The role of mannose-binding lectin in vitro and in vivo

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Safety and pharmacokinetics of plasma-derived mannose-binding lectin substitution in children with chemotherapy-induced neutropenia

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Chapter 8

Abstract

Purpose: Mannose-binding lectin (MBL) is a plasma protein that is part of innate immunity. MBL deficiency, i.e. genetically determined reduced MBL levels, is very common and has been associated with increased susceptibility to infections, especially in children and immunocompromized individuals. In a phase-I trial, MBL substitution has proven to be safe in healthy MBL-deficient adults. Data on MBL infusions in children are not yet available. Reasoning that children with cancer might benefit from MBL substitution during chemotherapy-induced neutropenia, we performed a phase-II study in MBL-deficient children with cancer. Here, we describe the safety and pharmacokinetics of MBL substitution in these children.

Patients and Methods: Twelve MBL-deficient children with cancer (aged 0-12 years) received infusions of plasma-derived MBL prepared by Statens Serum Institut (SSI, Copenhagen, Denmark), twice weekly during a chemotherapy-induced neutropenic episode (range: 1-4 weeks). Four patients received multiple (1-4) courses of MBL infusions. Target levels of 1.0 µg/ml were considered therapeutic.

Results: In total, 65 MBL infusions were given in 20 courses of MBL infusions. No MBL-related adverse reactions were observed and the trough level was 1.06 µg/ml (range: 0.66-2.05 µg/ml) during the study period. According to allometric scaling, pharmacokinetics were not related to age after correction for body weight. The half-life of MBL, for a child of 25 kg, was 36.4 hours (range: 23.7-66.6 hours). No anti-MBL antibodies were measured 4 weeks after each MBL course.

Conclusion: Substitution therapy with MBL-SSI twice weekly is safe and results in trough levels considered protective. Trial registration number: NCT00138736.

Introduction

Mannose-binding lectin (MBL) is a collagenous plasma protein that is part of the innate immune system. After binding to sugar residues on the surface of various micro-organisms, it activates the lectin pathway of the complement system through MBL-associated serine proteases (MASPs) (1).

MBL levels are genetically determined (2). MBL is encoded by the MBL2 gene (3). In general, individuals with a wild-type (denoted A) MBL2 gene have MBL levels above 1.0 µg/ml (4). Three single nucleotide polymorphisms (SNPs) in codon 52, 54, and 57 of exon 1 of the MBL2 gene (termed D, B, and C, respectively) induce reduced or deficient MBL levels (1). In addition, three polymorphisms at −550 (termed H/L), −221 (termed Y/X) and −66 (termed P/Q) in the promoter region influence MBL expression (2). The X variant is associated with reduced MBL
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Due to linkage disequilibrium only seven haplotypes are found: HYPA, LYPA, LYQA, LXPA, HYPD, LYPB and LYQC (6).

MBL deficiency is associated with increased infection susceptibility, particularly in children and immunocompromised patients (7, 8). Increased duration and severity of febrile neutropenia was seen in MBL-deficient children and adults with cancer (9-11). Neutropenic oncology patients were proposed to possibly benefit from MBL substitution, although infection susceptibility in MBL-deficient patients was not increased in more recent studies (12-14).

MBL substitution has proven to be safe in phase-I trials on both plasma-derived (n=20) and human recombinant MBL (n=40) in MBL-deficient adults (15-17) and a number (n=5) of additional patients (18-21). Therapeutic serum levels of > 1.0 μg/ml were reached after infusion of plasma-derived MBL. Peak levels were 1.2-4.5 μg/ml, but the half-life was highly variable with a mean of about three days (69.6 h; range: 14.6-114.9 h) (16, 21). Reanalysis of the pharmacokinetic data from this trial with a population pharmacokinetic approach enabled us to design a relatively small phase-II study to gather the data required for a future randomized placebo-controlled phase-III efficacy study. We performed an open, uncontrolled phase-II clinical trial on MBL substitution in 12 MBL-deficient pediatric oncology patients with chemotherapy-induced neutropenia. In this report, we describe the safety, pharmacokinetics and clinical course of these patients.

