Re: Folypolyglutamyl Synthetase Gene Transfer and Glioma Antifolate Sensitivity in Culture and In Vivo

The Journal recently published a report by Aghi et al. (1) and an editorial by Sirotnak (2) about the prospect for folylpolyglutamate synthetase (FPGS) gene transfer as a potentially novel strategy to enhance the therapeutic efficacy of antifolates against solid tumors. This proof of concept was demonstrated by a markedly enhanced therapeutic activity of two polyglutamatable antifolate inhibitors of dihydrofolate reductase, methotrexate and edatrexate, against human glioma cells stably transfected with human FPGS complementary DNA.

We agree with the authors that the outcome of this interesting study deserves further preclinical evaluation. Beyond this, however, we would like to address one important issue that has not received attention in the report by Aghi et al. (1). It is evident that when FPGS gene transfer enhances polyglutamylation of antifolate compounds, it does so as effectively as with natural reduced folate cofactors. Consequently, FPGS gene transfer will enhance the retention of natural folate cofactors and thus alter cellular folate homeostasis by expanding intracellular folate pools. These enhanced folate pools may diminish or even abolish polyglutamylation of antifolates.

This leads us to ask: Have natural reduced-folate cofactor pools been measured in the glioma cells in culture or in vivo after FPGS gene transfer? Recent evidence from preclinical (3–6) and clinical (7) studies indicated that an expansion of the intracellular folate pools can be a major factor in abolishing the activity of antifolates, in particular for antifolates that are highly dependent on polyglutamylation to exert their potent inhibitory effects. For instance, clinically active antifolates, such as ZD1694 (Tomudex™, raltitrexed) and MTA (multitargeted antifolate, LY231514), are marginally active as monoglutamate forms and require polyglutamylation to become biologically active. Impairment of polyglutamylation, in this case by di-
rect competition of antifolates with the expanded folate pools for polyglutamylation by FPGS, is a well-known mechanism of resistance to antifolates. Our studies (3,4) showed that the impact of a sevenfold expansion of intracellular folate pools in CEM human leukemia cells resulted in a marked loss in growth inhibitory effect by ZD1694 (865-fold) or by another antifolate dependent on polyglutamylation: 5,10-dideazatetrahydrofolate (270-fold). The activity of methotrexate and edatrexate was much less affected, even though their polyglutamylation was impaired. This effect may be consistent with the fact that, even in their monoglutamate forms, methotrexate and edatrexate remain potent inhibitors of dihydrofolate reductase and do not necessarily require polyglutamylation when given in an alternative schedule, such as by continuous administration. More important, our studies (3,4) also identified novel antifolates that display activity, regardless of the intracellular folate status; these include the nonpolyglutamatable antifolates ZD9331 and PT523.

In conclusion, FPGS gene transfer may be a double-edged sword for folate-based chemotherapy. For some types of antifolates, in particular, those that are highly dependent on polyglutamylation for their cytotoxic activity, the concomitant expansion of natural reduced folate cofactor pools may prevent optimal polyglutamylation and thereby confer resistance. For other antifolates—e.g., methotrexate and edatrexate—these possible adverse effects are of lesser importance, so that FPGS gene transfer can contribute to enhanced cytotoxicity (1).

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RESPONSES

The issue of whether or not enhanced polyglutamylation of natural folates counteracts enhanced polyglutamylation of antifolates is one that we considered during the study of folylpolyglutamyl synthetase (FPGS) gene transfer. This issue was not addressed in detail in our initial report (1), but our thoughts on the matter are presented here.

Jansen et al. (2) point out that the antifolate thymidylate synthetase inhibitors ZD1694 and LY231514 exhibit marked loss of their growth inhibitory effects when administered to human leukemia cell lines that have been mutated to overexpress the RFC1 gene. These studies differ from our work in that they involve increasing folate uptake, while our studies involved increasing polyglutamylation. The distinction is important because it has been shown that, at physiologic concentrations of folate (200 nM), folates are close to being maximally polyglutamylated, with FPGS active sites readily available for additional folate. In the 4-hour pulse protocol used in our study, most antifolates were employed at doses at which they are not close to being maximally polyglutamylated (3,4). Therefore, increasing the transport of folate, as was done in the ZD1694 and LY231514 studies (2), would provide for the continuous presence of additional intracellular folate that could be polyglutamylated by free FPGS active sites, which would, in turn, increase levels of the folate polyglutamates that can displace antifolates from their targets. On the other hand, increasing FPGS expression provides extra FPGS enzyme, which has a minimal effect on the already close to maximally polyglutamylated folate pools but can enhance the polyglutamylation of the submaximally polyglutamated antifolate pools during the brief 4-hour pulse. In fact, it has been shown (3) that using transfection to increase FPGS activity over a 10-fold range has minimal effect on the folate polyglutamate distribution at physiologic folate concentrations, with most folates having four to eight glutamates attached, regardless of FPGS activity. Over the same range of FPGS activities, methotrexate polyglutamate pools increase significantly after a 24-hour drug exposure (4).

