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Safety of milk and milk derivatives in relation to BSE: the lactoferrin example

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Abstract

Bovine Spongiform Encephalopathy (BSE) belongs to Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases. BSE is a feed borne infection of cattle. Epidemiological and laboratory data suggest that the BSE infectious agent is responsible for the variant form of Creutzfeldt-Jakob Disease (vCJD) and that the oral route is the most plausible way of infection. Therefore there is concern that the BSE agent can be transmitted to humans by biological materials (i.e. meat products, blood, milk) from susceptible BSE animal species (mostly cows but possibly, sheep and goats). Lactoferrin (LF) can be produced by purification from large volumes of cow's milk or whey. Therefore, a potential BSE risk for milk and milk products needs to be evaluated by risk assessment. The Committee for proprietary Medicinal Products - CPMP of the European Commission and the WHO have categorized risk tissues from TSE susceptible ruminant species in different classes in relation to the BSE risk for medicinal products. Milk, colostrum, and tissues of the mammary gland have been classified in the category of no detectable infectivity. A secondary contamination of milk can be virtually excluded (i.e. milk is taken from living animals). In the light of current scientific knowledge and irrespective of the geographical origin, milk and milk derivatives (e.g. lactoferrin, lactose) are unlikely to present any risk of TSE contamination provided that milk is sourced from healthy animals in the same conditions as milk collected from human consumption. So the risk of milk and milk derivatives in relation to BSE is negligible.

Introduction

Lactoferrin (LF) is an iron-binding basic glycoprotein (MW $\cong$ 80 kDa, pI = 8–9) with a natural antimicrobial and anti-inflammatory activity. LF is a food ingredient commonly used in a wide range of commercial products (i.e. infant and sports formulas, veterinary and feed specialities and personal care products) and can be produced both by recombinant technology and by purification from large volumes of cow's milk or whey (Vorland 1999). Many methods are available for isolating LF from milk. Among these membrane separation and column chromatography are the two principal methodologies. In the production process, no other materials from animal origin are used. There is concern that Bovine Spongiform Encephalopathy (BSE) infectious agent can be transmitted to humans by biological materials (i.e. meat products, blood, and milk) from susceptible BSE animal species (mostly cows but possibly, sheep and goats). Therefore, a potential BSE risk for milk and milk derivatives needs to be evaluated by risk assessment. BSE belongs to Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases. TSEs are fatal neurodegenerative disorders of the central nervous system (CNS) affecting animals (e.g. scrapie of sheep and goats) and humans (e.g. -variant form of Creutzfeldt Jakob Disease-vCJD). Clinical signs of these diseases take months, years or even decades to develop (Collinge 2001). The infectious agent responsible of these affections is unknown and it is apparently devoid of nucleic acid. The accumulation of an amyloid protein named PrPsc -sc is for scrapie- is the hallmark of the agent replication. According to the Prion theory self replicating PrPsc is the sole component of the infectious particle (Prusiner 1998). The conversion of PrPc -c

is for cellular- into the detergent insoluble, partially protease resistant and infectious isoform PrPsc is the key event in the pathogenesis of TSEs. PrPc and PrPsc are identical in aminoacid sequence but differ only in their conformational structure. Spectroscopic studies reveal that PrPc has a high alpha-helical content whereas PrPsc is rich in beta-sheets. In the laboratory diagnosis, PrPsc is the surrogate marker for the detection of infectivity (Ingrosso *et al.* 2002). Prions do not induce in the host any detectable immune response probably because they are 'self proteins'. Moreover they are extremely resistant to most of the chemical and physical procedures that inactivate conventional viruses. BSE is a feed borne infection of cattle described for the first time in the UK in 1986. From the first cases the epidemic spread to infect over 182.000 bovines and the epidemic peaked in 1992. The number of cases started to decline later that year and this decline continues now sharply (Bradley 2002, Smith 2003). The common practice of recycling animal protein as an ingredient of animal feed, introduced as inexpensive way to boost milk production and increase weight gain has been the cause of BSE and the ban on feeding meat and bone meal to ruminants has been the solution for the epidemic. The priority was and is to keep the BSE agent out of the food chain absolutely. It remains possible that other routes of transmission - other than oral- may occur infrequently, in particular maternal transmission from dam to calf. BSE cases have been detected in several countries outside the UK in all Europe (the total number is less than three thousand) also in USA, Canada and Japan. There is experimental evidence (i.e. epidemiological and biological) that shows that vCJD described for the first time in 1996 in UK is caused by the BSE infectious agent (Will *et al.* 1996). Identification of vCJD was based on novel neuropathological and clinical features of a series of ten young patients (i.e. vCJD patients are usually younger than CJD patients: median age at death 28 years). It seems very probable that vCJD results from ingestion of BSE infected meet products. In vCJD - differently from the other CJD forms - PrPsc is widespread in lymphoid tissues (i.e. tonsil, spleen, lymph node and appendix) (Bruce *et al.* 2001). Up to June 2003 142 individuals have been identified with a probable or confirmed vCJD diagnosis. We don't know how many people are incubating the disease. In more detail, 17 people died in UK from vCJD in 2002, compared with 20 in 2001 and 28 in 2000. The previously increasing trend is so slowing down as yet resulting from preliminary data for the in course year. These findings are reassuring but are not possible to exclude that mortality may increase again in the future (Andrews *et al.* 2003). Oral route is the most plausible way of vCJD infection. One gram of brain tissue from a BSE affected bovine can transmit the disease to another bovine by oral route. However we don't know the amount of BSE infectivity necessary to kill a man. With the exception of CNS only in distal ileum of orally challenged bovines has been detected infectivity. Prion diseases can have an infectious or hereditary origin, or can arise spontaneously (Clarke 2001). Infectious forms of human TSEs are Kuru, iatrogenic CJD and vCJD. Kuru is an epidemic of the middle of the last century of Fore people, Papua, New Guinea, related to cannibalistic rituals in which only women and children ate brain and offal of died relatives. Several thousand cases of the disease occurred during a period of several decades. No individual born after 1960 has ever-developed Kuru and disease disappeared in the 1990s (Goldfarb 2000). The milk of mothers suffering from Kuru was apparently not infectious, as shown by the fact that of the 450 Kuru affected cases that occurred among breast-feeding mothers, none of their children developed the disease. And yet the intracerebral injection (0.2 ml) of pooled milk from infected females ($n = 3$) into subhuman primates (i.e. chimpanzees, about one year of age) did not transmit the disease (Gibbs *et al.* 1972). Iatrogenic CJD is instead the accidental result of medical procedures, such as contaminated neurosurgical instruments, cadaveric dura mater graft or therapy with cadaveric pituitary-derived hormones. vCJD is the variant form of Creutzfeldt Jakob disease likely related to the consumption of BSE infected meet products. Noteworthy is that clinical and neuropathological features of vCJD are reminiscent of Kuru. So due to the verified possibility for the BSE infectious agent to cross the species barrier - i.e. bovine versus human - there is great concern that the BSE agent can also be transmitted to humans by biological materials - directly or indirectly by cross-contamination - derived from susceptible BSE animal species (mostly bovines but possibly, sheep and goats), utilized in the manufacture of cosmetic and medicinal products. To minimize the risk of transmission of TSEs to humans by medicinal products is necessary to conduct a risk assessment. It is mandatory the regulatory compliance of a generic medicinal product with the current CPMP/CVMP Note for Guidance (NfG) on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (TSE Guideline).

