5 μl containing 0.05 U/μl Taq, SYBR green and specific primers (0.2 μM). Primers used were as follows Human *β-actin* forward GCGCGGCTACAGCTTCACCA, reverse TGGCCGTCAGGCAGCTCGTA; Human *CXCR4* forward GCGCAAGGCCCTCAAGA, reverse GTGCGTGCTGGGCAGAGGTT; Human *Snail* forward CGAGTGGTTCTTCTGCGCTA, reverse CTGCTGGAAGGTAAACTCTGGA; Human *Integrin alpha 5* forward AATTTTACTGGCGAGCAG, reverse TTGGTGGCATGCTTCGAG; Human *PTHrP* forward GTCTCAGCCGCCGCCTCAA, reverse GGAAGAATCGTCGCCGTAAA Human *TNF-alpha* forward GCT CCA GTG GCT GAA CCG CC and reverse AGC ACA TGG GTG GAG GGG CA. Reaction conditions were 94 °C for 2 min, followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. The progress of the PCR amplification was monitored by real-time fluorescence emitted from SYBR Green during the extension time. At the end of each PCR run a melt curve analysis was performed to show the absence of non-specific bands. The relative quantification (RQ) value for each group was calculated by the instrument's software using cT values for non-treated controls normalized to the expression β-actin mRNA. Samples were analyzed in triplicate and experiments repeated separately three times.

2.3.1. Bone adhesion assay

To assess the effect of phytoestrogens on adhesion to bone matrix MCF-7 cells were pre-treated with phytoestrogens for 72 h and then seeded onto 9 mm^2 bovine bone slices in 96 well plates at a density of 2×10^4 cells per well. Cells were then incubated for 60–120 min at 37 °C 5% CO_2. After incubation non-adherent cells were removed from the surface of bone slices by vigorously washing three times in Dulbecco's phosphate buffered saline to remove non-adherent cells, fixed in 10% formalin and stained with 1% toluidine blue to enable visualization of adherent cells. After drying slices were mounted onto glass slides and the number of cells quantified by reflected light microscopy using an eyepiece graticule and magnification of ×40 on an Olympus BHB microscope with a Schott KL1500 light source.

2.3.2. Scratch cell migration assay

Cells were treated with phytoestrogens for 72 h before seeding onto 9 mm^2 bovine bone slices at 2×10^4 cells per well. Treatments were continued in these cultures for 3 days until confluency was reached. Scratches were created using a 100 μL micropipette tip on each slice and medium replaced. Closure was recorded at time 0, 5 and 24 h by removing bone slices washing three times in PBS, fixing in 10% formaldehyde before staining in 1% toluidine blue. Distance between the edges of the scratch at six points on each slice using a 1 mm eyepiece graticule and reflected light microscopy at a magnification of ×100 on an Olympus BHB microscope with a Schott KL1500 light source.

2.3.3. Effect of phytoestrogens on breast cancer and RANKL-induced osteoclastogenesis

To examine the impact of phytoestrogens on breast cancer induced osteoclastogenesis RAW264.7 osteoclast precursors were cultured in MCF-7 conditioned media with and without RANKL (30 ng/ml). Monocytic osteoclast precursors (RAW264.7 cells 1×10^4 cells/well) were incubated for 5 days with or without the osteoclast inducer RANKL (30 ng/ml) on bone slices in 50% conditioned media from MCF-7 cultures that had been pre-incubated with or without phytoestrogens for 72 h. Osteoclast differentiation was evaluated by staining for the specific osteoclastic marker tartrate resistant acid phosphatase (TRAP) using a

modification of the method of Burstone [23] using naphthol AS-BI phosphate as a substrate. The number of TRAP positive osteoclasts was counted using an eyepiece graticule at a magnification of ×100 and the results expressed as the number of TRAP positive cells per cm^2. All experiments were performed in triplicate.

