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一般的名称			研究報告の公表状況	Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation Vito Angelo Berardi, et al. Lancet 2006; 46: 652- 8	公表国 イタリア	
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研究報告の概要

英国で血液感染による変異型クロイツフェルト・ヤコブ病が 2 例報告されて以来，プリオンと関わる血液，血液成分，及び血漿由来製剤の安全性が大きく懸念されている。感染性海綿状脳症(TSE)に感染した齧歯類の脳の一部をスパイクした血液成分や血漿由来製剤からのプリオンの除去，あるいは不活性化を可能にする方法の開発が取り組まれた。しかしながら，スパイクには恐らく血液感染とは関連のない病原性プリオンタンパク質 (PrP^{TSE}) の凝集体が含まれており，この集合体の存在により試験が無効になる可能性がある。これを明らかにするため，筆者らは以下の実験を行った。
スクレーピーのハムスター263K 株の脳組織を 10%リン酸緩衝食塩水で懸濁し，低速遠心分離後，上澄み液を収集し，30 分間２５ 220,000×g で高速遠心分離を行った。高速遠心による浮遊物 (S^{HS}) と沈殿物を収集し，ウエスタンブロット法によりプロテイナーゼ耐性 PrP^{TSE} を，離乳ハムスターへ脳内接種し感染性を測定した。実験では，相当量のプリオン感染力(脳組織の 10%懸濁液の mL あたりの 50%致死量 105 以上)がスクレーピーのハムスター263K 株の S^{HS} 分画でみられたが，その効果は PrP^{TSE} の凝集体を含む分画よりも低かった。一方，この分画はなにも含んでいないか，含んだとしても僅か微量の PrP^{TSE} の凝集体であることが確認された。本実験で示された PrP^{TSE} の凝集体を含まない感染性物質を調製する簡便な方法は，ヒト血液成分及び血漿由来製剤におけるプリオン除去，または不活化確認試験に用いるのに適当なスパイク物質を調製する上で有用であると考えられた。

報告企業の意見	今後の対応
弊社の血漿分画製剤の製造工程において，2004 年の Position statement で欧州医薬品審査庁が推奨する，プリオン除去及び感染性評価の開発を行った。その結果，各製品の製造過程で，少なくとも4logのプリオン除去が可能であることが確認されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation

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BACKGROUND: Concern about the safety of blood, blood components, and plasma-derived products with respect to prions has increased since the report of two blood-related infections of variant Creutzfeldt-Jakob disease in the United Kingdom. Efforts were directed toward the development of procedures able to remove or inactivate prions from blood components or plasma-derived products with brain fractions of transmissible spongiform encephalopathy (TSE)-infected rodents as spiking materials. These spiking materials, however, are loaded with pathological prion protein (PrP^{TSE}) aggregates that are likely not associated to blood infectivity. The presence of these aggregates may invalidate these studies.

STUDY DESIGN AND METHODS: Brains from 263K scrapie–infected hamsters were suspended in 10 percent phosphate-buffered saline. After low-speed centrifugation, the supernatant was collected and ultracentrifuged at 220,000 × *g* at 25°C for 30 minutes. The high-speed supernatants (S^{HS}) and pellets were collected; the proteinase-resistant PrP^{TSE} was measured by Western blot and infectivity by intracerebral inoculation into weanling hamsters.

RESULTS: A substantial amount of prion infectivity (more than 10^5 LD_{50} per mL of a 10% suspension of brain tissues) is present in the S^{HS} fraction of 263K scrapie–infected hamster brains. Concomitantly, this fraction contains none or only traces of PrP^{TSE} in its aggregate form.

CONCLUSION: This study describes a simple and fast protocol to prepare infectious material from 263K scrapie–infected brains that is not contaminated with PrP^{TSE} aggregates. This S^{HS} fraction is likely to be the most relevant material for endogenous spiking of human blood in validation experiments aimed at demonstrating procedures to remove or inactivate TSE infectious agents.

The occurrence of two blood-related infections of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom[1,2] and the finding that approximately 10 percent of vCJD cases were blood donors before the appearance of clinical signs[1] are cause of increasing concern for the safety of blood transfusion and, as a consequence, of blood components or plasma-derived products. There is strong evidence that vCJD is caused by the consumption of bovine spongiform encephalopathy (BSE)-contaminated meat products, but the occurrence of human-to-human transmission of vCJD has now raised the possibility that other cases might be related to blood transfusions rather than meat consumption. BSE and vCJD, together with scrapie in sheep and goats, sporadic and genetic CJD, Gerst-

ABBREVIATIONS: BSE = bovine spongiform encephalopathy; LD_{50} = doses required to kill 50 percent of inoculated animals; NaPTA = sodium phosphotungstic acid; PK = proteinase K; P^{HS} = high-speed pellet; P^{NaPTA} = pellet after sodium phosphotungstic acid precipitation; PrP^{TSE} = pathological prion protein; $PrP^{27\text{-}30}$ = 27- to 30-kDa fragment of protease-resistant prion protein; S^{LS} = low-speed supernatant; S^{HS} = high-speed supernatant; TSE = transmissible spongiform encephalopathy; TBST = Tris-buffered saline (pH 8) with 0.05 percent Tween 20; vCJD = variant Creutzfeldt-Jakob disease.

