Coagulation function in fresh-frozen plasma prepared with two photochemical treatment methods: methylene blue and amotosalen

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BACKGROUND: Pathogen inactivation of plasma intended for transfusion is now the standard of care in Belgium. Two methods for treatment of single plasma units are available: amotosalen plus ultraviolet A light and methylene blue plus visible light. This study compared the quality and stability of plasma treated with these two methods.

STUDY DESIGN AND METHODS: Plasma units made from a pool of two ABO-matched fresh apheresis units were photochemically treated with either amotosalen (PCT-FFP) or methylene blue (MB-FFP). A total of 12 paired samples were evaluated. Plasma coagulation function was assessed at three time points: immediately after treatment, after 30 days of frozen storage, and an additional 24 hours at 4°C after thawing. Comparison between PCT-FFP and MB-FFP was assessed with the paired t test and a p value of less than 0.05 indicated statistical significance.

RESULTS: Based on statistical analysis, mean levels of factor (F)II, FXII, FXIII, von Willebrand antigen, ADAMTS-13, D-dimers, and protein C were equivalent between PCT-FFP and MB-FFP for all three time points. PCT-FFP exhibited shorter mean prothrombin time, activated partial thromboplastin time (two time points), and thrombin time and higher mean levels of fibrinogen, FXI, and protein S than MB-FFP. Retention of FV, FVII, FVIII, FX, or von Willebrand factor:ristocetin cofactor in PCT-FFP was either equivalent to or higher than MB-FFP. MB-FFP contained higher mean levels of plasminogen, antithrombin, and plasmin inhibitor than PCT-FFP. Retention of F IX in MB-FFP was higher than PCT-FFP only after the 4°C storage after thawing.

CONCLUSION: There is adequate preservation of therapeutic coagulation factor activities in both PCT-FFP and MB-FFP. The overall coagulation factor levels and stability of PCT-FFP were better preserved than MB-FFP.

The residual risk of selected transfusion-transmitted infections and the concerns about emerging pathogens have prompted some European countries to mandate additional practices for plasma. In Belgium, in addition to mandatory testing for a number of pathogens known to be transmitted by transfusion, pathogen inactivation of plasma intended for transfusion is now the standard of care. Since 2004, all individual therapeutic plasma units in Belgium have been treated with a disposable set for treatment of plasma (Theraflex, MacoPharma, Mouvaux, France), which utilizes methylene blue (MB) and visible light to inactivate viruses in plasma.[1] A new pathogen inactivation

ABBREVIATIONS: aPTT = activated partial thromboplastin time; MB-FFP = fresh-frozen plasma treated with the methylene blue method; PCT = photochemical treatment; PCT-FFP = fresh-frozen plasma treated with the amotosalen photochemical method; α2PI = α2 plasmin inhibitor; PT = prothrombin time; TT = thrombin time; TTP = thrombotic thrombocytopenic purpura; VWAg = von Willebrand antigen; VWcoR = von Willebrand factor:ristocetin cofactor.

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This study was supported in part by Cerus Corp. Three authors (JH, MR, LL) were affiliated with and held stock or stock options of Cerus Corp. during the conduct of this study.

Received for publication April 12, 2007; revision received June 12, 2007, and accepted June 15, 2007.

doi: 10.1111/j.1537-2995.2007.01488.x.

TRANSFUSION 2008;48:108-117.

system (the INTERCEPT Blood System, Cerus Europe BV, Leusden, Netherlands), which uses amotosalen and ultraviolet A (UVA) light to photochemically (PCT) inactivate viruses, bacteria, parasites, and white blood cells (WBCs) in platelets (PLTs), and plasma, has received CE Mark registration and is now available in Europe.[2-5]

These two technologies differ in their mechanism of action. The MB system is based on the production of nonspecific singlet oxygen. Visible light catalyzes the formation of reactive oxygen radical species, especially singlet oxygen via energy transfer, resulting primarily in guanosine oxidation and depurination. This photodynamic reaction is nonspecific, and singlet oxygen can diffuse and react indiscriminately with proteins, lipids, and nucleic acids.[6] The PCT system is based on the specific photochemical reaction of amotosalen and UVA light with nucleic acid.[4] Amotosalen molecules reversibly intercalate into the helical regions of DNA and RNA. Upon illumination with UVA light, intercalated amotosalen molecules form irreversible covalent bonds with pyrimidine bases present in DNA or RNA. Because of the nucleic acid reaction specificity, amotosalen–protein interaction is minimal.[7]

The PCT system has shown inactivation of high levels of a broad spectrum of bacteria,[2] viruses,[3] and parasites.[8-10] The MB system has also demonstrated the ability to inactivate a broad range of enveloped viruses[1] and some parasites.[11] The photodynamic effect of the MB system is not effective for inactivation of bacteria, cell-associated viruses, or WBCs.[12] The plasma filtration and/or freeze-thaw step incorporated into the MB system reduces the levels of contaminating WBCs and the associated viruses.

