Rapid inhibition of ongoing DNA synthesis in human glioma tissue by genistein

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Abstract. The effect of genistein, a protein tyrosine kinase and topoisomerase II inhibitor, on the DNA synthesis rate was studied in 21 human glioma specimens obtained at routine craniotomies for tumor resection. Ongoing DNA synthesis rate was determined by using a method based on the generation of tissue mini-units immediately after tumor resection and short incubation time (0-120 min) with [methyl- 3H]-thymidine. A 9-77% inhibition of DNA synthesis rate by 100 μM genistein was observed in 18/21 of the glioma specimens. In these cases, the average percentage of inhibition was 55±20% (mean ± SD, P<0.0001, Student’s t-test) and the inhibitory effect was >50% in 12/18 of the cases. In 3 cases genistein increased the DNA synthesis rate. The inhibitory effect of genistein had a short-time onset and was concentration-dependent. Additional experiments in 4 cases showed that herbimycin A had no effect on DNA synthesis rate while etoposide inhibited similarly to that of genistein. Our results suggest that the effect of genistein on DNA synthesis rate in gliomas is independent of protein kinase inhibition and probably mediated by topoisomerase II inhibition. In the RG2 model, 50 μM genistein inhibited ongoing DNA synthesis in glioma cells with little or no effect in normal tissue. The data also encourage further investigations on the therapeutic potential of genistein for gliomas.

Introduction

The uncontrolled growth of gliomas may result in part from excessive activation of protein tyrosine kinases. Consistent with this hypothesis, protein tyrosine kinase inhibitors reduce the proliferation of human glioma cell lines with IC50 proliferation values approximating those for inhibition of tyrosine kinase activity in cell free protein extracts (1) and block infiltration of glioblastoma spheroid cells into normal tissue in vitro by a mechanism involving inhibition of epidermal growth factor receptor (EGF-R) associated tyrosine kinase (2). The c-erbB gene encoding EGF-R is amplified and overexpressed in up to 50% of malignant gliomas (3). Tyrosine kinase activation of the EGF-R leads to phosphorylation of intracellular proteins, thus initiating an intracellular cascade that leads to cell division.

The isoflavone genistein is found in high amount in soy and has been shown to inhibit the growth of several normal and neoplastic cell lines (4) including human glioma cell lines (1,5). The inhibitory effect on cell proliferation (DNA synthesis) rate is generally attributed to inhibition of tyrosine kinases, especially the EGF-R associated tyrosine kinase activity, topoisomerase II (6) and histidine kinase activity (7) as well as other effects (8). Compared to etoposide (another topoisomerase II poison), the cytotoxic and genotoxic effects of genistein differ significantly (9). Genistein is a small molecule (M.W. 270.2 Da). When injected intraperitoneally to rats, it rapidly appears in brain tissue and in microdialysate fluid from the corpus striatum indicating that genistein is capable of crossing the blood-brain barrier (10).

These data suggest that genistein and other protein tyrosine kinase inhibitors might have a therapeutic potential for tumors expressing high levels of tyrosine kinase activities.

In a previous study, we found a strong inhibitory effect of genistein on ongoing DNA synthesis rate in the developing rat cerebral cortex and a much weaker inhibitory effect in adult rat cerebral cortex (11). Although ongoing DNA synthesis is one of the less studied targets for antineoplastic therapy, it might constitute a useful target to treat highly proliferative cancers, specially those arising in low proliferating tissues. In theory, the main advantage of ongoing DNA synthesis inhibitors is their ability to inhibit DNA replication within short period of time. Thus, higher concentrations of drugs might be able to be delivered locally into fast growing tumors with tolerable toxicity to the surrounding normal low proliferative normal tissue.

We studied the effect of relatively high concentrations of genistein on ongoing DNA synthesis rate in mini-units prepared from human gliomas as well as in mini-units prepared from the rat RG2 glioma model.
Materials and methods

All studies were approved by the Ethics Committee at Huddinge University Hospital. Tumoral tissue specimens were obtained at routine craniotomies for tumor resection. Genistein (Costa Mesa, CA, USA) was prepared as stock solution (100 mM) in dimethylsulfoxid (DMSO, Sigma, Sweden) and stored at -20˚C (12). The final dilutions (10–100 μM) were done in Dulbecco’s modified Eagle’s medium with glutamine and 4,500 mg/l D-glucose (DMEM; Gibco/Life Technologies, Sweden) keeping DMSO concentration below 0.1% (v/v). DMSO alone at the same concentration was used as control.

