Plasma prion protein and creatinine levels in dogs with renal deficiency

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SUMMARY
As prion protein acts as a substrate for conversion to the abnormal form associated with infectivity, the factors governing susceptibility to prion diseases might be highlighted by identifying those pathophysiological factors that increase PrPC levels. This study is aimed at characterizing the relationship between impaired kidney function and plasma PrPC concentration in dogs.

In the first experiment, the relationship between plasma creatinine and PrPC concentrations was evaluated in 21 healthy dogs and in 26 dogs with spontaneous renal failure. In a second experiment, the effect of experimental subclinical renal impairment on PrPC in 16 dogs was determined. Plasma PrPC was measured by ELISA and renal function markers were evaluated. Basal plasma prion protein concentrations were three fold higher in dogs with spontaneous renal failure and in dogs with surgically controlled renal alteration than in matched healthy dogs. The correlation between plasma PrPC and creatinine concentrations in dogs with chronic kidney disease was high. Renal dysfunction, by increasing the plasma PrPC level, might be a relevant physiopathological factor of susceptibility to prion diseases.

Keywords: Prion protein, renal dysfunction, creatinine, dog, kidney.

RÉSUMÉ
La protéine prion cellulaire est considérée comme un substrat nécessaire à la formation de la protéine prion anormale, associée au caractère infectieux de la maladie. C’est pourquoi, l’identification de facteurs physiopathologiques susceptibles d’augmenter les niveaux de PrPC est essentielle pour déterminer les facteurs de risque des maladies à prion.

L’objectif de cette étude est de caractériser la relation entre l’altération de la fonction rénale et la concentration de PrPC plasmatique chez le chien.

Dans une première expérience, la relation entre les concentrations plasmatiques de créatinine et de PrPC a été évaluée chez 21 chiens sains et chez 26 chiens présentant une insuffisance rénale spontanée. Dans une seconde expérience, l’effet de l’insuffisance rénale subclinique, induite chirurgicalement, comparativement aux chiens sains. Les concentrations plasmatiques de PrPC ont été déterminées sur 16 chiens. Les concentrations plasmatiques de PrPC ont été mesurées par ELISA et des marqueurs de la fonction rénale ont été évalués.

Les concentrations plasmatiques basales de protéine prion ont été multipliées par trois chez les chiens présentant une insuffisance rénale spontanée et chez les chiens présentant une altération de la fonction rénale contrôlée chirurgicalement, comparativement aux chiens sains. Les concentrations plasmatiques de PrPC ont été corrélées à celles de créatinine chez les chiens présentant une insuffisance rénale chronique. Le dysfonctionnement rénal, en augmentant le niveau de PrPC plasmatique, pourrait constituer un facteur physiopathologique de risque important des maladies à prion.

Mots clés : Protéine prion, insuffisance rénale, créatinine, chien, rein.

Introduction

The key event in prion disease pathogenesis is the conformational conversion of PrPC, a host-encoded cellular prion glycoprotein, into a pathogenic isofrom PrPSc [9]. PrPC is normally expressed at highest levels in the central nervous system and in peripheral tissues including blood cells [6]. It is now established that variant Creutzfeldt-Jakob disease can be iatrogenically transmitted from human to human by blood transfusion [18]. Recently, PrPSc was detected in the blood of scrapie-infected hamsters during most of the presymptomatic phase [10].

Most human blood PrPC is found in the plasma [6] and it was demonstrated that plasma prion protein levels show genotypic [14] and pathological variations [13, 15]. Because PrPC expression is necessary for PrPSc infection, the level of blood PrPC may be associated directly or indirectly with the regulatory mechanisms that determine susceptibility and resistance to prion disease. Factors governing prion disease susceptibility might thus be highlighted by identifying the physiopathological factors leading to elevated PrPC levels.

We previously showed that the kidneys greatly contribute to elimination of plasma prion protein in sheep [2]. We hypothesized that renal insufficiency, by altering plasma prion clearance, might be a risk factor affecting prion disease susceptibility and could be relevant to consider when evaluating the risk of prion disease transmission by blood transfusion. The dog species is characterized by a frequent incidence of renal disease. Although TSE has never been reported in dog, the similarities in the PrPC distribution in the brains observed in several domestic species including dog suggest that the biosynthetic pathway of PrPC is conserved across the species [1, 17]. Therefore, dog model would be of interest to
study the relationship between kidney insufficiency and plasma PrP<sub>C</sub> disposition.

**Materials and Methods**

The objective of the study was to characterize the relationship between renal glomerular clearance and plasma prion protein concentrations in dogs with spontaneous renal failure (Experiment 1) or with surgically controlled renal alteration (Experiment 2). This latter model allowed investigations in a homogenous group of dogs with sub-clinical renal failure.