Material and Methods

Study design and protocol

Between April 2004 and August 2006 a prospective, open, uncontrolled study was performed in 12 children admitted to the oncology unit of the Emma Children’s Hospital, Amsterdam, the Netherlands. All parents gave written informed consent in accordance with the Medical Research Involving Human Subjects Act (WMO). The study was conducted according to the declaration of Helsinki and Good Clinical Practice. The protocol was approved by the local ethics committee. Sanquin Plasmaproducts, Amsterdam, was responsible for monitoring the trial.

After the end of a chemotherapy course patients received a MBL infusion, which was repeated twice weekly until patients had recovered from chemotherapy-induced neutropenia (neutrophil count < 500 cells/μl) (Figure 1A). Dosages of 0.2 mg/kg alternated with dosages of 0.3 mg/kg. To increase the number of participants, the last 5 patients only received a single infusion according to an amendment in the study protocol. They were observed for three or four days (see below and Figure 1A). Patients were allowed to participate more than once.

Patient selection

Participants were children treated for cancer in the Emma Children’s Hospital, Amsterdam,
The Netherlands. Inclusion criteria were: 1) ≤ 12 years of age; 2) mutation in exon-1 of the MBL2 gene or plasma MBL level <0.10 μg/ml; and 3) cancer for which they were treated with chemotherapy expected to induce neutropenia. Exclusion criteria consisted of known allergic reactions against human plasma products, participation in other investigational drug studies within the last month, and clinically relevant abnormalities in serum immunoglobulins (IgG, IgA, IgM) or complement factors (measured by AP50 and CH50).

The clinical condition of 9 out of 34 eligible children did not allow them to participate in this clinical trial, e.g. palliative treatment setting. Parents of ten patients gave informed consent. The remaining parents (n=15) refused consent because of the required twice weekly visits to the hospital or because their child had not yet experienced infections during neutropenia.

Furthermore, two MBL-deficient patients were treated despite violation of the inclusion criteria. One patient had a plasma MBL level of 0.35 μg/ml, but no concomitant exon-1 mutation. Another patient was 15-years old. He was treated on compassionate grounds during Glivec therapy, by an amendment in the protocol.

Endpoints

The primary endpoints of our trial were 1). pharmacokinetics, i.e. determination of the half-life of MBL-SSI and the achievement of plasma trough levels > 1.0 μg/ml, 2). safety, i.e. lack of adverse events, and 3) biological efficacy, i.e. reconstitution of MBL-dependent complement activation and opsonophagocytosis in vitro. Data on the occurrence and duration of fever and infections, the use of antibiotics/antifungal medication, oxygen and/or immediate circulatory support were recorded. Due to the small number of patients, clinical efficacy was not considered a realistic endpoint.

Data collection

MBL levels were measured before infusion, 15 minutes (min), 2, 4, 6 and 16-24 hours (h.) after the first infusion, and before each following MBL infusion. All patients had a central venous catheter (port-a-cath), which was used for MBL infusions and blood sampling. Vital signs (blood pressure, temperature and heart rate) were measured before and after each MBL infusion. Full blood cell counts, creatinin, and liver enzymes were monitored before and 24 hours after infusion.

Blood cell products and granulocyte colony-stimulating factor were permitted during the study period. Patients measured their temperature twice daily at home and were hospitalized when fever >38.5°C developed.

MBL SSI production and dosage

Statens Serum Institut, Copenhagen, Denmark (SSI), produced MBL from a pool of plasma from non-remunerated voluntary Danish donors as described previously (22). Based on previously
collected data in MBL-deficient patients, adult volunteers and simulation studies, we calculated that administration of 0.2 mg/kg MBL-SSI for a 3-day interval between infusions and 0.3 mg/kg MBL SSI for a 4-day interval between infusions, would increase MBL serum level to the therapeutic level above 1.0 µg/ml (16).

**Assays**

MBL measurements were performed at Sanquin Research and the Landsteiner Laboratory, AMC, Amsterdam. Genotyping was performed by a Taqman assay, as previously described (23). For screening purposes, MBL plasma levels were measured by enzyme-linked immunosorbent (ELISA) assay technique as previously described (23, 24). Briefly, mannan was coated to the solid phase and incubated with plasma. Afterwards, biotinylated mouse-anti-MBL (anti-MBL-1, 10 µg/ml, Sanquin) was used as detection antibody (24). During the trial, MBL serum levels were determined by the same ELISA at the Department of Immunochemistry, Sanquin Diagnostics, Amsterdam.

