Apoptotic Mechanisms Increased by GluT Inhibitor DL-TBOA following Brain Cellular Damage

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Abstract:

Damage to CNS elements is caused by many neurologic diseases including cerebral palsy and multiple sclerosis, and has been attributed to dyshomeostasis of glutamate signalling. However, molecular mechanism remains elusive. This study was undertaken to elucidate the molecular role of glutamate transporter (GluT) signalling on injury mediated cellular damage using ex-vivo model of brain cultures. Cell injury was induced by transient deprivation of oxygen and glucose. Pharmacological inhibition of GluTs with DL-TBOA was initiated immediately after the end of cell injury. In this study, we detected cell mortality and apoptosis. Caspases, BCL-2 and BDNF transcripts were determined by Q-RT-PCR. Proliferation was detected by replicating cell DNA assay. GluT inhibition following injury was found to result in a further cellular damage as judged by ~ a 58% reduction in cell survival as well as ~ a 29% increase in apoptosis. There was an upregulation of caspase-1,-3,-8 and -9 transcripts. There was also further downregulation of BCL-2 and BDNF transcripts. In addition, there seemed to be proliferative cells increased significantly during the recovery phase. Our study shows that GluT signalling inhibition enhances cell death by a mechanism involving elaborating pro-death caspases or by suppressing anti-apoptotic BCL-2 and BDNF.

Keywords: CNS injury, Glutamate Transport, Glutamate excitotoxicity, Apoptosis.

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Introduction:

A major toxic insult implicated in the pathophysiology of many central nervous system (CNS) neurodegenerative diseases, including cerebral palsy and multiple sclerosis, is excitotoxicity (reviewed by Dong, Wang and Qin, 2009) and targeting this process is one potential therapeutic option [1]. Excitotoxicity is caused by excessive extracellular glutamate, an essential neurotransmitter in the CNS, and the subsequent over-stimulation of glutamate receptors (GluRs) [2-4]. Possible sources of glutamate are from the glutamate transporters (GluT) and cystine-glutamate antiporter [1, 5]. The GluTs appear to be especially abundant in cellular elements of CNS, such as glutamate aspartate transporter (GLAST); excitatory amino acid carrier-1 (EAAC1) [6-8]. Under physiological conditions, these GluTs maintain basal levels of extracellular glutamate in the range of 1-2 µM under physiological conditions [9]. Glutamate uptake from the extracellular space by GluTs is crucial for the terminating glutamate signaling and for the prevention of excitotoxic cell death owing to GluR activation [10]. Recently, GluT subtypes were implicated in cell survival and death. Thus, understanding how GluT signalling contributes to brain cell death is crucial for the development of therapeutic approaches to white matter disorders. While some studies suggest that GluT mechanism involving ameliorate cell death after primary cellular damage [1], others suggest that GluT inhibition influences injury [10]. Although GluTs are a highly studied target after CNS injury, the molecular mechanisms involved in CNS cell death are partially known at present. Accordingly, we aimed to further investigate the roles of GluT signalling at molecular level on primary injury mediated cell death in ex-vivo model of CNS injury, which might help to aid the search for potential therapeutic interventions. In the current study, we provide evidence indicating that inhibiting GluT signalling after primary injury is toxic to CNS elements ex-vivo, and that a similar mechanism operates in isolated optic nerves [11].

Materials and methods:

Organotypic cultures of rat brain slices:

Brain slices were established as described previously [12-14]. In brief, slice cultures were prepared from pups of Wistar rats (27 males). Postnatal day 7 (P7) animals were sacrificed by cervical dislocation, and the brain was dissected and transversely sliced at a thickness of 300-µm on a vibratome (Leica). Slices were carefully transferred onto each membrane inserts (Millipore-Falcon), and the inserts were placed in a 6-well plate (Falcon). Cultures were maintained in 1 ml of serum-based medium (50% Dulbecco's Modified Eagle Medium (DMEM, Invitrogen), 25% HBSS (hanks balanced salt solution, Invitrogen), 20% horse serum (Invitrogen), 4.6 mM (v/v) L-glutamine (Sigma), 21 mM (v/v) D-glucose (Fisher Scientific), 1% penicillin/streptomycin solution (Invitrogen), 4.2 µM (v/v) L-ascorbic acid (Aldrich-Sigma) and 11 mM (v/v) NaHCO3 at pH 7.2-7.4) in a humidified aerobic incubator (5% CO2) at 37 °C for 3 days. Thereafter, cultures were transferred to serum-free medium supplemented with 0.3 % B27 growth supplement (Invitrogen) and kept for up to 10 days at 37 °C in 5% CO2 with media changes performed twice a week.

