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# Etoposide sensitivity of radioresistant human glioma cell lines

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**Abstract** *Purpose*: Malignant gliomas display aggressive local behavior and are not cured by existing therapy. Etoposide, a topoisomerase-II-inhibitor agent, is one of the most active and useful antineoplastic agents. However, etoposide is not usually used on these tumors. We undertook an in vitro study to prove that etoposide is a useful drug for malignant gliomas. *Methods*: Five human glioma cell lines were the basis for this study. Following exposure to various concentrations of etoposide, the glioma cell lines were found to be sensitive; the median concentration inhibiting the number of cells by 50% ($IC_{50}$) was 8.76 μg/ml (range 8–15.8 μg/ml). Since topoisomerase II is the critical target for etoposide, it was of interest to determine the topoisomerase II activity (decatenation of kinetoplast DNA isolated from *Cryphtidia fasciculata*) and the etoposide-induced inhibition of topoisomerase II activity. *Results*: The topoisomerase II activity was homogeneous in glioma cell lines (average of 50% decatenation with 7,000 cells), and topoisomerase II was the target of the etoposide. *Conclusions*: Our results suggest that topoiomerase II-reactive agents may prove to be clinically useful drugs for patients with malignant gliomas.

**Key words** Etoposide · Human malignant gliomas · Topoisomerase II · Radioresistance

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## Introduction

Malignant gliomas are generally considered to be among the most radioresistant tumors [2, 3, 28]. Because of their volume and/or their localization, these tumors are often inaccessible to locoregional treatment such as surgery or radiotherapy [28, 37]. Despite intensive treatment such as combination radiation therapy and chemotherapy after surgery, the median survival of patients with malignant gliomas is generally less than 12 months and virtually no patient with glioblastoma multiforme survives for more than 5 years following treatment [2, 3, 13, 37]. Thus, numerous chemotherapy trials have been undertaken, proving to be rather inefficient but suggesting a significant survival advantage for patients receiving this therapy [2, 3, 13, 37]. Etoposide, a semisynthetic glucoside derivative of podophyllotoxin, is one of the most active and useful antineoplastic agents [17, 30]. The inhibition of DNA topoisomerase II is now known to be a major mechanism for the action of etoposide that stabilizes the cleavable complexes that trigger cell death. Etoposide is more active in tumor cells containing higher levels of topoisomerase II [17, 30]. The tumor growth rates of gliomas are generally high; thus, the level of the nuclear enzyme topoisomerase II could be high, because the level of topoisomerase II is correlated with proliferation of cells. To determine whether etoposide is a therapeutic agent for malignant gliomas we studied the in vitro sensitivity to etoposide of a panel of five characterized glioma cell lines. The activity of topoisomerase II and its inhibition by etoposide were also studied in these cell lines.

## Materials and methods

### In vitro primary cultures

Three primary cultures (G111, G142, and G152) established from glioma biopsies were used in these studies at passage levels lower than ten. In addition, an established glioma cell line (G5) and a

clone derived from this cell line (CL5) were also used. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin, streptomycin, and 15% fetal bovine serum (heat-inactivated at 56 °C for 30 min). The monolayers derived from primary culture in unsealed culture flasks (Primaria Falcon) were kept in a humidified atmosphere of 5% $CO_2$ and 95% air at 37 °C.

### Primary-culture-cell characterization

After one or two passages in culture the cell population was characterized. The determination of the tumorous origin of the culture was performed by karyotypic analysis [12, 34]. Chromosomal abnormalities were defined according to the International System for Human Cytogenetic Nomenclature [18]. The radiosensitivity of glioma cells was assessed as previously described [7, 29] (Mornex et al., submitted for publication).

### Etoposide-sensitivity assay

After the appropriate treatment, cells were removed from the dish with phosphate-buffered saline (PBS) containing 0.03% trypsin and 0.27 mM ethylenediaminetetraacetic acid (EDTA) and were diluted into culture dishes in appropriate numbers to yield between 20 and 200 colonies. Each measurement was performed in triplicate. After 12 days, cultures were fixed with methanol-acetic acid, stained with crystal violet, and scored for colonies containing more than 50 cells. The standard errors were typically less than 15% of the mean value unless otherwise stated.

### Topoisomerase II assay

Nuclear extracts were prepared. Nuclei were isolated as described previously [27, 33]. The activity of topoisomerase II was calculated from the percentage of decatenation obtained. Tritiated kinoplast DNA (KDNA 0.22 μg) was used as a substrate. Reactions to quantify topoisomerase II activity were carried out for 30 min at 37 °C and were stopped with 1% sodium dodecyl sulfate (SDS) and proteinase K (100 μg/ml) [27, 33]. Etoposide inhibition of topoisomerase II activity was carried out using the same procedure; etoposide and topoisomerase II were incubated for 30 min at 37 °C and reactions were stopped with SDS and proteinase K. The percentages of decatenation and inhibition of topoisomerase II by etoposide were obtained.