**Detection of anti-MBL antibodies**

MBL antibodies were assessed by ELISA (16). In brief, purified human MBL-coated microtitre plates (1 µg/well) were incubated for 2 h at RT with serial dilutions of the patient sera followed by a dilution series of rabbit anti-MBL. After washing, horseradish peroxidase-conjugated rabbit anti-human IgG or swine anti-rabbit immunoglobulins (DakoCytomation, Denmark) were added to the wells and incubated for 1 h. Anti-serum from rabbits immunized with purified human MBL served as positive reference. If the response of the 1/10 dilution of the patient’s sample was below the response of the 1/163840 dilution of the rabbit anti-MBL serum (the highest dilution showing a positive response), the patient was considered free of anti-MBL activity.

**Pharmacokinetics**

For the population pharmacokinetic analysis, NONMEM version VI (GloboMax LLC, Hanover MD, USA) was used, applying the first-order conditional estimation method with interaction throughout the analysis. An open, single-compartment model was used. Pharmacokinetic parameters estimated were: clearance, volume of distribution and baseline MBL level, which was assumed to be constant during the treatment period.

Precision of the parameters was estimated with the covariance step of NONMEM. Individual Bayesian parameter values were obtained with the posthoc step of NONMEM. Since data of several occasions were available from four patients, both interindividual and interoccasion variability was estimated with proportional models (25). Residual error was estimated with a proportional error model.

