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# CONTEMPORARY ASPECTS IN THE PATHOGENESIS OF BRAIN EDEMA IN PATIENTS WITH HEMORRHAGIC CEREBROVASCULAR INSULT

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**Abstract**

The worst neurological deterioration after hemorrhagic cerebrovascular insult (H-CVI) occurs due to the formation of perihematomal edema, a proven significant risk factor for poor prognosis. During the last several years, a vast number of studies have been focused on the pathogenesis of the brain edema.

The main objective of this review paper was the evaluation of the biochemical and molecular mechanisms involved in the pathogenesis of the edema, with a special focus on the inflammatory and oxidative mechanisms. We believe that this brief review could serve as a motivational boost for designing a comprehensive clinical study, in which the radiological and clinical variables, as well as the proinflammatory mediators and the oxidative stress markers will be simultaneously evaluated for their predictive roles in the formation of brain edema.

***Key words:*** hemorrhagic cerebrovascular insult, perifocal edema, pathogenesis, proinflammatory mediators, oxidative stress

## Introduction

Hemorrhagic cerebrovascular insult (H-CVI) accounts for 10-15% of all strokes [1]. The mortality rate of this severe type of stroke ranges between 30-50%, and as many as 74% of surviving patients remain functionally dependent for 12 months [2]. According to more recent literature, the incidence of H-CVI is 24.6 per 100,000 people per year, and is expected to double by 2050 [3]. The most significant risk factor for the occurrence of spontaneous H-CVI is the hypertension, the effect of which is significantly greater for deep hemorrhages than for lobar hemorrhage [4, 5, 6]. Other risk factors that have impact, but to a lesser extent, are smoking and alcoholism [7, 8], as well as the use of anti-aggregation therapy [9].

The worst neurological deterioration in patients after hemorrhagic cerebrovascular insult (H-CVI) occurs due to the formation of perihematomal edema, a proven significant risk factor for poor prognosis [10]. The occurrence of perihematomal edema increases the intracranial pressure, causes mass effect, and thus increases intrahospital mortality [11, 12]. The volume of edema begins to increase significantly as early as the first 24 hours after the appearance of H-CVI [10], continuing to increase over a period of 3 days, reaching its peak at the fourth or fifth day. These changes have been published in several studies based on monitoring the dynamics of CT and NMR findings during the first days after H-CVI has occurred [13, 14]. Therefore, it is very challenging to identify the major risk factors that influence the development of edema, which could be predicted by the fifth day on the basis of the parameters examined at admission.

During the last several years, a vast number of studies have been focused on the pathogenesis of the brain edema. The main objective of this review paper was the evaluation of the biochemical and molecular mechanisms involved in the pathogenesis of the edema, with a special focus on the inflammatory and oxidative mechanisms.

## Biochemical mechanisms involved in the pathogenesis of the perifocal brain edema

At different time intervals, brain edema has a different structure, composition and etiology, i.e. the formation of brain edema after H-CVI undergoes a certain "evolution" over time, encompassing at least three stages: hyperacute phase, acute phase and post-acute phase.

The hyperacute phase occurs a few hours after H-CVI has occurred and involves the formation of the so-called "early edema", also referred to as "ionic edema". It is due to the transcapillary efflux of electrolytes, osmotically active serum proteins and water from cerebral blood vessels, as well as from osmotic active substances originating from clot retraction; at this stage, the blood-brain barrier is

still intact [15]. In the later acute phase, perifocal edema can be defined as a mixture of formed "vasogenic edema" (which results from thrombin production, coagulation cascade activation, inflammatory process activation, and blood-brain barrier disruption) and "cytotoxic edema" (which results from neuronal death) [15, 16]. The combination of these processes results in damage to the blood-brain barrier and death of brain parenchymal cells [17].

The third phase, which begins approximately 72 h after H-CVI has occurred, involves erythrocyte lysis and hemoglobin-induced neurotoxicity [18]. The timing of the evolution of brain edema is described thoroughly in the following paragraphs.

**Early (hyperacute) phase**

At the earliest hyperacute phase, which occurs within the first few hours after H-CVI, a transcendental osmotic gradient is created and the blood clot is retracted, favoring the passage of osmotically active substances, and subsequently water, from the hematoma - into the surrounding brain interstitial tissue. This results in the formation of early, ionic edema, whose formation is independent of the blood-brain barrier, which is still intact at this time [15]. Several studies indicate that the presence of coagulum is necessary for the development of early, hyperacute edema. The retraction force of the coagulum causes insertion of the serum into the perichematic space [17]. Transcapillary efflux of electrolytes, osmotically active serum proteins and water from the cerebral blood vessels additionally contribute to increased volume of ionic edema.