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mann-Sträussler-Scheinker disease, and sporadic or familial fatal insomnia, belong to the group of transmissible spongiform encephalopathy (TSE) or prion diseases that are progressive degenerative disorders of the central nervous system with fatal outcome.[3] The majority of vCJD cases have occurred in the United Kingdom (n = 159)[4] or in patients who spent months in the United Kingdom before the development of disease. However, vCJD has also occasionally been reported in patients who were not British and never traveled to the United Kingdom, suggesting that these patients were infected in their own country.[5-8] Moreover, a preliminary prospective study in the United Kingdom has indicated that there are about 3000 individuals in the age group 10 to 30 years who might carry prion infectivity in the lymphoreticular tissues[9] and, possibly, in the blood. The findings that vCJD patients carry infectivity in blood up to 3 years before the appearance of clinical signs[1] and that no test is yet available for the screening of vCJD-infected people[10,11] have focused the efforts for the safety of blood toward procedures that may remove or inactivate the infectious agent in blood, blood components, or plasma products. These validation experiments are usually performed either with blood taken from TSE-infected rodents or with human blood spiked with TSE-infected rodent brains.[12] Blood of TSE-infected rodents, however, contains only up to 10^2 infectious doses per mL,[13,14] and even the complete removal of these low levels of infectivity does not guarantee the efficacy of the treatment and the safety of blood.[12] Spiking blood with TSE-infected brains greatly increases the amount of infectivity and therefore overcomes the low-level infectivity naturally carried in rodent blood, but the criticism of these validation studies is that the nature of prions in the brain may substantially differ from that present in the blood.[12] Most of the infectivity in the brain is associated with the abnormal prion protein (PrP^{TSE}) in its aggregate form, whereas in blood it is likely that infectivity is associated to a much more soluble fraction of PrP^{TSE}. This substantial difference in the physicochemical structure of PrP^{TSE}-associated infectivity may influence the efficacy of procedures able to inactivate or remove prion infectivity.

In this article, we show that fractions of 263K scrapie–infected brains retain a high level of prion infectivity without being associated with the aggregate form of PrP^{TSE}. This fraction might be useful in the validation studies of pharmaceuticals products derived from blood or urine collected from human or BSE-susceptible ruminants.

MATERIALS AND METHODS

Extraction of water-soluble scrapie infectivity

263K scrapie–infected hamster brains were suspended in 9 vol of sterile phosphate-buffered saline (PBS; pH 7.4) and homogenized by use of a Teflon-glass Potter tissue grinder. The homogenate was dispersed with 10 sonication pulses (Vibra Cell, Sonics & Materials Inc., Newtown, CT) while kept on ice and then centrifuged at $825 \times g$ for 15 minutes at 25°C (GS-6R, rotor GH-3.7, Beckman Coulter, Fullerton, CA). Low-speed supernatant (S^{LS}) was sonicated as above and ultracentrifuged at $220,000 \times g$ for 30 minutes at 25°C (Optima TL-100, rotor TLA 100.3, Beckman Coulter, Fullerton, CA). This high-speed supernatant (S^{HS}) was collected and the high-speed pellet (P^{HS}) was sonicated in sterile PBS to obtain a 10 percent suspension (gram-equivalents of brain/PBS). In Replicate 2, sonication was never performed. These three fractions (S^{LS}, S^{HS}, and P^{HS}) were stored at –70°C until assayed. The S^{HS} fraction of Replicate 3 was examined by transmission electron microscopy after negative staining.

Western blot measurement of the 27- to 30-kDa fragment of protease-resistant prion protein

Fractions S^{LS}, S^{HS}, and P^{HS} were thawed and treated for 60 minutes at 37°C with proteinase K (PK; Sigma Chemical Co., St. Louis, MO) at a final enzyme concentration of 50 µg per mL. The digestion was stopped by adding protease inhibitors (Complete, Roche Diagnostics GmbH Roche Applied Science, Mannheim, Germany) in accordance with the manufacturer's instruction.

Sodium dodecyl sulfate–polyacrylamide gel electrophoreses and Western blot assays were performed according to Lee and coworkers[15] with some modifications. After PK treatment, the samples were serially diluted in half-log steps in NuPAGE gel loading buffer, boiled for 10 minutes in a water bath, and electrophoresed on 12 percent NuPAGE Bis-Tris gels (Invitrogen Corp., Carlsbad, CA) for 60 minutes at 125 V. The nitrocellulose membrane (Hybond ECL, Amersham Biosciences Europe GmbH, Freiburg, Germany) was soaked in Towbin transfer buffer for 5 minutes before "sandwich" assembly and semidry transfer for 60 minutes at 125 mA at 4°C. The membrane was blocked for 60 minutes at 37°C in 3 percent nonfat dry milk (Bio-Rad, Hercules, CA), dissolved in Tris-buffered saline (pH 8) with 0.05 percent Tween 20 (TBST), and incubated overnight at 4°C with 3F4 monoclonal anti-hamster 27- to 30-kDa fragment of protease-resistant prion protein (PrP^{27-30}) antibody[16] (provided by H. Diringer) diluted 1:2000 in TBST. The membrane was rinsed with TBST (five changes of solution in 25 min), incubated for 90 minutes at room temperature with an alkaline phosphatase–labeled goat anti-mouse IgG (Perkin-Elmer Sciences, Wellesley, MA) diluted at 1:5000 in TBST, and rinsed again. Bands were revealed by the CDP-star chemiluminescence detection kit (Applied Biosystems, Foster City, CA) and

recorded onto sensitive films (Hyperfilm ECL, Amersham Biosciences).

Sodium phosphotungstic acid precipitation of the S^{HS} fraction

To recover PrP^{TSE} in fraction S^{HS}, the sample was mixed with 1 volume of 4 percent sarkosyl and processed with sodium phosphotungstic acid (NaPTA; 0.3%) and $MgCl_2$ (12.75 mmol/L) as published by Wadsworth and associates[17] with the only modification consisting in the precipitation of the final pellet by ultracentrifuge at 220,000 × *g* for 30 minutes at 25°C (Optima TL-100, rotor TLA 100.3). The pellet (P^{NaPTA}) was then sonicated in sterile PBS to obtain a 10 percent suspension (gram-equivalents of brain/PBS) and stored at –70°C until assayed.

