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Webinar EVER Pharma

The renaissance of neuroprotection in times of recanalization therapies

November 30, 2021

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Webinar EVER Pharma (November 30, 2021)

The renaissance of neuroprotection in times of recanalization therapies

Participants

Zdravka Poljakovic

Head of the Neurological Intensive Care Unit, Medical School Zagreb, University Hospital Zagreb

Moderator

Michael Chopp

Henry Ford Hospital, Department of Neurology, Detroit and Oakland University, Department of Physics, Rochester, Michigan, USA

Topic: **Exosome mediated post-stroke vasotherapeutics with Cerebrolysin**

Jacek Staszewski

Military Institute of Medicine, Warsaw, Poland

Topic: **Neuroprotective agents – can they improve outcome in recanalization treatment?**

Dina Khasanova

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Michael Kalinin

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Topic: **Innovative therapies reducing complications after recanalization**

Order of the webinar

Introduction

Zdravka Poljakovic

We are currently living in the exciting times of expansion of recanalization therapies. At the same time, a few key elements of acute stroke care remain unaddressed, as are the needs of patients not eligible for recanalization therapies. Dr. Poljakovic thanked EVER Pharma for educational efforts focused on much-needed continuous improvement in current stroke care standards. In acute stroke, the cases of futile recanalization and reperfusion injury are a constant source of frustration among stroke specialists. The chronic states related to neurodegeneration triggered by ischemia are localized around the border areas of an injury, not directly connected to hypo-perfused tissue. They are suspect as directly involved in the development of cognitive impairment and even post-stroke dementia. From a global standpoint, there is an urgent need and expectation for neuroprotection to impact the epidemiological picture of stroke positively. The research is ongoing in the so-called distant pathology area, which, albeit not directly associated with the occluded blood vessel, appears to seriously impact the outcome in stroke patients. Neuroinflammation, synaptic alterations, and neuronal loss are the prominent contributing factors. The neuroprotective agents, like Cerebrolysin, can alter processes underlying these pathophysiological phenomena. Finally, we must appreciate that this list of unmet needs is not just an index of wishes formulated against the neuroprotection concept. We already have proof of efficacy that should encourage the more comprehensive application of neuroprotection in our daily clinical practice. Today's webinar aims to overview the evidence at hand and discuss its practical implications for stroke care. The invited speakers will share their knowledge of the topic and their direct experience using Cerebrolysin as an add-on to the recanalization standard in the group of moderate-to-severe stroke patients.

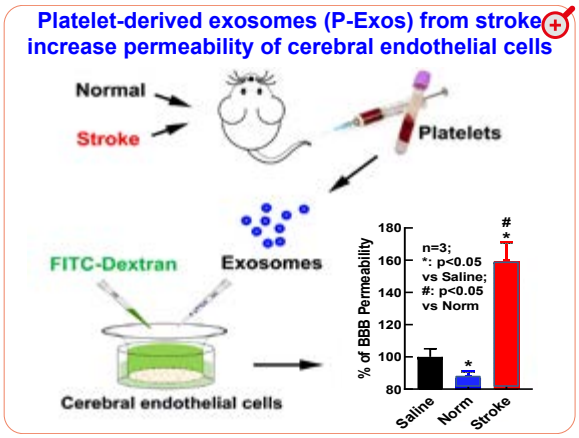
Exosome mediated post stroke vasotherapeutics with Cerebrolysin[1]

Michael Chopp

The question of why recanalization strategies in the clinic fail to deliver completely satisfying results (including inadequate tissue perfusion and incomplete recanalization) is of specific interest to Dr. Chopp. In particular, Dr. Chopp advocates vascular protection rather than neuroprotection as a therapeutic strategy to support and extend thrombectomy and thrombolysis. Stroke is a vascular disease that induces alterations in the brain's vasculature that play an essential role in developing the resulting complex neuropathology and functional deficits. The goal of Dr. Chopp's lecture was to explain how Cerebrolysin acts at the vascular level and the mechanism through which it exerts its therapeutic effects in stroke patients.

Dr. Chopp began by outlining the events happening after clot formation. Stroke triggers procoagulant, pro-inflammatory, and anti-fibrinolytic processes. First, the clot itself induces vascular damage in its direct vicinity. Downstream from the clot, within the brain's microvasculature, a cascade of pro-inflammatory and pro-coagulant events ensues, including fibrin deposition together with accumulation of inflammatory cells and platelets. This process, by itself, prevents complete reperfusion of the tissue. However, there is more to fibrin deposition than just creating a physical obstacle for blood circulation. Fibrin is toxic to the surrounding environment. It stimulates the production of pro-inflammatory and pro-thrombotic agents like PAI-1 (counteracting the effects of thrombolysis) and IRAK1 (an inflammatory cytokine). In a limited period after the initial event, the microvasculature becomes dysfunctional. In order to fully understand the remote effects of the blood clot to the downstream areas of the brain, one has to investigate the singling processes within the brain vasculature. These cell-to-cell communication mechanisms are subject to focused research conducted by Dr. Chopp's team at Henry Ford Medical System. Cells communicate with each other sending small packets of information called exosomes (also called small extracellular vesicles) into the bloodstream. These nanoparticles measure from 30nm to 150nm in diameter and contain varied numbers of proteins and nucleic acids (miRNAs and mRNAs). Among them, micro-RNAs (miRNAs) populations are potent physiological regulators. After a stroke, the clot produces and sends out exosomes that, to a great extent, determine the downstream ischemic events.

Dr. Chopp presented animal research and human-derived data substantiating this observation. In the in vitro study, animal-derived cerebral endothelial cells forming a layer representing the blood-brain barrier (BBB) were subject to the effects triggered during a stroke. The platelets taken from the clot of the laboratory animals after an induced ischemic stroke were used to isolate the exosomes, which were subsequently added to the BBB-forming cell cultures. The permeability of the BBB was assessed using a dye migration strategy. It increased significantly, suggesting that the clot-related changes in the composition of exosomes trigger the leakage of BBB. Additionally, and complementary to the observed effect at the level of BBB, the endothelial cells subject to post-stroke exosomes produced significantly increased amounts of pro-inflammatory and pro-coagulant cytokines, including ICAM1, TNFα, Tissue Factor, and PAI-1. Moreover, the concomitant reduction in the expression of the tight junctions proteins (occludin, ZO1, and claudin 5) occurred (**Fig. 1**).



