The Stroke Trial – can we predict clinical outcome of patients with ischemic stroke by measuring soluble cell adhesion molecules (CAM)?

Arnon Blum[1], Khaled Khazim[1], Monir Merei[1], Aviva Peleg[1], Nava Blum[2], Vladimir Vaispapir[1]

[1] Department of Internal Medicine A, the Neurological Unit, Baruch-Padeh Poria Medical Center, Lower Galilee 15208, Israel
[2] School of Public Health, Haifa University, Haifa, Israel

Correspondence : A. Blum
<navablum@hotmail.com>

ABSTRACT. ***Background.*** **Several studies have found that an increased concentration of haemostatic or inflammation markers was associated with worse prognosis in vascular disease. The inflammatory components in ischemic stroke are of current interest, and there is some experimental evidence that they may be linked.** ***Hypothesis.*** **The study was performed to determine the association between the neurological clinical outcome and levels of cell adhesion molecules in the first four days of hospitalization in patients with acute ischemic event.** ***Methods.*** **This prospective, pilot, case-controlled study examined the association between the clinical outcome and inflammatory markers within the first few days of hospitalization. The neurological evaluation was performed using the NIH score on admission and four days later, and levels of cell adhesion molecules were measured by ELISA methods on admission and four days later.** ***Results.*** **Twenty three patients with an acute cerebral event (mean age 71 ± 15 y, 12 women and 11 men) were examined neurologically on admission and four days later. Among 19 patients who improved, there was a significant decrease in the NIH neurological scale, from 3.8 ± 3.2 to 1.3 ± 1.8 (p = 0.01), which was accompanied by a significant decrease in the cell adhesion molecules that were measured (E-selectin, ICAM-1 and VCAM-1). Of the four patients who did not improve, their mean clinical NIH score was 10 ± 4.6 and worsened or remained unchanged after four days of follow-up. In this group, we could not demonstrate a significant change in levels of cell adhesion molecules between days one and four.** ***Conclusions.*** **Patients who improved clinically within the first four days of hospitalization demonstrated a remarkable inhibition of all three cell adhesion molecules that were measured (E-selectin, ICAM-1, and VCAM-1). Patients who did not improve had more severe cerebral infarcts, a higher NIH score on admission (10 ± 4.6), and no change was observed in levels of cell adhesion molecules during the follow-up period. Measuring cell adhesion molecule levels may predict objectively the clinical outcome in hospitalized patients with acute ischemic stroke.**

Keywords: CVA, cell adhesion molecules (CAM)

The onset of cerebral ischemia triggers a cascade of pro-inflammatory molecular and cellular events. Clinical studies suggest that the strength of this acute response is important in early and late clinical outcomes, early clinical worsening, and extent of brain damage [1]. A significant correlation was noted between high baseline, sensitive C-reactive protein (hs-CRP), ESR, and adhesiveness/ aggregation as well as the outcome of the ischemic neurological event as determined by the modified Rankin scale, 8-12 months following the insult [2].

In this preliminary study, the association between the clinical outcomes of hospitalized patients with acute cerebral ischemic attack and levels of cell adhesion molecules were examined, as well as the association between the change in the clinical outcome within the first four days and levels of cell adhesion molecules.

METHODS

This was a prospective, pilot, case-controlled study, in which 23 patients with an acute cerebral attack (mean age 71 ± 15 y, 12 women and 11 men) were studied and followed for four days. All signed a consent form before enrollment.

The inclusion criteria included patients who had suffered an acute ischemic cerebro-vascular accident (CVA). All underwent a brain computed tomography (CT) examination within the first 24 hours of admission.

Exclusion criteria included age above 75 years old, congestive heart failure or myocardial disease, acute myocardial infarction or acute coronary event, known malignancy and chronic renal failure (creatinine level above 2 mg), unconscious state, and a hemorrhagic stroke.

A neurological clinical score was performed according to the NIH score on admission and four days later. The neurological evaluation was performed by an experienced neurologist and the criteria for staging were in accordance with the NIHSS (NIH Stroke Score) guidelines [3].

The study was approved by the Internal Review Board of the hospital and all study participants signed a consent form before enrollment.

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METHODS

E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) were measured by ELISA (R&D Systems, Inc. 614 McKinley Place N.E. Minneapolis, MN 55413, USA). Fasting blood samples were taken on entry to the study and four days later. The blood was separated and frozen at -80^{0}C, and was processed later as one batch. The laboratory technician was blinded to patients' identity.

Statistical methods

The two-tailed, paired Student's t-test was used for analysis. A p-value of < 0.05 was considered statistically significant.

RESULTS

Twenty three patients with an acute cerebral event (mean age 71 ± 15 y, 12 women and 11 men) took part in a prospective, pilot, case-controlled study. They were all followed by a neurological examination on admission and four days later, while they were still hospitalized. Nineteen patients improved clinically (9 women and 10 men) within the first four days of hospitalization, while four patients deteriorated.

