TABLE 2. Factorial design for three variables (plasma, light intensity, and PLTs)*

Run	Plasma [%]	Light intensity (mW/cm²)	PLTs (cells/mL) × 10⁹	RF (log)
1	10	0.25	0	5.81
2	30	0.25	0	3.03
3	10	1.0	0	4.63
4	30	1.0	0	1.90
5	10	0.25	1.0 × 10⁹/mL	4.53
6	30	0.25	1.0 × 10⁹/mL	2.47
7	10	1.0	1.0 × 10⁹/mL	2.85
8	30	1.0	1.0 × 10⁹/mL	1.66
9	10	0.25	0	4.63
10	30	0.25	0	2.88
11	10	1.0	0	4.32
12	30	1.0	0	1.89
13	10	0.25	1.0 × 10⁹/mL	3.80
14	30	0.25	1.0 × 10⁹/mL	2.51
15	10	1.0	1.0 × 10⁹/mL	2.95
16	30	1.0	1.0 × 10⁹/mL	1.50

* Factorial design to determine the influence of three variables (percentage of plasma, light intensity, and absence or presence of PLTs) on the efficacy of UVC irradiation for the inactivation of BVDV in PCs. The percentage of plasma was tested at 10 and 30 percent, the light intensity at 0.25 and 1.0 mW per cm², and the PLT concentration at 0 and 1.0 (cells/mL) × 10⁹. The irradiation dose was fixed at 500 J per m², the depth was fixed at 1 mm, and orbital mixing was 50 r.p.m. The results for BVDV are shown as RF (log) calculated with the most probable number method.[19]

virus kill was somewhat lower, resulting in approximately 3 to 4 log at 500 J per m², but eventually more than 5 log at 1000 J per m² (medium-resistant viruses; Fig. 3B). For HIV and SV40, the virus kill was limited to approximately 1 log at 500 J per m² and approximately 2 log with doses up to 1000 J per m² (resistant viruses; Fig. 3C). Because cell-associated (CA) virus (intracellular and/or bound to the cellular membrane) may be more resistant to UVC damage, we also tested CA virus with HIV as a resistant virus and with VSV as a sensitive virus. In both cases, however, the CA-virus results mimicked those obtained with the corresponding cell-free viruses, that is, CA HIV was UVC-resistant and CA VSV was UVC-sensitive (Fig. 3D).

To further explore the resistance of HIV to UVC irradiation, we tested the effect of irradiation doses up to 4000 J per m². Although the virus kill slowly improved with increasing doses, infectious virus was still present even after irradiation at 4000 J per m² (RF = 3.5 log; data not shown).

Finally, we investigated the effect of UVC irradiation on the survival of bacteria. Experiments were performed with the same settings as for the virus studies. Irradiation at 250 J per m² resulted in greater than 4 log reduction for *S. epidermidis*, *S. aureus*, and *E. coli* and greater than 5 log at 500 J per m². In the case of *B. cereus*, the kill at 500 J per m² was limited to approximately 3 log (Fig. 4). An increase to 1000 J per m² resulted in a reduction of 3.7 log (data not shown). When after UVC irradiation, however, the *B. cereus* samples were incubated for 10 minutes at 80°C, all samples were below the detection limit (<0.5 log).

DISCUSSION

The risk of transmission of pathogens via cellular products, especially for PCs, is still a concern. Here we describe the potential of a UVC irradiation technique for pathogen inactivation in PCs.

Because of the high absorbance of UVC light by human plasma, we chose to study only PCs suspended in synthetic medium with 10 or 30 percent residual plasma. From the extinction coefficient per percentage of plasma (experimentally determined by us in a 1-cm cuvet as being close to 0.3), it can be calculated that, with a 1-mm light path as used in most of our experiments, 50 and 10 percent of the UVC light will reach the bottom of the suspension with, respectively, 10 or 30 percent plasma present. To avoid "dead" volumes not exposed to UVC, we chose the relative short light path of 1 mm, realizing that special containers of UV-permeable plastic of similar thickness would be required when the technique should be further developed into a blood bank procedure.