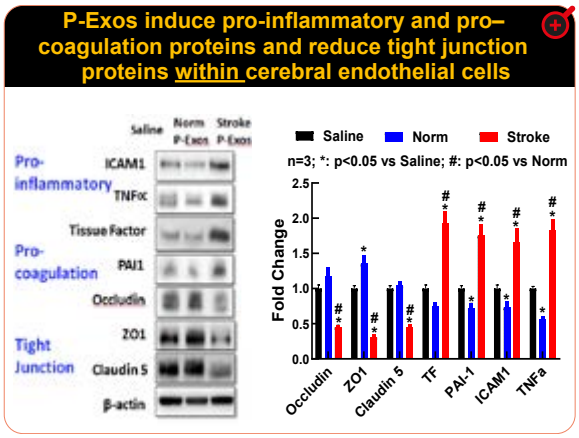
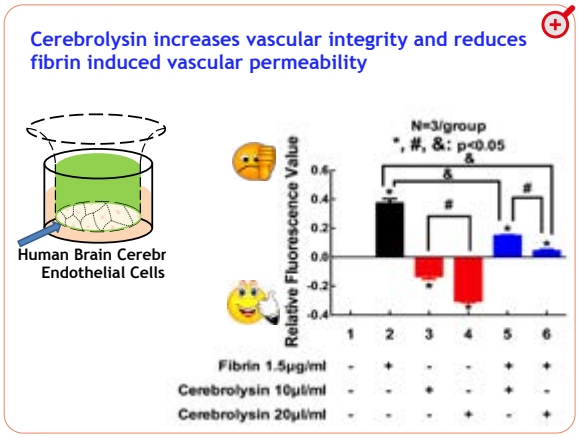


Fig. 1. The exosomes induced by the clot trigger processes underlying disruption of the BBB in an animal model of ischemic stroke.

Do these processes reflect the ischemic stroke pathophysiology observed in humans, asked Dr. Chopp? The exosomes derived from the clots removed from stroke patients were used for a similar BBB permeability experiment. In this case, the BBB formed with the human cerebral endothelial cells was subject to human-derived post-stroke exosomes. Again, and confirming the findings from the animal model, it appeared that the clot itself (via the exosomes) promoted the BBB leakage and the ensuing vascular damage. Interestingly, this pathological outcome was partially reversed by adding exosomes derived from the normal (non-ischemic) human endothelial cells. Next, Dr. Chopp wanted to investigate if Cerebrolysin (an agent known for its neuroprotective properties) can similarly protect the BBB. First, using his human cells-based BBB model again, Dr. Chopp and coworkers showed that Cerebrolysin reversed the BBB leakage induced by fibrin deposition. The mechanism through which Cerebrolysin protected the BBB seemed to reflect the Cerebrolysin-dependent reduction of pro-inflammatory and pro-coagulant cytokines produced by the endothelial cells exposed to fibrin (**Fig. 2**).



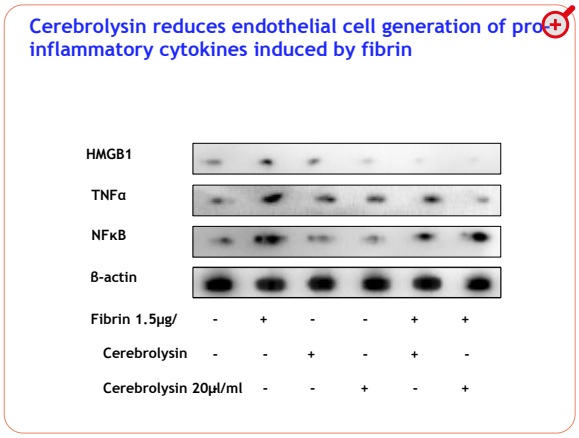


Fig. 2. Cerebrolysin prevents BBB leakage through reversing pro-inflammatory and pro-coagulant processes triggered by fibrin deposition in an experimental human cells-based model.

Summarising these experiments, Dr. Chopp stated that Cerebrolysin appears to be capable of reversing the vascular damage processes triggered by a stroke and protecting the vasculature from secondary ischemic damage. In another iteration of his BBB permeability experiment, Dr. Chopp showed that exosomes derived from human cerebral endothelial cells treated with Cerebrolysin could significantly reverse BBB leakage caused by the fibrin challenge. This protective effect was significantly more robust than the already significant protective effect recorded using primary exosomes derived from the normal, non-ischemic human endothelial cells. Accordingly, the Cerebrolysin-induced exosomes were able to reduce fibrin-promoted inflammatory cytokines while, at the same time, increasing the levels of tight junction proteins (**Fig. 3**).

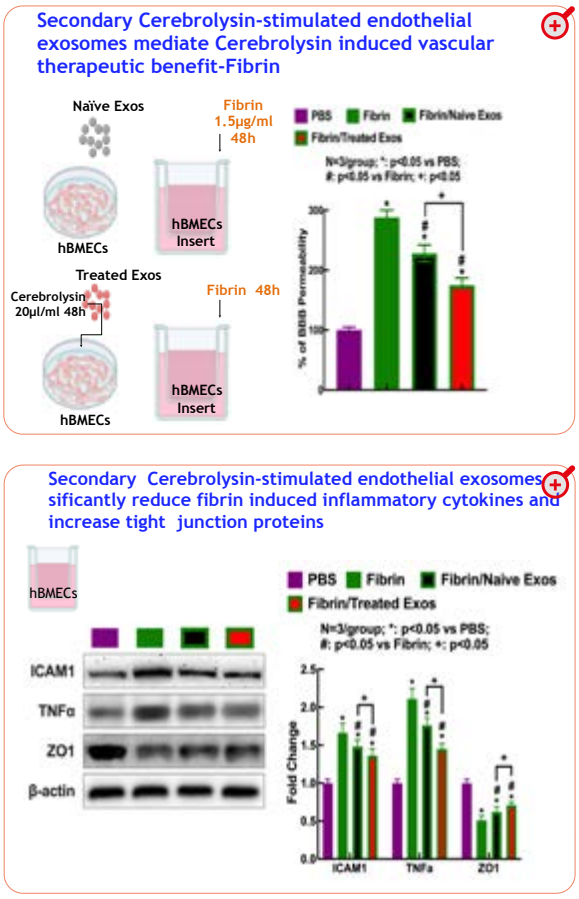


Fig. 3. Cerebrolysin reduces BBB leakage and vascular damage processes through exosomes-dependent singling pathway.

In another set of experiments, Dr. Chopp's team demonstrated that exosomes derived from the normal (non-ischemic) endothelial cells could reduce the infarct volume and improve functional outcomes in the animal model of the ischemic stroke. Likewise, using these exosomes as an add-on treatment to thrombolysis significantly reduced the infarct volume and improved the functional outcomes in the experimental animals undergoing recanalization (**Fig. 4**).

