Mode of Action
Booklet to video
Figure 1:
Cerebrolysin® reaches its place of action
Introduction

Cerebrolysin® reaches its place of action

When a brain injury occurs, Cerebrolysin®, a parenteral biological drug consisting of peptides and amino acids, is given to the patient by infusion. In this way, Cerebrolysin® reaches its place of action, the targeted tissue in the brain, directly and in full concentration. (see Figure 1)
Figure 2: **Blood vessels of the brain**

- Endothelial cell with tight junctions (BBB)
- Other drugs or molecules (e.g. Glucose Oxidase)
- Erythrocytes
- Leukocytes
- Cerebrolysin®
- Thrombocytes
The blood vessels of the brain are part of the bloodstream that supply our most important organ with oxygen, glucose and other nutrients and remove metabolic products and carbon dioxide. The endothelial cells of the blood vessel walls have a remarkable anatomical property – they form the blood-brain barrier, a selectively permeable barrier between brain tissue and bloodstream that controls the exchange of substances in the central nervous system. The most striking structural feature of that barrier consists of endothelial cells that are closely connected to each other by tight junctions (consisting of Claudin 5, Occludin and ZO1). The BBB protects the brain from the influence of foreign molecules and therefore the BBB is only permeable to a few molecules, for example water, sugar or nutrients that enter the brain, as well as for waste products that leave the brain. It also controls the access of neurotransmitters and hormones.\(^1\) In contrast, large molecules, including many drugs, are held back by this structure.

However, due to its molecular structure, Cerebrolysin® is small enough to pass the blood brain barrier (BBB).\(^2,^3,^4\) This enables Cerebrolysin® to act directly in the brain and its neuronal tissue! (see Figure 2) The final destination for Cerebrolysin® is the neurovascular unit which consists of blood vessels with endothelial cells, pericytes and extracellular matrix components, neurons, microglia cells, oligodendrocytes, astrocytes and myelin sheath etc. (see Figure 3)
Figure 3: Neurovascular Unit (NVU)
Blood vessels with endothelial cells, pericytes and extracellular matrix components

Neurons with axons and dendrite

Oligodendrocytes are a type of neuroglia whose main functions are to provide support and insulation to axons in the central nervous system. Oligodendrocytes do this by creating the myelin sheath.

Myelin sheath is an insulating layer, or sheath that forms around nerves. It is made up of protein and fatty substances. This myelin sheath allows electrical impulses to transmit quickly and efficiently along the nerve cells.

Astrocytes are the border and control point between the brain and the bloodstream. They supply neurons with nutrients and are responsible for disposing of the waste.

Microglia cells represent specialized population of macrophages-like cells in the central nervous system (CNS) considered immune sentinels that are capable of orchestrating a potent inflammatory response. Microglia are also involved in synaptic organization, trophic neuronal support during development, phagocytosis of apoptotic cells in the developing brain, myelin turnover, control of neuronal excitability, phagocytic debris removal as well as brain protection and repair.

Neurovascular Unit (NVU) The concept of the neurovascular unit detects the needs of neuronal supply and trigger necessary responses through their intimate anatomical and chemical relationship. It is composed of blood vessels with endothelial cells, pericytes and extracellular matrix components, neurons, oligodendrocytes and myelin sheath, microglia cells and astrocytes. In case of a stroke the NVU provides a modular framework where cell-cell signaling, and cell-matrix interactions mediate the overall tissue response. Cerebrolysin® supports this process and triggers specific activities in this area to normalize brain functions. The vascular protective/restorative role of Cerebrolysin® is critical for the therapeutic benefit and makes Cerebrolysin® unique and distinctive.
Preclinical studies, such as the studies by Frey et al. and Gschanes et al., demonstrated clearly that Cerebrolysin® molecules pass through the vessels into the neuronal parenchyma, and that Cerebrolysin® molecules can be detected in neuronal tissue. Additionally, numerous clinical studies have shown positive effect of Cerebrolysin® on motor and cognitive improvement of patients. This fact clearly demonstrates the ability of Cerebrolysin® to cross the blood-brain barrier and to exert its effect in neural and neurovascular tissues.