2.4. Statistical analysis

Differences between groups were assessed using a Tukey's post-hoc analysis of variance test for pairwise comparisons between means (Minitab, Minitab LLC, USA). The data corresponded to independent observations from three separate repeats each consisting of three to six replicates. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Estrogen-dependent actions of genistein and coumestrol decrease breast cancer cell number

MCF-7 cells were incubated with individual PE to establish concentrations able to significantly reduce cell number. Genistein (10^{-5}-10^{-6} M, $P < 0.05$ versus control) and coumestrol (10^{-5}-10^{-7} M, $P < 0.05$ versus control) significantly reduced MCF7 cell number (Fig. 1). Genistein causing a 25-21% decrease and coumestrol a 37-29% decrease in viable cell number compared to control. This suppressive action was lost when individually effective concentrations were combined (Fig. 1).

Genistein and coumestrol individually induced a 16-28-fold increase in executioner caspase 3/7 activity, which like the effect on cell number was not seen when PE were combined, suggesting a pro-apoptotic effect on breast cancer cells (Fig. 1). This is supported by the ability of the pan-specific caspase inhibitor Z-VAD-FMK to prevent the antitumorigenic action of both PE (Fig. 1). Furthermore, the suppressive effect of both PE was also prevented by the estrogen receptor antagonist ICI 182,780 suggesting an estrogen receptor-dependent action (Fig. 1).

3.2. Genistein and coumestrol decrease expression of genes promoting preferential metastasis and bone colonization

Skeletal metastasis is dependent on the coordinated expression of factors that target cells to bone, enable matrix attachment, and provide pro-osteoclastic signals that generate a niche conducive for colonization and survival. In the current studies genistein and coumestrol markedly