The PCT system for PLTs was introduced into Europe in 2003 and has been used on a routine basis in the Blood Transfusion Center of Mont Godinne since October 2003. With the recent introduction of the PCT system for plasma, there is now an alternative to the MB system for pathogen inactivation of plasma. As part of the decision process to evaluate new technology, we designed a study to assess and compare the in vitro coagulation factor activity, recovery, and stability after treatment with the PCT system and the MB system with matched plasma units. To our knowledge this is the first direct comparison between these two pathogen inactivation methods.

MATERIALS AND METHODS

Plasma collection and processing

Twenty-four units of apheresis plasma (500-600 mL) were collected from volunteer donors by plasmapheresis with an automated blood cell separator (Autopheresis-C, Baxter Healthcare Corp., Deerfield, IL) for 12 replicate experiments. Citrate anticoagulant was used at a ratio of 1:16. Donors with blood groups A, B, and O were represented in the study population. For each replicate experiment, two ABO-matched apheresis plasma units were pooled and divided into 3 units containing different volumes of identical plasma. A volume of 200 mL was used for the untreated control. A volume of 235 to 315 mL was transferred to a plasma container for the fresh-frozen plasma treated with the methylene blue method (MB-FFP) study arm, and a volume of 435 to 635 mL was transferred to a plasma container for the fresh-frozen plasma treated with the amotosalen photochemical method (PCT-FFP) study arm. Different volumes were used in the two methods. In routine use, individual whole blood–derived plasma units (235-315 mL) are treated with the MB process whereas apheresis plasma units or pooled whole blood–derived plasma units (435-635 mL) are treated with the PCT process.

PCT-FFP processing disposable sets

The PCT-FFP disposable set for treatment of plasma (Code INT310X, Cerus Europe BV) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen hydrochloride solution in saline packaged inside a plastic container (PL-2411, Baxter Healthcare Corp.) and protected from UVA light; a 1.3-L plastic container (PL-2410, Baxter Healthcare Corp.) for illumination of plasma; a flow compound adsorption device (CAD) to reduce the concentration of amotosalen and free photoproducts consisting of an adsorbent disk composed of a copolymer of polystyrene and divinylbenzene particles fused with an ultrahigh-molecular-weight polyethylene plastic enclosed in an acrylic housing; and three 400-mL plastic containers (PL-269, Baxter Healthcare Corp.) for storage of the treated plasma.

Amotosalen-UVA process and UVA illumination device

Each plasma unit with a volume ranging from 435 to 635 mL was treated with a single PCT-FFP processing set and resulted in 2 to 3 plasma units of approximately 200 mL. The manufacturer's instructions for processing were followed. Briefly, the plasma unit was sterile connected to the amotosalen container and the plasma content passed through the amotosalen container into the illumination container. The plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA light treatment. The illuminated plasma mixture was passed through the compound adsorption device by gravity in approximately 20 minutes and evenly distributed into two or three storage containers.

Illumination of plasma was performed in an INTERCEPT Illuminator (Code INT100, Cerus Europe BV, formerly known as R4R4008, Baxter Healthcare Corp.). The device was capable of illuminating 2 units of plasma (400-

650 mL) per processing cycle. The illuminator delivered a 3 J per cm^2 UVA (320-400 nm) treatment to each plasma unit in approximately 7 to 9 minutes. During illumination, plasma units were reciprocally agitated at approximately 70 cycles per minute.

MB-FFP processing disposable sets

The MB-FFP disposable set for treatment of plasma (Theraflex, MacoPharma) consisted of the following integrated components: a PLAS 4 membrane plasma filter, a tablet of MB hydrochloride (85 µg) situated between the plasma filter and the illumination container, a plastic container (400 mL) for illumination of plasma, a MB depletion filter to reduce the concentration of MB, and a plastic container (400 mL) for storage of the treated plasma.

MB process and visible light illumination device

Each plasma unit with a volume ranging from 235 to 315 mL was treated with a single MB-FFP processing set and resulted in one transfusable plasma unit. The manufacturer's instruction for processing was followed. Briefly, the plasma unit was sterile connected to the set and the plasma content passed through the PLAS 4 membrane plasma filter to remove cellular elements. The plasma content passed through the MB dry tablet into the illumination container. The plasma containing MB was illuminated with 180 J per cm^2 visible light. The illuminated plasma mixture was passed through the MB reduction filter by gravity into a single storage container.

