Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage: the immunoglobulin and selectin superfamilies

J J Nissen, D Mantle, B Gregson, A D Mendelow

Abstract

Objectives—Adhesion molecules are involved in the pathogenesis of cerebral ischaemia and may play a part in the pathophysiology of delayed ischaemic neurological deficit (DIND) after aneurysmal subarachnoid haemorrhage. It was hypothesised that after aneurysmal subarachnoid haemorrhage, adhesion molecules may play a part in the pathophysiology of DIND as reflected by significantly altered serum concentrations in patients with and without DIND.

Methods—In a prospective study, mean serum concentrations of ICAM-1, VCAM-1, PECAM, and E, P, and L-selectin were compared between patients without (n=23) and with (n=13) DIND in patients with World Federation of Neurological Surgeons (WFNS) grades 1 or 2 subarachnoid haemorrhage. Serum was sampled from patients within 2 days of haemorrhage and on alternate days until discharge. Concentrations of adhesion molecules were measured by standard procedures using commercially available enzyme linked immunoabsorbent assays.

Results—There were non-significant differences in serum concentrations of ICAM-1 (290.8 ng/ml v 238.4 ng/ml, p=0.0525), VCAM-1 (553.2 ng/ml v 425.8 ng/ml, p=0.053), and PECAM (22.0 ng/ml v 21.0 ng/ml, p=0.56) between patients without and with DIND respectively. The E-selectin concentration between the two patient groups (44.0 ng/ml v 37.4 ng/ml, p=0.33) was similar. The P-selectin concentration, however, was significantly higher in patients with DIND compared with those patients without DIND (149.5 ng/ml v 112.9 ng/ml, p=0.039). By contrast, serum L-selectin concentrations were significantly lower in patients with DIND (633.8 ng/ml v 897.9 ng/ml, p=0.013).

Conclusions—Of all the adhesion molecules examined in this study, P and L-selectin are involved in the pathophysiology of DIND after aneurysmal subarachnoid haemorrhage.

Keywords: adhesion molecule; delayed ischaemic neurological deficit; subarachnoid haemorrhage; selectin; immunoglobulin

Delayed ischaemic neurological deficit (DIND) remains the leading cause of death and disability after aneurysmal subarachnoid haemorrhage. Although nimodipine prophylaxis reduces the incidence of cerebral infarction from about 33% to 22% of patients with subarachnoid haemorrhage,1 further strategies are needed to ameliorate the devastating neurological effects of this poorly understood phenomenon.

In the pathogenesis of cerebral ischaemia and reperfusion injury, the importance of leucocyte mediated tissue damage has been shown in both animal and human studies.2 3 Recruitment of circulating leucocytes and their interaction with the vascular endothelium involves cellular adhesion molecules (CAMs)—a diverse set of macromolecules—the functions of which are pivotal in this process.

In leucocyte-endothelial interactions, the selectin superfamily, the immunoglobulin-like superfamily, and the integrins all play a part.

The selectins (P, E, and L-selectin) are transmembrane glycoproteins expressed on activated vascular endothelium (P and E), activated platelets (P), and leucocytes (L), and are involved in rolling and activation of leucocytes. The immunoglobulin-like superfamily (intercellular adhesion molecules, ICAM-1, ICAM-2, ICAM-3, vascular cell adhesion molecule VCAM-1, platelet-endothelial adhesion molecule, PECAM) are expressed by activated endothelium and act via binding to leucocyte transmembrane proteins—the integrins. This immunoglobulin-integrin interaction is responsible for leucocyte-endothelial firm adherence and subsequent leucocyte migration through the endothelium.