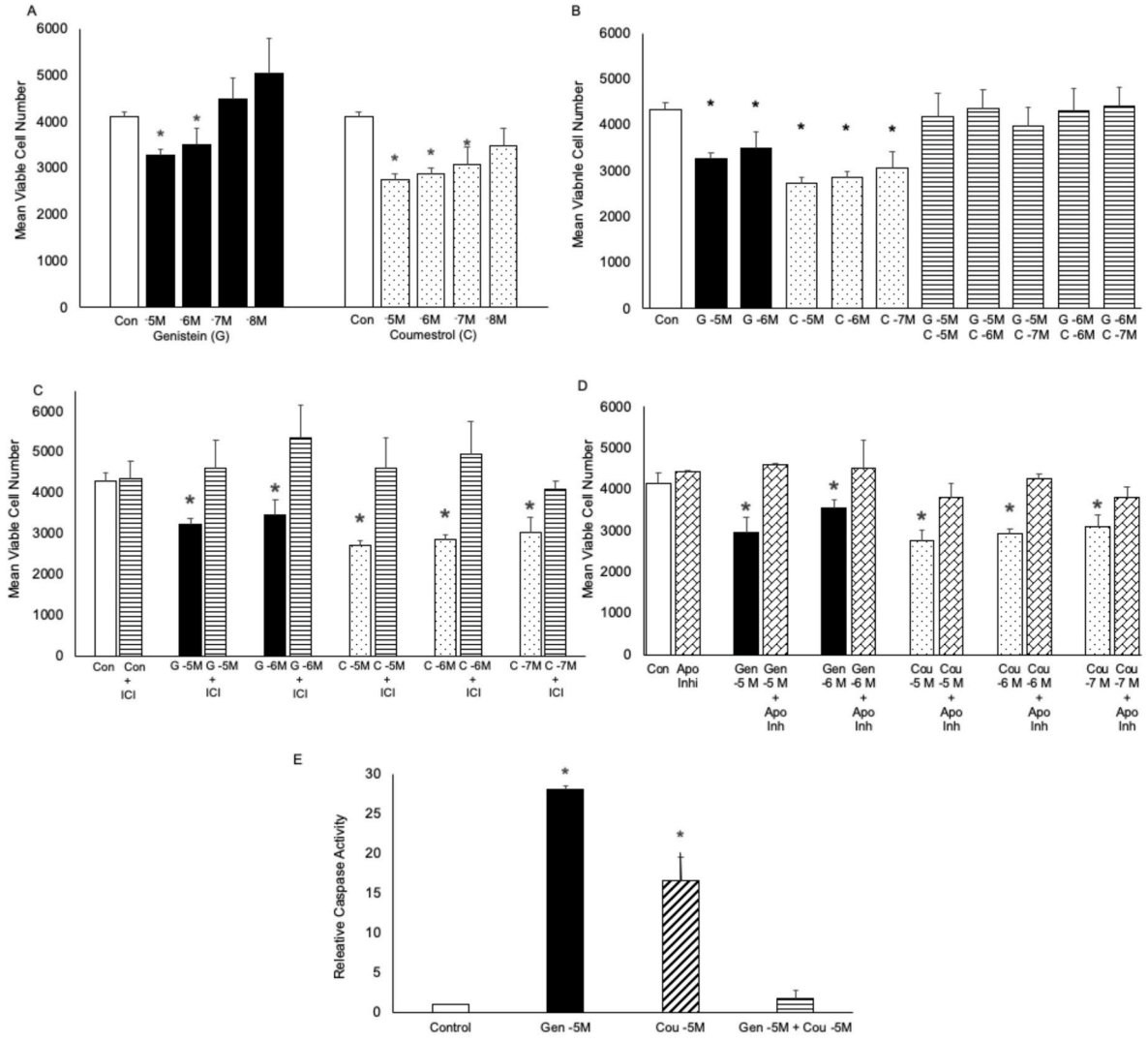

Fig. 1. Genistein and coumestrol significantly reduce viable MCF-7 cell number in an apoptosis and oestrogen receptor-dependent manner. A. genistein and coumestrol significantly suppress viable cell number. B. the inhibitory effect of genistein and coumestrol on viable cell number is lost when effective concentrations are combined. C. the oestrogenic receptor antagonist ICI 182, 780 (10^{-5} M) prevented the suppressive effect of genistein and coumestrol on viable cell number. D. the apoptosis inhibitor Z-VAD-FMK prevented the suppressive action of genistein and coumestrol on viable cell number. E. concentrations of genistein and coumestrol shown to decrease cell number significantly increased caspase activity. Results are the mean ± SEM for three repeat experiments. *$P < 0.05$ versus control.

reduced expression of genes central to this process including epithelial to mesenchymal transition (snail), preferential metastasis and matrix attachment (CXCR4 and integrin αV) and disruption of bone remodelling (PTHrP and TNF-α). The effect of genistein was more pronounced than coumestrol, genistein significantly decreased mRNA expression of snail (0.34–0.2 of control 10^{-5}M-10^{-6} M), CXCR4 (0.5–0.37 of control 10^{-5}M-10^{-6} M), integrin αV (0.09–0.12 of control 10^{-5}-10^{-6} M), PTHrP (0.11 of control 10^{-5} M) and TNF-α (0.60 of control 10^{-5} M). In contrast coumestrol only significantly reduced integrin αV expression (0.06–0.14 of control 10^{-5}-10^{-7} M) and had no significant effect on PTHrP, snail, CXCR4 or TNF-α (Fig. 2).

3.3. Genistein and coumestrol inhibit the attachment of breast cancer cells to bone

To determine if phytoestrogen-induced changes in cell adhesion molecule expression reduced the bone colonization potential of breast cancer cells the effect of phytoestrogen pre-treatment on bone matrix attachment and motility was assessed. Genistein and coumestrol (10^{-5} M) inhibited the adhesion of MCF-7 cells to bone matrix. Genistein significantly reduced attachment 3.29-fold and 4.11-fold at 1 h and 2 h, while coumestrol significantly reduced attachment 4.11-fold at 1 h and 7.60-fold at 2 h (Fig. 3). Attached cells pretreated with genistein or coumestrol had a more rounded morphology and were not as well spread as in control cultures, indicating that binding between treated cells and bone was weaker. The reduction in adhesion indicates a marked decrease in the ability to colonize bone matrix an important initial event in skeletal metastasis and niche formation and may relate to the reduction in integrin expression. In contrast once attached to bone, cell motility was not affected by genistein or coumestrol and there was no significant difference between control and PE treated cells in scratch closure at any time point (Fig. 3).

