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Hyperfibrinogenemia and its role during inflammation.

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Fibrinogen (Fg) is one of the biomarkers of inflammation and a high risk factor for many vascular disorders. It is involved in various physiological processes such as blood coagulation, platelet thrombogenesis, erythrocyte aggregation, and cell-cell interactions. High level of Fg in blood exacerbates circulatory complications during inflammatory diseases such as hypertension, diabetes, stroke, traumatic brain injury and other cardiovascular or cerebrovascular disorders. Enhanced blood content of Fg alters vascular reactivity and compromises endothelial cell layer integrity resulting in leakage of plasma substances from blood stream to interstitium. The purpose of this review is to demonstrate and discuss the effects of Fg causing a cerebrovascular leakage of blood plasma proteins and to offer possible mechanisms for this effect, which could enhance microcirculatory complications during inflammatory cerebrovascular disorders accompanied by an increased blood content of Fg.

Key words: fibrinogen, inflammation, caveolar transcytosis, vascular permeability, transcellular transport, paracellular transport.

Hyperfibrinogenemia and Inflammation

Most of the human diseases such as stroke, myocardial infarction, hypertension diabetes, atherosclerosis, and traumatic brain injury are associated with inflammation. Inflammation is accompanied with elevation of inflammatory mediators including high molecular weight (~ 340 kD) plasma adhesion glycoprotein fibrinogen (Fg), which is considered a high risk factor for many cardiovascular and cerebrovascular diseases 1. While Fg at the normal concentration (~2 mg/ml) has no discernible effects, at an elevated level (Fg level of ≥ 4 mg/ml, i.e. hyperfibrinogenemia, HFg) it is considered not only a marker of inflammation 1 but also a cause of inflammatory responses 2-5.

Changes in blood-brain barrier (BBB), which is the regulated interface between the peripheral circulation and the central nervous system (CNS), are also associated with inflammation. In general, the BBB consisting of the cerebrovascular endothelial cells (ECs) and vascular smooth muscle cells, together with astrocytes, pericytes, neurons, and associated extracellular matrix proteins represents a vasculo-neuronal unit responsible for changes along this axis. Majority of CNS diseases are associated with disruption of this unit. The regulation of ion balance, changes in oxygen and nutrition supply, homeostasis, transport of hormones and neurotransmitters are dependent on normal function of cerebral vessels and blood flow properties. Thus, vascular or blood flow dysfunction leads to or exacerbates neuronal abnormalities.

One of the indications of inflammation is increase in permeability of blood vessels, that result in leakage of blood plasma substances out of the blood stream into the interstitium and may cause edema. The brain edema may be characterized by intra- or extracellular fluid accumulation.

Intracellular edema occurs after cerebral ischemia, trauma, metabolic disorders and intoxications. Its main mechanisms are an enhanced sodium (Na+) permeability or a failure of active Na+ export via sodium-potassium ATPase (Na+/K+-ATPase) due to energy shortage. The main cause of extracellular edema is BBB damage and it is mostly featured by the accumulation of proteins in tissue 6. It occurs in brain tumors, infections, trauma, and hypertensive crisis 6.
Plasma components may pass through the endothelial barrier via two major transcellular and paracellular transport pathways 7 (Fig. 1). The paracellular transport occurs through gaps between ECs and involves alterations in all types of junction proteins (JPs) 7. It is implied, that low molecular weight molecules take this pathway as oppose to transcellular transport of high molecular weight molecules such as proteins, that occurs through the ECs and involves formation of functional caveolae and its motility 8. Thus, movement of proteins across the vascular wall via transcellular transport pathway can be defined as caveolar transcytosis. It has been found, that Fg enhances EC layer permeability 3 to proteins, and can itself leak through the EC layer 2. Fg extravasates at sites of inflammation or increased vascular permeability 2, where it is 4 immobilized and then converted to fibrin. These findings shed a new light on a role of Fg during inflammation-induced dysfunction of a microvascular bed.

Hyperfibrinogenemia and transport pathways

Paracellular pathway

Endothelial cells are connected to each other by JPs. There are three types of JPs: tight junction proteins (TJPs), gap junction proteins (GJPs) and adherence junction proteins (AJPs) 7.
It was shown, that HFg causes an increase in EC layer permeability 4. It causes downregulation of expression and translocation to cytosol of TJPs, such as occludin, zona occludin-1 (ZO-1), and zona occludin-2 (ZO-2) 3. We also showed, that increase in blood content Fg alters EC layer junctional integrity and its attachment to subendothelial matrix, causes downregulation of AJPs, particularly VE-cadherin and its disordered distribution along the cells' membranes 9. Connexins represent GJPs and are responsible for the cell-to-cell communications 10. They are organized in connexons, which can play role of channels for the intercellular passage for small molecular weight molecules 10. There is a lack of information on effect of Fg on GJPs.

