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研究報告の概要

○アモトサレンおよび紫外線A波による光化学的プロセス:欧州の血液センター3施設での検証

背景:治療用血漿中の病原体および白血球(WBC)を不活化する光化学処理(PCT)プロセスが開発された。欧州の血液センター3施設において通常の稼動状況下でプロセスバリデーション試験を実施した。

試験デザインおよび方法:各センターで、アフェレーシス血漿30、PCT用全血由来血漿製剤30～36を用意した。全血由来血漿はいずれも、適合する供血血液2～3を混合したものであった。未処置対照検体(対照新鮮凍結血漿C-FFP)を除いてから、6 mmol/Lのアモトサレン15mLおよびUVA 3J/cm²で血漿546～635 mLを処理し、吸着装置を用いて残存アモトサレンを除去した。プロセス後、血漿検体(PCT-FFP)を採取し、採血後8時間まで-60℃で冷凍保存し、凝固因子および残存アモトサレンの測定を行った。

結果:合計186本の血漿にプロセスを実施した。C-FFPと比較してPCT-FFPの平均プロトロンビン時間(12.2±0.6秒)および活性化部分トロンボプラスチン時間(32.1±3.2秒)は若干延長した。フィブリノゲンおよび第VIII因子は、PCTへの感受性がもっとも高かった(平均減少率26%)。しかし、PCT処理-FFPを実施しても、治療用血漿に必要なフィブリノゲン(217±43mg/dL)と第VIII因子(97±29 IU/dL)は十分保持された。PCT-FFP中の第II、V、VII、IX、X、XI、XIII因子の平均値はC-FFP(活性保持81～97%)と同等であった。抗血栓性のタンパク質は、PCTによる有意な影響を受けず、83%～97%の範囲で保持された。アモトサレン平均残存量は、0.6±0.1 μmol/Lであった。

結論:欧州の3つのセンターにおけるプロセスバリデーション試験は、治療用血漿に関する欧州規制およびそれぞれの国内基準の範囲内で、PCT-FFP中の凝固因子の活性は保持されていた。

報告企業の意見	今後の対応
欧州の3つの血液センターにおけるアモトサレンおよび紫外線A波による不活化工程のプロセスバリデーション試験は、処理済FFP中の凝固因子が治療用血漿に関する欧州規制および国内基準の範囲内に保持されることを示したとの報告である。	日本赤十字社では8項目の安全対策の一つとして、不活化技術の導入について、各不活化技術の効果、血液成分への影響、製造作業への影響などについて評価検討を行っている。外国での不活化実施状況や効果、新たな技術、副作用等の情報収集も含め総合的に評価し、導入について関係機関と協議しているところである。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
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血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(3)

Photochemical treatment of plasma with amotosalen and UVA light: process validation in three European blood centers

Peter Schlenke, Tor Hervig,* Hervé Isola, Marie-Louise Wiesel, Daniel Kientz, Linda Pinkoski, Yasmin Singh, Lily Lin, Laurence Corash, and Jean-Pierre Cazenave**

BACKGROUND: A photochemical treatment (PCT) process has been developed to inactivate pathogens and white blood cells (WBCs) in therapeutic plasma. Process validation studies were performed in three European blood centers under routine operating conditions.

STUDY DESIGN AND METHODS: Each center prepared 30 apheresis and 30 to 36 whole blood–derived plasma units for PCT. Each whole blood–derived plasma unit contained a mixture of two to three matched donations. After removal of pretreatment control samples (control fresh-frozen plasma [C-FFP]), 546 to 635 mL of plasma was treated with 15 mL of 6 mmol per L amotosalen, 3 J per cm^2 UVA treatment, and removal of residual amotosalen with a compound adsorption device. After processing, plasma samples (PCT-FFP) were withdrawn, frozen at –60°C within 8 hours of collection, and assayed for coagulation factors and residual amotosalen.

RESULTS: A total of 186 units of plasma were processed. The mean prothrombin time (12.2 ± 0.6 sec) and activated partial thromboplastin time (32.1 ± 3.2 sec) of PCT-FFP were slightly prolonged compared to C-FFP. Fibrinogen and Factor (F)VIII were most sensitive to PCT (26% mean reduction). PCT-FFP, however, retained sufficient levels of fibrinogen (217 ± 43 mg/dL) and FVIII (97 ± 29 IU/dL) for therapeutic plasma. Mean levels of FII, FV, FVII, F IX, FX, FXI, and FXIII in PCT-FFP were comparable to C-FFP (81%-97% retention of activity). Antithrombotic proteins were not significantly affected by PCT with retention ranging between 83 and 97 percent. Mean residual amotosalen levels were 0.6 ± 0.1 µmol per L.

CONCLUSION: Process validation studies in three European centers demonstrated retention of coagulation factors in PCT-FFP within the required European and respective national standards for therapeutic plasma.

