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To the editor:

Mutagenic potential of temozolomide in bone marrow cells in vivo

Therapy-related myeloid leukemias (t-MLs) occur in as many as 5% to 10% of patients who are otherwise cured of a primary neoplasm with aggressive multimodal regimens. Many patients with t-MLs or therapy-related myelodysplastic syndrome (t-MDS) have previously received chemotherapeutic agents that alkylate DNA, such as bis-chloroethyl-nitrosourea (BCNU) and cyclophosphamide (CP).[1] We and other investigators have shown CP to be mutagenic in preclinical studies both in vitro and in vivo.[2]

Temozolomide (TMZ) is a more recently developed alkylating agent that has been effective in the treatment of adult high-grade glioma and refractory leukemia.[3,4] TMZ is now being incorporated into initial therapy in more than 40 studies for a range of cancers, including glioblastoma and melanoma.[5] TMZ's cytotoxicity depends on the methylation of guanine bases at the *O6* position, resulting in *O6*-methylguanine and G:C→A:T transitions.[6] Despite initial hopes that TMZ would be less leukemogenic than traditional alkylating agents, 2 groups have recently reported secondary myeloid malignancies after TMZ treatment in clinical studies.[7,8] As TMZ moves into the front line of our chemotherapeutic armamentarium, further investigation of its in vivo mutagenic potential is warranted.

We used a transgenic mutation indicator mouse strain (small blue mouse) to compare the in vivo mutagenic potential of TMZ on bone marrow (BM) cells with that of CP. In this mouse model, the mutational target is the nontranscriptionally active lacZ portion of the plasmid pUR288. The mutation frequency was determined with a plasmid rescue procedure applied to genomic DNA derived from BM and with a subsequent selection for lac-Z negative clones, according to published protocols.[9] The type of mutation was further determined by PCR amplification and restriction digestion.

Animals were treated with TMZ (175 mg/kg/d intraperitoneally for 5 days), CP (200 mg/kg intraperitoneally either once or weekly for 6 weeks), or phosphate-buffered saline (PBS), and BM was harvested 10 days after the last treatment (Figure 1). TMZ and CP doses were chosen by treating C57BL/6 mice in groups of 10 to 20 until the development of neutropenia without mortality. Determination of the mutation frequency revealed that the 1-day CP treatment increased the mutational load in BM 2-fold over the control, whereas the TMZ regimen resulted in a 22-fold increase over control. BM cells in animals treated 6 times with CP did not show an increase in the mutation frequency over animals treated with only a single dose of CP. As we expected from TMZ's mechanism of action, over 90% of all mutations in response to TMZ treatment were point mutations.[10] Fewer than 30% of the mutations in BM cells from animals treated with CP were point mutations, with the remaining mutations being either translocations or deletions. These data emphasize TMZ's mutagenic potential for BM cells in vivo in the mouse model system and may indicate that TMZ's mutagenic potential is the underlying cause of the recently reported t-MLs in TMZ-treated patients. We suggest close long-term hematological monitoring of patients receiving TMZ in clinical trials. Further investigation is warranted to determine whether the increased mutation rate seen with TMZ exposure results in a comparable elevated risk of therapy-induced leukemia.

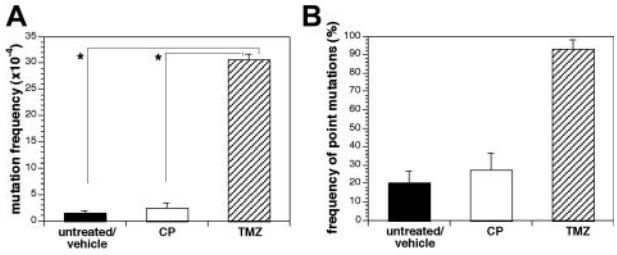

Figure 1. Strong mutagenic potential of TMZ in vivo. (A) Mutation frequency of BM cells derived from animals treated with either CP or TMZ in vivo, measured with the small blue mouse mutation indicator strain. (B) Frequency of point mutations (PM) in BM cells derived from animals treated with either CP or TMZ in vivo. Values shown are mean ± 1 SEM; n = 18 for untreated/vehicle, n = 4 for CP, and n = 4 for CP. *$P < .05$.

