QUALITY OF THERAFLEX® MB-PLASMA DURING STORAGE AND TREATMENT

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Introduction

Background: Although in the last decades thanks to the implementation of several methods like donor selection and testing procedures the risk of virus transmission from plasma has decreased, infection of patients still exists. Additionally new viruses like West Nile Virus enter the transfusion chain [1]. Therefore, the photodynamic treatment of therapeutic plasma with methylene blue (MB) is a technique used in several European countries for pathogen inactivation [2]. MacoPharma has developed the proprietary Theraflex® MB-Plasma bag system including a MB pill and a final MB filtration step.

Aim: Aim of the study is to show the quality of the MB plasma during the preparation procedure and during storage using the Theraflex® system (see Figure 1).

Materials & Methods

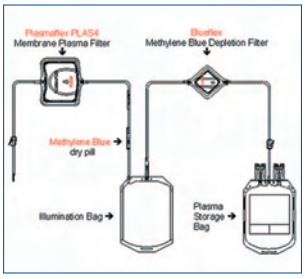


Figure 1: Theraflex® MB-Plasma bag system

Preparation Process
For the preparation process every single step was evaluated using 18 single donor plasma units. For the evaluation of the plasma factors 5 ml were drawn at different stages (see Figure 2). The samples were pooled after drawing and measured for the specified factors. Six samples of each stage were pooled at three days. A whole panel of plasma factors was measured for the resulting three pools (see Figure 3).

Stability
Stability data were generated using three plasma pools. Six plasmas were pooled and afterwards divided into six aliquots. Each was treated as single unit and then each was divided into six storage samples. The same plasma factors as for the manufacturing process were evaluated.
MB and photoproduct content was below the detection limit as previously described [3].

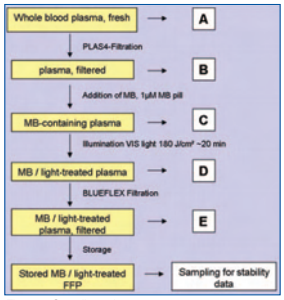
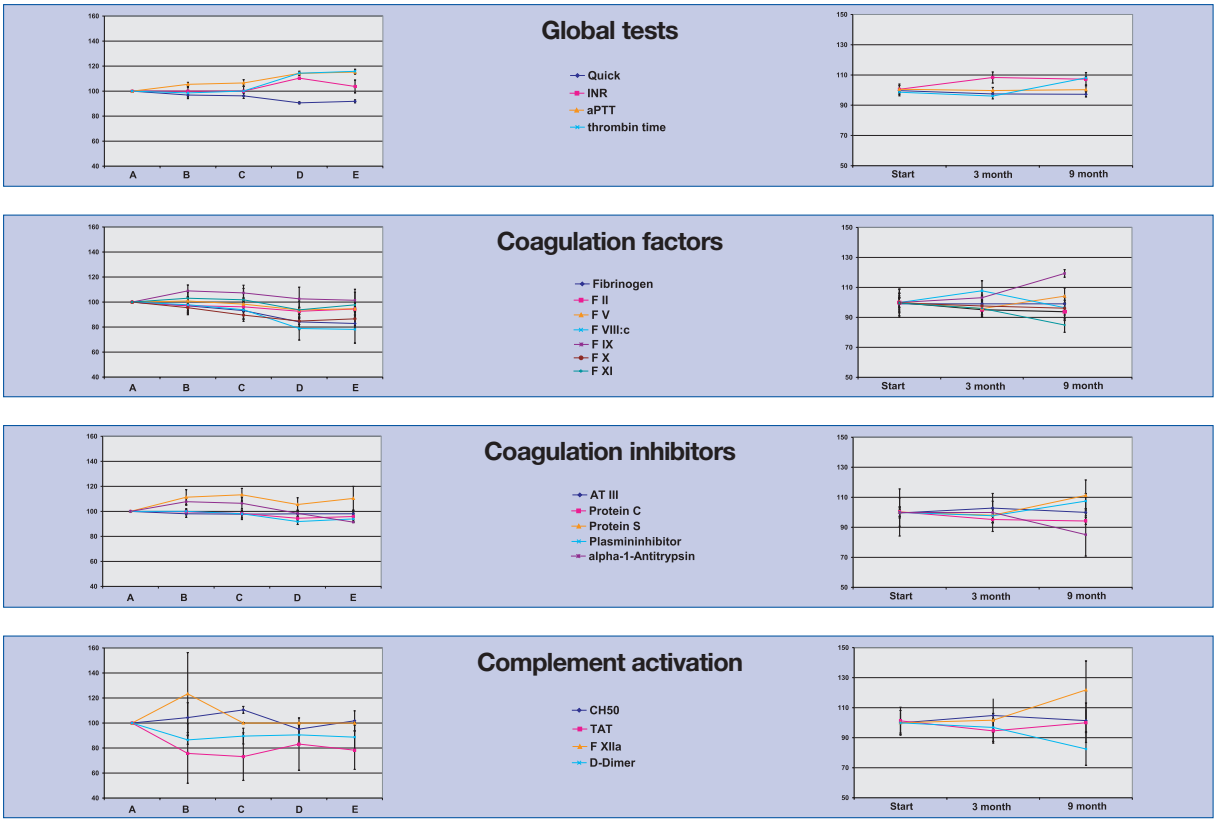