Weight was incorporated into the basic pharmacokinetic model according to allometric
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Trial no.</th>
<th>Sex</th>
<th>Age</th>
<th>MBL2 genotype</th>
<th>MBL level (µg/ml)</th>
<th>Infusion amount</th>
<th>Tumour</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>F</td>
<td>2.1</td>
<td>HYPA/LYPB</td>
<td>0.87</td>
<td>5</td>
<td>AML</td>
<td>MACE: methotrexate (MTX), amsacrine, cytarabine, etoposide (MRC12/DCLSG-ANLL-97)</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>M</td>
<td>8.1</td>
<td>LQYA/HYPD</td>
<td>0.66</td>
<td>2</td>
<td>Common ALL</td>
<td>FLAG-IDA: fludarabine, ara-C, MTX, idarubicine</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>M</td>
<td>12.5</td>
<td>LQYA/LXPA</td>
<td>0.35</td>
<td>2</td>
<td>T cell ALL</td>
<td>Induction: mitoxantrone, vincristine, asparaginase, MTX, ara-C (T-ALL 9)</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>F</td>
<td>1.1</td>
<td>LQYA/LYPB</td>
<td>0.51</td>
<td>3</td>
<td>Neuroblastoma</td>
<td>CADO: cyclophosphamide, doxorubicin, vincristine (AMRO neuroblastoma)</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td></td>
<td>2.0</td>
<td>=</td>
<td>=</td>
<td>4</td>
<td>=</td>
<td>N5: videsine, etoposide, cisplatin (High risk neuroblastoma)</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td></td>
<td>2.2</td>
<td>=</td>
<td>=</td>
<td>5</td>
<td>=</td>
<td>N5: videsine, etoposide, cisplatin (High risk neuroblastoma)</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td></td>
<td>2.3</td>
<td>=</td>
<td>=</td>
<td>3</td>
<td>=</td>
<td>N6: uromitexan, doxorubicine, vincristine, dacarbazine, ifosfamide (High risk neuroblastoma)</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>F</td>
<td>9.7</td>
<td>LXPA/LYPB</td>
<td>0.48</td>
<td>4</td>
<td>Ewing sarcoma</td>
<td>VIDE: vincristine, ifosfamide, doxorubicine, etoposide (EURO EWING 99)</td>
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<tr>
<td>F</td>
<td>6</td>
<td>M</td>
<td>1.7</td>
<td>LXPA/LYPB</td>
<td>0.09</td>
<td>5</td>
<td>B-ALL</td>
<td>COPADM: vincristine, cyclofosfamide, doxorubicine, MTX (LMB 2001)</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>M</td>
<td>0.5</td>
<td>HYPD/HYPD</td>
<td>0.08</td>
<td>8</td>
<td>Pro B-ALL</td>
<td>MARAM: MTX, asparaginase, 6-mercaptopurine, cytarabine (DCLSG Interfant 99)</td>
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<tr>
<td>G</td>
<td>13</td>
<td></td>
<td>1.3</td>
<td>=</td>
<td>=</td>
<td>2+5*</td>
<td>=</td>
<td>FOSFETO: cyclofosfamide, etoposide; THIME: 6-thioguanine, MTX (SNWLK ALL-relapse 98)</td>
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<tr>
<td>G</td>
<td>18</td>
<td></td>
<td>1.6</td>
<td>=</td>
<td>=</td>
<td>7</td>
<td>=</td>
<td>FLAG-IDA: fludarabine, ara-C, MTX, idarubicine</td>
</tr>
<tr>
<td>H</td>
<td>10</td>
<td>M</td>
<td>10.5</td>
<td>LXPA/LYPB</td>
<td>0.09</td>
<td>1</td>
<td>T cell lymphoma</td>
<td>Vincristine, doxorubicine (SKION EURO LB 02)</td>
</tr>
<tr>
<td>I</td>
<td>12</td>
<td>M</td>
<td>15.4</td>
<td>LXP/HYPD</td>
<td>0.47</td>
<td>1</td>
<td>Gastro-intestinal stromal tumour</td>
<td>Glivec</td>
</tr>
<tr>
<td>J</td>
<td>14</td>
<td>F</td>
<td>7.2</td>
<td>LXP/HYPD</td>
<td>0.38</td>
<td>1</td>
<td>Malign peripheral nerve sheath tumour (MPNST)</td>
<td>ICE: ifosfamide, carboplatin, etoposide (MPNST protocol)</td>
</tr>
<tr>
<td>J</td>
<td>16</td>
<td></td>
<td>7.3</td>
<td>=</td>
<td>=</td>
<td>1</td>
<td>=</td>
<td>ICE: ifosfamide, carboplatin, etoposide (MPNST protocol)</td>
</tr>
<tr>
<td>K</td>
<td>15</td>
<td>F</td>
<td>11.6</td>
<td>LXPA/LYPB</td>
<td>0.13</td>
<td>1</td>
<td>Osteosarcoma</td>
<td>Doxorubicine, cisplatin, cardiosane (EURAMOS)</td>
</tr>
<tr>
<td>K</td>
<td>17</td>
<td></td>
<td>11.7</td>
<td>=</td>
<td>=</td>
<td>1</td>
<td>=</td>
<td>Doxorubicine, cardiosane (EURAMOS)</td>
</tr>
<tr>
<td>K</td>
<td>19</td>
<td></td>
<td>11.8</td>
<td>=</td>
<td>=</td>
<td>1+2</td>
<td>=</td>
<td>Doxorubicine, cardiosane (EURAMOS)</td>
</tr>
<tr>
<td>L</td>
<td>20</td>
<td>M</td>
<td>9.6</td>
<td>LYP/LYPB</td>
<td>0.54</td>
<td>1</td>
<td>Ewing sarcoma</td>
<td>VAI: vincristine, aclimomyicine D, ifosfamide (EURO EWING 99)</td>
</tr>
</tbody>
</table>

F, female; M, male; =, same value as above; *There was an interval of 9 days between the second and third MBL infusion. THIME was given in between; †T-cell lymphoblastic non-hodgkin lymphoma; ‡ six days after a single MBL infusion, the patient was admitted with neutropenic fever and MBL infusions were resumed; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; MRC, UK Medical Research Council Adult and Children’s Leukaemia Working Parties; DCLSG: Dutch Childhood Leukemia Study; SKION: Dutch Society on Childhood Oncology; SFOP: French Society for Pediatric Oncology.
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Figure 1. Study protocol and inclusion of patients. (A) MBL treatment regimen. Each patient received a MBL infusion (---) on day 1. Seven patients received repeated MBL infusions afterwards (----). (B) Flowchart of included patients. Four patients were included multiple times. *Single infusion, followed by two extra infusions after 5 days.
Chapter 8

scaling (26). Weight was scaled at 25 kg to provide a relevant estimate of clearance and volume of distribution for a child of 25 kg. For diagnostic purpose, we attempted to evaluate the power estimates of the allometric scaling functions.

Age and gender were included in the pharmacokinetic model on clearance and volume of distribution and significance was evaluated with the likelihood ratio test (p-value of <0.01). The half-life and mean residence time were estimated from the primary pharmacokinetic parameters.

