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The pathophysiolgy of intracerebral haemorrhage

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Spontaneous intracerebral haemorrhage carries a high mortality rate and treatment of the disease raises more questions then answers. Mass effect, ischaemia and toxicity of blood components are responsible for brain tissue damage. Initially occurring disturbances of cerebral blood flow have a temporary character and do not play a key role in the pathology of intracerebral haematoma. Oedema formatting in the 24–48 hours after intracerebral bleeding is the result of multidirectional processes. The pathological mechanism that underlines it is the function of activation of systemic complement and cascade of coagulation. In the light of these findings, further clinical and experimental investigations should be focused on these factors.

Key words: intracerebral haemorrhage, oedema, systemic complement, inflammation, cytokines

INTRODUCTION

Spontaneous intracerebral haemorrhage (ICH) is the most destructive type of stroke [3]. ICH commonly occurs in the cerebral lobes, basal ganglia, thalamus, brain stem (predominantly in the pons) and cerebellum [5]. Perforation into ventricles develops in association with deep large haematomas [14].

Potential pathophysiological and pathochemical mechanisms that may be involved in ICH include mechanical trauma, mass effect and ischaemia, toxicity of blood components and excitotoxicity [25, 45]. Better understanding of the character and dynamics of the morphological, biochemical changes involved, with emphasis on those taking place within the perihaematomal zone, can provide a basis for the development of a more effective therapeutic strategy. The purpose of this study was to review recently published investigations into this problem.

As a result of ICH, serious impairment of the intracranial homeostasis develops. Primary injury after ICH is a result of the mass effect of the haematoma, causing local compression of the microvasculature and leading to disturbances of the cerebral blood flow (CBF) [23, 26]. Experiments have shown that perihaematomal CBF falls below 25 mL/100 g min but that the reduction lasts less than 10 min; CBF then returns to baseline within 3 h [25, 45]. Mass effect in the area of the caudate nucleus leads to disturbance of regional CBF not only in the lesioned region but also in the frontal cortex overlying the lesion [33].

According to Qureshi et al. [30], the ischaemic penumbra is not present around the clot in canine ICH investigation. It has been established that the CBF threshold for ischaemic injury is 15 mL/100 g min [41]. Wagner et al. [37] reported that the ATP level remained within the normal range, whereas brain phosphocreatine contents increased with time in the perihaematomal zone at 1, 3, 5 and 8 h after ICH. Most recently, Zazulia et al. [47] found that both CBF and the cerebral metabolic ratio of oxygen were

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reduced in the perihaematomal region, resulting in a reduced oxygen extraction fraction. These results indicate that energy deficit was not present around the haemorrhagic focus.

Secondary damage is thought to be caused by tissue reaction to the invasion of the products of blood breakdown in the perihaematomal region, initiating the formation of brain oedema [13, 19, 39]. Within the first 8 h of onset, this oedema is triggered by the accumulation of osmotically active substances and the movement of water across an intact blood-brain barrier (BBB) into the extracellular space [37]. The increase in the number of brain ions such as sodium and chloride accounts for most of the osmolar force driving water into the brain [37, 45]. Over the next 24 to 48 hours activation of the coagulation cascade and the induction of proteolytic enzymes leads to an inflammatory response, resulting in BBB disruption [20, 40].

Haemorrhage into brain tissue activates the coagulation cascade that produces a large amount of thrombin during the first day after ICH [19]. It is established that infusion of thrombin produces the same grade of increase in BBB permeability that is observed in intracerebral haematoma [20]. Inactivation of thrombin by antithrombin III and other plasma inhibitors limits brain oedema [20]. The specific mechanism of thrombin action that leads to disruption of the BBB is suggested by De Michele et al. [7] and Malik and Fenton [21]. According to these authors, direct modulation of endothelial cell permeability leads to the opening of the BBB.

Developing a few days later, red blood cell (RBC) lysis plays an important role in brain oedema following ICH [39, 40, 44]. Infusion of lysed RBCs evokes a 300% increase in BBB permeability compared with the 50% increase caused by the infusion of 5 U of thrombin [20]. One potential factor of erythrocyte lysis is the complement system. As a result of complement cascade activation, complement factors C5b, C6-C9 form a membrane attack complex (MAC), which is able to attach to the cell membrane forming a transmembrane pore that may cause cell lysis [12]. On an electron microscopy image MAC is seen as a ring-like structure on the erythrocyte membrane [4]. It is known that MAC occurs in neurons, causing necrosis, and destroys endothelial cells, leading to BBB leakage [41]. Limiting brain oedema in the course of ICH by the inhibition of complement using cobra venom factor indicates that systemic complement plays a key role in ICH-induced oedema [42].

Haemoglobin breakdown results in a release of the iron and bilirubin that cause the formation of brain oedema within 24 h [13]. A lipid peroxydation evoked by iron may contribute to BBB injury through free radical damage to the endothelial cells [40].

