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MECHANISMS OF ACTION IN NIH-3T3 CELLS OF GENISTEIN, AN INHIBITOR OF EGF RECEPTOR TYROSINE KINASE ACTIVITY

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Abstract—Genistein has been shown to inhibit specifically *in vitro* the epidermal growth factor (EGF)-receptor tyrosine protein kinase activity (Akiyama *et al.*, *J Biol Chem* **262**: 5592–5597, 1987). When the effects of genistein on NIH-3T3 cells were studied, a cytostatic effect was observed at low concentration (<40 μM) and a cytotoxic effect at higher concentration (>40 μM). Genistein was able to block the mitogenic effect mediated by EGF on NIH-3T3 cells (IC_{50} = 12 μM) or by insulin (IC_{50} = 19 μM). Genistein was also able to block the mitogenic effect mediated by thrombin (IC_{50} = 20 μM) although the thrombin receptor does not involve a protein tyrosine kinase activity. Genistein at cytostatic concentration (<40 μM) did not prevent the induction of c-*myc* mRNA synthesis caused by the activation of EGF receptor by EGF. Therefore the cytostatic effect of genistein on NIH-3T3 cells did not appear to be mediated by EGF receptor tyrosine kinase inhibition. This hypothesis was also supported by the absence of effect of genistein on the EGF-stimulated phosphorylation of several proteins and particularly of a cytosolic 80 kD protein. The stimulation of S6 kinase activity of cells treated by EGF was prevented by genistein. The stimulation by EGF of *in situ* S6 phosphorylation was also prevented by genistein. In addition, S6 kinase extracted from cells treated by EGF was inhibited by genistein. These effects occur at similar doses and maximal inhibition of S6 kinase was obtained at about 15 μM.

Tyrosine protein kinase activities are known to be associated with oncogene products of the retroviral "*src*" gene family [1–3] and with several cellular growth factor receptors such as epidermal growth factor (EGF‡) [4], PDGF [5, 6], insulin [7, 8] and insulin-like growth factors [9, 10]. Tyrosine protein kinases seem to play a key role in tumorogenesis. Therefore, inhibitors of protein tyrosine kinase activity might represent a new class of antitumor agents. Recently, two protein tyrosine kinase inhibitors, erbstatin [11] and genistein [12] have been isolated and characterized. Erbstatin, a compound chemically related to tyrosine elicited an antitumor activity on the mouse L1210 leukemia when injected together with a stabilizing agent [13]. Genistein, an isoflavone, has been reported to exhibit specific inhibitory activity against tyrosine kinases of EGF receptor (EGF-R), $pp60^{v\text{-}src}$ and $pp110^{gag\text{-}fes}$. The inhibition is competitive with respect to ATP and non competitive with phosphate acceptor. Treatment of A431 cells with high doses of genistein (IC_{50} = 120 μM) prevents the autophosphorylation of EGF-receptor mediated by EGF [12].

The EGF-R is a transmembrane glycoprotein, of relative molecular mass 170 kD, with an intrinsic ligand-dependent protein tyrosine kinase activity [4]. Binding of EGF to this receptor triggers a number of immediate biochemical events, such as alteration of intracellular free calcium, pH increase, transcription enhancement of several responsive genes among them the protooncogenes c-*fos* and c-*myc*, and phosphorylation of ribosomal S6 protein [14–21]. Several hours after these events, DNA replication and cell division occur. The activation of the tyrosine kinase activity of EGF-R is required to achieve cell response. NIH-3T3 cells transfected with an EGF-R of which the tyrosine kinase has been inactivated by mutation are not stimulated by EGF. In these cells the protooncogene expression of c-*fos* and c-*myc* [22], the phosphorylation of S6 ribosomal protein [23] and DNA synthesis [22] are not inducible by EGF.

Since it is established that the tyrosine protein kinase activity of EGF-R is directly responsible for most of the cellular events mediated by EGF, it was of interest to study the effects of a specific inhibitor of this activity, like genistein.

