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Growth-inhibitory Effects of the Natural Phyto-oestrogen Genistein in MCF-7 Human Breast Cancer Cells

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Genistein, a natural isoflavonoid phyto-oestrogen, inhibits the tyrosine kinase activity of growth factor receptors and oncogene products, as well as the *in vitro* growth of some tumour cell lines. The low incidence of breast cancer in countries with a flavonoid-rich soy-based diet and the protection afforded by soy-derived products against experimental mammary tumours in rats suggest that genistein and other isoflavonoid compounds may exert an anti-tumour activity. We analysed the effects of genistein on cell number and cell cycle progression (flow cytometric analysis of propidium iodide-stained nuclei) of human breast cancer cells (MCF-7) *in vitro*. Genistein produced a significant, dose-dependent inhibition of MCF-7 cell growth with an ID_{50} of ~40 μM after 72 h of incubation. Cell cycle analysis showed a reversible G_2/M arrest in cell cycle progression at 10 μM genistein concentrations, whilst a marked fall in S-phase cell percentage associated with a persistent arrest in G_2/M phase was observed in cultures treated with genistein doses equal to or greater than 50 μM. These effects were significant at 24 h of incubation; flow cytometric analysis at later times (48 and 72 h) revealed a population of cells with decreased DNA content and nuclear fragmentation characteristic of apoptosis. Thus, the growth inhibitory activity of genistein in MCF-7 cells results from the sum of cytostatic and apoptotic effects. Since the mitogenic action of insulin and insulin-like growth factor (IGF)-I in MCF-7 cells is a tyrosine kinase-dependent phenomenon, we analysed the genistein impact on S-phase entry produced by insulin in cultures partially synchronised in G_0/G_1 phase by serum deprivation. Insulin addition after a 36-h culture period in serum-free medium produced a strong increase in the percentage of S-phase cells (from 18.4 ± 2.3 to 46.2 ± 4.1 after 24 h) which was almost completely blocked by 100 μM genistein (20.1 ± 3.1). Immunofluorescence analysis with a fluoresceine isothiocyanate (FITC)-conjugated anti-phosphotyrosine antibody revealed a strong increase in MCF-7 cell staining after insulin stimulation, but not when genistein was added with insulin. In conclusion, the dietary phyto-oestrogen genistein inhibits *in vitro* growth of MCF-7 human breast cancer cells through blocks in the "critical checkpoints" of cell cycle control and induction of apoptosis. These effects are likely to depend on impairment in the signal transduction pathway from tyrosine kinase receptor(s).

Key words: genistein, isoflavonoids, MCF-7 cells, breast, apoptosis
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INTRODUCTION

GENISTEIN is a natural phyto-oestrogen present in plant foods including citrus fruits and soybean. Genistein and related flavonoid compounds, such as daidzein and equol, are absorbed from the gastrointestinal tract and eliminated in urine. Urinary levels are higher in people like the Japanese and western vegetarians, who eat soy or other vegetables [1]. Significant amounts of genistein circulate in human blood [2], and this molecule accumulates in various tissue, such as breast tissue and in milk [3, 4].

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There are three main reasons for the recent interest in genistein and related isoflavonoid compounds: (1) epidemiological data suggest that the low incidence of breast cancer in oriental women is related to the consumption of a flavonoid-rich diet [5]; (2) genistein and/or urine-derived flavonoid substances have a strong growth-inhibiting activity on tumour cells *in vitro* [6–9], protect animals from experimentally-induced mammary tumours and inhibit neo-angiogenesis [10]; (3) genistein has been shown to be a competitive inhibitor of ATP binding to the catalytic domain of tyrosine kinase and to inhibit tyrosine kinase actvity of both growth factor receptors [epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin and IGFs] and oncogene products (pp60v-src, pp110gag-fes) [11].

The *in vitro* growth of human breast cancer cell lines is potently inhibited by genistein [6], but little is known about the mechanisms of action of this molecule in counteracting breast

tumour cell growth. In this study, we analysed the effect of genistein on growth, cell cycle progression and proliferative response to insulin stimulation of *in vitro*-cultured MCF-7 human breast tumour cells.

MATERIALS AND METHODS

Media, sera, antibiotics, propidium iodide, sodium vanadate and other laboratory reagents were purchased from Sigma (St Louis, Missouri, U.S.A). Genistein (5,7,4'-trihydroxyflavone) was obtained from ICN-Flow Biomedicals (Bucks, U.K.). Stock solutions of the drug (10 mM) were prepared in dymethylsulphoxide (DMSO) and stored at −20°C in the dark. Dilutions were made in complete tissue culture medium.

Cells and cell culture

MCF-7 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/ml penicillin, 50 μg/ml streptomycin and 300 mg/l l-glutamine in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Cells were split every 4–6 days and seeded at 1×10^6/plate in 10-cm diameter plastic culture dishes. Immunocytochemical staining methods (ERICA, Abbot, Milan, Italy) showed that the cultures were strongly oestrogen receptor-positive and free from mycoplasm contamination.