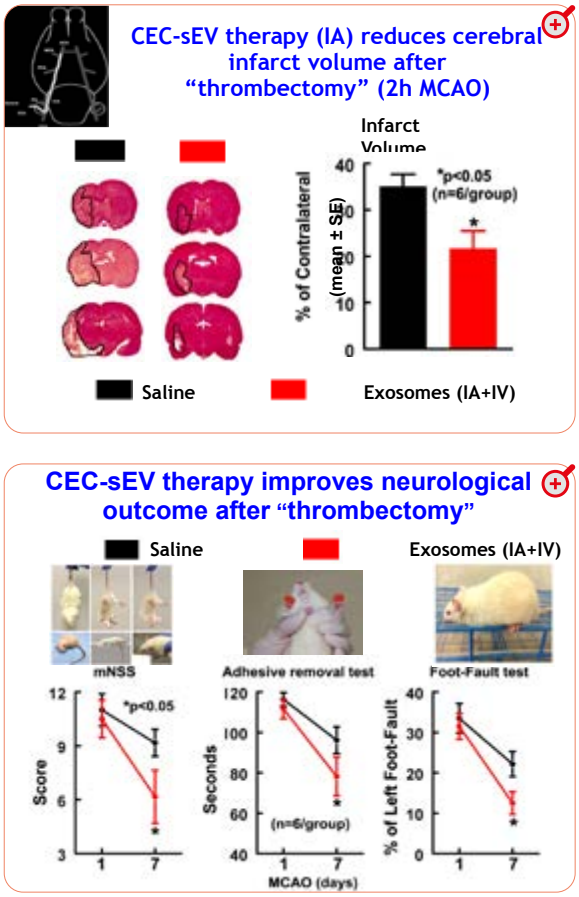


Fig. 4. The non-ischemic exosomes exert therapeutic effects in an in vivo animal model of ischemic stroke and thrombectomy.

Wrapping up his lecture, Dr. Chopp indicated that that the evidence presented here sheds light on the mechanisms by which Cerebrolysin develops its therapeutic effects in stroke patients. Cerebrolysin appears to reduce the BBB permeability and pro-inflammatory and pro-thrombotic protein expression that are increased in response to fibrin deposition after ischemic stroke. It also protects and restores endothelial cells function. Furthermore, Cerebrolysin mediates its therapeutic effects by stimulating the endothelial cells to generate secondary exosomes. These Cerebrolysin-induced exosomes are therapeutic and appear to be directly responsible for the pharmacological effects discussed in this presentation.

In conclusion, the experimental evidence gathered to date suggests that Cerebrolysin appears to be the justified add-on treatment option to recanalization therapies for further improving their related therapeutic benefits. Answering the question from Dr. Poljakovic, Dr. Chopp stated that the evidence gives us a strong rationale for using Cerebrolysin in various types of stroke, including subarachnoid hemorrhage (SAH). In addition, using it in various therapeutic windows, including in ambulance/pre-hospital administration, combination with thrombectomy and thrombolysis, and post-acute, longer-term treatment are all viable options.

Selected literature

1. All presented here results are the own data of Dr. Michael Chopp (published and unpublished)

Neuroprotective agents – can they improve outcome in recanalization treatment?

Jacek Staszewski

Dr. Staszewski expressed his conviction that we are currently witnessing a new era of stroke treatment in which supportive strategies can be re-approached for the benefit of stroke patients. However, just like Dr. Chopp argued for vascular protection instead of the neuroprotection concept, Dr. Staszewski pointed out that even a more comprehensive concept of cytoprotection should also be considered as an optimal adjunctive treatment approach in stroke. Dr. Staszewski gave an overview of the EVT (endovascular thrombectomy) standard as the perfect clinical backdrop for developing and effectively using cytoprotective (vasoprotective, neuroprotective) strategies in stroke patients. He also overviewed current development in the clinical research of neuroprotection as an add-on to the gold standard in stroke care: recanalization therapies and stroke rehabilitation.

During the last decade, the therapeutic landscape in stroke has changed significantly. Since 2016, when the results of five clinical trials assessing the efficacy and safety of EVT were published, we have a new effective tool in hands. The number needed to treat for the benefit (NNT) 3-7 for functional independence and NNT 2-3 for reducing disability by 1 point in mRS scale are the apparent signs of progress.[1] However, several recanalization-related issues remain unsolved. Among them is the timing of the treatment and the fact that substantial numbers of patients after early recanalization still deteriorate, experiencing symptomatic intracranial hemorrhages or distal re-occlusions. As the most up-to-date analyses show, we cannot achieve a success rate of above around 50% in EVT-treated patients. The total intracranial hemorrhage (ICH) frequency among EVT patients reaches 36.5% (COMPLETE Registry; patients treated in the longer time window and with the lower ASPECT scores).[2] ICHs have a complex nature and can impact the decision-making process for secondary stroke prevention. Apart from that, asymptomatic hemorrhages influence the 90-day functional endpoints, decreasing the likelihood of a positive outcome in affected patients. We suspect the involvement of several pathological mechanisms, such as neurotoxic effects related to blood cell breakdown and epiphenomenon secondary to lack of reperfusion or blood-brain barrier (BBB) dysfunction. However, we are probably not fully aware of the whole spectrum of associated pathological events as the currently used scales may lack the required sensitivity to detect all related complications. Therefore, it is a reasonable assumption that the pharmacological management targeting asymptomatic ICH as a primary endpoint might offer a valuable contribution to our best clinical standard (**Fig. 1**).

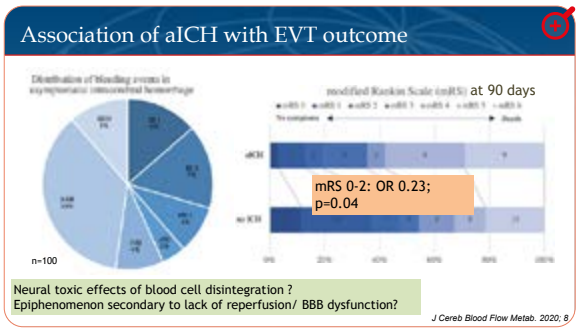


Fig. 1. Intracranial hemorrhage (ICH) as a primary endpoint for testing novel therapeutic strategies in stroke.

The measure of clinical success in stroke always relates to successful recanalization together with successful reperfusion. Unfortunately, only 50-80% of patients after EVT achieve good reperfusion. Dr. Staszewski indicated that about 30-40% of EVT patients have futile recanalization

(successful EVT without reperfusion) or experience the reperfusion injury (or both). These issues are probably closely related to the BBB dysfunction. Experimental studies show that, apart from the increased BBB permeability, the most prominent adverse effects associated with reperfusion are a production of ROS (reactive oxygen species), apoptosis, pro-inflammatory state, thrombosis, reactive hyperemia, loss of VMR (vasomotor reactivity), and cytotoxic edema. These reperfusion-related phenomena can lead to altered microvascular circulation (no-reflow) and hemorrhagic transformation. In stroke care, we respond to these challenges with supportive management (maintenance of cerebral perfusion pressure >70mmHg, normovolemia, normotension, avoidance of hypoglycemia). However, there is still a need for a complementary supportive pharmacological treatment: the hyperacute management of ischemic stroke should go beyond the recanalization therapy alone. In the past, the functional assessment of BBB was impossible in humans; we relied solely on animal models. However, recent developments in imaging modalities (MR, CT) changed that situation. The post-contrast flare imaging visualizes contrast extravasation through a damaged BBB. In Dr. Staszewski's opinion, the HARM (Hyperintense Acute Reperfusion Marker) may serve as a secondary injury endpoint in studies investigating adjunctive cytoprotective therapies (**Fig. 2**).