Cellular damage induction and DL-TBOA inhibitor application:

Cellular injury was modeled by oxygen-glucose-deprivation (OGD). The tissues were transferred into filter-sterilized, deoxygenated glucose-free culture medium for 20 minutes in an anaerobic airtight chamber with a mixture of 95% N2/ 5% CO2 gas flow, temperature maintained at 37 ± 0.5 °C. After treatment, the cultures were washed at least three times with fresh oxygenated culture medium containing 5 mg/ml D-glucose and supplemented with 2% B27 and returned to their culture conditions under normoxic atmosphere (5% CO2) at 37 °C. Control cultures were maintained for the same time under normoxic conditions. The non- or treated cultures were further incubated for 3 days as reperfusion period before being fixed for analysis. To assess the role of the GluT signalling, the selective GluT inhibitor DL-TBOA (200 µM) was added to the culture medium [1] for 20 minutes after injury,

then continued for 72 hours of recovery to mimic in-vivo conditions.

Cell mortality assessment:

Cell mortality (necrosis) was assessed by propidium iodide [PI] (Sigma; 5 µg/ml) and Calcein-AM uptake [Cal] (Molecular Probes, Invitrogen; 2 µM), according to the manufacturer's instructions. Cultures were incubated in the presence of a combined solution containing PI and Cal at 37 °C for 30-45 minutes, and were then fixed in 4% paraformaldehyde (PFA, Sigma) in phosphate-buffered saline (PBS, Oxoid) for 30 minutes at room temperature. Using microscopy, viable cells appeared bright fluorescent green of Cal, whereas dead (necrotic) cells were stained red of PI. Cell mortality is expressed as the ratio of PI+ cells /PI+ cells + Cal+ cells.

Apoptosis assessment:

Apoptotic cell death was determined by using terminal deoxynucleotidyl transferase-mediated dUTP digoxigenin nick-end labeling OH ends in genomic DNA with Fluorescein detection kit (Chemicon, ApopTag Fluorescein In-Situ Apoptosis Detection kit), according to the manufacturer's instructions. Nuclear dye 4`,6-dimidino-2-phenylindole (DAPI) (Vector Laboratories) was used to assess nuclear morphology as well as to count total number of cells.

Proliferation quantification:

Proliferative cells were counted by using the DNA replication marker 5-bromo-2`-deoxyuridine (BrdU, Aldrich-Sigma), according to the manufacturer's instructions. BrdU is a thymidine analog that incorporates into the DNA of all cells during the S-phase of the cell cycle [15]. Tissues were incubated in medium containing 20 µM BrdU for 24 hours prior to fixation. The fixed tissues were incubated with 1N HCL for 10 minutes on ice followed by 10 minute incubation with 2N HCL at room temperature before placing them in an incubator at 37 °C for 20 minutes. Tissues were incubated with borate buffer (0.1 M,

pH=8.5) for 12 minutes at room temperature. After washing in PBS with 1% Triton 100-X, the tissues were subsequently permeabilized in a solution containing PBS (1M) with 1% Triton 100-X, glycine (1 M) and 5% normal goat serum for 1 hour prior to incubation overnight with anti-BrdU mono-antibody (eBioscience) at a dilution 1: 50 in PBS.

Cell quantification:

Images were photographed using upright Zeiss LSM 510 Meta confocal laser scanning microscope. Cell counts were performed using imageJ software. Each immunoreactive cell in every field was counted only if their nuclei were visible with DAPI in the specific slice section. Additionally, in each experimental condition, the total number of cells (DAPI-stained nuclei) was compared to ensure that differences observed between the experimental groups were not attributable to a change in the number of cells.

