

NEUROPROTECTION AND NEUROPLASTICITY IN CRANIOCEREBRAL TRAUMA

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Abbreviations

ATP – adenosin 5'-triphosphate; BDNF – brain derived neurotrophic factor; CNTF – ciliary neurotrophic factor; TNF(R) – tumor necrosis factor (receptor); NGF – nerve growth factor; NMDA – N-methyl-D-aspartate; t-PA – tissue plasminogen activator; MMP – matrix metalloprotease; LRP – low density lipoprotein receptor-related protein

ABSTRACT

The concept of neuroprotection implies all mechanisms and strategies able to diminish the loss of nervous tissue caused by damaging agents. The main target of neuroprotection consists of limitation of neuronal death and conservation of neuronal function. The strategy of the neuroprotective treatment consists in interference with the molecular cascades that determine first neuronal dysfunction and then neuronal death.

Key words: neuroprotection, neural plasticity, cell death, brain trauma

Neuroprotection represents the totality of mechanisms and strategies used to increase the nervous cell's resistance to damaging agents.

The objective of neuroprotection is represented by the limitation of neuronal dysfunction/death due to different aggressions on the central nervous system and the struggle to maintain the integrity of the cellular interactions as high as possible so that the nervous functions should be conserved.

The strategy of the neuroprotective treatment consists in interfering with the molecular cascades that determine first neuronal dysfunction and than neuronal death. Several etiological agents or biological processes may trigger the same molecular cascades which will finally lead to neuronal death (1).

There are two main biological models of cellular death. The first is the necrosis during which a failure of cellular homeostasis installs after certain types of aggression (mechanical, anoxical, etc.). It is the result of the extracellular environment alterations occurred with such intensity that the cell's homeostatic machinery isn't anymore able to compensate them. The cell becomes excessively permeabilized and the content is eliminated into the extracellular space.

The second type of cellular death is called apoptosis and is an active suicide process during which

the cell is using its own mechanisms to initiate a series of events leading to digestion of several cellular components.

During necrosis the edema causes osmotic lysis, the cell dying passively. In the extracellular environment are liberated products that will initiate the inflammation.

Apoptosis is an active process, strictly controlled genetically, necessitating ATP. The cell is preparing itself, the final result consisting of apoptotic bodies. The apoptotic cell death doesn't trigger inflammation.

The last tendencies of the scientific world reserves the term "apoptosis" for the physiological process that helps the normal, healthy organism to control the number and quality of its cells. The programmed cellular death processes that occur in pathological situations – from degenerescence to citotoxicity – are called "apoptotic-like" processes. These entities form in fact the target for the cytoprotective therapy, a therapy gaining more and more popularity with the clarification of the intimate pathophysiology of many diseases that evolve with cellular loss.

The newest concept of cellular death was described in the nervous system: anoikis. Anoikis is defined as an apoptotic-like process induced by

inadequate cell-extracellular matrix interactions. It is considered today that neurons may die of “loneliness”. Indeed, the neurons are hyperspecialised cells with an internal structure and metabolism directed only for one purpose: management of information. The 100 billion cells in the human brain are spread between the three times more numerous glial cells and the blood vessels, everything being well-anchored on an extracellular matrix. The pieces of this huge puzzle – the nervous system – are all tightly interdependent, communicating permanently with the help of the signal molecules (nitric oxide being one of the most simple) and trophic factors. Deprivation from trophic factors or signal coming from the other components of the morphofunctional unit leads to initiation of the programmed cellular death. By this theory, all cells in the organism try permanently to die in apoptosis, but the phenomenon is prevented by the different signals received.

In some cases all the above-mentioned processes (necrosis, apoptosis, apoptosis-like, anoikis) take place in the same moment.

CONSIDERATIONS ON THE PATHOPHYSIOLOGY OF SOME NEUROLOGICAL CONDITIONS

Three major entities should be taken into consideration as being extremely important in the pathophysiology of neurological diseases:

- excitotoxicity;
- inflammation;
- apoptosis-like processes.

EXCITOTOXICITY

Excitotoxicity results from the excessive liberation and defective processing (by the astrocytes) of the excitatory neurotransmitter **glutamate**.

Glutamate activates receptors for N-methyl-D-Aspartate (NMDA), increasing the levels of intracellular calcium and leading to activation of proteases, lipases and other cellular lesions mediators. It results also membrane depolarization, increased energetic demands and high levels of extracellular glutamate.

Free radicals (partially reduced oxygen species) generated denatured DNA, proteins and fatty acids. It may also occur programmed cellular death (apoptosis).

High intensity lesions, such as those that occur in the core of the ischemic stroke lead to inflation and lysis (necrosis) of the neurons by massive NMDA receptors stimulation.

A more moderate hyperactivity of NMDA receptors, like in craniocerebral trauma, penumbral area of ischemic stroke or slow-onset neurodegenerative disease trigger the formation of free radicals and many other ways leading to apoptotic-like lesions.