Analysis of MCF-7 cell growth

Exponentially-growing MCF-7 cells from stock cultures were washed twice with sterile Hank's balanced salt solution (HBSS), and removed from the dish with 2 ml 0.05% trypsin and 0.02% EDTA in HBSS. The cells were then collected by centrifugation at 500 *g* for 3 min, washed three times with DMEM-1% BSA, and dispersed by gentle passages through a Pasteur pipette. Cells were seeded at 20000/ml in a special medium (DMEM/F-12 50:50 v/v with 10% FBS) either in 35-mm diameter culture dishes for flow cytometric analyses or in 24-well culture plates (5000 cells/ml) for 96-h experiments. After a 24-h preculture period to ensure attachment, medium was removed, the cultures washed twice with HBSS-1% BSA and fresh medium (DMEM/F-12) supplemented with 10% FBS alone or with the pre-established concentrations of genistein (from a 10 mM stock solution in DMSO) added. DMSO, at the same dilutions, was added in parallel cultures for control. Cells were counted directly in a haemocytometer after trypsinisation and dispersion because the 3-(4,5-dimethyl-thyazol-2-yl)-2,5-diphenyl-tetrazolium bromide colorimetric assay (MTT assay) routinely used in our laboratory proved unsuitable for evaluating the effect of genistein on *in vitro* MCF-7 cell growth [9].

Cell cycle analysis and flow cytometric measurement of apoptosis

A quantitative measure of cell cycle distribution was obtained by flow cytometric analysis of DNA histograms, as described previously [12]. Briefly, MCF-7 cells were cultured in 35-mm plastic wells in DMEM/F-12 medium supplemented with 10% FBS with or without the pre-established concentrations of genistein. In selected experiments, MCF-7 cells were partially synchronised in G_0/G_1 by 36-h preculture in serum-free medium and then restimulated with 10 μg/ml insulin with or without 100 μM genistein. At the pre-established times, the cells were washed twice with cold PBS and 2 ml fluorochrome solution (propidium iodide 0.05 mg/ml dissolved in 0.1% Na citrate with 0.1% Triton-X 100) added. The plates were placed at 4°C in the dark for 60–90 min, the adherent cells dislodged by repeated pipetting and the stained cells transferred to test tubes for DNA analysis.

DNA fragmentation, which characterises apoptotic cell death, was evaluated by a previously described flow-cytometric procedure [13, 14]. At the pre-established times, the monolayer cells and the overlying medium were collected and centrifuged at 500 *g* for 10 min. Complete removal of cells from the substrate was checked by phase contrast microscopy. After centrifugation, the cell pellet was washed in PBS and resuspended in 2 ml of the same fluorochrome solution for DNA staining.

Cell fluorescence was measured in a FACSCAN flow cytometer (Becton Dickinson, Mountain View, U.S.A.) by an argon ion laser at 488 nm for excitation. Red DNA fluorescence due to propidium iodide (PI) staining was read in the band above 620 nm. Data (forward scatter, right-angle scatter and DNA fluorescence of single nuclei) were recorded in a Hewlett-Packard (HP 9000 model 310) computer after an electronic gating of cell debris and nuclear aggregates by the Doublet

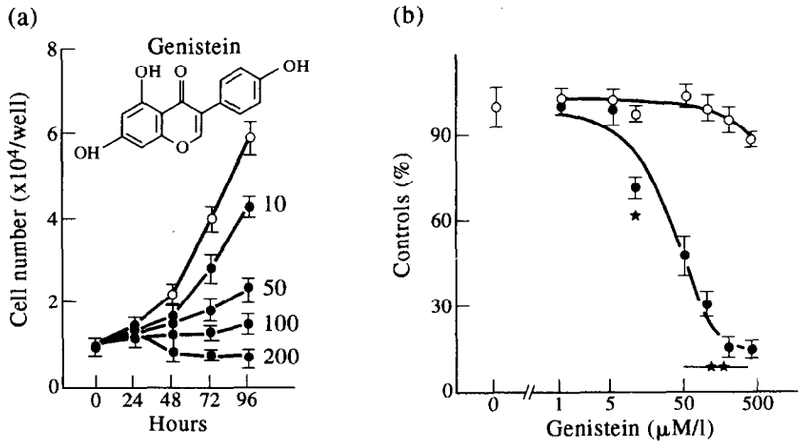

Figure 1. (a) Effects of different concentrations (10–200 μm, closed circles; untreated control, open circle) of genistein on MCF-7 cell growth in a 96-h time-course experiment. Genistein structure is reported in the upper left corner. (b) Dose-dependent inhibition of MCF-7 cell growth by genistein in a 72-h dose-response experiment (closed circles, genistein treated; open circles, untreated controls). Results are the mean ± S.E. of three experiments performed in triplicate. ⋆ $P < 0.05$; ⋆⋆ $P < 0.01$ by Kruskal–Wallis' analysis of variance versus controls.

