Consensus statement on the bio-safety of urinary-derived gonadotrophins with respect to Creutzfeldt–Jakob disease

Adam H. Balen1 and Ib Bo Lumholtz2,3*

1Professor of Reproductive Medicine and Surgery, Leeds General Infirmary, Leeds LS2 9NS, UK and 2BL Consult ApS, Ingersvej 4, DK-2920 Charlottenlund, Denmark
3To whom correspondence should be addressed. E-mail: Lumholtz@mail.com

Human transmissible spongiform encephalopathies (TSE) encompass a group of rare neurodegenerative diseases. In April 2004, a group of international experts and regulators met in Buenos Aires, Argentina, to review the safety and to reach consensus on the use of urinary-derived gonadotrophins with respect to TSE. Iatrogenic transmission of Creutzfeldt–Jakob Disease (CJD) from pituitary-derived gonadotrophins has been reported, no infectivity in urine has been demonstrated, and no definite cases of transmission via urine have been reported. It is currently not possible to monitor donor urine or finished product for the presence of prions. Therefore the assessment of risk has to be based on the likelihood of infection in urine, the source of the urine, and the capacity of the manufacturing process to remove any adventitious infection. Urine for the production of medicinal products should be obtained from sources that minimize the possible presence of materials derived from subjects suffering from human TSE. As no strong evidence for TSE infectivity in urine exists, it can be concluded that the risk of disease-generating prions and TSE infectivity being present in donor urine is low. Current evidence indicates that, with respect to the risk of TSE infection, urinary-derived gonadotrophins appear to be safe.

Key words: Creutzfeldt–Jakob disease (CJD)/ovulation induction/prion/transmissible spongiform encephalopathies (TSE)/urinary-derived gonadotrophin

Introduction

Urinary medicinal products have been utilized for many years. Their benefit is that they are a source of natural human proteins which can be prepared for therapeutic administration by partitioning and filtration processes, rather than by synthetic routes. The principal group of products produced in this way is the gonadotrophins, which are present at high concentrations in the urine of post-menopausal women (LH and FSH) or pregnant women (HCG).

All these products, prepared by several pharmaceutical manufacturers, have a long history of safe use. However, in recent years concerns have been expressed that these products could potentially be a source of viral transmission, and more recently a potential source of transmissible spongiform encephalopathy (TSE) infectivity. In both cases, the urinary products share these concerns with human blood and plasma products. In the case of viruses, it has been possible to demonstrate that for both blood and urine viruses are removed by the same fractionation procedure as is used for the purification of the products (Reichl et al., 2002). The use of process validation for removal of viruses has become a regulatory standard for human-derived products (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline Q5A, 1998). Likewise, it has been shown that a similar approach can be used for TSE infectivity potentially present in the source of human and animal blood and plasma products, and again regulatory guidelines are available covering the validation procedures (European Medicines Agency, 2004). Until recently there had not been a concern that TSE infectivity could be an issue for urinary products, and the World Health Organization (2003) listed urine as a Category IC ‘tissue’, that is tissue with no detected TSE infectivity. The ongoing variant Creutzfeldt–Jakob Disease (vCJD) crisis in the UK, and an article in the scientific literature suggesting...
that abnormal prion proteins might be present in the urine of TSE-infected animals and humans (Shaked et al., 2001), resulting in an initiative to hold a conference to perform an in-depth analysis of the bio-safety of urinary products. This conference took place at The FLENI Foundation, Buenos Aires, Argentina, April 26–27, 2004, and was attended by international experts in the field of prions and TSE, together with their counterparts from the regulatory authorities of the USA, Europe, Japan and Australia. Ferring Pharmaceuticals took the initiative to convene the conference. The full list of panelists and presenters is appended at the end of the paper. The selection of the panelists was done by an international board, comprising Professors Pocchiarì, Gambetti and Will, who advised on the scientists with expertise in prion disease. Dr Lumholtz invited regulators and Professor Taratuto invited the participants from South America. There were four panelists present from the field of reproductive medicine, two from North America and two from Europe (see list of panelists).

