Changes in MMP-9 and TIMP-1 Concentrations in Cerebrospinal Fluid after 1 Week of Treatment of Childhood Bacterial Meningitis

Irmeli Roine,a Tuula Pelkonen,b,c Anneli Lauhio,d Maija Lappalainen,e Manuel Leite Cruzeiro,b Luis Bernardino,b Taina Tervahartiala,f Timo Sorsag and Heikki Peltoła

Faculty of Medicine, Universidad Diego Portales, Santiago, Chile; bHospital Pediátrico David Bernardino, Luanda, Angola; cChildren’s Hospital, Helsinki University Hospital and University of Helsinki, Helsinki, Finland; dInfectious Diseases, Inflammation Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland; eVirology and Immunology, Laboratory Services (HUSLAB), Helsinki University Hospital, Helsinki, Finland; dDepartment of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; fDivision of Periodontology, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden

We explored the changes of the initially highly upgraded cerebrospinal fluid matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of MMP 1 (TIMP-1) response during recovery of childhood bacterial meningitis and their association with outcome. The sizes of these changes varied substantially, but a steeper decrease in the MMP-9 and an increase of the TIMP-1 concentrations augured a better outcome.

Matrix metalloproteinases (MMPs) are a group of zinc-dependent enzymes which, through their ability to degrade extracellular matrix and nonmatrix bioactive substances, can direct and modify multiple diverse biological and pathological processes, among them the inflammatory and immune responses (1). The MMP activities are inhibited by their specific endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs). Under physiological conditions, MMP activity is precisely regulated, whereas in disease, MMP activity is unbalanced (1).

In childhood bacterial meningitis (BM), the initial concentrations of MMP-9 and TIMP-1 in the cerebrospinal fluid (CSF), and their molar ratio (MR), are highly upgraded, and the highest values are associated with adverse outcomes (2–6). We studied how the response was shut down and whether this process influenced the outcome. A better understanding of the response kinetics may open novel opportunities for therapeutic interventions (1, 7).

The changes in the concentrations of MMP-9 and TIMP-1 and their MR from admission to 1 week of treatment (day 7) were analyzed using paired CSF samples taken, whenever enough CSF was available, on those days from patients of a previous BM study (8) (Table 1; see also Fig. S1 in the supplemental material). All patients were treated with intravenous cefotaxime but were randomized to receive it during the first 24 h either as a continuous infusion or as an every-6-hour bolus (8). No adjuvant dexamethasone was given, but all the patients received oral glycerol (9). The audiological outcome was tested on day 7 by brain stem auditory evoked potentials (Madsen Octavus). Deafness was defined as a hearing threshold >80 dB in the better ear. Severe neurological sequelae were defined as blindness, quadriplegia or paresis, hydrocephalus requiring a shunt, or severe psychomotor retardation at discharge.

The MMP-9 and TIMP-1 concentrations were determined by enzyme-linked immunosorbent assay (ELISA), as described previously (4). The changes in percent were compared with other data using the Spearman correlation for quantitative variables and the Mann-Whitney U test or Kruskal-Wallis test for nominal variables. The day-7 values also were examined in the same manner (see Tables S2 and S4 in the supplemental material). A P value of <0.05 was taken as significant.

Within 1 week, the MMP-9 concentration fell by 90% (interquartile range [IQR], 28%) (Fig. 1) from 476 ng/ml (IQR, 752 ng/ml) to 38 ng/ml (IQR, 62 ng/ml) (P < 0.0001). The children who later died (n = 3) (Table 1) had a smaller decrease of 26% (IQR, 10%; P = 0.02), and their day-7 MMP-9 concentration (see Table S2 in the supplemental material) remained high (202 ng/ml [IQR, 151]; P = 0.03). A high day-7 MMP-9 level also was associated (see Table S3 in the supplemental material) with a longer time to defervescence (rho, 0.30; P = 0.04) and a lower day-7 glucose concentration (rho, −0.54; P = 0.0002), and it was more frequent in malnourished children (n = 15; 63 ng/ml [IQR, 215 ng/ml]; P = 0.03) (see Table S2).