3.4. Genistein and coumestrol inhibit MCF-7 and RANKL induced osteoclast formation

Once established in bone breast cancer metastases elicit an osteolytic remodelling response, generating a tumor-promoting niche that supports further resorption. Osteoclast formation is a hallmark of many inflammatory disorders of bone due to high levels of pro-osteoclastic inflammatory cytokines such as TNF-α and elevated levels of the physiological regulator of osteoclastogenesis, RANKL. Interestingly we noted

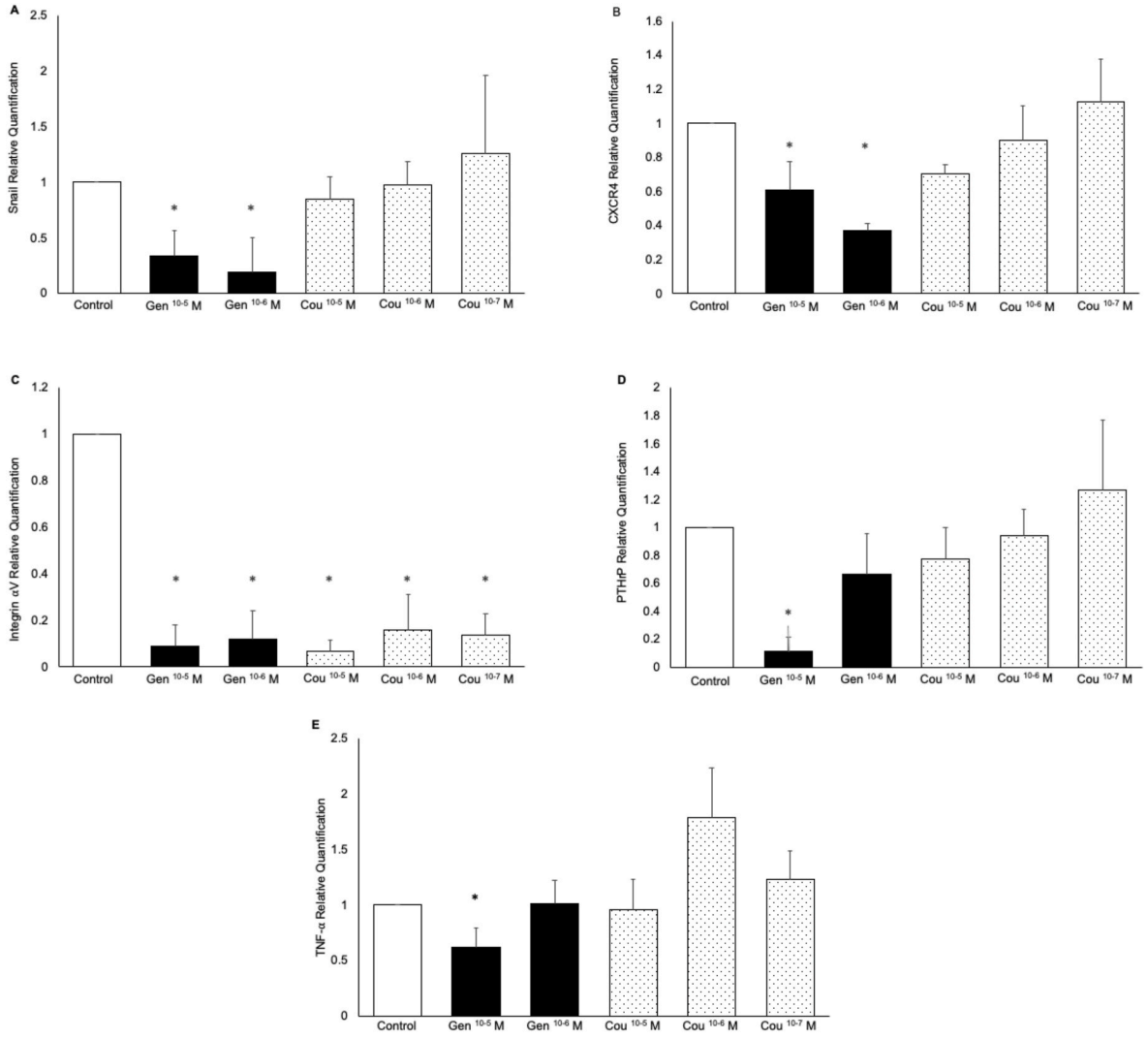

Fig. 2. Genistein and coumestrol suppress mRNA expression of factors implicated in epithelial to mesenchymal transition (A, snail), preferential metastasis (B, CXCR4) and (C, integrin α 5) and osteolysis (D, PTHrP) and (E, TNF-α). Cells were cultured with genistein or coumestrol for 72 h prior to mRNA isolation. Gene expression was then assessed using $\Delta\Delta C_T$ qPCR and the DNA-binding dye SYBR green for detection of PCR products. The relative quantification (RQ) value for each group was calculated using cT values for non-treated controls normalized to the expression β-actin mRNA. Samples were analyzed in triplicate and experiments repeated separately three times. *$P < 0.05$ versus control.