The agreed purpose of this meeting was to reach a consensus on the safety of urinary-derived products, and here we present that consensus, as well as providing some information on the background discussions which led to it. The consensus process was led by the chair of the meeting; all panelists and discussants contributed to the consensus statement which was discussed in open forum and subsequently circulated to and ratified by all panelists and presenters who were present at the meeting.

**Transmissible spongiform encephalopathies and prions**

Transmissible spongiform encephalopathies (TSE) are a group of invariably fatal neurodegenerative disorders affecting both humans and animals first identified in England in sheep in the 1730s (See Table I). The disease was called scrapie as the affected animals rub their coat as if it itches. The human form of the disease, Creutzfeldt–Jakob disease (CJD), was first described in the early 1920s by two Germans (Creutzfeldt, 1920; Jakob, 1921). Human-to-human transmission has occurred either by ritualistic cannibalism (Kuru) or through infected cornea and dura mater grafts, neurosurgical devices, and human-derived medicinal products (specifically, human pituitary growth hormone and human pituitary gonadotrophins) (iatrogenic CJD), and contaminated products (specifically, human pituitary growth hormone and mater grafts, neurosurgical devices, and human-derived medicinal products (specifically, human pituitary growth hormone and human pituitary gonadotrophins) (iatrogenic CJD). The infectious nature of the diseases was later documented by transmitting Kuru and sporadic CJD to chimpanzees and spider monkeys through intracerebral inoculation (Gajdusek et al., 1966; Gajdusek et al., 1998; Gibbs et al., 1968).

Creutzfeldt–Jakob disease (CJD) is a rare condition with an incidence of 1–2 per 10^5. The appearance of a variant form of CJD (vCJD) that is presumed to result from the ingestion of tainted beef products from cattle infected with the bovine spongiform encephalopathy (BSE) agent has increased the profile of TSE as a risk to human health. Both vCJD and the sporadically occurring form of the disease (sCJD) have long symptom-free incubation periods, making it impossible to predict how many people in the UK, the rest of Europe, and the rest of the world are incubating the diseases. The first recorded probable case of transmission of vCJD by blood transfusion had been described in humans (Llewelyn et al., 2004) shortly before the conference, and a further case was reported subsequent to the meeting (Peden et al., 2004). Unlike with vCJD, sporadic CJD has never been shown to be transmissible via the blood, for example in blood transfusion patients and haemophiliacs (Wilson et al., 2000).

Bovine spongiform encephalopathy was first recognized in British cattle in 1986 and current evidence suggests that it originated from the use of feed supplements containing meat-and-bone meal (MBM) contaminated with scrapie-infected sheep carcasses. In the UK a major BSE epidemic unfolded in the period 1986 to 2005 with >185 000 confirmed cases of BSE. Smaller outbreaks probably explained by MBM imports from the UK were seen in most other European countries totaling <4000 cases. Since 2002 the BSE epidemic has subsided; however, cases are still identified—also outside Europe, including recent cases in Japan, the USA and Canada. Very few countries are considered ‘BSE-free’, notably Argentina, Australia, Brazil and New Zealand—whether there is a totally BSE-free country is unknown.

The occurrence of BSE, unlike scrapie, has represented a significant risk to public health. Based on epidemiological, biochemical and transmission studies, a causal link has been established between BSE and a novel form of TSE (variant CJD, vCJD) first recognized in the UK in 1996 (Will et al., 1996; Hill et al., 1997; Bruce et al., 1997).