Here we would also like to emphasize that Cerebrolysin® plays a protective and restorative role for the vasculature in the brain, which is crucial for its therapeutic benefit. Cerebrolysin® makes a unique and distinctive contribution to parenchymal cells as well, giving Cerebrolysin® a vascular protective function. Thus, neurologists appreciate the concept of a cerebrovascular disease or injury and a therapy that aims at it. In the neurovascular unit Cerebrolysin® interacts with all different components and areas, triggering specific activities to normalize brain functions. This protective/restorative role on the brain’s vasculature of Cerebrolysin® is critical for the therapeutic benefit and makes Cerebrolysin® unique and distinctive!
FACT BOX:

- Cerebrolysin® passes the blood brain barrier (BBB)
- Cerebrolysin® acts in the neurovascular unit (NVU)
- Cerebrolysin® triggers specific activities to normalize brain functions
- Cerebrolysin® is unique and distinctive
Figure 4: Thrombus
The accumulation of risk factors like age, high blood pressure, diabetes, obesity, smoking, physical inactivity, to name just a few, are the main causes of stroke, particularly ischemic stroke.

An ischemic stroke occurs when a blood vessel is blocked by clots which have developed slowly over time and consist of substances transported through the blood stream. **Fibrin fibers**, with their adhesive properties, attach to the vessel walls and enable other components like erythrocytes, thrombocytes, leukocytes, lipids and proteins to form a **thrombus**. (see Figure 4)
Figure 5: Pro-inflammatory cytokines

- Ischemia
- Procoagulant, prothrombotic and inflammatory endothelia cells
- Proinflammatory cytokines
This thrombus is growing and can partly or completely block the flow of blood in a blood vessel. A blockage by clot formation may prevent oxygen and nutrients from reaching the brain tissue downstream of the site of blockage. This is called ischemia. Besides the deficiency and shortage of oxygen (O$_2$) and nutrients, there are also changes in gene expression$^{8,9}$ and alterations in molecular pathways in the affected tissue$^{10,11}$. If ischemia is not treated promptly, it can lead to tissue damage or death.

Due to a downstream deficiency of oxygen and nutrients, endothelial cells become procoagulant, prothrombotic and inflammatory$^1$.

In this phase, endothelial cells release pro-inflammatory cytokines. These cytokines are transported downstream with the slower blood flow and activate other endothelial cells. These cells become inflammatory as well and extend this process further. (see Figure 5) The released proinflammatory cytokines do not only act within the vessel but will be also released to the brain parenchyma. By secreting cytokines, the parenchymal cells, such as microglia cells, are attracted from the surrounding NVU. Due to their function as immune effector cells of the central nervous system and their role in the mononuclear phagocytic system, the migrated microglia accelerate the inflammation process of endothelial cells. In addition, microglial cells, astrocytes and other parenchymal cells are re-programmed to an inflammatory state and thus generate a pro-inflammatory environment in the brain.
With a continued aggravation of the inflammatory state, endothelial cells in the vessel wall swell and cause the gaps between these cells to widen. This inflammatory process leads to a deterioration of the cell-connecting tight junctions which are responsible for maintaining the blood brain barrier’s integrity. Consequently, this functional deterioration of the tight junctions leads to structural and functional damage of the blood brain barrier, and furthermore, due to cell swelling, the vessel walls become fragile and develop microbleeds. This “brittleness” of the vessel walls can cause a hemorrhagic transformation, resulting in intracerebral hemorrhage. (see Figure 6) Of course, not only inflammatory cytokines and the resulting inflammatory state of the vasculature, particularly its endothelial cells, can cause microbleeds, but also other mechanisms and substances such as collagen 4 can lead to micro-vessel leakage and hemorrhagic transformation. But in this booklet, we only focus on one aspect of structural and functional damage of the blood brain barrier.
Figure 6: Microbleeds
In addition to the inflammatory effect of cytokines, an upstream blockage in the vessel results in a downstream fibrin deposition, which detaches from the clot and can activate the inflammatory and prothrombotic status of the vasculature as well. Fibrin is toxic in itself and causes inflammation. This leads to further release of “inflammatory cytokines”, such as ICAM-1 (intercellular adhesion molecule 1), HMGB1 (high mobility group protein B1 / highly mobile group protein B1), TNFα (tumor necrosis factor α) and NFκB (nuclear factor ‘kappa-light-chain-enhancer’ of activated B-cells) of the endothelial cells of the vascular walls. Therefore, fibrin fibers, with their intrinsically toxic properties, can aggravate this process. (see Figure 7) Here are shown fibrin fibers detaching from the clot, attaching to other endothelial cells further downstream, inflaming these cells and causing these cells to release further cytokines. This is a perpetuating process.