All experimental procedures were performed in accordance with French legal requirements regarding the protection of laboratory animals.

In experiment 1, heparinised plasma was obtained from 21 control dogs and from 26 dogs with spontaneous renal failure during 2002, 2003 and 2004. Control dogs were presented at Toulouse Veterinary Teaching Hospital for pre-surgical assessment or suffering various diseases which do not interfere with renal function. Control dogs were matched for sixteen breeds, age and period of sampling with dogs with renal failure (Table I). Chronic kidney disease diagnosis was established from the usual clinical signs, i.e. polyuria-polydipsia, dehydration, associated with creatininemia above 130 µM. The control dogs were included in the trial on the basis of the absence of clinical signs of renal failure and creatininemia below 120 µM.

In experiment 2, sixteen adult Beagle dogs (10 females and 6 males), with renal impairment experimentally-induced for other purposes [4, 16], were considered retrospectively. They were aged 3-5 years and weighed 9.4-14.4 kg at the start of the experiment. Eight control Beagle dogs (4 females and 4 males) aged 2.9-6.4 years were also considered. The dogs were fed once daily with a commercial dog food. Water was given ad libitum.

Experimental renal failure was induced by nephrectomy-electrocoagulation as described previously [4]. Briefly, the right kidney was excised and portions of the left renal cortex were electrocoagulated to induce moderate renal impairment (i.e. about 60% of normal glomerular filtration rate). All animals recovered rapidly from surgery. Plasma PrP<sub>C</sub> concentrations were determined 2-3 months after the experimental renal injury.

Kidney function was assessed by measuring glomerular filtration rate with iohexol or creatinine plasma clearance as markers, as described previously [4, 16]. Glomerular filtration rate and plasma creatinine concentrations were assessed under control conditions 3-4 months before renal surgery and under renal-impaired conditions 15-30 days after surgery.

Blood samples were collected in heparinised vacutainers (Experiment 1) or in tubes containing EDTA (Experiment 2) and centrifuged at 3000 g for 10 minutes. The plasma was separated, assayed for creatinine and stored at −20°C until PrP<sub>C</sub> assay.

Plasma basal PrP<sub>C</sub> concentrations were measured by ELISA using SAF34, an antibody directed against the octapeptide region, and a C-terminal anti-PrP monoclonal antibody 12F10-AchE (Spi Bio, France), as previously described [8]. The PrP concentrations were compared with a standard curve obtained with a V136Q171 genetic variant of the ovine recombinant PrP protein. The level of quantification of the assay was 0.5 ng/mL. The mean intra-assay coefficient of variation was 8%. Signal specificity in dog plasma was checked by displacement with free SAF34 or unlabelled 12F10-AchE antibody.

The plasma creatinine concentrations were determined by enzymatic method with an analyzer (Ektachem 700 XR, Kodak, Johnson and Johnson Clinical Diagnostic Europe, Illkirch Graffenstaden, France in experiment 2, or Vitros 250, Ortho Clinical Diagnostics, France in experiment 1). Within- and between-day coefficients of variation were below 3%.

Results were reported as means ± SD. The relationship between plasma creatinine and PrP<sub>C</sub> concentrations was determined by regression with log-linear and polynomial functions (y = a Ln(x) + b or y = ax²+bx+c, respectively). The effect of renal insufficiency on mean parameters was analysed by Student t test.

**Results**

**EFFECT OF SPONTANEOUS RENAL DISEASES ON PLASMA PRP<sub>C</sub> CONCENTRATIONS**

Mean plasma PrP<sub>C</sub> concentration in dogs with renal failure (24.9 ± 12.5 µg/L, 8.1-50.9 µg/L) was 3.5 times higher than in control dogs (7.17 ± 2.76 µg/L, 2.6-13.9 µg/L, Figure 1). Mean creatininemia in dogs with chronic renal failure (440 ± 277 µmol/L, 131-1131 µmol/L) was also significantly greater than in control dogs (82 ± 16 µmol/L, 56-120 µmol/L). The effect of pathological status on plasma PrP<sub>C</sub> and creatinine concentrations was significant (P<0.001). Figure 2 shows the

<table>
<thead>
<tr>
<th>Pathological status</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 F (1 castrated), 11 M (1 castrated)</td>
<td>8.0 ± 4.2 (0.5-14)</td>
<td>20 ± 12 (5.2-39)</td>
</tr>
<tr>
<td>Spontaneous renal failure</td>
<td>10 F (3 castrated), 16 M (1 castrated)</td>
<td>9.5 ± 5.0 (0.5-18)</td>
<td>19 ± 7.0 (2.4-33)</td>
</tr>
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Table I: Sex, age and weight of 21 control dogs and 26 spontaneous renal failure affected dogs (mean ± SD and range).
relationship between plasma creatinine and basal PrP C concentrations in control dogs and those with renal failure (correlation coefficient 0.84 for the polynomial fitting and 0.74 for the log-linear model).