At this stage, the process of formation of cytotoxic edema begins, due to disruption of the membrane energy mechanisms of the sodium-potassium (Na + / K +) neuron pump [15, 19 - 21]. These disrupted mechanisms of early cytotoxic edema contribute to extracellular accumulation of excitotoxic metabolites such as glutamate, which subsequently cause an even greater disruption of mitochondrial Na + / K + energy mechanisms [22]

**Acute phase**

The second, acute phase occurs the first day after H-CVI has appeared, and is a consequence of thrombin production, which is activated by the coagulation cascade and neuroinflammatory mechanisms. Thrombin is a protease, which affects a number of processes that directly and indirectly contribute to the development of edema. Firstly, thrombin affects the increase of the permeability of the blood-brain barrier through activation of matrix metalloproteinases receptors (MMP) and vascular endothelial growth factor (VEGF) [23]. Matrix metalloproteinases (MMPs) are proteolytic enzymes that play a role in the reorganization of the extracellular matrix. Namely, MMP-9 and MMP-2 destroy the major components of the basal lamina on the cell wall, thereby damaging the blood-brain barrier. One study described the effect of MMP-9 on the development of brain edema in deep S-CVVs [24], while in another study the same effect of MMP-9 was confirmed [25].

At this stage, activation of the NF-kB transcription factor activates numerous neuroinflammatory mechanisms and mediators (cytokines, chemokines) that also affect brain edema and cellular damage [26]. Of proinflammatory cytokines, INTERLEUKIN 6 (IL-6) and INTERLEUKIN 10 (IL-10) play an important role at this stage. Both these cytokines can be activated and released by several types of cells, such as microglia, monocytes, endothelial cells, but in this case they are mainly secreted by microglia and monocytes [27]. In one study the effects of IL-6 and IL-10 were examined and correlated with the severity of clinical admission image measured by the Glasgow Coma Scale, while IL-6 also correlated with intracerebral hematoma volume [26] and with perifocal edema [28]. A similar effect has been shown by IL-11, which is a type of IL-6, and is correlated with intracerebral hematoma volume, perifocal brain edema, as well as with clinical outcome in H-CVI patients [29].

In essence, the initial inflammatory response to H-CVI is primarily orchestrated by two cytokines, namely tumor necrosis factor alpha (TNF-α) and INTERLEUKIN 1β (IL-1β), whose expression increases massively after several hours of H-CVI. Their increased concentration leads to an increased permeability of the blood-brain barrier and, thus, infiltration of peripheral immune cells into the brain parenchyma. These processes generally contribute to the development of vasogenic edema. TNF-α and IL-1β are present as early as the first day after H-CVI, suggesting that cytokine synthesis and secretion are probably part of the early microglial cell response to H-CVI [30].

TNF-α and IL-1β are secreted by thrombin-activated microglial cells and astrocytes, but their expression is dependent on other mediators [31]. For example, increased glucose levels

(hyperglycemia) may induce increased expression and increased levels of cytokines TNF-α and IL-1β in capillary endothelial cells [32, 33]. TNF-α has a direct effect on blood-brain barrier permeability and glutamate release as an excitotoxic neurotransmitter [34], thus TNF-α is one of the major causes of secondary brain damage and vasogenic edema formation [31].

In addition to mechanisms of inflammation, a number of mechanisms of excitotoxicity are also activated at this stage, including glutamate as the amino acid that accumulates in the extracellular tissue in the brain edema region. It is activated as a consequence of the postsynaptic N-methyl D-aspartate receptor (NMDA), leading to influx of Ca2 + and Na + ions into the cell, subsequent water uptake, cell infiltration and, ultimately, cell death [36]. High levels of glutamate released in the plasma within the first 24 hours after H-CVI had occurred, were associated with a poor clinical outcome and an increased volume of residual cavities [28].

During this period of development of brain edema, leukocyte infiltration, especially neutrophils, occurs in the peripheral brain tissue, as well as reactive oxygen species (ROS) activation. In summary, today it is thought that damage to the blood-brain barrier, activation of proinflammatory mediators, and production of ROS together contribute to secondary brain injury after H-CVI [37].

**Subacute phase**

The third or subacute phase in the development of edema is mainly due to the lysis of the red blood cells of the blood coagulum, i.e. the action of hemoglobin and iron, reaching its peak on day 3 after H-CVI has occurred. Iron is a key factor in the mechanisms of development of delayed brain edema, by stimulating lipid peroxidation and by creation of free radicals (ROS) [38, 39].