Illumination of plasma was performed in a commercially available illuminator (Maco-tronic, MacoPharma). The device was capable of illuminating 4 units of plasma per processing cycle. The illuminator delivered a 180 J per cm^2 visible light treatment to each plasma unit in approximately 20 minutes. During illumination plasma units were agitated.

Plasma processing after photochemical treatment

The two photochemical treatments (PCTs) were performed concurrently while the control unit was kept at ambient temperature for the duration of the PCT process. After completion of compound reduction, plasma units for three study arms (PCT-FFP, MB-FFP, and untreated control) were processed in an identical manner. Approximately 40 mL of plasma was removed from one plasma unit in each study arm. Plasma was aliquoted into polypropylene tubes and placed in a blast freezer at the same time as the plasma units. The three plasma units from each replicate experiment were frozen at the same time. All plasma units were frozen within 8 hours of collection.

Units were stored frozen for 30 days at –30°C. After 30 days, each plasma unit was thawed with a 37°C plasma warmer (DH8, Helmer, Noblesville, IN). Samples of plasma taken for analysis of coagulation function were transferred to polypropylene freezer tubes, frozen at –30°C, and stored at –30°C until assay.

All thawed plasma units were placed into refrigerator storage (4°C) for an additional 24 hours to assess relative stability of coagulation factors after thawing to model routine clinical practice of holding thawed plasma before transfusion. Samples of plasma from thawed units were withdrawn for coagulation function analysis, transferred to polypropylene freezer tubes, frozen at –30°C, and stored at –30°C until assay.

Measurement of in vitro coagulation function

Coagulation factors for each type of plasma were measured concurrently to minimize differences attributable to variability in assay conditions or substrates. Plasma samples were thawed with a 37°C water bath and assayed within 2 hours of thawing.

Each sample was assayed with a comprehensive panel of coagulation tests: prothrombin time (PT; Innovin, Dade Behring, Marburg, Germany); activated partial thromboplastin time (aPTT; PTT Automote, Diagnostica Stago, Vilvoorde, Belgium); thrombin time (TT; thrombin 10, Diagnostica Stago); fibrinogen (fibrinogen 5, Diagnostica Stago); D-dimers (Liatest Ddi, Diagnostica Stago); antithrombin (Stachrom ATIII, Diagnostica Stago); von Willebrand antigen (VWAg; Liatest VWF, Diagnostica Stago); von Willebrand factor:ristocetin cofactor (VWcoR; BC von Willebrand reagent, Dade Behring); Factor (F)II, FV, FVII, and FX (FII-, FV-, FVII-, or FX-deficient plasma, Diagnostica Stago; Innovin, Dade Behring); FVIII, F IX, FXI, and FXII (FVIII-, F IX-, FXI-, or FXII-deficient plasma, Precision Biologic, Dartmouth, Nova Scotia, Canada; CL'prest, Diagnostica Stago); FXIII (Berichrom FXIII, Dade Behring); protein C (Coamatic protein C, Chromogenix, Milan, Italy); protein S (Liatest protein S, Diagnostica Stago); plasminogen (Stachrom plasminogen, Diagnostica Stago); α2 plasmin inhibitor (α2PI; Stachrom antiplasmin, Diagnostica Stago); and ADAMTS-13 activity (ATS-13, GTI Diagnostics, Waukesha, WI). All assays were performed with clinically approved methods at the clinical laboratory of the Blood Transfusion Center of Mont Godinne. The reference ranges for each assay are indicated in Table 1.

Statistical analysis

The mean and standard deviation (mean ± SD, n = 12) were determined for each parameter. For each time point assayed, the ratio of the coagulation factor or antithrombotic protein activity remaining after pathogen inactiva-

TABLE 1. The reference ranges of each protein coagulation assay

Assay	Reference range
aPTT	26-38 sec
PT	8.5-10.2 sec
TT	19-26 sec
Fibrinogen	180-400 mg/dL
D-dimers	<500 ng/mL
Antithrombin	80-130 IU/dL
VWAg	60-130 IU/dL
VWcoR	50-150 IU/dL
FII, FV, FVII, or FX	50-150 IU/dL
FVIII	60-130 IU/dL
FIX	60-130 IU/dL
FXI	50-150 IU/dL
FXII	40-150 IU/dL
FXIII	50-150 IU/dL
Protein C	70-120 IU/dL
Protein S	65-140 IU/dL
Plasminogen	80-110 IU/dL
α2PI	50-150 IU/dL
ADAMTS-13	50%-150% of normal

tion with either MB or amotosalen to the activity in the corresponding untreated control was calculated and expressed as percentage of retention. For PT, aPTT, and TT, the prolongation of the clotting time after pathogen inactivation relative to the corresponding untreated control was determined. Significant differences between PCT-FFP and MB-FFP were evaluated with a t test for paired samples. A p value of less than 0.05 indicated significant differences.