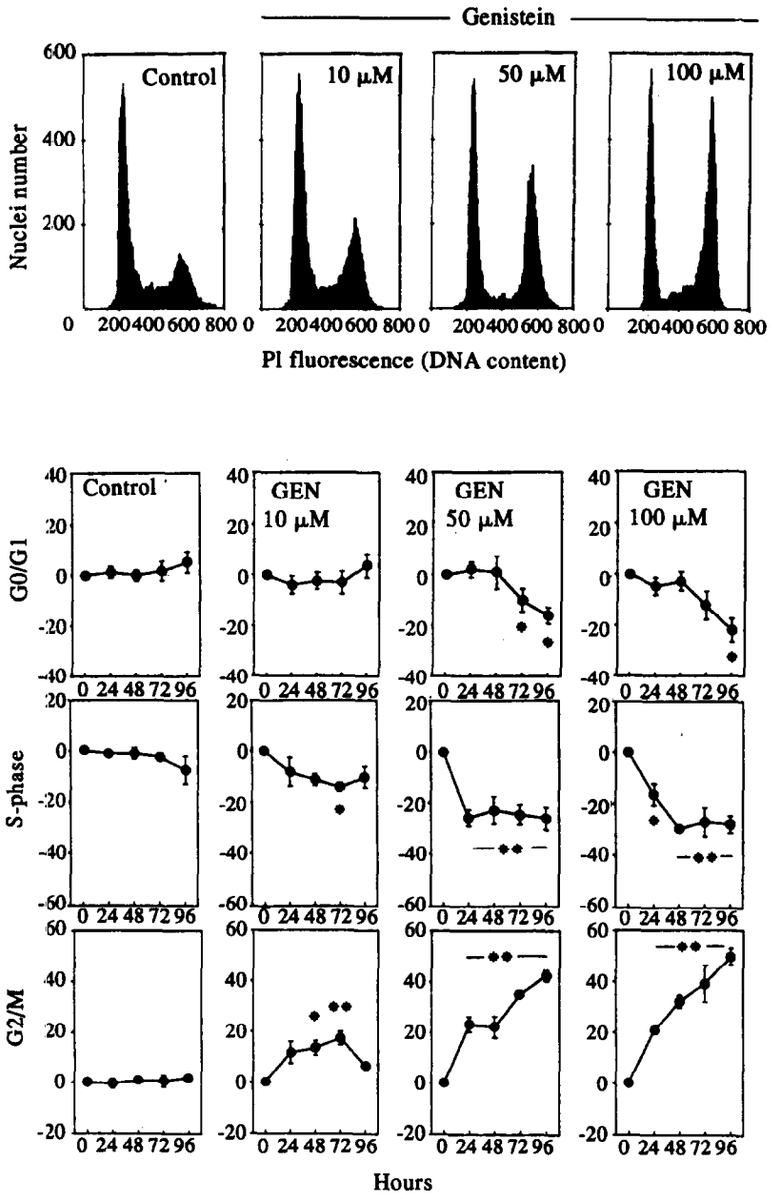

Figure 2. Flow-cytometric analysis of cell cycle distribution of MCF-7 exposed to genistein. The DNA histograms (upper panels) display the dose-dependent effect of a 24-h treatment with increasing genistein doses in a representative experiment (S-phase reduction and G_2/M arrest). The lower panels show the effect of different genistein concentrations on cell-cycle distribution of MCF-7 cells in a 72-h time-course experiment. Percentage of cells in the G_0/G_1 S and G_2/M cell cycle phase was calculated from the DNA histograms of propidium iodide-stained nuclei as described in Materials and Methods. The cells were incubated in 10% FCS-DMEM/F-12 with and without genistein and analysed at the indicated times. * $P < 0.05$; ** $P < 0.01$ by Kruskal–Wallis analysis of variance versus controls.

Discriminating Module (DDM, Becton Dickinson). The percentage of MCF-7 nuclei in the different phases of the cell cycle (G_0/G_1, S and G_2/M) was calculated from the histogram of the DNA fluorescence area with a specific DNA cell cycle analysis software (Cell-Fit, Becton Dickinson). A minimum of 10^4 cells/sample were analysed. Nuclei with DNA content more than 3 S.D. below the diploid DNA peak were considered apoptotic. Three-dimensional plots of DNA fluorescence versus side-angle scatter of individual nuclei were generated by the Lysis II software (Becton Dickinson).