Now the patient suffers from acute brain ischemia and the associated brain inflammation.
Figure 7: Fibrin
Cerebrolysin®, an agent with neuroprotective and anti-inflammatory properties, has been infused intravenously, travels through the blood stream and reaches its targeted tissue. There, Cerebrolysin® interacts with the endothelial cells and reduces the release of pro-inflammatory cytokines.¹

Cerebrolysin® shows a significant dose-dependent protective effect against fibrin-induced cellular leakage. For example, a set of experiments has shown that Cerebrolysin® reduces the production of the pro-inflammatory cytokines induced by fibrin. Additionally, Cerebrolysin® reduces the inflammatory response within the endothelial cells and supports the regeneration of fibrin-dependent damage to BBB. Concomitantly, the administration of Cerebrolysin® increases the numbers of the endothelial cell tight-junction proteins (reduced by fibrin deposits) and reduces the levels of ICAM1, the adhesion molecule responsible for the adhesion of inflammatory cells to the vasculature.³

Therefore the endothelial toxic factory ceases to operate. The vessels return to a normal state. The vasoprotective action of Cerebrolysin® is achieved by the ability of Cerebrolysin® to promote vascular integrity, by activating angiopoietin 1 (Ang1), Vascular Endothelia Growth Factor (VEGF) and the Sonic Hedgehog Pathway (Shh) ¹¹. These are the key molecules and are pivotal for vascular stabilization and maturation, as well as for the integrity of BBB and consequently are involved in angiogenesis and maintaining the function of the blood brain barrier. Angiopoietin 1 promotes the stabilization and maturation of the vessels and therefore angiopoietin 1 is one of THE protective, anti-inflammatory molecules. Additionally, Ang1 is a known restorative molecule active in the recovery processes post brain injuries.
Figure 8: Anti-inflammatory properties
For less damaged endothelial cells, Cerebrolysin® increases the number of tight junctions and thereby restores the integrity of the blood brain barrier and the tightness of the vessels. Thus, Cerebrolysin® also reduces the risk of hemorrhagic transformation. ¹

Another regulatory route ascribed to Cerebrolysin® involves the stimulation of micro-RNAs (miR17-92 cluster). Cerebrolysin® was shown to stimulate the production of the miR 17-92 cluster through a sonic hedgehog (SHH)-dependent pathway. These small regulatory molecules (miR17-92) are responsible for the concerted deployment and control of the endogenous neurorecovery processes. It was shown that miR 17-92 is responsible for the concerted regulation of the endogenous neurorecovery processes, including processes of brain plasticity (e.g. axonal growth) and stabilization of complex behavioral traits like depression and anxiety.

New unpublished data from Dr. Chopp’s laboratory has demonstrated that other miRs produced in platelets derived from the blood of the TBI/stroke-injured animals promote leakage of the BBB. The pro-inflammatory cascade leading to secondary brain injuries appears to be driven by multiple vascular events. Cerebrolysin® treatment promotes the reversal of these effects – a shift toward anti-inflammatory, pro-recovery processes within the microvasculature. This action prevents or at least limits the extent of the secondary brain injury and facilitates the recovery of lost functions. ¹²

Consequently, Cerebrolysin® protects not only the vascular system and subsequently the brain tissue, but also reverses vascular damage.
FACT BOX:

- Cerebrolysin® reduces the release of pro-inflammatory cytokines
- Reduction of the risk of hemorrhagic transformation after stroke with Cerebrolysin®
- Cerebrolysin® protects the vascular system and reverses vascular damage
Figure 9: Blockage of small vessels
Reduction of post-stroke dementia

Small vessel disease and mini strokes

A thrombus that dissolves after a large vessel occlusion releases larger and smaller fragments into the blood stream. Such circulating fragments consist of fibrin, erythrocytes, thrombocytes, leukocytes, lipids, and proteins.