RESULTS

Apheresis collections and processing

All apheresis plasma units were collected and processed without incident. Four of the 12 replicates were blood group A, 6 were blood group O, and 2 were blood group B. All units met the local requirements for red cells ($<6 \times 10^9$/L), WBCs ($<0.1 \times 10^9$/L), and PLTs ($<50 \times 10^9$/L).

Effect of pathogen inactivation on global coagulation assays

The mean values of aPTT and TT for control plasma, PCT-FFP, and MB-FFP remained within the reference ranges at all three time points. The mean values of PT for MB-FFP exceeded the upper limit of the reference range (10.2 sec) by 0.1-0.3 seconds at all three time points whereas the mean PT of the control plasma samples exceeded the upper limit by 0.3 seconds only at the final time point (Table 2).

The mean aPTT and TT for MB-FFP and PCT-FFP were increased over control values (Table 2). The prolongation was significantly shorter ($p < 0.05$) for PCT-FFP compared to MB-FFP at all test points for TT but only at the postthaw time point for aPTT (Table 3). The mean PT remained the same or decreased slightly for PCT-FFP whereas it increased for MB-FFP over control values after treatment and after thaw. After storage at 4°C after thawing, the mean PT of both PCT-FFP and MB-FFP decreased. The differences between PCT-FFP and MB-FFP reached significance at all three time points ($p < 0.05$; Table 3).

Effect of pathogen inactivation on coagulation factor activity

All mean values of coagulation factor levels throughout the testing periods fell within the reference ranges (Table 2). The mean fibrinogen levels were 326-333 mg per dL for PCT-FFP and 294-300 mg per dL for MB-FFP. The mean FVIII levels were 69 to 102 IU per dL for PCT-FFP and 69 to 91 IU per dL for MB-FFP. The mean retention relative to corresponding control plasma was generally in the range of 86 to 123 percent for factor activities (Table 2) with the exception for fibrinogen (79%-81% for MB-FFP) and FVIII (72%-81% for MB-FFP and 81%-83% for PCT-FFP).

No significant differences were seen in the mean retention of FII, FXII, FXIII, VWAg, and ADAMTS-13 between MB-FFP and PCT-FFP at all three test points (Table 3). The mean retention of fibrinogen and FXI was significantly higher ($p < 0.05$) in PCT-FFP than MB-FFP at all time points. The mean retention of FV, FVII, FVIII, FX, and VWcoR in PCT-FFP was either equivalent to or higher ($p < 0.05$) than MB-FFP. F IX was not different between the two groups for the posttreatment and postthaw test points; however, MB-FFP exhibited a higher F IX retention ($p < 0.05$) than the PCT-FFP group after 24 hours of storage at 4°C after thawing.

Effect of pathogen inactivation on inhibitors of coagulation

The mean protein C levels ranged from 107 to 111 IU per dL for both PCT-FFP and MB-FFP throughout the testing periods (Table 2). These values were within the reference range and were not statistically different from the control values (111-112 IU/dL).

The MB treatment process reduced the levels of protein S significantly compared to the PCT treatment process. The control plasma level of protein S had a mean value of 139 to 145 IU per dL. The mean protein S levels were 113 to 119 IU per dL for MB-FFP whereas the mean protein S levels were 137 to 139 IU per dL for PCT-FFP (Table 2). Even though these mean values were all within the reference range, the mean retention of protein S was significantly higher ($p < 0.05$) in the PCT-FFP group than the MB-FFP group at every time point (Table 3).