Patients who showed clinical improvement

Among the 19 patients who improved, there was a statistically significant decrease (improvement) in the NIH neurological scale, from 3.8 ± 3.2 to 1.3 ± 1.8 ($p = 0.01$). There was a statistically significant decrease in all cell adhesion molecules measured in this group on the 4th day compared with the levels on admission: E-selectin was decreased from 88.57 ± 40.69 ng/mL to 64.89 ± 26.93 ng/mL [$p = 0.003$], ICAM-1 was decreased from 316.84 ± 88.82 ng/mL to 282.31 ± 86.79 ng/mL [$p = 0.002$], and VCAM-1 was decreased from 668.31 ± 302.18 ng/mL to 583.94 ± 221.17 ng/mL [$p = 0.005$].

The individual data for each patient are presented in *table 1*.

Table 1
Patients who improved

VCAM-1			**ICAM.1**			**E-5ELECTIN**	
Entrv	**4 days**		**Entrv**	**4 dayS**		**Entrv**	**4 days**
470	400		300	260		70	64
480	440		260	240		114	94
450	400		420	420		82	68
608	480		340	340		116	80
880	640		350	240		40	36
480	360		260	200		68	50
600	575		320	340		112	86
560	440		340	240		180	36
400	480		260	320		54	50
900	800		300	320		78	62
660	540		420	340		154	124
500	480		280	220		36	30
460	460		320	280		140	100
840	880		280	220		46	41
1640	1200		460	400		68	40
460	460		50	44		95	82
480	420		360	320		82	74
1050	840		280	240		38	28
800	800		420	380		110	88
Mean							
668.31	**583.94**	**Mean**	**316.84**	**282.31**	**Mean**	**88.578**	**64.89**
SD 302.18	**221.17**	**SD**	**88.822**	**86.792**	**SD**	**40.695**	**26.93**
T-test	**0.005658**		**T-test**	**0.002499**		**T-test**	**0.003851**
Patients who did not improve							
VCAM -1			**ICAM -1**			**E-5ELECTIN**	
Entrv	**4 days**		**Entrv**	**4 days**		**Entrv**	**4 days**
450	460		360	360		36	37
260	360		260	260		100	100
460	420		250	220		45	52
500	520		350	300		28	34
Mean							
417.5	**440**	**Mean**	**305**	**285**	**Mean**	**52.25**	**55.75**
SD 107.19	**67.33**	**SD**	**58.02**	**59.72**	**SD**	**32.58**	**30.53**
T-test	**0.494046**		**T -test**	**0.200976**		**T-test**	**0.140243**

Patients who did not show clinical improvement

Among the four patients who did not show clinical improvement, their clinical NIH score was higher than 6 on admission (mean score was 10 ± 4.6 compared with 3.8 ± 3.2 in the group of patients who improved [$p \leq 0.05$]), and worsened or remained unchanged after four days of follow up (mean score of 12.5 ± 2; $p = 0.19$). In this group, all four patients had major infarcts and their NIH score was positively associated with the brain CT findings.

We could not demonstrate a significant change in levels of cell adhesion molecules between day 1 and 4: E-selectin levels increased from 52.58 ± 32.58 ng/mL to 55.75 ± 30.53 ng/mL, but this was not statistically significantly different [$p = 0.14$], ICAM-1 levels changed from 305 ± 58.02 ng/mL to 285 ± 59.72 ng/mL [$p = 0.20$], and VCAM-1 changed from 417.5 ± 107.19 ng/mL to 440 ± 67.33 ng/mL [$p = 0.49$].

The individual data for each patient are presented in *table 1*.

DISCUSSION

The results of our pilot study have shown that there were two groups of patients – patients who improved and patients who did not improve within the first few days of hospitalization.

A) An association was found between the clinical severity (NIH score) and the anatomical brain damage as demonstrated by CT scan.

B) Pf the patients who improved, all had a significant clinical improvement (NIH score changed from 3.8 ± 3.2 to 1.3 ± 1.8 [$p = 0.01$]) within the first four days of hospitalization, and they showed a remarkable inhibition of all three cell adhesion molecules that were measured (E-selectin, ICAM-1, and VCAM-1).

C) Patients who did not improve all had major cerebral ischemic infarcts, all had a high NIH score on admission (mean NIH score of 10 ± 4.6) that worsened in the first few days; no change was observed in levels of cell adhesion molecules during the follow-up period.

Inflammation and ischemic cerebrovascular accidents

Several studies have found that an increased concentration of haemostatic or inflammation markers was associated with a worse prognosis in vascular disease [4-7].

The inflammatory and thrombotic components in ischemic stroke are of current interest, and there is some experimental evidence that they may be linked [7-12].

