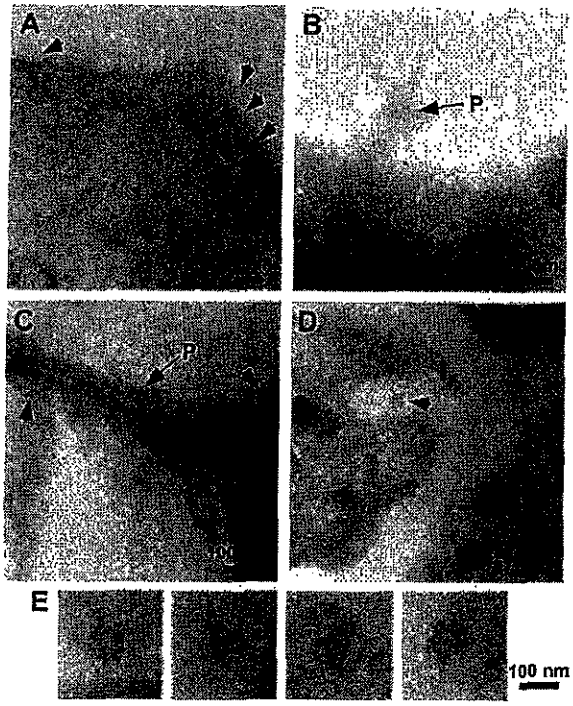

Figure 4. Immunogold labeling of resting and activated platelets. Resting and activated platelets were incubated with Abs to PrP^C (308 or FL253), followed by protein G or secondary antibodies conjugated to 10 nm gold (arrowheads). In resting platelets preincubated with Ab 308 before embedding, PrP^C is seen around the periphery of the cell (A). In activated platelets PrP^C is found at the periphery of the cell and is associated with pseudopods (P, arrows) (B, whole mount; C, frozen section). In frozen sections of activated platelets labeled with Ab FL253, PrP^C was also localized to released exosomes (seen between cells, D; and at higher magnification, E).

Discussion

The current study localizes PrP^C to platelet alpha granule, but not dense granule, membranes, confirming a recent study by Starke et al.[9] Thus, PrP^C is present with proteins such as the αIIb/β3 integrin, CD62 (P-selectin), CD36, and the GPIb/V/IX complex[32-35] inherent in the alpha granule membrane, and, in common with these other proteins, there is an activation-mediated increase in expression of PrP^C on the external platelet surface. The function of PrP^C in platelets is unknown; preincubation with anti-PrP^C Abs has a limited effect on platelet adhesion to a variety of matrices but no effect on agonist-induced aggregation (Robertson et al, unpublished); therefore, it is unlikely that PrP^C plays a significant role in either of these platelet functions. In contrast to the expression of other activation-associated proteins, the thrombin-induced expression of PrP^C on the platelet surface was transient and was followed by its release. Previous studies have shown that PrP^C is present in platelet releasates[10]; however, the current study demonstrates that the released PrP^C is associated with membranes, initially in small quantities on microvesicles and subsequently in higher levels on exosomes.

Exosomes are small (40-100 nm), membrane-bounded vesicles which are released from a variety of cells following exocytosis[36] and are present in human plasma.[37] Denzer et al[38] reviewed a large number of proteins and lipids that are associated with exosomes, which include members of the tetraspanin protein family, the immunoglobulin supergene family, as well as GPI-anchored proteins and cytosolic proteins. Exosomes have been implicated in cell-to-cell communication mechanisms by transferal of proteins directly from the exosomes to target cells, in a manner similar to the movement of GPI-anchored proteins from the plasma membrane of red blood cells to endothelial cells.[39,40] Furthermore, exosomes have been implicated in the activation of the immune system, including the stimulation of T lymphocytes and a potential interaction with follicular dendritic cells.[38] Reticulocyte-derived exosomes may participate in complement regulation.[41] Interestingly, Whiteside[42] has recently proposed that exosomes play a role in the evasion of tumor cells from the immune system.