On their journey through the vascular system, these fragments travel along increasingly narrowing vessels and finally block the very small vessels in the microvasculature. (see Figure 9) Eventually, the capillaries are getting too narrow, narrower than the diameter of the fragments of the thrombus. As a consequence, these particles get stuck and block the smaller vessels. This so-called downstream microvascular thrombosis (DMT), is an event associated with proximal occlusion and usually occurs in the venous compartment of the brain (in postcapillary microvessels). The microvessel lumina are obstructed with platelets, leukocytes, and fibrin-rich aggregates due to local activation of hemostasis in the ischemic microvascular bed. This causes, albeit to a lesser degree, once again an ischemic state.
Such micro-clots dissolve with time by naturally produced components of the body, like endogenous plasminogen and other immune components. However, due to the pro-inflammatory properties of the mini-thrombus, cells attached to the clot release pro-inflammatory cytokines and swell, which causes further obstruction of the capillary with reduced flow. (see Figure 10) Such reduced blood circulation leads to hypoxia, alters mechanisms of cerebral self-regulation, promotes the transcription of inflammatory genes, as well as the breakdown of the blood-brain barrier, and consequently the entry of inflammatory proteins into the vascular walls and the cerebral parenchyma. The released cytokines and fibrin get in contact with other endothelial cells and trigger more inflammation reactions. These blockages of the capillaries by the clot and the subsequent swelling of the endothelial cells can be considered as mini strokes. The resulting undersupply with oxygen and nutrients leads to atrophy and associated microbleeds. This results very often in disturbed cognitive functions, so-called small vessel disease, which can later on trigger complications such as post-stroke cognitive impairment or post-stroke dementia. 13
Figure 10: Swelling of endothelia cells
Cerebrolysin® is indicated for the treatment of all forms of vascular cognitive impairments and consists of small molecules with anti-inflammatory properties. The arriving Cerebrolysin® molecules reduce inflammation and reverse swelling. Normal blood flow is restored and activated endothelial cells stop their cytokine release. The affected micro-vasculature returns to normal function. (see Figure 11)⁴

Cerebrolysin® has a positive impact on inflammation in the vasculature and parenchyma and reduces microbleeds.¹ This, in turn, results in a proven effect on long-term prevention of post-stroke consequences such as small vessel disease and post-stroke dementia.¹⁴,¹⁵
Figure 11: Return to normal function
FACT BOX:

- Treatment for all vascular cognitive impairments
- Cerebrolysin® reduces inflammation
- Reduction of microbleeds with Cerebrolysin®
- Cerebrolysin® prevents post-stroke consequences, like post-stroke dementia
Figure 12: Ischemic stroke
Cerebrolysin® stimulates neuroplasticity

When a clot is formed in the capillary or a particle gets stuck due to the vessel’s decreasing diameter. It leads to a reduced blood flow or a complete blockage of the vessel and causes an ischemic condition.

Due to this undersupply, blood vessels and capillaries become atrophic, causing the vessels to contract and dissolve.
Figure 13: Consequences of an ischemic state
Consequences of an ischemic state (see Figure 13):

- some neurons lose their myelin sheath and are reduced in their functionality
- neurons slow down their communication with other neurons
- loosening of the connections of their dendrites and axons to neighboring cells
Figure 14: Glial cell

Neurotrophic factors

Glial cell
In order to counteract the impending neural death, a natural recovery process is initiated, which is unfortunately very limited in time. Glial cells begin the simultaneous expression of neurotrophic factors, such as BDNF, CTNF or NGF, to name just a few. 16 (see Figure 14)

These neurotrophic factors play an important role in the survival and regeneration of the neuronal network and start a natural recovery process, called neuroplasticity. 17
Figure 15: Formation of new connections
One of the most important mechanisms of neuroplasticity is the formation of new connections with intact, adjacent nerve cells. (see Figure 15) In these cells, the axons and dendrites begin to sprout, the density of dendritic spines increases, and new synapses are being built.