The INTERCEPT Blood System for plasma (Cerus Europe B.V., Leusden, the Netherlands) received CE Mark registration based on extensive studies demonstrating pathogen inactivation,[1] preclinical safety,[2] and clinical efficacy.[3-5] This system uses a photochemical treatment (PCT) process with amotosalen and long-wavelength ultraviolet UVA light (320-400 nm). Amotosalen is a synthetic psoralen molecule that reversibly intercalates into the helical regions of DNA and RNA. Upon illumination with UVA light, amotosalen forms irreversible covalent bonds with pyrimidine bases of the nucleic acid.[6] The genomes of pathogens and white blood cells (WBCs) modified by amotosalen can no longer replicate.

ABBREVIATIONS: aPTT(s) = activated partial thromboplastin time(s); AP = α2-antiplasmin; AT = antithrombin; CAD = compound adsorption device; C-FFP = control fresh-frozen plasma; PC = protein C; PCT = photochemical treatment; PCT-FFP = photochemically treated plasma samples; PS = protein S; PT(s) = prothrombin time(s).

From the University of Lübeck, Institute of Immunology and Transfusion Medicine, Lübeck, Germany; Haukeland University Hospital, Bergen, Norway; Etablissement Français du Sang-Alsace, Strasbourg, France; and Cerus Corp., Concord, California.

Address reprint requests to: Lily Lin, PhD, Cerus Corp., 2411 Stanwell Drive, Concord, CA 94520; e-mail: lily_lin@cerus.com.

*Peter Schlenke, Tor Hervig, and Jean-Pierre Cazenave contributed equally to this study.

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There are two components in the PCT system intended for commercial use: an integrated disposable set and a UVA illuminator. The integrated disposable set is a closed system composed of sequentially connected container with amotosalen, illumination container, a flowthrough compound adsorption device (CAD), and three plastic storage containers (Fig. 1). The UVA illuminator is a microprocessor-controlled device capable of delivering the target UVA dose and illuminating two plasma units simultaneously. The PCT system is capable of treating plasma units within the volume range of 385 to 650 mL.

Plasma units for PCT processing can be obtained by apheresis collection or by mixing 2 to 3 matched units of whole blood–derived plasma, analogous to whole blood–derived platelets (PLTs). After sterile connection to the PCT disposable set, plasma is passed through the series of containers. The PCT steps include addition of amotosalen to plasma, illumination of the plasma mixture with UVA light, and the removal of residual amotosalen. Although this system has been evaluated for preparation of plasma in specialized centers during clinical trials, process validation studies were conducted in blood centers to verify the performance of the PCT system under different routine operating conditions. Blood centers in three European countries (France, Norway, Germany) participated in these studies. Each center collected 30 units of apheresis plasma and prepared 30 to 36 units of whole blood–derived plasma with a target volume ranging from 600 to 655 mL. Residual amotosalen concentrations in photochemically treated plasma samples (PCT-FFP) were measured to assess CAD performance. The performance of the PCT system was assessed based on in vitro coagulation function assays in treated plasma (PCT-FFP) compared to the plasma before treatment (C-FFP) as well as to European and national regulatory requirements for therapeutic plasma.

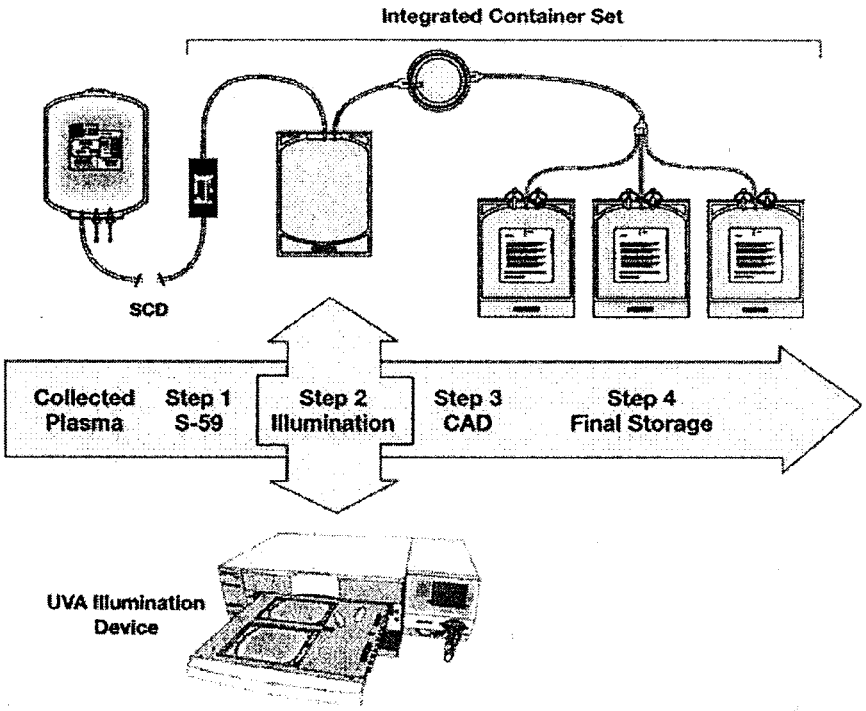

Fig. 1. The PCT system for plasma. The PCT system consists of a UVA illumination device and an integral disposable set. The UVA illuminator can illuminate 2 units of plasma per processing cycle. The disposable set provides a single-use, closed, integrated system for pathogen inactivation treatment of a plasma unit. The integrated disposable set is comprised of the following sterile components: a container with amotosalen (also known as S-59), a plastic illumination container, a CAD, and three plastic storage containers. The processing steps are as described under Materials and Methods section. SCD = sterile connection device.