Studies in platelets have shown the release of alpha granule membrane–derived exosomes following exocytosis.[29] Therefore, the presence of PrP^C on exosomes is entirely consistent with the alpha granule membrane source of these vesicles. The function of platelet-derived exosomes is unknown, although the low binding of factor X, prothrombin, and annexin V to their surface suggests that they do not have the same procoagulant activity as platelet-derived microvesicles.[29] The expression of CD62 on the surface of platelet-derived exosomes points to a possible role in adhesion, or cell-to-cell transfer of adhesive properties, because CD62 is known to mediate adhesion between leukocytes and endothelial cells.[43]

The presence of prion protein on exosomes has recently been highlighted by Fevrier et al,[44] who reported the presence of infectious PrP^{Sc} in exosomes derived from cultured epithelial and neuroglial cell lines after infection with scrapie. They subsequently proposed that exosomes may provide a vehicle for transport of PrP^{Sc} from cell to cell, thus providing a mechanism for transmission of infectious proteins in the body.[45,46] The current finding that PrP^C is present on platelet-derived exosomes strengthens the hypothesis that exosome release is a general mechanism for transport of proteins and inferentially pathogen transmission,

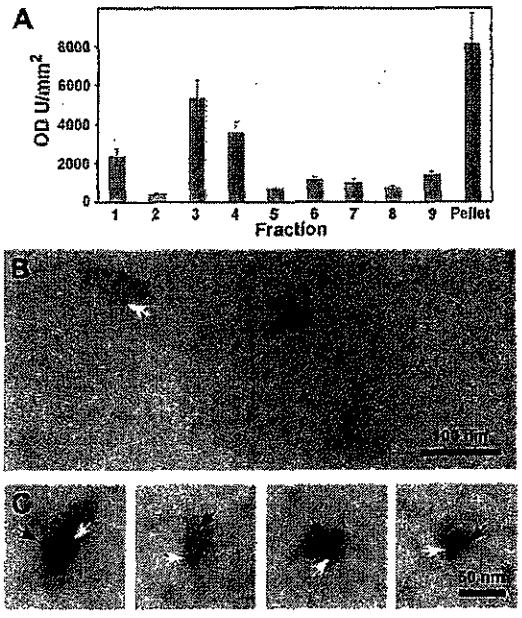

Figure 5. Immunoblotting and immunoelectron microscopy of isolated exosomes. Platelets were incubated with 1 U/mL thrombin for 120 seconds. Following termination, platelets were removed by centrifugation at 800*g*. Further centrifugation of the supernatant removed the microvesicles. Exosomes were isolated by differential centrifugation through a sucrose gradient. Fractions were collected from the top, and immunoblotting was carried out in each fraction using anti-PrP^C Ab 308. The blots were subjected to densitometry and are expressed as mean plus or minus standard error of the mean; n = 3 (A). Fractions 3 and 4 from the sucrose gradient were adsorbed onto formvar-coated grids and double labeled with anti-PrP^C Ab 308 followed by an anti-CD62 Ab (D541). The respective secondary Abs were conjugated to 5 nm (anti-PrP^C; black arrows) and 10 nm (anti-CD62; white arrows) gold (B-C).

including prions, between cells. Platelets contain a large proportion of circulating PrP^{C}[5,6]; therefore, platelet-derived exosomes could potentially act as an important source of protein for prion replication. In addition, the transferral of exosomes containing PrP^{C} to cell types in which it is normally absent may confer susceptibility to infection with prions. To date, this has not been addressed.

Although there is no biochemical evidence for the presence of PrP^{Sc} on platelets, a recent study by Cervenakova et al[23] identified prion infectivity in the platelet and plasma fractions of murine blood from mice infected with mouse-adapted vCJD. The present finding that PrP^{C} is released on exosomes from activated platelets therefore raises the possibility that PrP^{Sc} is similarly released from platelets. Although this has not been addressed in the current study, it is clearly plausible that the generation of PrP^{Sc}-containing platelet exosomes during preparation of blood products could account for the transmission of variant CJD by blood transfusion. Leukoreduction of plasma, a process which would not remove exosomes, reduced infectivity by only 42%[24] and, when taken in concert with the current study, suggests that further investigation into the possible role of platelet-derived exosomes as vehicles for prion transmission is clearly warranted.

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No. 14

医薬品 研究報告 調査報告書

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				2006. 5. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Ironside JW, Bishop MT, Connolly K, Hegazy D, Lowrie S, Le Grice M, Ritchie DL, McCardle LM, Hilton DA. BMJ. 2006 May 20;332(7551):1186-8.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			英国

