Mannose binding lectin and complement pathway in brain ischemic injury: studies in mouse models and patients

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RESULTS (Chapter II)

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Preface

The following body of work was performed at the Institute of Pharmacological Research “Mario Negri”, Milan, Italy, under the direction of Dr. Maria Grazia De Simoni and the supervision of Dr. Elisa R. Zanier.

Sources of Funding

This study was supported in part by an Italian Ministry of Health Young Investigators Award 2009 (to Dr Zanier) and by Cariplo 2009-2630.

List of publications related to this thesis


Lectin pathway of complement activation after subarachnoid hemorrhage. This paper is a work in progress
**List of main abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ARDS</td>
<td>acute respiratory distress syndrome</td>
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<tr>
<td>C1-INH</td>
<td>C1-inhibitor</td>
</tr>
<tr>
<td>CAMs</td>
<td>cell adhesion molecules</td>
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<tr>
<td>CCA</td>
<td>common carotid artery</td>
</tr>
<tr>
<td>CI</td>
<td>cerebral ischemia</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CR1</td>
<td>complement receptor 1</td>
</tr>
<tr>
<td>CRD</td>
<td>carbohydrate recognition domains</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computer tomography</td>
</tr>
<tr>
<td>DCI</td>
<td>delayed cerebral ischemia</td>
</tr>
<tr>
<td>ECA</td>
<td>external carotid artery</td>
</tr>
<tr>
<td>ECI</td>
<td>early cerebral ischemia</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunoassorbent assay</td>
</tr>
<tr>
<td>GOS</td>
<td>Glasgow outcome scale</td>
</tr>
<tr>
<td>Hgb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>Hp-Hgb</td>
<td>HaptoglobinHb protein</td>
</tr>
<tr>
<td>I/R</td>
<td>ischemia/reperfusion</td>
</tr>
<tr>
<td>ICA</td>
<td>internal carotid artery</td>
</tr>
<tr>
<td>ICP</td>
<td>intracranial pressure</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>MAC</td>
<td>membrane attack complex</td>
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</table>
MASPs  MBL-associated serine proteases
MBL  Mannose Binding Lectin
MBL-/-  MBL-A and MBL-C double knockout mice
MCP  and membrane cofactor protein
MRI  magnetic resonance imaging
OD  optical density
PAMPs  pathogen-associated molecular patterns
pMCAo  permanent middle cerebral artery occlusion
PWI  Perfusion-weighted Imaging
RBCs  Red blood cells
rhC1-INH  recombinant human C1 inhibitor
SAH  subarachnoid hemorrhage
SC5b-9  plasma levels of terminal complement complex
Sham  sham-operated
SNP  single nucleotide polymorphisms
sPRM  small pattern recognition molecule
TCC  terminal complement complex
tMCAo  transient middle cerebral artery occlusion
VSP  clinical vasospasm
WFNS  World Federation of Neurological Surgeons
Abstract

**Background.** The involvement of the complement system in brain injury has been scarcely investigated. Here we document the pivotal role of lectin pathway, initiated by mannose binding lectin (MBL) and ficolin-3, in brain ischemic injury in mice and humans.

We first evaluated the role of the lectin pathway in brain ischemic mice.

In order to investigate the clinical relevance of these experimental observations, the second aim was to evaluate the relevance of the lectin pathway in subarachnoid hemorrhage (SAH) patients. Brain ischemia is a main determinant of unfavorable outcome in SAH patients. It can play a role in the acute phase as a consequence of the initial intracranial bleeding and/or at delayed stages due to cerebral vasospasm.

**Methods and results: brain ischemic injury in mice.** Focal ischemia was induced in C57Bl/6 (WT) or in MBL-A and MBL-C double knockout mice (MBL-/-), by permanent or transient middle cerebral artery occlusion (pMCAo and tMCAo, respectively). Neurological deficits and infarct volume were measured 48 h after ischemia. MBL presence on cerebral vessels was assessed by immunostaining and confocal microscopy. The activation of lectin pathway after ischemia was analyzed by measuring circulating functional MBL/MASP-2 complexes by ELISA. Complement activation was assessed by western blot analysis of C3 fragments in plasma samples.

We first observed that MBL is deposited on ischemic vessels up to 48h after injury and that functional MBL/MASP-2 complexes and C3 complement fragments were significantly increased after tMCAo and pMCAo, indicating that the lectin pathway is activated in the ischemic injury.

Next, we demonstrated that MBL-/- mice were protected from anatomical and functional ischemic injury, showing 28% and 41% lesion reduction after tMCAo and pMCAo, respectively, compared to WT, thus suggesting a deleterious role of MBL and suggesting that inhibition of this protein could lead to neuroprotection.
Methods and results: subarachnoid hemorrhage patients.

Thirty-nine patients with SAH were enrolled. Clinical vasospasm (VSP) was defined as neuro-worsening with angiographic confirmation of vessel narrowing. Cerebral ischemia was defined as a hypodense lesion on computer tomography (CT) scan performed before Intensive Care Unit (ICU) discharge. Early cerebral ischemia was defined as a hypodense lesion on CT performed in acute phase (ECI), while delayed ischemia (DCI) was defined as a new hypodense lesion on CT scan performed in delayed phase. Six-month outcome was assessed using Glasgow outcome scale (GOS). Clinical severity, radiological status and outcome were studied in relation to lectin concentrations. Plasma levels of MBL, MBL/MASP-2 functional complexes, ficolin-3 and of complement factors (C3 and C5b-9) from patients and 20 healthy subjects, were determined in acute (1-3 day) and post acute phase (4-14 day) through western blot analysis and ELISA.

The plasma concentrations of MBL in SAH patients, however, was not significantly different from healthy subjects. Despite unaffected MBL levels, we have observed an acute and persistent reduction of MBL/MASP-2 levels.

On the contrary, a persistent increase in ficolin-3 was detected in SAH patients. Furthermore, ficolin-3 was related to brain injury severity. Namely, significantly lower levels of ficolin-3 were found in: severe patients, patients with VSP and patients with CT cerebral ischemia.

Conclusion: Our data show an important role for the lectin pathway in the pathogenesis of acute brain injury and provide a strong support to the concept that lectin pathway may be a relevant therapeutic target in humans with a wide therapeutic window of application.
References


Related links
American Heart Association. 2012 Heart and Stroke Statistical Update. Available at http://my.americanheart.org/professional/General/Heart-Stroke-2012-Statistical-Update_UCM_434526_Article.jsp


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