MATERIALS AND METHODS

Plasma collection

Plasma collections were performed in three European blood centers and the collections methods varied slightly. In the Etablissement Français du Sang-Alsace in Strasbourg, France (Site S), apheresis plasma was collected on the Haemonetics PCS platform (Haemonetics Corp., Braintree, MA) in acid citrate dextrose anticoagulant. In blood centers of the University of Lübeck, Institute of Immunology and Transfusion Medicine, Lübeck, Germany (Site L), and Haukeland University Hospital, Bergen, Norway (Site B), apheresis plasma was collected on the Autopheresis-C platform (Baxter Healthcare Corp., Deerfield, IL) in citrate or in half-strength citrate-phosphate-dextrose (CPD) anticoagulant, respectively. Each site collected 30 units of apheresis plasma. Of the 90 apheresis units processed for this study, 27 were blood group A, 24 group AB, 22 group B, and 17 group O. The target volume of plasma was 600 to 655 mL. Fresh apheresis plasma units were kept at ambient temperature before and during the PCT process.

Whole blood was collected in CPD anticoagulant. Plasma was prepared by centrifugation with local standard operating procedures. A leukofiltration step was incorporated in the whole blood process only in Etablissement Français du Sang-Alsace in Strasbourg. Sites S and B each prepared 30 units of whole blood–derived plasma and Site L prepared 36 units. Of the 96 units, 44 were

blood group O, 42 group A, 7 group B, and 3 group AB. The target volume for the whole blood plasma unit before removing baseline samples was 600 to 655 mL and was obtained by mixing the appropriate volume of 2 to 3 ABO-matched whole blood–derived plasma units with a pooling set (FTC 0061, Baxter Healthcare Corp.). The plasma units were maintained at ambient temperature before and during the PCT process.

PCT disposable sets and UVA illumination device

The PCT disposable set for treatment of plasma (INT 3103 and INT 3104, Cerus Europe B.V.) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen HCl 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 flowthrough CAD 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 to reduce the concentration of amotosalen and its photoproducts; and three 400-mL plastic containers (PL 269, Baxter Healthcare Corp.) for storage of the treated plasma.

Illumination of plasma was performed in a UVA illumination device (Model INT100, Cerus Europe B.V.). The device was capable of illuminating 2 units of plasma per processing cycle. During illumination plasma units were reciprocally agitated at approximately 70 cycles per minute.

PCT process

For these studies, the entire process was completed to allow frozen storage of treated plasma within 8 hours of the start of plasma collection. A Luer adapter was sterile-connected to each plasma unit. Baseline coagulation factor samples (C-FFP) of approximately 20 mL were collected from each unit before PCT.

During the treatment process, plasma was passed through each component of the PCT set in a series of steps (Fig. 1). In Step 1, the plasma unit was sterile-connected to the amotosalen container, and the entire plasma volume was passed through the amotosalen container into the illumination container. In Step 2, the plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA treatment. In Step 3, the illuminated plasma mixture was passed by gravity flow through the CAD into the storage containers.

After processing, plasma samples (PCT-FFP) were withdrawn and frozen in 1.5-mL aliquots in 2-mL polypropylene tubes at or below –60°C within 8 hours of the start of plasma or whole blood collection. The C-FFP and PCT-FFP samples were assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), Factor (F)I (fibrinogen), FII, FV, FVII, FVIII, F IX, FX, FXI, FXIII, protein C (PC), protein S (PS), antithrombin (AT), and α2-antiplasmin (AP). Samples were also withdrawn after addition of amotosalen before UVA illumination and after the entire PCT process (including CAD) for measurement of amotosalen concentrations. To minimize assay variability, samples collected from the three processing centers were shipped on dry ice to a single location for analysis. All assays were performed at Cerus Corp. with the exception of quantification of AP, which was performed by a reference laboratory (Esoterix Laboratories, Aurora, CO).