Immunofluorescence analysis of tyrosine phosphorylated proteins

For this analysis, cells were grown on poly-lysine precoated 20-mm round coverslips placed on the bottom of 35-mm culture plates. After a 24-h preculture period in DMEM/F-12 plus 10% FCS to ensure attachment, medium was removed, the cultures washed twice with HBSS-1% BSA and reincubated in serum-free fresh medium. After a 36-h period, cultures were stimulated with 10 μg/ml insulin or 10 μg/ml insulin plus 10 μM genistein. Sodium orthovanadate (final concentration 10 μM) was added to the culture medium to stabilise phosphotyrosine-containing protein by inhibiting tyrosine phosphatases. After a 12-h incubation, cell monolayers were washed three times with PBS containing 1 mM sodium orthovanadate and fixed for 1 h at 4°C with 2% paraformaldehyde. Cells were permeabilised with 0.15% Triton X-100, washed in 10 mM Tris (pH 7.4), 75 mM NaCl, 1 mM EDTA, 1 mg BSA/ml and stained with 2 μg/ml FITC-conjugated anti-phosphotyrosine monoclonal antibody

(PY20, ICN Biomedicals, Costa Mesa, California, U.S.A.) for 2 h at room temperature. Cell monolayers were then washed thoroughly with PBS-1% BSA, the coverslips mounted on slides, observed and photographed on a Leitz Orthoplan fluorescence microscope.

Statistical analysis

All data are the mean ± S.E. Due to the non-normal distribution of the data, non-parametric tests (Wilcoxon's rank sum test and Kruskal–Wallis analysis of variance) were adopted for statistical evaluation of the results.

RESULTS

Effect of genistein on growth and cell cycle progression of MCF-7 breast tumour cells

Cell growth was assayed in cultures exposed to 1–200 μM genistein in 10% FCS-supplemented DMEM/F-12. Under these conditions, genistein exerted significant dose-related inhibition on MCF-7 cell growth (Figure 1). Cell viability remained high (< 10% Trypan blue positive cells) in cultures treated with genistein concentrations of up to 50 μM, but there was a time- and dose-related increase in cell death in cultures incubated with genistein concentrations equal to or greater than 50 μM. ID_{50}, as measured by the number of viable cells 72 h after the addition of genistein, was seen at about 40 μM.

To analyse the genistein effect on cell cycle progression, the cell cycle phase distribution in MCF-7 cultures was measured by flow cytometric analysis of propidium iodide-stained nuclei. Exposure of MCF-7 cells to genistein concentrations greater than 10 μM resulted in a progressive reduction in S-phase DNA-synthesising cells and a significant increase in the proportion of cells in the G_2/M peak of the DNA histogram (Figure 2). Since fluorescence microscope observation of PI-stained nuclei did not reveal any augmentation in the number of mitotic cells, the increase in the percentage of tetraploid nuclei was dependent on a G_2/M arrest in the cell cycle progression [15]. The alterations in cell cycle distribution were transient in cells exposed to 10 μM genistein, but persisted during the overall culture period in cultures treated with concentrations of genistein equal to or higher than 50 μM. Therefore, genistein produces specific blocks in the two critical check-points of cell cycle control [15], namely from G_1 to the S-phase and from G_2 to mitosis, in MCF-7 cells.

At genistein concentrations above 50 μM, a fraction of cells with reduced DNA staining, represented on the DNA histograms as a hypodiploid peak characteristic of apoptotic nuclei [8, 12–14, 16, 17] was evident after 48 h of incubation (Figure 3). Cell viability was still high at this time, but fell rapidly in cultures which displayed this phenomenon. Fluorescence microscope examination of PI-stained cells confirmed the presence of a nuclear fragmentation pattern typical of apoptosis. However, DNA extracted from these cultures showed no evidence of DNA degradation into oligonucleosome-sized fragments (data not shown). Thus, the growth-inhibitory activity of genistein concentrations greater than 50 μM on MCF-7 cells results from the sum of cytostatic and apoptotic effects.

Effects of genistein on the proliferative action of insulin in MCF-7 cells

To verify whether the inhibition of receptor-associated tyrosine-kinases reported for genistein in isolated membranes [11] has a relevant biological counterpart in living cells, we investigated the effect of genistein on S-phase re-entry produced by insulin in MCF-7 cells growth-relented by serum deprivation [18]. For this purpose, MCF-7 cultures were partially synchronised in the G_0/G_1 cell cycle phase by 36-h incubation in serum-free medium, and then stimulated with 10 μg/ml insulin with and without different concentrations of genistein (Figure 4).