Generation of tissue mini-units, determination of [methyl-3H]-thymidine incorporation into DNA and ongoing DNA synthesis rate, protein quantitation and determination of effect of genistein were performed as previously reported (11,13). Briefly, mini-units of glioma tissue were generated immediately after tumor resection. These mini-units were then incubated in microwell plates (Nunc, Denmark) with DMEM containing 2 μCi/ml [methyl-3H]-thymidine (Amersham, UK) and genistein or DMSO. Each experimental point was in all but 2 cases determined by at least duplicate. The DNA synthesis rate was calculated as cpm/mg of protein/min. The effect of genistein was determined as change (%) of DNA synthesis rate compared to the corresponding control (Table I) or percentage of control DNA synthesis rate (Fig. 2-4).

Glioma model. The RG2 rat glioma model was performed as previously described (14).

Results

Effect of genistein on ongoing DNA synthesis in tissue mini-units prepared from human gliomas. As shown in Table I,
Tissue specimens were obtained from 15 males and 10 females with an age range of 23-66 years. The histopathological diagnoses were: glioblastoma (16 cases), anaplastic astrocytoma (4 cases), anaplastic oligodendroglioma (2 cases) and one case each of oligodendroglioma, oligoastrocytoma, and pilocytic astrocytoma. The interval between symptomatic onset and present surgery ranged from <1 month to >5 years. Previous or ongoing treatment was present in 8 patients.

Tissue mini-units generated from 6 different tumor specimens were incubated in DMEM containing 2 μCi/ml [methyl-3H]-thymidine during 0, 30, 60, 90 and 120 min (cases no. 1-4) and during 0, 30, 60 and 90 min (case no. 5 and 6). A quite linear incorporation of the radioactive precursor into DNA over time was observed up to 90 min despite the large intertumoral differences in the net rate of [methyl-3H]-thymidine incorporation (Fig. 1).

Figure 1. Temporal incorporation of [methyl-3H]-thymidine into DNA in mini-units from 6 different tumor specimens. Each point is the mean ± SEM of triplicates except for 0, 30 and 60 min of case no. 5 and 6 (duplicates). r = correlation coefficient.

Tissue mini-units generated from tumor specimens were incubated in DMEM containing 2 μCi/ml [methyl-3H]-thymidine plus DMSO alone (cases no. 1-25, controls/spontaneous DNA synthesis rate) or 100 μM genistein (cases no. 5-25) for 90 min. This concentration of genistein was chosen since it has been shown to inhibit cell proliferation (DNA synthesis) rate in different tissues (1,11,12). The standard incubation time of 90 min was chosen since this time gives a suitable difference in radioactive precursor incorporation between control and experimental samples. Furthermore, this short incubation time ensures minimal metabolic changes and allows monitoring of ongoing DNA synthesis rate (11,13,15). A high (around 250-fold) interspecimen variation of spontaneous DNA synthesis rate was observed (Table I). Genistein at 100 μM decreased the DNA synthesis rate in 18/21 of the specimens. The percentage of inhibition was 9-77% and was >50% in 12/18 of these cases. In the remaining 3 cases (13, 22 and 24) genistein increased the DNA synthesis rate. In the 18 cases where genistein decreased the DNA synthesis rate, the average percentage of inhibition was 55±20 % (mean ± SD, P<0.0001, Student’s t-test) (Fig. 2). The inhibitory effect of genistein was >50% in 8/9 cases with spontaneous DNA synthesis rates >100 cpm/mg of protein/min while this was found for only 4/12 cases with spontaneous DNA synthesis rates <100 cpm/mg of protein/min (Table I). However, the quite heterogeneous and limited material does not allow any conclusion regarding possible relations between DNA synthesis rate and effect of genistein.