Nevertheless, the recovery process includes much more. It comprises an orchestrated interaction of angiogenesis, neurogenesis, neurite growth and remyelination, because only this interaction is conducive to spontaneous recovery.  

Side note: This process of neuroplasticity is not only important in regeneration and post stroke recovery but is also the basic process of learning.
Figure 16: Sprouting and Synaptogenesis
One of the most important properties of Cerebrolysin® is the initiation and promotion of neuroplasticity, with its sprouting and synaptogenesis! (see Figure 16)

Cerebrolysin® itself has neurotrophic factor-like properties. It stimulates neurons and glial cells to produce neurotrophic factors like BDNF. These neurotrophic factors activate the outgrowth of axons and dendrites and trigger the formation of new synapses (neuroplasticity).19

Cerebrolysin® amplifies neuroplasticity and angiogenesis by facilitating this multi-layered process via multiple molecular pathways, by inhibiting factors that block some pathways and by inducing critical molecular restorative mediators within vascular and parenchymal cells, such as VEGF. Ang1, stimulated by Cerebrolysin®, again induces sonic hedgehog (SHH) a developmental morphogen which mediates a multiplicity of restorative agents. In addition, SHH induces the expression of miR-17-92 family which also mediates highly restorative neurite and axonal outgrowth and also has been shown to reduce anxiety and depression.20, 21, 22, 23 (see Figure 17)
Figure 17: Remyelination
FACT BOX:

- Cerebrolysin® has neurotrophic factor-like properties
- Cerebrolysin® stimulates neuroplasticity
- Cerebrolysin® enhances new neural networks
Figure 18: Neuroblasts

NTFs are secreted by the endothelial cells.

Neuroblasts
Neurogenesis

**Cerebrolysin® increases endogenous neurogenesis**

As discussed in the last chapter the ischemia provokes a cascade of vascular and neuro-degenerative processes that cause atrophy of blood vessels, whereby the vessels contract and dissolve. As well, the function of neurons and their communication pathways with neighbouring nerve cells deteriorate, and, in the worst case, the function of neurons and their communication die.

After neuronal death, *neurogenesis* is induced by glial cells. Surviving neurons start to produce neurotrophic factors (NTFs), in particular Brain Derived Neurotrophic Factors (BDNF). Later (after 1-2 weeks) the expression of BDNF mainly occurs in endothelial cells of the vessels.

Stroke-mediated BDNF and expression of other NTFs induce the recruitment of neuronal precursor cells from the subventricular zone into the ischemic stratum. On their way to the injured area, these de-routed neuroblasts use blood vessels as a physical scaffold for their migration. (see Figure 18) Once in the injured area, these neuronal precursor cells differentiate into neurons and integrate with other nerve cells into a new neural network.
Figure 19: Cerebrolysin® stimulates glial cells
However, this natural neurogenesis is slow and has a very limited time window!

After infusion, Cerebrolysin® reaches the endangered area of the brain. (see Figure 20)

Due to its neurotrophic factor-like activity

- Cerebrolysin® promotes and amplifies natural restorative processes like neurogenesis and angiogenesis.

- Cerebrolysin® stimulates neurons, glial cells, and endothelial cells to express neurotrophic factors, it catalyzes the conversion of proNGF to active NGF, and it mimics neurotrophic factor activities.

- Consequently, more neurotrophic factors are available to induce migration and differentiation of neuroblasts and the re-formation of a neuronal network.

Cerebrolysin® can be seen as a multi-targeted agent that amplifies multiple processes of protection and neurovascular recovery.

The whole process ends when a functional network is restored, which is the physiological basis for recovery and a successful rehabilitation therapy.
Figure 20: **Effects of Cerebrolysin®**

- Neurotrophic factors
- Reformation of the neuronal network
- Differentiation of neuroblasts
- Neurotrophic factors
FACT BOX:

- Cerebrolysin® stimulates neurons, glia cells and endothelial cells to express neurotrophic factors
- Cerebrolysin® re-forms the neuronal network
- Cerebrolysin® amplifies multiple processes of protection and neurovascular recovery
- Cerebrolysin® increases neurogenesis
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References


12. Exosome study, M. Chopp, not yet published
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.