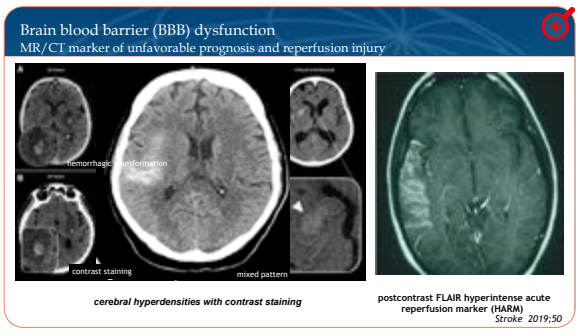


Fig. 2. The HARM - an effective marker of BBB disfunction in the ischemic stroke patients.

Another important consideration for developing future supportive therapies in EVT patients remains the timing of the treatment. Reversible penumbra can be found within 6 hours post-stroke and sometimes even in a broader time window. However, while time is essential, it is not the only factor. The individual predisposition for a successful recovery of the penumbral tissue is often paramount. The penumbral tissue loss and infarct growth are probably a function of collateral circulation, as a lack of collaterals was associated with "progressive stroke" and aICH. Accordingly, the EVT patients can be divided into fast and slow progressors, with rapid and slow infarct growth, respectively. From this point of view, time appears to be the secondary, not the primary factor. About 50% of stroke patients belong to the slow progressors category, characterized by good collaterals. We know that deterioration of collaterals in fast progressors is associated with factors such as BP variability, hypotension, hypovolemia, hypocapnia, general anesthesia, edema, and fever. Therefore, the vascular protection drugs that could facilitate the collateral flow would be a critical element of any remediation strategy in the fast progressing stroke. (**Fig. 3**).

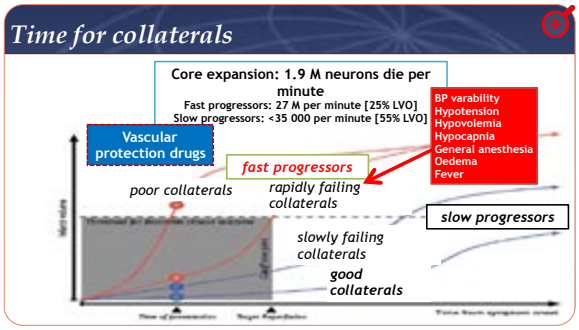


Fig. 3. The relationship of time and collaterals defines the treatment window for neuroprotection.

Dr. Staszewski underlined that the timely performed EVT alone does not guarantee an excellent short-term and long-term outcome. The Mr. CLEAN extended follow-up study demonstrated that the most valuable predictor of 1-2 years outcome in EVT patients is the 3-month status. This period constitutes the general opportunity window for improved care, rehabilitation, and supportive cytoprotective strategies.

Summarizing the current state-of-the-art in reperfusion after stroke, Dr. Staszewski implied that we need more treatment opportunities to

improve the short- and long-term outcomes. There is a need for effective cytoprotective strategies to counteract pathological mechanisms induced by ischemia, like neuronal death, endothelial damage, and BBB leakage. At the same time, we have to support the endogenous repair mechanisms initiated very early post-stroke. Effective supportive treatment for EVT should employ a multimodal agent that works at the level of general cytoprotection within the complex neurovascular unit and the level of neurorestoration enhancing neuronal activity in the peri-infarct regions and the intact hemisphere.

Why is then EVT a perfect clinical setup for assessing the effectivity of cytoprotective adjunctive therapies? First, EVT defines the optimal therapeutic time window. Second, it works in the presence of penumbra and (in some patients) collaterals. Third, it allows for effective drug delivery through successful reperfusion (or at least recanalization). Finally, effective recanalization due to EVT represents a human model of transient ischemia that can be investigated within a homogenous stroke population. In such a population, the treatment effect size can be maximized.

Dr. Staszewski went on to overview current developments in this field. The first effort to combine EVT with a neuroprotective strategy was recently published. In a trial assessing the efficacy and safety of Nerinetide (inhibitor of excitotoxicity) as an add-on to EVT, the results showed no improvements in the functional outcome as measured with mRS in the whole study population. However, it suggested benefits in patients who did not undergo thrombolysis: reduction in mortality by 7.5% and an 18% increase in the chance of achieving an mRS score of 0-2. Unfortunately, the nullifying effect of the alteplase on the efficacy of Nerinetide appears to limit its practical clinical usage.[3]

Cerebrolysin is known to possess broad cytoprotective properties. It also enhances neurogenesis, inhibits apoptotic processes, and reduces neuroinflammation (**Fig. 4**).

These mechanisms were confirmed in experimental studies and acute and subacute stroke in humans. The PubMed research conducted by Dr. Staszewski delivered >500 reports on

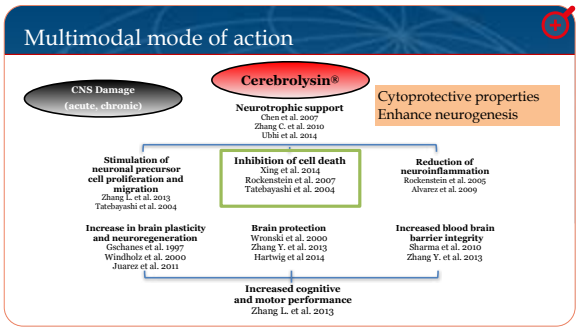


Fig. 4. Cerebrolysin is a multimodal agent with well established neuroprotective properties.

Cerebrolysin, with many of them published in recent years. Dr. Staszewski presented te results of clinical trials in stroke investigating Cerebrolysin as an add-on to the current gold standard in recanalization and rehabilitation.

Dr. Poljakovic and her team from Zagreb Medical University tested the hypothesis that Cerebrolysin (given up to 24 hours post-stroke for 14-21 days) improves the outcome in severe stroke patients (NIHSS≥8) after futile recanalization (with tPA or with tPA plus thrombectomy).[4] This pilot trial included a small group of patients (n=44). It demonstrated a trend toward better outcome at seven days (measured with NIHSS) and a trend toward better outcome after one year as measured with mRS. However, the most important discovery related to Cerebrolysin was preventing the hemorrhagic transformation after the futile recanalization. Furthermore, this result correlated with a significant reduction in mortality after one year (**Fig. 5**).