Insulin addition produced a strong increase in the percentage

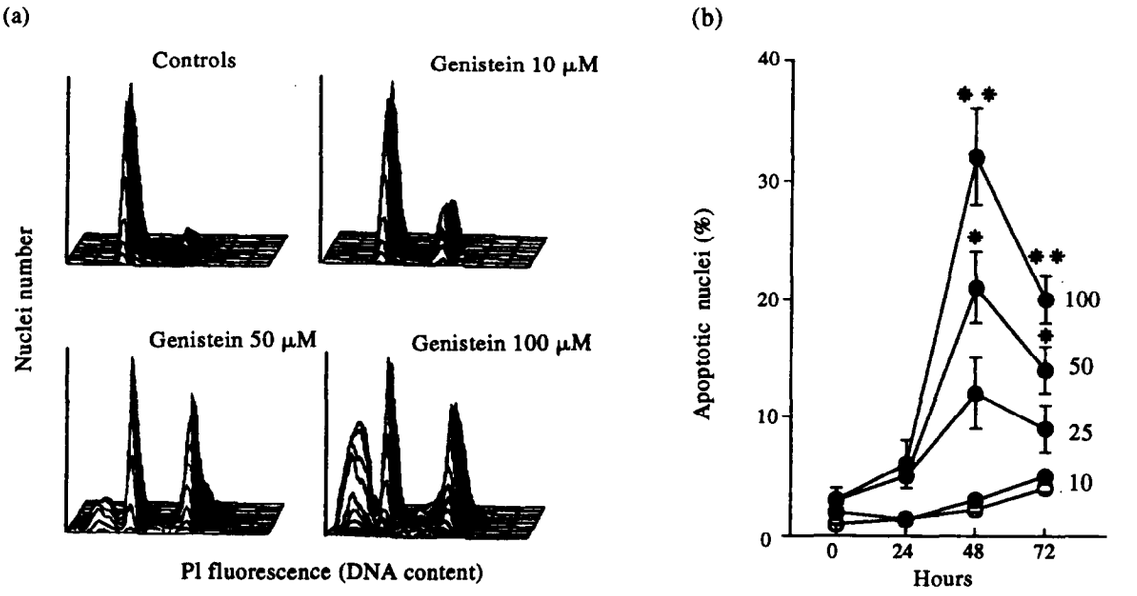

Figure 3. (a) Flow cytometric evaluation of apoptosis in MCF-7 cells incubated with increasing concentrations of genistein in a representative experiment. Computer-drawn 3-D histograms show an hypodiploid DNA peak, corresponding to the apotic nuclei, after 48 h of incubation with 50 and 100 μM genistein. (b) Apoptosis produced by different genistein concentrations (10–100 μmol) in MCF-7 cells. Cells were cultured in 10% FCS-DMEM/F-12 with and without the indicated genistein concentrations and analysed at the indicated times. Percentage of apoptotic nuclei was measured by flow-cytometric technique as described in Materials and Methods. Results are the mean ± S.E. of three experiments performed in duplicate. * $P < 0.05$, ** $P < 0.01$ versus controls.

