The Effect of Intravenous Methylprednisolone Therapy on Adhesion Molecules in Relapsing Remitting Multiple Sclerosis

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ABSTRACT

Multiple sclerosis is an autoimmune disease of the central nervous system with multifocal areas of inflammatory demyelination. Immune cell entry into the site of inflammation is mediated by adhesion molecules. **Objective:** To determine the serum levels of adhesion molecules in blood samples from patients with relapsing remitting multiple sclerosis (RRMS) in exacerbation before and after intravenous methylprednisolone therapy. **Subjects and Methods:** This study was carried out on 21 patients (8 males and 13 females) with definitive relapsing remitting multiple sclerosis in exacerbation; in addition to 10 subjects (4 males and 6 females) as healthy controls. The concentrations of circulating adhesion molecules; intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in were measured in blood samples of all subjects using enzyme-linked immunoassay kits and immunophenotyping. In all patients, the ICAM-1 and the VCAM-1 concentrations were measured before and 2 weeks after a high-dose methylprednisolone therapy course 1gm intravenous infusion daily for 5 days. All patients underwent studies on the same 1.5-tesla magnetic resonance imaging (MRI) unit. **Results:** Both ICAM-1 and VCAM-1 were significantly higher in lymphocytes and non-significantly higher in monocytes of patients compared to the controls (p<0.05). Serum concentrations of soluble ICAM-1 and soluble VCAM-1 were non-significantly higher in patients than the controls (p>0.05) before the start of methylprednisolone therapy. After the course of high dose intravenous methylprednisolone therapy, the serum concentrations of soluble ICAM-1 and soluble VCAM-1 were significantly reduced compared to the controls (p<0.05). A significant reduction (p<0.05) of the serum concentrations of soluble ICAM-1 and soluble VCAM-1 was shown after the course of intravenous methylprednisolone therapy when compared to those before treatment. The serum concentrations of the soluble adhesion molecules in patients were correlated with the corresponding volumes of T2-weighted plaques before the steroid course (r=0.69; p<0.05). On the other side, no significant correlation was found between MRI abnormalities and the expression of adhesion molecules in the blood of patients of the present study. The Expanded Disability Status Scale (EDSS) showed a significant reduction 2 weeks after the high dose intravenous methylprednisolone therapy when compared to that during relapse before the start of this therapy. **Conclusion:** Therapies interfering with cell adhesion should be considered as a potential objective in RRMS. The importance of such adhesion molecules modulating therapies has been demonstrated with eventually subsequent restriction of inflammatory cell invasion to the CNS. This may provide an important tool in the effort to suppress MS. (Egypt J. Neurol. Psychiat. Neurosurg., 2006, 43(1): 615-622)

INTRODUCTION

Multiple sclerosis is an autoimmune disease characterized by multifocal areas of inflammatory demyelination within the central nervous system (CNS). The binding of circulating autoreactive T cells and macrophages to the CNS endothelial cells and subsequent migration through the blood brain-barrier is an essential step in the initiation of the brain inflammatory process.¹². This step of immune cell entry into the site of inflammation is mediated by adhesion molecules.³ In the CNS, T cells are exposed to the sensitization antigen that triggers inflammation by cytokines released during immunorecognition.⁴ Adhesion molecules induced by such cytokines on brain cells and immune cells promote interaction between these cells, leading to damage of myelin and oligodendrocytes with subsequent demyelination.⁵
Two adhesion molecule pathways have been well defined: the intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and leukocytes together with its ligand on leukocytes, the lymphocyte function associated antigen-1 (LFA-1), and the vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells and macrophages and its ligand on monocytes and lymphocytes, the very late activation antigen-4 (VLA-4). Increased expression of various cell adhesion molecules has been found in the CNS in multiple sclerosis (MS). The adhesion molecules are detected especially in and around cerebral microvessels and on inflammatory cells, including macrophages, microglial cells and lymphocytes in MS lesions. Under normal conditions, cerebrovascular endothelium exhibits only low levels of ICAM-1, and no constitutive expression of other adhesion molecules has been reported.