Transcellular pathway

Caveolae, fenestrae, transendothelial channels, vesiculo-vacuolar organelles and endothelial pockets are the main components involved in transcellular transportation 8. Caveolae, are distinct flask-shaped invaginated structures present at the surface of many cell types including ECs. Its outer diameter is around 70 nm and at the neck opening is about 25 nm 7. Therefore, they can take up larger molecular weight proteins such as albumin 7 Caveolar walls are enriched in cholesterol, glycosphingolipids, and sphingomyelin 11. Caveolae can be defined by the presence of caveolin-1 (Cav-1), a main building component of caveolae wall 12. It is the major protein of endothelial caveolae and is necessary for caveolae assembly 13. It is considered as a biochemical marker and a structural protein of caveolae in most types of cells 11.

Another marker of caveolae is plasmalemmal vesicle associated protein-1 (PV-1). PV-1 is an integral membrane-associated protein whose expression is associated with caveolae formation 8. It is also considered as a functional biomarker for altered vascular permeability following central nervous system trauma. In central nervous system and thus in brain, PV-1 formation is limited.

Movement of caveolae is governed by activation of Na+/K+-ATPase 11, 14. That is a crucial membrane-bound enzyme responsible for regulation of the electrochemical gradient across the cell membrane. It plays a critical role in regulation of membrane potential in neurons and synapses 6. This olygomeric enzyme consists of α and β subunits. Subunit α has catalytic properties and exists in different isoforms. Isoforms α1 and α 2 are mostly in glial cells, while α 2 and α 3 are in neurons and astrocytes 6. 5 Subunit β regulates activity and conformational stability of α subunit. It is known, that plasma membrane expression of the Na+/K+-ATPase requires the assembly of its α and β subunits. Their interaction is required to accomplish an ion transport. In association with α and β dimmers there is a third subunit y, which modulates a transport function of the enzyme 6. Recent evidence suggests that cells contain two functionally different pools of Na+/K+-ATPase, an ion pumping pool and a signaling pool. Moreover, the non-pumping Na+/K+-ATPase has been shown to co-localize in caveolae and it interacts directly with multiple proteins such as protein kinases, ion transporters, and structural proteins to exert its non-pumping functions including regulation of Cav-1 membrane trafficking 11. Na+/K+-ATPase utilize about 30% of the total cellular energy to maintain intracellular ionic concentration which allows transport of various ions, glucose, and amino acids against their concentration gradient. An increase in Na+/K+-ATPase activity would strongly suggest an increase in overall transport activity.

The role of Na+/K+-ATPase in BBB integrity and particularly in function of vasculo-neuronal unit is very important. We already showed that most of the neurotransmitters (noradrenaline, dopamine, serotonin, and acetylcholine) inhibit the synaptic membrane Na+/K+-ATPase activity 15. However, some neurotransmitters such as glutamate, histidine, and glycine do not influence this system 15. It is important to underline the role and significance of Na+/K+-ATPase during different cerebral disorders such as Alzheimer's disease, epilepsy, ischemia, brain injury, in addition to different cognitive deficits, stress, depression, mania or mood disorders. It is also known that the inhibition of Na+/K+-ATPase activity impairs learning and memory. Involvement of Na+/K+-ATPase in regulating carcinoma cell motility has been reported 6. It is directly involved in the migration of cancer cells in general and of glioma cells in particular 6.

Recently we showed that during HFg formation of caveolae or fenestrae was evinced by the enhanced expression of PV-1 5, 8 and Cav-1 16 in mouse brain vessels and ECs. These results were associated with Fg-induced increase in cerebrovascular permeability 5. Our data also indicate that at high content Fg can activate Na+/K+-ATPase in ECs 14. All these results indicate a strong involvement of Fg binding to ECs in caveolae formation, its motility, and the resultant protein transcytosis.

Excessive extravasation of Fg also leads to a strong association with Amyloid beta (Aβ) peptide forming Fg-Aβ plaque that is the hallmark of Alzheimer's disease (AD). This emphasizes the significant contribution of Fg in development of AD and therefore in loss of memory.

The caveolae-mediated transcytosis and paracellular transport are interconnected. Since we already found that HFg causes greater leakage of plasma components through both transcellular and paracellular transport (Fig. 1), we used a newly developed dual-tracer probing method to define prevailing role of one or another pathway during various pathologies 17. The data showed that increased Fg concentration enhances microvascular permeability via both pathways, however, it mainly activates transcellular transport while only transiently - paracellular transport (Fig. 1). 6

Regulation of blood content of fibrinogen

Fg is synthesized and assembled inside hepatocytes and fibroblasts 18. While cytokines such as IL-1, IL-6, and oncostatin promote its synthesis 18, IL-3, IL-4, and IL-10, which have a protective effect against vascular injury causing atherosclerosis downregulate the biosynthesis of Fg 18. Studies on defibrinogenating effects of batroxobin 19 and peroxisome proliferator-activated receptor-α produced some positive outcomes and are still ongoing. Ancrod, a thrombin-like α-fibrinogenase enzyme purified from the venom of Agkistrodon rhodostoma has been used for defibrination in animal studies 20 and in humans 21, 22. While some studies report promising effects of Ancrod in treatment of acute ischemic stroke 22, others indicate, that Ancrod did not improve its outcome 21. The problem associated with Ancrod treatment is an enhanced bleeding 21, 22. Besides the Fg, Ancrod down regulates other high molecular weight blood proteins such as factor VIII, vWF 23 and may be others, that are involved in coagulation and thrombogenesis. Thus, defibrinating treatment of HFg should be conducted with extreme caution because of the resultant excessive bleeding.