In this work, we have studied the action of genistein on several biochemical events mediated by EGF, like protooncogene induction, DNA synthesis, S6 kinase activation and *in situ* S6 phosphorylation.

Our results suggest that biological effects of genistein at cytostatic concentrations on NIH-3T3 cells are not mediated through the inhibition of EGF protein tyrosine kinase activity and that S6 kinase may represent one of the genistein target.

MATERIALS AND METHODS

Genistein was purchased from Extrasynthese

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‡ Abbreviations used: EGF, Epidermal growth factor; PDGF, Platelet derived growth factor; ED_{50}, the dose which reduced the cloning efficiency by 50%; IC_{50}, the dose which reduced the incorporation of [^{3}H]thymidine by 50%; PBS, phosphate-buffered saline [NaCl 8 g/KCl 0.4 g/Na_2HPO_4 12 H_2O 0.39 g/KH_2PO_4 0.15 g/L]; TPA, phorbol 12-myristate 13-acetate.

(Lyon, France). Genistein solutions were made in DMSO and in all experiments the final concentration of DMSO was 1%. EGF, insulin and α-thrombin were from Sigma Chemical Co. (Poole, U.K.). [^{3}H]TdR, [α-^{32}P]dCTP, [α-^{32}P]UTP, [^{32}P]orthophosphate were products of the Radiochemical Centre (Amersham, France).

Cell culture and DNA synthesis. The mouse fibroblast line NIH-3T3 was obtained from Dr H. Jacquemin-Sablon (Villejuif) and cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and antibiotics.

For measurement of DNA synthesis re-initiation, cells were grown to sub-confluence in 60-mm Petri dish. They were made quiescent by a 24 hr incubation in a serum-free medium. [^{3}H]TdR (0.5 μCi/mL) was added 6 hr after growth factor and genistein addition. [^{3}H]TdR incorporation was terminated 24 hr after mitogen addition. Radioactivity incorporated into trichloroacetic acid precipitable material was assayed by liquid scintillation spectrometry [23].

Cell survival and drug exposure. For the determination of cloning efficiency, NIH-3T3 fibroblast-cells were seeded in 60-mm diameter petri dishes at a density of 3×10^5 cells/dish and left overnight for attachment. The medium was then replaced either with 3 mL of fresh medium (controls) or with 3 mL of medium containing the drug at the indicated concentrations. After 3 hr of incubation at 37°, the drug was washed of by rinsing the dishes twice with 3 mL of medium and cells were trypsinized. About 500 cells were plated in triplicate in 60-mm diameter Petri dishes containing 5 mL of medium. The colonies were counted about 10 days later. To establish the kinetics of exponentially growing cells in the presence of drug, cells were seeded at 3×10^4 cells/60-mm Petri dishes in the presence of drug and the cell number was determined after trypsinization at different times.

Preparation of RNA and Northern Blot analysis. Cells grown to subconfluence in 10-cm culture dishes were arrested by 24 hr of serum deprivation. Cells were then treated either with different concentrations of drug 1 hr prior to EGF addition, or pretreated with a unique dose of drug for different periods before the addition of the growth factor (30 min, 1, 3, 6 hr). Cells were maintained with EGF for 30 min and then rinsed twice with PBS at room temperature before lysis with 5 mL of solution containing: 5.5 M guanidinium isothiocyanol, 5 mM Na-citrate, 0.1 M 2-mercaptoethanol, 0.5% sarkosyl. Total RNA was centrifuged on a 5.7 M CsCl cushion in SW41 rotor at 85,000 *g* for 20 hr at 20°. Total cellular RNA was recoved by phenol-chloroform, chloroform extraction and ethanol precipitation. Four μg of total RNA per lane were submitted to electrophoresis through 1.2% agarose gell containing formaldehyde. Transfer on uncharged nylon membrane (Amersham hybond N) was performed in 150 mM ammonium acetate.