Therapy of acute exacerbations of MS with high-dose intravenous methylprednisolone has shortened the recovery period after relapses, but the mechanisms responsible for the beneficial effects of intravenous methylprednisolone in attacks have not been clearly established.

The aim of this study was to evaluate the levels of soluble ICAM-1 and soluble VCAM-1 during exacerbation in relapsing remitting multiple sclerosis (RRMS) before and 2 weeks after intravenous methylprednisolone therapy.

SUBJECTS AND METHODS

This study was carried out on 21 patients (8 males and 13 females) with definitive relapsing remitting multiple sclerosis in exacerbation according to the criteria of Poser et al. Recruited from Neuropsychiatry department, Tanta University Hospital, and 10 subjects (4 males and 6 females) were healthy controls. The presence of exacerbation was confirmed in all patients. This was defined as a worsening of neurological impairment or the appearance of a new symptom or abnormality attributable to MS, lasting at least 24 hours, and preceded by stability for at least 1 month. The severity of the disease was scored using the Expanded Disability Status Scale (EDSS). Patients with acute viral or bacterial infection or patients who had received corticosteroids in the 3 months before the study were excluded. Patients were neither under interferon therapy nor had they received any other immunosuppressive treatment in the previous 6 months. Blood samples were obtained by routine procedures, with the informed consent of the patients. The levels of circulating adhesion molecules were measured in frozen serum stored at -70°C.

All patients underwent studies on the same 1.5-tesla magnetic resonance imaging (MRI) unit (Signa Horizon SR 120; General Electric Medical Systems, Milwaukee, WI), using a standard head coil. Axial T1-weighted, axial T2-weighted, sagittal T1-weighted, proton density-weighted, gadolinium enhanced T1-weighted, and fluid attenuation inversion recovery-weighted images were obtained. In addition, the imaging protocol included axial T1- and T2-weighted fast spin echo images for volumetric analysis.

The concentrations of soluble ICAM-1 and VCAM-1 in the serum were measured in all subjects. In the patients these concentrations were measured before the start and 2 weeks after the end of a course of high-dose methylprednisolone therapy 1gm intravenous infusion daily for 5 days. These concentrations were measured using enzyme immunoassay kits (BBE 1B and BBE 3 respectively; R & D systems, Minneapolis, Minn). Serum samples diluted 1:20 for soluble ICAM-1 and 1:50 for soluble VCAM-1 were added to microtiter wells and assayed according to manufacture procedures. For phenotyping the mononuclear cells, the 3-layer indirect immunoperoxidase technique was applied.

The primary antibodies used were ICAM-1 (CD54) and VCAM-1 (CD106) (0544 and 1244 respectively; Immunotech, Marseille, France). The dilution used for blood specimens was 1:200. The secondary antibody was a peroxidase-conjugated rabbit anti-mouse used in dilution of 1:10 (P0161; Dako, Glostrup, Denmark) and the
third was a peroxidase-conjugated goat anti-rabbit antibody used in dilution of 1:20 (L42007; Caltag, San Francisco, Calif). The specimens were analyzed at x100 magnification. A total of 400 cells were analyzed for each cell surface marker. The results were expressed as the percentage of positively stained monocytes in the total number of monocytes counted.

Statistical analysis of the data of our results was performed by using: (1) independent 2-tailed "t" test in the group mean comparisons with normal distribution, as well as the paired "t" test, (2) the X² was used for comparison of qualitative data, and (3) the Pearson correlation coefficient in correlation analyses. The level of significance adopted was at p<0.05.

RESULTS

The clinical characteristics of the patients of RRMS and the controls were expressed in table (1).

The proportions of the adhesion molecule expression on the blood lymphocytes and monocytes in all patients with RRMS in exacerbation are presented in table (2). In lymphocytes, both ICAM-1 and VCAM-1 were significantly higher in patients compared to the controls (p<0.05). On the other hand, in monocytes, both ICAM-1 and VCAM-1 were non-significantly higher in patients compared to the controls (p>0.05).