Infectivity bioassay

Groups of 7 to 10 Syrian hamsters were anesthetized and then inoculated intracerebrally with 50 µL of fractions S^{LS}, S^{HS}, P^{HS}, and P^{NaPTA}. Animals were maintained in coded plastic cages with water and food ad libitum and regularly scored for clinical signs of scrapie disease as previously described.[18] Incubation periods (mean ± SD) were measured and infectivity titers were estimated by applying these values to a dose incubation curve drawn after an endpoint titration.[19] An inverse relation exists between dose and incubation period in the 263K strain in hamsters, which gives a mean incubation period of 155.5 days for 1 LD_{50} intracerebral unit in 0.05 mL of a 10 percent brain homogenate.[19] Animals were housed at the animal facility of the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) under the supervision of the Service for Biotechnology and Animal Welfare of the ISS who warrants the adherence to national and international regulations on animal welfare.

RESULTS

Western blot analyses of 263K scrapie–infected brain fractions

As expected, a great amount of partially PK-resistant PrP^{TSE} (PrP^{27-30} Fig. 1) and infectivity (approx. 8 log LD_{50}/mL 10% brain suspension) is present in the supernatant (S^{LS}) after low-speed centrifugation of 263K scrapie–infected brain homogenates in PBS. The majority of PrP^{27-30} and infectivity is then recovered in the pellet after ultracentrifugation (P^{HS}). As shown in Table 1, the difference

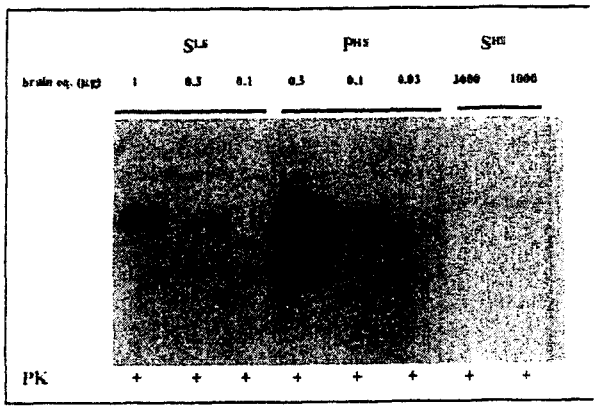

Fig. 1. Western blot analysis of PrP^{27-30} in low-speed (S^{LS}) and high-speed (P^{HS} and S^{HS}) fractions prepared from 263K scrapie-infected brain (Replicate 3). The samples were treated with PK, diluted in half-log steps in NuPAGE gel loading buffer, and resolved on a 12 percent NuPAGE Bis-Tris gels. After the transfer to nitrocellulose membrane and incubation with monoclonal antibody 3F4, PrP^{27-30} was visualized by chemiluminescence on sensitive films. PrP^{27-30} was not measurable in S^{HS}, indicating that virtually all the aggregate form of PrP^{TSE} was precipitated after centrifugation at 220,000 × *g* for 30 minutes.

TABLE 1. Endpoint titration of PrP^{27-30} in different fractions (S^{LS}, S^{HS}, and P^{HS}) of 263K scrapie–infected brain

Replicate number	Fraction	Log dilutions (weight-equivalents of brain tissue) 0.5 (3 mg)	1 (1 mg)	4 (1 µg)	4.5 (0.3 µg)	5 (0.1 µg)	5.5 (0.03 µg)	6 (0.01 µg)	Difference (log) between S^{LS} and S^{HS}
1	S^{LS}			+	+	–			≥4.5
	S^{HS}	–	–						
	P^{HS}			+	+	+	–		
2*	S^{LS}			+	–				≥4.0
	S^{HS}	–	–						
	P^{HS}			+	–				
3	S^{LS}			+	+	+	–		≥5.0
	S^{HS}	–	–						
	P^{HS}			+	+	+	+	–	
4	S^{LS}			+	+	+	–		4.5
	S^{HS}	+	–						
	S^{NaPTA}†	–							
	P^{NaPTA}	+	–						

* No sonication was performed in this replicate.
† S^{NaPTA} = supernatant after sodium phosphotungstic acid precipitation.

between the amount of PrP^{27-30} in S^{LS} and P^{HS} was either null (Replicate 2) or no more than 0.5 log (Replicates 1 and 3) and it was not influenced by the use of sonication for the dispersion of samples (compare Replicates 1 and 3 with Replicate 2). Concordantly, PrP^{27-30} was either not measurable (Fig. 1, Replicate 3) or present at a very low amount (Fig. 2, Replicate 4) in the supernatant after ultracentrifuge (S^{HS}, Table 1), indicating that virtually all the aggregate form of PrP^{TSE} was precipitated by 220,000 × *g* for 30 minutes. In other words, the ultracentrifuge reduces the amount of PrP^{TSE} aggregates in the supernatant of more than 10,000 times (Table 1). Examination of the S^{HS} fraction of Replicate 3 revealed amorphous proteinaceous material and no delimiting membranous structure. In Replicate 4, the small amount of PrP^{TSE} was completely precipitated by NaPTA precipitation (P^{NaPTA}; Fig. 2). In the other replicates, PrP^{TSE} was not recovered even after NaPTA precipitation.