Radiolabeled probes. For detection of c-*myc* expression, a pMYCME3 plasmid (obtained from Dr F. Dautry) which contains a 1 kb RsaI-Hind III fragment from c-*myc* [24] (exon 3 and part of intron 2) cloned in bluescript (Stratagene, La Joola, U.S.A.) was used. BamHl linearized pMYCME3 was used for direct synthesis of radiolabeled RNA, using T3 RNA polymerase and [α-^{32}P]UTP. The obtained probe had a specific activity $\geq 10^7$ cpm/μg RNA. The 9Cl probe was isolated by Dr F. Dautry (unpublished results) from a c-DNA library of Interleukin 2-stimulated murine lymphocytes. The expression of this gene is not induced by growth factors and it was used as control. The 0.9 kb EcoRI fragment of this gene was inserted into bluescript at the EcoRI restriction site. DNA probe 9Cl was synthetized by using random-priming technique (Multiprim Amersham) with specific activity $\geq 10^8$ cpm/μg DNA. RNA was first hybridized with pMYCME3 probe at 60° for 20 hr in a buffer containing 50% deionized formamide, 750 mM NaCl, 150 mM Tris pH 8, 200 mM sodium phosphate buffer pH 6.8, 10 mM EDTA, 0.1% SDS and 500 μg/ml heparin. The filter was rinsed twice and washed for 45 min at 68° in 90 mM NaCl, 5 mM NaH_2PO_4, 0.5 mM EDTA, 0.1% SDS, 0.1% sodium pyrophosphate. Autoradiography was performed and filter dehybridized twice in boiling buffer (10 mM Tris–HCl, 1 mM EDTA, 0.1% SDS) for 20 min. The membrane was then hybridized with the 9Cl probe for 20 h at 42° in a buffer containing 50% deionized formamide, 1% SDS, 50 mM sodium phosphate buffer pH 6.8, 5 × SSC, 0.1 mg/mL salmon sperm DNA, 0.1% Denhart buffer [24]. Filter was rinsed twice in 2 × SSPE/0.1% SDS at 50° for 15 min. Autoradiography was performed at −70° for 2 days.

Measurement of S6 kinase activity. NIH-3T3 cells were seeded (10^6 cells per 15-cm Petri dishes). After 10 days without change of medium when mitosis was no longer observed [25], cultures were treated with different concentrations of genistein 1 hr before a 30 min stimulation by EGF. Cells were washed in buffer A (80 mM β-glycerophosphate, 15 mM $MgCl_2$, 20 mM EGTA Na_2, pH 7.4), and scraped off in 0.3 mL/dish of buffer A containing 10 μg/mL leupeptine, 1 mM phenylmethylsulfonyl fluoride and were homogenized with a tissue homogenizer. The homogenate was centrifuged at 2° a 100,000 *g* for 1 hr. The supernatant was aliquoted and stored at −70° and used for S6 kinase activity assay. S6 protein kinase activity was measured by using 40S ribosomal subunit as previously described [26]. For measuring the inhibitory effect of genistein on the S6 kinase *in vitro*, the supernatant of the EGF stimulated cells were prepared as described above and the drug was added in the reaction mixture.

In situ *phosphorylation experiments.* NIH-3T3 cells were seeded (10^6 cells per 15-cm Petri dishes). After 10 days without change of medium, cells were washed with 5 mL of phosphate free medium and incubated for 90 min with 50 μCi/mL of [^{32}P]orthophosphate in 5 ml of phosphate free DMEM. Genistein at 15 μM was added at this time. EGF (12 ng/mL) was added for the last 30 min of incubation.

Cells were solubilized in buffer A (50 mM Tris HCl pH 7.8, 2.5 mM KCl, 1 mM $MgCl_2$, 1% Triton X-100, 100 μM sodium vanadate 10 μg/mL leupeptin and 20 mM sodium fluoride) containing 1% sodium deoxycholate. The supernatant obtained by centrifugation at 36,000 *g* for 20 min was then centrifuged at 150,000 *g* for 120 min. The pellet contained