Measurement of the levels of amotosalen

The initial and residual amotosalen levels in each plasma unit were quantified. A 200-µL volume of plasma was diluted to 1 mL with 35 percent methanol in buffer. After centrifugation, the supernatant was filtered and 100 µL were analyzed on a C-18 (Zorbax) reverse-phase column (Agilent Technologies, Palo Alto, CA) with a gradient of increasing methanol in KH_2PO_4 buffer. Amotosalen was detected by optical absorption (300 nm).[7]

Measurement of in vitro coagulation function

Clottable fibrinogen (FI) was measured with a modified Clauss assay. Coagulation factors were assayed with one-stage PT-based clotting assays (FII, FV, FVII, FX) or one-stage aPTT-based clotting assays (FVIII, F IX, FXI). The clotting time of a mixture of diluted test plasma and plasma deficient in the factor being quantified was compared with a reference curve constructed from the clotting times of 5 dilutions, ranging from 1:5 to 1:320, of plasma with known activity mixed with deficient plasma. These coagulation assays, as well as the PT and aPTT, were performed on an automated coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co., Lexington, MA). Reagents included brain thromboplastin (Hemoliance, Instrumentation Laboratory Co.), Platelin L (bioMérieux, Durham, NC), and congenitally depleted factor-deficient substrate (Helena Laboratories, Beaumont, TX; George King Bio-Medical, Overland Park, KS). The endpoint of all tests was the formation of a clot detected photooptically and measured in seconds. Factor Assay Control Plasma (FACT; George King Bio-Medical) was used as the reference standard for the procoagulation factor assays.

FXIII was measured with a commercially available FXIII kit (Berichrom, Dade Behring, Marburg, Germany). FXIII, activated by thrombin, releases an activation product that leads to a series of reactions resulting in a decrease in nicotinamide adenine dinucleotide, detected by monitoring absorbance at 340 nm. The assay was per-

formed on a Behring Clot Timer (BCT, Dade Behring), and standard human plasma (Dade Behring) was used as the reference standard.

PC and PS were measured with commercially available PC and PS kits (Staclot, both from Diagnostica Stago, Asnieres, France). PC and PS assays were based on prolongation of the aPTT resulting from inactivation of FV and FVIII by activated PC. The activator in the PC assay was an extract of *Agkistrodon contortrix* snake venom; the activator in the PS assay was activated PC. The tests were performed on the Behring Clot Timer. Unicalibrator (Diagnostica Stago) was used as the reference standard.

AT was measured with a commercially available ATIII kit (Stachrom, Diagnostica Stago). Plasma containing AT was incubated with a known excess of thrombin. A chromogenic substrate, imidolyzed by the remaining thrombin, was detected photooptically on a coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co.). Factor assay control plasma was used as the reference standard.

α2-AP was quantified with reagents from Diagnostica Stago. In this chromogenic method, plasmin was added in excess to the test plasma, resulting in the formation of antiplasmin–plasmin complexes. The concentration of residual plasmin is measured by its amidolytic activity with a chromogenic substrate measured at 405 nm. α2-AP concentration is inversely proportional to the residual plasmin concentration and is determined by color intensity. This analysis was performed by Esoterix Laboratories (Aurora, CO) with an STA analyzer (Diagnostica Stago).

The mean and standard deviation (SD) were determined for each coagulation variable. All factor activities were expressed in IU per dL with the exception of fibrinogen, which is expressed in mg per dL. The activity of each coagulation variable remaining after PCT was also expressed as proportional (%) retention compared to the pretreatment (baseline) activity. Comparison of the PT and aPTT was based on the prolongation of the clotting time in seconds after PCT relative to baseline. Significant differences were determined by the t test at a p value of 0.05. Reference ranges for each assay were defined as the mean ± 2 SD for untreated plasma samples.[1]

RESULTS

Processing of plasma

A total of 186 units of plasma of approximately 600 to 655 mL was prepared in this study. After removal of control samples, the volume per plasma unit for PCT processing ranged from 546 to 635 mL. The mean pretreatment amotosalen concentration was 143 ± 8 µmol per L (Table 1). The illumination of a 3 J per cm^2 UVA treatment took 7 to 9 minutes. The mean time required for the plasma mixture to completely pass through the CAD was 21 ± 3 minutes (range, 15-30 min). After CAD, the mean residual amotosalen concentration was 0.6 ± 0.1 µmol per L (Table 1). All values were below the target performance value of less than 2.0 µmol per L. The PLT and RBC concentrations for both apheresis and whole blood–derived plasma before treatment and after treatment were less than 50×10^9 and less than 6×10^9 per L, respectively, and were within the European guidelines for therapeutic FFP.[8] The WBC concentrations for apheresis plasma were less than 0.1×10^9 per L before and after treatment. The WBC concentrations for nonleukofiltered whole blood plasma in Site L and Site B were higher before treatment ($6 \times 10^9 \pm 7 \times 10^9$ and $38 \times 10^9 \pm 22 \times 10^9$/L, respectively); however, after treatment, the WBC concentrations in PCT-FFP were within the European guidance limits.

