Temozolomide and Resistant Glioma Cells

TO THE EDITOR: We read with interest the article by Uzzaman et al. (Uzzaman M, Keller G, Germano IM: Enhanced proapoptotic effects of tumor necrosis factor-related apoptosis-inducing ligand on temozolomide-resistant glioma cells. J Neurosurg 106:646–651, April, 2007) reporting that the tumor necrosis factor-related apoptosis-inducing ligand enhanced apoptosis in temozolomide-resistant glioma cells.

Abstract

Object. Death receptor targeting is an attractive approach in experimental treatment for tumors such as malignant gliomas, which are resistant to radiation and chemotherapy. Among the family of cytokines referred to as death ligands, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has attracted clinical interest. The aim of this study was to assess whether TRAIL can be used as an adjuvant to temozolomide (TMZ) for apoptosis induction in malignant glioma cell lines.

Methods. Six human malignant glioma cell lines (A172, U87, U251, T98, U343, and U373) were exposed to human (h)TRAIL, TMZ, or an hTRAIL/TMZ combined treatment. Cell viability was assayed using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide and phase-contrast microscopy. Cell apoptosis was detected using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling technique and quantified using flow cytometric analysis. The apoptosis signaling cascade was studied with Western blotting.

The additive effects of hTRAIL and TMZ resulted in a significant decrease in cell viability and an increased apoptotic rate. Expression of the death receptors DR5 and DR4 in two cell lines (A172 and U251) upregulated significantly when they were used in combination hTRAIL/TMZ treatment (p < 0.05 compared with baseline control), leading to activation of caspase-8 and caspase-3 (p < 0.05 compared with baseline control) and confirming an extrinsic apoptotic pathway. A cell intrinsic pathway through mitochondrial cytochrome c was not activated.

Conclusions. Based on this work, one may infer that hTRAIL should be considered as an adjuvant treatment for TMZ-resistant human malignant gliomas.

The authors exposed cells from 6 established human malignant glioma cell lines to human TRAIL, TMZ, or a combination of TRAIL and TMZ, and found that the additive effect of TRAIL and TMZ resulted in a significant decrease in cell viability and an increased apoptotic rate. They reported that this effect of the combination of TRAIL and TMZ was associated with upregulated expression of the death receptors DR5 and DR4 in 2 cell lines (A172 and U251). The intrinsic apoptotic pathway was not activated. They concluded that TRAIL may be considered as an adjuvant treatment for TMZ-resistant malignant gliomas.

In mammalian cells, 2 important pathways of apoptosis have been described: the extrinsic pathway mediated by death receptors, mainly DR4 (TRAIL-R1) and DR5 (TRAIL-R2), and the intrinsic pathway controlled by members of the Bcl-2 protein family. In cancers, TRAIL may selectively kill tumor cells by inducing TRAIL-R1 and TRAIL-R2. We have previously reported that TRAIL alone activated both the extrinsic and intrinsic pathways in glioma cells but there was no significant upregulation of TRAIL-R2 expression. Thus, the contributions of the upregulation of TRAIL-R2 and of the intrinsic pathway in the induction of apoptosis during TRAIL treatment of glioma cells are not clear yet. As Uzzaman et al. report in their paper, however, the combination of TRAIL and TMZ enhanced apoptosis in glioma cells resistant to temozolomide alone.

Interestingly, we have constructed a recombinant replication-deficient adenovirus vector carrying the TRAIL-R2 cDNA. It would be interesting to see in primary and established glioma cells if this adenovirus has an additional effect when used with the combination of TMZ and TRAIL.

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References


RESPONSE: We thank Dr. Kyritsis and colleagues for their interest in our work. These authors had previously shown activation of both intrinsic and extrinsic apoptotic pathways after exposure to TRAIL in 8 glioma cell lines, including U251. In our study, we did not demonstrate activation of either pathway when TRAIL was used alone in U251 and A172 cell lines. On the other hand, when TRAIL was used in combination with TMZ, we showed activation of the extrinsic pathway.

The results of the studies concurred in showing lack of significant upregulation of TRAIL-R2 (DR5) receptors, previously thought to have a role in TRAIL-induced apoptosis. We agree with Dr. Kyritsis and colleagues that the recombinant replication-deficient adenovirus carrying TRAIL-R2 cDNA, developed by their group, should be tested on malignant glioma cell lines using TMZ and TRAIL in combination. We certainly hope to collaborate with them on these interesting experiments.

