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 up-regulation 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.

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

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.

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 Scale score ≥ 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.1 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.2 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.3 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.4 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


To THE EDITOR: We read with interest the recent article by Stiefel et al. The authors evaluated 29 patients with severe TBI after resuscitation with parenchymal oxygen monitoring (PbtO2; LICOX). They found that in spite of optimal resuscitation by current standards, with ICP < 25 mm Hg and CPP > 60 mm Hg, that cerebral hypoxemia was seen, with PbtO2 < 20 mm Hg in 47% and < 10 in...
21% of patients. They point out that current emphasis on ICP and CPP management may be inadequate to assure adequate cerebral oxygenation and avoidance of secondary brain injury.

Stiefel et al. cited the work of several authors who have demonstrated similar results with the use of jugular venous oxygen saturation (SjO2) or parenchymal monitoring. We presented similar results at the 2001 Annual Meeting of the Congress of Neurological Surgeons. We studied a group of 56 patients who had severe TBI and were monitored with ICP, CPP, and SjO2 monitoring and found that the SjO2 was within the normal range (55–75%) in 30 (54%). Thirteen of these patients had low ICP (23%) and 17 had high ICP (31%). The combination of low SjO2 and high ICP (>20 mm Hg) was found in 7 patients (13%); low SjO2, and low ICP (<20 mm Hg) were found in 19 patients (34%). All patients with low ICP had acceptable CPP (>60 mm Hg).

It is in this last subgroup of 19 patients (34% of our study group) that cerebral hypoxemia would be found when neurocritical care is guided only by ICP and CPP. The work of Stiefel et al. confirms our findings that approximately one third of patients are at risk for secondary brain injury when conventional neurocritical care is used without monitoring of oxygen levels. We congratulate Stiefel and colleagues on their continued efforts supporting the use of cerebral oxygen monitoring to improve neurocritical care.

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To The Editor: We read with great interest the recent paper by Stiefel and colleagues.

Clarification of several issues would facilitate interpretation of the authors’ results. The basis for their suggestion that we consider factors other than CPP when managing patients with TBI was a finding in 25 patients that “brain O2 level did not correlate with ICP (r2 = 0.0663; Fig. 3) or CPP (r2 = 0.2221).” The legend for the referenced figure reads, “The ICP and brain O2 values represent the mean of each patient’s values obtained during the first 1-hour period after brain tissue O2 probe stabilization,” which indicates that each diamond in Fig. 3 represents a mean value for 1 patient. Thus there should be 25 diamonds in the scattergram. There are, however, about 132 diamonds in the figure. What data set do those diamonds represent? And which data set was used to derive the correlation coefficient of 0.26 (r, as distinct from r2, which is the coefficient of determination)?

More important, since CPP incorporates ICP to create a variable that affects flow, and so PbtO2, why did the authors present a scattergram of ICP × PbtO2, instead of CPP × PbtO2? Physio-logic aside, if one draws a negative inference—in this instance, a lack of correlation—why present the weakest evidence for that correlation instead of the strongest evidence?

With regard to the relationship between CPP and PbtO2, what data points were used to derive the nonsignificant correlation of r = 0.47? What was the probability value of that relationship? Was it calculated from 25 points or 130-some points? Was the statistical power of the test sufficient to warrant concluding that CPP does not correlate with PbtO2, . . . as distinct from concluding that, based on the obtained results, the best guess about the correlation between CPP and PbtO2 is r = 0.47, but the confidence interval (CI) around that guess is not narrow enough to warrant a conclusion about the utility of conventional neurocritical care?

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RESPONSE: We thank Drs. Fountas, Hartung, Cottrell, and Palmer, and Ms. Bader for their careful reading of our paper and their thoughtful comments and analysis. They raise several important questions, in particular: 1) Where should brain oxygen tension (PbtO2) monitors be placed? 2) What other factors (for example, temperature and Hb) may influence PbtO2? 3) Can we conclude anything about the utility of conventional neurocritical care in early resuscitation? Drs. Hartung and Cottrell also pointed out a typographical error in 1 of our figure legends.