CALPAIN SYSTEM

CANP represents the acronym for Calcium Activated Neutral Proteases. There are two isoforms: μ -calpain and m-calpain. Both isoforms set up a largely distributed and strictly regulated proteolytic system which influences cellular functions (Fig. 1).

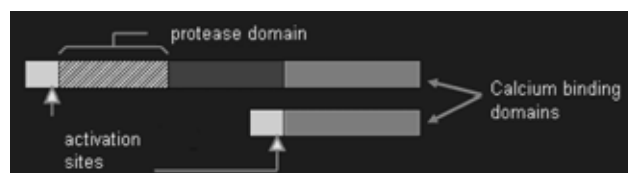


Figure 1
The structure of calpain

Calpain has two subunits (80 kDa and 30 kDa); m-calpain necessitating micromolar and μ -calpain milimolar calcium concentrations, both being inactivated by calpastatin (Fig. 2).

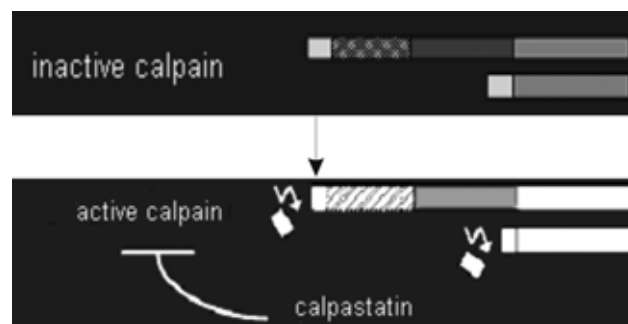


Figure 2
Activation of calpains

The main function is to controllably destroy structural cytoskeleton proteins and to activate signal transduction cascades and phosphorylation pathways (protein kinase C). Remodeling the elements of the cytoskeleton, of the membrane architecture and of the connection between membrane and cytoskeleton contributes to neuroplasticity by promoting:

- neurite growth;
- synaptic remodeling;
- dendritical remodeling.

The pathological activation of calpain (as result to the intracellular influx of Ca^{++}) leads to: uncontrolled degradation of the cytoskeleton elements, alteration of signaling ways (PK-C) and of the function of CDK-5, these phenomena resulting in neuronal structure alterations and cytoskeletal

lesions leading to necrotic and apoptotic death. Calpain is inhibited by calpastatin.

APOPTOSIS

Essentially apoptosis may be triggered in two ways:

- intrinsic activation (mainly mitochondrial) triggered by high intracellular calcium concentrations, oxygen reactive species, glutamate, etc.
- extrinsic activation (binding to the receptors of cellular death); for example the TNF α binds the Fas receptor

Both ways lead directly or indirectly to the activation of caspases – a hierarchical group of 14 proteases that are cystein-dependent and aspartate-specific. Caspases are essential mediators of programmed cellular death. A multitude of substances are either activated either inactivated by caspases, taking part in the regulation of cellular death process. Substrates may be DNA repairing enzymes like PARP (poli-ADP-ribose polymerase). PARP is attacked by caspases during apoptosis loosing thus its capacity to bind and repair the DNA. Other substrates are proteins of the cellular cycle, members of Bcl-2 family, transcription factors, protein kinases and proteins of the cytoskeleton (τ proteins associated to microtubules).

Apoptosis is regulated by the availability of the neurotrophic factors which offer trophic support for neurons:

- deprivation of growth factors induces apoptosis
- cellular survivals based on a continuous stimulation with neurotrophic factors
- cells are under permanent survey from the “cellular community”

In case of craniocerebral trauma the apoptotic-like processes are induced by:

A. alterations in calcium homeostasis

- inductor caspases associated to the endoplasmic reticulum
- increase in the concentration of calcium ions produces stress in the endoplasmic reticulum and activates the caspases

B. liberation of the citocrom from the mitochondria

- craniocerebral trauma induces apoptosis by liberating pro-apoptotic factors from the mitochondria

Between the calpain system and the caspase system exist interconnections: calpastatin is cleaved by caspase 3, so that the induction of apoptosis will induce activation of caspase 3 and consequently

the cleavage of calpastatin which will increase the activation of calpain.

THE THERAPEUTIC CONTROL OF APOPTOSIS

The therapeutic control of apoptosis can be made using caspase inhibitors like:

1. IAP group (Inhibitors of Apoptosis Proteins). IAP holds Zink-Finger domains, CAR domains, ubiquitin-dependent domains with enzymatic activity, nucleotide-binding domains, BIR domains - important for the apoptosis suppression. IAP inhibits the effects of caspases 3 and 7 and initiator caspase 9. IAP acts either indirectly by inhibiting the apoptosome or directly by inhibiting the caspase 3. IAP may be themselves blocked by other proteins such as DIABLO (Direct IAP – binding protein with low pI) – in mice or SMAC (second mitochondria derived activator of caspases) – in humans.