Comparison between the levels of soluble adhesion molecules in patients of the present study with RRMS in exacerbation and the controls before the start of high dose intravenous methylprednisolone therapy is shown in table (3). Serum concentrations of soluble ICAM-1 and VCAM-1 were non-significantly higher in patients than the controls (p>0.05).

Two weeks after the end of the course of high-dose intravenous methylprednisolone therapy, the serum levels of soluble ICAM-1 and soluble VCAM-1 were significantly reduced compared to the controls (p<0.05) as shown in table (4). Table 5 showed a significant reduction (p<0.05) of the serum concentrations of soluble ICAM-1 and soluble VCAM-1 after the course of high dose intravenous methylprednisolone therapy when compared to those before treatment.

A significant improvement in the EDSS score 2 weeks after intravenous methylprednisolone therapy completion was detected in patients of the present study when compared to that before treatment (p<0.05), as shown in table (6).

The concentrations of the soluble adhesion molecules measured in the sera of patients of the present study were correlated with the corresponding volumes of T2-weighted MS plaques before treatment with high-dose intravenous methylprednisolone (r=0.69; p<0.05). The mean (±SD) concentration of soluble ICAM-1 in the patients was 295±62 nanogram / milliliter, and the median volume of T2-weighted plaques was 7.5 cm³.

Table 1. The clinical characteristics of the patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=21)</th>
<th>Controls (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean ± SD)</td>
<td>33 ± 9</td>
<td>36 ± 8</td>
<td>&gt;0.05 (t = 0.89)</td>
</tr>
<tr>
<td>Male : female (number)</td>
<td>8 : 13</td>
<td>4 : 6</td>
<td>&gt;0.05 (X² = 0.01)</td>
</tr>
<tr>
<td>Duration of MS in months (mean ± SD)</td>
<td>45 ± 10</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>EDSS (mean ± SD)</td>
<td>3.1 ± 1.9</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

EDSS = Expanded Disability Status Scale.
Table 2. Adhesion molecules expression in the blood of patients versus control.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=21)</th>
<th>Controls (n=10)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1 (mean ± SD)</td>
<td>19.2 ± 2.8</td>
<td>5.8 ± 1.6</td>
<td>14</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1 (mean ± SD)</td>
<td>22.7 ± 4.9</td>
<td>20.1 ± 4.3</td>
<td>1.43</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* = significant.

Table 3. Serum levels of soluble adhesion molecules (in nanograms per milliliter) in patients before methylprednisolone therapy versus controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=21)</th>
<th>Controls (n=10)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble ICAM-1 (mean ± SD)</td>
<td>295 ± 62</td>
<td>281 ± 51</td>
<td>0.62</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Soluble VCAM-1 (mean ± SD)</td>
<td>585 ± 119</td>
<td>579 ± 117</td>
<td>0.13</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 4. Serum levels of soluble adhesion molecules (in nanograms per milliliter) in patients after methylprednisolone therapy versus controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=21)</th>
<th>Controls (n=10)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble ICAM-1 (mean ± SD)</td>
<td>197 ± 48</td>
<td>281 ± 51</td>
<td>4.47</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Soluble VCAM-1 (mean ± SD)</td>
<td>481 ± 91</td>
<td>579 ± 117</td>
<td>2.56</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

* = significant.

Table 5. Serum levels of soluble adhesion molecules (in nanograms per milliliter) in patients before and after intravenous methylprednisolone (IVMP) therapy.

<table>
<thead>
<tr>
<th></th>
<th>Before IVMP</th>
<th>After IVMP</th>
<th>Paired t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble ICAM-1 (mean ± SD)</td>
<td>295 ± 62</td>
<td>197 ± 48</td>
<td>5.73</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Soluble VCAM-1 (mean ± SD)</td>
<td>585 ± 119</td>
<td>481 ± 91</td>
<td>3.18</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* = significant.

Table 6. Expanded Disability Status Scale (EDSS) during relapse versus 2 weeks after high-dose intravenous methylprednisolone therapy (IVMP).