Gene expression studies using Q-RT-PCR:

Total RNA was extracted as described in [16]. Purified RNA was stored at -70 ºC till are being used. For each sample, cDNA was reverse transcribed from 1 µg total extracted RNA in 50 µL RT reaction mix at 37°C for 2 hours using 0.5 µl (0.25 µg) random hexamer primer (Promega) and 0.5 µl (0.25 µg) Oligo dTprimer (Promega) and this mixture was annealed by heating to 65 °C for 5 minutes then allowed to cool in ice. Annealed RNA and primers were made up to a volume of 20 µl with final concentrations of the following reagents: 200 units (0.2 µl) M-MLV reverse transcriptase (Promega), 1x M-MLV RTase buffer, 0.2 mM dNTPs. RNA: DNA hybrid was degraded using 0.2 µl Rnasin® RNase inhibitor (Promega). This reaction was incubated at 37 °C for 1 hour. For each sample, an additional reaction was done without RTase - the 'No RT' control. Resulted cDNA was purified using a PureLinke™ PCR Purification kit (Invitrogen), according to the manufacturer's instructions. Primers were designed using online software (https://www.roche-applied-

science.com/sis/rtpcr/upl/index.jsp?id =uplct_ 030000) and BLAST search was then used. All primer sequences used in this study are described in table 1. Quantitative real-time polymerase chain reactions (Q-RT-PCR) were carried out in duplicate using 5 µl of each cDNA sample, 10 µM of each primer (usually intron-spaining), and 10 µl SYBR Green PCR mix (Invitrogen) in 20 µl reaction using Rotor Gene 6000 PCR analyzer (Corbett Research). Q-RT-PCR parameters: first cycle was 95 °C for 10 seconds, 65 °C for 15 seconds, 72 °C for 30 seconds followed by 45 cycles. PCR product levels were determined using SYBR Green fluorescence collected during Q-RT-PCR. To ensure the signal was not derived from contaminated genomic DNA, similar Q-RT-PCR was done on No RT samples. Specificity of PCR amplification was confirmed by analysis of cycling and melting curves. Amplicons were also analyzed using gel electrophoresis and sequencing. Linearity and specificity of PCR amplification were validated before quantification. Target genes were normalized to levels of the house keeping gene, U6 (18S RNA; Eurogenetic). Data are expressed as fold change in gene expression compared with control.

Table 1. Rat mRNA primer sequences for Q-RT-PCR.

Primer	Reverse primers (5`-3`)	Forward primers (5`-3`)	Amplicon	Source
U6	aacgcttcacgaatttgcgt	ctcgcttcggcagcaca	120 bp	Eurogenetic
Cas-1	cacaagaccaggcatattctttc	tgctttctgctcttcaacacc	110 bp	Sigma
Cas-3	Catgacccgtcccttgaa	ccgacttcctgtatgcttactcta	70 bp	Sigma
Cas-8	tcacatcatagttcacgccagt	agagcctgagggaaagatgtc	72 bp	Sigma
Cas-9	gagcatccatctgtgccata	cgtggtggtcatcctctctc	81 bp	Sigma
BCL-2	ggggccatatagttccacaa	gtacctgaaccggcatatg	76 bp	Sigma
BDNF	ccttttctggtttgcaatgag	cgaggttggaacctaacagc	68 bp	Sigma

Statistical analysis:

Statistical analysis was performed using SPSS software (version 20). All data represent the mean ± SEM of at least three independent experiments performed in triplicate. Statistical differences were assessed by Student's t test or ANOVA test with

Tukey post hoc analysis as appropriate. Results were considered to be statistically significant at $P < 0.05$.

Results:

Cell mortality is increased by GluT signalling inhibition after primary injury

To evaluate the contribution of GluT signalling on primary injury induced cell death, ex-vivo tissue cultures were treated or non-treated with DL-TBOA, a potent competitive Na+-dependent GluT-inhibitor after transient injury episode. At 24 hours post-injury, cell mortality was significantly (41.1±2.73 %, P = 0.012) increased by up to 1.4-fold compared to control cultures as evidenced by immunostaining for cell death-marker propidium iodide (PI) and cell survival-marker calcein-AM (Cal) (Figure 1A and B). Incubation with DL-TBOA resulted in a significant increase in the percentage of dead cells 2.9-fold (55.75 ± 4.18 %, P = 0.034) compared to non-treated cultures with DL-TBOA (Figure 1A and B), demonstrating the necrotic nature of cell death.