Model evaluation was based on both numerical and graphical diagnostics. The R-based model building aid Xpose (version 4, Uppsala, Sweden) (27), was used for graphical model diagnostics. The model was evaluated with basic goodness-of-fit plots (e.g. predicted versus observed level and several residual based diagnostics). Furthermore, a case-deletion procedure was executed to evaluate whether the parameter values were driven by a single influential individual. Finally, a visual predictive check was performed (28).

Since not all patients received MBL infusion during the whole neutropenic period, the time above 1.0 μg/ml was estimated for each individual, assuming that patients received the twice-weekly dosing strategy, by means of the individual pharmacokinetic parameters of each patient. Similarly, the trough level and the maximal level were estimated.

Based on the population pharmacokinetic model developed, a simulation study with >10,000 individuals was conducted to investigate whether the proposed dosing strategy would yield adequate MBL substitution. The time above 1.0 μg/ml was estimated.

Statistical analysis

Continuous variables were presented by descriptive statistics, whereas categorical variables were summarised by frequency counts. Because of the limited number of patients, data were analysed descriptively. The occurrence of (serious) adverse events possibly related to the study drug was described.

Results

Baseline characteristics

The median age of the 12 patients (7 males) was 8.8 years (range: 6 months-15.4 years). The underlying malignancy varied (Table I). Median baseline MBL plasma level was 0.40 μg/ml (range: <0.04-1.0 μg/ml).

Each patient received a unique identification letter, and each inclusion a unique identification number. Seven patients (A-G) received repeated MBL infusions, varying from 2 to 8 infusions in total (Table I, Figure 1B). Five patients (H-L) received single MBL infusions (Figure
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1B). Patient C and I were excluded in the per-protocol analysis due to violation of the original inclusion criteria. Patient D was included four times, patient G and K three times and patient J two times. In total, 65 MBL infusions were given in 20 MBL courses (trial number 1-20) to 12 patients (A-L).

Safety

No MBL-related serious adverse events were reported during 20 MBL courses. Two patients experienced serious adverse events considered unrelated to the study drug, both three days after the last MBL infusion. Patient C developed permanently disabling convulsions and aphasia. The MRI showed two infarctions, as a result of thrombosis, a possible complication of concomitant sepsis or chemotherapy. Patient G experienced an anaphylactic reaction to asparaginase. Two mild, one moderate and two severe MBL-unrelated adverse events (port-a-cath removal) occurred. Another mild adverse event was considered ‘possibly’ MBL-related. This patient developed a short-lasting temperature of 38°C without allergic reactions one hour after the second MBL infusion. No adverse events were suggestive of infusion reactions or resulted in either discontinuation or reduction in the dose of MBL. The MBL infusions did not affect vital signs or laboratory parameters in any of the patients. None of the patients withdrew from the study. No anti-MBL antibodies were detected four weeks after each final MBL infusion.

MBL concentrations

Figure 2 shows MBL level versus time after the first course of MBL infusions in 12 patients. Patients D, G, J, and K were included multiple times (Figure 3). Two of these patients received

Figure 2. Observed MBL levels versus time after the first course of MBL infusion(s) of each patient. After day 4 trough levels of alternate dosages are shown of patients A-G, who received repeated infusions of MBL.
multiple MBL courses with an interval of one year. Their peak levels were lower at younger age (Figure 3A and B, dotted versus straight lines).

Pharmacokinetics

In the per-protocol pharmacokinetic analysis, data on all occasions (multiple inclusions) of 10 patients (A-B, D-G, J-L) were simultaneously included. Table II shows the final parameter estimates of the basic model. Estimation of the power coefficients of the allometric scaling did not improve the model. The final estimates of the power coefficients were near the expected values of 0.7 and 1 for clearance and volume of distribution, respectively. Inclusion of interoccasion variability on clearance did not improve the fit, and the estimate of interoccasion variability was not significantly different from 0. Basic goodness-of-fit plots did not reveal any relevant structural model misspecification (data not shown).

