Adhesion molecules of immunoglobulin gene superfamily in stroke

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Stroke-induced inflammatory reaction leads to the accumulation of leukocytes in the brain ischaemic region, where they exert a detrimental effect - promotion and extension of cerebral damage. Intracerebral infiltration of peripheral blood leukocytes requires prior endothelial-leukocyte interactions that are mediated by such cell surface proteins as adhesion molecules. Among adhesion molecules, it is the immunoglobulin gene superfamily (IgSF) that is responsible for strong attachment and transendothelial migration of leukocytes. The principal members of IgSF are: intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion molecule-1 (PECAM-1). In this review the following issues were described and discussed: an increased expression of ICAM-1 and VCAM-1 in ischaemic brain as well as a detection of their soluble(s) forms in sera of stroke victims. The presented data suggest the involvement of both ICAM-1 and VCAM-1 in the sequence and timing of the infiltration of leukocytes into the brain ischaemic zone after stroke. They have also revealed changes in serum concentrations of sICAM-1 and sVCAM-1 that are characteristic for stroke. Recently, increase in sPECAM-1 levels in serum and cerebrospinal fluid (CSF) has been shown within 24 h of the onset of stroke, having indirectly suggested involvement of the molecule in the inflammatory events during the early phase of stroke.

key words: stroke, inflammation, brain, serum, CSF, ICAM-1, VCAM-1, PECAM-1

THE EFFECT OF INFLAMMATION ON STROKE

Current concepts of the pathomechanism of stroke indicate the significance of cerebral ischaemia-induced local inflammatory reaction to the brain damage. Endotheliocytes of ischaemic brain microvasculature secrete inflammatory mediators attracting peripheral blood leukocytes to the cerebrovascular milieu [23, 60]. Adhesion of leukocytes to the endothelium occludes cerebral capillaries impairing microcirculation [42, 59]. Phospholipase activation in leukocytes results in synthesis of prostaglandins, leukotrienes, eicosanoids, and platelet-activating factor, which can cause vasoconstriction and increase platelet aggregation.

Ischaemic brain-infiltrated leukocytes produce a cohort of biotoxic compounds like proteolytic enzymes, toxic oxygen metabolites and cytokines that contribute to the cerebral tissue injury, including secondary lesion of the ischaemic neurones [7, 24, 25].
With regard to the above-mentioned involvement of inflammatory cells in promotion and extension of brain ischaemic damage, the central role of endothelial-leukocyte interactions mediated by such bioactive substances as adhesion molecules appears ever more critical in stroke-induced inflammatory reaction.

AN OUTLINE OF ADHESION MOLECULES NATURE

Adhesion molecules are cell surface proteins involved in interactions between cells. They participate in many biological processes, such as attachment, proliferation, migration and cellular growth. They are divided into three primary groups: selectins, immunoglobulin gene superfamily (IgSF) and integrins. Soluble forms of adhesion molecules exist as a result of proteolytic cleavage from the cell surface and may be measured in body fluids such as serum or cerebrospinal fluid (CSF). Adhesion molecules mediate attachment and transendothelial migration of leukocytes, being an important phenomenon involved in the inflammatory processes, including stroke-induced inflammation [2, 8, 24, 45]. Interactions between cell adhesion molecules of leukocytes and the endothelium in the region of evolving inflammation appear in the order of sequence [6, 15]. Selectins mediate initial attachment of flowing leukocytes to the blood vessel and rolling them along the endothelial surface [8]. These interactions are transient and reversible and do not lead to firm adhesion and transendothelial migration of leukocytes unless members of the IgSF are involved [15, 58]. Thus, we focused on IgSF, which — together with their counterparts, i.e. the integrins — have a key significance in leukocyte adhesion, migration and brain infiltration in stroke-induced inflammatory reaction.

IgSF is the most abundant family of cell surface molecules, accounting for 50% of leukocyte surface proteins. Their structure is characterised by repeated domains, similar to those found in immunoglobulins, built from a tightly packed barrel of beta strands. By mutation and selection, the Ig domain has evolved to serve many different functions including: receptors for growth factors; receptors for the Fc region of Ig; and as adhesion molecules, which now seems to be the function of the majority [28].

The principal members of IgSF are: intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion molecule-1 (PECAM-1).

ICAM-1

ICAM-1 is a 90–115 kDa protein expressed in several organs, including cerebral endothelium and leukocytes [46]. The expression can be stimulated by such proinflammatory cytokines as tumour necrosis factor-alpha (TNF-alpha), interleukin-1-beta (IL-1-beta), and interferon-gamma (IFN-gamma) [1, 54]. Proinflammatory cytokines TNF-alpha and IL-1-beta are induced following brain ischaemia earlier than adhesion molecules [52]. The development of inflammatory response in cerebral ischaemia, therefore, appears to follow the sequence: expression of TNF-alpha and IL-1-beta, adhesion molecules cascade, leukocyte adhesion followed by migration [52]. ICAM-1 upregulation in cultured human cerebromicrovascular endothelial cells has been demonstrated by 4–24 h exposure to TNF-alpha or IL-1 beta, and by a 4 h exposure to stimulated in vitro ischaemia [48].