Figure 2: Sampling scheme

Results

In-process control



Storage stability

Figure 3: Percentage of deviation from the source plasma for different plasma factors during storage and treatment A: before treatment; B: after PLAS4 filtration; D: after MB addition; D: after illumination; E: after Blueflex filtration

All investigated plasma factors remained stable during the investigated storage time. A moderate reduction for some coagulation factors during the preparation was found in the illumination step but not in the other preparation stages. This was mainly fibrinogen (17,5 %), factor VIII (22,2 %), and factor X (13,4 %). Despite this reduction the values were within the ranges found in non-treated plasma.

Conclusions

Plasma treated with the Theraflex procedure showed slight reduction during treatment and no reduction during storage. All plasma factors remained within the threshold values. The treatment of therapeutic plasma with MB is a valid technique of pathogen inactivation.

[1] **West Nile virus in plasma is highly sensitive to methylene blue-light treatment** Mohr et al, **Transfusion** 2004;44:886-890
[2] **Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety?** LM Williamson et al, **Transfusion** 2003;43:1322-1329
[3] **Filtration of Methylene Blue and Photoproducts after Photodynamic Treatment of Plasma using BLUEFLEX** T Verpoort et al, 2003 VIII European ISBT Congress P245

PREPARATION OF METHYLENE BLUE –TREATED PLASMA UNDER WORST-CASE CONDITIONS - INFLUENCE ON QUALITY AND STABILITY-

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INTRODUCTION

Treatment with methylene blue (MB) and light is a well-known procedure for the inactivation of blood-borne viruses in Fresh Frozen Plasma (FFP). The purpose of this study was to assess the quality and stability of MB/light-treated plasma (MB plasma) processed by the MacoPharma Theraflex MB-Plasma® system. Preparation was done under worst case conditions for routine processing to evaluate the worst plasma quality to be expected during production.

METHODS

12 single donor units of MB/light treated plasma were prepared using the MacoPharma Theraflex MB-Plasma® system. Preparation included leukocyte depletion (Plasmaflex-filter), addition of methylene blue (MB-pill) prior to illumination and depletion of MB and photoproducts (Blueflex-filter) after treatment. Samples were taken before treatment and from the final product. For the assessment of stability, plasma from four different plasma pools was photodynamically treated and stored for up to 9 months. Treatment was done under worst-case conditions for the preservation of product quality: maximum MB concentration during illumination (1.15 µmol/l), maximum storage time of whole blood before separation (4°C, 17 h), maximum storage time of MB plasma before freezing (1 h).

RESULTS

Thrombin time, fibrinogen (Clauss), factors V, VIII, XI and protein C were significantly altered by MB/light treatment, while anti-thrombin III (AT III), vWF:RCo, vWF cleaving protease (vWF CP), plasmin inhibitor and α_1-antitrypsin remained unchanged (Fig. 1). There was no activation of the coagulation markers (F 1+2, D-dimers) attributed to the virus inactivation procedure including the filtration steps for leukocyte depletion and MB and photoproduct depletion. The influence of each manufacturing step on the activity of coagulation factors was investigated using three plasma pools. Most of the activity was lost during illumination (Fig. 2). After illumination MB and its photoproducts (azure A, azure B, azure C) were depleted by Blueflex filtration (Fig 3) to a final concentration of <0.1 µmol/l (MB + sum of photoproducts). Stability of MB-Plasma was tested during storage at -30°C for up to 9 months (Fig 4). Stability testing will be continued for a total of 27 months.