We try to place a PbtO2 monitor into normal-appearing white matter (based on the admission computed tomography) in the frontal lobe closest to the lesion. We confirm this placement by means of follow-up CT and physiological challenge (fraction of inspired oxygen 100%). If an appropriate response does not occur and the probe is in an abnormal area (for example, a contusion), it is replaced. Certainly the site where any monitor, including an ICP or PbtO2 monitor, is located can be a limitation. Nevertheless, there is evidence to suggest that when a PbtO2 monitor is located in white matter that appears normal on CT, it provides information about global oxygenation. While PbtO2 is lower at the margin of a focal lesion, increases in PbtO2 may still occur when CPP is increased. As with all monitoring, these trends or changes over time ultimately may be more important than absolute values or thresholds. In some of our patients we have performed xenon-enhanced CT or perfusion CT studies and found a close correlation between cerebral blood flow and PbtO2 in all parts of the brain independent of probe placement.

Temperature is an important variable that can influence oxygen–Hb dissociation. In addition there is evidence to suggest that hyperthermia may exacerbate neuronal damage and outcome after TBI. At present, however, there is no direct evidence for a cause-and-effect relationship between fever and worse outcome after TBI, and the precise reason why outcome is worse has not been fully elucidated. In addition, the role of hyperthermia in TBI is far from clear. We measured brain temperature in our study, and the mean brain temperature was 97.9°F (95% CI 96.7–99.0°F). This study represented only 1 hour of care among patients who underwent monitor placement within the first 6 hours after injury. During this short period we did not observe a relationship between changes in brain temperature and PbtO2. We have used PbtO2 monitors in nearly 400 patients and have further explored the relationship between body temperature, brain temperature, and PbtO2 during a patient’s intensive care unit (ICU) course after severe TBI. Brain temperature correlates moderately well with core body temperature over a wide range of core body temperatures (34.6–40.4°C) when ICP is normal or elevated. Hyperther-
mia (systemic or brain) does not seem to reduce PbtO₂ or increase the number of episodes of brain tissue hypoxia in patients with severe TBI (unpublished data). We tend to base our ICU therapies and prognoses on clinical condition, radiographic findings, PbtO₂, and ICP, among other variables, and infrequently use brain temperature as a management guide. We do, however, use several techniques, such as administration of acetaminophen and application of cooling blankets (Artic Sun, Cincinnati Sub-Zero) to restore systemic normothermia in those with markedly elevated core body temperatures, and in rare cases we have used moderate systemic hypothermia as a second-line treatment for increased ICP. It is important, however, to ensure that shivering does not occur, because there are anecdotal reports that it may adversely affect brain oxygen.

As Dr. Fountas suggests, a decrease of brain temperature relative to body temperature, called temperature reversal, has been described as an early marker of irreversible brain damage and impeding brain death. Although temperature reversal may be an ominous sign, our experience suggests that it is extremely rare and is a relatively late marker of brain injury. In one case in which we observed temperature reversal (in our database but not included in this submission), the phenomenon only developed after 5 hours of intractable intracranial hypertension and very low CPP. In this patient, PbtO₂ was severely hypoxic (<5 mm Hg) long before temperature reversal.