2. PACAP (pituitary adenylate cyclase activating polypeptide) is a 38 aminoacids polypeptide which inhibits directly the caspase 3 with a neurotrophic effect. Experimental intravenous administration one hour after the onset of a cerebral infarction in animals reduces significantly the volume of the ischemic lesion.

3. Bcl-2 blocks the apoptosis induced by ceramides.

4. Nonsteroidal antiinflammatory medication reduces the conversion of sphingomyelins to ceramide. The ceramides are mediators of TNF-induced apoptosis.

5. The cascade leading to apoptosis may be activated also through the path of MAP kinase.

In 2001 the National Institutes of Neurological Disorders and Stroke meeting emphasized the importance of dynamic actions between endothelial cells, smooth muscle cells, astrocytes, microglia, neurons and tissue matrix proteins, defining the concept of “neurovascular unit”.

The dynamics of vascular, cellular, matrix signals is involved in the maintenance of the cerebral tissue integrity both in the white substance and in the grey substance, the importance in the pathophysiology of conditions like stroke, craniocerebral trauma, vascular dementia, migraine, multiple sclerosis or, possibly, elderliness, being overwhelming.

After ischemia, the functional neurovascular alterations initiate multiple lesional cascades.

Signals like oxidative stress together with neutrophil's and platelet's interactions with activated endothelium stimulate matrix metalloproteinases, the plasminogen activators and other proteases which will degrade the matrix and lead to interruption of the blood-brain barrier.

The inflammatory cells and mediators infiltrate through the blood-brain barrier and amplify the cerebral lesions. Additionally the alterations of cell-matrix homeostasis may also trigger an anoikis-like cellular death both in the parenchymatous and in the vascular compartment. Superposition with excitotoxicity is also documented by the interactions with NMDA receptors mediated through t-PA which increases the ionic imbalance and lead to cellular death.

The neurovascular unit places the stroke and the neurotrauma in the context of an integrated tissular response where all the cellular and matrix elements, not only neurons, are involved in the evolution of the tissular lesion.

The efficiency of blood-brain barrier is dependent of the interactions between endothelial cell astrocyte and matrix.

The interruption of the neurovascular matrix, including components of the basal membrane such as type IV collagen, heparan-sulphate, laminin or fibronectin, will cause troubles in the signaling between cells and between the cells and matrix, signaling that maintain the neurovascular homeostasis.

Although many proteases contribute to the proteolysis of the extracellular matrix, in the context of stroke and neurotrauma the plasminogen activator and the matrix metalloproteinases are perhaps the most important. t-PA was used successfully as a therapy in stroke.

New data emphasize the importance of the connections between t-PA, matrix metalloproteinases, edema and hemorrhage after stroke and craniocerebral trauma (2).

Matrix metalloproteinases (MMP) are endopeptidases produced by all types of cells in the neurovascular unit:

- gelatinases (MMP-2 and -9)
- collagenases (MMP-1, -8, -13)
- stromelisin (MMP-3, -10, -11)
- membrane-type MMPs (MMP-14, -15, -16, -17)
- other MMPs (MMP-7, -12)

The system of plasminogen activators together with the matrix metalloproteinases play a central role in the brain development and plasticity, modulating the extracellular matrix to allow the growth of neuritis and cellular migration. MMP activators are:

- MAP kinase pathway
- oxidative stress

- oxidation and nitration stress
- caspase-mediated cellular death
- excitotoxicity
- neuroinflammation

The new therapies could target the t-PA - LRP - MMP pathway improving the safety profile of t-PA in patients with stroke and craniocerebral trauma.

Parallel between the treatment strategies in craniocerebral trauma and stroke:

Craniocerebral trauma	Stroke
Quick resuscitation after the event	Prevention
Prompt evacuation of large lesional masses	Reperfusion
Adequate intensive care	
Neuroprotection	Neuroprotection
Long term recovery (some cases)	Recovery

The main purpose of neuroprotection in craniocerebral trauma is to prevent and reduce secondary lesions, as well as the recovery, while the purpose of neuroprotection in stroke is to prevent the neuronal death in the penumbral area (3).

There are absolute and relative mechanisms in neuroprotection.

Relative mechanisms include:

- calcium channels modulators
- sodium channels modulators
- NMDA receptors antagonists
- GABA receptors antagonists
- antioxidants
- free radical scavengers
- adhesion molecules
- adenosine agonists and antagonists

The absolute mechanisms include:

- neurotrophic factors
- neurotrophic factor-like molecules
- some cytokines classes

The craniocerebral trauma produces lesions and dysfunctions both by primary mechanism (bio-mechanical effects) and by secondary effects (due to the activation of pathophysiological cascades).

The secondary lesion consists from several complex biochemical and cellular pathways that influence the progression of the primary lesion. In reality, the cerebral lesion develops sequentially (about 1/3 of the patients deceased after a craniocerebral trauma were conscientious and cooperative at a certain moment after impact), the neuroprotective treatments initiated having to interrupt the pathophysiological mechanisms that lead to secondary cerebral lesions.