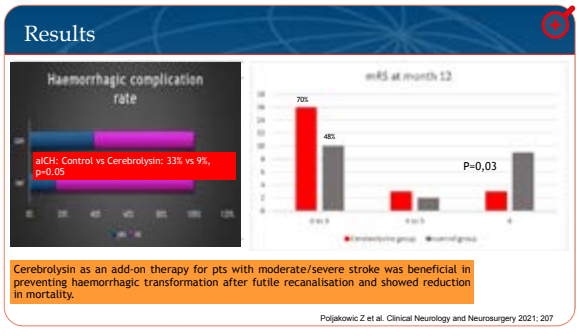


Fig. 5. Cerebrolysin prevents hemorrhagic transformation and increases survival rate after futile recanalization in stroke patients.

Another study published in 2013 by the LYSE Study group tested the hypothesis that Cerebrolysin is effective in patients when administered immediately after rtPA. One hundred nineteen patients received this therapy versus placebo. The study demonstrated a faster recovery in patients receiving Cerebrolysin than patients receiving placebo as an add-on to thrombolysis (measured with NIHSS and mRS).[5] The most noticeable results were evident in the more severely affected stroke patients (**Fig. 6**).

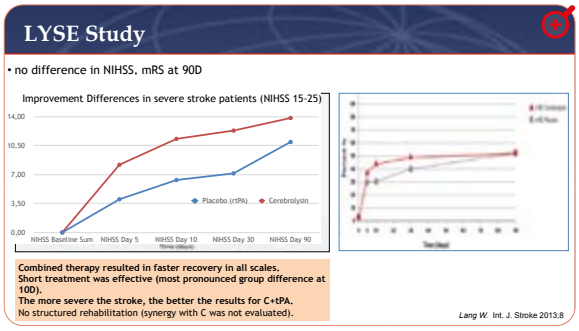


Fig. 6. Cerebrolysin supports faster recovery of stroke patients undergoing thrombolysis.

Recently, Dr. Staszewski and coworkers from the Military Institute of Medicine in Warsaw initiated a trial that tests a hypothesis that adding Cerebrolysin in selected patients (baseline small ischemic core, good collateral status, significant reperfusion following EVT, but persisting significant deficit with cortical signs) may increase the effectiveness of EVT by initiating cytoprotective effects and preventing reperfusion injury and delayed cell death. Cerebrolysin was administered in combination with EVT for 21 days and then rehabilitation in the post-acute period. Currently, 15 patients have been enrolled in this ongoing study. The results gathered from 10 patients indicate a trend toward improvement in NIHSS for the Cerebrolysin group compared to the placebo group, with no discernible effect in mRS scores. Interestingly, already at this early stage of the trial, the striking effect of Cerebrolysin on reduction of secondary ICH at 24 hours (30% reduction with Cerebrolysin vs. 0% reduction with placebo) was recorded (**Fig. 7**).

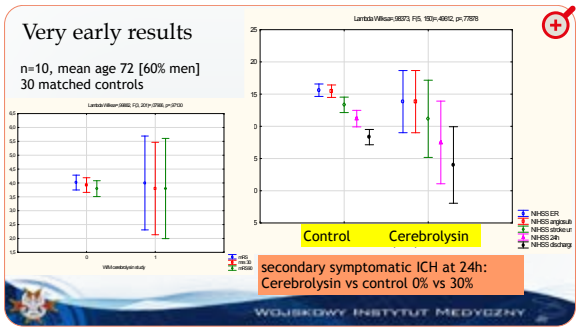
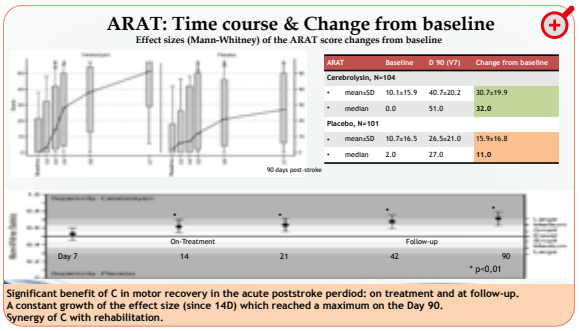


Fig. 7. The yet unpublished results of a trial performed at Military Institute of Medicine (Warsaw), investigating Cerebrolysin as an add-on to EVT, confirm its role in preventing hemorrhagic transformation.

In a pre-hospital treatment setup, another study in Thailand investigates Cerebrolysin in patients with suspected stroke. As shown by the FAST-MAG trial a few years ago, we already know that this treatment strategy is feasible. It would be interesting to see if, unlike magnesium, Cerebrolysin can support very early treatment of stroke patients, including its potential impact on the effectiveness and safety of recanalization therapies.

It is of utmost importance for all stroke patients, including those with unfavorable outcomes after EVT, to receive effective rehabilitation. The vital trial was published in Stroke in 2016 (CARS trial). It tested the hypothesis that Cerebrolysin given 24-72 hours post-stroke for 21 days, as an add-on to standardized rehabilitation, will benefit patients with severe stroke recovering from motor deficits of the upper limb. The efficacy of the treatment was tested with ARAT, which is a sensitive tool for detecting differences in arm movement. This trial demonstrated the benefit of Cerebrolysin for motor recovery in the acute and post-acute stroke period. Furthermore, the trial reported a constant growth of the beneficial effect size up to the day 90 observation point. Notably, the synergy between the pharmacological treatment with Cerebrolysin and the structured rehabilitation program was evident (**Fig. 8**).



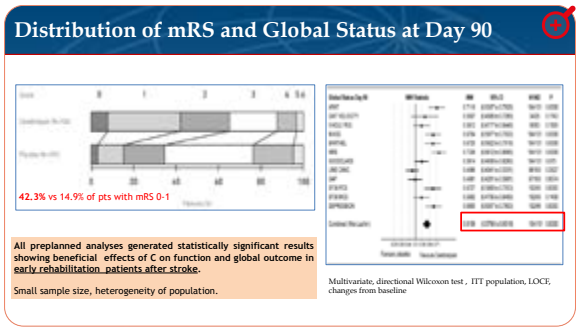


Fig. 8. The CARS trial evidenced the synergy between the pharmacological action of Cerebrolysin and structured motor rehabilitation.

The follow-up trial, the CARS-2, performed in a less severe stroke population (NIHSS 6.8 vs. 9.2 in CARS), did not demonstrate the beneficial effect of Cerebrolysin, probably due to the ceiling effect in the placebo group. Nevertheless, the importance of this follow-up study was proven in the meta-analysis of both CARS trials.[6] It confirmed the efficacy of Cerebrolysin in motor function rehabilitation after 14, 21, and 90 days as measured with NIHSS and after 90 days as measured with ARAT. Furthermore, all patients benefited from the treatment, with the most pronounced effect observed in the more severely affected stroke population.

