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Review

Iatrogenic prion diseases in humans: an update

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A B S T R A C T

Although Creutzfeldt–Jakob disease (CJD) was first identified in 1920, prevention of transmission raised particular concern all over the world when a new variant of the disease was first described in 1996. There is good evidence of iatrogenic transmission of this new variant among human beings through blood, blood components, tissues and growth hormone. Furthermore, four cases of iatrogenic transmission of CJD through fertility treatment with human pituitary-derived gonadotrophins have been reported.

It is important to distinguish the categories of infectivity and categories of risk, which require consideration not only of the level of infectivity of a given tissue or fluid, but also the amount of tissue/fluid to which a person is exposed, the duration of exposure and the route by which infection is transmitted.

The potential presence and infectivity of prion proteins in human urinary gonadotrophin preparations is a matter of debate. Differences in the sensitivity of bioassay methods are of paramount importance when considering the infectivity of a tissue. Some new methods might detect small amounts of agent in some tissues currently thought to be free of infectivity.

No cases of human prion disease due to the use of urinary gonadotrophins have been recognized to date. However, the detection of prions in the urine of experimental animals and in some urine-based preparations, and the young age of fertility drug recipients, require the application of the precautionary principle to urinary preparations.

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1. Introduction

The year 2012 marks 25 years from the first identified bovine spongiform encephalopathy (BSE) in cows, and 15 years from the first description of variant Creutzfeldt–Jakob disease (vCJD) in humans, being both different forms of transmissible spongiform encephalopathy (TSE) [1].

Concerns about potential infectivity of human biologics and biotherapeutics arise from time to time. Risk assessments provide a basis for examining the adequacy of different measures to minimize the risks to humans from human-derived materials and provide a framework for developing regulatory health actions. As new technologies, such as better testing for prions, are developed, however, risk assessments can be further refined and risk-mitigating measures will unavoidably be revised. Until more is known about possible alternative routes of vCJD transmission we should "hope for the best and protect against the worst" [2]. Even if the relative risk is low, the population attributable risk may be high [3].

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In this field, the potential presence and infectivity of prion proteins in human urinary gonadotrophin preparations is a matter of debate. The detection of prions in the urine of experimental animals and in some urine-based preparations, and the young age of fertility drug recipients, requires the application of the precautionary principle for urinary derived preparations. This precautionary principle suggests that when the potential risk to public health is substantial there is no case for sitting back to wait for indisputable evidence.

In the 1990s, according to an editorial by Wickham [4], bovine pancreas was the source of bovine insulin, and gut and lung (also of bovine origin) were the sources of some heparins. Theses sources were considered at that time of low or no detectable infectivity concerning TSE. Furthermore, in statements issued by the manufacturers of bovine insulins available in Britain in 1996, the risk to patients was assessed as negligible. Nevertheless, nowadays production of both heparin and insulin is based on recombinant technology. Although no case of transmission of TSE has been documented, a rational precautionary principle was followed to change the source of these hormones.

Thousands of donors are required for production of urine-derived preparations due to the huge increase in demand for fertility products. Even assuming adequate management and tracing, it has been demonstrated that prionuria may exist before the onset of clinically detectable disease. Moreover, the demonstration that not all methods used are able to detect prion proteins remains a cause of concern [5,6].

2. What is a prion disease?

Prion diseases or TSE include different fatal neurologic diseases. BSE in cattle; scrapie in sheeps and goats; and CJD, Gerstmann–Sträusler–Scheinker syndrome, and kuru in humans [6,7].

Genetic forms (gTSE), classified as gCJD, fatal familial insomnia (FFI) or Gerstmann–Sträuler–Scheinker syndrome (GSS) are linked to mutations in the prion protein gene (PRNP gene). Sporadic CJD (sCJD) of unknown etiology and three acquired forms have also been described. The latter include the iatrogenic disease (iCJD), mainly linked to dura mater implants or treatments with hormones of human origin (growth hormone); vCJD associated with dietary exposure to BSE as well as being transmitted by blood transfusion, and kuru, the form attributed to cannibalism among the Fore people in New Guinea [8,9].

New variant CJD (vCJD) was first described in 1996. This new disease is believed to be caused by the same abnormal prion protein (PrP^{Sc}) that causes BSE and is thought to result from eating contaminated beef products [10].

All forms of prion diseases are associated with the accumulation in the central nervous system of an abnormal and protease-resistant form of prion protein known as scrapie protein (PrP^{Sc}) that is derived from its normal counterpart (PrP^{C}) [10]. This misfolded prion protein is infectious and has been identified in humans and in a variety of animal species [11–16].