Release of soluble adhesion molecules (sCAMs) into the circulation is known to occur in many diseases.4 Although the function of these “shed” molecules is unknown, serum concentrations may correlate with outcomes in various clinical circumstances including severe trauma5 and septic shock.6 Adhesion molecules have been demonstrated in the pathogenesis of various CNS disorders including bacterial meningitis,7 encephalitis,8 multiple sclerosis,9 and cerebral ischaemia,10 but little work has investigated the role of adhesion molecules in delayed ischaemia after subarachnoid haemorrhage. Leucocytes have been found in the structural arteriopathy associated with DIND11-14 and in brain infarction,15 16 and therefore adhesion molecules may play a role in the pathophysiology of delayed ischaemia.
There is evidence from both animal and human studies to support roles for ICAM-1, VCAM-1, PECAM, and E, P, and L-selectins in cerebral endothelial and ischaemic brain injury\textsuperscript{14,15} and so the aim of this study was to evaluate the concentration of the immunoglobulin and selectin families of adhesion molecules in DIND. The hypothesis was that after aneurysmal subarachnoid haemorrhage, the selectin and immunoglobulin superfamilies of adhesion molecules may play a part in the pathophysiology of DIND as reflected by significantly altered serum concentrations in patients with DIND versus patients without DIND.

In a prospective study, we have compared serum concentrations of ICAM-1, VCAM-1, PECAM, and E, P, and L-selectin in patients without (group A) and with (group B) delayed ischaemia after aneurysmal subarachnoid haemorrhage.

**Patients and methods**

Ethical approval for the study was obtained from the joint ethics committee of the Newcastle and North Tyneside Health Authority. Informed written consent was obtained for all patients included in the study.

**PATIENTS**

We studied patients referred to the Neurosurgery Unit at Newcastle General Hospital from July 1997 to November 1998 with subarachnoid haemorrhage established by CT or lumbar puncture who were grade 1 or 2 on the World Federation of Neurological Surgeons (WFNS) scale.\textsuperscript{7} All patients were given oral nimodipine prophylaxis for DIND from admission and all had a craniotomy with clipping of intracranial aneurysms.

**DELAYED ISCHAEMIC NEUROLOGICAL DEFICIT**

We defined delayed ischaemic neurological deficit as a new, focal delayed onset neurological deficit not attributable to other causes, with an appropriate rise in middle cerebral arterial transcranial Doppler velocity to greater than 120 m/s, or MCA/ICA ratio of 3 or greater. Angiography was not routinely used for the diagnosis of DIND. Patients with DIND were treated with hypervoleaemia and induced hypertension using inotropes where necessary (DIND refractory to hypervoleaemia). Non-symptomatic rises in Doppler velocities were not considered to be diagnostic of DIND. Clinicians were not aware of sCAM concentrations and analysis of the samples was performed blind to clinical details.

We excluded patients with intercurrent medical conditions or nosocomial infection in which adhesion molecules may play a part. In addition, patients with significant postoperative intracerebral haemorrhage or treatment with endovascular obliteration of ruptured aneurysms were excluded.

**SERUM SAMPLES**

For each patient, initial serum samples were taken within 48 hours of admission (and always before craniotomy) and subsequent samples on alternate days thereafter until discharge. Specimens were allowed to coagulate and then were centrifuged at 3000 rpm for 12 minutes and the supernatant stored at −40°C before analysis.

We assayed serum concentrations of adhesion molecules using commercially available enzyme linked quantitative sandwich immunoabsorbant assay (ELISA) kits (R and D Systems, Abingdon, UK) in accordance with the supplier’s instructions. The assays involved the simultaneous reaction of sCAM (in sample or standard) with monoclonal antibody pre-coated on the walls of microtitre plate wells, and to an unbound second antibody directed against a different molecular epitope. The second antibody was conjugated to horseradish peroxidase. After removal of unreacted reagents, bound sCAM-HRP antibody was detected by reaction with a horseradish peroxidase specific substrate (tetramethylbenzidine), which yielded a coloured product, proportional to the concentration of sCAM (determined relative to an appropriate standard curve). Absorption measurements were carried out at 450 nm (and correction wavelength of 650 nm to eliminate optical imperfections in the plate) using a microtitre plate reader (Dynatech MR5000). We diluted serum samples 20–100 fold (depending on the analyte) before analysis.

**STATISTICAL ANALYSIS**

The mean of means were compared between the two groups. Unpaired $t$ tests were used to assess the null hypothesis. A $p$ value <0.05 was used to indicate statistical significance.

**Results**

We included 36 patients in the study out of a possible total of 46 (10 were excluded, eight for nosocomial infection and two for postoperative intracerebral haemorrhage). Twenty three patients (group A) did not develop DIND and 13 patients did (group B). There were 23 women and 13 men, age range 25–74 years. The median number of serum samples/patient was five (no DIND) and eight (DIND). This reflects the longer stay in hospital of patients with DIND.

