

Traumatic brain injury: Current endeavours and trends for neuroprotection and related recovery

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Abstract

This paper aims to give a brief overview on traumatic brain injuries’ (TBIs) main pathophysiological mechanisms, to summarize the current available therapeutic strategies and offer a glimpse at possible future advancements in the field.

The impact of TBI on health care systems and society is tremendous. While the surgical and intensive care management are relatively well standardized for acute TBI, subsequent, therapeutic interventions remain at the physician’s choice/professional skills, and their clinical utility is still questionable.

Previous experience and numerous trials have failed to demonstrate a consistent benefit from therapies like corticosteroids, lazaroids, nsoids vasodilators, glutamate blockers, immune modulators, or general hypothermia.

Novel achievements in neuroprotection are now expected from developing anti-apoptotic agents (such as caspase and/or calpain inhibitors), from more potent antioxidants, cholinergic agents, alpha blockers from researching various physiologic substances, like melatonin, protirelin, inosine, progesterone, and including from regenerative medicine and psychiatric, assisstive technology/bioengineering advancements.

Keywords: neuroprotection, primary brain damage, secondary brain injury, traumatic brain injury

Introduction

In head traumas, the primary brain damage occurs at the time of initial impact, and is refractory to most prophylactic/therapeutic actual strategies. The secondary injuries’ ”cascade” is the result of complex (quite similar in traumatic injuries of the central nervous system (CNS), i.e. both, the brain and the spinal cord; they have been detailed, in some of our previous articles, so we shall not further develop them here) pathophysiological events that unrel over a longer period of time and are, at least in theory and partially, preventable and treatable.

The main physical mechanisms of the primary brain injuries are:

- Direct impact/ physical contact with the under-skull tissue: penetrating head injuries - the dura is pierced (bullet or knife wounds to the head are such examples).
- Indirect impact/ physical contact with the under-skull tissue: closed head injuries. Closed or blunt head injuries represent the majority of cases in civilians; many of these injuries are produced by motion without impact and/ or external evidence of injury to the head. The most common causes are

sudden acceleration or deceleration. The dura remains intact.

The physical factors influencing – mainly through inertial and/ or contact forces – the type and severity of a closed injury to the brain, include energy load per unit area, duration, velocity and direction of the force, configuration of various parts of skull, rate of acceleration or deceleration, and diffusion of forces resulting in agitation of the entire cerebrum.

More precisely, three principal physical mechanisms are described: impact loading (percussion of the head with a solid object, at a significant speed - may involve coup and contrecoup injuries, if the moving head hits a fixed object), impulsive loading (abrupt motion/ cessation of motion of the head, without significant contact with other objects) and static or quasistatic loading (external forces applied gradually – therefore the importance of the impact velocity is not always determinant – with a crushing effect; it is a rare occurrence). (21, 37)

As a bottom line it is stated that the amount of brain damage is proportional to the rotational acceleration achieved and the mass of the brain, because contact and/or inertial forces may strain the brain tissue – i.e. the amount of its deformation - beyond its structural tolerance, thus leading to injury.

There are described three basic types of tissue deformation: by compression (compressive), by tissue stretching (tensile) and by tissue distortion, when sliding over other tissue/s (shearing) (21, 37).

Considering the aspects described above, primary brain injuries can be classified as focal injuries (penetrating wounds - with skull fractures -, intracranial hemorrhages, lacerations, contusions, multiple possible

trauma at auditory-vestibular level – in striking of the temporal region) or diffuse ones (diffuse axonal injuries – DAI, including a form considerate mild: concussions)(21, 37)

The major lesional / pathophysiological conditions involved in the secondary brain damages are currently considered to be (94):

- brain tissue swelling
- hemorrhage (extradural, subdural, subarachnoid, intracerebral, intraventricular)
- loss of the local/ regional blood flow – generating ischemia
- (and possibly/ added) infection

They correspond, at cellular and subcellular level, to (what modern research has identified as a cascade of intimate events leading, in vicious circles, to the secondary injury): failure of cellular energy metabolism, local intense generation of reactive oxygen species/ oxidative stress (followed by biological membranes' lipid peroxidation, DNA and proteins damage/ misfolding), immune shifts/ inflammatory processes/ acidosis, global and/or focal multifactorial ischaemia, ischemic penumbra and alteration of regional micro-circulation, ionic disturbances (leading to cells swelling including massive edema – followed, because of suddenly installed osmolysis – by cell induced necrosis/ passively dying off), excitotoxicity (excess of neurotransmitters – especially glutamate – and also enhanced intracellular influx of calcium ions) and related activation of apoptotic genes and thus of different pathways of “delayed mechanisms of cell death”: apoptosis and apoptosis-like processes (60, 94).

Hence, the secondary events' ”cascade” entails an extremely complex and extended

reaction, practically of the entire body: from its gene level to the "macroscopic"/ clinical, one.

Therefore, the concept of secondary brain injuries has become the basis – many of them being intimate targets – for developing an array of neuroprotective modern therapies.

Neuroprotective therapies

Below we give an extensive list of potential neuroprotective therapies, classified by mechanism of action and clinical use. This classification paradigm also appeared in one of our previous papers, dedicated to neuroprotection in spinal cord injury (SCI), published in 2009, the October issue of Spinal Cord journal.