During the last decades the understanding of the craniocerebral trauma pathophysiology progressed quickly and, based on this understanding, numerous pharmacological treatments were developed, tested and proved efficient on experimental models.

The new data regarding the neurobiology and neuropharmacology of craniocerebral trauma should not distract the attention from the absolute importance of combating hypoxia, hypotension, increased intracranial pressure and other causes of secondary ischemic lesion.

Also it should not discourage the negative results and the difficulties met during the clinical trials, but continuous researches for neuroprotective drugs in craniocerebral trauma should be performed, and the clinical studies more realistically approached.

If the main mechanisms of the neuronal death in the penumbral area and the neuroprotective strategies are well delimited nowadays, the mechanisms of neuronal death and neuroprotective strategies in craniocerebral trauma are still unclear, the poor results in clinical studies not offering much additional data.

The complex pathophysiology of craniocerebral trauma offers numerous targets for potential neuroprotective agents. Many of these agents were or will be investigated on experimental neurotrauma models. Unfortunately the clinical trials involving craniocerebral trauma were, up to this date, unsuccessful.

ANTAGONISTS OF EXCITATORY NEUROTRANSMITTERS

High concentrations of extracellular glutamate were discovered both in experimental neurotrauma models and in human craniocerebral trauma patients.

Experimental studies identified a number of glutamate antagonists acting on either presynaptic or postsynaptic ionotropic receptors (NMDA, AMPA, etc.) or metabotropic, competitive or uncompetitive, or modulator (4).

Receptors for glutamate are important for normal functioning of neurons, so that the antagonism must be made with minimum interferences to the physiological mechanisms.

Some NMDA antagonists with strong neuroprotective effects were not evaluated in clinical trials because of the possible secondary psychotropic

effects, while for other compounds the clinical trials were terminated prematurely because of an excess of mortality in concomitant trials involving stroke (7).

Taxoprodil, a second generation NMDA antagonist that targets selectively the NMDA receptors that contain NR2B subunit was evaluated in a clinical trial. The molecule was well tolerated and despite the fact that the results were not statistically significant, a better state of patients was observed together with a reduction of mortality in the most severely affected patients.

Dexanabinol is a synthetic cannabinoid with no psychotropic activity, but with a strong neuroprotective activity (due to anti-excitotoxic, antioxidant and anti-inflammatory properties). This drug was recently evaluated in a phase III study, the results indicating the fact that it is safe, but not efficient in the treatment of craniocerebral trauma (6).

The efficacy in blocking the excitotoxic response consequent to craniocerebral trauma and other central nervous system lesions remains still unproved.

Magnesium plays an important role in the normal functioning of the cell and it was proved to have neuroprotective properties in experimental studies on neurotrauma and cerebral ischemia animal models. One of the main advantages of magnesium is its pleiotropic effect. While other compounds interfere usually with one pathophysiological mechanism, the magnesium exerts its neuroprotective effects by blocking uncompetitive the NMDA receptors, inhibiting the presynaptic liberation of excitatory neurotransmitters, suppressing the cortical depression and blocking the voltage-dependent calcium channels (5).

Despite all this evidence regarding the neuroprotective effects of magnesium, a recent double blind, randomized clinical trial evaluating the efficacy of a continuous 5 days magnesium administration in 499 patients with severe and moderate craniocerebral trauma couldn't emphasize neuroprotective effects and even indicated the possibility that magnesium has a damaging effect. No eventual statistical or methodological causes for the negative results were detected (8).

Other studies with the purpose of elucidating the relation between total and ionized magnesium concentration in serum and cerebrospinal fluid at different moments after craniocerebral trauma could eventually offer an explanation to the negative results of the recent clinical studies.

MITOCHONDRIAL DYSFUNCTION

Mitochondrial dysfunction can be attenuated by mitochondrial permeability transition inhibitors such as cyclosporine A and its derivatives (9).

Based on preclinical data, cyclosporine A was evaluated in two phase II clinical trials, being emphasized its properties of enhancing the cerebral perfusion pressure and metabolism, as microdialysis showed.

Cyclosporine A is considered safe in patients with craniocerebral trauma and its pharmacokinetics in the injured central nervous system was elucidated, sustaining the initiative for a phase III clinical trial (10).

During experimental research, blocking the type N voltage-dependent calcium channels by **ziconotide (SNX-111)** led to partial restoration of mitochondrial function, but a clinical trial was prematurely terminated because of increased mortality in the treatment group.

Type N voltage dependent calcium channels blockers like **SNX-185** have a better bioavailability and neuroprotective properties on experimental models, but need an additional preclinical evaluation.

ERYTHROPOIETIN

The abundant expression of erythropoietin and its receptors in the central nervous system exists and, as a response to hypoxia or excitotoxicity, this expression increases suggesting a central role in endogenous protection against lesional stimuli.