**Focus on oxidative stress and inflammatory mechanisms**

Oxidative stress describes the state in which the body responds to various harmful stimuli by producing excessive amounts of reactive free oxygen radicals (ROS) and reactive nitrogen radicals (RNS). Under normal conditions, there is a certain balance between the amount of antioxidants present and the produced radicals, with the cell managing to effectively protect against the toxic effects of the oxidants. Oxidative stress is defined as an imbalance between the production of radicals (including other highly reactive oxidants, such as reactive oxygen species, ROS, which are not necessarily chemical in nature), and their elimination by cellular protectorates. Simply put, oxidative stress is a condition in which the cell is unable to effectively deal with large amounts of accumulated radicals and highly reactive oxidants, leading to a gradual damage of key cell components and, death of the cell itself.

Oxidative stress plays an important role in secondary brain injury after H-CVI [40]. Oxidative stress has been included not only in the pathological process of H-CVV, but also in the various stages of the pathophysiological response to H-CVI [41]. Different metabolic pathways can lead to the production of ROS and RNS by H-CVI, two of which are the most significant. Firstly, decomposition products from blood cells, such as iron ions, heme, and thrombin, can cause the production of free radicals. Experimental results show that bivalent iron ions (Fe2 +) can interact with lipids and lead to the production of radicals, leading to nerve damage [42, 43]. Secondly, immune cells, such as microglial cells and neutrophils, can generate ROS. During the inflammatory response to H-CVI, neutrophils are stimulated and activated, resulting in a respiratory chain eruption, releasing large amounts of ROS and nitric oxide (NO), as well as excessive consumption of superoxide dismutase (SOD) and excessive lipid peroxidation [44].

Nerve cell damage caused by free radicals is manifested in a variety of ways, with free radicals triggering a plethora of detrimental events, most notably cell membrane damage, DNA breakage induction and induction of programmed cell death [45]. ROS-induced cell damage is due to the induction of lipid peroxidation. Lipid-rich brain tissue is particularly sensitive to oxygen free radicals that can enhance lipid peroxidation, cause membrane damage and increase cell membrane permeability and calcium ion influx [45]. Meanwhile, crosslinking and polymerization of membrane lipids occurs after lipid peroxidation, which indirectly inhibits the activity of membrane proteins such as calcium pumps, sodium pumps, and Na + / Ca2 + exchangers [46]. This leads to a further increase in intracellular calcium concentration, which then stimulates mitochondrial calcium pumps to take up calcium. Calcium and phosphates in mitochondria combine to form insoluble calcium phosphate, which interferes with mitochondrial oxidative phosphorylation and leads to decreased ATP production

[47]. Meanwhile, the increased intracellular calcium ion concentration can activate phospholipase, promoting the breakdown of membrane phospholipids and causing damage to the structure of cell and organelles [48, 49]. In summary, free radicals are the main "killers" of hemorrhagic brain tissue, i.e. they are closely linked to brain injury and bleeding disorders.

Inflammation and oxidative stress following H-CVI are closely related. Oxidative stress induces inflammation, and inflammation causes neurological damage through oxidative stress [50]. The emergence of ROS induces the expression of acute proinflammatory cytokines, primarily TNF-α and IL-10, and also leads to the activation of NF-κB, which plays a crucial role in the inflammatory process [51, 52]. On the other hand, proinflammatory cytokines can induce ROS production [51]; in this way, a positive feedback loop is formed on the so-called "circulus vitiosus", in which oxidative stress stimulates inflammation and vice versa. Oxidative stress can also initiate an increased expression of MMP-9 in the brain after H-CVI [53], which triggers numerous acute-phase detrimental events, such as basal lamina destruction, VEGF activation, and apoptosis process activation. Additional studies have suggested involvement of prostaglandin-mediated inflammatory mechanisms in secondary brain injury after H-CVI, as well as their association with oxidative stress [53].

**Conclusion**

It is very challenging to identify the major risk factors that influence on the development of edema, which could be predicted by the fifth day on the basis of the parameters examined on admission to hospital.

However, so far most studies focusing on inflammatory mechanisms and oxidative stress after X-CVI have been performed on animal models of H-CVI, and detailed clinical studies explaining their predictive role in the development of edema are scarce. Therefore, we believe that this brief literature review would serve as the motivation for conducting a comprehensive study that would simultaneously monitor and evaluate the predictive role of radiological and clinical variables, as well as proinflammatory mediators and markers of oxidative stress in the occurrence of brain edema.

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