In a typical case of sCJD, the invariably progressive, rapid clinical evolution and the short illness duration (median 4 months), readily distinguish the disease from most other dementing illnesses. It is predominantly a disease of late middle-age with a mean age at death in the late 60s. By contrast,

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<td><strong>BSE</strong></td>
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<td><strong>CJD, sCJD</strong></td>
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<td><strong>PrP, PrP^C</strong></td>
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a typical case of vCJD occurs in adolescents and young adults with a mean age at death in the late 20s, an illness duration that is greater than that for sCJD (median 14 months), and a clinical picture in the early stages of the disease that is dominated by psychiatric symptoms (Spencer et al., 2002).

The prion protein
After several years of controversy it is generally believed that the infectious agents responsible for TSE are self-replicating abnormal, misfolded variants of normal protein isofoms referred to as cellular prions (prion protein cellular, PrP\textsuperscript{c}). The abnormal prion proteins are referred to as PrP\textsuperscript{sc} (scrapie) or PrP\textsuperscript{res} (proteinase resistant), although the possibility remains that PrP\textsuperscript{sc} itself represents a surrogate marker of TSE (Aguzzi and Weismann, 2001).

The PrP\textsuperscript{sc} proteins are normally metabolized by proteases, but after they change conformation they also become proteinase-resistant and infectious, and therefore the abnormal proteins steadily accumulate in cells, particularly those of the CNS where there are the highest concentrations of normal PrP\textsuperscript{c} proteins. This accumulation of abnormal prions eventually leads to the symptoms of TSE (DeArmond et al., 2003). In cases of vCJD, the abnormal prions accumulate in the CNS, following transport through the body primarily via the lymphoid system. In cases of sCJD and iatrogenic CJD there is rarely or never any deposition of infectivity in the lymphoid system. In both vCJD and sCJD there is only minimal accumulation in other tissues and body fluids.

The pathogenesis of TSE is linked to simultaneous expression of normal and abnormal prion protein and seems to be promulgated by a peculiar process of ‘template folding’ that spreads by direct molecule-to-molecule transmission. In this, a PrP\textsuperscript{sc} molecule which has become folded into the abnormal PrP\textsuperscript{sc} shape (‘beta pleat’) is able to impress its shape on neighbouring normal PrP\textsuperscript{c} molecules and cause them to assume the thermodynamically more stable abnormal PrP\textsuperscript{sc} shape and by subsequent aggregation form destructive amyloid-like plaques.

Detection of prions and infectivity
Formation of a pathological prion protein is a unique feature of TSE and its presence represents a diagnostic marker for prion infectivity for which a number of commercial tests based on the immunological detection of PrP\textsuperscript{sc} are available, mainly using western blot or enzyme-linked immunosorbent assay. The current methods perform well for diagnosis of BSE on bovine brain specimens due to the high titre of PrP\textsuperscript{sc}. The current methods, however, do not qualify as screening tests in humans for detecting individual incubating TSE as they have limited sensitivity and as it has been difficult to achieve high specificity with available antibodies—not only to discriminate between the abnormal prion protein and the normal prion protein, but also to distinguish between different strains of prions (Korth et al., 1997).

With increasing sensitivity of new assays it becomes likely that a more widely extraneural distribution of PrP\textsuperscript{sc} in the body than previously realized for sporadic CJD will be detected (Glazel et al., 2003). The ability of the individual tests to discriminate between positive cases and non-cases, and at the same time between different types of human TSE therefore could become more important than the sensitivity of the individual assays. This development would also impact the risk assessment and tissue classification (World Health Organization, 2003). This would have implications for pharmaceuticals that contain, or are manufactured, using bovine- or human-derived materials. Screening of source materials such as blood and urine for the presence of PrP\textsuperscript{sc} is not possible with current methods.

PrP\textsuperscript{sc} is a surrogate marker for prion disease but whether or not PrP\textsuperscript{sc} is present does not represent the final proof of infectivity (Lasmezas, 1997). The current ‘gold standard’ of prion diagnostics therefore is based on bioassays in rodents, chiefly a mouse or hamster bioassay, in which test material is inoculated intracerebrally into the indicator animals and the clinical onset of disease noted. This procedure suffers from inaccuracy and is limited by the requirements for large numbers of experimental animals and an often extended duration.