ICAM-1 mRNA expression has been shown in brain microvascular endothelial cells during hypoxia/reoxygenation [27]. Upregulation of ICAM-1 in microvessels in the ischaemic zone of focal brain ischaemia has been reported in experimental [38] or post mortem [46] studies. Clark et al. [12] showed that the expression of ICAM-1 has been significantly elevated on the microvasculature within 1 h after global ischaemia in rats and that this persisted for several days. They also demonstrated an association between the time of ICAM-1 brain microvessel expression and the time of leukocyte subsets infiltration to the ischaemic forebrain (neutrophils within the first 24 h and monocytes up to 7 days after ischaemia). Lindsberg et al. [31] performed human brain autopsy study of patients who died within 15 h to 18 days of an acute stroke, demonstrating ICAM-1 upregulation on microvessels in areas of infarction by 1.8 days after ischaemia. Moreover, they found that neutrophils accumulation in the brain infarcted region was observed as early as 15 h in amounts of 11.3 cells/mm² compared with 0.5 cells/mm² in control areas and exceeded 200 cells/mm² by 2.2 days, depicting a correlation between ICAM-1 expression and leukocyte migration.

These data indicate the involvement of ICAM-1 in the sequence and timing of the infiltration of inflammatory cells to the ischaemic brain region after stroke, which was well documented in both animal models [30] and in humans [3]. Within hours of cerebral infarction, neutrophils are the first peripheral blood leukocytes that begin to migrate into the ischaemic tissue [30] and their response reaches its
maximum usually 24–48 h after stroke [3]. An influx of monocytes begins within 24 h and its maximum value is observed several days later [30].

Increased sICAM-1 levels have been found in sera of patients within the first 24 h of stroke in comparison with healthy age-matched control individuals [44]. Bitsch et al. [9] determined serum sICAM-1 levels in patients with cerebral ischaemia at the study entry (the sudden emergence of a focal neurological deficit of shorter than or equal to 12 h duration has been the criterion for inclusion), after 12 h, 24 h, 5 days for transient ischaemic attack (TIA), and additionally after 14 days for stroke. They revealed that in patients with stroke but not in patients with TIA, sICAM-1 peaked in serum within 24 h of the onset of neurological signs. This may reflect the pathogenetic differences between these two conditions with respect to a more prominent endothelial activation and subsequent molecule expression in stroke compared with TIA.

Fassbender et al. [16] serially measured sICAM-1 levels in sera of stroke patients from 4 h to 5 days after the disease onset as well as in sera of control group, which consisted of non-stroke patients with risk factors for atherosclerosis and healthy subjects. They reported that concentrations of sICAM-1, already increased in subjects with vascular risk factors, did not elevate further in stroke patients. This is consistent with reports of serum increased levels of sICAM-1 in diabetes mellitus [19, 40] and with a strong upregulation of ICAM-1 observed in atherosclerotic vessels [14]. It may indicate involvement of ICAM-1 not only in stroke-induced acute inflammatory reaction but also in chronic endothelial inflammation, which has appeared already during atherogenesis.

Clark et al. [11] reported decreased serum levels of sICAM-1 and increased neutrophils adhesion to laminin (major glycoprotein of endothelial basement membrane) [56] which is used to attach neutrophils to collagen [49] in patients with acute (< 72 h after onset) ischaemic stroke compared with controls or individuals with vascular risk factors. They hypothesised that neutrophils in patients with acute stroke may have a high number of adhesion molecule receptors and hence a higher degree of sICAM-1 binding. Indeed, increased adhesiveness of neutrophils was detected in patients within the first three days of ischaemic stroke [21].

In genetically engineered mice that lack ICAM-1, brain infarct volume is significantly reduced after transient middle cerebral artery occlusion compared with normal animals [13]. This effect seems to be elicited with elimination of ICAM-1-mediated adhesion and transmigration of leukocytes; as Pozzilli et al. [39] demonstrated, the elevation of peripheral blood leukocytes counts observed after cerebral infarction reflected the degree of the inflammatory response in the acute phase of ischaemic stroke and was related to the extent of the local cerebral damage measured with computed tomography of the brain.

**VCAM-1**

VCAM-1 is a 90–110 kDa protein. In contrast to ICAM-1, VCAM-1 is absent in resting endothelial cells, but — similarly to ICAM-1 — can be upregulated by several proinflammatory cytokines like TNF-alpha, IL-1-beta or interferon-gamma [43]. Cultured cerebral endothelial cells express VCAM-1 after in vitro stimulation by TNF-alpha and IL-1-beta or simulation of ischaemia in the manner similar to ICAM-1 [48].