Erythropoietin was proven neuroprotective on experimental models of stroke and neurotrauma, a decrease in lesional volume and an increase in functional results being possible by limiting the inflammatory reaction.

A double-blind clinical trial did not record any adverse events, suggesting a functional results improvement for patients treated with erythropoietin. These results need further clinical research, like a multicentric, phase II/III clinical trial performed on stroke patients and a pharmacokinetics study on the erythropoietin level in the cerebrospinal fluid after systemic administration (11).

For craniocerebral trauma, a phase II randomized trial is currently ongoing in Wisconsin, USA. This trial is primarily focused on moderate craniocerebral trauma patients and, instead of using the Glasgow Outcome Score it evaluates markers of neuronal death as primary results.

HORMONES

A vast metaanalysis suggests the fact that there are no differences between the evolutions of male and female patients after a craniocerebral trauma.

Both progesterone and allopregnenolone enhance neuronal survival and functional recovery after experimental neurotrauma (12).

Based on the experimental researches the progesterone exerts its neuroprotective effects by a variety of mechanisms:

- the decrease of edema formation because of modifications in the blood-brain barrier and GABAergic neurotransmission modulation with the result of decreased excitotoxicity
- apoptosis inhibition
- gliosis reduction
- reduction of the inflammatory response

Allopregnenolone, a progesterone metabolite, also has neuroprotective properties and could be even more efficient than progesterone.

Experimental data on the neuroprotective effects of progesterone correlated with adequate pharmacokinetic studies resulting from a phase II clinical trial concluded that there are no serious adverse effects after administration to patients with craniocerebral trauma. In patients with moderate neurotrauma treated with progesterone the evolution was better compared with those treated with placebo. A large multicentric study was initiated to prove these observations (13).

BRADKININE ANTAGONISTS

The B2 receptors of bradikinin proved their importance in what is considered to be a promising strategy for neuroprotection. A phase I clinical trial to investigate the pharmacokinetics for **Anatibant** was conducted and published. Right now, a phase II study regarding the safety of the administration is conducted on 500 patients with craniocerebral trauma (14).

NITRIC OXIDE AND NITRIC OXIDE SYNTHASE INHIBITORS

Nitric oxide is a key factor in the development of secondary lesions, being potentially neurotoxic since it contributes to:

- neuronal excitotoxic death
- generates cytotoxic peroxinitrites

- directly damages the DNA
- inhibits DNA synthesis
- inhibits mitochondrial respiration
- was associated with apoptotic cellular death

Nitric oxide is synthesized from L-arginin by nitric oxide synthetase (NOS) for which were discovered four isoforms. Three of these are constitutive and one inducible.

The three constitutive isoforms are the neuronal, endothelial and mitochondrial NOS. The fourth form is inducible – inducible NOS – and is expressed in pathological conditions.

Nitric oxide accumulates in the brain immediately after the lesion and a few hours or days after the lesion. Experimental studies with NOS inhibitors showed benefic effects, especially for neuronal and inducible NOS inhibitors.

Clinical studies do not support these benefits and even show deleterious effects. These differences may reflect different effects of the investigated agents on NOS isoforms, and also the importance of the administration moment.

A new drug currently investigated is VAS203, a structural analogue of 5678-tetrahydrobiopterin, the endogenous cofactor of NOS and one of the most powerful NOS inhibitors discovered until now.

NANOTECHNOLOGY

Nanotechnology represents the design and elaboration of new materials and structures with a functional organization of nanometric order at least in one dimension. The possibility of manipulation and control of self-organising or self-assembling substrates determines material properties which couldn't result from the properties of the constitutive molecules alone. At biological level the functional integration between artificial materials and biologic systems could reach new borders. Nanotechnology may be applied in the neuroprotection of the central nervous system concomitantly with other procedures.

One of the nanotechnological solutions applicable nowadays is the fullerene based carbon 60. The fullerenes are molecules composed of three-dimensional equidistant carbon similar with soccer balls. Fullerenols are fullerene derivatives in which a hydroxyl group was added and which presents antioxidant and antiexcitotoxic properties. Fullerenols are free radical scavengers and are capable to reduce glutamate-, NMDA-, AMPA- or kainate-mediated excitotoxicity also inhibiting concomitantly the

apoptotic cellular death. Neuroprotective mechanisms mediated by fullerenol are, at least partially, due to the inhibition of glutamate channels, because neither GABA(A) receptors nor taurin receptors were affected. Additionally the fullerenols decrease the concentration of calcium ions that was increased by glutamate (15, 16, 17, 18).