In our experimental setup, we then investigated whether varying a number of variables like percentage of plasma, irradiation dose, and light intensity would result in conditions with good pathogen inactivation in combination with good PLT quality. An acceptable compromise was found for 10 percent plasma in Composol, in combination with a depth of 1 mm, a light intensity of 0.25 mW per cm², and a total dose of 500 J per m². These conditions resulted in good (3-4 log) inactivation for the majority of pathogens tested with only limited effects on in vitro PLT quality. Evidently, this set of conditions can only be taken as a rough indication, because in this explorative study neither the irradiation nor the storage resembled blood bank conditions and the set of PLT quality variables was limited. The only quality variable for PCs mentioned in guidelines is the pH, which should be between 6.4 and 7.4 (at 22°C) according to European blood bank regulations.[21] Although this condition was met under all conditions tested, the results of the other in vitro quality variables indicated that in 10 percent plasma with doses higher than 500 J per m², PLT quality was seriously affected. An increase of 10 to 30 percent in CD62P-positive PLTs has been reported for standard PC at the end of their shelf life (5-7 days),[14,22,23] whereas with CD62P values higher than 50 percent the in vivo survival seems affected.[24] PS exposure during storage of standard PC usually remains below 20 percent at the end of the

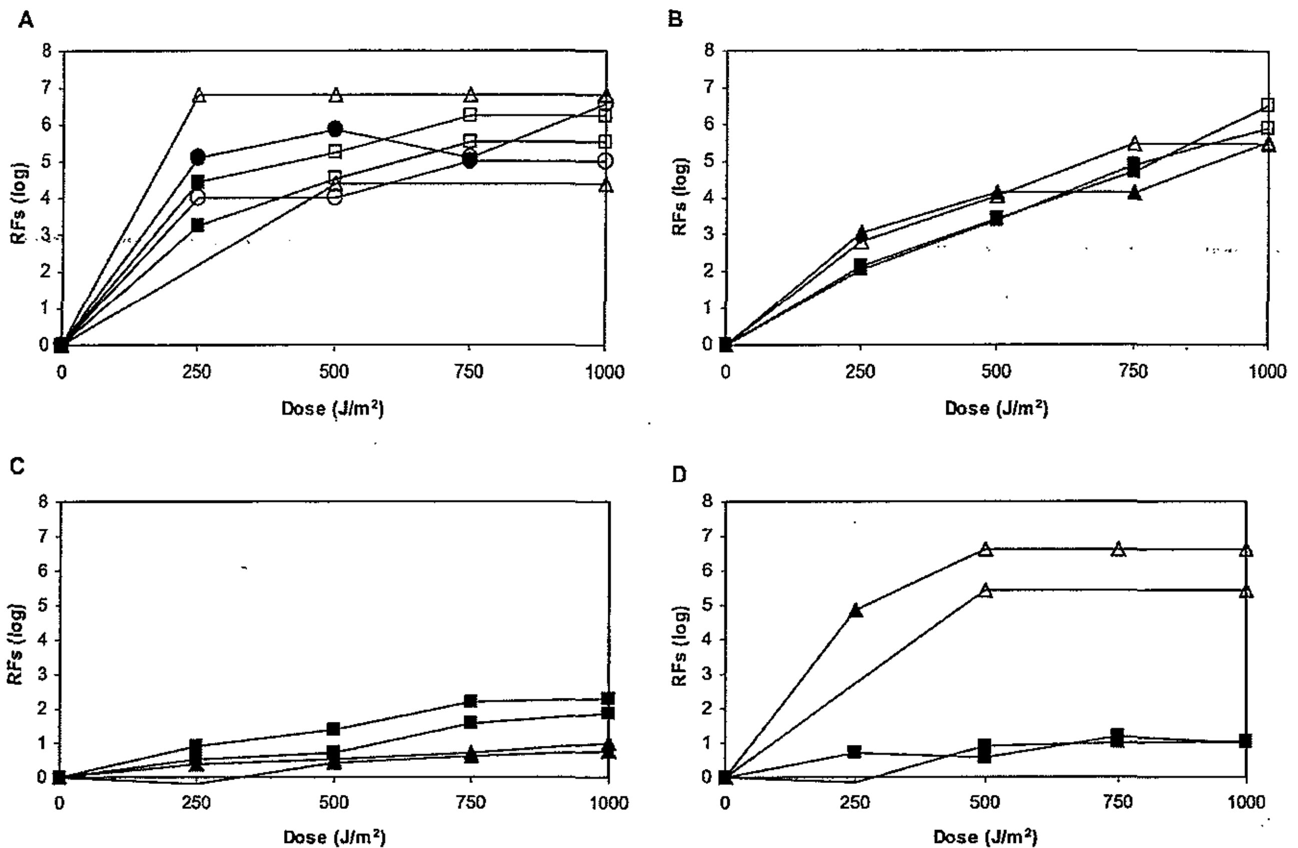

Fig. 3. Inactivation of different viruses by UVC irradiation. Virus inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. (A) Sensitive viruses (■ = CPV; ▲ = TGEV; ● = VSV); (B) medium-resistant viruses (■ = BVDV; Δ = PRV); (C) resistant viruses (■ = HIV; ▲ = SV40); (D) CA viruses (■ = CA HIV; ▲ = CA VSV). Open symbols indicate maximal reduction. Results shown are representative for at least two experiments.

shelf life,[22,23] but so far there are no data about correlation of PS exposure and in vivo survival.

