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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
変異型クロイツフェルト・ヤコブ病(vCJD)が発症し得るヒトのウシ海綿状脳症(BSE)に対する曝露量は，経口感染の効率およびウシからヒトへの伝播に対する生物学的防御機構の程度についての知識が不十分であるために明らかにされていない。BSEの霊長類への経口伝播について調査するため，2頭のマカクザル(プリオン遺伝子のコドン129でメチオニンホモ接合性)にBSE感染ウシ由来の脳ホモジェネート5gを経口投与した。1頭は，投与60ヵ月後にvCJD様の神経症状を発症したが，もう1頭は76ヵ月間経過しても発症しなかった。本試験とこれまでの他の試験のデータを併せて，ヒトにおける食物を介した曝露リスクについて予備的な推定値を求めた。著者らは，リスクは非常に低いと考え，現行の公衆衛生上の措置でヒトへのBSE伝播は予防可能であると結論した。しかしながら，ウシからヒトへのBSE経口伝播について確認するためには，最大50年以上の潜伏期間があることを考慮しておくことが望ましいと提唱している。	

報告企業の意見	今後の対応
本試験はわずか2頭のサル類を用いた予備的な試験であり，BSEが経口から感染するかどうかの結論を得るためにはフランス原子力庁BSE研究チーム(CEA)で現在実施されているより大規模な研究の結果を待たなければならない。本研究報告では弊社の血漿由来製剤の安全性に影響するような情報はないと考える。	引き続き，vCJDのヒトへ伝播に関する情報の収集に努める。引き続き関連情報の収集に努める。

Risk of oral infection with bovine spongiform encephalopathy agent in primates

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The uncertain extent of human exposure to bovine spongiform encephalopathy (BSE)—which can lead to variant Creutzfeldt-Jakob disease (vCJD)—is compounded by incomplete knowledge about the efficiency of oral infection and the magnitude of any bovine-to-human biological barrier to transmission. We therefore investigated oral transmission of BSE to non-human primates. We gave two macaques a 5 g oral dose of brain homogenate from a BSE-infected cow. One macaque developed vCJD-like neurological disease 60 months after exposure, whereas the other remained free of disease at 76 months. On the basis of these findings and data from other studies, we made a preliminary estimate of the food exposure risk for man, which provides additional assurance that existing public health measures can prevent transmission of BSE to man.

Up to 400 000 cows with undiagnosed bovine spongiform encephalopathy (BSE) infection are estimated to have been slaughtered for food before brain and spinal cord were banned from human consumption in 1989. More restricted exposure to BSE could have continued through 1995 from consumption of processed meat products containing mechanically recovered meat contaminated with central nervous system (CNS) tissue and spinal ganglia.[1] The discovery of BSE in Canada and the USA, where consumption of brain and other viscera was allowed until 2003, and of secondary cases of variant Creutzfeldt-Jakob disease (vCJD) in the UK, possibly attributable to contaminated blood donated by people with pre-clinical primary infection, reinforces the need for an experimental assessment of the risk of oral exposure to BSE. We therefore investigated oral transmission of BSE to non-human primates.

We chose cynomolgus macaques for the study because these old-world monkeys have a digestive physiology similar to that of human beings, are methionine homozygous at codon 129 of the *PRNP* gene, and have a BSE neuropathology similar to that of vCJD.[2,3] We gave two 4-year-old adult macaques a 5 g oral dose of brain homogenate from a BSE-affected cow. We tested for proteinase-resistant prion protein (PrP^{res}) in this homogenate with a commercial BSE-testing ELISA kit (Bio-Rad, Marnes-la-Coquette, France). A sample of the 100% homogenate brain paste inoculum that was fed to the primates was rehomogenised at 20% weight-per-volume in the kit buffer. Serial dilutions were made with a pool of 20% weight-per-volume BSE-negative brain homogenate in the same buffer. Testing was done according to the manufacturer's instructions and results were confirmed by a western blot test (Bio-Rad) with a similar process of PrP^{res} dilution. With both methods, dilutions of up to 1 in 300 provided a positive signal (figure A).

One macaque developed neurological disease 60 months after exposure and was killed at 63 months because of recumbency. Histopathological examination of the brain of this animal showed the typical pathology of vCJD (figure B) and an accumulation of PrP^{res} associated with the follicular dendritic cells in tonsils (figure C), spleen, and intestine. A western blot showed similar patterns of PrP^{res} in a brain sample from the macaque and the BSE-infected bovine inoculum (figure D). The other macaque remained free of clinical signs 76 months after exposure, and a tonsil biopsy done at 72 months was negative (figure E).