3. What is a prion?

Prions are pathogenic forms of proteins naturally produced by nerve cells and other cells. The normal protein isoform is known as prion protein cellular (PrP^{C}) [10,17]. The change of prion protein may occur in three different ways. In sporadic disease, prion protein changes to the insoluble form spontaneously. Sporadic CJD accounts for most cases of CJD. In familial vCJD, an abnormality in the gene coding for prion protein produces a protein that is unstable and changes into the abnormal protein. Prion diseases can also be experimentally transmitted by different routes [1].

The prion protein (PrP^{C}) is expressed by a host gene predominantly in brain tissue and detected at low levels in other types of tissues [7,18]. It is a membrane-bound sialo-glycolipoprotein with a glycophospatidylinositol moiety, associated with transmembrane-signaling functions [7]. PrP^{C} has been found in various types of cells and tissues including the central nervous system, myoblasts, vascular endothelial cells, reproductive tissues and hemopoietic cells [16,19–22]. The physiological function of PrP^{C} is unknown. Previous studies have shown that PrP^{C} has different cellular and molecular activities including protection of cells against apoptotic and oxidative stress, transmembrane signaling, formation and maintenance of synapses, adhesion to extracellular matrix as well as activation, differentiation and maturation of dendritic and thymocyte cells [23,24].

PrP^{Sc} is a misfolded form of its normal counterpart PrP^{C}. The protein sequences of PrP^{C} and PrP^{Sc} are identical, but the two isoforms differ in physicochemical properties [5]. PrP^{Sc} is rich in beta sheets whereas PrP^{C} contains abundant alpha helices [25]. PrP^{Sc} is devoid of nucleic acids [26] and is known to deposit and aggregate in the brain [25] leading to loss of neurons [16]. The normal PrP^{C} isoform exists as a soluble dominantly α-helical monomer and is almost completely degraded by a proteolytic enzyme such as proteinase K (PK) [5]. In contrast, PrP^{Sc} has a β-sheet-rich conformation and when subjected to PK, a large C-terminal 27–30 kDa segment of PrP^{Sc} resists further degradation allowing detection by Western blotting [5,7]. The unusually stable conformation of PrP^{Sc} is believed to be responsible of its resistance to conventional physical and chemical decontamination and inactivation procedures [16].

4. How could a prion based disease be transmitted?

The BSE crisis years ago demonstrated that prion diseases can cross the barrier into humans. More than 200 people contracted the new vCJD caused by oral exposure to BSE prions.

Concerns exist, however, about the potential transmission of subclinical vCJD by blood products [27] or the suggested potential risk factor of surgery-connected blood transfusion with more than 10 years latency in sCJD [28]. So the so-called sCJD could be in some cases an unidentified case of iCJD linked to some medical procedures, either surgical or pharmacological. There is a good evidence of iatrogenic transmission of the BSE agent and the vCJD agent among human beings through blood, blood components, tissues and growth hormone [16]. In December 2003 the UK health secretary, John Reid, told the British Parliament of the death of the first probable victim of vCJD after being transfused blood in 1996 from a donor who had been incubating vCJD [2]. This was the first probable case of transmission of vCJD following blood transfusion. Furthermore, 194 individuals were infected through intramuscular administration of pituitary growth hormone [29]. Case reports of CJD have been reported not only after treatment with human growth hormone but also after neurosurgical procedures and corneal transplantation [8]. Finally, four cases of iatrogenic transmission of CJD through fertility treatment with human pituitary-derived gonadotrophins were reported in Australia [30]. Japanese workers have found infectious material in placental and umbilical cord blood [9,22]. No cases of human prion disease secondary to the use of urinary gonadotrophins have been reported to date, but taking into account that iatrogenic transmission may occur via organ transplantation, surgical instruments and human-derived therapeutic products, new outbreaks of vCJD might occur [6].

Influencing the interpretation of epidemiologic studies are certain genetic factors affecting the host. Mutations and polymorphisms in the gene that encodes the prion protein can significantly alter that the disease will develop. There is a polymorphism at codon 129 of the gene consisting of a methionine/methionine

(MM), valine/valine (VV) or methionine/valine (MV) combination. In human populations it appears that homozygosity predicts either increased susceptibility or a shortened incubation period [31].