In a factorial design, it was shown that the percentage of residual plasma was the major variable affecting the outcome of the UVC irradiations. As expected, the percentage of plasma resulted in opposite effects on pathogen inactivation and PLT quality. To a lesser extent, this opposite effect was also found for the light intensity, whereas the presence or absence of PLTs had a relatively minor, although significant, effect on pathogen inactivation. The presence of PLTs, resulting in a decrease of approximately 1 log of pathogen inactivation, should be taken into account in interpreting much of our inactivation data. Furthermore, the necessity to lower the residual plasma concentration to guarantee sufficient pathogen inactivation will require adjustment of current procedures to produce suitable PCs, but it has been shown in earlier studies[25,26] that this may be achievable. Because UVC irradiation results in extra glucose consumption, additional measures should be taken to ensure provision of glucose.

It was anticipated that in case of bacteria, high inactivation values would be observed[6] and this was indeed the case for all bacteria tested, with the exception of *B. cereus*. The reason for the decreased sensitivity of *B. cereus* is not quite clear, but one might speculate that formation of spores plays a role. It has been shown that *Bacillus* spores are 10 to 20 times more resistant to UVC irradiation;[27] thus formation of spores can cause a suboptimal kill of *Bacillus*. Because freshly prepared bacteria cultures were used containing relatively low amounts of spores (as determined by specific staining of spores, data not shown), however, a higher resistance toward UVC irradiation of *B. cereus* itself compared to other bacteria is also a possible explanation.[28,29] Moreover, the UVC-surviving bacteria were killed upon incubation at 80°C for 10 minutes, a treatment that spores will survive, also indicating that *B. cereus* itself has a higher resistance toward UVC.

We found a broad spectrum of viral sensitivity to UVC irradiation. For CPV, TGEV, and VSV we found very high inactivation, whereas the inactivation for BVDV and PRV

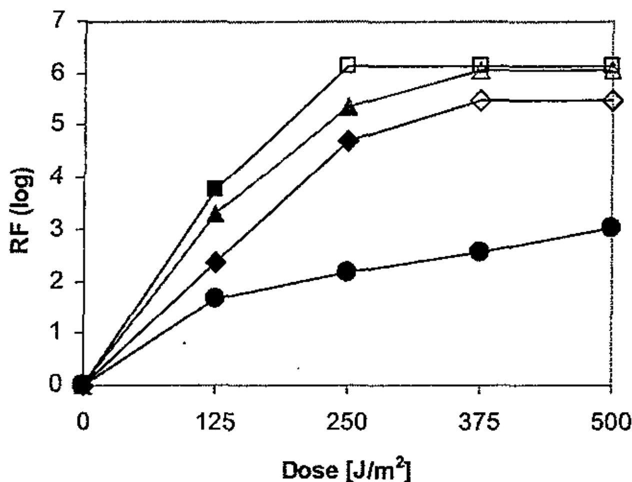

Fig. 4. Inactivation of different bacteria by UVC irradiation. (◆) *S. epidermidis*; (■) *S. aureus*; (▲) *E. coli*; and (●) *B. cereus*. Bacteria inactivation by UVC irradiation was tested in Composol containing 10 percent plasma; RF values (log) are shown. Experimental conditions were as described in the legend to Fig. 1. Open symbols indicate maximal reduction. Results shown are representative of two experiments.