In contrast, the levels of antithrombin were higher in MB-FFP than PCT-FFP (Table 2), although all individual

TABLE 2. The effect of pathogen inactivation on factors of the coagulation system immediately after treatment, after frozen storage, and after subsequent storage at 4°C (mean ± SD, n = 12)

	(T1) Immediately after treatment			(T2) After frozen storage			(T3) +24 hr at 4°C after thawing		
Assay	Control-1*	MB-FFP-1†	PCT-FFP-1‡	Control-2	MB-FFP-2	PCT-FFP-2	Control-3	MB-FFP-3	PCT-FFP-3
aPTT (sec)	30.1 ± 1.5	31.8 ± 3.8	31.7 ± 1.6	30.0 ± 1.6	33.3 ± 2.1	32.0 ± 1.7‡	31.7 ± 1.6	33.5 ± 2.6	33.7 ± 2.7
PT (sec)	9.9 ± 0.4	10.5 ± 0.3	9.9 ± 0.4‡	10.1 ± 0.4	10.4 ± 0.5	9.9 ± 0.4‡	10.5 ± 0.4	10.3 ± 1.2	9.6 ± 0.8‡
TT (sec)	20.8 ± 0.7	24.7 ± 1.1	22.0 ± 0.8‡	21.0 ± 0.6	24.9 ± 1.1	22.1 ± 0.8‡	21.1 ± 0.7	25.2 ± 1.3	22.6 ± 1.2‡
Fibrinogen (mg/dL)	372 ± 57	294 ± 40	330 ± 56‡	373 ± 57	300 ± 45	333 ± 55‡	367 ± 57	296 ± 42	326 ± 58‡
D-dimers (ng/mL)	323 ± 105	286 ± 80	301 ± 95	318 ± 120	301 ± 93	299 ± 83	308 ± 100	310 ± 101	315 ± 105
Antithrombin (IU/dL)	106 ± 6	108 ± 6†	105 ± 6	109 ± 6	108 ± 6†	104 ± 6	109 ± 6	108 ± 5†	105 ± 5
VWAg (IU/dL)	125 ± 54	121 ± 52	122 ± 52	128 ± 58	120 ± 51	121 ± 50	124 ± 53	121 ± 53	121 ± 51
VWcoR (IU/dL)	73 ± 33	66 ± 28	73 ± 34‡	69 ± 30	64 ± 27	71 ± 35	69 ± 32	64 ± 28	70 ± 30‡
FII (IU/dL)	119 ± 9	112 ± 10	111 ± 9	118 ± 9	114 ± 8	112 ± 9	117 ± 10	111 ± 11	111 ± 10
FV (IU/dL)	124 ± 11	115 ± 8	121 ± 11‡	122 ± 13	112 ± 7	119 ± 9‡	123 ± 13	113 ± 9	116 ± 7
FVII (IU/dL)	104 ± 11	107 ± 10	114 ± 14‡	103 ± 10	111 ± 17	114 ± 13	93 ± 7	101 ± 14	115 ± 17‡
FVIII (IU/dL)	122 ± 30	91 ± 23	102 ± 30‡	116 ± 32	82 ± 20	94 ± 27‡	86 ± 26	69 ± 24	69 ± 19
F IX (IU/dL)	128 ± 15	115 ± 13	114 ± 18	130 ± 17	115 ± 13	112 ± 16	124 ± 17	113 ± 13†	109 ± 14
FX (IU/dL)	112 ± 14	105 ± 15	108 ± 14‡	111 ± 13	105 ± 14	106 ± 13	110 ± 13	103 ± 14	106 ± 14‡
FXI (IU/dL)	114 ± 16	101 ± 19	106 ± 16‡	116 ± 17	100 ± 18	106 ± 15‡	112 ± 18	100 ± 20	106 ± 17‡
FXII (IU/dL)	110 ± 17	101 ± 17	98 ± 13	112 ± 15	101 ± 15	100 ± 14	112 ± 17	102 ± 18	98 ± 15
FXIII (IU/dL)	121 ± 21	118 ± 18	117 ± 21	123 ± 21	118 ± 18	119 ± 24	124 ± 20	119 ± 24	119 ± 25
Protein C (IU/dL)	111 ± 16	108 ± 16	107 ± 15	111 ± 15	107 ± 15	107 ± 15	112 ± 15	111 ± 16	109 ± 16
Protein S (IU/dL)	145 ± 31	113 ± 21	139 ± 28‡	139 ± 28	113 ± 21	137 ± 27‡	140 ± 28	119 ± 24	138 ± 27‡
Plasminogen (IU/dL)	109 ± 11	109 ± 12†	102 ± 11	110 ± 11	109 ± 13†	104 ± 10	111 ± 10	108 ± 12†	104 ± 12
α2PI (IU/dL)	108 ± 5	101 ± 7†	93 ± 6	107 ± 5	102 ± 8†	94 ± 5	107 ± 5	101 ± 5†	93 ± 7
ADAMTS-13 (%N)	122 ± 14	114 ± 12	119 ± 16	119 ± 15	115 ± 17	119 ± 19	118 ± 20	118 ± 11	115 ± 19

* Control plasma was not treated. The units for factors are as indicated. The ADAMTS-13 results are expressed as the percent of normal. The numbers (1,2,3) indicate the time points at which samples were taken: T1 = immediately after pathogen inactivation treatment; T2 = 30 days after frozen storage and thawed; T3 = an additional 24 hours storage at 4°C postthawing.
† MB-FFP is superior to PCT FFP.
‡ PCT FFP is superior to MB-FFP.