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To the editor:

Fibrinogen containing γ′ chains

A recently published paper by Siebenlist et al[1] presents data that apparently contradicts results published previously by our laboratory[2] and others.[3] Siebenlist et al's data show that factor XIII activation is more rapid in the presence of γ_A/γ_A fibrinogen than in the presence of γ_A/γ' fibrinogen.[1] These findings are in contrast to our previous results showing that factor XIII activation is more rapid in the presence of γ_A/γ' fibrinogen. Siebenlist et al[1] reconciled this discrepancy by proposing that our fibrinogen 2 (γ_A/γ' fibrinogen) preparations "contained unaccounted for factor XIII." [1(p2734)] Curiously, they did not present any analysis of contaminating factor XIII in their own fibrinogen preparations. But the fibrinogen preparations in our paper were assayed for contaminating factor XIII activity that can copurify with γ_A/γ' fibrinogen.[4] Although these data were not included in the final version of our paper,[2] they are presented here in Figure 1.

These data clearly demonstrate that our fibrinogen preparations did not contain sufficient contaminating factor XIII to account for the increased rate of factor XIII activation that we observed when γ_A/γ' fibrinogen was added to factor XIII. Therefore, the reason for the discrepancies between our results and those of Siebenlist et al[1] is still unclear. One possibility is that Siebenlist et al[1] measured the rate of factor XIIIa-subunit activation peptide cleavage, whereas our laboratory measured the appearance of factor XIIIa activity. These two assays do not, in fact, measure the same thing. While factor XIII activation peptide cleavage is necessary for factor XIII activation under physiologic conditions, it is not sufficient. A second step that must occur prior to activation is the dissociation of the b-subunits of factor XIII.[7] The paper by Siebenlist et al[1] measured the first step in factor XIII activation, activation peptide cleavage, whereas our paper measured the last step in factor XIII activation, the expression of catalytic activity. Our hypothesis is that γ_A/γ' fibrin(ogen), by virtue of its binding to factor XIIIb-subunits through the γ' chain,[4] increases the rate of factor XIII activation by increasing the rate of factor XIIIb-subunit dissociation from the catalytic a′-subunits.

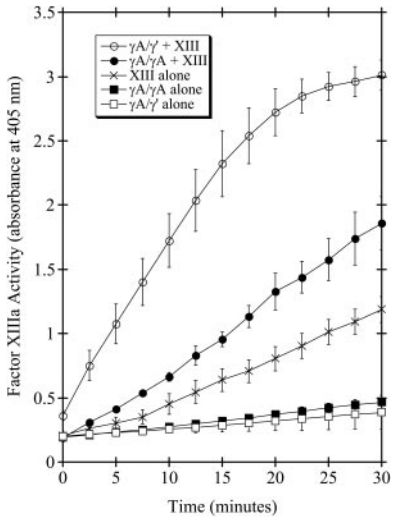

Figure 1. Factor VIII activation in the presence or absence of fibrinogen. One NIH unit/mL α-thrombin in 100 μL buffer containing 1 mM $CaCl_2$, and 1 mM GPRP peptide to prevent fibrin polymerization,[5] was incubated at room temperature with 43 nM factor XIII (×), 86 nM γ_A/γ_A fibrinogen (■), 86 nM γ_A/γ' fibrinogen (□), 86 nM γ_A/γ_A fibrinogen + 43 nM factor XIII (●), or 86 nM γ_A/γ' fibrinogen + 43 nM factor XIII (○). Thrombin was inactivated at the indicated times with 0.5 mM PPACK. Factor XIIIa activity was measured by the incorporation of 0.5 mM 5-(biotinamido)pentylamine into immobilized *N, N′*-dimethylcasein, and detected at 405 nm by *P*-nitrophenyl phosphate hydrolysis following incubation with streptavidin-conjugated alkaline phosphatase.[6]

Another possibility for the discrepancy in results is that subtle differences in fibrinogen purification methods among different laboratories may result in different amounts of contaminants or different amounts of fibrinogen degradation that may influence these assays. Such differences could potentially account for the discrepancies between the paper by Siebenlist et al[1] and our paper, and possibly the discrepancies between their paper and a previous paper by Cooper et al.[3] The data of Cooper et al[3] indicate that fibrinopeptide A release from γ_A/γ_A and γ_A/γ' fibrinogen is identical but that fibrinopeptide B release is delayed from γ_A/γ' fibrinogen. In contrast, the data presented by Siebenlist et al shows that fibrinopeptide A release from γ_A/γ' fibrinogen is delayed compared to γ_A/γ_A fibrinogen, as well as fibrinopeptide B release. The reason for these discrepancies is still unclear.

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