Reports in recent years indicate that evacuation of the clot, formerly liquefied by tissue plasminogen activator (t-PA), markedly decreases the degree of cerebral oedema and BBB disruption and improves cerebral perfusion pressure [38]. On the other hand, Rohde et al. [31] discovered that t-PA employed during evacuation of the haematoma results in an increase in oedema volume. The inhibitor of tissue plasminogen activator (PAI-1) suppresses both t-PA and thrombin [31]. By application of t-PA and an increase in its concentration, inhibition of PAI-1 production is observed with the effect of decreased thrombin inhibition. The role of thrombin in oedema formatting in the course of ICH has been discussed above.

The astroglial and microglial cells, which are sensitive markers of changes in the cellular microenvironment of the central nervous system, are important factors in the processes which take place when ICH leads to injury of the nervous tissue [27]. However, in the first hour after ICH the expression of proinflammatory cytokines TNF-α, IL-1β, and IL-6 does not produce any significant changes [29].

In resting form astrocytes have small round cell bodies with long slim processes (Fig. 1A). Following brain injury after ICH the cell bodies of astrocytes become markedly enlarged, and their processes become short and thick (Fig. 1B). This activation of astroglial cells is likely to be mediated by signals from damaged neurons and induced by subtle changes in the extracellular milieu.

In resting (ramified) form microglial cells are highly branched, usually having between two and six primary branches that radiate from the soma and that have many thin protrusions, which extend from the primary branches (Fig. 1C). During the early phase of ICH the microglial cells undergo morphological and metabolic changes towards an activated form; they retract their processes and become pseudopodic (Fig. 1D). Their branches typically recede at a rate of 0.5–1.5 μm/min [34]. The achievement of an amoeboid form by the microglial cells (Fig. 1E) is preceded by absorption of the tips of the retracting processes by the cell body. These changes are accompanied by the upregulation of the expression of receptor complement 3, major histocompatibility complex class I and inducible nitric oxide synthase [27, 35, 36, 46].