Another trial assessing the efficacy of Cerebrolysin in combination with rehabilitation after stroke was published in BMC Neurology by a group of Dr. Kim from Seul, Korea.[7] It tested the hypothesis that adding Cerebrolysin to motor rehabilitation would benefit stroke patients as measured with functional MRI, apart from the Fugl-Meyer Assessment for the motor function recovery. The results demonstrated that the symmetric functional connectivity index of the brain's hemispheres was more pronounced in the patients treated with Cerebrolysin, indicating improved recovery of motor functions (**Fig. 9**).

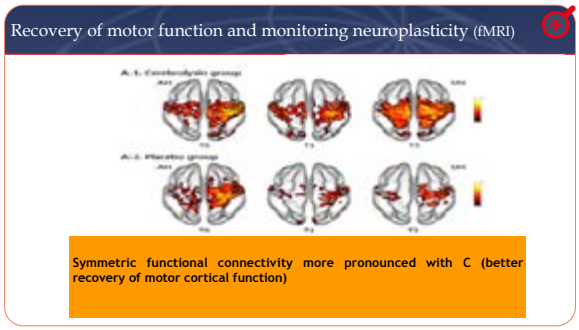


Fig. 9. Cerebrolysin induces changes in the sensorimotor network of stroke patients that lead to increased symmetric functional connectivity between the bilateral primary sensorimotor cortices.[7]

These trials were essential, underlined Dr. Staszewski, as they demonstrated that Cerebrolysin given in a post-acute period would positively impact the motor performance in stroke patients. The European Academy of Neurology analyzed the results. It issued a recommendation for using Cerebrolysin in post-acute stroke, especially in the treatment of moderate-to-severe stroke patients (Grade 2A, level of evidence A). Similar recommendations have been issued by the Canadian ERABI group, the German Society for Neurorehabilitation, and the Polish Society of Neurology.[8-11]

Summarizing his extensive overview of Cerebrolysin clinical data, Dr. Staszewski underlined that Cerebrolysin appears to be an attractive pharmacological candidate for use in acute and post-acute stroke periods. Its manifold effects on the nervous system are well documented in pre-clinical research. The reported results suggest cytoprotective and neurorestorative activity of Cerebrolysin that may enhance the beneficial effects of reperfusion and rehabilitation strategies. Combining multimodal treatments (like Cerebrolysin) with reperfusion and rehabilitation as a complementary, supportive strategy could have additive, if not synergistic, clinical effects. Stroke is the only neurological disorder amenable to complete reversal of disabling deficits. To achieve this goal, we should observe, critically evaluate, and improve the milestones of stroke care: reperfusion, early rehabilitation, secondary prevention, and neuro-(or cyto)-protection.

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Innovative therapies reducing complications after recanalization

Dina Khasanova, Michael Kalinin

Dr. Khasanova and Dr. Kalinin devoted their lecture to discussing strategies for preventing hemorrhagic transformation, a frequent complication after reperfusion therapies. This topic is increasingly relevant in light of current trends to expand the inclusion criteria for these life-saving procedures. Additionally, the increasing number of stroke patients justifies an ongoing reassessment in this field.

Recently, there was a paradigm shift in reperfusion therapy. Time is no longer considered a decisive limiting factor for effective reperfusion as it used to be. Several studies showed that the traditional window of 4.5 hours post-stroke can be extended with the help of modern imaging modalities. At the same time, the prevention of reperfusion injuries became a primary focus of modern stroke care. The ideal approach to stroke management should encompass several vital elements: reperfusion strategies directed at arterial recanalization, inhibition or modulation of inflammatory processes, prevention and treatment of complications, and cytoprotective strategies aimed at cellular and metabolic targets (**Fig. 1**).

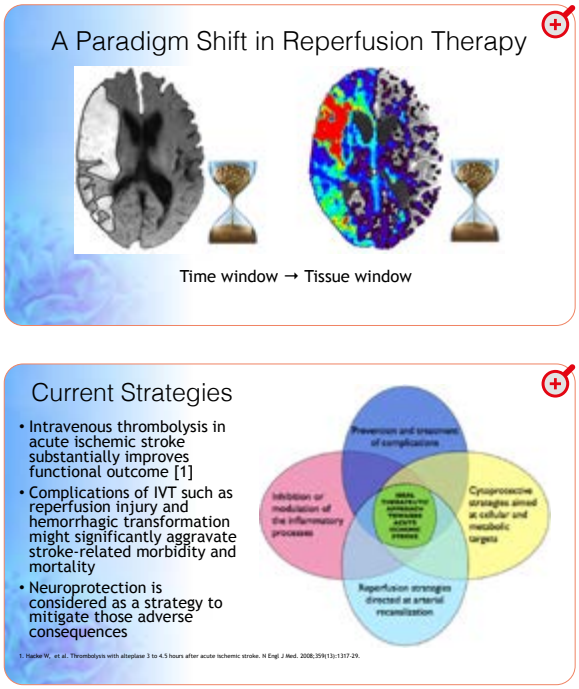


Fig. 1. A paradigm shift in reperfusion therapy requires complementary supporting strategies.

Preventing hemorrhagic transformation (HT) requires an understanding of its pathophysiology. The underlying processes involve ischemic injury, reperfusion injury, neuroinflammation, vascular remodeling, and direct rtPA toxicity, which negatively affect the BBB integrity. The hemorrhagic transformation is a consequence of this pathological development. Several studies explored simultaneous use of rtPA and various agents, which can potentially reduce hemorrhagic transformation and exert additional neuroprotective effects. The conclusions arising from these efforts indicated that therapies directed at single targets isolated from the complex pathophysiological milieu of stroke are insufficient to achieve desired clinical results. Therefore, the focus of research shifted to cell therapies and pleiotropic agents, which might have a broader impact on hemorrhagic transformation mechanisms. Several experimental stroke models, including those published by Dr. Michael Chopp's research team, were used to investigate the multimodal agent Cerebrolysin. They demonstrated the neuroprotective effect of Cerebrolysin, including its ability to protect BBB against ischemic injury. Additionally, several clinical trials and meta-analyses have suggested that Cerebrolysin (also in combination with rtPA) decreases neurological deficit after stroke (**Fig. 2**).[1-2]

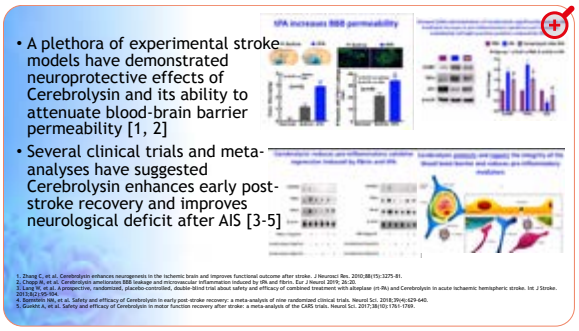


Fig. 2. The experimental evidence as well as clinical trials data point to Cerebrolysin as an effective adjunct treatment in stroke.