Amphiphilic peptidic molecules (with a hydrophobic tail and a hydrophilic head) were designed to self-assemble in nanofibrillary structures in physiologic ionic conditions. The surface of nanofibers is formed by the hydrophilic groups of the aligned peptides and may contain physiologically active amino acid sequences involved in cellular signaling as well as surface receptors ligands (19, 20, 21). For example, using a sequence like isoleucin-lysin-valin-alanin-valin, derived from the neuron-specific matrix lamina promoted the growth and development of neurites. Amphiphilic peptidic molecules solubilized in an aqueous solution keep their structure intact until they come in contact with the calcium ions in physiological concentrations. In this moment, the peptidic molecules organize as nanofibres, forming a gel-like substrate. In this gel may be encapsulated progenitor neural cells which, due to the nanofibres properties, may functionally signal three-dimensionally. The differentiation of these cells into mature neuronal types is faster and more complete (β -tubulin III is expressed after 7 days in 50% of the neurons), the differentiation into astrocytes being almost absent (under 5% after 7 days). These new technologies could limit reactive gliosis after traumatic or degenerative events and could be a real help for pluripotent cell transplantation (22, 23).

NEUROTROPHIC FACTORS

The neurotrophic factors play roles in:

- ontogenetic development - control the cellular proliferation and differentiation (expression of the mediator phenotype, of ionic channels, growth of neurites)
- promotion of neuronal survival (if there are no damaging agents) throughout the life and maintaining the phenotype
- increase of the resistance of nervous cells to the action of damaging agents (hypoxia, ischemia, hypoglycemia, excitotoxicity, toxic substances, trauma), neuroprotection
- plasticity and synaptic activity (learning process), neuroplasticity

The neurotrophic factors modulate neuronal and synaptic plasticity, determining the rebuild of neuronal circuits and synaptic transmission.

Nerve Growth Factor (NGF) was discovered by Rita Levi-Montalcini in 1951 when it was detected in large quantities in the submandibular glands of male mice; later it was identified in other organs and in their species, including man. In 1986 Rita Levi-Montalcini received the Nobel prize for discovering the NGF. NGF is synthesized and secreted by the target organs innervated by sympathetic and sensitive neurons derived from the neural crest, it binds to these neurons receptors and it's trapped and retrograde transported to their cellular body. Here they regulate the development and functional differentiation of neurons by incompletely elucidated mechanisms.

Brain Derived Neurotrophic Factor (BDNF) is the most widely spread neurotrophin in the brain. BDNF was evidenced in the neocortex, piriform cortex, amygdala complex, hippocampus, claustrum, some thalamic and hypothalamic nuclei, substantia nigra, some structures in the brainstem. mRNA encoding BDNF was discovered in the lateral septum, terminal stria, medial preoptic nucleus, olivary pretectal nucleus, lateral paraventricular nucleus or the dorsal cornu of the spinal chord. Kainate treatment (an excitatory amino acid, aromatic analogue of the glutamic acid which destroys neurons when given in high doses) determines an intense expression of BDNF in CA1 pyramidal cells, dentate gyrus, CA2 and CA3 hippocampus regions, proving that BDNF has a strong neurotrophic action to maintain neurons and synapses.

Neurotrophin 3 (NT-3) promotes neurite outgrowth both in sensitive neurons derived from the neural crest (spinal ganglia) and in those derived from the placoda (nodous ganglia).

Ciliary neurotrophic factor (CNTF) is part of the neurokinins family together with the leukemia inhibiting factor, interleukin 6, cardiotrophin 1. Expression of CNTF is limited especially to nervous system and skeletal muscle cells; in physiological concentrations CNTF acts only on these types of cells. CNTF sustains the survival of nervous cells in the ciliary ganglia and induces synthesis of acute phase proteins. All neurons in the central and peripheral nervous system express CNTF alpha in certain stages and the majority respond to CNTF *in vitro* with a minimum 24 hours survival.

Leukemia inhibiting factor (LIF) is a factor with known actions in the immune system that acts also on the cultured sympathetic neurons determining the modification of the neurotransmitter phenotype from noradrenergic to cholinergic and regulates the expression of neuropeptides in these cells. LIF acts as a trophic factor for oligodendrocytes and promotes the survival and differentiation of astrocytes.

The effects of NGF are multiple and exert both on the nervous system and other organs and tissues.

In the peripheral nervous system NGF acts as a neurotrophic factor on the noradrenergic neurons in the sympathetic ganglia and nociceptive neurons in the spinal ganglia.

In the central nervous system the main target for NGF is represented by the cholinergic neurons.