Parameter		Before treatment	After treatment	Percentage of loss (-) or increase (+)
Thrombin time	[s]	15.8 +/- 0.7	19.1 +/- 1.5	+20.6 §
Fibrinogen (Clauss)	[mg/dl]	279.9 +/- 53.6	222.5 +/- 41.8	-20.3 §
Factor V	[%]	120.7 +/- 30	101.8 +/- 29.8	-16.4 §
Factor VIII	[%]	115.2 +/- 23.9	89.5 +/- 21.3	-22.2 §
Factor XI	[%]	94.6 +/- 20.1	82.1 +/- 18.7	-13.3 §
Antithrombin III	[%]	87.6 +/- 7.4	87.3 +/- 6.0	-0.3 ns
Protein C	[%]	105.3 +/- 21.3	94.5 +/- 17.4	-9.8 §
Protein S, free	[%]	94.2 +/- 11.2	94 +/- 12	-0.2 ns
vWF:RCo	[%]	98.8 +/- 23.5	98.8 +/- 23.5	-5.0 ns
vWF CP	[%]	52.8 +/- 13.9	50.2 +/- 9.7	- 1.8 ns
Plasmin inhibitor	[%]	94.2 +/- 9.1	93 +/- 8.7	-1.2 ns
α_1-Antitrypsin	[mg/dl]	95.8 +/- 19.3	95.3 +/- 19	-0.5 ns
F 1+2	[nmol/l]	1.02 +/- 0.25	1.03 +/- 0.27	+1.0 ns
D-Dimers	[mg/l]	0.20 +/- 0.04	0.18 +/- 0.04	-9.2 ns
CH 100	[U/ml]	618 +/- 115	633 +/- 169	+4.1 ns

n = 12, § significant , P < 0.01 (Wilcoxon Rank Sum Test) ns not statistically significant

Fig.1 Influence of the MB/light treatment on plasma quality (data from 12 single donor units, treatment under worst-case conditions)

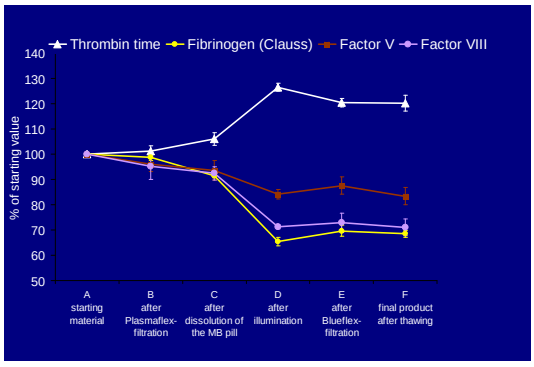

Fig.2 Influence of the individual manufacturing steps on plasma quality (data from three pools)

Methylene Blue	µmol/l	Depletion (C / A)
A (before illumination)	0.97 +/- 0.06	
B (after illumination)	0.65 +/- 0.15	
C (after Blueflex filtration)	0.01 +/- 0.01	98.8 %
Azure A	**µmol/l**	
A	0.01 +/- 0.01	
B	0.06 +/- 0.03	
C	0.01 +/- 0.01	
Azure B	**µmol/l**	
A	0.11 +/- 0.01	
B	0.24 +/- 0.04	
C	0.01 +/- 0.01	
Azure C	**µmol/l**	
A	0.00 +/- 0.00	
B	0.03 +/- 0.03	
C	0.01 +/- 0.02	
Phenothiazine total	**µmol/l**	
A	1.09 +/- 0.07	
B	0.99 +/- 0.08	
C	0.04 +/- 0.03	96.4 %

Fig.3 Depletion efficacy of the Blueflex filter (data from 12 single donor units)