We will further discuss some of these therapeutic drugs and procedures. Our color code serves to achieve a balanced, selective approach; the present paper refers to the drugs effective/ researched in TBI and to some equally effective/ researched in both TBI and SCIs; considering both conciseness and a selective review of recent and/or less discussed matters, not all items will be elaborated:

Colour code:

- Mainly used/researched for brain injuries
- Rather equal efficiency in SCI and TBI
- Mainly used/researched for spinal cord injuries

"Classical" drugs, with a long history of clinical use

- Peptide mixtures with neurotrophic actions (Cerebrolysin®, Actovegin®)
- Musculotropic vasodilators

- **Nootropic agents**
- **Anesthetic agents** (including Xenon gas, with anti-excitotoxic properties)
- **(Other) Hormones**
 - Sex hormones (progesterone)
 - TRH (thyrotropin-releasing hormone) and analogues
- **Anti-inflammatory drugs and immune modulators:**
 - SAIDs (Methylprednisolone)
 - Lazaroids (21-aminosteroids)
 - NSAIDs (COX2 inhibitors)
 - IL 10 (Interleukin 10)
 - Glatiramer acetate (Copaxone®)
- **Anti-excitotoxic agents (glutamate blockers)**
 - Xenon gas
 - NMDA blockers
 - AMPA blockers
 - KDI tripeptide
- **Iron kelators** (deferoxamine, 2,2'-bipyridine, quercetin)
- **Vitamins and other nutritional supplements** (B vitamins, folic acid, selenium, zinc, magnesium, etc.)
- **Ca²⁺ channel antagonists** (nimodipine)
- **Lithium**
- **Statins** (Simvastatin) – acting as antagonists of growth inhibitory signals in central nervous system
- **Dopamine agonists:**
 - Bromocriptine
 - Lisurid
- **Cholinergic agents**
 - Citicoline
 - Rivastigmine (Exelon)

New / experimental drugs

- KDI tripeptide
- Monosialoganglioside GM 1

- **IGF-1 (Insulin-like Growth Factor)**
- **Neurotrophins – characterized by pleiotropic effects**
 - GDNF (Glial Derived Neurotrophic Factor)
 - BDNF (Brain Derived Neurotrophic Factor)
 - NGF (Nerve Growth Factor)
 - NT-3 (Neurotrophin-3)
 - CNTF (Ciliary Neurotrophic Factor)
- **Apoptosis inhibitors**
 - Protease inhibitors (caspase inhibitors, calpain inhibitors)
 - PARP (poly(ADP-ribose) polymerase) inhibitors
- **DMSO (dimethyl sulfoxide)**
- **Antioxidants**
 - Glutamate transporters (EAAC1 protein)
 - Free radical scavengers: ascorbate, vitamin E, beta-carotene, alpha-tocopherol, penicillamine, superoxide dismutase, Q10 coenzyme
 - L-cysteine
 - Selenium, zinc, magnesium
- **Cell adhesion molecules (L1-CAM)**
- **Erythropoietin**
- **Melatonin**
- **Inosine (Axosine™)**
- **AIT-082 (leteprinim potassium, Neotrofin)**
- **4-aminopyridine (Fampridine)**
- **Riluzole**
- **Antagonists of the growth inhibitory signals in CNS**
 - Monoclonal antibodies to Nogo
 - Phosphodiesterase inhibitors (Rolipram)
 - Dibutyryl cyclic AMP (db cAMP)

- **Inhibitors of Rho signaling (Cethrin)**
- **Scar preventing substances** (chondroitinase, cordaneurin, EphA4 antagonists)

New / experimental procedures

- **Local hypothermia**
- **Combinatory ('COMBO') strategies**
- **Hyperbaric oxygen therapy**
- **Fusion technology** (Polyethylene glycol)
- **Physical therapy**
 - Low-level LASER therapy
 - Oscillating field stimulator (OFS)
 - Functional electrical stimulation
- **Immunotherapy with activated macrophages or lymphocytes**

Pharmacological and physiatriac stimulation of the spinal central pattern generator (CPG)

Neurotrophic peptide mixtures

Cerebrolysin

Cerebrolysin is a mixture containing 85% free amino acids and 15% biologically active low-molecular weight peptides, prepared by enzymatic lysis of lipid-free pig brain products. Its exact mechanisms of action are not known, but the drug has been proven useful in a large number of conditions, including neurological trauma of any type (3, 108), stroke (48), Alzheimer's and other degenerative disorders of the brain. The most numerous clinical studies with Cerebrolysin® were conducted in patients with dementia (Alzheimer's and vascular) and they yielded good results, with significant improvement of cognition in the treated subjects (35). To date, there are no reported significant adverse reactions to Cerebrolysin®, except

for allergies and seldom CNS excitability enhancements.

The small molecules contained by the mixture readily cross the blood-brain barrier. They exert neuroprotective, neurotrophic, nootropic and neuromodulating – i.e. multimodal – effects on the CNS, similar to those of the naturally occurring growth factors, especially the Nerve Growth Factor. Many experimental studies (67, 74, 82) and clinical trials (3, 48, 108) suggested a variety

of mechanisms for these beneficial effects:

- An increase in the efficiency of the aerobic neuronal metabolism
- Stimulation of protein synthesis in the CNS
- Stimulation of neuronal differentiation
- Inhibition of reactive oxygen species formation
- Inhibition of lipid peroxidation (67)
- Anti-excitotoxic actions (74)
- Anti-apoptotic effects (32)
- Immunoactive properties (84)