The development of an in vitro system that uses highly susceptible cloned neural cell lines permissive to mouse scrapie prions (Klohn et al., 2003) might represent a technology that will evolve into a more efficient diagnostic approach for prion infectivity. The method published can be completed in 2 weeks, as compared with 20 weeks in the most rapid mouse bioassay, and may have similar sensitivity.

Prions and TSE infectivity in urine
There is no evidence of blood-related iatrogenic transmission of the infectious agent of prion disease in humans (Cervenakova et al., 2002); however, experimental rodent models have consistently shown that blood and blood components contain infectivity (Kuruda et al., 1983). Transmission of prion disease by blood transfusion has been demonstrated in sheep (Hunter et al., 2002), and probably in humans (Llewelyn et al., 2004; Peden et al., 2004). It is not surprising that, as the kidneys filter the blood, kidney tissue has been shown to contain infectivity; however, even in the presence of infectivity in the kidney and blood, infectivity in the urine has not been confirmed and is considered unlikely (World Health Organization, 2003). This might be explained by the lack of sensitivity and specificity of the assays available but urine is regarded as a tissue with no detected infectivity in contrast to blood that is classified by the World Health Organization (2003) as a lower-infectivity tissue.

A report of a previously unknown abnormal proteinase-resistant prion protein in urine of TSE-infected hamsters, cattle and humans (Shaked et al., 2001) therefore created considerable interest, as it represented the potential for the development of a pre-symptomatic test for prion diseases. However, TSE infectivity was not detected in the urine of the infected hamsters from this study, leading to the conclusion that this abnormal prion (referred to as uPrP\textsuperscript{sc}) was not associated with infectivity. This did not mean, however, that uPrP\textsuperscript{sc} could not be a potential biomarker for prion diseases.

The significance of the findings remains to be determined, but the publication was the main catalyst for the subsequent concern that urine could harbour TSE infectivity, with obvious implications for the safety of urinary-derived pharmaceuticals.
Recently two publications have shed further light on the nature of the protein found in urine. One suggests that the protein might be derived from bacterial contamination (Furukawa et al., 2004) and the other demonstrates that the positive band detected previously was due to cross-reactivity of the anti-mouse IgG antibody used with IgG light chains and possibly heavy chain fragments in urine, and was not PrP (Serban et al., 2004). Head et al. (2005) also failed to confirm the presence of PrPSc in the urine of patients with sporadic, variant and familial forms of CJD. In their study, using the method of Shaked et al. (2001) protease-resistant material in the correct molecular mass range for PrPSc was detected but this signal appeared to derive from cross-reactivity with immunoglobulins (κ and λ light chains) (Head et al., 2005). The group that published the original findings on an abnormal prion protein in urine has published very recently that the proteinase resistant protein detected in urine by their method might represent several proteins, among them light chain immunoglobulin and that whether abnormal prion protein is present in urine is still unclear (Karir-Inbal et al., 2005).

Thus there is currently no published evidence that prion proteins which might be associated with TSE infectivity are present in urine of infected animals and humans. Therefore as no strong evidence for TSE infectivity in urine exists, it can be concluded that the risk of disease-generating prions and TSE infectivity being present in donor urine is low.

Urinary gonadotrophins
Several biological and pharmaceutical products, including recombinant and urinary-derived gonadotrophins, use human or ruminant materials in the manufacturing process or as starting materials. It is difficult currently to assess the risk associated with BSE and variant CJD as the number of BSE-infected herds or BSE-exposed individuals that may be incubating the disease is unknown, as is the virulence of the BSE prion protein in humans. The regulatory authorities have developed policies to obtain reliable information to assess risk and evaluate product safety. It is prudent that manufacturers address the concern that medicinal products that contain or are manufactured using bovine-derived or human-derived materials might spread the TSE infectious agent. There is no evidence to suggest a difference in safety with respect to TSE infectivity between recombinantly derived or urinary-derived gonadotrophins (Balen, 2002; Reichl et al., 2002).