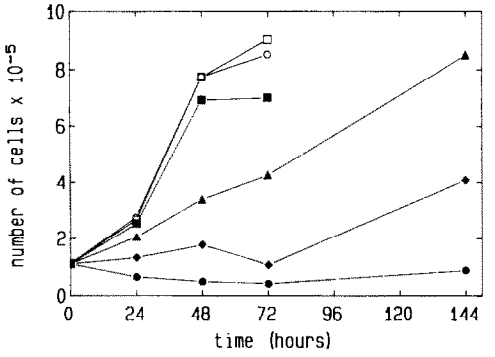

Fig. 1. Effect of genistein on cell growth. Cells were grown in the absence (□) in the presence of DMSO (○) or in the presence of different concentrations of genistein 4 μM (■), 20 μM (▲), 40 μM (♦) and 75 μM (●) for different times. At the indicated time the number of cells was determined. In some cases, after 72 hr in the presence of genistein, the drug was removed from the medium and the cell number was determined 72 hr after.

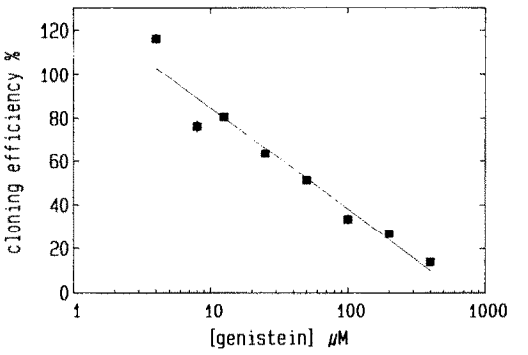

Fig. 2. Effect of genistein on cell survival as a function of drug concentration. After 3 hr of drug treatment, the cell cloning efficiency was measured as described in Materials and Methods.

crude ribosomes and the supernatant cytosolic proteins. The supernatant was kept at −20°.

Ribosomal pellet was resuspended in 500 μL buffer A containing 0.5% sodium deoxycholate and ribosomes were centrifuged at 65,000 *g* for 18 hr through 1 mL of 1.5 M sucrose cushion made in buffer A. To remove non-structural ribosomal proteins, ribosomes were incubated in 50 mM Tris pH 7.5, 12.5 mM $MgCl_2$, 0.88 M KCl, 20 mM 2-mercaptoethanol and 0.1 mM puromycin for 15 min at 37° and centrifuged at 150,000 *g* for 2 hr at 28°. Ribosomal proteins are extracted as described in Ref. 27.

Finally, the labelled ribosomal proteins were dissolved in urea 8 M/electrophoresis buffer (1/1) and separated by electrophoresis [27].

Cytosolic proteins were precipitated with 10% trichloroacetic acid, washed as described in Ref. 27 and submitted to electrophoresis in the same condition than for ribosomal proteins.

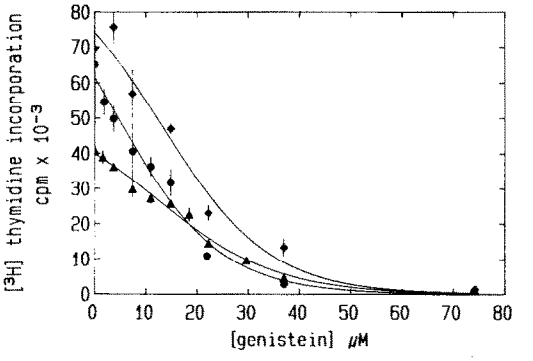

Fig. 3. Effect of genistein on growth factor-induced [^{3}H]thymidine incorporation. [^{3}H]Thymidine incorporation was measured as described in Materials and Methods, after treatment either EGF (●—●) insulin (▲—▲) or thrombin (♦—♦) and different concentration of genistein.

RESULTS

Effect of genistein on cell growth and survival

As shown in Fig. 1, NIH-3T3 cell growth was inhibited by genistein at concentrations from 4 to 75 μM. At concentrations below 40 μM, cells were able to recover growth after the drug was removed. In contrast, at 75 μM and above, cells were unable to recover growth. Cytotoxicity assessed by cloning efficiency measured after 3 hr of treatment yielded an ED_{50} of 35 μM (Fig. 2).