Effects on the central nervous system include:

- neuriteogenesis stimulation (in culture, on PC12 cells)
- collateral spreading of the cutaneous nociceptive fibres (laboratory animals)
- phenotype modification for the sympathetic innervation of the sweat glands in transgenic mice expressing more NGF
- NGF has an important role in mature neurons in the spinal ganglia, ligation of a spinal nerve leading to an increase of the NGF mRNA
- prevention of neurite outgrowth in the spinal chord dorsal cornu after axotomy having thus an impact on neuropathic pain
- perivascular axonal growth after administration in the lateral ventricle in rats
- growth of the small neurons in the spinal ganglia after intrathecal administration in rat
- increase of the transmitting peptides contents in primary sensitive afferent synapses
- aberrant growth of sympathetic and sensitive fibres consequent to postnatal NGF hyper-expression

Apart from the effects on the nervous system NGF exerts a strong influence on other organs and tissues:

- it has been identified in different organs like spleen, lymphatic ganglia, thymus as well as in mastocytes, eosinophils, B and T lymphocytes
- NGF blood level increases during inflammatory reactions, in several autoimmune diseases, parasitic infestations and allergic conditions

- stress determines an increase of NGF expression
- during inflammatory arthropathies NGF increases in synovial fluid
- NGF plays a major role in the reepithelisation of cutaneous lesions and in skin lesions prevention consequent to ultraviolet irradiation
- during hypophyse development NGF stimulates the differentiation and proliferation of lactotropic, the lack of control over this mechanism being a cause for prolactinoma occurrence and progression
- pancreatic b cells express NGF receptors, the blocking of which significantly delays the morphogenesis of Langerhans islands
- NGF intensifies the expression of insulin
- NGF regulates the growth of several tumor cells of nervous origin by inducing a differentiated phenotype and consequently inhibiting the proliferation velocity of the tumor

The effects of BDNF administration are evident both on the nervous system and other tissues. On the nervous system:

- experiments on cultured mesencephalic nervous tissue slices containing substantia nigra showed that adding BDNF to the culture medium determines an increase in the number of neurons and thyrozinhydroxylase immunoreactive fibres. BDNF suppresses the apoptotic cellular death taking place in the substantia nigra in postnatal rat, indicating a potential effect of BDNF for the treatment of Parkinson's disease
- continuous BDNF infusion in the supranigral region for 3 hours prevented the death of GABAergic neurons caused by the destruction of the caudate-putamen and globus pallidus with ibotenic acid
- BDNF prevents the atrophy of rubrospinal neurons, stimulates the expression of regeneration-associated genes (GAP-43, α -1 tubulin) and increases the regenerative capacity of axotomized rubrospinal neurons
- pretreating cortical neuron cultures with BDNF prevents the inactivation of protein-kinase C consequent to the exposure to lethal doses of glutamate or NMDA

On other tissues:

- BDNF lowers the glicemia level in diabetic obese mice improving the insulin resistance

by intensifying the effects of insuline on the peripheral tissues

- in obese mice BDNF determined a decrease of the glycogen in the liver and of the serical hepatic enzymes
- modulators of AMPA receptors interfere with BDNF synthesis. Two of the four classes of receptors modulators (ampakines and biaril-propylsulphonamides) have the property to increase the BDNF expression. A marked increase in BDNF expression was noticed in forward brain neurons after the administration of ampakines (CX614 and CX546) (27).

Neurotrophin 3 (NT-3). In experiments on rat, after sectioning and resuturating the sciatic nerve it was locally administrated NT-3. After 8 months it was concluded that:

- the gastrocnemius muscle significantly increased in weight
- the proportion and the diameter of rapid muscular fibers MHC2b was increased after sectioning the spinal chord at T8 in rat, were administrated NT-3, BDNF, NGF, CNTF. Applying NT-3 and not other neurotrophic factors, saved 50% of the neurons in the nucleus of Clarke, which otherwise die and vanish.

CNTF sustains the motor neurons survival and reduces the atrophy induced by skeletal muscle denervation. CNTF is a strong activator of astrocytes and is responsible for the installation of the persistent glial hypertrophy observed after lesions and diseases of the central nervous system (27).

HYPOTHERMIA

Lesions determined by craniocerebral trauma are aggravated by posttraumatic inflammatory processes. As a method to suppress the inflammatory reactions was proposed the hypothermia. If immediately after the neurotrauma is induced a whole-body hypothermia (32°C, 4h) it takes place an attenuation of the posttraumatic increase of interleukin-1 β (a cytokine determining the initiation of the inflammatory cascades) and a block in the increase of mRNA for NGF (27).

CONSIDERATIONS ON THE CLINICAL STUDIES INVOLVING NEUROPROTECTIVE AGENTS

The most of the clinical studies in stroke involving neuroprotective agents are destined to fail,

mainly because of problems starting in the pre-clinical development stage and continuing in the design phase or after.

THE PRECLINICAL PHASE

In the preclinical stage the therapeutic agents are often tested on young and healthy animals in strictly controlled laboratory conditions. The treatment is not tested adequately (for example by more investigators on different models of craniocerebral trauma and stroke) before included in clinical trials.

The laboratory animals are raised for genetic homogeneity – genetic differences and other factors like elderly and co-morbidities (arterial hypertension, diabetes mellitus) present in some patients may alter the therapeutic response. Despite similarities between basic stroke pathophysiology in several species, there are important structural, anatomical and functional differences in the cerebral vessels.