The concern for urinary gonadotrophins is whether prions or TSE infectivity could reach the urine of a donor who has pre-symptomatic sCJD or vCJD. As mentioned previously, there is only limited distribution of infectivity to non-neural tissues. However, infectivity has been shown to enter the blood, although it has not been possible to demonstrate the presence of PrPSc. It has been shown that in human CJD cases there is a low incidence of infectivity in the kidneys (five out of 28 patients), but no infectivity was found in urine (Brown et al., 1994). If infectivity is in the circulation, then it is not surprising that it can also reach the kidneys.

A single report of infectivity in urine from a sCJD case (Tateishi, 1985) has never been confirmed, and is considered improbable (World Health Organization, 2003). It has also never been shown that urine from animal species infected with TSE (e.g. sheep, cattle, monkeys, etc.) is infectious.

After the conference, a case of vCJD was reported in a woman who had received gonadotrophin treatment for infertility, 20 months before the onset of clinical symptoms (Ward et al., 2004). The infertility treatment included both recombinant and urinary gonadotrophins. It was concluded that the development of vCJD was unrelated to the infertility treatment, because the earliest onset of symptoms of various types of iatrogenic CJD after transmission is 4.5 years (Brown et al., 2000). It is most likely that the woman was exposed via diet at an earlier stage of her life.

A considerable problem with TSE is the lack of suitable diagnostic tests which can detect the infection prior to the onset of clinical signs. Many academic and commercial groups are attempting to develop tests using blood or urine, but a sufficiently sensitive and rapid test continues to be elusive. For this reason it is not possible to monitor donor urine or finished product for the presence of prions or infectivity. Therefore the assessment of risk has to be based on the likelihood of infection in urine, the source of the urine, and the capacity of the manufacturing process to remove any adventitious infection.

Sourcing of donor urine
Many years of unevenful use of urinary-derived gonadotrophins vouch for their safety, and regulatory authorities require rigorous screening of donors. The risk of contamination of urine should be achieved by judicious selection of the source. In this respect, it is necessary to consider sCJD and vCJD separately.

The incidence of sCJD worldwide is relatively constant, with one to two cases per million per year in all countries with systematic evaluation. Therefore controlling geographical sourcing has no benefit in minimizing the risk of human TSE contamination of urinary-derived products. However, the risk of transmitting sCJD can be minimized using rigorous donor exclusion criteria, e.g. excluding donors suffering from any type of neurological disease (and also those where any blood relatives have suffered such diseases), and excluding any donors who have received any type of brain or transplant surgery. Donors that have had blood product transfusions from countries with intrinsic cases of vCJD (i.e. Italy, France, The Netherlands and the UK) should also be excluded from donating urine.

With vCJD the best approach is to source from countries where BSE does not occur. The Office International des Epizooties (OIE) monitors the worldwide incidence of BSE, and categorizes countries into those which have reported the disease in indigenous animals, and those that have only found BSE in imported animals (Office International des Epizooties: http://www.oie.int/eng/info/en_statesh.htm). Further, on request, the OIE evaluates countries where BSE has not been reported with a view to conferring the status of ‘free’ or ‘ provisionally free’ from BSE. To date, four countries have received the provisionally free of BSE status: Argentina, Iceland, Singapore and Uruguay. In addition, again on request, an assessment has been performed on various countries by the EC Food Safety Scientific Steering Committee to assess their BSE status (EC Food
Safety Scientific Steering Committee: http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html). A geographical BSE risk (GBR) code is assigned based on the likelihood that domestic cattle are (clinically or pre-clinically) infected with the BSE agent: GBR-I status is ‘highly unlikely’, GBR-II ‘unlikely, but cannot be excluded’, GBR-III ‘likely, but not confirmed’, and GBR-IV ‘confirmed’. The four countries that are provisionally free from BSE in the OIE assessment are also in the GBR-I category, and 15 other countries also have GBR-I status, the majority in South and Central America, but also Australasia and a small number of countries in Africa, Europe and Asia.