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Cerebral Oxygenation


Abstract

Object. Control of intracranial pressure (ICP) and cerebral perfusion pressure (CPP) is the foundation of traumatic brain injury (TBI) management. In this study, the authors examined whether conventional ICP- and CPP-guided neurocritical care ensures adequate brain tissue O2 in the first 6 hours after resuscitation.

Methods. Resuscitated patients with severe TBI (Glasgow Coma Scale score ≤ 8 and Injury Severity Score scale ≥ 16) who were admitted to a Level I trauma center and who underwent brain tissue O2 monitoring within 6 hours of injury were evaluated as part of a prospective observational database. Therapy was directed to maintain an ICP of 25 mm Hg or less and a CPP of 60 mm Hg or higher.

Data from a group of 25 patients that included 19 men and six women (mean age 39 ± 20 years) were examined. After resuscitation, ICP was 25 mm Hg or less in 84% and CPP was 60 mm Hg or greater in 88% of the patients. Brain O2 probes were allowed to stabilize: the initial brain tissue O2 level was 25 mm Hg or less in 68% of the patients, 20 mm Hg or less in 56%, and 10 mm Hg or less in 36%. Nearly one third (29%) of patients with ICP readings of 25 mm Hg or less and 27% with CPP levels of 60 mm Hg or greater had severe cerebral hypoxia (brain tissue O2 ≤ 10 mm Hg). Nineteen patients had both optimal ICP (< 25 mm Hg) and CPP (≥ 60 mm Hg); brain tissue O2 was 20 mm Hg or less in 47% and 10 mm Hg or less in 21% of these patients. The mortality rate was higher in patients with reduced brain tissue O2.

Conclusions. Brain resuscitation based on current neurocritical care standards (that is, control of ICP and CPP) does not prevent cerebral hypoxia in some patients. This finding may help explain why secondary neuronal injury occurs in some patients with adequate CPP and suggests that the definition of adequate brain resuscitation after TBI may need to be reconsidered.

The authors must be congratulated on their well-designed clinical study, which emphasized the importance and the clinical significance of monitoring and adequately maintaining cerebral tissue oxygenation in patients suffering severe TBI. Their stimulating study, however, generates further questions regarding tissue oxygenation and its relationship with other physiological parameters in these patients.

It is well known that the hemoglobin (Hb)–oxygen dissociation curve and consequently tissue oxygen delivery are greatly influenced by local tissue temperature. Moreover, it has been demonstrated that temperature reduction can prevent neuronal cell death by diminishing apoptosis via inhibition of both caspase-dependent and caspase-independent pathways. Therefore, it would be beneficial if the authors could provide any data regarding intracranial temperature and its variations in their patients, since the monitoring device utilized in their study (LICOX, Integra LifeSciences) could be coupled with an intraparenchymal temperature probe providing real-time information for intracranial temperature. Did the authors record intracranial temperatures in their study? Did they notice any tissue oxygen changes when intracranial or even systemic temperature changes occurred? They mentioned in their article that an effort was made to maintain normothermia in their patients. Do they refer to intracranial or systemic temperature? How did they manage to keep the patients’ temperature within normal range? It has been previously reported that intracranial temperature significantly dropped several hours before any intracranial and CPP changes. Did they notice any tissue oxygenation changes preceding or following the previously described drop in intracranial temperature in those patients with bad outcomes in their study?

It has also been demonstrated that increases in patients’ Hb levels could result in increased cerebral tissue oxygenation, especially in anemic patients with TBI. Could the authors provide any data regarding the Hb or the hematocrit values of their patients? Did any of their patients receive any blood transfusion, and if so, did that have an impact on tissue oxygenation?

Finally, Stiefel et al. stated that they “tried to place the [monitoring] probes close to the worst area of injury observed on admission head computed tomography (CT) scans.” Did the authors have any additional imaging (magnetic resonance imaging) or biochemical (microdialysis) data guiding them for placing their monitoring probes? If not, the possibility of monitoring an ischemic penumbra zone surrounding the “worst area of injury” cannot be ruled out and needs to be identified as a limiting factor of their tissue oxygenation monitoring methodology.

I would like to thank and congratulate the authors for their interesting clinical study.

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References