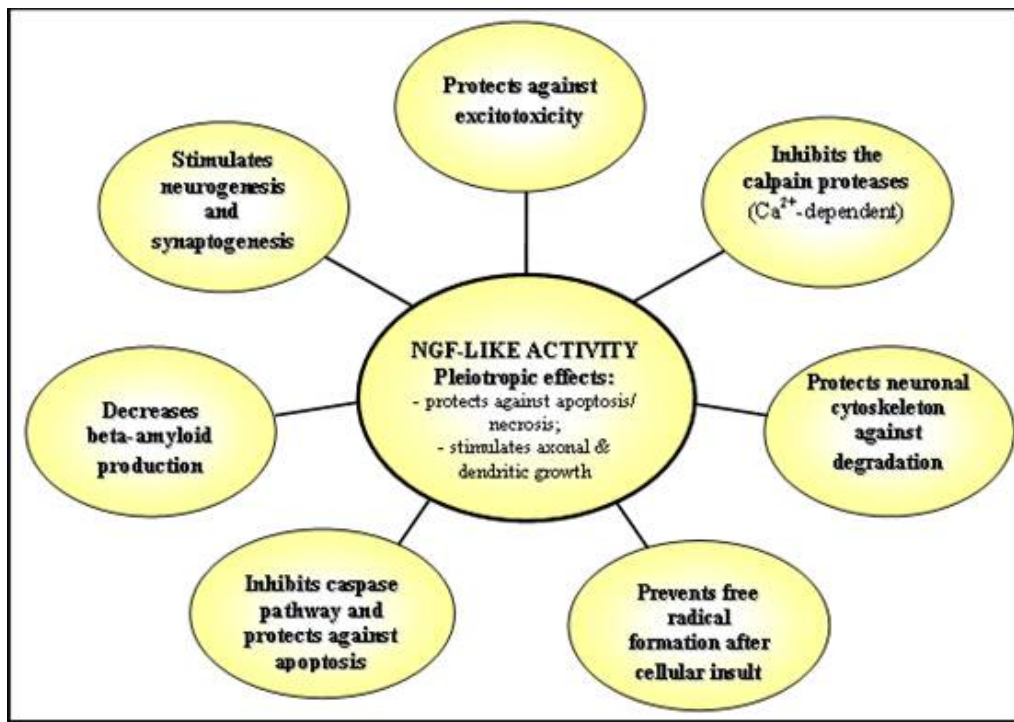


Figure 1 Correspondence between Cerebrolysin’s main effects and pathways of the secondary injuries cascade it targets/ counteracts: pleiotropic, but by stimulating neuro-/ synaptogenesis and respectively, neuroplasticity, it results in a multimodal way of action, too (G. Onose et al. Neuroprotective and consequent neurorehabilitative clinical outcomes, in patients treated with the pleiotropic drug cerebrolysin, *Journal of Medicine and Life*, 2(4): 350-61, 2009)

Actovegin

Actovegin is a deproteinized hemoderivative of calf blood, obtained by ultrafiltration. It contains electrolytes, essential trace elements and 30% organic components: amino acids, oligopeptides,

nucleosides, inositol phospho-oligosaccharides (IPOs), intermediary products of the carbohydrate and of the lipid metabolism, and components of the cellular membranes, such as glycosphingolipids. The molecular weight

of the organic components is below 6000 Da.

The main effect of Actovegin® is to increase the cellular energy metabolism, by increasing the respiratory capacity of mitochondria, the oxygen and the glucose uptake. Also, the IPO fraction of Actovegin® demonstrated a positive effect on glucose carrier activity (GLUT1): it stimulates glucose uptake by the cerebral tissues, as well as other tissues and activated glucose oxidation. The IPO fraction acts indirectly on the citric acid cycle, by increasing acetyl coenzyme A synthesis(12).

Several small clinical studies found benefits of Actovegin® administration in elderly patients with organic brain syndrome(45), in dyscirculatory encephalopathy(55, 100) and in TBIs, where the investigators found some positive effects of the drug on the EEG changes(92). Actovegin® is very well tolerated, and few adverse reactions have been described; allergies are the most frequent side effects and severe anaphylaxis has been reported in one case(56).

Musculotropic asodilators

Papaverine

Cerebral vasospasm is a severe complication that can develop in 5% to 50% of patients with TBI(97), especially in relation to subarachnoid hemorrhage (SAH) or other intracranial hemorrhages. The posttraumatic cerebral vasospasm can lead to massive stroke, increasing the morbidity and the mortality after head injuries, which is why treating it is one of the main neuroprotective measures in these patients.

Papaverine (an opium alkaloid) is a potent smooth muscle relaxant, but hypotension limits its systemic application.

It has been used in topical (with a syringe, gelfoam, cotton pledgets, or controlled-release drug pellets) application on arteries during surgery(85). Local application in a controlled-release matrix has been clinically tested in a study involving 117 patients. The results showed that the procedure effectively prevented the development of symptomatic vasospasm and improved neurological outcome in treated patients, with no adverse effects(17).

It has also been administered as intra-arterial injections, but the effectiveness of this method in reversing vasospasm-associated cerebral hypoperfusion is controversial. Some authors found it useful(52), but some found no beneficial effect of intra-arterial papaverine(96). Also, there is one case report of transient severe brain stem depression during intra-arterial papaverine(6).

Vinpocetine (Cavinton)

Vinpocetine is a synthetic derivative of vincamine, an alkaloid derived from vinca minor. It was discovered during the late 1960s and has been used in the treatment of cerebrovascular and cognitive disorders for decades. Its primary mechanism of action is direct vasodilation of cerebral vessels, enhancing the cerebral blood flow, but hypotension is a rare side effect. It also reduces platelet aggregation (by inhibiting its capacity to take in adenosine), increases platelet plasticity and decreases blood viscosity. More recent studies suggested that vinpocetine and vincamine may decrease the mitochondrial dysfunction induced by glutamate excitotoxicity (63, 22, 93). In vitro and in vivo experiments demonstrated the the compound inhibits phosphodiesterase 1, blocks voltage-operated calcium channels and voltage-dependent sodium channels (13), and has

antioxidative action(68). A clinical trial with vinpocetine in newborns with intracranial birth trauma demonstrated that the compound inhibits posttraumatic epileptic activity (25).

A small Hungarian clinical study showed that intravenous administration vinpocetine in stroke patients increases the cerebral blood flow and the glucose metabolism, especially in the thalamus and caudate nucleus(90).

All this accumulating data suggests that intravenous vinpocetine might a useful therapeutic tool for head injury treatment, but further evidence is needed.