In addition to controlling the source country of donors, an additional exclusion criterion is whether a potential donor has lived outside their home country for a prolonged period (>6 months) coinciding with the BSE crisis in the UK (January 1, 1980 to December 31, 1996).

Controlling the donor pool in this way further minimizes the very low risk of TSE infectivity attributable to sCJD or vCJD being present in urine used for the production of gonadotrophins.

Production process validation

The final factor which further minimizes the risk of TSE infectivity being present in urinary gonadotrophins is the purification process itself. It has been demonstrated in several publications that for blood and plasma products the various partitioning and filtration steps used in the production process are capable of reducing or removing any abnormal prions or prion infectivity present in the source material (Foster et al., 2000; Vey et al., 2002). It has therefore become a standard procedure for a scaled-down version of the production process to be validated for its potential to remove a TSE-infective spike. The production process used for urinary gonadotrophins is very similar to that used for blood and plasma products, and therefore it can be assumed that it would be equally effective in removing infectivity if it were present. To date there have been no publications of a validation of a urinary product, but in an article written by Reichl et al. (2002), six separate steps in the purification process of menotrophins were identified as having the potential to remove prions and infectivity.

In response to the concerns of the medical profession and patients, the producers of urinary products are currently performing validations of their purification processes to demonstrate that in the unlikely event that abnormal prions and TSE infectivity are present in the source material, it would be removed during production. As mentioned earlier, there are no sufficiently sensitive assays currently available to measure infectivity in the source material or final product. It is therefore necessary to perform bioassays in mice or hamsters by assessing clinical signs of TSE or material or final product. It is therefore necessary to perform bioassays currently available to measure infectivity in the source material, the potential to remove prions and infectivity.

Summary: consensus from the Buenos Aires Conference

The first consensus conference on the Bio-safety of Urinary Derived Medicinal Products brought together international experts on prions and their colleagues from the regulatory agencies in Australia, Japan, Europe and USA to review the current available data on TSE infectivity in urine. All of the participants took part in a debate with the purpose of arriving at a consensus opinion on the safety of urinary gonadotrophins. The following five points constitute the consensus opinion:

(i) Human TSE encompass a group of rare neurodegenerative diseases. Whereas the majority of cases are sporadic, these diseases may be transmitted to new hosts. The infectious agent appears to be a protein, but the exact nature is not yet established.

(ii) Transmission of human TSE infectivity has been reported via consumption of infected neural tissue, dura mater transplants, corneal transplants, pituitary-derived gonadotrophins and growth hormone, and brain electrodes. A single case of probable transmission via blood transfusion has been reported (plus a further case subsequent to the conference). No infectivity in urine has been demonstrated, and no cases of transmission via urine or urine-derived medicinal products have been reported. Since epidemiological evidence has allowed the identification of cases of iatrogenic transmission of CJD from pituitary-derived gonadotrophins, it could be expected that transmission from urinary-derived gonadotrophins would have been detected if it had occurred.

(iii) Urine for the production of human-derived medicinal products should be obtained from sources that minimize the possible presence of materials derived from subjects suffering from, or at greater risk of, human TSE.

(iv) Although clearance of TSE infectivity is not currently mandatory for urinary-derived medicinal products, the manufacturing process for urinary-derived medicinal products should be scrutinized to identify steps that may result in the removal of the TSE agent.

(v) Whilst further research is needed to resolve the uncertainty as to whether urine in human TSE contains infectivity, current evidence indicates that, with respect to the risk of TSE infection, urinary-derived gonadotrophins appear to be safe.

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European Commission Food Safety Scientific Steering Committee: http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html


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