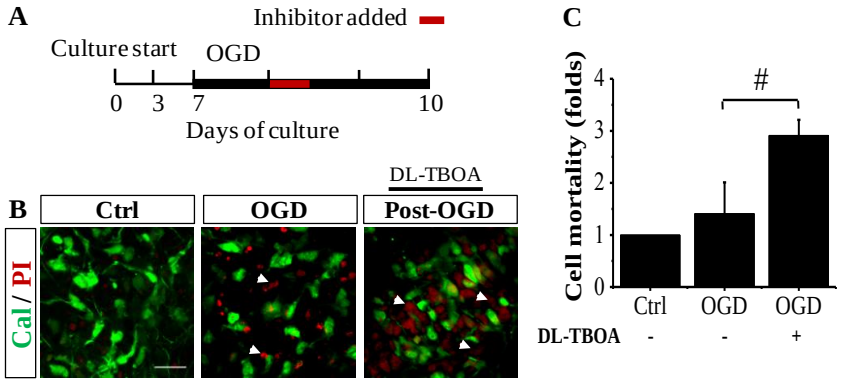

Figure 1. Quantitative analysis of cell mortality. (A) Schematic of experimental paradigm. Tissues were cultured and exposed to conditions of Ctrl (control), OGD, OGD+DL-TBOA. (B) Confocal merged images of immunofluorescence for viable cell (Cal, green) and dead/necrotic cells (PI, red). Sale bar: 30 µm. (C) Quantification of cell mortality. Data are expressed as fold change in cell mortality compared with Ctrl and are represented as mean ± SEM for at least 5 independent biological replicates. # *vs*. (versus) OGD. #$P \leq 0.05$. One-way ANOVA followed by Post-Tukey test.

To find out whether the of GluT inhibition also involves alterations in apoptosis after insult, the TUNEL assay in combination with DAPI-nuclei labelling was carried out (Figure 2A). In contrast to control condition, there was a significant ($P < 0.001$) increase in the percentage of TUNEL+ cells in slices after (Figure 2B), demonstrating the apoptotic nature of cell death (data not shown). We observed that inhibition of GluT signalling caused a significant ($P < 0.05$) increase in the percentage of apoptotic cells elucidated by insult, suggesting higher cells with overt DNA damage. Taken together with data presented in Fig1., the results suggest that necrotic and apoptotic cell death are attributed to inhibition of GluT signalling pathway in this ex-vivo tissue model of brain cellular injury.

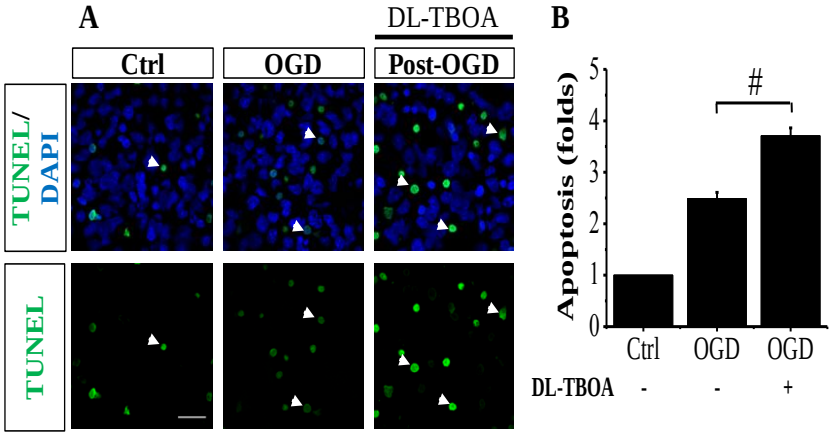

Figure 2. Quantitative analysis of apoptosis. (A) Tissue cultures were subjected to conditions of Ctrl, OGD, or post-OGD treated with DL-TBOA, and then TUNEL assay was performed. TUNEL labelling is shown green, whereas DAPI-stained nuclei appear in blue. Sale bar: 20 µm. (B) Quantification of apoptosis. Treatment with DL-TBOA increased injury induced apoptotic cell death. Data are expressed as fold change in cell mortality compared with Ctrl and are represented as mean ± SEM for at least 5 independent biological replicates. # *vs*. OGD. #$P \leq 0.05$. One-way ANOVA followed by Post-Tukey test.