TABLE 1. Amotosalen concentrations before illumination and after CAD treatment*

Measure	Amotosalen (µmol/L) Before illumination	After CAD
Target range	110-225	<2
Apheresis plasma		
Site S† (n = 30)	139 ± 4	0.5 ± 0.1
Site B† (n = 30)	151 ± 6	0.5 ± 0.1
Site L† (n = 30)	142 ± 2	0.7 ± 0.1
Whole blood–derived plasma		
Site S (n = 30)	136 ± 8	0.6 ± 0.1
Site B (n = 30)	136 ± 4	0.5 ± 0.1
Site L (n = 36)	150 ± 3	0.6 ± 0.1
Overall (n = 186)	143 ± 8	0.6 ± 0.1

* Data are reported as mean ± SD.
† Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.

The effect of PCT on global coagulation assays (PT and aPTT)

The mean C-FFP and PCT-FFP PT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP PTs were prolonged by a mean of 0.3 ± 0.2 seconds for apheresis plasma (n = 90) and 0.5 ± 0.1 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4).

Similarly, the mean C-FFP and PCT-FFP aPTT for both apheresis plasma and whole blood–derived plasma for all three processing sites were within reference ranges (Tables 2 and 3). PCT-FFP aPTTs were prolonged by a mean of 3.5 ± 1.3 seconds for apheresis plasma (n = 90) and 4.6 ± 0.9 seconds for whole blood–derived plasma (n = 96) compared to C-FFP measurements (Table 4). The overall (apheresis and whole blood combined for an n = 186) mean PCT-FFP PT and aPTT compared to C-FFP was prolonged by 0.4 ± 0.2 and 4.1 ± 1.2 seconds, respectively (Table 4).

TABLE 2. Apheresis plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	12.2 ± 0.6	12.7 ± 0.7	0.5 ± 0.2	12.3 ± 0.7	12.5 ± 0.7	0.2 ± 0.2	12.0 ± 0.4	12.4 ± 0.5	0.3 ± 0.2
aPTT (sec)	23-35	27.9 ± 2.5	32.4 ± 3.2	4.5 ± 1.1	26.1 ± 2.5	28.5 ± 2.7	2.3 ± 1.0	27.8 ± 2.5	31.5 ± 3.1	3.8 ± 0.8
FI (mg/dL)	200-390	295 ± 71	208 ± 55	70 ± 5	313 ± 48	261 ± 43	83 ± 3	287 ± 57	216 ± 43	75 ± 3
FII (IU/dL)	80-120	102 ± 17	89 ± 15	88 ± 7	117 ± 15	107 ± 16	91 ± 3	103 ± 14	91 ± 12	88 ± 3
FV (IU/dL)	95-170	120 ± 29	116 ± 26	97 ± 3	144 ± 23	142 ± 20	99 ± 5	125 ± 19	119 ± 17	95 ± 3
FVII (IU/dL)	70-175	116 ± 25	93 ± 21	80 ± 4	112 ± 27	92 ± 22	82 ± 3	111 ± 23	91 ± 19	81 ± 3
FVIII (IU/dL)	85-235	140 ± 34	99 ± 25	70 ± 3	161 ± 42	130 ± 35	81 ± 4	123 ± 39	91 ± 30	74 ± 3
F IX (IU/dL)	75-145	90 ± 13	75 ± 10	84 ± 4	101 ± 18	88 ± 14	87 ± 3	93 ± 12	78 ± 11	84 ± 4
FX (IU/dL)	75-130	108 ± 21	95 ± 19	88 ± 4	117 ± 20	106 ± 19	91 ± 3	112 ± 14	99 ± 12	88 ± 2
FXI (IU/dL)	60-150	93 ± 16	79 ± 13	85 ± 8	111 ± 19	101 ± 7	91 ± 6	103 ± 20	90 ± 18	87 ± 2
FXIII (IU/dL)	85-135	114 ± 22	110 ± 22	97 ± 9	126 ± 18	121 ± 18	96 ± 5	115 ± 21	108 ± 20	94 ± 3
PC (IU/dL)	80-140	122 ± 25	102 ± 24	84 ± 9	122 ± 20	107 ± 19	88 ± 4	119 ± 22	101 ± 20	85 ± 2
PS (IU/dL)	85-135	108 ± 25	107 ± 25	100 ± 9	108 ± 18	103 ± 17	95 ± 6	92 ± 20	88 ± 19	96 ± 3
AT (IU/dL)	85-105	102 ± 9	97 ± 9	95 ± 4	105 ± 9	101 ± 8	96 ± 4	95 ± 10	91 ± 10	95 ± 3
AP (IU/dL)	80-150	97 ± 11	81 ± 8	83 ± 10	108 ± 13	89 ± 8	83 ± 6	101 ± 12	82 ± 6	82 ± 8

* Site S = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 3. Whole blood–derived plasma: clotting times and coagulation factor activity before and after PCT (mean ± SD)