Vincamine (Ceredia, Oxybral, Oxicebral, Cetal, Devincan, Pervincamin) appears to have the same pharmacological characteristics, except the risk of hypotension and cardiac arrhythmias is higher with intravenous administration.

Clinical trials found orally administered vincamine beneficial in primary degenerative and vascular dementia, but there are no studies vincamine for CNS trauma.

Ginkgo biloba extract (Tanakan)

Ginkgo biloba is the best-selling phytomedicine on the European market. It has been widely used to treat a variety of conditions, including peripheral vascular disease, vertigo, ischemic heart diseases, eye diseases, depression, ischemic stroke, demetia and TBI. The extract contains flavone glycosides, ginkgolides, bilobalides and other constituents. Pharmacological effects include peripheral arterial dilation, free radical scavenging, inhibition of reactive oxygen species formation, inhibition of lipid peroxidation, activation of energy metabolism, decreasing platelet aggregation.

In therapeutic doses, the side effects are

mild: gastro-intestinal irritation, headache, allergic skin reactions. Very large doses have been reported to cause diarrhea, nausea, vomiting and restlessness. A few case reports described spontaneous bleedings related to Ginkgo biloba.

Experiments with Ginkgo biloba on rat models of TBI showed that the extract improved the behavioural outcome in the injured animals, and histology demonstrated that the treated rats developed less cerebral edema in response to injury (4).

Nootropics

Nootropic drugs form a large group of substances known to enhance cognitive abilities in cerebrovascular, involutive and post-traumatic disorders. Currently, they are successfully used as part of the rehabilitation therapy for the patients with chronic TBIs. No significant adverse reactions have been reported.

Pyritinol (Encephabol, Enerbol)

The compound is chemically affined to pyridoxine and penicillamine. It facilitates the passing of glucose across the blood-brain barrier and increases its metabolism in neuronal tissue; also has antioxidant properties (44). Pyritinol is successfully used as an immune-enhancer in rheumatoid arthritis (50).

It has been tested in small clinical studies for comatose patients (after head traumas). The results showed slightly improved neurological outcome and a significant decrease in mortality (18, 102). The drug is generally well tolerated; adverse reactions are more frequent with prolonged administration and high doses: agitation, insomnia, gastrointestinal symptoms, nausea, headache, and rarely allergies. A survey of six cases suggested a link between

pyritinol and severe cholestatic hepatitis (58); another rare side effect is acute pancreatitis (89).

Racetams

Piracetam (Pyracebral, Nootropil, Pyramen, Gabacet) is a cyclic derivative of GABA (gamma-aminobutyric acid). Its proposed mechanisms of action are: improvement of cross-hemispheric information transfer, stimulation of the cholinergic system (via muscarinic receptors), decrease of neuronal oxygen consumption, increase of glucose oxidation, with subsequent ATP formation. Clinical studies indicate a weak protective effect against hypoxia.

Piracetam has been extensively studied for its beneficial effects in dyslexia (probably due to the enhancement of cross-hemispheric communication). Yet, a recent clinical study found no significant improvements on speech disorders after ischemic stroke with prolonged (6 months), high doses of Piracetam (36). A Polish clinical trial, which included 100 patients, concluded that high doses of piracetam improved clinical outcome in head injury patients, if started immediately after the trauma (33). Still, a recent paper classifies Piracetam among the drugs detrimental for neuroprotection (40).

Pramiracetam (Pramistar®) is a liposoluble racetam derivative. Recent clinical trials indicate that it might be the most potent nootropic substance. It accelerates the acetylcholine turnover by activating the HACU (High Affinity Choline Uptake) system and the nitric oxide-synthase, it inhibits the cerebral neuropetidase, it has antidepressant actions. Reports suggested that the drug improved cognition in TBI patients (57).

Anaesthetic agents

Anaesthetic agents are used during brain surgery or in head traumas refractory to conventional therapy. Some authors used them prophylactically, in an attempt to improve outcome in TBI patients. Anaesthetics make the object of another of our articles, and are only briefly discussed in the present paper.

We will mention that of all the clinically available anaesthetics, the barbiturates seem to have the greatest neuroprotective potential (26). They reduce the functional activity of the brain, the cerebral metabolic rate of oxygen (CMRO₂), therefore lowering the cerebral metabolic demands (94), the cerebral blood flow and the intracranial pressure (ICP). This results in an increase of global cerebral perfusion and oxygenation.

There have been many published clinical trials with barbiturates in head injury patients, but none of them demonstrated that this therapy has significant benefits. Moreover, barbiturates were associated important complications, like hypotension, infection, hypothermia (81) and severe hypokalaemia with rebound hyperkalaemia (62).

In conclusion, analysis of currently available data on the use of barbiturates shows that they might be beneficial in selected, hemodynamically stable, patients with important, intractable elevations of ICP.

Sex hormones

The observation that female laboratory animals recover better than males after traumatic and ischemic brain injuries (5, 51, 78) led to the hypothesis that progesterone and estrogens might have neuroprotective properties. Subsequent studies showed that

both estrogen and progesterone reduce the effects of TBI when administered to males or ovariectomized females (64). While estrogens were also shown to exacerbate the brain damage, especially in animal models of ischemic stroke (16, 38), progesterone had no significant side effects in preclinical studies. It could be given to both males and females, without affecting gender differentiation and sexual functions (88).

Intensive research during the last decade demonstrated that sex steroid hormones are up-regulated in the CNS following traumatic injuries (31) and exert an important neuroprotective and neuroregenerative effect. The mechanisms for these beneficial effects are complex, diverse and not completely understood.

It has been demonstrated on animal models that progesterone markedly reduces edema after brain injury (16), modulates glial cell activity (31), decreases lipid peroxidation, reduces the expression of pro-inflammatory genes, attenuates mitochondrial dysfunction (87), decreases pro-apoptotic and increases anti-apoptotic enzymes (24), enhances remyelination, synaptogenesis and dendritic arborization (75).