Most cases of sCJD, and until recently all cases of vCJD, have been homozygous for methionine at codon 129 of the prion protein gene (the MM genotype found in around 40% of general population). There was a case, however, in which someone with vCJD infection, but not the illness, linked to a blood transmission had the MV genotype (found in 50% of general population). Furthermore, the identification of prion proteins in two samples of appendix tissue of two homozygous valine genotype (VV) individuals in 2006 [32] may rekindle fears that a larger epidemic is an ongoing threat. This suggests that a wider group of people may be at risk, perhaps with different incubation characteristics [1,33–35].

No cases of human prion disease due to the use of urinary gonadotrophins have been recognized to date. Epidemiological data alone, however, do not seem a sufficient basis to confirm the safety of such products. In fact, for vCJD the epidemiological experience is too limited, given the long incubation periods and the low incidence rates, to reach conclusions on whether or not vCJD could be transmitted by urine-derived products [8]. A major problem in the production of human-derived gonadotrophins is the need for large quantities from postmenopausal women. When urinary gonadotrophins were first produced, there were three urine-collecting centers (in the Netherlands, Spain and Italy) with about 600 donors who produced 120,000 l of urine annually. In the 1990s, it was estimated that 120 million l of urine were needed to satisfy the worldwide demand of FSH, requiring the services of 600,000 donors. Furthermore, each year millions of women worldwide are prescribed urine-derived preparations for fertility.

The report that stirred up the debate on the potential infectivity of urine was the finding of a protease-resistant isoform of PrP in the urine of scrapie-infected hamsters, BSE-infected cattle and humans suffering from CJD [36].

It is now clear that infectious prions are excreted into the urine in renal disease states. Aguzzi et al. reported in 2005 the presence of fully infectious prion particles in the urine of scrapie mice with co-existing renal inflammation [37]. The reported levels of infectivity in urine seem to be lower than in blood but are in comparable ranges. Experiments in scrapie-infected hamsters [38] and in lymphocytic nephritic mice [37] found infectivity titers. Recent findings, however, suggest that urinary prion excretion may occur without renal pathology [38,39]. Prionuria was demonstrated by inducing a prion disease in naïve hamsters through inoculation of scrapie urine [38]. These findings may change opinion about the safety of urinary products. As an example, blood products were considered safe, based on the lack of detectable prions in vCJD using an inadequately sensitive mouse assay [40].

It is important to distinguish the categories of infectivity and categories of risk, which require consideration not only of the level of infectivity of a given tissue/fluid, but also the amount of tissue/fluid to which a person is exposed, the duration of exposure and the route by which infection is transmitted. The 2010 updated World Health Organization report moved urine from the category of "tissues with no detectable infectivity" to the category of "low infectivity tissue". Blood is included in this latter category [41]. Unlike blood products, urine-derived preparations are not essential in medical treatments, given the fact that recombinant products do exist.

5. Have prion proteins been identified in urine derived products? Is it possible that a given method were unable to detect the presence of a prion?

Aguzzi et al. reported the presence of fully infectious prion particles in the urine of scrapie mice with co-existing renal inflammation. PrP^{TSE} levels were below the limits of detection by immunoblotting, but intracerebral inoculation of concentrated urine did transmit scrapie in host mice. This implies that these authors failed to detect PrP^{TSE} in urine subsequently proven to be infectious [37]. These findings underline the importance of reliable detection methods to prevent the transmission of prion diseases, given that the number of asymptomatic infected individuals remains unknown and the perceived incubation period time for human prion diseases could be decades [16].

Differences in the sensitivity of bioassay methods are of paramount importance when considering the infectivity of a tissue. Some new methods might detect small amounts of agent in some tissues currently thought to be free of infectivity. In 2007 Muruyama et al. [42] published the detection of PrP^{Sc} in urine from TSE infected animals using PMCA. Homogenate of brains from hamsters infected with prion strain Sc237 were injected intracerebrally or administered orally. Following oral administration, PrP^{Sc} was present in plasma samples during the symptomatic phase. Also, PrP^{Sc} was excreted in urine for a few days after oral administration and at the terminal stage of the disease. After intracerebral inoculation, urinary PrP^{Sc} was detected both in the symptomatic and terminal stages of the disease.

Dabaghian et al. [43] reported the results of a western blotting analysis of concentrated urine samples of CJD affected individuals. By using anti-PrP-antibodies, they were able to detect PK resistant bands in several samples of urine from sCJD patients, which did not appear in healthy control samples.