As calculated from the primary pharmacokinetic parameters, the half-life of MBL for a typical child of 25 kg was 36.4 h (range: 23.7-66.6 h). Variability was moderate for clearance and volume of distribution (up to 27%). The alternate dosing strategy resulted in an adequate substitution of MBL in the included patients, since the median fraction of time above the threshold of 1.0 μg/ml was 1 (range 0.8-1.0) for a two-week treatment period (Table II). Only patient G had a somewhat lower fraction of time above 1.0 μg/ml, but this patient was still above this threshold for 80% of the time. Median trough and maximum MBL levels were 1.1 and 5.8 μg/ml, respectively (Table II). No significant relation between pharmacokinetics of MBL and sex or age was demonstrated. In the simulation study, the median time above 1.0 μg/ml MBL was over 99%, indicating adequate MBL substitution with the dosing strategy as proposed in the study protocol. After inclusion of patient C and I the half-life remained similar: 34.0 h (range: 22.2-62.9 h).

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Value</th>
<th>IIV (%)</th>
<th>IOV (%)</th>
<th>RSE (%)</th>
<th>Rangea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (L/h)a</td>
<td>0.0329</td>
<td>22.1</td>
<td>8.39</td>
<td>0.0122</td>
<td>0.0513</td>
</tr>
<tr>
<td>V (L)b</td>
<td>1.73</td>
<td>27.5</td>
<td>27.0</td>
<td>14.1</td>
<td>0.736</td>
</tr>
<tr>
<td>Baseline MBL (μg/ml)</td>
<td>3.29</td>
<td>85.7</td>
<td>28.8</td>
<td>0.07</td>
<td>1.12</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>29.8</td>
<td>7.6</td>
<td>43.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>129</td>
<td>66</td>
<td>152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean residence timeb (h)</td>
<td>52.6</td>
<td>34.1</td>
<td>96.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>36.4</td>
<td>23.7</td>
<td>66.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction of time above 1.0 μg/mlc</td>
<td>1</td>
<td>0.804</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal MBL level (μg/ml)</td>
<td>5.75</td>
<td>4.89</td>
<td>7.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trough MBL level (μg/ml)d</td>
<td>1.06</td>
<td>0.66</td>
<td>2.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IIV=interindividual variability, IOV=interoccasion variability, RSE=relative standard error of estimate, V=volume of distribution, t1/2=half life

aTypical values reported for a patient with a body weight of 25 kg, bSecondary parameters are calculated from primary parameters, therefore, no parameter precision can be reported, cMedian value of individual estimates provided, dRange of individual values as obtained from Bayesian estimation
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Repeated infusions:

(A) Patient D (n=4). D-9/ D-11: no MBL levels measured at time points 15 min. through 6 h.

(B) Patient G (n=3).

(C) Patient J (n=2). J-16: no MBL levels measured at time points 2 h. through 6 h.

(D) Patient K (n=3). K-19: Two repeated MBL infusions five days after single MBL infusion.

Figure 3.
Clinical parameters were evaluated in 11 MBL courses of 6 patients (A-B, D-G) who received repeated infusions during a neutropenic episode (Figure 1B). In 8 out of 11 MBL courses (73%), neutropenic fever occurred (Table III). In 4 patients fever resolved within 72 hours after starting antibiotics and 4 needed prolongation or change of antibiotic therapy after persistence of fever. Two patients (A-1 and G-18) had positive blood cultures (Comamonas acidovorans and Streptococcus mitis) and persistent fever, for which their port-a-cath was removed. For the latter infection immediate circulatory support was required. None of the patients needed oxygen support or intensive care during the study. All patients used oral prophylactic antibiotic and antifungal medication (selective gut decontamination consisting of co-trimoxazole, amphotericin or nystatine and colistine or ciprofloxacin).

None of the four patients with one or more single MBL infusions developed fever or infection within three or four days after the MBL infusion.

Discussion

We demonstrated that twice-weekly infusions of plasma-derived MBL resulted in therapeutic MBL trough levels in neutropenic children with cancer. The results of our population