In a previous study, two macaques orally dosed with 5 g of brain from a macaque with terminal clinical BSE became ill after 44 and 47 months.[4] The results of the present study suggest that the incubation period for interspecies transmission of BSE can be considerably

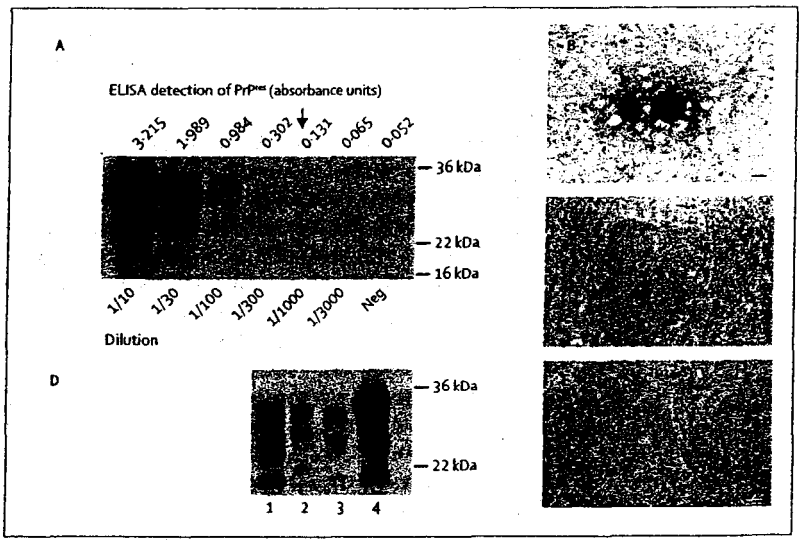

Figure: **PrP^{res} content of brain homogenate and histopathological assessment of macaque tissues**
(A) Results of in-vitro testing for PrP^{res} in BSE-infected inoculum by ELISA and western blot. Neg=normal bovine brain material. (B) Typical florid plaque in the occipital cortex of the macaque that developed disease. PrP^{res} detected by proteinase K treatment with SAF32 anti PrP monoclonal antibody (kindly provided by Jacques Grassi, CEA Saclay). The dense core of PrP^{res} is surrounded by several vacuoles in a fibrillar proteinaceous corona; bar=10 μm. (C) Positive PrP^{res} staining in tonsil (>80% of follicules stained positive) of the macaque that developed disease; bar=50 μm. (E) Negative PrP^{res} staining in tonsil of the macaque that did not develop disease; bar=50 μm. (D) Western blot showing similar PrP^{res} patterns in samples from a patient with vCJD (lane 1), the macaque that developed disease (lane 3), and the bovine BSE inoculum (lane 4). By contrast, a macaque inoculated intracerebrally with material from a patient with sporadic CJD showed a different PrP^{res} pattern (lane 2).

	BSE bovine brain inoculum								
	100 g	10 g	5 g	1 g	100 mg	10 mg	1 mg	0·1 mg	0·01 mg
Primate (oral route)*			1/2 (50%)						
Cattle (oral route)*	10/10 (100%)	7/9 (78%)		7/10 (70%)	3/15 (20%)	1/15 (7%)	1/15 (7%)		
Riff mice (ic + ip route)*						17/18 (94%)	15/17 (88%)	1/14 (7%)	
PrP^{res} biochemical detection						+	+	+	–

The comparison is made on the basis of calibration of the bovine inoculum used in our study with primates against a bovine brain inoculum with a similar PrP^{res} concentration that was inoculated into mice and cattle.[3,4] *Data are number of animals positive/number of animals surviving at the time of clinical onset of disease in the first positive animal (%). The accuracy of bioassays is generally judged to be about plus or minus 1 log. ic + ip=intracerebral and intraperitoneal.

Table 1: Comparison of transmission rates in primates and cattle infected orally with similar BSE brain inocula

longer than that of intraspecies transmission (60 months *vs* 44 and 47 months, representing 36% and 28% increases, respectively). The interval between the period of peak exposure to infectious BSE tissue and the hitherto peak incidence of vCJD is about 10–15 years, but incubation periods of up to 40 years have followed oral infection with kuru between human beings.[5] Therefore, maximum incubation periods might exceed 50 years in cases of oral transmission of BSE from cattle to man.

The present data do not provide a definitive minimum infective dose for transmission of cattle BSE to primates, but they do give enough information for a preliminary assessment of the adequacy of existing measures to protect the human food chain. Results of ongoing experiments provide a rough estimation of the intraspecies transmission rates in cattle. The BSE brain inoculum to which the cattle were exposed had an infectivity titre of $10^{3.5}$ mouse infectious (intracerebral and intraperitoneal) units ID_{50} per g (ID_{50} is the dose at which 50% of animals become infected). Interim results at 6 years after exposure suggest that the oral ID_{50} in cattle may be between 100 mg and 1 g (table 1; S A C Hawkins, T Konold, G A H Wells, unpublished data).