Variable†	Reference range†	Site S* (n = 30)			Site B* (n = 30)			Site L* (n = 30)		
		C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡	C-FFP	PCT-FFP	% Retention‡
PT (sec)	11.1-13.5	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.5 ± 0.3	12.0 ± 0.3	0.4 ± 0.1	11.5 ± 0.4	12.0 ± 0.4	0.6 ± 0.1
aPTT (sec)	23-35	29.5 ± 1.8	34.2 ± 2.4	4.7 ± 0.7	28.6 ± 1.8	32.9 ± 2.4	4.3 ± 0.9	28.2 ± 1.4	33.1 ± 2.1	4.9 ± 0.8
FI (mg/dL)	200-390	290 ± 25	207 ± 23	71 ± 3	291 ± 30	222 ± 24	76 ± 3	272 ± 40	191 ± 30	70 ± 3
FII (IU/dL)	80-120	106 ± 8	95 ± 9	89 ± 3	100 ± 9	89 ± 8	89 ± 3	101 ± 7	89 ± 7	88 ± 3
FV (IU/dL)	95-170	123 ± 14	119 ± 13	97 ± 2	125 ± 15	121 ± 14	97 ± 3	119 ± 18	114 ± 17	96 ± 3
FVII (IU/dL)	70-175	113 ± 15	91 ± 12	80 ± 3	108 ± 14	86 ± 11	80 ± 3	114 ± 17	90 ± 14	79 ± 3
FVIII (IU/dL)	85-235	127 ± 24	91 ± 19	71 ± 3	118 ± 20	91 ± 17	77 ± 4	119 ± 23	84 ± 19	71 ± 4
F IX (IU/dL)	75-145	93 ± 10	78 ± 8	84 ± 3	92 ± 11	78 ± 10	85 ± 2	95 ± 9	78 ± 6	82 ± 4
FX (IU/dL)	75-130	108 ± 9	94 ± 8	87 ± 1	106 ± 12	92 ± 11	87 ± 3	105 ± 10	90 ± 9	85 ± 2
FXI (IU/dL)	60-150	83 ± 14	69 ± 12	83 ± 4	101 ± 11	87 ± 12	86 ± 6	94 ± 9	81 ± 9	85 ± 3
FXIII (IU/dL)	85-135	104 ± 12	101 ± 12	97 ± 2	121 ± 10	111 ± 10	92 ± 3	113 ± 14	106 ± 12	94 ± 3
PC (IU/dL)	80-140	115 ± 14	97 ± 12	85 ± 5	114 ± 19	102 ± 16	90 ± 5	116 ± 16	99 ± 14	85 ± 7
PS (IU/dL)	85-135	111 ± 15	107 ± 14	96 ± 3	117 ± 14	114 ± 14	97 ± 4	114 ± 16	109 ± 15	96 ± 5
AT (IU/dL)	85-105	101 ± 7	97 ± 7	96 ± 2	96 ± 4	93 ± 4	96 ± 2	91 ± 6	86 ± 6	95 ± 2
AP (IU/dL)	80-150	100 ± 8	85 ± 5	85 ± 5	95 ± 5	78 ± 4	82 ± 4	93 ± 6	76 ± 4	82 ± 3

* Site S: = EFS Alsace, Strasbourg, France; Site B = Blood Bank of Haukeland University Hospital, Bergen, Norway; Site L = University of Lübeck, Lübeck, Germany.
† The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
‡ For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.

TABLE 4. Combined clotting times and coagulation factor activity before and after PCT (mean ± SD): comparison apheresis plasma and whole blood–derived plasma

		Apheresis plasma (n = 90)			Whole blood plasma (n = 96)			Overall (n = 186)		
Variable	Reference range*	C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†	C-FFP	PCT-FFP	% Retention†
PT (sec)	11.1-13.5	12.2 ± 0.6	12.5 ± 0.6	0.3 ± 0.2	11.5 ± 0.3	12.0 ± 0.4	0.5 ± 0.1	11.8 ± 0.6	12.2 ± 0.6	0.4 ± 0.2
aPTT (sec)	23-35	27.3 ± 2.6	30.8 ± 3.4	3.5 ± 1.3	28.7 ± 1.7	33.4 ± 2.3	4.6 ± 0.9	28.0 ± 2.3	32.1 ± 3.2	4.1 ± 1.2
FI (mg/dL)	200-390	299 ± 60	228 ± 52‡	76 ± 7‡	284 ± 34	206 ± 29‡	72 ± 4‡	291 ± 49	217 ± 43	74 ± 6
FII (IU/dL)	80-120	107 ± 17‡	95 ± 16	89 ± 5	102 ± 8‡	91 ± 8	89 ± 3	105 ± 13	93 ± 13	89 ± 4
FV (IU/dL)	95-170	130 ± 26‡	126 ± 24‡	97 ± 4	122 ± 16‡	118 ± 15‡	97 ± 3	126 ± 22	122 ± 20	97 ± 3
FVII (IU/dL)	70-175	113 ± 25	92 ± 21‡	81 ± 4‡	112 ± 15	89 ± 12‡	80 ± 3‡	112 ± 21	90 ± 17	81 ± 3
FVIII (IU/dL)	85-235	141 ± 41‡	107 ± 35‡	75 ± 6‡	121 ± 23‡	88 ± 18‡	73 ± 5‡	131 ± 34	97 ± 29	74 ± 5
F IX (IU/dL)	75-145	95 ± 15‡	80 ± 13‡	85 ± 4‡	94 ± 10‡	78 ± 8‡	84 ± 3‡	94 ± 13	79 ± 11	84 ± 4
FX (IU/dL)	75-130	112 ± 18‡	100 ± 17‡	89 ± 4‡	106 ± 10‡	92 ± 9‡	86 ± 2‡	109 ± 15	96 ± 14	88 ± 3
FXI (IU/dL)	60-150	102 ± 19‡	90 ± 18‡	88 ± 7‡	93 ± 13‡	79 ± 13‡	85 ± 5‡	97 ± 17	84 ± 17	86 ± 6
FXIII (IU/dL)	85-135	119 ± 21‡	113 ± 21‡	96 ± 6	113 ± 14‡	106 ± 12‡	94 ± 3	116 ± 18	110 ± 17	95 ± 5
PC (IU/dL)	80-140	121 ± 22	103 ± 21	85 ± 6	115 ± 16	99 ± 14	87 ± 6	118 ± 20	101 ± 18	86 ± 6
PS (IU/dL)	85-135	103 ± 22‡	99 ± 22‡	97 ± 7‡	114 ± 15‡	110 ± 15‡	96 ± 4‡	109 ± 20	105 ± 19	97 ± 5
AT (IU/dL)	85-105	100 ± 12‡	95 ± 12	95 ± 3	95 ± 7‡	91 ± 7	96 ± 2	98 ± 9	94 ± 9	96 ± 3
AP (IU/dL)	80-150	102 ± 12‡	83 ± 8‡	82 ± 7	96 ± 7‡	79 ± 6‡	83 ± 4	99 ± 11	82 ± 7	83 ± 6