<table>
<thead>
<tr>
<th></th>
<th>EDSS during relapse</th>
<th>EDSS 2 weeks after IVMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>3.1 ± 1.8</td>
<td>2.0 ± 1.6</td>
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</table>

Paired t = 2.1, p < 0.05

* = significant.
DISCUSSION

Multiple sclerosis is an acute relapsing inflammatory disease of the central nervous system myelin, white matter tracts are affected including those of the cerebral hemispheres, infratentorium and spinal cord. It is the most common disabling neurological disease of young people. The primary pathogenic process is unclear, however, postulated to be a T-cell mediated autoimmune process in which autoaggressive T cells with specificity to myelin antigens traffic to the CNS across the blood-brain barrier to initiate the inflammatory process.

It is hypothesized that in MS the autoantigen-specific T cells are sensitized initially peripherally and then leave the systemic circulation by a transendothelial migration process and access the CNS to initiate local autoimmune responses. It is now clear that the vascular endothelium plays a central active role in controlling the leukocytes migration into tissue and in regulating the development of inflammatory responses.

Upon activation by inflammatory cytokines, the endothelium expresses an ensemble of adhesion molecules which are reciprocally expressed on leukocytes.

Adhesion molecules induced by the cytokines released during immuno-recognition on brain cells and immune cells promote interaction between these cells, leading to damage of myelin and oligodendrocytes with subsequent demyelination. Expression of adhesion molecules on the surface seems to underlie the ability of these inflammatory cells to penetrate the blood-brain barrier.

The exact mechanisms involved in the exacerbations and remissions during the course of MS have not been established. It has been proposed that cytokines produced by activated T lymphocytes play an important role in the initiation and regulation of an autoimmune response during the course of MS.

The interaction of ICAM-1 and VCAM-1 with their respective ligands promotes strong adhesion to endothelium before transendothelial migration occurs. Activation of endothelial cells and immune cells in the blood in patients with MS results in rapid up-regulation and possible shedding of adhesion molecules from the surface of activated endothelium into the serum and cerebrospinal fluid in a soluble form. Elevated serum and cerebrospinal fluid levels of certain adhesion molecules have been associated with varying activity and clinical course of MS.

Elevated levels of soluble adhesion molecules in MS have been reported by many investigators, but much less information is available on the cell surface expression of these proteins in the blood and cerebrospinal fluid. In our study, we found increased proportions of serum soluble ICAM-1 and soluble VCAM-1 in patients with relapsing remitting MS in relapse compared to healthy controls. This indicates leukocyte - endothelial activation in acute MS.

Previous reports on the soluble endothelial adhesion molecules in the serum of patients with MS have been conflicting, showing elevated and normal levels. This indicates that serum levels of adhesion molecules do not consistently reflect the activity of MS disease. However, our results showed the level of serum soluble ICAM-1 and soluble VCAM-1 in patients of the present study to be higher than the controls. Elevated levels of soluble ICAM-1 and soluble VCAM-1 in the cerebrospinal fluid during MS relapse seem to be more closely related to disease activity.

The discrepancies between our results and previous reports may be explained by differences in methods or timing of sampling in relation to disease activity. Moreover, the cellular source of adhesion molecules and the kinetics of cleavage of endothelial cell surface molecules to their soluble form may differ in patients with RRMS in exacerbation.

In our study, it is reported that high-dose intravenous methylprednisolone therapy reduced the serum concentrations of soluble ICAM-1 and soluble VCAM-1 in patients with RRMS. This observation is in harmony with the current knowledge that corticosteroids suppress many
In the study, there was a correlation between the serum levels of soluble ICAM-1 and ICAM-302. These molecules modulating therapies has been demonstrated with eventually subsequent restriction of inflammatory cell invasion to the brain. This may provide an explanation for the exacerbation of MS disease activity. Therapies interfering with cell adhesion should be considered as a potential objective in relapsing remitting multiple sclerosis. The importance of such adhesion molecules in the central nervous system. Cell; 85: 299-87.

In conclusion, serum adhesion molecules may represent reliable markers of multiple sclerosis and an important tool in the effort to suppress MS. Significant correlations were found between the numbers of T2 weighted lesions and the volume of brain atrophy. In a study of patients with RRMS in July 2004 and immunopathology of its lesions? correlation with neurological disability in secondary progressive multiple sclerosis. J Neurol Sci; 165: 36-42. New diagnostic criteria for multiple sclerosis: an expanded disability status scale (EDSS). Neurology; 33: 1444-1452.