Effect of genistein on DNA synthesis

As previously described [28], EGF induces DNA synthesis in quiescent cells. In our experiments, addition of 12 ng/mL EGF to quiescent cells increased 2.5-fold the thymidine incorporation level (not shown). When genistein is added at increasing concentrations to EGF stimulated cells, the thymidine incorporation decreased as shown in Fig. 3. The observed IC_{50} is 12 μM.

The same inhibition pattern of genistein was obtained when cells were stimulated with insulin (IC_{50} = 19 μM). The inhibitory effect of genistein could therefore involve other targets stimulated by growth factors. Since thrombin is thought to enhance DNA synthesis through inositol phosphate pathway [29], the effect of genistein on DNA synthesis stimulated by thrombin was studied in NIH-3T3 cells. The inhibition of thymidine incorporation observed in this case was comparable to that observed with EGF and insulin (IC_{50} = 20 μM).

These results show that genistein can block growth factor induced mitogenesis without specificity for growth factor receptors possessing tyrosine kinase activity. This suggests that genistein could act on sites other than EGF-R tyrosine kinase. Otherwise, genistein, also inhibited thymidine incorporation in unstimulated cells but at higher concentrations (IC_{50} = 35 μM) where a cytotoxic effect of the drug was important as shown in Fig. 2.

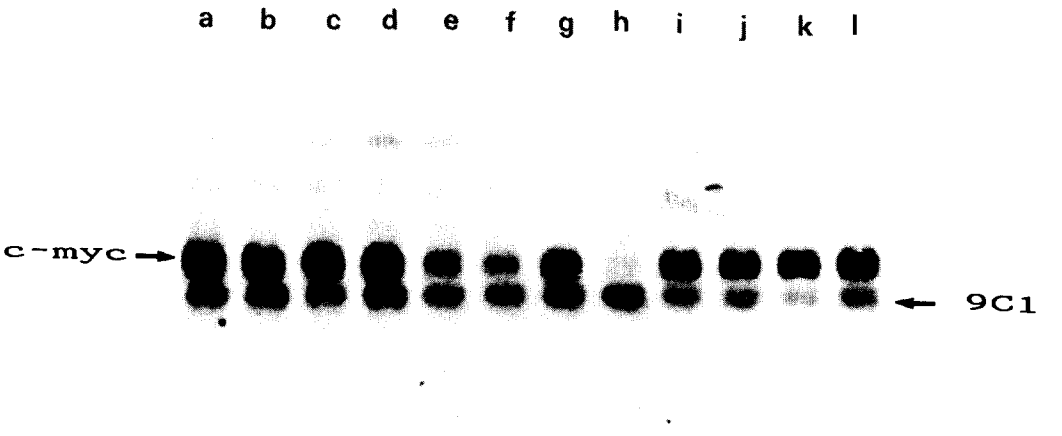

Fig. 4. Effect of genistein on the c-*myc* mRNA synthesis stimulated by EGF. Total mRNA was isolated from quiescent cells treated 1 hr with 1% DMSO (lane a) or with 4 μM (lane b), 20 μM (lane c), 40 μM (lane d), 75 μM (lane e) of genistein before EGF (12 ng/mL) treatment. mRNA extracted from quiescent cells which were treated with 40 μM of genistein (lane f), with EGF (lane g) without any treatment (lane h). mRNA was also isolated from quiescent cells treated with a single dose of genistein 40 μM for 30 min (lane i), 1 hr (lane j), 3 hr (lane k), 6 hr (lane l) before EGF treatment. The membrane was first hybridized to c-*myc* RNA probe. Afterwards the labelled probe was removed and the blot was rehybridized to 9Cl cDNA probe.