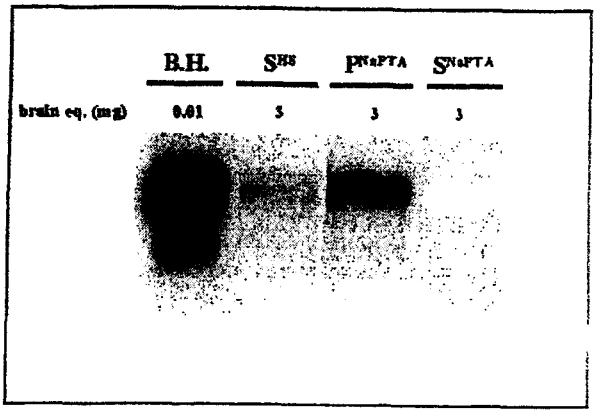

Fig. 2. Western blot analysis of PrP^{27-30} in S^{HS} fraction before (S^{HS}) and after (S^{NaPTA}) NaPTA precipitation (Replicate 4). The samples were treated with PK, resolved on a 12 percent NuPAGE Bis-Tris gels, and transferred to nitrocellulose membrane. After incubation with monoclonal antibody 3F4, PrP^{27-30} was visualized by chemiluminescence on sensitive films. PrP^{27-30} traces in the S^{HS}, were recovered in the pellet after NaPTA precipitation (P^{NaPTA}). PK-treated 263K-infected Syrian hamster brain homogenate (B.H.) was loaded as positive control.

Infectivity measurement of 263K scrapie–infected brain fractions

Virtually all infectivity present in the S^{LS} fraction is recovered in the pellet after ultracentrifugation (P^{HS} fraction). A substantial amount of infectivity is also found in the S^{HS} fraction, however. The mean incubation periods of hamsters inoculated with aliquots of S^{HS} fractions were, respectively, 15.9, 18.5, and 25.6 days longer than the corresponding S^{LS} fractions (Table 2), which corresponds to an estimate lost of infectivity titer ranging from 30 to 200 times.

The enrichment factor for infectivity versus PrP^{TSE} (calculated as the difference between the reduction factor for PrP^{TSE} and the reduction factor for infectivity) ranged from more than 200 times in Replicate 2 up to 1000 times in Replicate 1 (Table 2). Virtually all infectivity in S^{HS} was recovered in the pellet (P^{NaPTA}) after NaPTA precipitation.

DISCUSSION

In blood of TSE-affected rodents or sheep, a substantial proportion of infectivity is associated with plasma.[20-22] If the distribution of infectivity in human blood is the same as in animals, then plasma-derived products might be at risk of transmitting vCJD. Usually, precautions against the risk of infection in medicinal products, including plasma-derived pharmaceuticals, consist of source deferrals, screening of donors, and inactivation or removal of the infectious agent. In TSE diseases, however, the first two lines of defense are poorly practicable because blood is infectious during the long asymptomatic phase of

TABLE 2. Bioassay measurement of infectivity in different fractions (S^{LS}, S^{HS}, and P^{HS}) of 263K scrapie–infected brain

Replicate number	Fraction	Number of days (± SD) in incubation periods (number)	Estimated titer (log LD_{50}/mL of 10% brain suspension)	Difference (log) between S^{LS} and S^{HS}	Enrichment factor (infectivity/PrP^{27-30})
1	S^{LS}	55.8 ± 1.0 (8)	8.4	1.5	$\geq 10^{3.0}$
	S^{HS}	71.7 ± 2.3 (9)	6.9		
	P^{HS}	56.4 ± 2.7 (10)	8.3		
2*	S^{LS}	56.5 ± 2.0 (10)	8.3	1.7	$\geq 10^{2.3}$
	S^{HS}	75.0 ± 0.0 (9)	6.6		
	P^{HS}	59.1 ± 6.9 (10)	8.0		
3	S^{LS}	62.3 ± 0.7 (7)	7.7	2.3	$\geq 10^{2.7}$
	S^{HS}	87.9 ± 1.9 (7)	5.4		
	P^{HS}	Not done			
4†	S^{HS}	85.6 ± 4.2 (7)	6.6		
	P^{NaPTA}	87.7 ± 4.6 (7)	6.4		

* No sonication was performed in this replicate.
† Samples were 10-fold diluted respect to other replicates.

disease[1,2] and no tests are yet available for an early preclinical diagnosis[3,8] or for the screening of blood.[10,11] Thus, efforts are directed to implement procedures able to remove or inactivate TSE agents. Validation studies performed in the past years suggest that TSE agents can be removed by the processes used to manufacture plasma products. There is uncertainty, however, on the complete validity of these experiments mainly because it has been questioned whether the TSE agents in exogenous infectious materials used to spike human blood share the same physicochemical characteristics of the vCJD and other TSE agents in blood.[12,23] A comparison of different spiking preparations showed that brain homogenate, caveolae-like domains, and microsomes partition similarly, whereas purified PrP^{TSE} had significantly different partitioning properties.[23] Obviously, the best spiking material would be infectious human plasma,[24] but all attempts to transmit the disease with whole blood or buffy coat from human patients to experimental animals have so far failed.[25,26] The next best is to use blood from TSE-infected rodents. There is long-lasting evidence that blood of hamsters with experimental scrapie,[14,27] mice with experimental Gerstmann-Sträussler-Scheinker disease[20,28] or vCJD,[13] and sheep with natural scrapie[21] or experimental BSE[21,29] is infectious. Their blood contains too little infectivity to ensure the efficacy of removal procedures, making mandatory the use of exogenous spiking materials to perform reliable validation studies. Then, considering that removal may be influenced by the state of prion aggregation,[30] what is the most appropriate spiking material for the validation of the processes used for manufacturing plasma products? Brain homogenate may not be relevant because it contains large cell and membrane debris, high lipid content, and other brain molecules. Neither are highly purified PrP^{TSE} aggregates since they are not likely to be present in blood. Any attempt to measure PrP^{TSE} in blood or concentrates of blood components, such as buffy coats, has been frustrating, and claims of success have not been successively confirmed.[10] PrP^{TSE} in buffy coat of diseased 263K scrapie-infected hamsters is detectable after at least 144 cycles of protein misfolding cyclic amplification.[31] Theoretically, microsomal membrane fraction is a better spiking material,[12] although data from rodents infected with a mouse-adapted strain of human Gerstmann-Sträussler-Scheinker disease have shown that plasma is free of membranous structures,[32] that filtration or high-speed centrifugation does not eliminate infectivity from plasma,[22] and that in plasma of vCJD-infected mice, infectivity is reduced by PK treatment.[33] These data suggest that in plasma the infectious agent is very small, unsedimentable, and poorly aggregated.