## Results

### Primary-culture cell characterization

The tumorous origin of all cultured cells was proved by karyotypic abnormalities. A near diploid modal number was observed in three cell lines (G111, G142, and CL5), G5 was near-triploid, and G152 was bimodal. Double-minutes (dmins), which are the cytogenetic expression of gene amplification, were detected in all glioma cell lines (the mean percentage of methaphase with dmins was 43.4%). Three glioma lines (G5, G142, and G152) showed a gain of chromosome 7, loss of chromosome 10 was observed only in G142 and G152, and structural abnormalities of chromosomes 1, 2, 3, 6, and 9 were found in G5, CL5, G142, and G152. Surprisingly, G5 was the only glioma cell line with two copies of chromosome 10. All five glioma cultures were highly radioresistant to SF2, with values of 83.1%, 87.3%, 89.3%, and 94.3% being recorded for G5, CL5, G111, G152, and G142, respectively (Mornex et al., submitted for publication).

### Etoposide sensitivity

The five glioma cell lines were exposed to doses of etoposide ranging from 4 to 40 μg/ml for 1 h. Four glioma cell lines displayed homogeneous sensitivity to

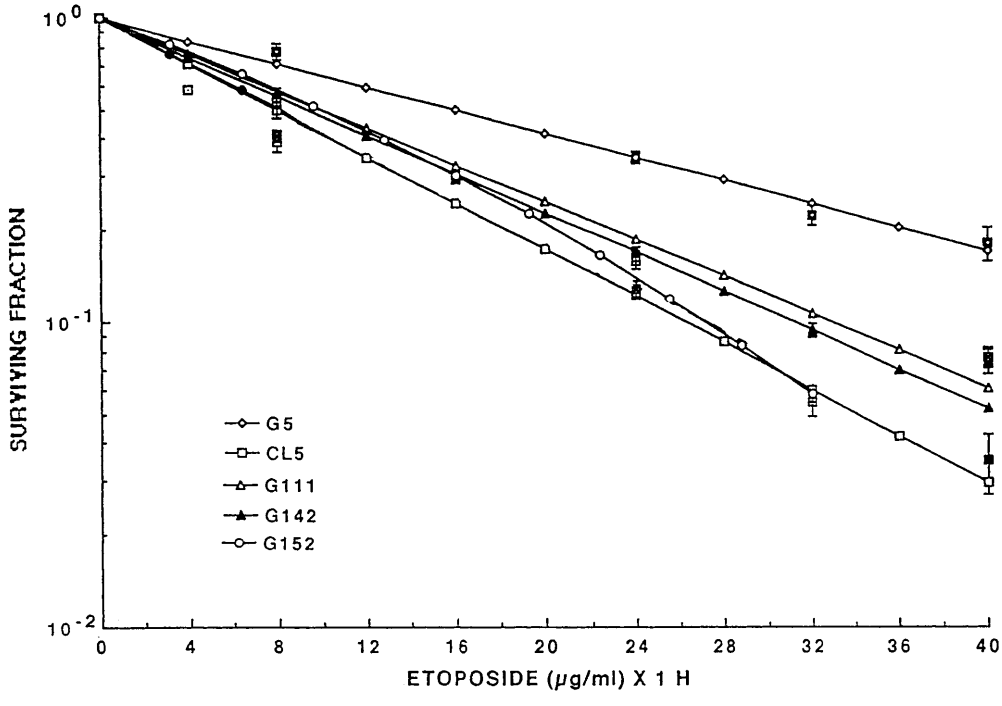


**Fig. 1** Survival curves generated for the 5 glioma cell lines exposed to etoposide for 1 h. Etoposide sensitivity was examined by colony-formation assay. Mean values for 3 independent experiments are presented