Mean serum concentrations of ICAM-1 and VCAM were lower in patients with DIND than in those without DIND, but this did not reach

---

**Table 1 Mean serum concentrations of adhesion molecules in patients without and with delayed ischaemic neurological deficit (DIND)**

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1 (ng/ml)</th>
<th>VCAM-1 (ng/ml)</th>
<th>PECAM (ng/ml)</th>
<th>E-selectin (ng/ml)</th>
<th>P-selectin (ng/ml)</th>
<th>L-selectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DIND (SE)</td>
<td>290.8 (16.8)</td>
<td>553.2 (42.6)</td>
<td>22.0 (1.1)</td>
<td>44.0 (4.3)</td>
<td>112.9 (8.1)</td>
<td>897.9 (53.6)</td>
</tr>
<tr>
<td>DIND (SE)</td>
<td>238.4 (19.0)</td>
<td>425.8 (42.6)</td>
<td>21.0 (1.4)</td>
<td>37.4 (4.9)</td>
<td>149.5 (17.0)</td>
<td>633.8 (93.7)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.0525</td>
<td>0.052</td>
<td>0.56</td>
<td>0.33</td>
<td>0.039*</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

*Statistically significant.
Table 2  Mean serum concentrations of adhesion molecules in patients not requiring and requiring dopamine in delayed ischemic neurological deficit (DIND)

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1 (ng/ml)</th>
<th>VCAM-1 (ng/ml)</th>
<th>PECAM (ng/ml)</th>
<th>E-selectin (ng/ml)</th>
<th>P-selectin (ng/ml)</th>
<th>L-selectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No dopamine</td>
<td>230.6</td>
<td>385.5</td>
<td>460.3</td>
<td>20.59</td>
<td>19.9</td>
<td>37.0</td>
</tr>
<tr>
<td>Dopamine</td>
<td>245.1</td>
<td>460.3</td>
<td>19.9</td>
<td>37.0</td>
<td>110.9</td>
<td>462.3</td>
</tr>
<tr>
<td>p Value</td>
<td>0.72</td>
<td>0.41</td>
<td>0.84</td>
<td>0.94</td>
<td>0.005*</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

*Statistically significant.

statistical significance (table 1). The concentration of PECAM demonstrated no significant difference (22.0 ng/ml v 21.0 ng/ml, p=0.56) and mean serum concentrations of E-selectin showed no significant difference between group A (44.0(SE 4.3) ng/ml) and group B (37.4(SE 4.9) ng/ml) (p=0.33).

There were, however, significant differences in P-selectin and L-selectin concentrations between the two groups. For P-selectin, serum concentrations were significantly higher for patients with DIND than patients without DIND (149.5 (SE 17.0) ng/ml v 112.9 (SE 8.14) ng/ml, p=0.039). For L-selectin, serum concentrations were significantly lower for patients with DIND (897.9 ng/ml (SE 53.6 v 622.8 ng/ml (SE 93.7, p=0.013, table 1).

In patients with DIND, there was no significant difference in serum concentrations of L-selectin between samples taken before DIND onset, versus samples after onset of DIND (606.2 (SE 87.8) ng/ml v 667.8 (SE 104.8) ng/ml, p=0.1). Although there was a trend towards higher P-selectin concentrations after onset of DIND, this did not reach significance (125.5 (SE 13.5) ng/ml v 160 (SE 21.6) ng/ml, p=0.16).

In 12 patients with DIND, seven required dopamine for induced hypertension. There was no statistical difference in ICAM, VCAM, PECAM, and E-selectin between patients requiring dopamine and those not requiring dopamine (table 2). Dopamine use decreased both P-selectin concentration (195.3 ng/ml v 110.9 ng/ml, p=0.005) and L-selectin concentration (833.9 ng/ml v 462.3 ng/ml, p=0.04).

The mean peripheral white cell count showed no significant difference (10.9 v 11.0) whereas mean platelet count showed a significant difference between groups A and B (262 (SE 14.9) v 349 (SE 30.8), p=0.008), (no DIND v DIND respectively).