In this scenario, the S^{HS} fraction purified from 263K scrapie-infected hamster brains may be an appropriate spiking material for these studies. S^{HS} fraction contains at least 10,000-fold the infectivity found in blood of TSE-infected rodents; it is virtually free from PrP^{TSE} aggregates, membranous fractions, and detergent contaminants, which interferes with the efficacy of TSE removal during the production of plasma derivatives.[12,34,35] The finding that in S^{HS} fraction TSE infectivity is dissociated from PrP^{TSE} aggregates is not surprising, although the primary consequence of an infection with a TSE agent is the conformational change of the cellular PrP into a pathological conformer (PrP^{TSE}), rich in β-sheet structures, which tends to aggregate into amyloid fibers[36] and cosegregates with infectivity.[37] Exceptions to this rule, i.e., the presence of infectivity without the formation of PrP^{TSE} aggregates, have been reported,[38-42] and blood might simply be another condition where this divergence occurs. Likely, the infectivity in S^{HS} is associated with dimer or small aggregates of PrP^{TSE} that remain in solution after ultracentrifuge, but precipitate in the presence of NaPTA and Mg^{2+}, which form complexes with PrP^{TSE} but not with cellular prion protein.[43]

It is therefore likely that an efficient removal of infectivity through nanofilters or depth filtrations[44-46] is achieved only when infectivity is associated to PrP^{TSE} in its aggregate form, but that these procedures may not be so effective when applied to naturally infected blood or plasma units. In conclusion, this study shows a simple and fast method for preparing a suitable spiking material to use in validation experiments aimed at proving removal or inactivation of prion infectivity in the preparation of blood components or pharmaceuticals derived from human plasma.

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別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.mhlw.go.jp/houdou/2006/08/h0824-3.html	公表国 日本	
販売名(企業名)	－					

研究報告の概要

変異型クロイツフェルト・ヤコブ病(vCJD)は、献血の際に血液で検査する方法が未だ実用化されていないため、例えば、欧州滞在歴のある方などvCJD伝播のリスクが否定できない方について、問診により献血制限を行う暫定的な措置が講じられている。
今般、ヒト胎盤エキス(プラセンタ)注射剤を使用した方の取扱いについても、以下の措置を講じることとなった。
(1) 同注射剤による vCJD 感染事例は報告されていないが、輸血や臓器移植と同様にヒト由来の臓器から製造されていることから、vCJD伝播の理論的なリスクが否定できないため、念のための措置として、その使用者について問診により献血を制限する。
(2) 日本赤十字社においては、1カ月後を目途に措置を実施する予定。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
ヒト胎盤エキス(プラセンタ)注射剤 からの vCJD伝播の理論的なリスクが否定できないため、念のための措置の情報である。 現時点まで血漿分画製剤からのvCJD伝播の報告はない。	今後ともvCJDに関する安全性情報、規制情報等に留意していく。

報道発表資料

平成18年8月24日
（連絡先）
医薬食品局血液対策課
課長 関 英一
（内） 2900
（直通） 03-3595-2395

ヒト胎盤エキス（プラセンタ）注射剤使用者の献血制限について

平成18年8月23日に開催された薬事・食品衛生審議会血液事業部会安全技術調査会において、ヒト胎盤エキス（プラセンタ）注射剤を使用した方の献血を制限する措置を日本赤十字社が実施することが了承された。

1. 経緯等

(1) 変異型クロイツフェルトヤコブ病（vCJD）は、献血の際に血液から検査する方法が未だ実用化していないため、例えば、欧州滞在歴のある方などvCJD伝播のリスクが否定できない方について、問診により献血制限を行う暫定的な措置を講じてきているところである。

（※） 暫定的な措置の内容
- 平成17年2月に国内でvCJD患者が確認され、英国滞在歴を有していたことを踏まえ、同年6月より、特定の期間に1日以上英国滞在歴のある方の献血を制限。
- 輸血及び臓器移植（ヒトの臓器に由来するもの）を受けた方からの献血を制限。

(2) ヒト胎盤エキス（プラセンタ）注射剤を使用した方の取扱いについても、安全技術調査会において平成16年10月から審議されてきたところであり、今般、以下の措置を講じることとしたものである。

2. 新たな措置の内容

(1) 同注射剤によるvCJD感染事例は報告されていないが、輸血や臓器移植と同様にヒト由来の臓器から製造されていることから、vCJDの伝播の理論的なリスクが否定できないため、念のための措置として、その使用者について問診により献血を制限することとする。