* The reference range was calculated from the mean ± 2 SDs of untreated, conventional plasma. n = 80 for fibrinogen, FV, FVII, FVIII, F IX, and FXI; n = 50 for FII and FX; n = 25 for FXIII, AT, PC, and PS. The reference range for AT was established by Esoterix Laboratories.
† For PT and aPTT, the reported values = (PCT-FFP – C-FFP) in seconds.
‡ Significant differences between apheresis and whole blood plasma were detected at a p value of less than 0.05.

The effect of PCT on procoagulant factors

The mean procoagulant factor activities in apheresis C-FFP obtained in each processing site were within reference ranges (Table 2). Apheresis plasma processed with the PCT system retained mean factor activity ranging from 70 to 83 percent and 70 to 81 percent of C-FFP for fibrinogen (FI) and FVIII, respectively. Retentions of FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 80 to 99 percent (Table 2). After PCT, the mean procoagulant factor activities were also within the reference ranges. Mean FVIII activities were 99 ± 25, 130 ± 35, and 91 ± 30 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

Similarly, the mean activity levels of the procoagulant factors in whole blood–derived plasma were within reference ranges (Table 3). Whole blood plasma processed with the PCT system retained mean coagulation factor activities ranging from 70 to 76 and 71 to 77 percent of C-FFP for FI and FVIII, respectively. The mean retentions for FII, FV, FVII, F IX, FX, FXI, and FXIII ranged from 79 to 97 percent (Table 3). After PCT, the mean factor activities were also within the reference ranges with the exception of FI and FVIII in Site L. Mean FVIII activities were 91 ± 19, 91 ± 17, and 84 ± 19 IU per dL in Site S, Site B, and Site L, respectively, meeting national (70 IU/dL) and European Pharmacopoeia (50 IU/dL) requirements for therapeutic plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). Whole blood–derived plasma generally exhibited statistically significantly lower factor activities compared to apheresis plasma (see Table 4). All mean values, however, fell within the reference ranges. The mean FVIII activity in apheresis PCT-FFP (107 ± 35 IU/dL, n = 90) and whole blood–derived plasma (88 ± 18 IU/dL, n = 96) met the national and European Pharmacopoeia requirements for therapeutic plasma.

The overall (n = 186) mean level of fibrinogen in PCT-FFP was 217 ± 43 mg per dL, which is 74 ± 6 percent of the C-FFP values. The overall (n = 186) mean activity of FVIII in PCT-FFP was 97 ± 29 IU per dL, which is 74 ± 5 percent of the C-FFP values. The retention of other factors in PCT-FFP was consistently higher (81%-97%; Table 4).

The effect of PCT on antithrombotic and fibrinolytic protein activity

All pretreatment activities of PC, PS, AT III, and α2-AP of apheresis plasma (Table 2) and whole blood–derived plasma (Table 3) were within reference ranges. After PCT, the mean activities of these antithrombotic proteins were still within the reference ranges with two exceptions: the

mean levels of AP activity in PCT-FFP from Site B (78 ± 4 IU/dL) and Site L (76 ± 4 IU/dL) were slightly outside the lower limit (80 IU/dL) of the reference range.