GluT signalling pathway and Caspase mediated apoptosis:

To investigate the molecular injury pathways involved in GluT signalling after primary injury-mediated apoptosis, mRNA expression levels for caspase-1, -3, 8, and -9 were measured. Data of Q-RT-PCR showed that insult triggered an upregulation of expression of caspase-1, -8, -9, and -3 transcripts at 20 minutes after the end of the insult (Figure 3). Inhibition of GluT signalling resulted in a further upregulation of the transcript levels of pro-death caspase-1, -8, -9, and -3 (Figure 3), suggesting that caspase mediated apoptosis. Our results suggest further caspase expression may be mediated by GluT signalling pathways induced by altering glutamate homeostasis in this model.

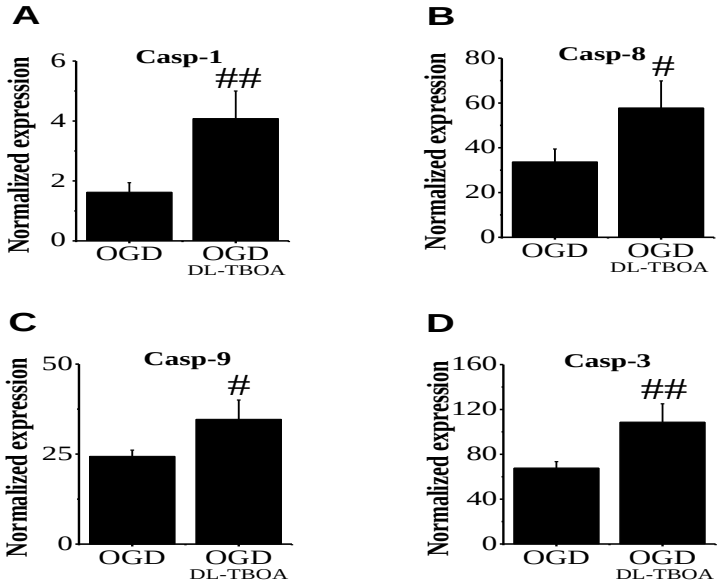

Figure 3. Injury-mediated caspase signalling. (A-C) Relative mRNA expression levels for caspase-1 (A), -8 (B), -9 (C), and -3 (D) at 20 minutes period in Ctrl, OGD, and OGD+ DL-TBOA cultures. Expression levels were calculated relative to the housekeeping gene, U6, and then normalized to the level of Ctrl. Data are expressed as fold change in gene expression compared with Ctrl and are represented as mean ± SEM for at least 4 independent biological replicates. # *vs*. OGD. #$P \leq 0.05$, ##$P \leq 0.01$. Student's *t* test.

BCL-2 and BDNF expression is downregulated by inhibition of GluT Signalling:

To further characterize the overall role of GluT signalling on cell death in ex-vivo model of brain injury, mRNA expression of cell survival genes: anti-apoptotic gene BCL-2 (B-cell CLL/lymphoma 2) and BDNF (brain-derived neurotrophic factors) were measured at serial time points. Q-RT-PCR data revealed that injury triggered a significant down regulation in the mRNA expression levels of BCL-2 and BDNF transcripts at 0, 20 minutes and 72 hours periods, suggesting an ongoing cell survival disturbance (Figure 4A and B). Under these conditions, GluT inhibition had differential impacts on the mRNA expression levels of these pro-survival genes. For example, at 0 minutes post-injury, GluT inhibitor DL-TBOA largely down-regulated expression levels of BDNF, but not BCL-2 transcripts (Figure 4A and B). At 20 minutes post-injury, GluT inhibitor DL-TBOA down-regulated expression levels of BCL-2, but not BDNF transcripts (Figure 4A and B). However, at 72 hours post-injury, GluT inhibitor DL-TBOA down-regulated expression levels of BCL-2 and BDNF transcripts (Figure 4A and B). Since BCL-2 and BDNF are likely to reflect cell survival *in-vivo*, this response may be linked to reduction percentage of cell survival appearing under these circumstances (Figure 1 and Figure 2). Together, these results suggest that the inhibition of GluT signalling may influence secondary cellular damage through suppressing BCL-2 and BDNF mRNA expression levels in this model system.