The overall change in the TIMP-1 concentration was an increase (Fig. 1) by 51% (IQR, 551%) from 326 ng/ml (IQR, 889 ng/ml) to 557 ng/ml (IQR, 859 ng/ml); the TIMP-1 increased in 58% of patients (36 of 62 patients), whereas it decreased in 42% (26 of 62 patients). A greater increment was associated with a better Glasgow outcome score on day 7 (rho, 0.27; P = 0.03) (see Table S4 in the supplemental material). The 8 patients who survived with severe neurological sequelae had a TIMP-1 decrease of −42% (IQR, 25%; P = 0.04) (Table 1). On day 7, the TIMP-1 concentration was higher in the patients who received cefotaxime as an infusion (907 ng/ml [IQR, 881 ng/ml]; P = 0.03) (see Table S2 in the supplemental material). The sizes of the changes in the MMP-9 and TIMP-1 concentrations did not correlate with each other (rho, −0.09; P = 0.55).
TABLE 1 Change from baseline to 1 week of treatment in cerebrospinal fluid MMP-9 and TIMP-1 concentrations and their molar ratio compared with qualitative data

<table>
<thead>
<tr>
<th>Variable</th>
<th>MMP-9 (n = 50)</th>
<th>TIMP-1 (n = 62)</th>
<th>MMP-9/TIMP-1 MR (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR) change</td>
<td>Median (IQR) change</td>
<td>Median (IQR) change</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>P</td>
</tr>
<tr>
<td>Female (n = 31)</td>
<td>−88 (28)</td>
<td>−90 (37)</td>
<td>0.95</td>
</tr>
<tr>
<td>Severe malnutrition (n = 15)</td>
<td>−80 (44)</td>
<td>−91 (26)</td>
<td>0.31</td>
</tr>
<tr>
<td>HIV positive (n = 5)</td>
<td>−91 (73)</td>
<td>−89 (26)</td>
<td>0.85</td>
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Etiology 0.10 0.31 0.03

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The MMP-9/TIMP-1 MR decreased by 91% (IQR, 20%) from 0.22 (IQR, 1.79) to 0.02 (IQR, 0.03; P < 0.0001) (Fig. 1). The size of the fall was associated with the baseline TIMP-1 and glucose concentrations (rho, 0.53 [P = 0.0004] and −0.48 [P = 0.001], respectively) and the CSF white cell count (rho, 0.31; P = 0.03). Patients with a pneumococcal etiology had a larger decrease, of respectively) and the CSF white cell count (rho, 0.31; P = 0.0004] and −0.48 [P = 0.001], respectively) and the CSF white cell count (rho, 0.31; P = 0.03). Patients with a pneumococcal etiology had a larger decrease, of 98% (IQR, 11%; P = 0.03), than the others.

We acknowledge that depicting the behaviors of two inflammatory mediators in a relatively small number of patients offers only a narrow vision of the very complex inflammatory response (1). In this context, the relevance of our results consists of depicting the substantial differences in the shutdown process of the initially high MMP-9 and TIMP-1 responses (Fig. 1) and identifying specific trends associated with a poor outcome. Although the high MMP-9 level was extinguished in most patients within 1 week, the failure to achieve this was associated with forthcoming death or a slower recovery. The changes in the TIMP-1 concentrations were complex, with a bigger increase auguring a better outcome and a decrease in survival with neurological sequelae. The change in the MMP-9/TIMP-1 MR reflected its individual components but was not by itself significantly associated with outcome.

In comparison with previous data on MMP-9 kinetics in the CSF in a mere 5 adults with BM (5), our results coincide with the wide variation in the size of the changes and the association of death with the lack of MMP-9 reduction observed in one of those patients. Regarding the TIMP-1 or the MR kinetics, no previous data from the CSF in BM were available for comparisons.

Because TIMP-1 inhibits MMP-9 in 1:1 molar relation (1), one would expect a more favorable outcome had there been enough TIMP-1 to block the deleterious MMP-9 (2). In agreement with this hypothesis, a bigger TIMP-1 increase protected our patients from severe sequelae. Contrary to the expectation, the sizes of the TIMP-1 and MMP-9 changes did not correlate, although MMP-9 is supposed to directly trigger the inhibitory activity of TIMPs. Possibly, an association was missed, because only TIMP-1, and not the other TIMPs, was measured (1).

Because none of the patient or disease characteristics, with the exception of severe malnutrition, explained the wide variations observed in the MMP-9 and TIMP-1 changes, this issue should be further explored, possibly by genetic analysis of the inflammatory response. Also, the promising effect of a cefotaxime infusion in incrementing the day-7 TIMP-1 level (see Table S2 in the supple-
mental material) in comparison with the bolus administration, and the role of etiology in the changes, should be further examined. Our number of patients limited a meaningful analysis.

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REFERENCES