研究報告の概要

○変異型クロイツフェルトヤコブ病(vCJD):有病率の後方視的研究から得られた陽性虫垂組織のプリオン蛋白の遺伝子型解析
目的:疾患関連プリオン蛋白陽性を示した虫垂組織から抽出したDNAのプリオン蛋白遺伝子(PRNP)コドン129の解析。
デザイン:英国における、変異型クロイツフェルトヤコブ病の連結不可能匿名化した後方視的有病率試験で判明した陽性例の再解析。
試験サンプル: 疾患関連プリオン蛋白の検査を実施した虫垂及び扁桃検体12,674件のうちの陽性例3検体。これらの検体を採取した患者の手術時(1996-1999年)の年齢は20-29歳であった。
実施場所:イングランド及びスコットランドの2箇所の第三次センターの病理学部門。
結果:3件のうち2件の検体について、適切なDNAが採取できたが、いずれもPRNPコドン129の遺伝子型はバリンのホモ接合体であった。
結論:PRNP中のコドン129がバリンのホモ接合体であるサブグループがvCJD感染に対する感受性があることが初めて示された。これまでvCJDの検査を受けた症例は、すべてメチオニンのホモ接合体サブグループであり、医原性vCJDと推定される1例のみがメチオニン/バリンのヘテロ接合だった。PRNPコドン129がバリンのホモ接合体であるvCJD患者の潜伏期間はより長期である可能性があり、輸血あるいは無症候の期間に患者に使用した外科用器具の汚染を介して、水平感染を起こす可能性がある。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「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
vCJD有病率の後方視的研究から、PRNPのコドン129がのホモ接合体であるサブグループがvCJD感染に対する感受性があることが初めて示されたとの報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。さらに、感染リスク低減の目的から、血液製剤の保存前白血球除去の導入を進めている。今後も、CJD等プリオン病に関する内外の新たな知見及び情報の収集に努める。

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Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

James W Ironside, Matthew T Bishop, Kelly Connolly, Doha Hegazy, Suzanne Lowrie, Margaret Le Grice, Diane L Ritchie, Linda M McCardle and David A Hilton

BMJ 2006;332;1186-1188; originally published online 10 Apr 2006; doi:10.1136/bmj.38804.511644.55

What is already known on this topic

The evidence base for prescribing drugs to children lacks sufficient pharmacokinetic and pharmacodynamic data

Adult doses are often extrapolated to children without taking account of potential differences in drug handling with age or dose requirements for effectiveness

Licensing data for paediatric dosing are often sparse, and subsequent studies may result in important changes to recommended doses

What this study adds

HIV infected UK and Irish children have been underdosed with antiretrovirals in the past nine years

Poor pharmacokinetic data at licensing results in incorrect drug dosing until important pharmacokinetic results emerge after licensing and inform revision of dosage recommendations

Guidelines stating alternative dosage strategies (by weight or surface area) for the same drug lead to different and inconsistent doses

Inadequate dosing also arises through failure to adjust for ongoing growth

the United States have recently committed to promoting research specific to children's medicines while protecting children as participants in clinical trials. The UK Department of Health has launched the Medicines for Children Research Network (www.liv.ac.uk/mcrn), which aims to develop closer links between the drugs industry, regulators, families, and paediatricians, links that will be needed to meet the challenges of developing and manufacturing appropriate paediatric drugs (www.hivforum.org).

The Collaborative HIV Paediatric Study (CHIPS) is a collaboration between the Medical Research Council Clinical Trials Unit, UK, and the National Study of HIV in Pregnancy and Childhood (NSHPC) at the Institute of Child Health, London. Committees and participants are on bmj.com.

Contributors: See bmj.com

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Competing interests: None declared.

Ethical approval: UK multicentre research ethics committee and relevant local research ethic committees.

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Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

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Editorial by Wilson and Ricketts

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continued over

BMJ 2006;332:1186–8

Abstract

Objective To perform prion protein gene (*PRNP*) codon 129 analysis in DNA extracted from appendix tissue samples that had tested positive for disease associated prion protein.

Design Reanalysis of positive cases identified in a retrospective anonymised unlinked prevalence study of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.

Study samples Three positive appendix tissue samples out of 12 674 samples of appendix and tonsil tested for disease associated prion protein. The patients from whom these samples were obtained were aged 20-29 years at the time of surgery, which took place in 1996-9.

Setting Pathology departments in two tertiary centres in England and Scotland.

Results Adequate DNA was available for analysis in two of the three specimens, both of which were homozygous for valine at codon 129 in the *PRNP*.

Conclusions This is the first indication that the valine homozygous subgroup at codon 129 in the *PRNP* is susceptible to vCJD infection. All tested clinical cases of vCJD have so far occurred in the methionine homozygous subgroup, and a single case of probable iatrogenic vCJD infection has been identified in one patient who was a methionine/valine heterozygote at

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