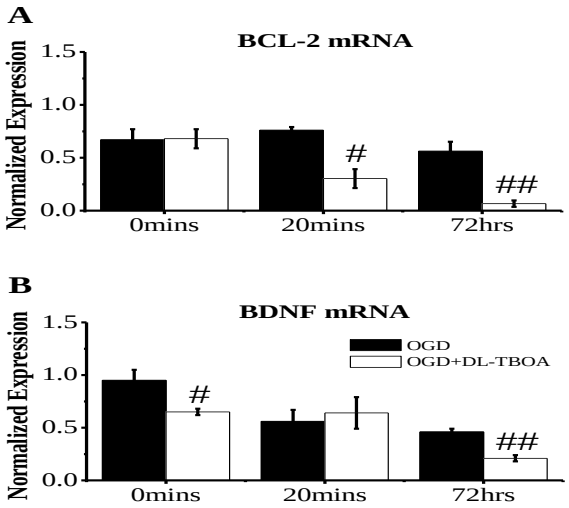

Figure 4. Gene expression measurement of BCL-2 and BDNF transcripts. Relative mRNA expression levels for BCL-2 (A) and BDNF (B) at 20 minutes period in Ctrl, OGD, and OGD+DL-TBOA. Expression levels were calculated relative to the housekeeping gene, U6, and then normalized to the level of Ctrl. Data are represented as mean ± SEM for at least 3 independent biological replicates. # *vs*. OGD. #$P \leq 0.05$, ##$P \leq 0.01$. Student's *t* test.

Cell proliferation is increased by GluT inhibition after injury:

To investigate the contribution of GluT inhibition on cell proliferation in an ex-vivo model of neonatal brain injury, cultures were incubated with the mitotic marker BrdU 24 hours prior to each fixation (Figure 5A) to determine cell proliferation, since BrdU would be incorporated only by replicating cell DNA. At 72 hours after injury, there was a significant decrease in the average number of cells undergoing proliferation. Under high magnification, BrdU+ cells appeared proliferative, as indicated by arrowheads (Figure5). Immunocytochemical studies revealed that injury induced a ~ 50% (40.7 ± 2.99, P = 0.00091) reduction in the mean number of BrdU+ cells compared to Ctrl (13.14 ± 1.25) (Figure 5). Incubation with GluT inhibitor DL-TBOA had opposite

effect on the average number of BrdU+ cells elucidated by OGD insult. After injury, the average number of BrdU+ cells was significantly ($P < 0.001$) increased by up to 2.1-fold (80.2 ± 8.64) in cultures treated with DL-TBOA compared to non-treated cultures (40.7 ± 2.99), demonstrating that the majority of cells underwent proliferation (Figure 5). Together with the cell survival and apoptosis data (Figure 1 and 2), the reduction in the cell survival with enhancement in the cell proliferation (Figure 5) as early as 72 hours post injury, suggesting that newly born cells that we saw might be produced to substitute some that degenerate during injury-reperfusion periods.

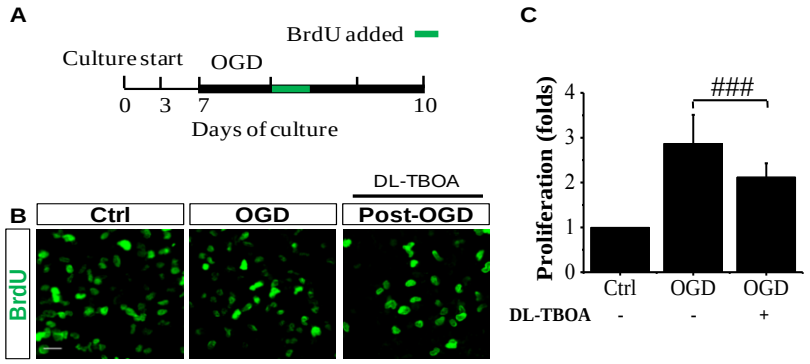

Figure 5. Quantitative analysis of cell proliferation. (A) Schematic of experimental paradigm. (B) Representative confocal micrographs of BrdU labelling in organotypic cultures of brain slices. BrdU is shown green. Scale bar: 20 µm. (C) Quantification of proliferative cells. Data are represented as mean ± SEM for at least 5 independent biological replicates. # vs. OGD. ###$P \leq 0.001$. One-way ANOVA followed by Post-Tukey test.