EFFECT OF MILD EXPERIMENTAL RENAL FAILURE ON PLASMA PrP C LEVELS

No clinical signs, except mild polyuria and polydipsia, were observed in dogs with surgically induced renal failure. Surgery induced a significant decrease (P<0.001) in glomerular filtration rate, determined by iohexol plasma clearance (from 3.8 ± 0.6 mL/kg/min to 1.6 ± 0.3 mL/kg/min in 10 female dogs) and by creatinine plasma clearance (from 3.3 ± 0.16 mL/kg/min to 1.7 ± 0.3 mL/kg/min in 6 male dogs). The mean decrease in glomerular filtration rate induced by surgery was 54% (range 35-68%). Renal impairment was confirmed by a two-fold significant (P<0.001) increase in creatininemia (65.8 ± 7.4 μM before and 123.3 ± 23.7 μM after surgery, respectively) but most of the post-operative values remained below the upper limit of the canine reference interval (130 μM). The mean basal plasma PrP C concentration in experimentally-induced renal failure dogs (7.84 ± 1.61 ng/mL, 5.3-12.1 ng/mL) was 2.8 times higher than in control dogs (2.78 ± 0.95 ng/mL, 1.6-4.6 ng/mL, P<0.001).

Discussion

Our results show that chronic renal failure is associated with elevated concentrations of plasma PrP C. Our clinical data agree with those obtained from patients suffering from extensive renal insufficiency [13]. It should be noted that although the dog species used in our study was not sensitive to TSE, these results obtained with dog model, characterized by a frequent incidence of renal disease, may provide useful
information for plasma PrP\textsuperscript{C} elimination in others species because the renal filtration of proteins is almost conserved across the mammalian species. In our study, the increase in plasma PrP\textsuperscript{C} concentrations was observed both in dogs with spontaneous chronic renal failure and sub-clinical experimental renal insufficiency. It is noteworthy that the two dogs with highest creatininemia had the lowest plasma PrP\textsuperscript{C} in the group of spontaneously affected dogs and also presented proteinuria. The relevance of this observation remains unclear but it cannot be excluded that in some affected dogs, the glomerular filtration barrier was sufficiently altered to increase bulk protein filtration. This gross protein leakage led to a decrease of plasma PrP\textsuperscript{C} so that the curve of the relationship between creatinemia and plasma PrP\textsuperscript{C} was an inverted U-shape (see Figure 2B). In agreement with this hypothesis, proteinuria was recently reported in scrapie mice with chronic nephritis [11]. Moreover, alterations in PrP\textsuperscript{C} disposition in spontaneous renal-impaired dogs may result both from reduced renal elimination and from changes in PrP\textsuperscript{C} metabolism or distribution. The advantage of the surgically-induced renal failure model was its selectivity and reproducibility. Tissue destruction was confined to the kidney, suggesting that the plasma PrP\textsuperscript{C} variations were solely attributable to renal function. The high correlation between plasma PrP\textsuperscript{C} and creatinine concentrations reinforces the hypothesis that the kidney plays a major role in prion protein elimination. Prion protein of approximately 35 kDa could pass through the glomerulus and then be catabolized by proteases located in the renal tubules. In agreement with this hypothesis, the urinary excretion of PrP\textsuperscript{C} was reported in healthy humans [7] and prion infectivity was evidenced in kidney of prion affected–sheep and in urine from scrapie-infected hamsters [3, 5, 12]. We also previously showed with the ovine model that the kidneys contribute to about 50% of the clearance of recombinant PrP produced in \textit{E. Coli} [2]. It would therefore be interesting to see whether modified prion protein can be detected in the urine of dogs with renal failure.

For the prion assay used in this investigation in dogs, we took advantage of the cross reactivity between species exhibited by antibodies produced against well-conserved structural regions characteristic of PrP\textsuperscript{C} [17].

In conclusion, chronic renal failure is associated with elevated concentrations of plasma PrP\textsuperscript{C}. Our results suggest that renal dysfunction, by determining plasma PrP\textsuperscript{C} levels, may increase the likelihood of PrP\textsuperscript{Sc} propagation upon infection and thus contribute to prion disease susceptibility.

Acknowledgements

This study was supported by grants from the French National Institute for Agronomical Research (INRA), from GIS prion and DGER. The authors declare that there is no conflict of interest relative to the major sources of funding that would prejudice their impartiality relative to the results of the study.

References