Methylprednisolone also stabilizes the blood brain barrier by regulating the expression of histocompatibility antigens at the blood brain barrier. The effects cause inhibition of inflammatory cell recruitment to sites of inflammation, decrease in the numbers of circulating B lymphocytes and the level of serum immunoglobulins as well as reemigration of vascular addressins and ICAM-1 on mononuclear cells from blood.

The multiple effects of intravenous methylprednisolone on the immune system include inhibition of T cell recruitment to sites of inflammation, decrease in the numbers of circulating B lymphocytes and the level of serum immunoglobulins as well as reemigration of vascular addressins and ICAM-1 on mononuclear cells from blood. This may provide an explanation for the exacerbation of MS disease activity. Therapies interfering with cell adhesion should be considered as a potential objective in relapsing remitting multiple sclerosis. The importance of such adhesion molecules in the central nervous system. Cell; 85: 299-87.

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تأثير العلاج بميثيل بريدنيزيلون الوريدى على جزيئات الالتصاق في مرض التصلب المتعدد الإنتكاسي-النكوصي

بعد مرض التصلب المتعدد من الأمراض المناعية الغير محددة الأسباب والتي تصيب الجهاز العصبي المركزي كما تعتبر جزيئات الالتصاق جزئى الالتصاق بين الخلايا-1 وجزء الالتصاق الخلوي الوعائي-1 من الدبالات المناعية التي يتم دراسة العلاقة بينها وبين النشاط الإنتكاسي في مرض التصلب المتعدد في السنوات الأخيرة. ويدفع هذا البحث إلى دراسة نسبة جزيئات الالتصاق بالدم في مرض التصلب المتعدد أثناء الإنتكاسي قبل وبعد العلاج بميثيل بريدنيزيلون الوريدى. تم إجراء هذا البحث على 21 مريض بمرض التصلب المتعدد أثناء الإنتكاسي بالإضافة إلى 10 من الأصحاء كمجموعة ضابطة. تم قياس نسبة جزء الالتصاق بين الخلايا-1 وجزء الالتصاق الخلوي الوعائي-1 في الدم لكل المرضى قبل وبعد العلاج بميثيل بريدنيزيلون 1 جرام بالتنقيط في الوريد يوميا لمدة 5 أيام بالإضافة إلى قياسهم في الأصحاء. كما تم عمل أشعة بالرنين المغناطيسي على المخ لكل المرضى. وقد تم التوصل إلى بعض النتائج وإتلافة في نسبة هذه الجزيئات في كل من الخلايا الليفاوية (وكان ذلك دالة إحصائية) والخلايا الأحادية (وكان دون دالة إحصائية) في المرضى مقارنة بالأصحاء. وقد وجد أيضاً ارتفاع ليس ذو دالة إحصائية في نسبة هذه الجزيئات الالتصاق في المرضى أثناء الإنتكاسي المرضية مقارنة بالأصحاء قبل بدأ العلاج بميثيل بريدنيزيلون والتي تتسبب مع حجم الإصابات الموجودة بالمخ في أشعة الرنين المغناطيسي والتي سرعان ما خفف استنفادها ذا دالة إحصائية بعد انتهاء العلاج بسبيون وعلى الجانب الآخر لم يتم التوصل إلى أي علاقة بين حجم الإصابات الموجودة في أشعة الرنين المغناطيسي وبين نسبة هذه الجزيئات في الخلايا الليفاوية أو الأحادية في المرضى. ومن هذا يتبين أنه يمكن القول بأن نسبة جزيئات الالتصاق الذاتية بالمصل قد تمثل دلالات يعتمد عليها في الوقوف على نشاط مرض التصلب المتعدد، كما أن العلاجات التي تعارض مع الالتصاق الخلوي لابد وأن تؤخذ في الاعتبار أثناء علاج التصلب المتعدد الإنتكاسي-النكوصي أثناء الإنتكاسة.