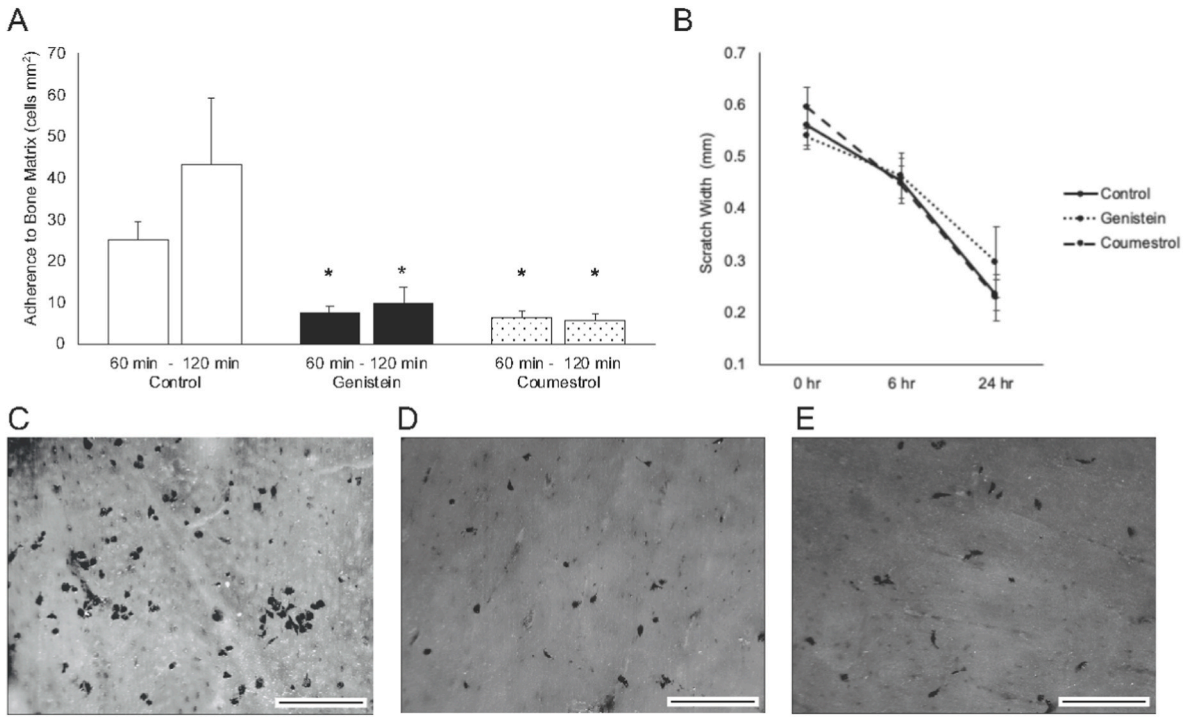

Fig. 3. Genistein and coumestrol (10^{-5} M and 10^{-7} M) inhibit breast cancer cell attachment to bone matrix but have no effect on motility. A: MCF-7 cells were pre-treated with phytoestrogens for 72 h and then seeded onto bovine bone slices for 60–120 min at 37 °C 5% CO_2. After incubation non-adherent cells were removed from the surface of bone slices by vigorously washing three times in Dulbecco's phosphate buffered saline, fixed and stained with 1% toluidine blue. Genistein and coumestrol significantly reduced the number of adherent cells at both time points in comparison to control *$p < 0.05$. B: Genistein and coumestrol had no effect on wound closure at any time point. C-E images of cell attachment to bone at 60 min in C control, D genistein 10^{-5} M treated and E coumestrol 10^{-5} M treated cells. Scale bars 0.2 mm.

that MCF-7 cells produce TNF-α and this expression was suppressed by genistein (Fig. 2). Culturing osteoclast precursors with MCF-7 conditioned media promoted osteoclast formation in a RANKL independent manner as OPG a soluble decoy receptor for RANKL had no effect. Interestingly both genistein and coumestrol significantly decreased the ability of MCF-7 conditioned media to promote osteoclast formation (Fig. 4). Furthermore, both also significantly suppressed osteoclast formation when exogenous RANKL was added to cultures treated with MCF-7 conditioned medium.