TABLE 3. Comparison of PCT-FFP and MB-FFP with the paired t test (n = 12)

	PCT-FFP (mean ± SD)*			MB-FFP (mean ± SD)*			p values (PCT vs. MB)†		
Assay	Posttreatment/ Control 1	Postfreeze/ Control 2	+24 hr at 4°C/ Control 3	Posttreatment/ Control 1	Postfreeze/ Control 2	+24 hr at 4°C/ Control 3	Posttreatment/ Control 1	Postfreeze/ Control 2	+24 hr at 4°C/ Control 3
aPTT (sec prolonged)	1.7 ± 1.0	2.0 ± 0.6	2.1 ± 0.9	1.7 ± 3.5	3.4 ± 0.8	1.8 ± 2.5	0.97	<0.05	0.61
PT (sec prolonged)	–0.1 ± 0.3	–0.2 ± 0.3	–1.0 ± 0.8	0.5 ± 0.2	0.3 ± 0.5	–0.3 ± 1.1	<0.05	<0.05	<0.05
TT (sec prolonged)	1.2 ± 0.3	1.0 ± 0.5	1.6 ± 1.0	3.9 ± 0.9	3.9 ± 0.9	4.1 ± 1.2	<0.05	<0.05	<0.05
Fibrinogen (%)	89 ± 2	89 ± 3	89 ± 5	79 ± 5	81 ± 5	81 ± 5	<0.05	<0.05	<0.05
D-dimers (%)	94 ± 11	97 ± 13	103 ± 10	90 ± 9	97 ± 9	101 ± 14	0.18	0.89	0.78
Antithrombin (%)	99 ± 4	95 ± 5	96 ± 5	102 ± 3	99 ± 5	99 ± 5	<0.05	<0.05	<0.05
VWAg (%)	98 ± 4	95 ± 4	98 ± 3	97 ± 3	94 ± 3	98 ± 4	0.10	0.081	0.78
VWcoR (%)	98 ± 8	103 ± 8	103 ± 8	91 ± 9	96 ± 11	95 ± 7	<0.05	0.054	<0.05
FII (%)	93 ± 4	95 ± 3	95 ± 3	94 ± 2	97 ± 2	95 ± 4	0.69	0.13	0.65
FV (%)	98 ± 6	98 ± 6	95 ± 7	93 ± 4	92 ± 5	93 ± 8	<0.05	<0.05	0.12
FVII (%)	109 ± 7	110 ± 8	123 ± 5	103 ± 4	108 ± 22	108 ± 8	<0.05	0.74	<0.05
FVIII (%)	83 ± 7	81 ± 3	82 ± 7	75 ± 7	72 ± 9	81 ± 9	<0.05	<0.05	0.73
F IX (%)	89 ± 5	86 ± 5	88 ± 4	90 ± 4	89 ± 7	92 ± 7	0.36	0.16	<0.05
FX (%)	96 ± 3	95 ± 3	96 ± 3	94 ± 5	95 ± 6	94 ± 3	<0.05	0.60	<0.05
FXI (%)	93 ± 6	91 ± 5	95 ± 7	88 ± 7	86 ± 9	89 ± 6	<0.05	<0.05	<0.05
FXII (%)	89 ± 6	89 ± 4	88 ± 5	92 ± 6	90 ± 6	91 ± 5	0.11	0.34	0.087
FXIII (%)	97 ± 11	97 ± 6	96 ± 10	98 ± 9	97 ± 7	96 ± 7	0.74	0.93	0.88
Protein C (%)	96 ± 3	96 ± 3	98 ± 4	97 ± 2	96 ± 2	99 ± 5	0.52	0.77	0.27
Protein S (%)	96 ± 5	99 ± 5	99 ± 7	79 ± 10	82 ± 9	85 ± 11	<0.05	<0.05	<0.05
Plasminogen (%)	94 ± 3	94 ± 2	93 ± 3	100 ± 2	99 ± 3	97 ± 3	<0.05	<0.05	<0.05
α2PI (%)	86 ± 4	88 ± 4	87 ± 5	94 ± 5	95 ± 4	94 ± 4	<0.05	<0.05	<0.05
ADAMTS-13 (%)	97 ± 11	100 ± 7	98 ± 13	94 ± 10	97 ± 8	101 ± 13	0.11	0.15	0.48

* The values are ratios of treated samples to the corresponding control samples except for the clotting times, where the values are calculated by subtracting the control from the treated. The numbers (1, 2, 3) indicate the time points at which samples were taken: 1 = immediately after pathogen inactivation treatment; 2 = 30 days after frozen storage and thawed; 3 = an additional 24 hours storage at 4°C after thawing.

