THE HEMATOPOIETIC GROWTH FACTORS – A NEW PERSPECTIVE IN THE NEUROPROTECTIVE THERAPY OF THE ISCHEMIC STROKE

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ABSTRACT

The hematopoietic growth factors (HGF) have been known for 20 years, named for their role in the proliferation, differentiation and survival of hematopoietic progenitor cells. They (erythropoietin, G-CSF and GM-CSF) have been used many years in clinical practice in oncological and hematological pathology. Recent studies suggest that HGF have also important non-hematopoietic functions in the central nervous system.
HGF and their receptors are expressed by neurons in many brain regions and are up-regulated after focal ischemia, indicating an autocrin protective response of the injured brain. The neuroprotective function of HGF has been suggested by the effect of decreasing infarct volumes in different experimental models in rodents and has been attributed to their anti-apoptotic activity (by activating several protective pathways, PI3K/Akt being the most important). Moreover, HGF induces neurogenesis and angiogenesis, possible the substrate of improving recovery post-stroke.
There is emerging data from recent studies suggesting that EPO, G-CSF and GM-CSF are potential new agents, a novel type of multifactorial drugs and candidates for neuroprotection in ischemic stroke. Here we discuss this new property of HGF behind the known function in hematopoietic system, summarize data from literature concerning HGF's actions in cerebral ischemia and highlight clinical implications.

Key words: hematopoietic growth factors, ischemic stroke, neuroprotection

Despite of the sustained efforts regarding the researches of the ischemic stroke etio-pathophisiology and of the significant improvement of medical care for the patients with this pathology in the last decades, the therapy of ischemic stroke is insufficient in the acute and chronic phase.

The only confirmed specific treatment is the thrombolysis with rt-PA, but because of the narrow time window of 3 hours and the potentially dangerous side effects represented by hemorrhagic complications, only 3-8% of all stroke patients receive rt-PA and the remaining 92-97% of patients receives no specific therapy.

The ischemic stroke is an undertreated pathology demanding the improvement of the existing therapy and research of new therapies.

The future of the stroke therapy will be represented by multimodal approach targeting the main key of the multiple mechanism involved simultaneously in and after of the development of stroke.

One of these modalities of therapeutic approach for the acute phase (saving the ischemic penumbra zone) and for the chronic phase too (regarding the functional rehabilitation) is the neuroprotective therapy, especially the exploitation of endogen neuroprotectiv systems (1-3).

A few therapy candidates for neuroprotection were studied, some of them passed the experimental phase on animal model, but the majority failed to show significant therapeutic benefit or demonstrated

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serious side effects, such are the growth factors: bFGF (brain-derived neurotrophic factor), IGF-1 (insulin-like growth factor), VEGF (vascular endothelial growth factor), glial cell line-derived neurotrophic factor.

Recently the attention of the neuroresearchers was turned toward another class of growth factors: the hematopoietic growth factors (HGF), of which the activity in the central nervous system was unknown until the last decade.

HGF stimulates and controls the proliferation, differentiation, maturation and survival of the hematopoietic lineage cells.

Recent studies demonstrated that the HGF have an important and unexpected role in the central nervous system, the activity of apoptosis inhibition (1,4,5) being analogous with the activity from hematopoietic system.

The first studied factor from this series was the erythropoietin (EPO, the critical modulator of the erythropoiesis), followed by granulocyte colony stimulating factor (G-CSF) and the lesser studied granulocyte-macrophage colony stimulating factor (GM-CSF), the last two being the counterpart of the EPO to the neutrophilic and monocytic lineage (1, 2, 6).

HGF and their receptors are not expressed only on the hematopoietic system cells. They are broadly expressed on a variety of cells type (neurons, glial cells, endothelial cells) in the rodent brain (demonstrated by immunohistochemistry): hippocampus (CA3 region), subgranular zone and hilus of the dentate gyrus, entorhinal cortex and olfactory bulb, pyramidal cells, Purkinje cells and cerebellar nuclei, brainstem nuclei.

Moreover, postmortem studies revealed the presence of HGM and their receptors in the human brain (frontal cortex) (3-15).

Recent researches on rodents revealed that acute focal cerebral ischemia (as acute cerebral lesion model) and the global cerebral ischemia induce an up-regulation of the HGF and their receptors in the brain.

Moreover, the neurons from the penumbra zone express in a high quantity both receptors and ligands, suggesting the existence of a protectiv autocrin mechanism in these neurons at risk (2, 4, 5, 9, 13, 16). Regarding G-CSF, was reported an increase more then 100-fold and 8-fold for his receptor in ischemic conditions.