In addition, as was mentioned in the original report (1), some antifolates would be suboptimal for therapeutic use with FPGS gene transfer. The best antifolates for use with FPGS gene transfer are probably substrates that have an intermediate affinity for FPGS. Drugs that are very good substrates for FPGS may be close to being maximally polyglutamylated at physiologic levels of FPGS activity, and drugs that are poor substrates for FPGS would not experience greater polyglutamylation following the expansion of FPGS activity by the degree achieved through transfection. Studies (5,6) of antifolates as FPGS substrates have produced the results shown in Table 1. Thus, it appears that edatrexate possesses the desirable intermediate kinetics sought in a therapeutic substrate of FPGS because it experiences greater increases in polyglutamylation from FPGS gene transfer than do weaker FPGS substrates, such as methotrexate, or better substrates, such as ZD1694 and LY231514.

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Table 1. Relative efficiency of various antifolates as substrates for folypolyglutamyl synthetase (FPGS)

| Substrate | \(V_{\text{max}}/K_m\) (relative) for FPGS
<table>
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<tr>
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<tbody>
<tr>
<td>LY231514</td>
<td>549</td>
</tr>
<tr>
<td>ZD1694</td>
<td>495</td>
</tr>
<tr>
<td>Folate</td>
<td>100</td>
</tr>
<tr>
<td>Edatrexate</td>
<td>12</td>
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<tr>
<td>Methotrexate</td>
<td>4</td>
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</tbody>
</table>

*Data are relative to folate, which is set as 100, and are combined from two references: (5) provides LY231514 and ZD1694 data from hog liver FPGS, and (6) provides edatrexate and methotrexate data from murine sarcoma cells. \(V_{\text{max}}\) = maximum volume; \(K_m\) = Michaelis constant.

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Jansen et al. raise an interesting pharmacologic question, but one that does not, however, appear to be immediately relevant to the report by Aghi et al. The notion that they propose, that complementary DNA (cDNA)-mediated elevation of folypolyglutamyl synthetase (FPGS) can increase folate pools in target tumor cells as a result of folate polyglutamylation, is inconsistent with the enhanced cytotoxicity and therapeutic efficacy of folate analogues already observed in these cells by such transfection. Moreover, Jansen et al. themselves agree that these results represent a definite “proof-of-concept” for this approach to gene therapy. Although Aghi et al. achieved their results by enhancing FPGS gene expression exogenously, the idea that alterations in intracellular levels of FPGS will influence the cell’s cytotoxicity and therapeutic sensitivity to folate analogues is certainly not new. There is earlier documentation of this effect in the literature in the form of reports (1–3) of tumor cell variants with either decreased or increased levels of FPGS that display corresponding differences in resistance to folate analogues. Also, let us not forget the earlier study by Kim et al. (4), in which FPGS cDNA transfection restored cytotoxic sensitivity of an FPGS-deficient auxotroph to methotrexate. It is difficult to imagine how these consequences of altered FPGS gene expression could occur if there was a commensurate expansion in folate pools as a result of their polyglutamylation.

The evidence that Jansen et al. cite in support of their notion emanate from studies (5–7) that use tumor cell variants with alterations at the plasma membrane affecting either the mediated entry or egress of folates. These studies provided interesting conclusions and demonstrate the impact of expanded folate pools on the cytotoxicity to folate analogues. However, these alterations in folate pool sizes, in the main, would appear to reflect the changes in membrane transport of folates and less so folate polyglutamylation. For this reason, the bearing of these studies on that by Aghi et al. is questionable.

Given the information available on the relative saturability and capacity of mediated transport of folates and their analogues in tumor cells by means of the reduced folate carrier-1 encoded system, it is clear that the concentration of folates normally found in tissue culture medium or in plasma is severely limiting to folate polyglutamylation (folic acid is a very poor substrate and competitive inhibitor for FPGS as well). This is not the case for the folate analogues used in studies by Aghi et al. The pulse exposure in cell culture and bolus dosing in mice that were used result in the presentation of micromolar concentrations of these analogues to the target glioma cells. As a consequence, one would expect FPGS cDNA transfection to produce substantially greater enhancement of intracellular levels of methotrexate and edatrexate polyglutamates (and more so of ZD1694 and MTA polyglutamates) as compared with enhancement of folate polyglutamate levels. In light of these considerations, the concerns expressed by Jansen et al. would appear to be unfounded.

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