The concentration-dependent and temporal effects of genistein could, due to the limited amount of tumor tissue, only be studied in a few cases. Tissue mini-units generated from one tumor specimen (case no. 19) were also incubated with DMEM containing 2 μCi/ml [methyl-3H]-thymidine plus DMSO alone or 10, 50 and 100 μM genistein for 90 min. The results showed that genistein had a concentration-dependent

Figure 2. Effect of 100 μM genistein (G) on ongoing DNA synthesis in mini-units prepared from human gliomas. DMSO at equivalent concentration was used as control (C). Data are the mean ± SE of 46/47 (n) determinations from mini-units prepared from cases 5-16 (see Table I for details).
effect on DNA synthesis rate (Fig. 3). Tissue mini-units generated from 2 tumor specimens (case no. 5 and 6) were incubated with DMEM containing 2 μCi/ml [methyl-3H]-thymidine plus DMSO alone (controls) or 100 μM genistein for 0, 30, 60 and 90 min. The results showed that genistein decreased the DNA synthesis rate within 30 min (Fig. 4). Finally, in 4 cases (no. 10, 19, 21 and 23), the experiments included exposure to 10 μM herbimycin A [a potent tyrosine kinase inhibitor (16)] and/or 250 μM etoposide [a selective inhibitor of topoisomerase II (6)]. Herbimycin A had no effect on DNA synthesis rate while etoposide inhibited the DNA synthesis rate to a similar extent as genistein (data not shown).

**Effect of genistein on ongoing DNA synthesis in tissue mini-units prepared from the RG2 rat glioma model.** The tissue mini-units were prepared from tumor specimens obtained from the rat RG2 glioma model and incubated for 90 min in DMEM containing 2 μCi/ml [methyl-3H]-thymidine + 0 or 50 μM genistein. As control, tissue mini-units obtained from normal cerebral cortex of the opposite hemisphere of the same animal was used. Fig. 5 shows the percentage of DNA synthesis inhibition induced by 50 μM genistein in glioma cells as well as in adult rat cerebral cortex. The result show that the incorporation of [methyl-3H]-thymidine in glioma cells is very high when compared to normal rat cerebral cortex and that 50 μM genistein produced a strong inhibitory effect in glioma cells with no significant effect on normal tissue probably due to the low proliferative activity of the adult normal brain tissue.

**Discussion**

To the best of our knowledge, this is the first report on the effect of genistein on ongoing DNA synthesis rate in tissue specimen from human gliomas and from the rat RG2 glioma model. More than 50% inhibition of DNA synthesis rate by 100 μM genistein was observed in 12/21 of the tumor specimens (Table I). The inhibitory effect was shown to be concentration-dependent (Fig. 3) and of short-time onset (Fig. 4) as previously reported for normal rat cerebral cortex (11). Genistein increased the DNA synthesis rate in 3 cases. Although this enhancement is in contrast with the results for the major part of tumor specimens and the existing literature, an enhancement of experimental colon cancer by dietary genistein was reported by Rao et al (17). The authors emphasize that the biological effects of genistein may be organ specific and inhibit cancer development in some sites while showing no effect or an enhancing effect on the tumorigenesis at other sites. Our results suggest that the effect of genistein may also show interindividual variation even for tumors of the same histopathological type.

The tumors included in this study showed a considerable intertumoral variation in the spontaneous DNA synthesis rate
in cells derived from slowly proliferating tissue such as adult rat cerebral cortex (11). Genistein can be given in vivo with tolerable toxicity (12). Moreover, recent data indicate that genistein at pharmacological doses does not give any reproductive or developmental alterations even after chronic treatments (25-27). Asians consuming a traditional diet with high soy product content may show high plasma concentrations (up to 2.4 μM) of genistein (28). This concentration is close to the IC$_{50}$ (2.7 μM) for the autophosphorylation of the EGF-R associated tyrosine kinase and the IC$_{50}$ (5 μM) for topoisomerase II (6). In male Wistar rats, the plasma level of total genistein after an oral dose of 2,000 mg/kg was maintained at levels of ~40 μM for around 9 h. Even at these high levels no genotoxic effect was observed using different mutagenic and clastogenic tests (29). In a human prostate cell line genistein inhibits cell proliferation only at high concentration (50-100 μM) (30). New delivery methods of drugs such as intracerebroventricular injection (31,32), might allow the application of high concentration of genistein into the brain while preserving potential systemic toxicity in other high proliferating tissue. Considering the fact that the median survival time is 9-14 months in patients with high malignancy-grade gliomas (33), our results encourage further studies in order to investigate the chemotherapeutic and chemopreventive potential of genistein for these tumors.

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References