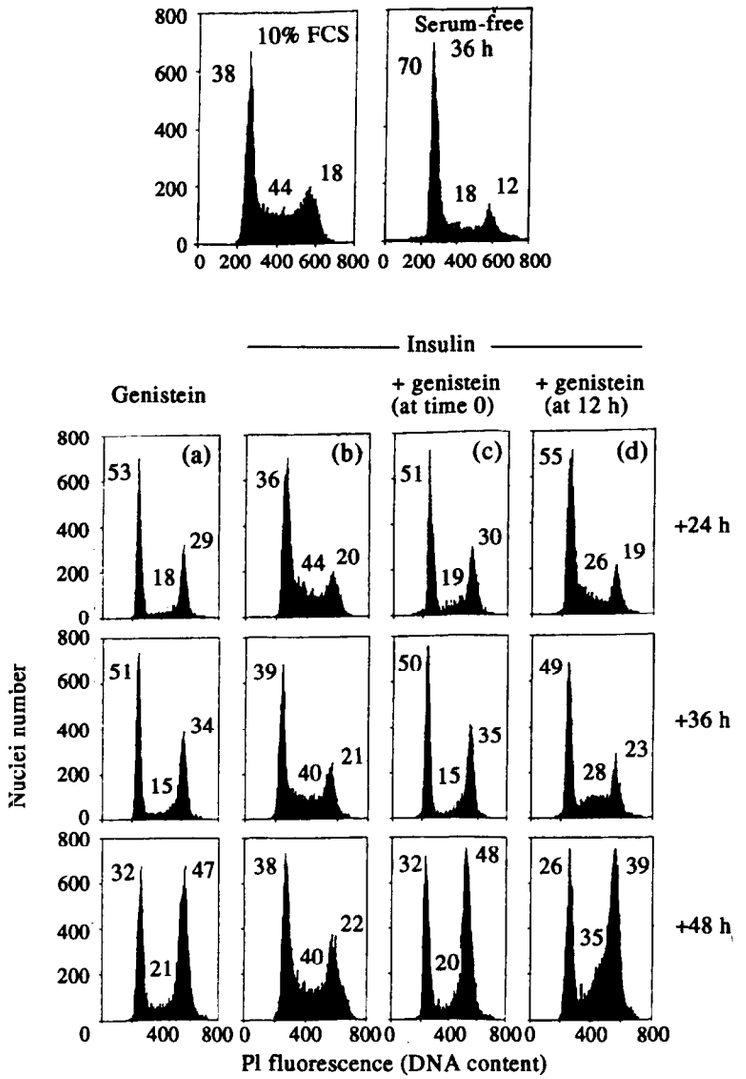

Figure 4. Effect of 10 μM genistein on insulin-induced S-phase re-entry in MCF-7 cells, in a representative experiment. Cells precultured in 10% FCS-DMEM/F-12 medium (upper panel, left) were partially synchronised in G_0/G_1 phase by 36-h serum deprivation (upper panel, right). At this time (time 0), 100 μM genistein (a), 10 μg/ml insulin (b) or 100 μM genistein plus 10 μg/ml insulin (c) were added to the culture medium. MCF-7 cell cycle distribution was measured by flow cytometry after 24, 36 and 48 h. In a parallel experiment (d), genistein was added 12 h after insulin stimulation. The numbers are the per cent distribution of cells in the different cell cycle phases in a representative experiment of three.

of S-phase cells (from 18.4 ± 2.3 to 46.2 ± 4.2 after 24 h of insulin stimulation, $P < 0.01$), which was almost completely abolished by 100 μM genistein (20.1 ± 3.1 after genistein plus insulin; Figure 4a–c). To exclude that the genistein antagonism on insulin effect was dependent on a non-specific toxic activity, and to verify whether the genistein block was really exerted on the G_1-S transition, genistein was added 12 h after insulin stimulation in a parallel experiment. Cells which entered the S-phase before genistein was added showed a normal progression through the S-phase and accumulated later in G_2. However, a progressive reduction in S-phase cells become evident again ater genistein addition, thereby reconfirming the G_1-S cell cycle block (Figure 4d).

To investigate whether the interactions between insulin and genistein in regulating cell cycle progression were associated with variations in the cell content of tyrosine phosphorylated substrates, we performed immunofluorescence analysis of serum-deprived, insulin-stimulated and insulin-stimulated genistein-treated MCF-7 cultures. Cells were fixed and immunostained with a FITC-conjugated anti-phosphotyrosine antibody after 12 h culturing in medium containing 10 μM Na-orthovanadate to prevent tyrosine dephosphorylation by endogenous phosphatases. Insulin treatment led to a marked increase in fluorescence of MCF-7 cells with respect to untreated control cultures (Figure 5a and b). However, the increase in the cell immunostaining produced by insulin was almost totally inhibited by addition of 100 μM genistein (Figure 5c).

DISCUSSION

Protein tyrosine kinases (PTK) play a key role not only in normal cell differentiation, but also in tumour cell proliferation. They are associated with both growth factor receptors (EGF-, PDGF-, insulin and IGFs-receptors [19, 20]) and several oncogene products [21]. Ligand-induced activation of receptor PTK and overexpression of PTK-oncogenes result in a pleiotropic