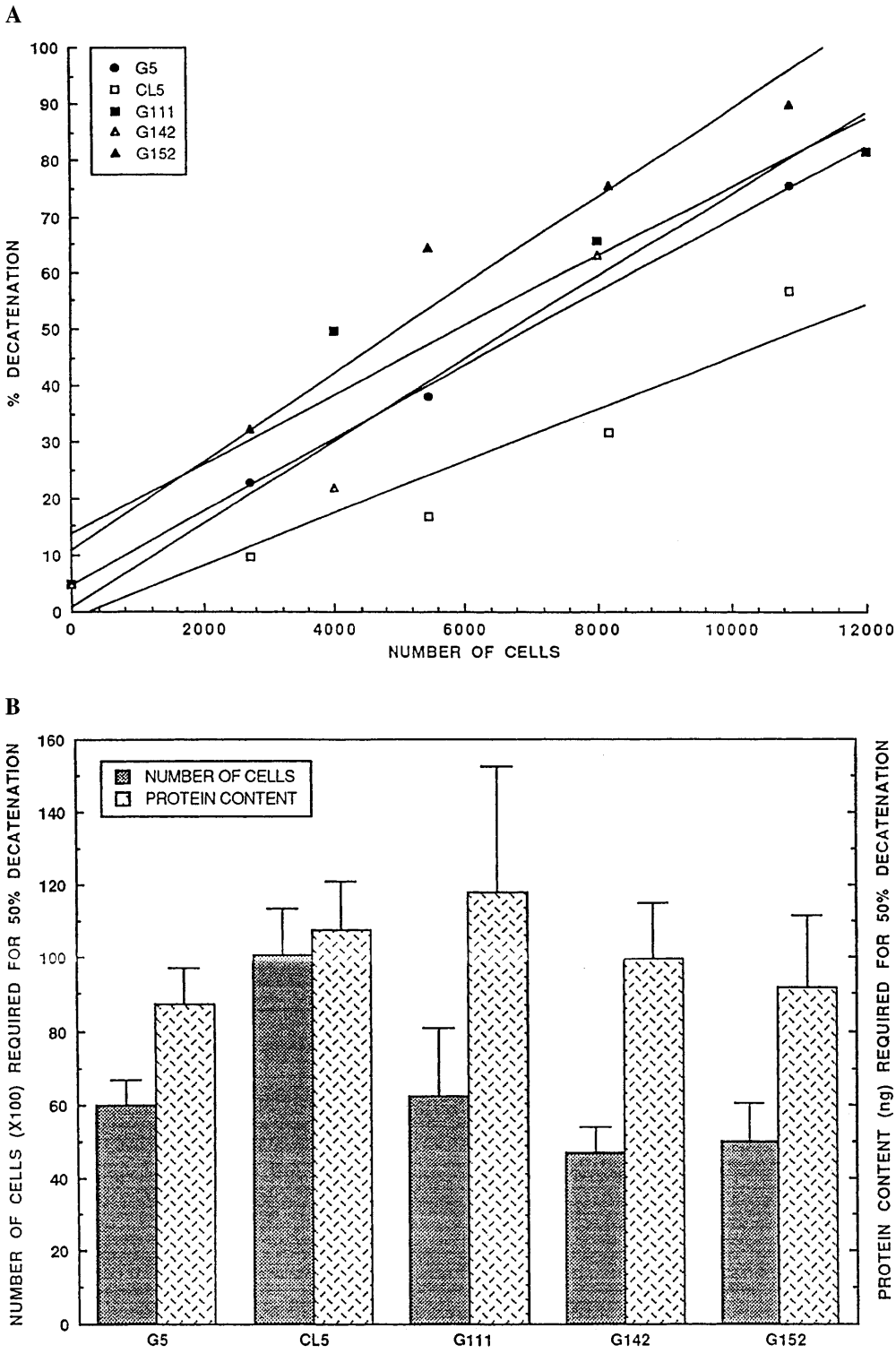


**Fig. 2A,B** Topoisomerase II activity determined in the 5 glioma cell lines. Topoisomerase II catalytic activity was quantified by the decatenation assay with a tritiated kDNA substrate (see Materials and methods). Reactions were run for 30 min at 37 °C. Mean values for 4 independent experiments are presented. **A** The amount of extract required to decatenate 50% of the kDNA substrate was estimated by number of cells for each glioma cell line. **B** The topoisomerase II activity of the 5 glioma cell lines was examined with respect to the number of cells and the cellular protein content

etoposide, but the G5 cell line seemed more resistant than the other glioma lines. The $IC_{50}$ (drug concentration reducing cell numbers by 50%) was 8, 9, 9.8, 10, and 15.8 μg for CL5, G142, G152, G111, and G5, respectively. The $IC_{90}$, corresponding to the dose required to reduce the initial population to 10%, was attained in cell lines CL5, G152, G142, and G111 at 26, 27, 32, and 33 μg etoposide, respectively, but could not be determined for the G5 cell line, despite the use of etoposide at a dose as high as 40 μg (Fig. 1).