It has been raised the question if today's models of neurotrauma adequately mimic the human craniocerebral trauma. Many aspects of human craniocerebral trauma, either focal or diffuse, are reflected through different experimental models, but these models can not reproduce entirely the heterogeneous spectrum of craniocerebral trauma. Extrapolating the results obtained in animals to the clinical frame remains a problem (24).

THE CLINICAL TRIALS PHASE

Comparison between the management of stroke and craniocerebral trauma:

Stroke	Craniocerebral trauma
Type of stroke	Type of craniocerebral trauma
Severity of stroke	Severity of craniocerebral trauma
Identification of ischemic penumbra	
Time window for drug administration	Time window for drug administration
Combined therapy	Combined therapy
Dosing regimen	Dosing regimen
Statistical power	Statistical power
Study results	Study results

TYPE OF CRANIOCEREBRAL TRAUMA

The first trials included patient based on the Glasgow coma Score at admission, no matter the mechanism type.

The presence of some pathophysiological mechanisms in several patients should be the base for inclusion in a clinical trial evaluating compounds that interfere with certain pathways. In other words patients with diffuse axonal lesions and those with subdural hematoma should not be included in the same trial even if their Glasgow score at admission is the same.

In trials should be selected and included patients who may benefit more probably from the evaluated treatment.

THE SEVERITY OF CRANIOCEREBRAL TRAUMA

Mild and moderate craniocerebral trauma may be considered as being a completely different condition when compared with the severe craniocerebral trauma. Unsuccessful clinical trials during which the moderate neurotrauma subpopulation responded to treatment raises the question if clinical trials should not be focused more on the moderate or even mild craniocerebral trauma where the brain can still be saved. This population remains under-represented in clinical trials, but constitutes the majority of craniocerebral traumas around the world.

TIME WINDOW FOR DRUG ADMINISTRATION

Preclinical studies on stroke indicate the fact that most of the neuroprotective drugs are efficient when given 2-4 hours after the arterial occlusion. One of the major limitations in the failed clinical trials on stroke and neurotrauma is related to the time window for drug administration.

Because there are many similarities between the pathophysiological cascades, the most logical sequence for the neuroprotective therapies both in stroke and in craniocerebral trauma should be:

- anti-excitotoxicity
- anti-inflammation
- anti-apoptosis

That is why the best solution for neuroprotection is the administration of pleiotropic drugs that inhibit excitotoxicity, decrease the deleterious effect of inflammation (stimulating the positive effects) and does not inhibit the neuroplasticity offered in the second phase by the excitatory amino acids.

The only class of molecules capable to offer concomitant neuroprotection (by stopping the effects of the lesional cascades, pleiotropically targeting several mechanisms) and neuroplasticity (precocious

neurorepair) is represented by the neurotrophic factors and neurotrophic factor-like molecules.

That is why the drug Cerebrolysin has proven effective in stroke and neurotrauma trials. Cerebrolysin is the only product on the market containing neurotrophic factors active fragments.

COMBINED THERAPY

Taking into consideration the fact that many pathways leading to cellular death are activated during the evolution of cerebral ischemia and craniocerebral trauma, an efficient neuroprotection should combine drugs targeting different distinct pathophysiological pathways.

Unlike stroke, experimental and clinical trials on neurotrauma were focused on a single drug at a time, becoming obvious the fact that only one “magic bullet” can not improve the evolution in all neurotrauma subtypes (with complex pathophysiological cascades). It should be seriously taken into consideration the possibility of combined therapy, where multiple compounds are given concomitantly or sequentially.

Different neuroprotective combinations were used with some success on animal stroke models, in most cases NMDA receptors antagonists plus:

- GABA receptors agonists
- free radical scavengers
- cytidine-5'-diphosphocholine (Citicoline)
- proteic synthesis inhibitors (cyclohexamide)
- caspases inhibitors
- growth factors like basic fibroblast growth factor (bFGF)

- two different antioxidants
- cytidine-5'-diphosphocholine plus bFGF

DOSING REGIMEN

Concrete pharmacokinetics data from phase I trials should establish the maximal tolerable dose. Phase I studies or the first phase II studies should insure that therapeutic plasmatic concentrations may be reached within acceptable tolerance limits. Pharmacokinetics is considerably altered in craniocerebral trauma patients in comparison with the physiological situation.

In recent phase III studies the drug administration was based on specially elaborated phase II studies. More detailed pharmacokinetics studies are necessary to monitor the substance concentration in the cerebrospinal fluid and cerebral interstitial space. In this case microdialysis will probably become an important monitoring and diagnosis instrument.

STUDY RESULTS

Most of the studies performed had as main objective the evaluation of Glasgow score (favorable or unfavorable).

The use of this scale together with the hypothesis that in most studies a 10% increase of positive results is considered as sufficient and lead to the already known negative results.

Although being reported as negative, most of the trials showed in fact statistically insignificant evolution improvements, indicating that it was proved neither the efficacy nor the inefficacy of the tested products.

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