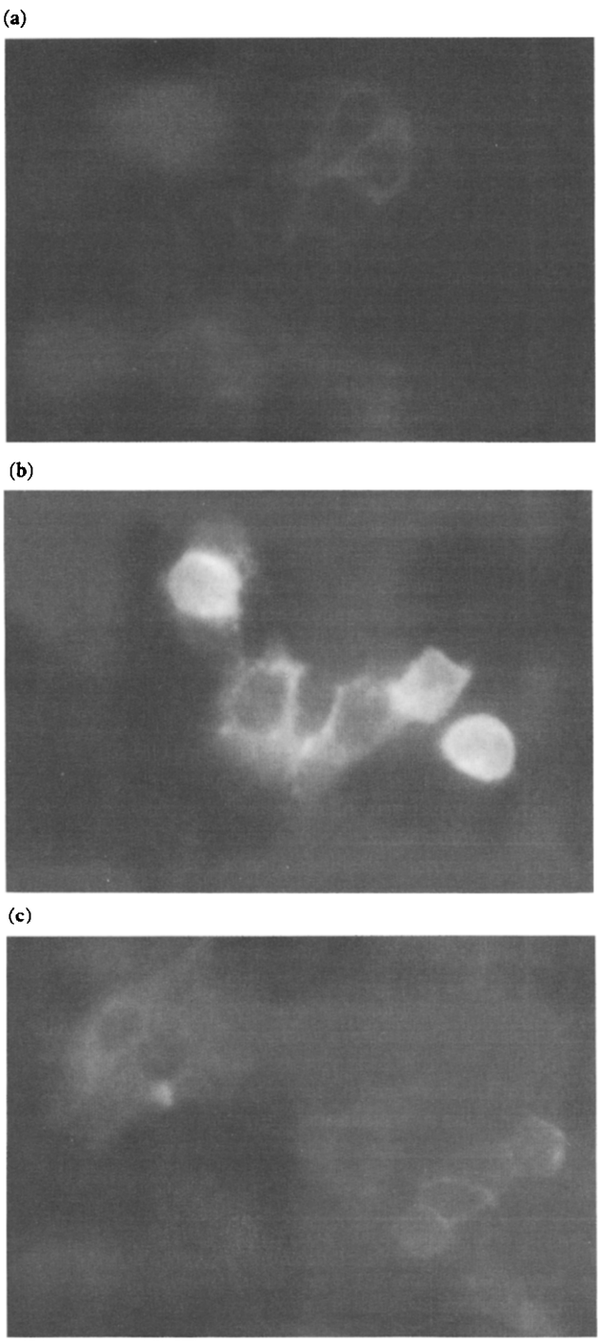

Figure 5. Immunofluorescence photomicrograph of MCF-7 cells stained with an FITC-conjugated anti-phosphotyrosine monoclonal antibody. (a) Cells incubated for a 36-h period in serum-free DMEM/F12. (b) Cells precultured in serum-free medium and stimulated with 10 μg/ml insulin 12 h before immunostaining. (c) Cells precultured in serum-free medium and stimulated with 10 μg/ml insulin plus 100 μM genistein 12 h before analysis. Results of a representative experiment of two. Original magnification ×800.

signal that is transmitted to the nucleus through a cascade of regulatory events [20].

A number of PTK inhibitors, both natural and synthetic, have been discovered and investigated as promising new therapeutic agents for the treatment of cancer [22]. Genistein is a flavonoid phyto-oestrogen with a strong and specific activity against EGF- and PDGF-receptor PTK, as well as the oncogenic tyrosine kinases pp60v-src and pp110gag-fes [11]. Genistein and genistein precursors are present in soy and citrus fruits and absorbed by the gastro-intestinal tract from dietary sources [1–4]. Genistein itself is eliminated at high concentrations in the urine of people who traditionally consume a soy-rich diet, such as the Japanese [2] and western vegetarians [7]. Furthermore, genistein plasma levels in the micromolar range have been measured in some Japanese people [2]. Interestingly, the incidence of, and mortality from, breast cancer is low among Japanese women. Moreover, a soy-rich diet reduces experimentally-induced mammary tumours in rats [5]. These data suggest that genistein and/or related compounds protect against some types of cancer.

Our present data confirm tha genistein exerts a dose-dependent inhibition of MCF-7 human breast cancer cell growth *in vitro* [6], and demonstrate that arrests in the two critical check points of the cell cycle control and induction of apoptosis are responsible for the anti-tumour effect of this molecule. Cell cycle arrest is a well-known effect of protein kinase inhibitors [23], since a series of kinase-mediated phosphorylations are essential for cell cycle progression [15]. In normal cells, inhibitors of tyrosine and other protein kinases produce arrest in the G_0/G_1 phase, whereas transformed cells are mainly arrested in the G_2 compartment [24]. An accumulation in the G_2 phase after genistein treatment has already been reported in HL-60 human myelogenous leukaemia and MOLT-4 lymphocytic leukaemia cells, but stathmokinetic analysis showed that G_1-S transition and S-phase progression were also prolonged by genistein in these lines [8]. Our present data confirm that genistein exerts a strong inhibitory effect on the progression of MCF-7 breast tumour cells through two critical points of cell cycle control, namely G_1-S transition and M-phase entry. A number of mechanisms have been put forward to account for genistein-induced tumour cell growth inhibition [8, 25] and its capacity to inhibit receptor-associated PTK(s) has recently been called into question [26]. Because the tyrosine kinase activity of insulin receptors is known to be essential for triggering DNA synthesis in sensitive targets [27], we analysed the effect genistein exerts on cell cycle progression and tyrosine-phosphorylated protein content in insulin-stimulated MCF-7 cells after 36 h preculturing in serum-free medium. Insulin treatment produced a marked rise in both the percentage of cells in S-phase and phosphotyrosine content, as revealed by the more intense fluorescence of cells immunostained with a FITC-conjugated anti-phosphotyrosine antibody. Genistein (100 μM) almost completely blocked the S-phase entry provoked by insulin, and also abolished the insulin-dependent increase in tyrosine-phosphorylated proteins, thereby providing evidence for antagonism on insulin-dependent pathways. The cell cycle arrest was not due to an aspecific toxicity, since cells exposed to genistein after they had passed the G_1-S check point(s) (when genistein was added 12 h after insulin stimulation) completed the DNA synthesis and accumulated later in G_2. Although our present results do not provide direct biochemical evidence for an antagonism on insulin receptor-linked PTK(s), they demonstrate that biological effects of insulin on MCF-7 cells are strongly counteracted by genistein. Studies are now in progress in our laboratory to identify the kinase(s) and/or substrates which are the genistein targets in MCF-7 cells.

Incubation with genistein concentrations equal to or greater than 50 μM produced apoptotic death of MCF-7 cells. There is some controversy on the mode of breast cancer cell death and apoptosis has been observed in MCF-7 and other breast cell

lines by some authors [12, 28–30] but not by others [31, 32]. Although conclusive proof is lacking, it is likely that biological heterogeneity of MCF-7 cell lines [33], due to different culture conditions or prolonged subculturing, may be responsible for the otherwise difficult to explain discrepancies. As reported by Warri and colleagues after toremifene [29], genistein-treated MCF-7 cells displayed morphological and flow cytometric aspects of apoptotic death, but not the pattern of DNA fragmentation detectable as ladders in agarose gels.