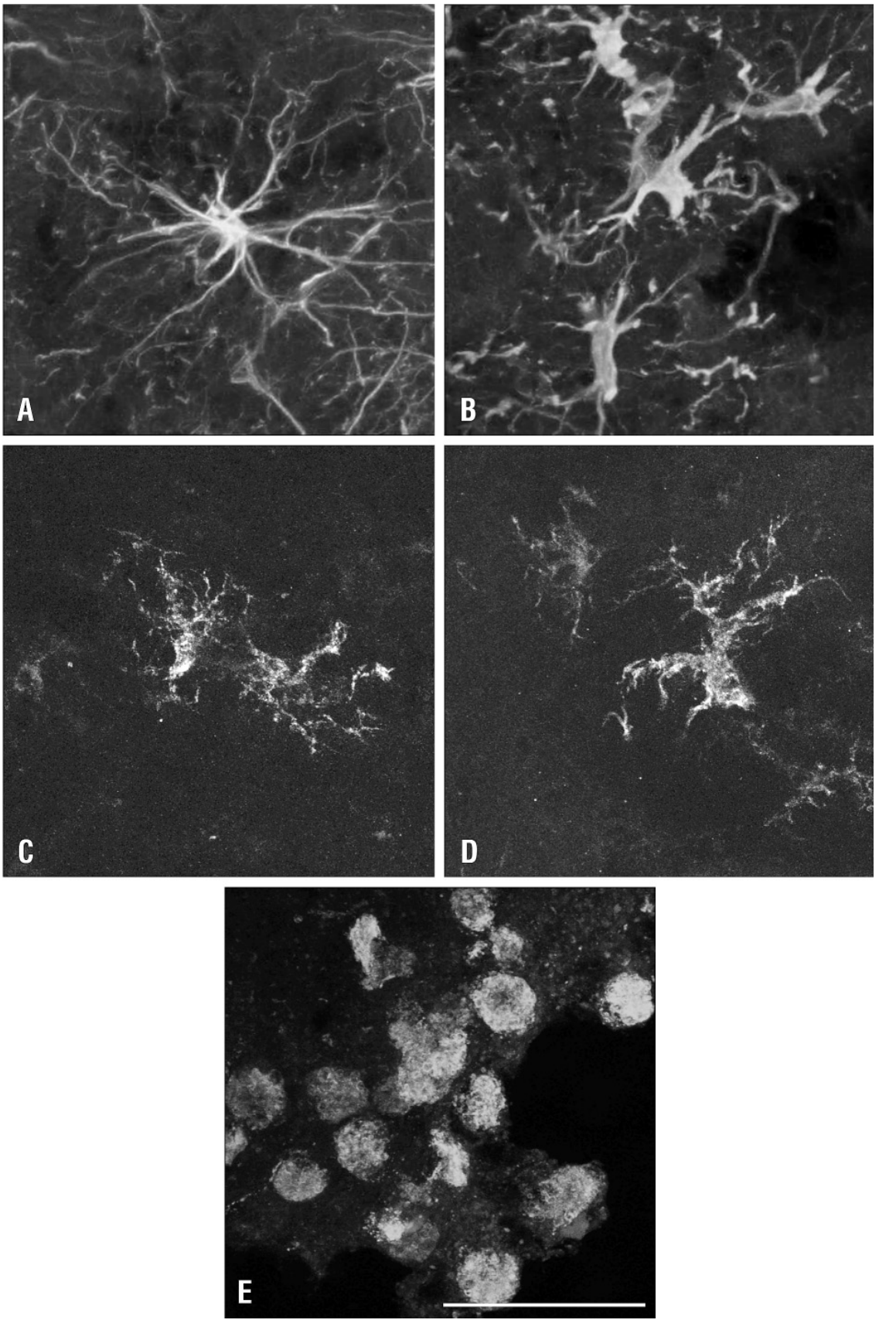

Figure 1. Types of glial cells. **A.** Resting form of astrocyte; **B.** Activated form of astrocyte; **C.** Resting form of microglia; **D.** Activated form of microglia; **E.** Amoeboid form of microglia. Scale bar = 25 μm.

The acute phase is reached within the first day of injury and is determined by events such as neuronal degeneration, disruption of the BBB and leukocyte extravasation, which trigger important changes in glial cell morphology toward fully reactive forms and metabolically activated cells. These cytoskeletal changes are also accompanied by phagocytic activation [32]. Moreover, during this stage astrocytes proliferate and migrate, contributing to restoration of the structural integrity of the tissue. A recently published outcome of laboratory studies has revealed that astrocytes demonstrate the morphological features of activation on the third day after ICH and that these remain for the following three weeks [17]. After damage reactive astrocytes maintain the activation nuclear factor κB (NF-κB). Focal perivascular activation of the NF-κB was seen as early as 2 h after ICH, while more widespread perilesional activation was observed at 8 h [10]. The peak increase in the level of protein subunits of NF-κB was observed around the haemorrhagic focus 4 days after ICH [10]. Activated astrocytic cells synthesise and secrete TNF-α, IL-1β and IL-6 [1, 2, 6].

During the acute phase the presence of neuronal debris, massive tissue disruption and BBB breakdown induces brain microglial cells to become phagocytic cells [34], showing MHC class II expression and upregulation of TNF-α, IL-1β [28]. Activated microglial cell are able to move at a speed of 118 μm per hour [34]. Kowiański et al. [18] pointed out the marked activation of microglial-macrophage lineage cells starting during the first day after ICH.

Intracerebral administration of IL-1β increases the water content in the brain [9]. It has been shown that administration of TNF-α induces the BBB to open *in vivo* [24]. Holmin and Mathiesen [11] observed that IL-1β caused vasogenic oedema that was transient within 72 h, whereas TNF-α caused a less pronounced but more long-lasting vasogenic oedema. Peroxynitrite, which forms by reacting nitric oxide with superoxide, and high concentration cytokines (IL-1β, TNF-α) activate matrix metalloproteinases (MMPs) [43]. Activated MMPs are able to proteolyse the components of the tight adherent junction (occludins, cadherins, claudins) [43]. The result of these changes is increased endothelial permeability. A recently published clinical investigation demonstrates that a high concentration of cytokines, MMPs and products of the degradation of basal membrane components are associated with the early stage of ICH [32].

Clinical investigation supports the occurrence of a late progression of oedema in the third week after ICH [48]. According to the authors, new vessels around the haematoma, developing between the second and third weeks, lack a complete BBB and are responsible for this phenomenon.

The formation of gliotic tissue, the so-called glial scar, is the result of the repair response of the glia. The glial scar is composed of hypertrophied astrocytic processes, macrophages and extracellular matrix [1, 27]. Some reactive astroglial cells of the glial scar contain NF-κB in their nuclei and express IL-1β, IL-6 and TNF-α [1, 2, 6, 27]. In addition, they modulate their own activation by expressing transforming growth factor β1 (TGF-β1) [15]. Furthermore, this factor increases the expression of several collagen types (fibronectin, tenascin, thrombospondin) and promotes the synthesis of protease inhibitors, providing a reinforcement of glial scar formation [22].

Macrophages play an important role in formatting glial scar. These derive from endogenous microglial cells and express IL-1β and secrete TGF-β1, undergoing reduction with time [2].

As a result of apoptosis, the number of microglial cells in the glial scar undergoes a gradual reduction. A recently published study has shown DNA fragmentation observed in the clot as well as in the region surrounding the clot during 3 weeks of observation [16]. Gong et al. [8] observed TUNEL-positive cells over a period of 14 days after ICH. Most of them were neurons, while some were astrocytes or endothelial cells. This leads to the conclusion that neurons are more vulnerable than astrocytes or endothelial cells in the course of ICH.

In summary, the pathological processes involved in ICH have a multidirectional and multidimensional character. At the beginning they destruct and than try to restore the structural and functional integrity of the nervous tissue. It seems that the initially occurring reduction in CBF does not play an important role. The results of the investigations presented suggest that the therapeutic option should be concentrated on the early stages of ICH, before the activation of NF-κB and the expression of inflammatory substances. Early evacuation of the haematoma, within the first few hours of haemorrhage, should be taken into consideration. Future studies in this domain will reveal new insights into the pathophysiological links between disturbances of the CBF, the products of blood decomposition and secreted cytokines and should provide new clues for the treatment of these complex diseases.

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