A phase II randomized, double-blind, placebo-controlled clinical trial (called 'ProTECT') was conducted at the Emory University, Atlanta, to test the effects and assess the safety of intravenous progesterone in acute TBI. The study enrolled 100 adults with blunt head injury. The progesterone administration was started within the first 11 hours of initial trauma and patients were re-assessed at 30 days. The results of the study showed that no significant adverse effects were found, and progesterone reduced the 30-day mortality to less than half of the controls. In

the treated group, the patients with moderate TBI had better functional scores, but those with severe head injury had longer duration of coma (109). Other small clinical studies gave encouraging results with the use of this hormone in both, male and female patients (86) and several phase II and III clinical studies are currently ongoing or recruiting, to study the effects of intravenously administered progesterone in acute TBI (42).

TRH and analogs

The tripeptide thyrotropin-releasing hormone (TRH, protirelin) and some of its analogues have long been recognized as neuroprotective factors. Studies showed that TRH administration improves neurological outcome in animal models of CNS trauma (29, 99). Protirelin and its analogues seem to antagonize a variety of mechanisms that lead to the secondary damage in TBI: the opiate receptor activation by injury-induced endorphin release, the glutamate toxicity (99), the lipid peroxidation, the inflammatory cytokines.

A major drawback for the clinical use of these compounds as neuroprotectives are their undesired endocrine, autonomic and analeptic effects. Also, TRH has a poor blood-brain barrier penetration and this raises the problem of the route of administration.

A small clinical trial was conducted in California with intravenous TRH in patients with acute spinal cord injuries. They found no significant side effects; the treated group showed a better neurological outcome, but these results are difficult to interpret, given the small number of patients included in the study (72). There have been no clinical trials to test TRH in TBI.

Several analogues with better brain penetration and lesser endocrine effects have been synthesized, and some of them are currently under evaluation for possible entry into future trials (27, 28).

Xenon gas

Xenon is a naturally occurring gas that may prevent or ameliorate acute neuronal injury, as it seems to inhibit the activity of glutamate receptors (106).

Brain damage, with neurocognitive deficit, is for instance, a potential postoperative-complication following coronary artery by-pass grafting (CABG). One study showed that xenon administration before CABG, while on hypothermic cardiopulmonary bypass,

using both a standard anesthetic breathing circuit and the oxygenator had no harmful effects (53).

Anti-excitotoxic agents

The recognition of the role of excessive amounts of glutamate in the intracellular calcium accumulation in CNS lesions has opened an entirely new research field. The role of excitatory neurotransmitters in the progression of various neurologic disorders, including trauma, stroke, dementia, multiple sclerosis, epilepsy, glaucoma is attracting more and more attention (65). Currently, the glutamate-receptor antagonists are probably the most important issue in neuroprotection research.

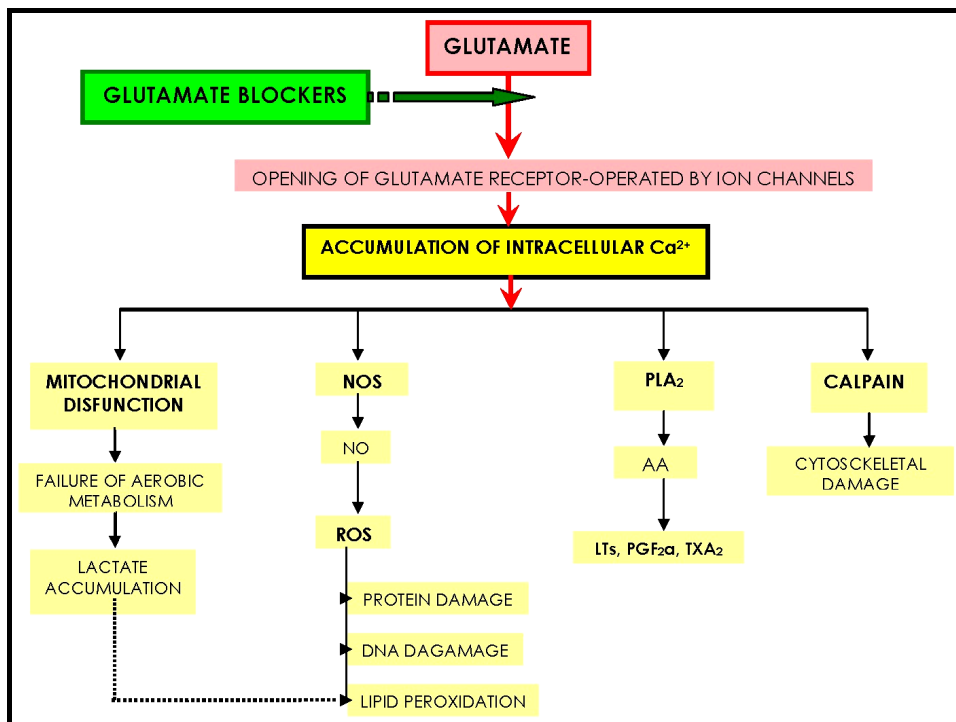


Figure 2 Schematic synthesis of the main effects of glutamate and, consequently, of its blockers. Legend: Ca^{2+} – calcium ions; NOS – nitric oxide synthase; NO – nitric oxide; PLA_2 – phospholipase A_2 ; ROS – reactive oxygen species; AA – arachidonic acid; LTs – leucotriens; $\text{PGF}_{2\alpha}$ – prostaglandin $\text{F}_{2\alpha}$; TXA_2 – thromboxane A_2 (G. Onose et al. Integrative emphases on intimate, intrinsic propensity/ pathological processes – causes of self recovery limits and also, subtle related targets for neuroprotection/ pleiotropicity/ multimodal actions, by accessible therapeutic approaches - in spinal cord injuries. *Journal of Medicine and Life*, 3(3): 262-74, 2010)

Glutamate has 4 types of receptors: NMDA (N-methyl-D-aspartic acid), AMPA (alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), kainic acid and the metabotropic receptors, linked to the G protein. A large number of glutamate receptors has been identified, and as a consequence, a huge variety of glutamate antagonists were developed and studied.