This scientific and clinical background gave the impulse to design a new trial investigating the impact of Cerebrolysin on hemorrhagic transformation at the Kazan State Medical University. The rationale for the study stated that rtPA increases the hemorrhagic transformation by degrading the BBB integrity and promoting neuroinflammation and excitotoxicity. At the same time, Cerebrolysin was shown to ameliorate rtPA-related adverse effects and, therefore, can potentially prevent rtPA-induced HT. "Cerebrolysin as an Early Add-on to Reperfusion Therapy: Risk of Hemorrhagic Transformation after Ischemic Stroke (CEREHETIS)" is a pilot, randomized, open-label, active control, multicenter, parallel-group study that enrolled patients across 8 Russian centers. The study's primary goal was to assess the HT rate, drug safety, and functional outcome after an early Cerebrolysin add-on treatment to reperfusion therapy in AIS patients. The inclusion criteria for the study were similar to those defined for the thrombolysis trials. Three hundred forty-one patients were included, and Cerebrolysin was administered for 14 days (once daily), starting concomitantly with rtPA, via the separate infusion line (30 ml for 20 min). Both groups received standard rtPA treatment within 4.5 hours from the onset of the symptoms (**Fig. 3**).

The primary endpoints were any and symptomatic HT verified on a follow-up CT scan, while the secondary endpoints included the functional outcome and the drug safety. In selected patients, the extensive advanced brain imaging analysis was conducted as a marker for in vivo assessment of the pharmacological impact of Cerebrolysin. On days 1 and 14, a routine brain MRI was acquired and followed by an axial diffusion tensor imaging scan. On day 14, a brain CT perfusion scan was also obtained. The DTI and CTP data were further processed: the infarcted area was outlined and mirrored to the contralateral hemisphere, and the values of fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and permeability surface-area product (PS) were assessed within each region of interest. These methods allowed for a surrogate in vivo assessment of the axonal and myelin damage and the assessment of treatment-related changes in the overall brain micro-architecture. Notably, PS is a known marker of BBB permeability, and its increase correlates directly with hemorrhagic transformation.

Three hundred eighteen patients constituted the dataset for per-protocol analysis. For the primary endpoint, the Cerebrolysin group showed statistically significant improvement compared to the rtPA-alone group. Cerebrolysin significantly reduced any HT with a rate of 13.7% versus 22.9% in the control group and a corresponding OR of 0.417 (95% CI: 0.200-0.871; p = 0.032). In category of symptomatic HT, Cerebrolysin treatment resulted in a significant reduction of this post-stroke complication (2.6% compared to 9.0%) with an OR of 0.171 (95% CI: 0.040-0.726; p = 0.022). The NNT for treatment benefit was 10.86 (95% CI, 5.50-420.68) and 15.65 (95% CI, 8.33-129.10), re-

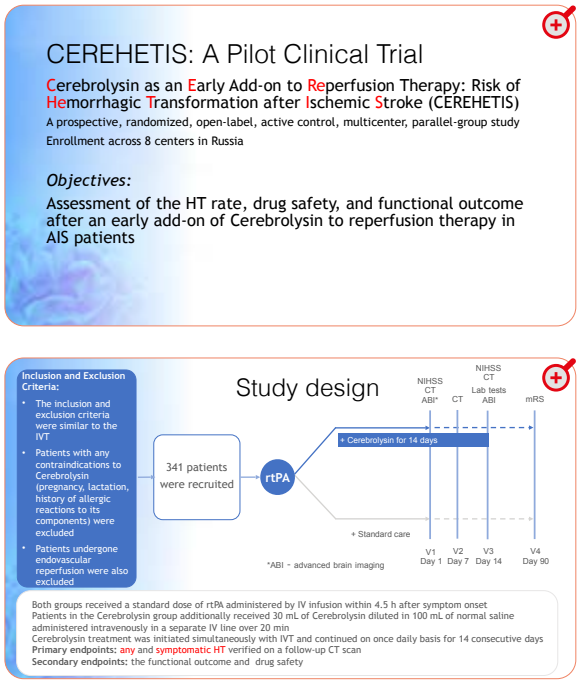
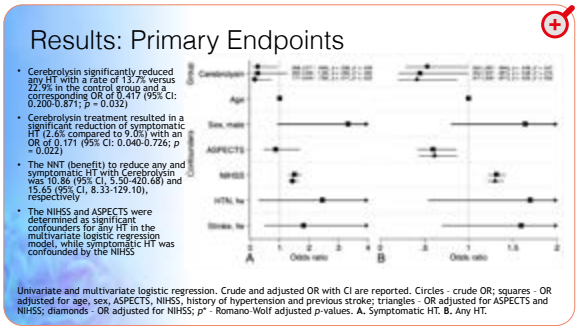
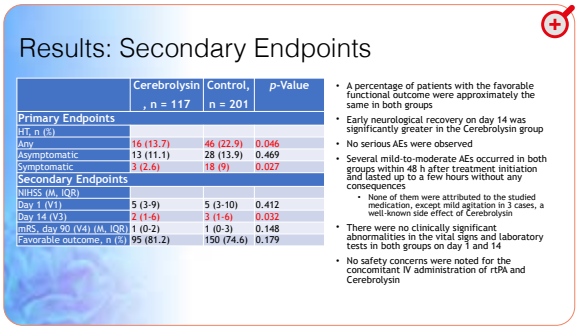


Fig. 3. The CEREHETIS trial investigating Cerebrolysin as add-on to reperfusion therapy.[3]

spectively. Concerning the secondary endpoints, while a percentage of patients with the favorable functional outcome were approximately the same in both groups, the early neurological recovery on day 14 was significantly greater in the Cerebrolysin group. This result confirmed the size of the effect reported earlier in a recent meta-analysis. For the Cerebrolysin group, a strong positive trend toward improved functional outcome on day 90 (mRS) for both ITT and PP populations was also reported (**Fig. 4**).