† The comparison was performed with the t test between PCT-FFP and MB-FFP. $p < 0.05$ indicates significant differences.

measurements remained in the reference range. The differences in retention between MB-FFP and PCT-FFP reached significance ($p < 0.05$) throughout the three test time points (Table 3).

Effect of pathogen inactivation on fibrinolytic proteins and thrombin activation

The mean levels of plasminogen and α2PI were higher in MB-FFP than PCT-FFP at all test times (Table 2). Even though these mean values were all within the reference range, the difference in retention between MB-FFP and PCT-FFP reached significance ($p < 0.05$) at every test time (Table 3). For measurements of D-dimers, there were no differences in the mean levels of D-dimers between the two study groups.

DISCUSSION

Pathogen inactivation of plasma intended for transfusion is now the standard of care in Belgium. Plasma treated with MB and visible light has been in clinical use since 2004. PCT with MB has been shown to inactivate a range of viruses.[1] Reported retention of the coagulation factors after treatment varied depending on the factors and among studies. Published retention values were between 61 and 79 percent for fibrinogen, 92 percent for FII, 90 and 95 percent for FV, 96 percent for FVII, 71 and 76 percent for FVIII, 89 percent for F IX, 85 percent for FX, 85 and 87 percent for FXI, and 84 percent for FXIII.[13-15] The commercial system treats previously frozen plasma as well as fresh plasma. The differences in the observed factor activities after treatment may represent differences between fresh and frozen plasma.

An alternative PCT method for single plasma units was recently CE Mark–approved. PCT with amotosalen and UVA light has been shown to inactivate a broad spectrum of pathogens including viruses, bacteria, parasites, and WBCs in plasma prepared as FFP.[5] The impact of PCT on coagulation activities varied among individual factors. In the studies described by Singh and colleagues,[5] the retention percentages for fibrinogen, FVII, and FVIII were 72 to 78 percent. All other coagulation parameters retained at least 82 percent of baseline activity, with FV, FXIII, and VWcoR retaining at least 92 percent. Antithrombotic proteins (protein C, protein S, antithrombin) demonstrated a retention ranging between 95 and 98 percent. α2PI was retained at 80 percent of baseline activity. The factor activities were within the reported reference ranges for conventional plasma and were suitable for transfusion support.[16,17]

The current study is the first paired study to compare the performance of these two pathogen inactivation systems with commercial processes in a blood bank environment. We prepared matched plasma units and simultaneously treated these units with either the PCT system or the MB system, whereas a control unit was not treated.

The results of this study show that all measurements of coagulation factor parameters are similar to published values.[1,5,17] Neither system had a major effect on the mean levels or retention of FII, FXII, FXIII, VWAg, ADAMTS-13 activity, and protein C throughout the treatment and storage periods. The proteins most affected by both systems are fibrinogen and FVIII. The MB system reduced the levels and retention of fibrinogen more than the PCT system. The MB system also reduced the levels and retention of FVIII to a greater extent compared to the PCT system immediately after treatment and after thawing. The residual FVIII levels after 24 hours of storage at 4°C after thawing, however, were similar between the two systems and within therapeutic ranges. For FV, FVII, and FX, which are important for support of coagulopathy of chronic liver disease, the activity levels and retention in PCT-FFP were either equivalent to or higher than those in MB-FFP. Of all factors assayed, only F IX in MB-FFP was higher than PCT-FFP at the final test time point. F IX was equivalent between MB-FFP and PCT-FFP immediately after treatment and after freeze-thaw.

In both systems, the levels of the anticoagulant protein C were relatively unaffected after treatment and storage. The MB system reduced significantly the levels and retention of protein S compared to the PCT system. Previous studies have shown that the solvent/detergent (S/D)-treated plasma also resulted in a significant loss of protein S.[16,18] The low levels of protein S in S/D plasma have been linked to some sporadic cases of hepatic artery thrombosis in association with liver transplantation after treatment with S/D plasma prepared with the US treatment process (PLAS+SD, Vitex Technologies, Watertown, MA)[19] although this association has not been reported with S/D plasma prepared with the European process (Octaplas, Octapharma, Lachen, Switzerland). Protein S deficiency has also been linked to venous thrombosis in some patients undergoing plasma exchange with S/D plasma (prepared with either the US or European process) as the exchange fluid for thrombotic thrombocytopenic purpura (TTP).[20,21] MB-FFP, however, is prepared by an entirely different process and has not been clinically associated with thrombosis.