Effect of genistein on the expression of c-myc *after stimulation by EGF*

Treatment of quiescent NIH-3T3 cells by EGF increases expression of c-*myc* [17] and this expression requires the tyrosine kinase activity of EGF-R. In our experiments, using 12 ng/mL EGF, c-*myc* mRNA level reached a maximum 1 hr after stimulation (lanes a and g). When genistein was added at 4, 20, 40 μM to the cells 1 hr prior to stimulation by EGF, no decrease of c-*myc* expression is observed (Fig. 4, lanes b–d) although DNA synthesis was inhibited. At a cytotoxic concentration of genistein (75 μM), a decrease of c-*myc* expression was observed (Fig. 4, lane e). No decrease of c-*myc* expression was observed when cells were treated with a single dose of genistein (40 μM) for different periods (0.5, 1, 3, 6 hr) (Fig. 3, lanes i–l) before addition to EGF.

These observations indicate that genistein below 40 μM did not inhibit the EGF receptor tyrosine kinase. This strengthens the hypothesis, suggested above that genistein interacts with other targets to promote its effect on DNA synthesis stimulated by various growth factors. As S6 kinase appears to be a common step taking place rapidly after binding of various growth factors to their receptors, the S6 kinase seemed a candidate.

Effect of genistein on S6 kinase activity and in situ *S6 phosphorylation*

Within minutes of addition of EGF to quiescent NIH-3T3 cells, there is a specific enhancement of 40S ribosomal protein S6 phosphorylation [21] which is associated with an increase S6 kinase activity [25]. The same phenomenon is observed when cells are stimulated by insulin [30]. In the case of thrombin

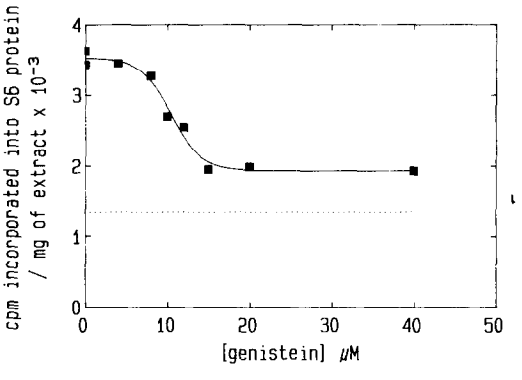

Fig. 5. Modification of EGF-stimulated S6 kinase activity in NIH-3T3 cells treated by genistein. Cellular extracts from quiescent cells treated with different concentrations of genistein before EGF stimulation were used to measured the S6 kinase activity. (· · ·) represents the S6 kinase activity in extracts of unstimulated cells in absence of genistein.

[31] the increase in S6 phosphorylation was observed, but the stimulation of S6 kinase activity has not been reported. The effect of genistein was investigated on EGF-stimulated S6 kinase. As shown in Fig. 5, the S6 kinase activity increases in the presence of EGF as compared to untreated cells. Genistein partially prevents the increase of S6 kinase activity. A reproducible effect was observed from 6 μM and the maximal effect was found at 15 μM. We also observed that activation of S6 kinase by TPA [32] was inhibited two-fold by 20 μM genistein (not shown).