	Cerebrolysin, n = 117	Control, n = 201	p-Value
Primary Endpoints			
HT, n (%)			
Any	16 (13.7)	46 (22.9)	0.046
Asymptomatic	13 (11.1)	28 (13.9)	0.469
Symptomatic	3 (2.6)	18 (9)	0.027
Secondary Endpoints			
NIHSS (M, IQR)			
Day 1 (V1)	5 (3-9)	5 (3-10)	0.412
Day 14 (V3)	2 (1-6)	3 (1-6)	0.032
mRS, day 90 (V4) (M, IQR)	1 (0-2)	1 (0-3)	0.148
Favorable outcome, n (%)	95 (81.2)	150 (74.6)	0.179

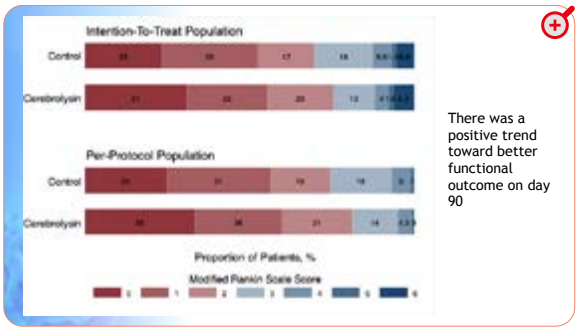


Fig. 4. The results of CEREHETIS trial - the primary and secondary endpoints.[3]

No differences in DTI metrics and the infarct volume between compared groups were detected at day 1. However, patients treated with Cerebrolysin showed significant improvement at day 14. The two-week treatment course with Cerebrolysin reduced the BBB permeability and CT-derived infarct volume by more than 1.5-fold. In addition, no safety issues were reported confirming the favorable safety profile of Cerebrolysin in this patient population (**Fig. 5**).

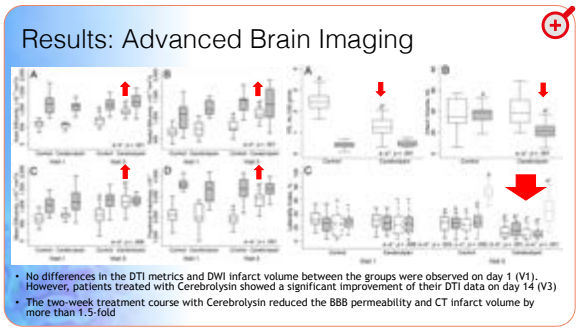


Fig. 5. The brain imaging analysis indicated reduced BBB permeability and reduced size of infarct volume in the Cerebrolysin group.[3]

Dr. Kalinin went on to present exemplary clinical cases representing the study population. As seen on imaging analysis at day 14, the BBB permeability returned to normal levels in the Cerebrolysin treated patient while remaining abnormally high in the patient from the control group, even though both patients presented with very similar topography and size of the infarct. This result suggests BBB stabilizing properties of Cerebrolysin. In addition, significant improvements in other measured DTI parameters were also detected in the Cerebrolysin treated patients on Day 14. The observed changes indicate a probable neuroprotective effect of Cerebrolysin in these patients (**Fig. 6**).

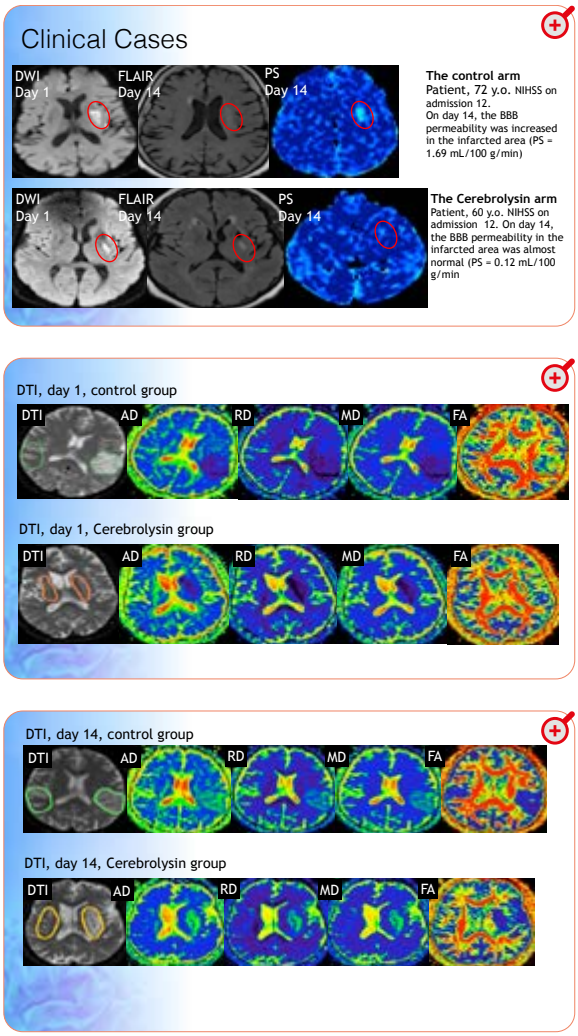


Fig. 6. The exemplary imaging analyses of patients included in the CEREHETIS trial.[3]

Concluding their lecture, Dr. Kalinin indicated that early add-on treatment with Cerebrolysin was safe and significantly decreased the rate of hemorrhagic transformation and early neurological deficit after reperfusion therapy. In addition, a positive trend was detected toward better functional outcomes on day 90. Together with the imaging metrics of the infarcted area, these results suggest clinical benefits that could be attributed to the neuroprotective properties and the blood-brain barrier stabilizing activity of Cerebrolysin. Dr. Kalinin indicated that the limitations of the presented study included a short course (14 days) of Cerebrolysin treatment and a relatively short follow-up period (90 days). A separate study is needed to establish the optimal treatment duration in this patient population.

Selected literature

1. Bornstein NM, et al., Neurol Sci. 2018; 39(4):629-640
2. Guekht A, et al., Neurol Sci. 2017;38(10):1761-1769
3. Unpublished results

ABBREVIATED PRESCRIBING INFORMATION. Name of the medicinal product: Cerebrolysin - Solution for injection. Qualitative and quantitative composition: One ml contains 215.2 mg of Cerebrolysin concentrate in aqueous solution. List of excipients: Sodium hydroxide and water for injection. Therapeutic indications: For treatment of cerebrovascular disorders. Especially in the following indications: Senile dementia of Alzheimer's type. Vascular dementia. Stroke. Craniocerebral trauma (commotio and contusio). Contraindications: Hypersensitivity to one of the components of the drug, epilepsy, severe renal impairment. Marketing Authorisation Holder: EVER Neuro Pharma GmbH, A-4866 Unterach. Only available on prescription and in pharmacies. More information about pharmaceutical form, posology and method of administration, special warnings and precautions for use, interaction with other medicinal products and other forms of interaction, fertility, pregnancy and lactation, effects on ability to drive and use machines, undesirable effects, overdose, pharmacodynamics properties, pharmacokinetic properties, preclinical safety data, incompatibilities, shelf life, special precautions for storage, nature and contents of the container and special precautions for disposal is available in the summary of product characteristics.

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