Unfortunately, several clinical trials of glutamate antagonists failed to confirm the encouraging data from in vitro and in vivo studies.

Selfotel (CGS19755), a competitive NMDA blocker, was the first glutamate antagonist that underwent phase III clinical trial in TBI, but the research project had to be shut down due to an increased mortality report from the simultaneously running stroke trials (7, 65).

Dexanabinol (HU-211) is a cannabinoid and a non-competitive NMDA antagonist. It also acts as a free radical scavenger inflammatory cytokine inhibitor. In phase II trials, results showed if administered within 6 hours of injury, it reduced mortality, it lowered ICP and had no significant side effects. But the recently published results of a phase III multicentric clinical trial, involving 861 patients with severe TBI demonstrated that though it is safe, Dexanabinol has no therapeutic benefits in head injuries (54).

Traxoprodil (CP-101,606) is a non-competitive NMDA antagonist, highly selective for the NR2B subunit. Phase II clinical trials found it to be safe, but with no consistent therapeutic benefits. A phase III randomized, double-blind, placebo-controlled trial tested the efficacy of a 72-hour infusion of traxoprodil, started within 8 hours of the initial brain injury. It

provided no substantial evidence of the drug's efficacy in TBI (115). Eliprodil also failed to show any therapeutic efficacy.

Aptiganel (cerestat) another non-competitive NMDA antagonist, entered a clinical trial, but the study was stopped following negative reports from the concurrently running ischemic stroke trial (65).

D-CPP-ene (SDZ EAA-494) a competitive NMDA antagonist, failed to demonstrate any benefit in a phase III clinical trial.

Xenon, an anaesthetic gas, has also been identified as a non-competitive NMDA receptor antagonist. Studies on neuronal cultures and lab animals demonstrated that it provides protection against hypoxia-induced excitotoxicity (41, 69, 70). The advantage of xenon is its long history of clinical use (as an anaesthetic) which has proven it safe in humans.

Nutritional supplements

Magnesium is a non-competitive NMDA antagonist and it may exert neuroprotective actions through several other mechanisms: increased cerebral blood flow to ischemic areas, competitive antagonist at all voltage-sensitive calcium channels, amendment of cellular energy metabolism, enhanced mitochondrial calcium buffering.

Studies on animal models of brain trauma showed that magnesium sulfate infusion, administered immediately after injury, significantly reduces the tissue loss and improves neurological outcome (14, 39).

A pilot clinical trial was conducted to test the safety and efficacy of field administration of intravenous magnesium sulfate in acute stroke patients (the FAST-

MAG trial). The results were encouraging: magnesium significantly ameliorated recovery with no severe side effects (one patient with skin flushing) (80). Currently, the FAST-MAG trial is recruiting patients for phase III (42).

Magnesium appears to be a promising neuroprotective tool in TBI, but further evaluation is needed.

Ca²⁺ channel antagonists

The proposed mechanisms of action for calcium-channel blockers in TBI are the reduction of the calcium cellular influx and the prophylaxis/ lysis of the cerebral vasospasm that may accompany acute traumas, especially when subarachnoid hemorrhage (SAH) is associated (7).

Nimodipine (Nimotop) is dihydropyridine calcium channel blocker. Since it has greater lipid solubility than the other similar compounds and consequently some selectivity for the cerebral vasculature, the drug is already established for the prevention and treatment of cerebral vasospasms. It is approved by FDA, in oral administration, for the therapy of cerebral ischemia following SAH (2, 23).

The use of Nimodipine in unselected TBIs is however questionable, given the known side effects of calcium-channel blockers: systemic hypotension, cerebral vasodilation, decreased cerebrovascular reactivity. Large clinical studies, led by the Head Injury Trial Group (HIT I, II and III) were carried on in unselected TBI patients in Europe and they found some outcome improvements in injuries associated with traumatic SAH (61, 95).

A review of six randomised controlled trials with Nimodipine in unselected patients with acute TBI concluded that the drug may only have some beneficial effects

in selected subjects, with SAH, while being potentially harmful in other brain injuries (49). Another review, of four randomised controlled trials with Nimodipine for traumatic SAH, found no beneficial effects (98).

Dopamine agonists

Dopamine agonists are used as cortical stimulants, to treat a variety of neurological symptoms: aphasia, akinetic mutism, amotivational syndrome/ apathy/ abulia/ anergia, attention disorders, neglect, depression (34). Recent studies suggested that these compounds may also provide neuroprotection by inhibiting glutamate neurotoxicity (83).

Bromocriptine, an ergot alkaloid is a potent agonist for D2 dopamine receptors and some serotonin receptors, currently used in the treatment of pituitary tumors (with hyperprolactinaemia or acromegaly), Parkinson's disease, neuroleptic malignant syndrome, and type 2 diabetes. It has been shown to interfere with the activity of astroglial glutamate transporter GLT-1 (EAAT2) (83).

Bromocriptine has been used with good clinical results in the paroxysmal autonomic instability with dystonia (PAID)/ "diencephalic seizures" – a syndrome consisting in tachycardia, tachipnea, hyperthermia, diaphoresis and decerebrate posturing, following severe TBIs (10, 15). Also, the administration of Bromocriptine to posttraumatic vegetative state and minimally conscious state patients led to improvements of the motor and cognitive outcomes (66).

