available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Short Communication

Safety and pharmacokinetics of plasma-derived mannose-binding lectin (MBL) substitution in children with chemotherapy-induced neutropaenia

Florine N.J. Frakking[a,], Nannette Brouwer[b], Marianne D. van de Wetering[a], Ilona Kleine Budde[c], Paul F.W. Strengers[c], Alwin D. Huitema[d], Inga Laursen[e], Gunnar Houen[e], Huib N. Caron[a], Koert M. Dolman[a,b], Taco W. Kuijpers[a,b]*

[a]*Emma Children's Hospital, Academic Medical Center (AMC), Room G8-205, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*
[b]*Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, AMC, University of Amsterdam, The Netherlands*
[c]*Medical Department, Sanquin Plasma Products, Amsterdam, The Netherlands*
[d]*Department of Pharmacy & Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands*
[e]*Statens Serum Institut, Copenhagen, Denmark*

ARTICLE INFO

Article history:
Received 17 August 2008
Received in revised form 29 October 2008
Accepted 24 November 2008
Available online 31 December 2008

Keywords:
Mannose-binding lectin
Phase II trial
Chemotherapy
Pharmacokinetics

ABSTRACT

Mannose-binding lectin (MBL)-deficient children with cancer may benefit from substitution of the innate immune protein MBL during chemotherapy-induced neutropaenia. We determined the safety and pharmacokinetics of MBL substitution in a phase II study in MBL-deficient children.

Twelve MBL-deficient children with cancer (aged 0–12 years) received infusions of plasma-derived MBL once, or twice weekly during a chemotherapy-induced neutropaenic episode (range: 1–4 weeks). Four patients participated multiple times. Target levels of 1.0 μg/ml were considered therapeutic.

In total, 65 MBL infusions were given. No MBL-related adverse reactions were observed, and the observed trough level was 1.06 μg/ml (range: 0.66–2.05 μg/ml). 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 h (range: 23.7–66.6 h). No anti-MBL antibodies were measured 4 weeks after each MBL course.

Substitution therapy with MBL-SSI twice weekly was safe and resulted in trough levels considered protective.

1. 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 concentrations 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

* *Corresponding author:* Tel.: +31 (0) 6 41514481; fax: +31 (0) 20 691 2231.
E-mail address: f.n.frakking@amc.uva.nl (F.N.J. Frakking).
0959-8049/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2008.11.036

1.0 μg/ml.[4] Three single nucleotide polymorphisms (SNPs) in codons 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 levels.[5]

MBL deficiency is associated with increased infection susceptibility, particularly in children and immunocompromised patients.[6,7] Duration and severity of febrile neutropaenia were increased in MBL-deficient children and adults with cancer.[8–10] Therefore, neutropaenic oncology patients were proposed to possibly benefit from MBL substitution. 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.[11,12] 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 3 d (69.6 h; range: 14.6–114.9 h).[11] 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 randomised placebo-controlled phase III efficacy study. We performed an open, uncontrolled phase II clinical trial on MBL substitution in 12 MBL-deficient paediatric oncology patients with chemotherapy-induced neutropaenia. In this report, we describe the safety, pharmacokinetics and clinical course of these patients.

2. Material and methods

2.1. 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 Ethical Committee. Sanquin Plasma Products, Amsterdam, were responsible for monitoring the trial. Trial registration number: NCT00138736.

After the end of a chemotherapy course patients received an MBL infusion, which was repeated twice weekly until patients had recovered from chemotherapy-induced neutropaenia (neutrophil count <500 cells/μl) (Fig. 1). Dosages of 0.2 mg/kg alternated with dosages of 0.3 mg/kg. Patients were allowed to participate more than once. To increase the willingness of patients to participate, the study protocol was changed after the first seven patients. Interim analysis had demonstrated that single MBL infusions would be sufficient to determine pharmacokinetic parameters. Therefore, patients could also participate with only a single infusion followed by an observation period of 3 or 4 d (Fig. 1).

2.2. Patient selection

Participants were children treated for cancer in the Emma Children's Hospital, Amsterdam, The Netherlands. Inclusion

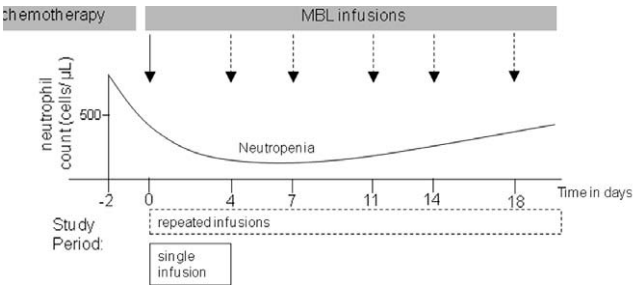

Fig. 1 – MBL treatment regimen. Each patient received a MBL infusion (—) following a neutropaenia-inducing chemotherapy course (day 0). Seven patients received repeated MBL infusions afterwards (- - - -).

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 neutropaenia. Exclusion criteria consisted of the 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 and IgM) or complement factors (measured by AP50 and CH50).

The clinical condition of 9 of 34 eligible children did not allow them to participate in this clinical trial, e.g. palliative treatment setting. Parents of 10 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 neutropaenia.

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.

2.3. End-points

The primary end-points 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 and oxygen and/or immediate circulatory support were recorded. Due to the small number of patients, clinical efficacy was not considered a realistic end-point.

2.4. Data collection

MBL levels were measured before infusion, 15 min (″), 2, 4, 6 and 16–24 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