（注） ヒト胎盤エキス（プラセンタ）注射剤については、国内では2製剤が薬事法の承認を受けている。

[1] メルスモン（注射薬）（メルスモン）
効能・効果 更年期障害・乳汁分泌不全

[2] ラエンネック（注射薬）（日本生物製剤）
効能・効果 慢性肝疾患における肝機能の改善

※ 美容形成（シミ・シワ・ニキビ等）に一部使われていることも知られている。

(2) 日本赤十字社においては、1ヶ月後を目途に措置を実施する予定である。

→ 関連資料 … 薬事・食品衛生審議会 平成18年度第1回血液事業部会安全技術調査会

トップへ

報道発表資料 厚生労働省ホームページ

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2006 年 2 月 28 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Emerging viral diseases and infectious disease risks Tapper, M.L. Haemophilia 12, (Suppl. 1), 3 - 7 (2006)	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
西ナイルウイルス感染症，鳥インフルエンザ，重症急性呼吸器症候群(SARS)に特に重点をおいた「新規感染症」の概念，及びそれに関連すると思われる血液製剤とその使用患者の安全性について取り上げている。1992年以来，米国の医学研究所(IOM)は「新規感染症は，新型の，または再興する，または薬剤耐性の感染症であり，ヒトへの罹患率が過去20年以内に増加しているか，近い将来増加する恐れがある疾患」と定義している。海外旅行や国際商取引，人口統計学上及びそれに付随した行動の劇的な変化により感染因子は世界的に蔓延し，加速している。 2002年以来，輸血に関連する西ナイルウイルス感染症報告を受けて，米国においてウイルス検査を行った結果，多くの感染供血者(献血時には無症候であった)が特定された。その一方，SARSウイルスとトリインフルエンザウイルスでは，現時点で安全な血液供給に影響を及ぼす事態は生じていない。しかし，血液供給と血液由来製剤の安全性を脅かす新興病原体の検出と除去に対して厳重に警戒するよう提言している。	BYL-2006-0220-1

報告企業の意見	今後の対応
血漿分画製剤を介した西ナイルウイルス感染は現在までにまったく報告されていない。また，2003 年 5 月 1 日の FDA ガイドラインでは，血漿分画製剤に使用する血漿プールに対しては西ナイルウイルスの検査は必要ないとしている。さらに，弊社のウイルス不活化処理は血漿プールにおいて，西ナイルウイルスを不活化させるのに十分であることが証明されている。インフルエンザウイルス及びコロナウイルスに関しても同様に，弊社のウイルス不活化処理またはウイルス除去処理により安全性が確保されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Haemophilia (2006), 12, (Suppl. 1), 3–7

Emerging viral diseases and infectious disease risks

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Summary. New pathogens and antimicrobial-resistant forms of older pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Pathogens can emerge either through introduction into a new population or when the interaction with the vector changes; emergence is also influenced by microbiological adaptation and change, global travel patterns, domestic and wild animal contact and other variants in human ecology and behaviour. Quick, decisive action to detect and control novel pathogens, and thereby contain outbreaks and prevent further transmission, is frequently hampered by incomplete or inadequate data about a new or re-emerging pathogen. Three examples of pathogens that are current causes for human health concern are avian influenza, West Nile virus (WNV) and the severe acute respiratory syndrome (SARS) coronavirus. Pathogens directly or indirectly transmitted by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges. Rapid screening tests for other newly described pathogens such as WNV require time for development and may be <100% reliable. The importance of vigilance in the detection and control of newly recognized infectious threats cannot be overstressed. The presence of infectious agents in the blood supply could again have a significant impact on the safe use of both blood and blood-derived products in the care of patients with haemophilia, as did the human immunodeficiency virus in the 1980s. Emerging pathogens will continue to be a reality requiring the collaborative efforts of public health and individual healthcare providers worldwide to contain outbreaks and prevent transmission.

Keywords: avian influenza, haemophilia, human immunodeficiency virus, pathogens, severe acute respiratory syndrome, West Nile virus

Introduction

The emergence of new infectious pathogens and the recurrence of older pathogens in unique settings have become common topics in the medical literature and lay media, indicating an increasing concern among healthcare providers and the general public alike. The presence of infectious agents in the blood supply, for example, has had – and could again have – a profound influence on the safe use of both blood and blood-derived products in the care of patients with haemophilia. This article provides an overview of emerging infectious diseases in general and discusses some examples of viral pathogens that are currently cause for concern, including West Nile virus (WNV), severe acute respiratory syndrome (SARS) and avian influenza. It also lays the foundation for discussions about the implications of emerging infectious diseases for the safety of the blood supply and for the care of patients who depend on the safety of the blood supply, such as those with haemophilia.

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Infectious disease outbreaks of the last decade

In the last decade there have been a number of major global infectious disease outbreaks that have had the potential to be major health threats. Many of these rapidly spreading viruses, including SARS and avian influenza, appear to have originated as zoonoses in Asia [1]. These viruses have also demonstrated an extraordinary capacity to move quickly (and often surreptitiously) between animal and human populations and across continents.

Definition of an emerging infectious disease

Defining an emerging infectious disease is not necessarily straightforward. Morbidity and mortality from

emerging infectious diseases are understood to be a continual threat, yet the exact nature of that threat is not well defined. One widely accepted definition was proposed in 1992 by the Institute of Medicine (IOM) in the USA, which defined an emerging infectious disease as a new, re-emerging, or drug-resistant infection whose incidence in humans has increased within the past two decades or whose incidence has threatened to increase within the near future [2]. Based on this definition, a spectrum of potential infectious diseases becomes apparent.