## Topoisomerase II activity

The activity of this enzyme with respect to the number of cells or the cellular protein content was examined in the

five glioma cell lines noted above. It was found that the five glioma cell lines expressed similar topoisomerase II activity, although the topoisomerase II activity measured in the CL5 cell line was inferior to the other values. To obtain 50% decatenation, corresponding to topoisomerase II activity, the ranges recorded for the cellular protein content and the number of cells were 90–140 ng and 4,700–10,000 cells, respectively. The average values for these findings were 115 ng protein and 7,000 cells (Fig. 2). Furthermore, inhibition of topoisomerase II was studied in the glioma cell lines as described above. It was noted that the etoposide inhibition of topoisomerase II was homogeneous. The average inhibition rates noted for the five glioma cell lines were 15%, 21.8%, 31.8%, 41.5%, and 49.5% for 1, 2, 4, 8, and 16 μg etoposide, respectively.

## Discussion

The data obtained in our study show an effective experimental activity of etoposide against a panel of five malignant glioma cell lines. The response corresponds to type III, which means that the tumor is sensitive with a low initial sensitivity threshold [22]. The average $IC_{50}$ is 10.52 μg etoposide/ml, although, some cell lines, as exemplified by G5 cells, may be more chemoresistant to etoposide. Generally experimental studies of testis-tumor cell lines show the range of sensitivities to etoposide and the mean $IC_{50}$ to be respectively 0.0104–0.0549 and 0.0187 μg/ml: in one study, Chresta et al. [10, 14, 25] found an average $IC_{50}$ for etoposide of 8.14 μg/ml. The acute leukemia cell lines are 7.62 times more sensitive than our glioma cell lines (mean etoposide $IC_{50}$ 1.38 μg/ml) [6]. For human lung-cancer cell lines, one experimental study displayed heterogeneous sensitivity to etoposide; therefore, two others have considered these cell lines to be highly sensitive to etoposide [15, 21, 23]. Some data in the literature demonstrate heterogeneity (especially regional heterogeneity) of malignant gliomas reflecting the current chemoresistance of these tumors [11]. Previous workers have shown an etoposide sensitivity of glioma cell lines, such as Abe et al. [1] who found five of seven glioma cell lines to be sensitive to etoposide; however, Jordan et al. [20] showed only two of seven glioma cell lines studied to be sensitive to etoposide. Thus, in the light of data derived both from the literature and from our results, etoposide seems to be an efficient drug against malignant glioma cell lines.

We detected topoisomerase II activity in all five glioma cell lines. The expression of this activity was homogeneous in the five glioma cell lines, although the topoisomerase II activity was lower in cell line CL5 than in the others, and the average 50% decatenation was obtained with 7,000 cells. In their study, Mousseau et al. [31] observed no expression of the topoisomerase II gene in 8 of 11 malignant glioma biopsy specimens. It should be stressed that we used primary cell lines and not tumor-biopsy specimens and that we evaluated topoisomerase II activity. The absence of topoisomerase II gene expression in Mousseau et al.'s work could have been due to the presence of necrosis and the high proportion of quiescent cells. Except for the CL5 cultured line, a correlation was found between topoisomerase II activity and etoposide sensitivity for four further cell lines. This finding is consistent with previous reports [6, 15, 21]. Inhibition of topoisomerase II activity by etoposide was homogeneously observed in the five glioma cell lines, which suggests that this mechanism is effectively the target of this anticancer drug [17, 30]. The percentage of topoisomerase II inhibition by etoposide correlated with the doses of etoposide. Finally, this inhibition of topoisomerase II activity by etoposide could be a predictive factor for evaluation of hypothetic glioma-cell sensitivity to etoposide.

Some experimental data have demonstrated etoposide to be a radiosensitizer and raised interest in the resulting supra-additivity effect [16, 26]. This supra-additivity phenomenon was proved for a human cancer cell line: a glioma cultured line [30]. Some clinical trials have demonstrated the cytotoxic action of etoposide on glioma tumors and are thus in accordance with our experimental results, but many trials have used combination chemotherapy [4, 5, 9, 19, 24, 32, 36]. After the normally given dose of etoposide (100 $m/m^2$), the plasma concentration of this drug is 15 μg/ml [8, 35]. Brain-tumor concentrations of etoposide ranged from 2% to 75% of the concurrent plasma concentration as compared with 5% in the normal brain [8, 35]. Hence, we anticipate using etoposide in the treatment of patients with malignant gliomas. In the future we will encourage the development of clinical trials testing a concomitant combination of radiotherapy and etoposide to confirm this finding.

In summary, this work demonstrates that malignant glioma cell lines seem to be sensitive to etoposide. The target of this drug is actually the nuclear enzyme topoisomerase II. Finally, the evaluation of glioma-cell sensitivity to etoposide may be assessed by topoisomerase II activity and/or the inhibition of this activity by etoposide. Thus, etoposide seems to be a useful agent for patients with high-grade gliomas.

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