The inefficiency of some methods to detect urinary PrP while being able to detect brain PrP may rely on the fact that the former is a truncated form of the latter. Narang et al. [5] first described a method capable of detecting PrP in urine by using the anti-C antibody. They showed that PrP was detectable in less than 1 ml of urine in all healthy individuals examined. An earlier report by Shaked et al. [36] showed that the use of 3F4 mAb was able to detect urinary PrP, but their findings were challenged by others [44]. A non-specific cross-reactivity to other contaminating proteins was reported as the reason of PrP urinary detection claimed by Shaked et al. [36]. The detection of PrP^{C} using the C antibody is not due to contamination with bacterial outer membrane proteins or endogenous IgG as observed by some authors when using 3F4 mAb. Because 3F4 is well known for its ability to recognize brain PrP [45], its failure to detect urinary PrP^{C} suggests that the 3F4 epitope located in the N-terminal region is absent in urinary PrP^{C}. This conclusion of Narang et al. [5] was consistent with the fact that the anti-C antibody against the C-terminus or PrP readily detects urinary PrP^{C}. They demonstrated that the size of urinary PrP^{C} shifted from 28 to 30 kDa of brain PrP^{C} to 18 KDa. The truncated form of PrP^{C} in human urine is likely the result of proteolytic processing that occurred in vivo before the excretion, a normal metabolic event previously shown in human neuroblastoma cells and the brain [46], possibly by a calpain-dependent proteolytic process [47].

The data from Narang et al. [5] suggest the potential of their method for detection of urinary PrP^{Sc} in TSEs which might so far evaded detection due to its extremely low concentration. These findings not only highlighted the unique structural properties of urinary PrP that are apparently different from brain PrP, but also helped clarify the former controversy involving attempts to detect urinary PrP using 3F4 [36,44].

A recently published paper demonstrated the presence of prion proteins (among other non-gonadotrophin proteins) in urine-derived fertility products containing hCG, hMG and hMG-HP by using electrophoresis and mass spectrometry methods [6]. In this paper, prion protein was detected in two u-hCG preparations among the 33 different non-gonadotrophin proteins identified as contaminants in these pharmaceutical products. It was also

reported that the two hMG tested were less pure than hMG-HP, containing a large number of contaminant proteins. The use of a sensitive MS-based quantification technique, however, demonstrated that all urine-derived preparations tested, produced by different manufacturers, showed the presence of human prion proteins in varying amounts. When experiments were performed using one vial per product, prion proteins were not detectable in HMG-HP preparations, but when the experiment was repeated with 6 pooled vials, prion presence was confirmed [6]. This experiment confirms the paramount importance of the cumulative doses administered in terms of potential prion protein transmission. Prion proteins could also be detected in HMG preparations by using two-dimensional gel electrophoresis and mass spectrometry [48].

These findings are very important in two ways. First of all, the purification processes for different urine-derived preparations are unable to remove prion proteins from the source material. Secondly, the process controls employed in the production of these preparations did not permit the identification of this contaminant.

The presence of a prion protein in theses preparations does not necessarily mean that a risk of a TSE transmission exists, but in neither paper could prion proteins be identified in the recombinant preparations assessed [6,48].

6. In which countries/regions have prion diseases been detected?

Prion diseases are widespread in animals around the world, including scrapie in sheep, and are under-tested in some countries. BSE has been detected, however, in most European countries, USA, Canada and Japan [1]. Although the UK remains the country at greatest risk from past exposure to the BSE agent, vCJD has also been identified in an increasing number of other countries in recent years. In most of those countries only single cases have been identified. Furthermore, some countries might have unrecognized cases of vCJD. It would be therefore prudent for national authorities to prepare, in advance, plans to reduce the risk of secondary transmission by blood components, plasma-derived products, and other human derivates even if BSE and vCJD have been not recognized in their country.

The estimation that 237 people per million population could be silent carriers of vCJD [49] suggest that there is the possibility that asymptomatic citizens, incubating the disease for decades, may be distributed in many countries [6,38,40,43].

A recently published review of guidelines for prevention of CJD transmission [8] confirms that there is a great degree of variation among the measures proposed control of CJD transmission in the European Union. The definition of a person at risk of CJD is far from clear. Furthermore, because of long latencies in humans, identification of new risk factors would have major implications for new strategies to minimize the risks inherent in treatments with blood products or other body fluid-based preparations. The expected benefits may be apparent only after a considerable delay, and the lack of alternatives (for blood products) requires careful weighing and tailoring to different circumstances. Unlike blood-derived products, there exist alternative preparations in the case of urine-based preparations.

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