Blood transfusions to increase Hb are used to avoid the deleterious effects of oxygen debt; for years the traditional threshold for transfusion has been an Hb level of ≤10 g/dl. This practice has been challenged in recent years by an accumulating body of evidence suggesting that transfusion may exacerbate outcome in general medical and surgical ICU patients, including trauma patients, and contribute to a variety of medical complications.⁵,⁶,¹⁴ None of our patients received a blood transfusion during our study since ongoing blood loss was an exclusion criterion. The mean Hb level among the study patients at the start of the study was 11.6 g/dl (as shown in Table 1 of our article). The Hb level, however, is only part of a complex relationship between fluid status, blood pressure, and oxygenation. Furthermore, the results of recent studies by Manley and colleagues suggest that PbtO₂ is more likely to represent the diffusion limits to oxygen delivery in the brain—than total oxygen delivery or metabolism (presented to the AANS, 2007; unpublished data). This may be important in TBI, because findings of magnetic resonance imaging studies suggest that cellular edema is the predominant form of brain edema after TBI whereas the results of positron emission tomography studies suggest that there also are diffusion limits to oxygen delivery in the brain.⁸ We have previously examined the relationship between transfusion and PbtO₂ in volume-resuscitated patients.¹⁰ An increase in PbtO₂ was observed in 26 (74%) of 35 patients, and in those patients the increase was associated with a mean increase in hemoglobin of (1.4 g/dl ± 1.1 g/dl [standard deviation]). In a subsequent study we observed that transfusion of a second unit of red blood cells does not further increase PbtO₂.¹⁴

Drs. Hartung and Cottrell point out a typographical error in the legend to Fig. 3. They are correct that there are >130 data points—each point represents the mean of each patient’s ICP and PbtO₂ values calculated for each 10 minutes during the first hour of observation. There are some data points missing because of patient transport. These data were used to derive the correlation coefficient “r” and the coefficient of determination “r²” that are related to each other by squaring. In theory, they measure the same thing—that is, the strength of linear correlation between the response variable and the predictor. The interpretation of r² is easier to understand since it is the percentage of variation in the response that is explained by the predictor. For example, when r = 0.2, r² = 0.04, implying that only 4% of the variation in the response is explained by the predictor.

We chose to illustrate the relationship between ICP and brain oxygen levels because CPP is a derived value and there is a general consensus on a threshold above which to treat elevated ICP, whereas the question of what is the optimal CPP level for patients with TBI remains unanswered. Consequently, guidelines about CPP have changed over the years; current guidelines suggest that CPP <50 mm Hg or aggressive attempts to keep CPP >70 mm Hg should be avoided.³ Cerebral perfusion pressure is one of many variables that may influence PbtO₂; however, the relationship between CPP and PbtO₂ remains poorly defined. Although increasing CPP can increase brain oxygen (measured using a jugular catheter or direct parenchymal monitor), this relationship may only exist when CPP is in the normal range. Furthermore, cerebral hypoxia can be observed even when CPP is normal.³⁵,⁵⁵,¹¹ In our analysis, the correlation between CPP and PbtO₂ (r) was 0.47 (95% CI 0.16–0.6). We draw no conclusion about the utility of conventional neurocritical care but rather as indicated in the manuscript, “we suggest that brain tissue O₂-guided management will determine the appropriate CPP for each patient.”

We thank Dr. Palmer and Ms. Bader for sharing their findings that one third of TBI patients have cerebral hypoxia despite control of ICP and CPP when cerebral oxygenation is measured using SjO₂ monitoring. These results are similar to ours and again emphasize that normal ICP and CPP may not mean that there is no risk of secondary neurological injury. Similarly, the results of clinical cerebral microdialysis studies also have demonstrated that an increase in the lactate/pyruvate ratio (that is, anaerobic metabolism) can be independent of CPP in TBI.⁹

As we stated in our paper, our results should be regarded as preliminary. Furthermore, our findings do not suggest that conventional neurocritical care is lacking; rather they suggest that we need to rethink our definitions of adequate brain resuscitation and consider measures other than ICP and CPP alone in the care of patients with TBI. What Dr. Fountas indicates is that there are many variables that can influence cerebral metabolism and brain oxygen, and this is consistent with our findings of how ICP and CPP are related to brain oxygen tension. Indeed we have found that over 20 different treatments that are in current use in the ICU can have an effect on PbtO₂ (presented to the Neurocritical Care Conference, 2006; unpublished data). With respect to facilitating the care of patients with TBI, a PbtO₂ monitor can be used along with our current monitors to help define adequate resuscitation and can help tailor management to the individual patient and his or her specific pathological condition. (DOI: 10.3171/JNS/2008/108/01/0198)

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References