The results of this study also showed that PCT-FFP generally fared better on global coagulation tests (PT, aPTT, and TT) compared to the MB-FFP. Pock and colleagues[22] recently reported that thrombin generation capacity was significantly impaired in MB-treated plasma (approx. 70% reduction in peak thrombin levels, 38% reduction in AUC, and 11-min prolonged lag time) compared to levels in conventional FFP. The clinical consequence of insufficient thrombin generation on clot formation is unknown, however. In contrast, MB-FFP

delivered significantly higher levels of coagulation inhibitor antithrombin as well as plasminogen and α2PI than PCT-FFP, but remained within the range described for therapeutic efficacy.

In summary, with matched plasma units and treating these units with pathogen inactivation processes under similar settings, there is adequate preservation of relevant coagulation factor activities in both PCT-FFP and MB-FFP. Both products are suitable for support of patients requiring plasma transfusion. More detailed analysis, however, revealed that the overall coagulation factor levels and stability of PCT-FFP were better preserved than in MB-FFP.

Since the introduction of MB plasma, more than 3 million units have been transfused with no serious adverse events reported although few randomized controlled trials with MB plasma have been published. A postmarketing 5-year surveillance in one center showed a significant reduction in adverse reactions for MB-FFP compared to untreated conventional FFP.[23] Several earlier reports have suggested reduced efficacy when used as the exchange fluid in TTP.[24,25] Atance and colleagues[26] also noted increased blood component usage after conversion to MB plasma, perhaps due to decreased therapeutic efficacy of MB plasma. PCT-FFP has been evaluated in three Phase III clinical trials with 203 patients for all major indications: congenital[27] and acquired coagulation[28] deficiencies and TTP.[29] The results of these trials demonstrated the efficacy and safety of PCT-FFP in the settings for which FFP transfusion is indicated. This study compared the MB system with the PCT system on the effect of pathogen inactivation treatment on protein function with in vitro assays. Additional paired studies are needed to determine whether the observed differences are clinically relevant.

For both systems, there are published studies addressing the safety of adding a substance to a blood component for pathogen inactivation. Toxicology assessment in conformance with the International Conference on Harmonization (ICH) standards has been conducted with nonphotoilluminated amotosalen and amotosalen photochemically treated plasma. Results of these studies show large safety margins relative to the expected clinical exposure.[30,31] In published toxicology studies on MB, large safety margins have also been shown, but the toxicologic assessment available in the literature is limited to MB, not photoilluminated MB in plasma.[32,33] The safety of the MB system is largely supported by years of clinical use experience and several million units of MB-plasma transfused.

For implementation of a new pathogen inactivation technology, although it is important to evaluate the mechanism of action, pathogen inactivation efficacy, coagulation factor activity, toxicology, clinical efficacy, and safety, a blood center must take into consideration the processing logistics. The MB illuminator is a stand-alone floor model that illuminates 4 units (235-315 mL) of plasma simultaneously, whereas two PCT UVA illuminators can be stacked and illuminate four jumbo units (650 mL) of plasma. In the same time frame, under routine blood bank operation conditions, 12 PCT-FFP products (200 mL) can be manufactured compared to 4 MB-FFP products (200-300 mL). It should be noted that the MB system is capable of treating single-donor products from whole-blood or apheresis procedures. The apheresis plasma units, if more than 315 mL, must be split before treatment. The PCT system is capable of treating single-donor jumbo apheresis plasma units. For centers that do not collect enough apheresis plasma for therapeutic transfusions, whole blood–derived plasma can be treated with PCT by pooling 2 to 3 units before treatment. This requirement may pose a problem for blood collection establishments where such pooling is not authorized.

The synergy of using the same technology and same system for PLTs and plasma is important in our institution. Our center introduced the PCT system for PLTs in October 2003; adding the PCT system for plasma is logistically efficient because the same UVA illuminators are used to treat PLTs and plasma with very similar processing procedures.

ACKNOWLEDGMENTS

The authors thank Ms Edith Goossenaerts for support in the coordination and supplies for the study, Ms Rebecca Spurling for technical assistance with the ADAMTS-13 activity assay, and Dr Laurence Corash for his review of the manuscript.

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