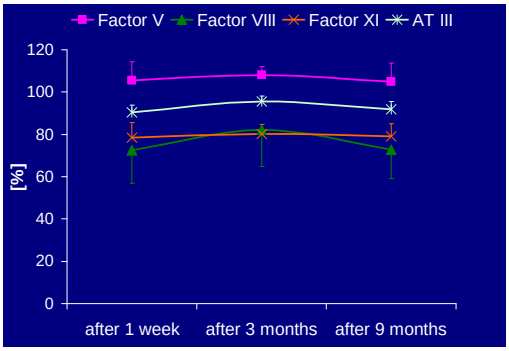

Fig.4 Stability of MB/light-treated plasma (data from 4 pools, , treatment under worst-case conditions)

CONCLUSIONS

Even under worst-case conditions, photodynamic treatment of FFP using the Theraflex MB-Plasma® system only moderately affects the activities of coagulation factors. The Blueflex-filter depletes MB and its photoproducts by over 90% after photodynamic treatment. Storage of MB plasma for up to 9 months had no effect on coagulation factors.

THERAFLEX – MB PLASMA

Coagulation factors and activation parameters

						For each stage in the preparation		
Parameter	**Limits**	**Samp.A (n = 40)**	**Samp.B (n = 40)**	**Samp.C (n = 10)**	**Samp.D (n = 10)**	**Samp.A (n = 10)**	**Samp.Ab (n = 10)**	**Samp.B (n = 10)**
Prothrombin rate (%)	70 - 130	80.8	71.8	87.7	76.6	73.5	68.2	65.7
INR	1	1.21	1.33	1.11	1.24	1.3	1.39	1.43
Activated partial thromboplastin time test (ratio)		1.12	1.26	1.05	1.18	1.13	1.22	1.27
Fibrinogen	2 - 4	3.11	2.29	2.94	2.04	3.03	2.28	2.36
Factor II (%)	70 - 120	102	98	98	95.1	95.1	86.7	92.3
Factor V (%)	70 - 120	102.2	94.1	89.5	83.2	105.7	98.1	96.4
Factor VII (%)	70 - 130	113.1	101.9	106.1	92.1	107.6	100.6	104.2
Factor VIII (%)	60 - 150	100.6	73.5	101.1	76.1	114	89.5	86.1
Factor IX (%)	60 - 150	96.5	78.8	98.6	86	99.2	78.9	85.7
Factor X (%)	70 - 120	105.9	95.3	106.9	92.6	97.3	91.7	92.3
Factor XI (%)	60 - 140	90.9	75.3	86.7	64.4	85.5	70.8	65.7
Factor XII (%)	60 - 140	103.7	92.6	110.5	99.9	98.4	96	93.9
Antithrombin III (%)	80 - 120	104.8	89.9	104.4	101.9	105.8	103.7	93.9
Protein C (%)	70 - 140	110.9	105.3	108.4	103	120.7	112.9	112.6
Protein S (%)	70 - 140	82.6	78.6	81.6	71.2	83.8	77.6	81.4
V Willebrand Factor CoF ristocetin (%)	60 - 150	97.6	92.9	87.4	84	143.6	137.4	138.6
Von Willebrand Factor Ag (%)	60 - 150	134.6	118.2	130.3	128	143.6	121.8	122.2
Plasminogen (%)	80 - 120	102.7	100.9	100.8	96.9	103.6	101.6	101.6
α2-antiplasmin (%)	80 - 120	111.9	107.7	106.2	104.2	112.5	106.5	107.7
C3a (mg/l)	100 - 400	134.8	134.1	146.8	143.1	124.2	125.5	124.0
C5a (µg/l)	0.9 - 15.4	10.9	14.1	15.1	23.9	7.5	8.3	10.9
Factor XIIa (ng/ml)	< 3.0	2.39	2.22	2.87	2.63	2.57	1.79	2.49
F1 + 2 prothrombin (nmol/l)	0.4 - 1.1	0.99	1.20	0.88	0.87	0.79	0.72	0.78
Platelet factor 4 (UI/ml)	56 - 805	317.2	301.1	413.9	375.4	186.9	197.2	208

A : Plasma before contact with methylene blue

Ab : Plasma after visible light (plasmas 31 to 40)

B : MB-removed Plasma before freezing

C : MB Plasma after 6 months at - 30° C

D : MB-removed Plasma after 6 months at - 30° C

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