After PCT, the mean retention of antithrombotic proteins ranged from 84 to 90 percent for PC and 82 to 85 percent for AP (Tables 2 and 3). No differences were observed between apheresis and whole blood–derived plasma. Similarly, the mean retention in PCT-FFP ranged from 95 to 100 percent for PC and 95 to 96 percent for AT (Tables 2 and 3). The results were comparable between apheresis and whole blood–derived plasma.

To compare the processing characteristics between apheresis plasma (n = 90) and whole blood–derived plasma (n = 96), the results from all three sites were combined for analysis (Table 4). There were no significant differences in the activity of PC of either C-FFP or PCT-FFP between apheresis and whole blood–derived plasma. Whole blood–derived plasma contained significantly higher levels of PS before and after PCT than apheresis plasma (see Table 4). In contrast, the levels of AT and AP were generally lower in whole blood–derived plasma compared to apheresis plasma and reached significance (see Table 4).

The overall (n = 186) mean activities of the antithrombotic proteins in C-FFP and PCT-FFP were within the reference ranges. After PCT, mean retention of PC and AP was 86 and 83 percent, respectively, whereas mean retention of PS and AT was 97 and 96 percent (Table 4).

DISCUSSION

The PCT process for preparation of pathogen-inactivated FFP involves the addition of amotosalen to a nominal concentration of 150 µmol per L (range, 110-225 µmol/L), illumination of the plasma mixture with a 3 J per cm² UVA treatment, and removal of residual amotosalen to less than 2.0 µmol per L by a flow CAD. Three European centers participated in this study to validate the process under routine blood bank operation conditions. Each center processed 30 units of apheresis plasma and 30 to 36 units of whole blood–derived plasma with integral disposable sets that have received CE Mark approval and commercial UVA illuminator. The PCT process was completed within the time frame for FFP, allowing units to be frozen within 8 hours of collection.

The mean pretreatment amotosalen concentration from all three sites was 143 ± 8 µmol per L (n = 186), which is well within the target system performance range of 110 to 225 µmol per L. The use of the microprocessor-controlled UVA illuminator ensured delivery of the UVA treatment dose of 3 J per cm². All three centers demonstrated the addition of the correct amotosalen concentration, combined with a 3 J per cm² UVA treatment dose, thus ensuring robust pathogen inactivation. The mean amotosalen level after the CAD treatment for all centers was 0.6 ± 0.1 µmol per L (n = 186), which is significantly below the target performance value of 2.0 µmol per L. The mean residual amotosalen levels among the three sites ranged from 0.5 ± 0.1 to 0.7 ± 0.1 µmol per L with no units having residual amotosalen higher than 1.2 µmol per L, demonstrating the consistency and the efficacy of the CAD. These results demonstrate that the PCT process can be performed consistently under blood bank conditions.

The quality of PCT-FFP was assessed for activity of FVIII with respect to meeting national and European regulatory guidelines.[8] The consistency of the PCT process was assessed by the retention of all factor activities in PCT-FFP compared to levels in pretreatment plasma samples.

The factor most affected by PCT was FVIII with a mean of 26 percent reduction in activity. However, residual activity is within the current requirement for FFP as the level of FVIII is greater than 50 IU per dL in the European Pharmacopoeia standard for therapeutic FFP.[9] The mean FVIII activity after PCT was 107 ± 35 IU per dL (n = 90) for apheresis plasma and 88 ± 18 IU per dL (n = 96) for whole blood–derived plasma or an overall mean of 97 ± 29 IU per dL (n = 186). All units had FVIII activity greater than 50 IU per dL. In France, greater than 90 percent of quality control samples must have greater than 70 IU per dL in FVIII. Preliminary studies measuring the thrombin generation time for PCT-FFP have shown no difference from untreated plasma for peak thrombin levels, lag time to start of thrombin generation, or total thrombin produced.[10] These observations suggest that the reduction in FVIII levels are not critical to generation of thrombin and the ultimate conversion of fibrinogen to fibrin. These observations of normal thrombin generation are in contrast to those recently reported for plasma prepared with methylene blue and visible light.[11]

Fibrinogen was also affected by PCT with a mean of 26 percent reduction in the clottable fibrinogen levels. Although there is no required standard for the level of fibrinogen in FFP, the mean levels retained in PCT-FFP (217 ± 43 mg/dL, n = 186) were within the reference range. Prior clinical studies with PCT-FFP for support of liver transplant with massive transfusion have shown no increased requirement for plasma or cryoprecipitate, indicating that the levels of fibrinogen in PCT-FFP are sufficient.[4] These patients have a significant period of fibrinolytic activity after unclamping of the transplanted liver. The study examined the use of conventional FFP, cryoprecipitate, and PCT-FFP for support of these patients and observed no differences to indicate that the reduced levels of fibrinogen in PCT-FFP were clinically relevant. In addition, the levels of AP activity are reasonably conserved in PCT-FFP.[1] Although the levels of fibrinogen are reduced by the treatment, the levels appear adequate to support hemostasis in patients with active fibrinolysis.