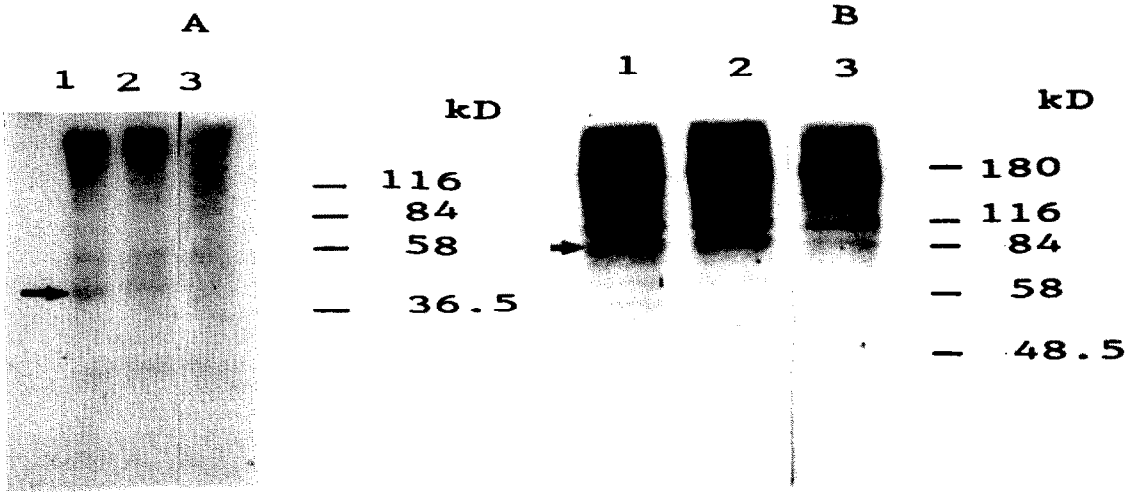

Fig. 6. Effect of genistein on the phosphorylation *in situ* of S6 ribosomal protein (A) and cytosolic 80 kD protein (B). NIH-3T3 cells were labelled with [^{32}P]orthophosphate during 90 min in the absence (lane 1) or in the presence (lane 2) of genistein. EGF was added for the last 30 min. Control experiment without EGF and genistein in lane 3. The arrows indicate the position of S6 ribosomal protein in (A) and the position of the 80 kD cytosolic protein in (B), respectively.

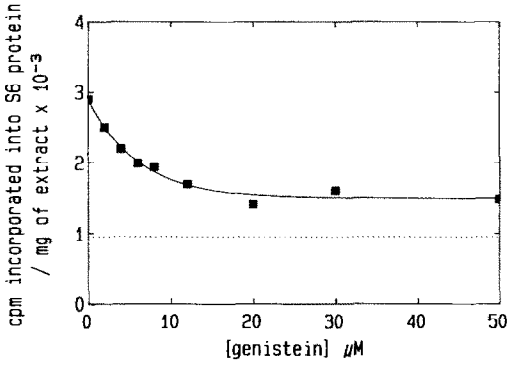

Fig. 7. Effect of genistein on S6 kinase activity *in vitro*. Different concentrations of genistein were added to cellular extracts stimulated by EGF and the S6 kinase activity was assayed. (· · ·) represents the S6 kinase activity in extracts of unstimulated cells in the absence of genistein.

The observed inhibition of S6 kinase activation reflected effectively a decrease of S6 phosphorylation in the cells as illustrated in Fig. 6A. EGF stimulated *in situ* S6 phosphorylation and this effect was prevented by genistein. In the same experiment, we observed that other EGF receptor mediated events proceeded normally in the presence of 15 μM genistein as shown in Fig. 6B. Indeed phosphorylation stimulated by EGF of several proteins and particularly of 80 kD protein was not blocked by genistein. This observation confirms our proposal that genistein did not inhibit the EGF dependent tyrosine kinase.

Then we looked for a direct effect of genistein on S6 kinase in extracts. For this purpose, the post-microsomal fraction of NIH-3T3 cells stimulated by EGF was incubated with various concentrations of genistein, and the activity of S6 kinase was measured (Fig. 7). Addition of genistein to the incubation mixture reduced the level of the S6 kinase activity. At 20 μM, the level of S6 kinase observed is greatly reduced but did not reach the basal level. A slight effect of genistein was also observed on the S6 kinase of non-stimulated cells (not shown).

DISCUSSION

Genistein has been shown to elicit specific inhibitory effect on protein tyrosine kinase *in vitro* [12]. Its action on other protein kinases only appeared at doses more than 100-fold higher. It was therefore of interest to check whether such inhibition could take place in intact cells and what was the effect on the mitogenic response. A specific protein tyrosine kinase inhibitor might represent a new class of anti-tumor agent.

We have examined in this paper the effect of genistein on early and late events of the mitogenic response. It was found that genistein is a cytostatic drug when used at low concentrations (<40 μM) and a cytotoxic drug at higher concentrations (>40 μM). DNA synthesis stimulated by EGF was inhibited by genistein. Mitogenic response induced by insulin and thrombin was also inhibited by genistein in a similar way and in the same range of concentrations.