Since the brain of a cow weighs 500 g and a spinal cord 200 g, CNS tissues from a cow with clinical signs of BSE could contain enough infective agent to transmit disease orally to 490–1400 cows (70% of 700 g if 1g is needed, or 20% of 700 g if 100 mg is sufficient), or to 70 primates (50% of 700 g if 5 g represents the oral ID_{50}).

The accuracy of estimates of the oral ID_{50} for man will not be improved until completion, several years from now, of a large dose-response European study (QLK1-2002-01096) in macaques, in which the minimum dose is 50 mg. However, because similar inocula were used in both the cattle and macaque studies,[6] a tentative comparison can be made between the efficiency of oral infection in cattle and that in primates. On this basis, a factor of 7–20 could be considered as the range of magnitude of a bovine-to-primate species barrier for oral BSE infection (70 primates infected compared with 490 or 1400 cows, with a similar dose).

Elimination from the human food chain of CNS tissues from cows with clinical BSE is estimated to have reduced the risk of human exposure to the disease by about 90%.[7] Risk was further reduced in continental Europe by systematic screening for the diagnostic presence of PrP^{res} in the brainstem of all cattle older than 30 months, and in the UK by the total interdiction of cows older than 30 months. In an oral exposure study to assess the pathogenesis of BSE in cattle, in which the same European Union-evaluated test as we used in the present study was applied to CNS tissues, some preclinical cases of the disease were diagnosed.[8]

Using the same test, pooled brainstem from cows with clinical BSE has yielded a endpoint titre of PrP^{res} corresponding to a 1-in-300 to 1-in-1000 dilution of positive brainstem.[6,9] If people were to eat CNS tissues from a cow with preclinical BSE with a concentration of PrP^{res} just below the test detection limit of 1 in 300, they would need to ingest at least 1·5 kg to reach the degree of exposure equivalent to that in the 5 g of brain used for oral transmission to the macaque in the present study. If the oral ID_{50} for man was one log below this dose (ie, similar to that in cattle, and not accounting for any species barrier between cattle and man; see table), 150 g of CNS tissue that tested falsely negative could represent an infective dose. Because use of cattle brain and spinal cord for human consumption is prohibited, and in view of the existing mechanically recovered meat regulations, a person would be very unlikely to ingest this amount of cattle CNS tissue.

The minimum sensitivity of screening tests to detect 100% of BSE-infected animals has yet to be ascertained. However, our results provide reassurance that BSE screening procedures combined with CNS removal are effective measures to protect the human food chain.

Contributors

J-P Deslys, C Lasmézas, and E Comoy were responsible for design and management of this study. G Wells, S Hawkins, and T Konold were responsible for the pathogenesis study in ruminants. C Lasmézas, C Herzog, and N Lescoutra-Etchegaray were in charge of the primate experiments. F Auvré undertook the biochemical analyses. N Salès was responsible for the immunohistochemical analyses, which were done by E Correia. C Lasmézas, E Comoy, F Mouthon, G Wells, P Brown, and J-P Deslys drafted the manuscript.

Conflict of interest statement

Commissariat à l'Energie Atomique owns a patent covering the BSE diagnostic test commercialised by Bio-Rad. All authors had full access to all data and had responsibility to submit for publication. The funding sources had no role in the collection, analysis, and interpretation of data, writing of the report, or decision to submit the paper for publication.

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References

1 Anderson RM, Donnelly CA, Ferguson NM, et al. Transmission dynamics and epidemiology of BSE in British cattle. *Nature* 1996; 382: 779–88.

2 Lasmézas CI, Deslys JP, Demaimay R, et al. BSE transmission to macaques. *Nature* 1996; 381: 743–44.

3 Lasmézas CI, Fournier JG, Nouvel V, et al. Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health. *Proc Natl Acad Sci USA* 2001; 98: 4142–47.

4 Herzog C, Salès N, Etchegaray N, et al. Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. *Lancet* 2004; 363: 422–28.

5 Klitzman RL, Alpers MP, Gajdusek DC. The natural incubation period of kuru and the episodes of transmission in three clusters of patients. *Neuroepidemiol* 1984; 3: 3–20.

6 Deslys JP, Comoy E, Hawkins S, et al. Screening slaughtered cattle for BSE. *Nature* 2001; 409: 476–78.

7 European Commission. Opinion of the Scientific Steering Committee on the Human Exposure Risk via food with respect to BSE. Adopted on 10 December 1999. http://europa.eu.int./comm/food/fs/sc/ssc/out67_en.pdf (accessed Jan 17, 2004).

8 Grassi J, Comoy E, Simon S, et al. Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue. *Vet Rec* 2001; 149: 577–82.

9 Moynagh J, Schimmel H. Tests for BSE evaluated. Bovine spongiform encephalopathy. *Nature* 1999; 400: 105.