4. Discussion

Breast cancer has a predilection to spread to the skeleton and is associated with severe pain, hypercalcemia, pathological fracture, and poor prognosis. Bone metastasis is dependent on the aberrant expression of genes that target malignant cells to bone, facilitate bone matrix adhesion and then elicit a self-perpetuating osteolytic response that forms a pro-tumorigenic niche. Current treatments for breast cancer skeletal metastases, such as bisphosphonates, limit resorption but are typically palliative and don't impact colonization. Therefore, outcomes could be improved by identifying therapeutic interventions that not only suppress resorption but also limit the potential for skeletal metastases. There is considerable evidence that phytoestrogens, a diverse group of dietary compounds with a structure and function like estradiol, reduce breast cancer incidence and progression [24]. In addition, there is evidence that PE decrease metastatic potential in animal models of breast cancer [25–27]. However, the mechanism through which PE modify skeletal metastasis and their impact on the pro-tumorigenic osteolytic response is not well understood. The current study indicates that genistein and coumestrol have multiple actions that could limit breast cancer skeletal complications.

Similar to the studies of Kabala-Dzik [28] a pro-apoptotic effect of genistein and coumestrol was noted. This was lost when individually effective concentrations were combined. Similar antagonistic relationships have been noted previously [22] and are attributable to distinct affinities for ERα, ERβ, and ER-independent actions which can have opposing effects on tumour activity [29]. This generates an interplay between PE and tumors with the overall response being cell, PE, and concentration specific. Whatever the explanation it is clear individually genistein and coumestrol are more effective than in combination and therefore if used therapeutically dietary sources of PE should be monitored to limit the potential for antagonistic interactions.

The benefit of individual PE is underlined by the modification of genes implicated in malignant transformation and skeletal metastasis. Genistein suppressed snail expression, a transcription factor involved in the promotion of breast cancer epithelial to mesenchymal transition, dissemination and migration [30]. In keeping with this genistein also reduced expression of the snail-regulated chemokine receptor CXCR4. Binding of CXCR4 to its bone marrow derived ligand CXCL12 promotes chemotactic migration towards the skeleton and underpins preferential metastasis [31]. Minimising this bone trophic signal would limit bone dissemination and thereby decrease morbidity and mortality associated with skeletal secondaries, and this may mediate the bone trophic effects of genistein noted *in-vivo*. However, this does not prohibit malignant cells randomly metastasising to the skeleton, but for these cells to survive tumour-bone interactions are needed that enable colonization and initiate a pro-tumorigenic remodelling response. The first stage of successful colonization is the binding of aberrantly expressed cell adhesion molecules such as integrins to RGD containing proteins in the bone matrix. This sequesters tumour cells to bone, provides additional proliferative stimuli and promotes VEGF-induced angiogenesis, contributing to the disproportionate impact of skeletal metastases in late-stage disease. Integrin αV in particular is highly expressed in breast cancer bone metastases compared to other tissues and is associated with shorter