This is the strongest regulation of any gene in cerebral ischemic events that has been reported, implicating an important adaptative response in neurons (1, 6, 9).

Based on this experimental findings is justified the hypothesis that supporting this endogenous system by exogenous administration of HGF would be followed by a robust neuroprotection.

This hypothesis was confirmed by studies in vitro (on neuroblastoma cells cultures) and in vivo by studies on different animal models of focal cerebral ischemia (middle cerebral artery occlusion combined or not with ipsilateral internal carotid artery occlusion, photothrombotic ischemia model).

A prerequisite for the direct action of HGF on the brain is the permeability of the blood-brain barrier (BBB) for these proteins (2, 5, 13, 17)

Studies with EPO in animal models of focal ischemic stroke had reported a reduction of the cerebral infarct volume until 75% and an increase of the survival rate after a month almost of 3-fold (18).

Minnerup&al published in 2008 a meta-analysis based on 13 studies regarding the efficacy of G-CSF on animal model (rodent), between parameters being the dosage, the therapeutic window and the outcome assessment by infarct size and sensori-motor deficit. G-CSF reduce considerably both parameters: the volume of the cerebral infarct by 42% (95% CI, 3% la 49%) and the sensori-motor deficit until 40% (95% CI, 9% la 61%), in function of the evaluation test used (6).

Schabitz&al demonstrated a decrease of cerebral infarct with 34% in the GM-CSF administration too (2).

In vitro, on rodent cortical neurons and on human neuroblastoma cells was highlighted that HGF counteracts the apoptosis camptothecin-induced and NO-induced and protect the neuronal cultures by the glutamate-induced excitotoxicity.

The anti-apoptotic effect of HGF is performed through 3 intracellular signaling pathways: ERK or MAPK (especially ERK 5) pathway, JAK2/STAT (STAT 5 for EPO and STAT 3 for G-CSF) pathway, Akt/ PI3K pathway (the most important anti-apoptotic pathway activated by these factors).

The result of the activation of this anti-apoptotic pathways is the inactivation of pro-apoptotic proteins (like BAD or caspase 9) and activation of anti-apoptotic gene transcription (like the BCL 2 family) (2, 4, 5, 8).

There are studies that suggest the existence of an underexplored pathogenetical mechanism in the ischemic stroke: a systemic immunodeficiency and HGF have immunomodulatory effects in this setting.

HGF has anti-inflammatory effects too, decreasing the release of pro-inflammatory cytokine in the reperfusion phase (19, 20).

Last year researchers shows that cortical lesions stimulate the proliferation of the neural progenitors cells (NPC) from the lateral ventricles wall.

NPC was revealed by immunofluorescence against doublecortin (DCX – a microtubule-associated protein which is specifically expressed by neural progenitor cells and immature neurons).

The development into new neurons was detected by colabeling of DCX with the mature neuronal marker NeuN and β III tubulin.

By the same method it was demonstrated the migration of NPC trough the ischemic zone in neocortex and a visible recruitment of NPC in the ischemic area.

The proliferation and migration of the NPC is enhanced by HGF administration (5, 8). Stimulating the hippocampal neurogenesis, HGF can enhance the structural repair after cerebral lesions providing a new therapeutic strategy for the patients with ischemic stroke in chronic phase (5, 21).

The angiogenetic effect, with new vessels formation, contributes to the reorganization of the infarcted brain with importance in rehabilitation on long term (6, 19, 22-24).

EPO and G-CSF were studied only in small clinical trials with improvement of NIHSS and Barthel index.

Certainly it is early for any conclusion regarding their efficacy in ischemic stroke but demonstrate for the first time the feasibility and safety of HGF in patients with stroke.

Other clinical trials, bigger and with more parameters (the volume of the ischemic lesions evaluated MRI, functional scores) are on going (AXIS and RAIS for the acute phase, STEMS for the chronic phase) (25-27).

As a conclusion, the neuroprotective function of HGF demonstrated in vitro and in vivo in the cerebral ischemia (the most studied cerebral injury) is based on a pleiotropic effect including anti-excitotoxicity, the anti-apoptotic effect, the anti-inflammatory effect, the stimulation of angiogenesis and neurogenesis.

The HGF are attractive candidates for the neuroprotectiv treatment of the ischemic stroke due their history of safe use in the hematological and oncological pathology, the capacity to penetrate the blood brain barrier and their multimodal activity, enhancing endogen neuroprotective mechanisms.

The future preclinical and clinical researches will define the profile of each of them and will select the most promising candidate for a further development in a protocol for the treatment of ischemic stroke.

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