The immunocytochemical study of brain tissue from patients who died following acute ischaemic stroke has shown intense expression of VCAM-1 by endothelial cells and astrocytes from the infarcted, but not the non-infarcted, areas as well as only weak expression of ICAM-1 [10, 29]. Moreover, Blann et al. [10] reported increased serum sVCAM-1 but not sICAM-1 levels in patients within acute phase of stroke (the measurements were performed below 12 h from the onset of neurological signs) compared with healthy controls and subjects with carotid atherosclerosis as well as still high levels at 3-month follow-up. These findings not only suggest VCAM-1 expression and release in both the acute and chronic stages of ischaemic stroke but they also indicate involvement of VCAM-1 in complex pathophysiological responses to infarction and repair of brain tissue following stroke.

Fassbender et al. [16] reported that patients with acute stroke displayed significantly increased sVCAM-1 levels in serum compared with subjects with vascular risk factors. This may reflect acute upregulation of the molecule during stroke early phase and its shedding at sites of ischaemic cerebral tissue lesion. Bearing in mind that VCAM-1 mediate firm adhesion, activation and subsequent passage of leukocytes into the region of evolving inflammation [20, 53], the early increase in sVCAM-1 levels in serum of stroke patients is in accordance with observations of the neutrophil extravasation as early as 30 to 60 minutes in microvessels after experimental middle cerebral artery occlusion [22, 32, 59].
Fassbender et al. [16] added the remark that the increase in sVCAM-1 concentrations in serum of stroke patients observed as early as at 4 h after the disease onset persisted until day 5. Bitsch et al. [9] also reported that sVCAM-1 reached a maximal level in serum of stroke patients 5 days after the onset of neurological signs. Moreover, they emphasised that this characteristic peak of sVCAM-1 level in serum was only found in patients after stroke but not after TIA. It is consistent with a study performed by Sörnäis et al.[47], who demonstrated that peak monocyte accumulation in cerebrospinal fluid (CSF) occurred between 3 and 7 days after stroke in humans. These data support a consideration that VCAM-1 may contribute not only to the early infiltration of neutrophils into the brain ischaemic region but also to the second invasion of inflammatory cells, which involves primarily the migration of monocytes which begin to invade the brain within 24 h of stroke and reaches a maximum several days later [30].

**PECAM-1**

PECAM-1 is a 130-kDa protein constitutively expressed on endothelial cells, platelets, neutrophils, monocytes, lymphocytes and basophils [18, 35, 50]. It appears in large amounts as a major constituent of the endothelial cell intercellular junctions [5, 33, 34], where up to 10^6 of PECAM-1 molecules [36] are concentrated. In contrast to other members of IgSF, expression of PECAM-1 is not altered by cytokines TNF-alpha and IL-1-beta treatment [26, 41, 55]. This is in accordance with the concept suggesting that the involvement of PECAM-1 in adhesion molecules cascade depends at least in part on integrin signalling during endothelial-leukocyte interactions [4, 20].

A number of studies indicate PECAM-1 to be the molecule directly engaged in the process of transendothelial migration of leukocytes [35, 37, 50, 51]. Müller et al. [35] were the first to show, in a quantitative in vitro assay of transendothelial migration, that pretreating neutrophils or monocytes with antibodies specific for PECAM-1 inhibited their migration across the endothelial cell monolayer. They also showed that blocking endothelial cell junctional PECAM-1 effectively inhibited leukocyte transmigration, indicating that PECAM-1 molecules on both the endothelial cell as well as the leukocyte side contributed to the transmigration process.

PECAM-1, in contrast to other adhesion molecules, has an ability to bind to itself [17]. Therefore the process of transendothelial migration of leukocytes through intercellular junctions of endothelial cells — where the molecule is largely present — is mediated at least in part by homophilic adhesive interactions that take place between leukocyte and endothelial cell junctional PECAM-1 molecules [51].

So far, investigations on PECAM-1 in experimental animal models of cerebral ischaemia or in infarcted human brain have not been performed. However, we have recently studied sPECAM-1 levels in serum and CSF in 23 patients with first-ever in a lifetime completed ischaemic stroke within 24 h of the onset of neurological signs. Serum and CSF from 15 individuals, who were age- and sex-matched with the stroke patients, served as a control group, which consisted of 9 patients diagnosed with tension headache and 6 patients with anxiety neurosis. Stroke patients displayed statistically significantly higher levels of sPECAM-1 in sera and CSF in comparison with control group. Furthermore, the stroke patients presented significantly higher levels of sPECAM-1 in sera than in CSF and these levels were correlated between each other. The results of our study directly suggest that PECAM-1 may play a role in the inflammatory events during the early phase of ischaemic stroke [57].

**CONCLUSIONS**

We believe that the data of IgSF so far obtained from stroke patients depict only a part of events appearing in stroke-induced inflammatory reaction. Nevertheless, the data described above indicate the involvement of IgSF in the pathomechanism of stroke and show that these dynamically changing molecules play an important role in the regulation of the inflammatory process after stroke.

**REFERENCES**