The most common side reactions of bromocriptine are: orthostatic hypotension, nausea, headaches and vomiting, liver toxicity (107).

Cholinergic agents

Cholinergic agents (Rivastigmine – Exelon) are currently used in the treatment of mild to moderate dementia in Alzheimer’s and Parkinson’s diseases, with some beneficial results in cognitive functions. Their use is based on the hypothesis that the cholinergic system stimulates brain areas related to learning and memory.

Citicoline – cytidine diphosphate-choline (CDP-Choline) is an intermediate product in the generation of phosphatidylcholine from choline. It is sold in many countries as a psychostimulant/nootropic dietary supplement. The presumed mechanisms for its neuroprotective effects include: counteracting excitotoxicity, maintaining cellular adenosine 5'-triphosphate levels, stimulating neuronal plasticity (43). It has been used for many types of cognitive impairment, especially of vascular and degenerative etiology (30). A few small clinical studies reported significant improvements with the use of this compound (20), but larger trials are needed to establish the efficacy of Citicoline.

Apoptosis inhibitors

Traditionally, neuroprotective therapies have been mainly focused on reducing necrotic cell death, by antagonizing various factors that eventually lead to the failure of cellular metabolism. But after CNS trauma, necrosis is an early event, and the therapeutic window for these strategies is short. Recent experimental work substantiated the important role of apoptosis as a secondary injury mechanism. In contrast to necrosis, apoptosis is a more delayed process, that enreels for days, even weeks after the initial trauma, which is why

modulating it is likely to be a more effective approach.

Apoptotic inhibitors are currently in the experimental phase, but they are a dynamic and very promising research field.

Calpain inhibitors

Calpains are constitutively expressed intracellular, non-lysosomal cysteine proteases. They are classified as ubiquitous and tissue-specific. The ubiquitous isoforms, μ -calpains (or calpains I) and m-calpains (or calpains II), are abundantly expressed in the central nervous system (CNS). Their activation requires the presence of Ca^{2+} (in micromolar concentrations for calpains I and in millimolar concentrations for calpains II) (73).

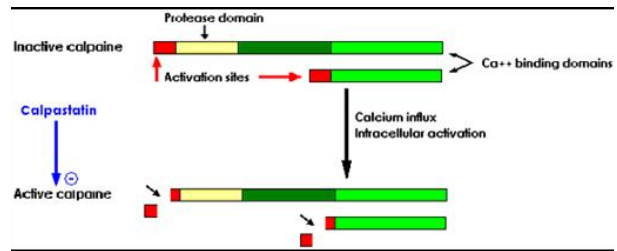


Figure 3 Activation of calpains - within the calpains pathway of apoptosis (G. Onose et al. Integrative emphases on intimate, intrinsic propensity/ pathological processes – causes of self recovery limits and also, subtle related targets for neuroprotection/ pleiotropicity/ multimodal actions, by accessible therapeutic approaches - in spinal cord injuries. *Journal of Medicine and Life*,3(3): 262-74, 2010)

Although various calpain substrates have been identified (cytoskeletal proteins, growth factor receptors, adhesion molecules, transcription-related proteins), the precise function of these enzymes in vivo is still poorly understood (111). The massive cellular Ca^{2+} influx associated with CNS injury inevitably activates calpains, which, in conjunction with caspases, promote irreversible damages to

key cellular structures that finally lead to apoptosis.

The endogenous specific inhibitor of calpains is calpastatin (CAST), but overactivation of calpain may degrade calpastatin. A number of cell-permeable calpain inhibitors (e.g. epoxysuccinate derivatives, aldehydes, and alpha-keto carbonyl compounds) (71) have been synthesized and tested on animal models of TBI and other CNS injuries, and some of them showed important neuroprotective effects (47, 73).

Caspase (cysteiny] aspartic acid-protease) inhibitors

Based on their substrate specificity, there have been identified 14 mammalian caspases (110). These enzymes are translated as zymogen proforms and upon activation, caspases may cleave their own precursors or other procaspases, resulting in a caspase activation cascade (112).

Caspases are involved in several interrelated apoptotic pathways, which are not yet completely understood. Caspase-3 has received most of attention, since it appears to be the major effector in neuronal apoptosis. Initial strong evidence supporting the specific role for this protease came from studies on caspase-3 knock-out mice, in which brain development was severely altered (110). Later, in vivo and in vitro experiments with semispecific peptide caspase inhibitors (Z-DEVD-FMK, Z-IETD-FMK, Z-LEHDFMK) (8) established the role of this protease in injury-induced neuronal loss (47).

Cathepsin inhibitors

Like calpains, cathepsins also belong to the papain superfamily of cystein proteases. Cathepsins are found predominantly in the lysosomes, but also in the cell nuclei and cytosol. These enzymes have also been

implicated in neuronal injury, and inhibitors such as CA-074 and E-64c have been proven to significantly decrease neuronal death(113).

PARP-1 [Poly(ADP-Ribose) Polymerase -1] inhibitors

Recent research shows that while mild damages to DNA activate the repair mechanisms, severe insults induce PARP-1 overactivation and cell death (11).

PARP-1 is an abundant nuclear protein, functioning as a DNA damage-sensor and signaling molecule. Upon binding to DNA breaks, activated PARP-1 cleaves NAD⁺ into nicotinamide and ADP-ribose and polymerizes the latter into branched nucleic acid-like polymers, covalently attached to nuclear acceptor proteins. The negative charge of these covalently attached ADP-ribose polymers severely alter the function of target proteins (such as histones, topoisomerases, DNA polymerases, DNA ligases, transcription factors and PARP-1 itself) (11).