The cell death by apoptosis [34] that followed the cell cycle derangements produced by genistein is evidence that the cell cycle block and apoptotic death are closely interrelated. Apoptosis follows both the G_2 arrest produced by topoisomerase-II-reacting drugs [16], platinum [35] and kinase inhibitors [8] in a number of tumour cell lines, and the G_0/G_1 arrest induced by either growth-factor deprivation or enhancement in p53 gene expression [34, 36]. The realisation that tumour cell growth may also be related to the suppression of normal cell death by oncogenes and growth factors [37, 38] has opened up new directions for both basic and clinical cancer research. A series of recent reports demonstrate that both receptor-linked and oncogenic PTKs are able to promote cell growth through inhibition of apoptotic cell death. PDGF and IGF-I protect oligodendrocytes from apoptosis during optic nerve development [39] and tumour cells that overexpress the pp185Her2/neu oncogenic tyrosine kinase are resistant to the apoptosis produced by TNF-α and cytotoxic T-cells [40]. In contrast, the dephosphorylation of specific proteins, induced by irradiation, heat shock [41] or activation of receptor tyrosine phosphatases [12, 30] leads to apoptosis in sensitive targets. It can, therefore, be hypothesised that genistein and similar tyrosine kinase inhibitors exert their anti-tumour effect through a negative interference with survival signals; the resulting effect being the induction of apoptosis. These data indicate that genistein and related isoflavonoid compounds can be promising molecules in cancer chemoprevention and treatment.

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Somatostatin Receptor Expression in Lung Cancer

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Experimental evidence suggests that somatostatin analogues may have a role to play in the management of lung tumours. We evaluated membrane preparations of nine small cell lung cancer (SCLC) cell lines and of tumour samples from 3 patients with non-small cell lung cancer (NSCLC), 1 patient with an atypical carcinoid and another with a bronchial carcinoid for the presence of specific binding sites for RC–160, a potent growth inhibitory octapeptide analogue of somatostatin. Specific binding was noted on six of nine SCLC lines. Radio-receptor assay on the cell line NCI H 69 showed evidence of two specific binding sites for RC–160, one with high affinity and the other with low affinity. Binding sites were also found on all five tumour samples. Scatchard analysis indicated the presence of a single class of receptors with high affinity in each case. Histological assessment of the resected specimens before binding assay showed them to be comprised of tumour cells and necrotic tissue, stroma and/or inflammatory cells. Therefore, the specific binding of RC–160 may be to tissues other than the tumour cells. In 3 patients, from whom the tumour samples were obtained, radiolabelled somatostatin analogue scintigraphy using [^{111}In] pentetreotide was performed prior to surgery. In all cases, the radiolabel localised the disease. This study demonstrates the presence of specific binding sites for RC–160 in SCLC. Furthermore, the detection of specific binding *in vitro* and *in vivo* in NSCLC and intrapulmonary carcinoids demonstrates that these tumours contain cells which express specific binding sites for somatostatin. These results suggest that RC–160 may have a role to play as a therapeutic agent in lung cancer.

Key words: somatostatin, receptor, RC–160, [^{111}In] pentetreotide, lung cancer
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INTRODUCTION

IN VITRO studies have demonstrated that 50–75% of small cell lung cancer (SCLC) tumours express specific high affinity binding sites for somatostatin. Somatostatin analogues inhibit vasoactive intestinal polypeptide-induced cyclic adenosine monophosphate accumulation in SCLC cells, the clonal growth of SCLC cell lines in culture and the growth of SCLC xenografts in athymic nude mice. These results suggest that somatostatin receptors on SCLC are functional, and that the effect of somatostatin on SCLC proliferation is exerted through interference with growth pathways within the cell [1–6]. As a result of the receptor and growth inhibitory studies, somatostatin analogues are currently being evaluated as radiodiagnostic and therapeutic agents in SCLC. Employing radiolabelled somatostatin analogues as the scintigraphic agent, SCLC tumours have been successfully imaged in 62.5–100% of patients evaluated to date [1, 7, 8]. One small study, on the value of octreotide in the treatment of SCLC prior to chemotherapy, yielded equivocal results and demonstrated that this agent did not interfere with the subseqent chemoresponsiveness of the disease [4].

Whilst *in vitro* studies have failed to demonstrate the presence of somatostatin receptors in non-small cell lung cancer (NSCLC), somatostatin analogue treatment has inhibited NSCLC xenograft growth *in vivo* [1–6].

RC–160 is a potent octapeptide analogue of somatostatin. Like octreotide, it is a highly potent inhibitor of growth hormone