Discussion:

In this study we employed ex-vivo tissue culture techniques and molecular biology approaches to elucidate the molecular injury pathways involved in dyshomeostasis of GluT signalling following cellular injury, which might aid the search for therapeutic interventions. In so doing, a number of important insights were

gained. The authors conclude that primary injury involves: 1) increased necrotic and apoptotic cell death; 2) up-regulation of pro-death genes caspases; 3) down-regulation pro-survival gene BCL-2 and BDNF. In general, pharmacological inhibition of GluT signalling after a period of OGD did not alter this scenario into one leading to reduce primary cellular damage. Critically, the inhibition of GluT signalling had a significant effect on the developmentally important processes, including cytogenesis (cell proliferation) when initiated under this circumstance as judged by an~ 65% increase in the mean number of proliferating cells.

Primary cell culture model systems have been used to investigate the mechanisms of ischaemic injury [17], but our ex-vivo model system provides a more appropriate environment of the CNS with a mixture of cell types, which homogeneous cell cultures do not. The CNS neurodegeneration implicates a complex interaction between CNS elements (neural cells), so using intact brain slices maintains such a mix of different cell types and the interplay between them.

Consistent with previous studies [17-19], we observed that neonatal brain elements are vulnerable to transient ischaemic-insult and that the majority of white matter cells were killed by necrosis as early as 24 hours post-injury as evidenced by immunostaining for cell death-marker propidium iodide and cell survival-marker calcein-AM. Prior reports using tissue preparations have investigated short-term effects of an ischaemic insult, however, it is well documented that delayed cell death occurs where the affected individual survives following an ischaemic insult [3, 20-23]. Using apoptotic-marker TUNEL, we found that injury significantly increased the percentage of apoptotic cells at 72 hours post-brain injury.

Given that presence of GluTs on cellular elements of developing brain [6, 10, 24], we aimed to further investigate the molecular roles of GluT signalling on injury induced cell death in ex-vivo model of brain injury. Cell mortality studies showed that pharmacological inhibition of GluT signalling with DL-TBOA

inhibitor after primary injury caused further cellular damage. Furthermore, this early effects appear to result in a long-term reduction in cell survival and increase in cell death (necrosis and apoptosis), in this ex-vivo model, a consequence of white matter injury and injury related CNS disorders seen in premature infants.

One key question about GluT signalling on primary injury to CNS elements following primary injury is the route by which cell death is triggered, and accordingly, which of the cell death signalling pathways could be chosen as a therapeutic target. Activation of excitotoxic cascades mediated by injury mediated glutamate release and subsequent intracellular Ca2+ overload upon GluRs prolonged activation is considered to be one of the most likely mechanisms leading to damage to white matter elements [25-27]. Indeed, a cytotoxic accumulation of intracellular Ca2+ is thought to initiate a series of cytoplasmic and nuclear events, including the initiating of the apoptotic cascade [28-30]. Activation of caspases is considered a commitment to apoptosis [31-32]. Gene expression studies using Q-RT-PCR, showed that GluT signalling inhibition resulted in further upregulation of pro-death genes caspase-1, -8, -9 and -3 transcripts compared with non-treated cultures, confirming caspase-dependent cell death.

The expression of the anti-apoptotic genes BCL-2 and neurotrophin BDNF are involved in the modulation of the cell survival and death [33-34]. For example, BCL-2 plays a crucial role in the cell death control through the stabilization of the mitochondrial membrane potential, thereby preventing cytochrome c and APAF-1 release in the cytosol, which recruits pro-apoptotic pathways [35-36]. In the current study, Q-RT-PCR data showed that transient episode of injury significantly downregulated mRNA expression levels of BCL-2 and BDNF. Inhibition of GluT signalling with DL-TBOA resulted in further downregulation of expression of BCL-2 and BDNF transcripts. The downregulation of BCL-2 and BDNF mRNA expression was accompanied by an increase in the cellular damage and caspase expression. The findings described above suggest that reducing pro-survival genes