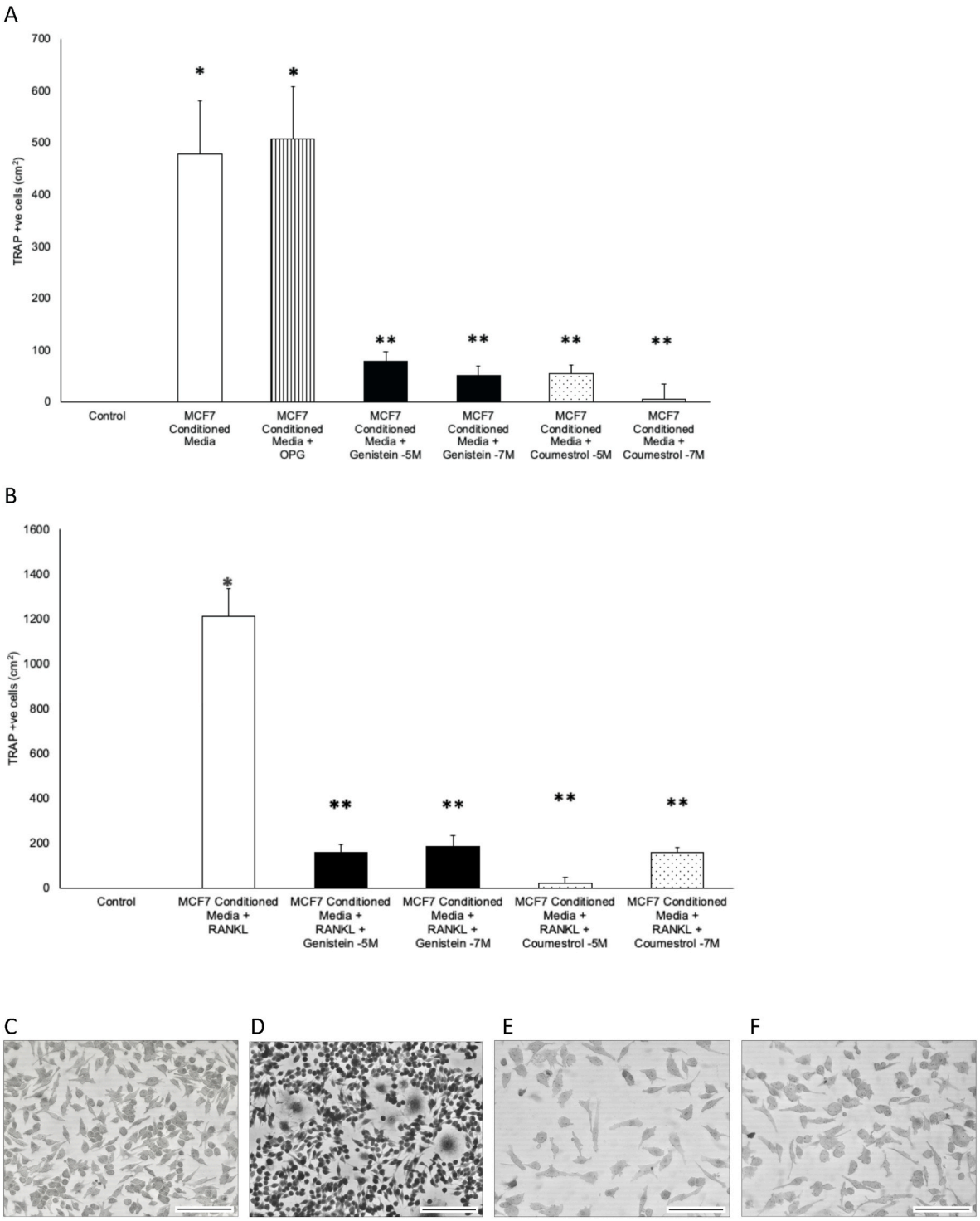

Fig. 4. Genistein and coumestrol significantly inhibit MCF-7-induced osteoclast formation in the presence or absence of RANKL. RAW264.7 osteoclast precursors were cultured for 5 days in 50% conditioned media taken from MCF-7 cultures that had been incubated with genistein or coumestrol (10^{-5} and 10^{-7} M) for 3 days. A: in the absence of RANKL (30 ng/ml) and B: with RANKL (30 ng/ml). Osteoclast differentiation was evaluated by staining for the specific osteoclastic marker tartrate resistant acid phosphatase (TRAP) using naphthol AS-BI phosphate as a substrate. The number of TRAP osteoclasts was counted using an eyepiece graticule at a magnification of ×100 and the results expressed as the number of cells per cm^2. C–F images of TRAP stained cultures in C control D: MCF7 media and RANKL 30 ng/ml, E: MCF7 media, RANKL and genistein 10^{-5} M, F MCF7 media, RANKL and coumestrol 10^{-5} M. Scale bars 0.2 mm. All experiments were performed in triplicate * $p < 0.05$ versus control, **$p < 0.05$ versus MCF7 conditioned media or MCF7 conditioned media plus RANKL.