The TSE Guideline has been given force of law by virtue of the Commission Directive 1999/82/EC (http://www.emea.eu.int/pdfs/vet/regaffair/041001en.pdf). TSE Guideline states that reduction of risk is based on a multifactorial approach. So it is necessary to evaluate *source of animals* (i.e. geographical origin of animals according OIE or SSC classification, age), *nature of animal tissue used in manufacture and production process* (including the risk of cross contamination, traceability and auditing system). Materials covered by the TSE guideline are active substance, excipients, raw or source materials and reagents. Careful selection of source animal materials is the most important criterion for the safety of medicinal products. The most satisfactory source of materials is from countries that have not reported indigenous cases of BSE and have a compulsory BSE notification system (i.e. compulsory clinical and laboratory verification of suspected cases and an active surveillance program). The potential BSE risk for milk and milk products needs to be evaluated by risk assessment. The Committee for Proprietary Medicinal Products (CPMP) of the European Commission and the WHO in relation to the BSE risk for medicinal products have categorised risk tissues from TSE susceptible ruminant species in different classes. Colostrum, milk and tissues of the mammary gland have been classified in the category of no detectable infectivity. The demonstration of infectivity is usually by the inoculation of suspect tissue into target species or laboratory animals (i.e. bioassay). On the basis of epidemiological and experimental evidence it seems possible to exclude BSE transfer via milk to the calves (in absence of evident species barrier). Moreover milk from BSE affected cows given to mice ($n = 275$) by drinking (i.e. average consumption of 300 ml per mouse for a total of 40 days) or combined intracerebral (0.02 ml) and intraperitoneal (0.1 ml) routes produced no clinical disease and no neuropathological lesions after a period of observation of about two years (Taylor *et al.* 1995). Although these results are reassuring, the decrease in transmissibility to mice due to the bovine/murine 'species barrier' is not well known and may differ from that between bovines and humans. Data from a suckle herd study (started in 1994) in the UK further support the conclusions concerning the safety of milk. No BSE cases out of offspring born to BSE-infected cows have occurred (Wilesmith 1997). Further experimental work is in progress to verify the absence of even extremely low level of infectivity in milk taken from BSE affected cows (i.e. to take account of possible infrequent or irregularly distributed packets of infectivity in milk) (Domingo 2002). There is evidence from other animal (i.e. scrapie) and human spongiform encephalopathies (i.e. CJD, Kuru) to suggest that milk do not transmit these diseases (Haltia *et al.* 1979, van Duijn *et al.* 1998, E.C. 2001).

In the light of current scientific knowledge and irrespective of geographical origin of animals milk is unlikely to present any risk of TSE. Therefore - as reported in NfG - 'milk and materials derived only from milk are excluded from the scope of the guideline provided the following two conditions are satisfied: milk is sourced from healthy animals in the same conditions as milk collected for human consumption; the milk derivatives are prepared without the use of other ruminant materials'. Derivatives of milk from ruminants prepared with the use of other ruminant materials are not excluded from the scope of NfG, because of the use of these other ruminant materials. Milk and milk products, even in countries with a high incidence of BSE, are therefore considered safe. In conclusion for milk and milk derivatives (e.g. lactoferrin, lactose) there is a negligible BSE risk.

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