In a prospective study, CRP, fibrinogen, and D-dimer were found to be significant predictors of the risk of future cardiovascular events. However, only CRP was independent of other clinical, laboratory, and neuro-radiological prognostic factors in predicting the risk of recurrent cardiovascular events with a time-to-event dependency. It was found that elevated levels of CRP, rather than of D-dimer and fibrinogen, are related to the risk of new cardiovascular events following ischemic stroke [13].

In another study, 60 patients with an acute ischemic neurological event were examined within 24 hours of the appearance of symptoms. A significant correlation was noted between baseline (on admission) hs-CRP concentrations, sedimentation rate, as well as adhesiveness/aggregation and the outcome of the ischemic neurological event as determined by the modified Rankin scale 8-12 months following the insult [14].

Another study determined the expression of p-selectin and lysosome-associated membrane protein (CD63) on platelets using flow cytometry at 10 time points between day 1 and 90 in 50 patients after ischemic stroke, in 30 healthy subjects, and in 20 risk factor, control subjects. Leukocyte count, C-reactive protein, and fibrinogen levels were correlated with platelet activation markers. It was found that p-selectin and CD63 expression were higher on day 1 after stroke than in either control groups. P-selectin expression declined rapidly, whereas CD63 expression remained high until day 90. Stroke severity and different medication for secondary stroke prevention did not influence p-selectin or CD63 expression. Platelet activation markers and inflammatory parameters did not correlate at any time point after stroke [15].

Inflammation, cerebral stroke, and genotyping

Serum levels of interleukin 6 (IL-6) increase markedly in stroke. IL-6 is a key regulator of inflammatory mechanisms that play an important part in stroke pathophysiology. The action of IL-6 is modified by its soluble receptor subunits sgp130 and sIL-6R. Serum levels of IL-6, sgp130 and sIL-6R were measured by ELISA in 48 patients with acute stroke and in 48 age and sex-matched control subjects. Serum concentrations of IL-6 significantly increased, while sgp130 levels were transiently reduced after stroke, and sIL-6R levels remained unchanged.

The common haplotype A-G-8/12-C was associated with low IL-6 levels after stroke and a reduced induction of IL-6 transcription on stimulation with an adenovirus analog *in vitro*. The data have demonstrated that there might be a genetic variation for the expression of IL-6 levels in stroke. It had been shown that induction of the inflammatory response by IL-6 might be enhanced by transient regulation of the IL-6 antagonist sgp130. [16] Interleukin 1 (IL-1) is one of the key modulators of the inflammatory response, and its activity is critically regulated by its receptor antagonist (IL-1Ra). It was found that the frequency of the IL-1Ra 1/1 genotype was significantly higher in stroke survivors with respect to age-matched controls [17]. CD44 is a trans-membrane glycoprotein known to be involved in endothelial cell recognition, lymphocyte trafficking, and regulation of cytokine gene expression in inflammatory diseases. A potential role of CD44 in ischemic brain injury was investigated using CD44-deficient (CD44-/-) mice. Over 50% and 78% reduction in ischemic infarct size was observed in CD44-/- mice compared with that of wild-type mice following transient (30 minutes ischemia) and permanent (24 hours) occlusion of the middle cerebral artery, respectively. The marked protection from ischemic brain injury in CD44-/- mice was associated with normal physiological parameters, cytokine gene expression, astrocyte and microglia activation as compared with wild-type mice. In CD44-/- mice, significantly lower expression of soluble interleukin-1 protein was noted after brain ischemia [18].

Anti-inflammatory treatment for ischemic stroke

One hundred and sixty seven patients that were studied received 1000 units of unfractionated heparin or 300 mg of aspirin within six hours of admission with acute ischemic stroke. Repeated plasma level measurements of interleukin 6 (IL-6), IL-10, IL-4, tumor necrosis factor (TNF-alpha), soluble intercellular adhesion molecule 1 (ICAM-1), and soluble vascular cell adhesion molecule 1 (sVCAM-1) were compared in both groups using multivariate analyses. TNF alpha and sICAM-1 decreased at 48 hours, but IL-6, IL-4, and sVCAM-1 were increased compared with baseline values. The rise of sVCAM-1 levels at 48 hours was significantly lower in patients that were treated with unfractionated heparin. These results suggest that high dose unfractionated heparin might have anti-inflammatory effects which might improve recovery if administered early after stroke onset [19].

CONCLUSION

Patients who experienced significant clinical improvement within the first four days of hospitalization showed remarkable inhibition of all three cell adhesion molecules that were measured (E-selectin, ICAM-1, and VCAM-1). Patients who did not improve had more severe cerebral infarcts, had a higher NIH score on admission (mean NIH score of 10 ± 4.6), and no change was observed in levels of cell adhesion molecules during the short follow-up period. In patients who improved clinically, the significant inhibition of cell adhesion molecule levels within the first days of hospitalization may suggest a decrease in the inflammatory state that accompanies acute vascular events and reperfusion injury.

The reduction in cell adhesion molecule levels within the first few days of hospitalization may predict a favorable outcome in patients with acute cerebral events.

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