Conclusion:

HFg increases blood viscosity and therefore increases shear stress, that activates ECs and upregulates expression of various plasma adhesion molecules and integrins including Fg receptors α5β1 and αvβ3 integrins and intercellular adhesion molecule-1 (ICAM-1)5. Enhanced binding of Fg to ICAM-1 24 activates extracellular signal-regulated kinases 1 and 2 (ERK-1/2) 25, which increases production of endothelin-1 (ET-1) 25, that in turn is involved in vascular tone modulation 24.

HFg causes translocation and downregulation of JPs, and increase in formation of caveolae. These effects transiently enhance gap opening between the ECs junctions and increase caveolae formation and motility leading to an enhanced microvascular permeability to blood proteins. Thus HFg increases microvascular permeability affecting both paracellular and transcellular pathways, but mainly caveolar transcytosys (Fig. 1). Since Fg plays such an important role in vascular permeability and in general in inflammation, it is suggested to carefully monitor plasma levels of Fg.

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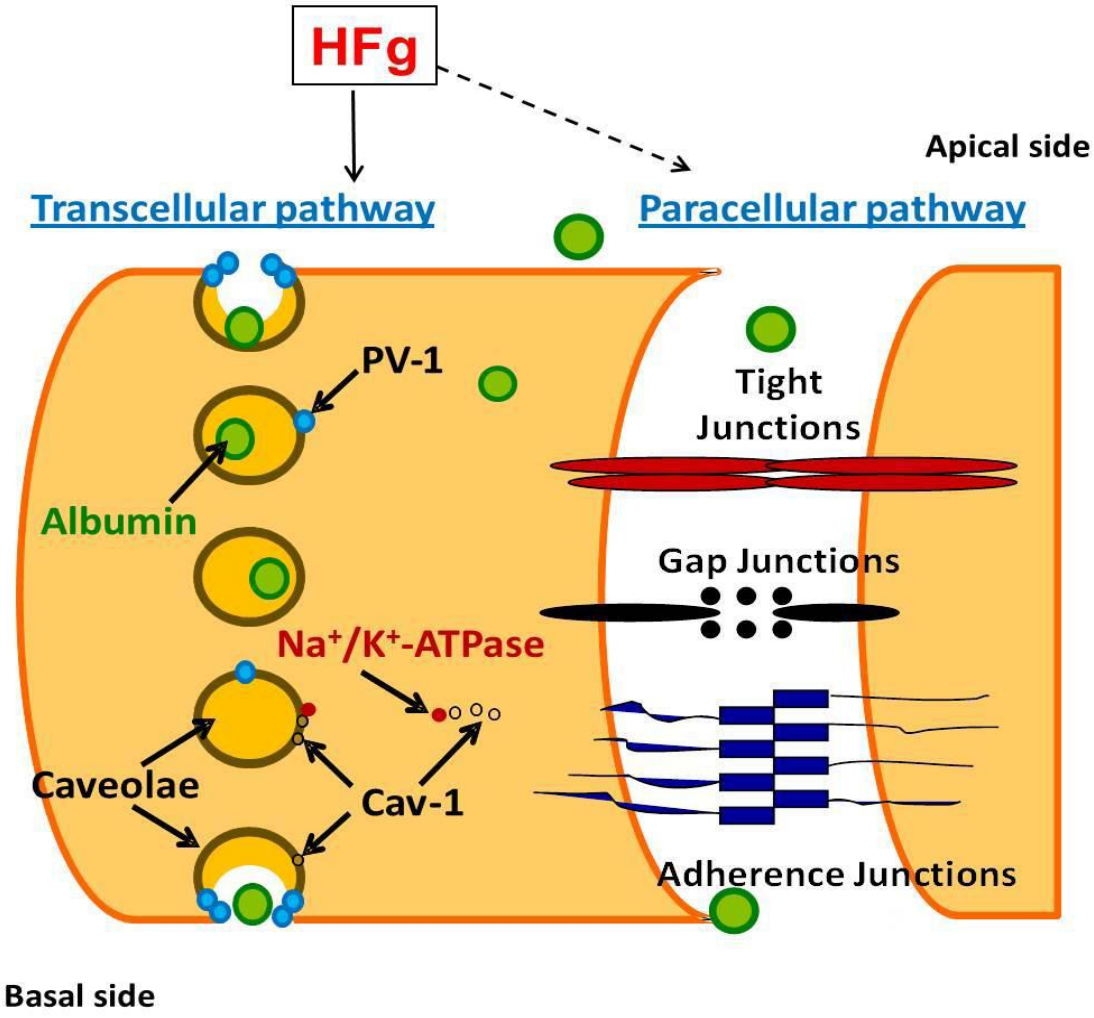
Figure 1. Possible mechanism of hyperfibrinogenemia-induced increased microvascular permeability 8



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