Potential infectious disease threats

A continuum exists in types of pathogens that emerge and infect new populations. The continuum includes infectious diseases such as SARS that appear to be newly introduced to humans from animals as well as bioengineered organisms that produce disease in unforeseen ways, such as the transmission of anthrax by contaminated mail in the USA in 2001. Outbreaks of disease once thought to be well controlled may be associated with a breakdown in core public health measures such as treatment of established infection (e.g. tuberculosis) or routine childhood immunizations (poliomyelitis) The continuum of potential disease threats also includes new antimicrobial-resistant forms of established pathogens, such as methicillin-resistant *Staphylococcus aureus*. In addition, scientists continue to recognize previously unidentified infectious origins of some chronic diseases, such as Lyme borreliosis [3].

Factors contributing to emerging infections

In 1992 the IOM identified numerous factors that contribute to emerging infectious diseases, all of which may impact the safety of the blood supply [2]. These factors include:

1 human demographics and behaviour;
2 technology and industry;
3 economic development and land use;
4 international travel and commerce;
5 microbiological adaptation and change;
6 breakdown of core public health measures.

In 2003, the IOM published an update to the 1992 report in which additional contributing factors were identified [3]:

1 human susceptibility to infection;
2 climate and weather;
3 changing ecosystems;
4 poverty and social inequality;
5 war and famine;
6 lack of political will;
7 intent to harm.

Many of these factors are interdependent. International travel and commerce and human demographics and behaviour, for example, are closely related and have undergone considerable change in the last century. Over the last 150 years as the global population has increased dramatically, the length of time required to circumnavigate the globe has decreased dramatically (Fig. 1) [4]. International travel and commerce have affected the size and mobility of human populations, bringing some environments, humans and other animal species into contact with each other for the first time. These changing human demographics may enable an infectious agent to become adapted to and disseminated within a new host population, often resulting in an expansion of the agent's geographic range [5]. The combination of these factors has accelerated the global spread of infectious agents.

Route of transmission of emerging infectious disease

Emergence of an infectious disease can occur either through its introduction into a new population or when the interaction with the vector of a disease changes. The latter scenario is the likely manner in which viruses such as WNV and Lyme borreliosis have spread [5]. The WNV strain found in the USA, for example, is believed to have spread from the Middle East and be a variant of the virus first isolated in 1937 in the West Nile District of Uganda in Africa. It is uncertain how WNV spread to the USA. It has been hypothesized that the strain in the USA was

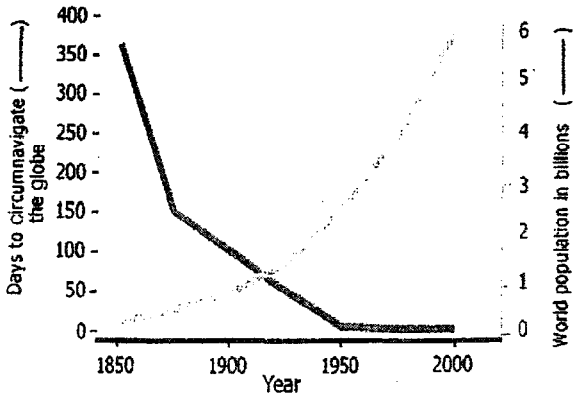

Fig. 1. Speed of global travel in relation to world population growth [4].

transported in an infected bird illegally imported from the Middle East or Central Europe where the disease had previously been endemic. Mosquito transmission subsequently resulted in transmission to birds, horses and humans in the USA. After its initial appearance in New York City in 1999, WNV spread to the lower 48 states in the US in <2 years [6].

Recent infectious disease concerns

New, emerging infectious diseases and disease agents continue to be discovered and described. While incomplete, the list in Table 1 provides an indication of the variety and quantity of pathogens that confront public health officials and present potential threats to human health [3].

West Nile virus

In 1999, the first cases of WNV infection were recognized in New York City. Over the next several years, the virus spread throughout the northeastern part of the country and subsequently spread west to the Mississippi River and south into Florida. By 2002, cases were being reported across most of the Midwest, and by 2005 every state in the continental USA had reported cases of WNV in humans, birds, mammals or mosquitoes [7].

Since 2002, following reports of transfusion-associated WNV infections, the US blood supply has been screened for the virus. As of 15, November 2005, 382 presumptively viremic blood donors had been identified and reported to the US Centers for Disease Control and Prevention (CDC). These donors were generally asymptomatic for WNV infection at the time of blood donation but tested seropositive when pooled samples were screened using nucleic amplification technology (NAT). Some of these individuals subsequently developed clinical symptoms [8].

Table 1. Partial list of emerging infectious diseases and disease-causing agents*.

HIV/AIDS
Tuberculosis
Dengue
Malaria (resistant *Plasmodium falciparum*)
Severe acute respiratory syndrome
Cholera
Meningococcal meningitis
Cryptosporidiosis
Filoviruses (Ebola/Marburg)
Legionella pneumophila
Lyme disease
Poliomyelitis
Toxin producing streptococci and *Staphylococcus aureus*
Human Herpesvirus-8
Parvovirus B19
Hepatitis C
Arenaviruses (Lassa)
Cyclospora cayetanensis
Hantavirus (Sin Nombre)
New variant CJD (BSE)
Bunyaviruses (Rift Valley)
Rotavirus
Escherichia coli 0157:H7
Bartonella henselae (cat scratch disease)
Community acquired MRSA
Avian influenza (H5N1)
West Nile virus
Salmonella enteritidis

AIDS, acquired immunodeficiency syndrome; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; HIV, human immunodeficiency virus; MRSA, methicillin-resistant *Staphylococcus aureus*.
*Data adapted from Smolinski *et al.* [3].

Severe acute respiratory syndrome

At the outset of the SARS epidemic in Asia, a number of small mammals commonly maintained in open food markets in Canton were found to be infected with the SARS coronavirus. More recent data have suggested that certain species of bats native to China may be the definitive host of the virus in nature [9].