Discussion
CEREBRAL ISCHAEMIA, LEUCOCYTES, AND ADHESION MOLECULES
Evidence for a pathological role of leucocytes and adhesion molecules in cerebral ischaemia-reperfusion injury exists in both animal and human studies. Animal studies have shown that induced neutropenia can protect against ischaemia-reperfusion injury.21 Okada et al have shown transient expression of ICAM-1 and persistent upregulation of P-selectin in a primate focal ischaemia-reperfusion model. P-Selectin was detected from both postcapillary microvascular endothelium, and from platelets. Non-endothelial, platelet derived P-selectin was significantly correlated with platelet accumulation.22 In other rat models, persistent ICAM-1 expression has been shown in a non-reperfusion model,13 and Clark et al demonstrated early (6 to 24 hours) endothelial and late (4 to 7 days) parenchymal expression of ICAM-1 in forebrain ischaemia-reperfusion and these correlated temporally and topographically with leucocyte accumulation. Wang et al24 showed increased E-selectin mRNA in ischaemic rat cortex compared with non-ischaemic cortex and E-selectin upregulation has been shown in a rat MCA occlusion-reperfusion model.25 Using immunohistochemical staining, Lindsberg et al26 found upregulation of endothelial ICAM-1 expression in adult human infarcts compared with both non-infarcted hemispheres and control brains.

By using antiadhesion strategies, a role of CAMs in cerebral ischaemia has also been shown using experimental models of both permanent and transient cerebral ischaemia. Anti-adhesion therapies to ICAM-1 and integrin subunits have been shown in these models to be protective, with reduction in infarct size.27–29

SERUM ADHESION MOLECULES AND CEREBRAL ISCHAEMIA
Circulating sCAMs have been investigated after human stroke. Shyu et al4 found raised ICAM-1 but normal E-selectin within 24 hours of ischaemic stroke in adults. Bitsch et al2 found a peak in expression of ICAM-1 at 24 hours, VCAM at 5 days, and a fall in E-selectin over 5 days. By contrast, ICAM1 and VCAM were not raised in the study by Frijns et al, but E and P-selectin were raised 24 hours after stroke.15

ADHESION MOLECULES IN SUBARACHNOID HAEMORRHAGE
The role of adhesion molecules in subarachnoid haemorrhage is less clear. Few studies have investigated the changes in adhesion molecules in subarachnoid haemorrhage. In a rat cisterna magna model of subarachnoid haemorrhage, Handa et al50 showed induction of ICAM-1 expression on the endothelial and medial layers of the basilar artery with a correlation between the degree and timing of basilar spasm and leucocyte inflammatory response within the vessel. Sills et al51 demonstrated in a rat femoral artery model of vasospasm that there was early induction of ICAM-1 on the endothelium and showed a direct correlation with an inflammatory response within the vessel wall. Anti-ICAM-1 monoclonal antibodies were given via an intraperitoneal route in the same rat femoral artery model by Oshiro et al who was able to demonstrate reduced arterial narrowing and less inflammatory infiltrate within the periadventitia. More recently, Polin et al52 have shown significantly increased concentrations of ICAM-1, E-selectin, and VCAM-1 in the CSF in 17 patients with subarachnoid haemorrhage undergoing craniotomy, compared with 16 controls. Concentrations of L-selectin were slightly but not significantly increased. Three patients developed severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with severe or moderate angiographically demonstrated vasospasm and had higher concentrations of E-selectin than other patients with.
subarachnoid haemorrhage. After cisternal subarachnoid haemorrhage in rabbits, Bavbeck et al4 showed that cisternal administration of anti-ICAM-1 antibody or anti-CD18 antibody attenuated vasospasm. When given in combination, the effect was additive.

In this study, we have shown significant differences in serum selectin concentrations between patients who do, and do not develop delayed ischaemic neurological deficits after aneurysmal subarachnoid haemorrhage. Although ICAM-1 and VCAM differences did not reach significance, this may reflect the relatively small patient population. E-Selectin did not show any significant difference between the two groups, but P-selectin concentration was significantly higher and L-selectin concentration significantly lower in those patients with delayed ischaemia.