bone metastasis free survival and osteolysis; and is a potential therapeutic target for disseminated disease [32]. Genistein and coumestrol both significantly inhibited integrin αV expression and reduced attachment of breast cancer cells to bone matrix. Reduced colonization potential of breast cancer cells would be expected to further decrease bone metastasis, slow disease progression and improve patient outcome. Current anti-integrin therapies have limited clinical benefit due in part to difficulties with delivery to bone niches that prevent effective integrin blockade [33]. This could be overcome however by combining anti-integrins with phytoestrogens which would decrease the

concentration of inhibitor needed to block tumour integrin activity.

Bone due to its mineralised nature is a hostile environment for tumour growth but once established skeletal metastases have an elevated proliferation rate. This paradox is explained by the ability of micro-metastases to disrupt the mechanisms that regulate bone remodelling, generating an environment that is conducive for activation and growth. During normal remodelling the coordinated action of formative osteoblast and resorptive osteoclast creates a bone structure that is optimised for preceding mineral and mechanical requirements [34]. At metastatic sites tumour derived and induced factors disrupt this balance skewing remodelling in favour of resorption. Several mechanisms account for this including the release of pro-osteoclastic inflammatory mediators such as TNF-α which directly stimulation osteoclast formation from monocytic precursors [35]. Tumors also secrete high levels of parathyroid hormone related protein (PTHrP) which indirectly stimulates osteoclast formation by activating osteoblastic RANKL expression to regulate osteoclastogenesis. The RANKL-RANK system is central to the physiological regulation of osteoclast formation with parathyroid hormone released in response to low circulating serum calcium promoting osteoblastic RANKL expression, which in turn stimulates osteoclastogenesis by activating RANK receptors on osteoclast precursors [36]. Interestingly, we found that genistein significantly reduced TNF-α and PTHrP expression which would be expected to suppress direct and in-direct RANKL mediated osteoclastogenesis. In keeping with this genistein and coumestrol significantly inhibited osteoclast formation from monocytic precursors treated with MCF-7 conditioned media with or without RANKL supplementation. This suggests that PE inhibit both direct and in-direct RANKL mediated pathways for osteoclastogenesis, which would reduce skeletal related morbidities such as osteolytic fracture, bone pain and hypercalcemia of malignancy. In addition, as matrix remodelling is essential for the initiation and progression of skeletal niches this would limit the ability of skeletally disseminated cancer cells to survive in bone tissue and if micro metastases do establish these would develop at a slower rate.

Skeletal metastases are also common in late-stage prostate cancer (PC). However, the remodelling response elicited by PC differs to that induced by breast cancer, with PC secondaries typically inducing an osteoblast-mediated osteosclerotic rather than lytic response. Treatment for PC metastases is even more limited and typically involves analgesics or radiotherapy. Interestingly, like breast cancer a recent meta-analysis indicates that genistein but not coumestrol has a beneficial impact on prostate cancer incidence [37] and similar to the current study the beneficial action is attributed to reduced tumour cell number [38]. There may also be further effects to reduce dissemination as genistein reduces PC markers of epithelial to mesenchymal transition and invasive potential a key event in early-stage metastasis [38]. This suggests that genistein could act in combination alongside standard therapy to limit metastasis. However the impact of genistein on bone tumour interactions is unclear, as unlike its effect on breast cancer genistein may increase PTHrP expression which would be expected to promote osteoclastogenesis, however genistein may also increase tumour OPG/RANKL ratio which may counteract this [39]. There is also evidence that genistein promotes osteoblast differentiation which could worsen the sclerotic response [40]. Therefore, the net effect of genistein on PC metastasis and bone remodelling is unclear and warrants further investigation.

5. Conclusions

Data from the current study suggests that the phytoestrogens genistein and coumestrol possess multiple actions that could limit the spread of breast cancer to bone. Both reduced cell number, inhibited the expression of mediators of preferential metastasis, adhesion to bone and tumour and RANKL-induced osteoclastogenesis. These compounds therefore represent a potential avenue to limit the detrimental impact of skeletal complications in breast cancer.

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Declaration of competing interest

The authors report no conflict of interest.

Data availability

Data will be made available on request.

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