Severe acute respiratory syndrome was first recognized in Hanoi, Vietnam in February 2003, although it is now believed to have originated in the Guangdong Province in southeast China in November 2002 [10]. In late February 2003, the first case of SARS in Hong Kong was reported in a physician from the Guangdong Province, who travelled to Hong Kong for a wedding. While staying overnight in a local hotel, it appears he transmitted the virus to 12 people on his floor. Subsequent generations of infection from the physician (who died in a Hong Kong hospital 2 days after arriving at the hotel), his relatives and others staying in the hotel involved more than 95 healthcare workers and 100 close contacts in the city of Hong Kong [11].

The global spread was rapid. Other infected hotel guests subsequently travelled to Vietnam, where 37 healthcare workers and 21 close contacts became infected, and to Singapore, where 34 healthcare workers and 37 close contacts were infected [11]. Another returned to Canada, where a cluster of infections commenced in a local hospital, involving family members, healthcare workers and other patients. Ultimately, over 200 people in Canada were infected, approximately one-third of whom died [12].

Avian influenza

Avian influenza is a major potential threat to the populations of the world and may be the source of the next flu pandemic [13]. There were three major flu pandemics in the last century: the so-called 'Spanish flu' in 1918–1919, potentially responsible for up to 50 million deaths worldwide; the Asian flu in 1957–1958, responsible for approximately 70 000 deaths in the USA; and the Hong Kong flu in 1968–1969, responsible for 34 000 deaths nationwide. Many epidemiologists believe that the human population is overdue for a pandemic [14]. Figure 2 illustrates a timeline of the emergence of several strains of the influenza virus.

Since 1918 there have been a number of shifts in the influenza virus's haemagglutinin and neuraminidase components, its key antigens. Fifteen types of haemagglutinin (H1–H15) and nine types of neuraminidase (N1–N9) have been recognized. Combinations involving subtypes H1–H3 and N1–N2 have been responsible for both seasonal and epidemic outbreaks in humans. The definitive hosts of influenza in nature are non-domesticated birds, particularly ducks that carry H1–H15 type viruses. Direct bird-to-human (and to date, rare instances of human-to-human) transmission of avian influenza has been reported [15] with increasing frequency in the last two and a half years.

Mechanism of influenza antigenic shift

Influenza viruses undergo constant subtle evolution and mutation of their principal proteins, a process referred to as antigenic drift. In addition to this naturally occurring and random process, influenza strains from different host species can periodically recombine. Swine may serve as hosts for both human and duck influenza strains and hence can function as ideal mixing vessels for major antigenic recombination and the emergence of novel influenza strains. When such shifts or recombinations occur and result in a virus with the capacity to maintain ongoing transmission between humans, a major pandemic may occur [16].

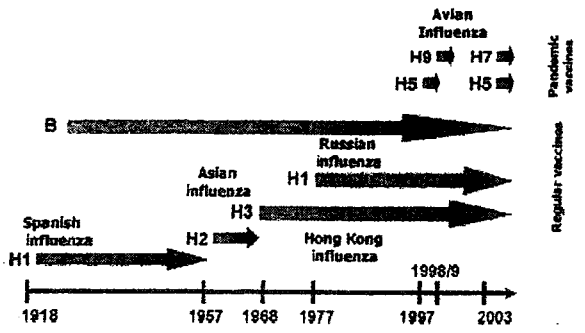

Fig. 2. Timeline of emergence of influenza viruses in humans. (Figure courtesy of the Centers for Disease Control and Prevention.)

In 1997 in Hong Kong, the first evidence emerged that avian viruses could directly infect humans without going through this interim mixing step [15,16]. In 1997, there was an outbreak of influenza associated with an avian (H5N1) strain in humans that was preceded by an outbreak of the same strain in poultry [17]. With six deaths among 18 hospitalizations, H5N1 exhibited unusual lethality and was considered by some public health officials and epidemiologists as a pandemic warning call.

By December 2003, confirmed cases of avian influenza among humans were reported in Vietnam and Thailand, and since January 2004, human cases have been reported in Vietnam, Thailand, Cambodia, Indonesia and the People's Republic of China. The total number of cases as of 17, November 2005 was 130, with 67 deaths [18]. Sustained outbreaks among domestic poultry flocks in Asia preceded these human cases.

While the major outbreaks of avian influenza have occurred among domestic poultry flocks, evidence of avian influenza viral infection in migrating birds throughout Asia (and more recently in Europe) has also been demonstrated. It has been suggested that migratory birds may be responsible for the widespread introduction of avian influenza into other bird populations, both domestic and wild [19].

Conclusion

New pathogens continue to emerge, some with the potential for rapid, global spread and high morbidity and mortality. Laboratory tests for viral detection can be developed once a virus is identified, but their development takes time and their reliability may be <100%.

Pathogens spread by aerosolized droplets, such as avian influenza and SARS, pose considerable containment challenges, although neither pathogen appears to clearly impact the safety of the blood supply. In the case of SARS, patients can be screened, but the exact mode of human-to-human transmission remains uncertain. In contrast, reasonably (although not universally) effective screening exists for some newly described blood-borne pathogens such as WNV. Nonetheless, the hard-learned lesson from the human immunodeficiency virus (HIV) experience in the 1980s is that the importance of vigilance in the

detection and elimination of newly recognized threats to blood safety cannot be overstressed. For these reasons, emerging pathogens will continue to be a reality requiring the best efforts of both public health officials and individual healthcare providers worldwide to identify emerging pathogens in a timely fashion, contain outbreaks and prevent transmission.

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