was less and slower. In the case of HIV and SV40, the inactivation was very limited and only 1 to 2 log was observed. Increasing doses of UVC irradiation also induced increased damage to HIV. Even a dose of 4000 J per m^2, however, did not result in complete inactivation. Interestingly, the efficacy of UVC was not different for cell-free and CA virus because CA HIV and CA VSV showed susceptibilities very similar to the corresponding cell-free virus. This indicates that the presence of infected cells is not an impediment for virus inactivation with UVC irradiation, provided that the virus in question is sensitive to UVC irradiation.

Based on our results we would rank the viruses in the following order with respect to UVC sensitivity: TGEV > VSV > CPV > PRV > BVDV > HIV > SV40. This ranking is exactly in line with previous observations and theoretical considerations postulating that UVC is especially effective on viruses with large genomes (i.e., PRV)[30] and on viruses with single-stranded nucleic acid genomes (i.e., TGEV, VSV, and CPV).[31] Furthermore, it has been described that RNA is less severely damaged than DNA, because pyrimidine dimers and more specifically thymine are the most frequent lesions caused by UVC irradiation.[7] Our results are also in line with Caillet-Fauquet and coworkers[6] who determined a sensitivity of MVM > EMC > BHV and Li and colleagues[32] who showed a sensitivity of CPV > BVDV > HAV > PRV. Wang and coworkers,[33] however, reported SV40 to be highly sensitive, more or less comparable to parvovirus. In contrast, we found that SV40 is very resistant, similar to the resistance found for HIV, as was also predicted by Lytle and Sagripanti.[34] The reason for this discrepancy remains unclear, especially because the possible explanation of cell line–dependent repair can be ruled out, because both studies propagated the virus in the cell line BSC.

Considering the ranking of virus inactivation as observed in this study and as predicted by Lytle and Sagripanti,[34] it can be concluded that single-stranded DNA or RNA viruses are effectively inactivated by UVC irradiation. This confirms that UVC is distinct from several other techniques with respect to its capacity to inactivate the NLE viruses like parvovirus B19 and HAV. This effective elimination of NLE viruses, combined with B19 contamination in several blood and plasma products, renders this technique interesting for further consideration.

SV40 has been regarded to be a very resistant virus and was often used in the past as a general model for NLE viruses with a DNA genome (like parvovirus B19). At present, however, specific model viruses for parvovirus B19 are applied and/or parvovirus B19 itself. Given the fact that CPV is very effectively inactivated by UVC irradiation, the relevance of SV40 as model virus for parvovirus B19 can be questioned. We do recommend, however, continuing studies with SV40 as a general model virus as this virus may be representative for new currently unknown threats to the blood supply. Indeed, the inability of UVC irradiation to inactivate viruses with small double-stranded genomes like SV40 illustrates possible limitations of this treatment.

BVDV and PRV are effectively inactivated, although the kinetics are slower compared to the sensitive group of viruses. Therefore, it is expected that UVC is capable of inactivating problematic blood-borne viruses like HBV and HCV. The inability of UVC irradiation to sufficiently inactivate HIV, a very relevant virus, however, is a major disadvantage. There seem to be several reasons for the resistance of HIV to UVC irradiation. HIV is a retrovirus with a small RNA genome. It has a single-stranded genome, but each virion encapsulates two copies of the viral RNA that are tightly linked and might serve as each other's back-up in case of UVC-induced damage. Indeed, strand transfers during reverse transcription are an integral part of the HIV life cycle.[35]

The observation that UVC does not effectively inactivate HIV may be partially compensated by careful and efficient donor screening for HIV. Both specific antibody and NAT are routinely performed and the risk of HIV transmission via cellular products is estimated to be less than 1 in 1 million.[36,37] One should again keep in mind, however, that new viruses may emerge with similar characteristics as HIV that would not be affected by this treatment in its current state. Given the broad inactivation of bacteria and viruses, we believe, however, that UVC irradiation for PCs is a promising technology that warrants further investigation.

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医薬品 研究報告 調査報告書

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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。

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」
照射赤血球濃厚液-LR「日赤」

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

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。