(BCL-2 and BDNF) and increasing pro-death genes (caspases) may be a mechanism by which inhibition GluT signalling confer damage to CNS elements following primary injury. However, it will be necessary to determine if these responses are translated into changes in functional proteins by using protein expression techniques, such as western blotting and immunocytochemistry. Furthermore, it is essential to note that cell proliferation was significantly decreased after injury was completely altered by inhibition of GluT signalling, while changes in BCL-2 were readily evident. Therefore, it seems unlikely that alterations in mRNA levels of pro-survival gene are simply a function of increased numbers of proliferating cells surviving due to GluT inhibition. The present study cannot address change in cell proliferation from our data. Unlike the in-vivo literature, documented changes in cell proliferation as assessed in ex-vivo model system of developing brain "ischaemia relevant" challenges are far from clear. Others have shown that the neurotransmitter glutamate may influence proliferation and differentiation in a cytoarchitecturally intact system of CNS [37].

Conclusion:

Understanding the molecular mechanisms responsible for long-term damage evolving from a CNS injury event is an essential step in identifying potential therapeutic interventions for the treatment of many neurodegenerative diseases including cerebral palsy. We show that pharmacological inhibition of GluT signalling after induced cell injury using ex-vivo model, results in a further increase of the cell death rate, and hypothesized that the GluT signalling could alteri it by elaborating pro-death caspases or by suppressing pro-survival BCL-2 and BDNF. Furthermore, the work presented in this study has not only deepened our understanding of molecular mechanisms underlying developmental brain injury, but it has also raised some interesting issues regarding the use of different techniques and approaches to study transcriptional regulation in brain in health and disease.

آليات الموت المبرمج بعد تلف خلايا الدماغ تزداد بواسطة مثبط ناقل الجلوتاميت (DL–TBOA)

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مايكـــــــــل سولتر**

المستخلص:

إن الأضرار الناجمة عن تلف أنسجة الدماغ قد يكون سببها العديد من الأمراض التي تصيب الجهاز العصبي مثل الشلل الدماغي وتصلب الأنسجة المتعدد والتي تُعزى إلى خلل في إشارات الجلوتاميت. على الرغم من هذا لا تزال الآليات الجزئية المسؤولة عن حدوث مثل هذه الأمراض غير معروفة. تهدف هذه الدراسة إلى توضيح بعض الآليات الجزيئية المسببة في حدوث موت الخلايا. استخدم مثبط ناقل الجلوتاميت الـ DL–TBOA مباشرة بعد تلف الخلايا والذي تم تحفيزه من خلال حرمان الخلايا من الأكسجين والجلوكوز لفترة قصيرة. استخدمت كلا من calcein–AM وpropidium iodide للكشف عن موت الخلايا. استخدمت تقنية الـ Q-RT-PCR لقياس مستوى الـ caspases وBCL-2 وBDNF. استخدمت تقنية قياس معدل تكرار الحمض النووي الـ DNA في الخلية (replicating cell DNA assay) لقياس معدل انقسام الخلايا. أشارت نتائج هذه الدراسة أن التثبيط الدوائي باستخدام الـ DL-TBOA بعد فترة قصيرة من تلف الخلايا تسبب في حدوث انخفاض كبير في نسبة الخلايا الحية إلى 58.6% وكذلك زيادة في معدل الموت المبرمج للخلايا إلى 29%. أظهرت النتائج أيضا زيادة في مستوى الـ caspases وانخفاض في مستوى كل من الـ BCL-2 وBDNF. بالإضافة إلى كل ذلك، أوضحت نتائج هذه الدراسة أنه توجد زيادة كبيرة في عدد الخلايا المنقسمة خلال مرحلة الانتعاش. أظهرت دراستنا أن تثبيط إشارات الجلوتاميت بعد فترة قصيرة من تلف الخلايا يعزز من موت الخلايا بواسطة آلية تنطوي على زيادة مستوى الـ caspases التي تلعب دوراً هاماً في زيادة معدل الموت المبرمج أو بخفض معدل الـ BCL-2 الذي له دور في منع أو خفض معدل الموت المبرمج.

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