### Table III. Infectious outcome of the 6 patients with 11 cycles of repeated MBL infusions.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Trial no.</th>
<th>Infection description</th>
<th>Duration fever (days)</th>
<th>Antibiotics Type</th>
<th>Duration (days)</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Neutropenic fever: Comamonas acidovorans sepsis</td>
<td>2+1*</td>
<td>V/G/fortum</td>
<td>12</td>
<td>54</td>
</tr>
<tr>
<td>D</td>
<td>4</td>
<td>Neutropenic fever e.c.i.</td>
<td>4</td>
<td>V/G</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>Neutropenic fever e.c.i.</td>
<td>3</td>
<td>V/G</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>F</td>
<td>6</td>
<td>No fever</td>
<td>0</td>
<td>none</td>
<td>0</td>
<td>x</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>No fever</td>
<td>0</td>
<td>none</td>
<td>0</td>
<td>x</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>Neutropenic fever e.c.i.</td>
<td>7</td>
<td>V/G</td>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>Neutropenic fever e.c.i.</td>
<td>1</td>
<td>V/fortum</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td>Neutropenic fever e.c.i.+ superficial skin candida infection</td>
<td>1</td>
<td>V/G</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>G</td>
<td>13</td>
<td>No fever</td>
<td>0</td>
<td>none</td>
<td>0</td>
<td>x</td>
</tr>
<tr>
<td>G</td>
<td>18</td>
<td>Neutropenic fever: Streptococcus mitis sepsis, superficial skin candida infection</td>
<td>3</td>
<td>V/G</td>
<td>19</td>
<td>158</td>
</tr>
</tbody>
</table>

*This patient had 2 days of fever, for which the port-a-cath (PAC) was removed 6 days later. After the surgery, fever was observed once, probably due to reactive bacteremia. Nr, number; V, vancomycin; G, gentamycin; e.c.i., e causa ignota; x, not measured.
pharmacokinetic analyses are in agreement with the retrospective population pharmacokinetic analysis of MBL in 20 healthy MBL-deficient adults (16, 21). The clearance we reported for a patient of 25 kg translates into a clearance of 0.071 L/h for a 75 kg patient, which is close to the estimate we obtained from adults (0.052 L/h), keeping in mind that both study populations were relatively small. The half-life of 36 hours is also close to the half-life in adults of 45 hours, as calculated from the results of our pharmacokinetic analysis. According to allometric scaling, pharmacokinetics were not related to age after correction for body weight. This indicates that calculation of the optimal dose based on body weight is a reasonable strategy for MBL substitution in MBL-deficient patients. In the simulation study the proposed dosing strategy led to adequate MBL substitution.

The half-life range (23.7-66.6 h) in our trial is smaller than that observed in the phase-I trial in healthy adults and closer to that observed with recombinant MBL, i.e. approximately 30 hours (16, 17), which can be explained by weight-adjusted dosing. Plasma-derived MBL differs from recombinant MBL because it contains MASPs and a higher degree of oligomerization (22, 29).

In agreement with the experience in adults (15-19), the infusion of plasma-derived MBL appears to be safe in children. Dosages up to 14 mg can be injected within 10 minutes without any adverse effects. The only adverse event possibly related to the study drug was probably caused by a viral upper respiratory tract infection. The absence of anti-MBL antibodies up to one year after MBL substitution suggests that no immune response against the plasma-derived MBL was initiated.

MBL substitution appeared to be beneficial in case reports and pre-clinical studies in knock-out mice (18, 30). Although MBL infusion in vivo resulted in complement activation and opsonophagocytosis in vitro (31), the number of patients included is too small to demonstrate clinical benefit. Moreover, MBL substitution in pediatric oncology patients is debated because of the variable results with respect to infection risk and severity in MBL-deficient neutropenic children (9, 23, 32). This variation may be related to the depth and duration of chemotherapy-induced bone-marrow suppression (14, 3), disabling an optimal phagocytic function in the host, as affirmed to be necessary for MBL-induced opsonophagocytosis (9, 23, 34). MBL substitution therapy may be more suitable in other pediatric patient groups, such as neonates or children with sepsis or recurrent (airway) infections (19, 35-38).

In sum, we have demonstrated that therapeutic MBL trough levels can be predicted and attained with twice-weekly infusions with plasma-derived MBL in children with cancer. Repeated MBL substitution treatment appears to be safe. The pharmacokinetics of MBL in MBL-deficient children are comparable to adults, after correction for body weight. The half-life of MBL was estimated to be 36 hours, with a smaller range than in adults. After definition of a suitable target patient group, clinical efficacy of MBL should be investigated in multicenter phase-III clinical trials.
Chapter 8

Acknowledgements

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Reference List

Safety and pharmacokinetics of plasma-derived MBL substitution in children