As this is the first report of serum concentrations of adhesion molecules after subarachnoid haemorrhage, it is difficult to relate our findings to previous CAM studies in subarachnoid haemorrhage. In comparison with studies of sCAM in human stroke, our findings are consistent with raised P-selectin demonstrated by Frijns et al18 and with the reduction in L-selectin in patients with stroke over time as demonstrated by Passbender et al.13 For the other CAMs, comparison is more difficult as for example, ICAM-1 has been shown to both rise,16 fall,17 and remain unchanged18 after stroke.

Serum concentrations of shed adhesion molecules may not reflect true local tissue concentrations,3 and measurable sCAM will depend on the complex balance between production of de novo CAM or release of preformed CAM, cleavage from the cell membrane, adhesion to counter-receptors, and clearance from the circulation. On the basis of serum concentrations of the selectins and immunoglobulin superfamilies analysed, we conclude that P and L-selectins may be involved in the pathophysiology of delayed ischaemia. Whether these changes are causal in DID, an epiphenomenon or simply a part of the complex cascade of events in DID requires further study. Similarly, the contribution of platelet derived versus endothelial derived P-selectin is unknown in our patients and further research is needed to ascertain the origin of the P-selectin. Certainly, a platelet origin would be consistent with both the significant rise in peripheral platelet count seen in our patients with DIND and with known evidence suggesting platelet involvement in the pathogenesis of DIND.16–17 The reduction in measurable L-selectin may reflect reduced availability in serum due to binding to receptor ligands by activated leucocytes. It is also possible that this results from the hyper-volaemia used as part of the treatment for DIND. This is, however, unlikely as no other sCAM concentration fell significantly. Seven of 12 patients with DIND also required dopamine for induced hypertension. Catecholamine inotropes are known to be anti-inflammatory and may alter the expression of adhesion molecules.38–40 For ICAM, VCAM, PECAM, and E-selectin, no difference was found in sCAM between patients requiring and not requiring dopamine. However, significant reductions in serum concentrations of P and L-selectin were seen. This may be confounded due to the small numbers of patients but may be due to an anti-inflammatory effect of dopamine. Unfortunately, patients requiring dopamine were refractory to haemodilution alone and may therefore have more “severe” DIND. In the absence of cerebral blood flow studies, we cannot comment further on this and further research is needed to investigate the effects of inotropes and cerebral expression of CAMs.

Interestingly, there were no statistically significant differences in the sCAM concentrations for P and L-selectins before the development of symptomatic DIND compared with after the onset of symptoms. This may therefore reflect early activation of endothelial cells, leucocytes, or platelets in patients before the onset of clinical symptoms of DIND.

Due to the complex factors determining serum concentrations of adhesion molecules, we cannot exclude a role for ICAM-1, VCAM-1, PECAM, and E-selectin, and further research is required to ascertain this.

The importance of these findings is that they offer a potential therapeutic target for patients with subarachnoid haemorrhage. Because DIND typically occurs between 4 and 14 days after onset of SAH, the opportunity exists for prophylactic anticipatory therapy. Identifying effective methods of prophylaxis obviously depends on knowledge of the pathophysiology of DIND and we present the first human serum evidence of a change in concentration of adhesion molecules in delayed cerebral ischaemia.

In conclusion, on the basis of serum concentrations of adhesion molecules, P and L-selectins may be involved in the pathophysiology of delayed ischaemic neurological deficit after aneurysmal subarachnoid haemorrhage. Further study is required to elucidate the relevance of serum concentrations of adhesion molecules compared with local cerebral concentrations, to determine the relative contributions of platelets, leucocytes, and endothelium in CAM production, and to determine whether antiadhesion therapy will attenuate delayed ischaemia after aneurysmal subarachnoid haemorrhage.

We acknowledge the contributions of Tom Wooldridge, Alison Blackburn, and Jane Barnes to the study. This research was supported by research grants from the Royal Victoria Infirmary Special Trustees and the Northern Brainwave Appeal.

Immunoglobulin, selectin adhesion molecules, and delayed ischaemic neurological deficit

6 Newman W, Beall LD, Carson CW. Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in serum of patients with septic shock. J Immunol 1993;150:54–52.