The effect of genistein was also examined on the EGF-stimulated induction of c-*myc* mRNA levels in NIH-3T3 cells. At a low concentration of genistein although the EGF-stimulated DNA synthesis was inhibited, we did not observe any modification of the level of c-*myc* mRNA, whether the cells were treated for 1 hr or for different periods of time prior to the mitogen addition. Only an inhibition was observed with a cytotoxic concentration (75 μM) of genistein.

These data clearly indicate that the cellular action of genistein below 40 μM is not mediated through an inhibition of the tyrosine protein kinase. This conclusion agrees with observations made by Ogawara *et al.* [33] who do not find close correlation for

several synthetic derivatives of genistein between their effects on tyrosine kinase activity and on cell growth. An inhibition of the EGF-R tyrosine kinase should prevent the c-*myc* mRNA induction by EGF. Such a lack of c-*myc* mRNA induction is well observed in cells of which the tyrosine kinase of EGF-R has been inactivated by mutation. Also, genistein inhibits the mitogenic effects of thrombin, although the thrombin receptor does not involve a protein tyrosine kinase activity.

Our observations suggest that EGF-R tyrosine kinase of NIH-3T3 cells is inhibited by 75 μM genistein since induction of c-*myc* mRNA is decreased. This agrees with experiments on A431 cells in which the half maximal effect of genistein on autophosphorylation of EGF-R tyrosine kinase is observed at 120 μM [12].

Our results raised the hypothesis that genistein, at cytostatic concentration, can impair another step of the transduction process. The S6 phosphorylation may be this step. It is generally believed that phosphorylation of the S6 ribosomal protein modulates protein synthesis [30, 34]. Such a phosphorylation has been shown to be involved in the cellular response of cells to a variety of hormones, growth factors, transforming viruses, tumor promotors etc... It could represent a common step in the cellular response to various mitogenic agents. We therefore tested the effect of genistein on the cytosolic S6 kinase activity involved in cells treated by EGF. We observed that *in vitro* genistein inhibits S6 kinase activity of cellular extracts. The inhibition takes place at concentrations which are about two to three-fold higher than that able to inhibit *in vitro* the human EGF-R tyrosine kinase [12].

Furthermore, the S6 kinase activity of cells treated by EGF and genistein is maximally inhibited at doses close to 15 μM, the concentration which also prevents the EGF stimulated S6 phosphorylation *in situ*. However, the stimulated enzyme is not completely inhibited as also observed for the *in vitro* experiments. Moreover the S6 kinase activity of unstimulated cells is not strongly inhibited by genistein. This observation is not surprising since the basal level does not only represent the activity of the non-stimulated growth factor S6 kinase but also the residual activity of other kinases such kinase A or kinase C which also phosphorylate S6. Efficient concentrations of genistein on the cells are similar to that which promotes maximal inhibition *in vitro* on S6 kinase. This concentration (15 μM) can be compared to the dose of genistein which is necessary to inhibit the autophosphorylation of EGF-R in human A431 cells (~120 μM) [12].

In vivo, there is a strong qualitative association between S6 phosphorylation and DNA synthesis in 3T3 cells [35]. In addition, all mitogens, for 3T3 cells, including serum, fibroblast growth factor, insulin, EGF and insulin-like growth factor [21, 36] stimulate S6 phosphorylation. A similar correlation between stimulation of growth and S6 phosphorylation has been shown for growth factors in other cells [37]. Since phosphorylation of S6 ribosomal protein by S6 kinase must probably modulate mRNAs translation [21, 34], the inhibition of S6 kinase by genistein may lead to inhibition of translation of some mRNA coding for proteins required for DNA synthesis or mitosis. The effect of genistein on the cytosolic S6 kinase activity provides a reasonable explanation for the inhibition by the drug of the EGF effect on DNA synthesis.

It is possible that transduction under EGF action involved other serine protein kinases as shown for other growth factors such as insulin-stimulated MAP 2 kinase [38] or PDGF-stimulated Raf-1 associated serine-threonine kinase [39]. These kinases might be eventually inhibited by genistein.

On the other hand, we do not exclude that the inhibition of topoisomerase II by genistein [40, 41] contributes also to the inhibition of DNA synthesis.

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