Researchers tested a large number of PARP-1 inhibitors (nicotinamide, 3-aminobenzamide, monoaryl amides, bi-, tri-, or tetracyclic lactams), most of them acting as competitive inhibitors that block the binding of NAD⁺ to the catalytic domain of the enzyme. Some benzamides have also been shown to inhibit the binding of PARP to DNA(104). Many of these compounds have been shown to protect neuronal tissue against various insults, in vitro and in vivo (91, 101).

A recent study demonstrated that minocycline and other tetracycline derivatives, previously researched for their neuroprotective properties, also inhibit PARP-1 activity and reduce neuronal death (1).

Antioxidants

Oxidative stress is one of the key mechanisms of secondary injury in CNS traumas and an attractive target for neuroprotection. The oxygen free radicals cause peroxidation of membrane phospholipids and oxidation of cellular proteins and nucleic acids, damaging both neurons and cerebral vasculature.

Superoxide dismutase (SOD)

SOD is a physiological antioxidant, an enzyme that reduces the superoxide anion concentration by dismutation to hydrogen peroxide, which is then converted to water.

SOD has a very short half-life (of about 5 minutes), which limits its clinical utility, but conjugation with polyethylene glycol (PEG) extends its half-life to approximately 5 days (105).

The polyethylene glycol-conjugated SOD (PEG-SOD, pegorgotein) proved to be safe in phase II clinical trials and seemed to improve the outcome of head-injured patients (59). As a consequence, it underwent a phase III, multicentric, randomized, placebo-controlled trial. The study enrolled 463 patients with severe, closed head injury. The treated groups received a single intravenous dose of either 10 000 U/kg, or 20 000 U/kg of pegorgotein. No significant difference in neurologic outcome or mortality was found at 3 or 6 months between the treated groups and the placebo group (114).

New / experimental procedures

Local hypothermia

Hypothermia (the intentional reduction of core temperature below 36°C) is thought to protect the blood-brain barrier and prevent inflammation, the development of vasogenic edema and intracranial hypertension, while decreasing cerebral

metabolic rate for oxygen and energy requirements (94, 106). Whole body hypothermia has been intensively studied as a neuroprotective intervention, especially in newborn. A number of clinical trials studied the effects of general hypothermia in ischemic stroke, neonatal hypoxic-ischemic encephalopathy and ischemic stroke (42). Though experimental and clinical results showed that this procedure exerts some neuroprotective effects, the use of whole body cooling is hindered by serious complications (e.g. sedation, arrhythmia, coagulopathies, increased risk of infection, tremor, hypotension, hyperkalaemia) (79, 94).

Local hypothermia is a newer and apparently safer intervention. Experiments showed that even profound local hypothermia does not damage cortical neurons (106). A phase III clinical trial is currently on going, to assess the feasibility and efficacy of discrete cerebral hypothermia in TBI (42).

Hyperbaric oxygen therapy

Oxygen is largely used as a therapeutic agent. Hyperbaric oxygen therapy consists in administering 100% oxygen at pressures higher than 1 atmosphere (0,1Mpa) using a small hyperbaric chamber or a mask (9). This procedure achieves hyperoxia, leading to an increase of the physically dissolved blood oxygen, which is proportional to the partial pressure of the oxygen in the ambient.

Hyperoxia exerts a variety of beneficial effects: improvement of tissue oxygenation, decrease of inflammation, stimulation of tissue repair mechanisms, antibacterial effects and consequently it has a broad range of clinical applications, for conditions that involve ischemia, impairment of tissue repair, inflammation, infection. Hyperbaric

oxygen is an established therapy, approved in many countries for many indications such as: monoxide poisoning, decompression sickness, gas embolism, necrotizing soft tissue infections, diabetic wounds, osteoradionecrosis. (9). The main concern with the use of hyperbaric oxygen therapy is systemic vasoconstriction (9), but the extensive clinical experience with this procedure proved its safety if application parameters are rigorously controlled (duration, pressure).

A consistent body of research exists for the use of this intervention in TBI and it has been suggested that, despite the cerebral vasoconstriction it produces, hyperoxia has many beneficial actions that may sum up to better rehabilitative outcomes: increasing the partial pressure of oxygen in brain tissue, restoring the mitochondrial redox potential (enhancing damaged mitochondrial recovery) (19, 76), decreasing intracranial pressure (9,77). A prospective, randomized clinical trial compared the effect of hyperbaric to normobaric hyperoxia when applied to severe TBI patients within 24 hours of injury; while both procedures increased brain oxygenation and improved indices of oxidative metabolism (in comparison to the control group), only hyperbaric oxygen lowered intracranial pressure. However, these effects were transient, lasting only a few days after the intervention. No significant adverse reactions were recorded (77).

A phase II clinical trial is presently on going to assess the feasibility of hyperbaric oxygen therapy in patients with chronic sequelae following brain injury (42).

Conclusions

In experimental conditions, various pharmacological agents lead to impressive reductions in the extent of brain damage and consequent significant neurological recovery after TBI, inciting high expectations of a clinical benefit.

But to date, all the clinical trials failed to demonstrate a radical improvement with any of these agents. Furthermore, many of these substances (like many glutamate receptor antagonists) were found to have serious side effects. Yet, on one hand, compound products such as Cerebrolysin (more and more largely used and tested in standardized, multicentric clinical trials – including our involvement for one of them) and on the other, citicoline (which, as previously emphasized, is considered in many countries a supplement – and therefore is easily accessible and very widely) seem to be in a recent prestigious review (40) the most efficient substances used in connection with neurorehabilitation practice. Novel achievements in neuroprotection are now expected from developing anti-apoptotic agents (such as caspase and/or calpain inhibitors), from more potent antioxidants, cholinergic agents, alpha blockers, from researching various physiologic substances, like protirelin, inosine, progesterone, and including from regenerative medicine and psychiatric, assistive technology/bioengineering advancements, all within multidisciplinary and intricate endeavours – on a still long and windy road.

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