Evaluation of drugs for treatment of prion infections of the central nervous system

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Prion diseases are fatal and at present there are neither cures nor therapies available to delay disease onset or progression in humans. Inspired in part by therapeutic approaches in the fields of Alzheimer’s disease and amyotrophic lateral sclerosis, we tested five different drugs, which are known to efficiently pass through the blood–brain barrier, in a murine prion model. Groups of intracerebrally prion-challenged mice were treated with the drugs curcumin, dapsone, ibuprofen, memantine and minocycline. Treatment with antibiotics dapsone and minocycline had no therapeutic benefit. Ibuprofen-treated mice showed severe adverse effects, which prevented assessment of therapeutic efficacy. Mice treated with low- but not high-dose curcumin and mice treated with memantine survived infections significantly longer than untreated controls (P<0.01). These results encourage further research efforts to improve the therapeutic effect of these drugs.

Transmissible spongiform encephalopathies (TSEs) or prion diseases are ultimately lethal progressive neurodegenerative disorders. Prion diseases are usually associated with the appearance of a misfolded, insoluble and protease-resistant form of a normal host-encoded protein, the prion protein (PrP). Hence, the accumulation of misfolded PrP (termed PrPSc) is likely to play a central role in disease development (Prusiner, 1998). However, underlying pathomechanisms in prion infections of the CNS are still elusive (Burwinkel et al., 2004; Caughey & Baron, 2006). Moreover, at present neither cures nor palliative therapies are known/available to delay onset or progression of disease in humans (Trevitt & Collinge, 2006). A number of therapeutic approaches have shown effectiveness against prion replication in cell culture, but failed in vivo or showed only some benefit in slowing down peripheral prion spread (Kocisko et al., 2004; Larramendy-Gozalo et al., 2007; Trevitt & Collinge, 2006). In part, these results can be attributed to the charged composition of drugs, preventing their passage through the blood–brain barrier (BBB). We report here the evaluation of drugs for treatment of intracerebrally (i.c.) prion-challenged mice, which (i) efficiently pass the BBB, (ii) are orally available and (iii) have acceptable safety profiles. The focus on well-characterized drugs was anticipated to be of particular interest for possible treatment of cases of known genetic predispositions for development of Creutzfeldt–Jakob disease (CJD). Furthermore, drug selection was in part influenced by therapeutic approaches developed in the fields of Alzheimer’s disease (AD) and amyotrophic lateral sclerosis (ALS), because we reasoned that anti-inflammatory and/or neuroprotective strategies might be similarly applicable to the treatment of prion diseases.

Groups of animals (4–6-week-old female C57BL/6 mice) were infected i.c. with 10−5 and/or 10−4 diluted 10% brain homogenates [108 lethal doses 50% (LD50) per brain] prepared from terminally ill scrapie strain 139A-infected mice as described previously (Schultz et al., 2004). Each animal was exposed to 400 or 40 LD50, respectively. Drug treatment commenced 100 days post-infection (dpi). Drugs were administered as ingredients of the chow pellets (prepared by ssniff), and drug dosages (given as mg kg−1 body weight daily per os) were adjusted to previously described ranges: 50 and 500 mg curcumin (Sigma-Aldrich) (Lim et al., 2001); 16.75 mg dapsone (kindly provided by Fatol) (Manuelidis et al., 1998; Guenther et al., 2001); 30 mg memantine (Merz) (Jin et al., 2006); 200 mg minocycline (kindly provided by Hexal) (Kriz et al., 2002) and 100 mg ibuprofen (Biomol) (Lim et al., 2000). Mice were monitored twice weekly for clinical signs of disease development and were finally sacrificed when the gravity of symptoms indicated a likely death due to the disease within the next 48 h (Mok et al., 2007). Statistical evaluations were carried out using the unpaired t-test and the log-rank test for analysis of differences between Kaplan–Meier survival curves.

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With the exception of ibuprofen, a cyclooxygenase-1/2 inhibitor and peroxisome proliferator-activated receptor gamma agonist, none of the drugs elicited adverse effects during the treatment period. Scrapie-infected ibuprofen-treated mice developed large erthema, followed by ulcerations of the outer genital region and massively enlarged urinary bladders (data not shown). About half of the ibuprofen-receiving mice had to be sacrificed due to their poor condition before they displayed typical symptoms of a late-stage scrapie infection. Uninfected ibuprofen-treated mice remained completely normal, indicating that the combination of drug treatment and ongoing prion disease development triggered these adverse responses. Interestingly, bladder enlargement has also been described for scrapie infections in combination with treatment with the drug dapsone (Guenther et al., 2001); however, this was not observed during the experiments described here. Generally, in our C57BL/6-mouse/scrapie strain 139A model, cases of moderate bladder enlargement and/or incontinence were occasionally observed. This might indicate that prion infections affect brain areas involved in central control of micturition and urinary continence, like Barrington’s nucleus, but presently it is not clear how ibuprofen could exaggerate this process. We currently plan to study ibuprofen treatment of scrapie-infected CD-1 mice to clarify the influence of mouse strain on this phenomenon, and to reassess the therapeutic potential of this drug.

Survival data are summarized in Table 1. Dapsone and minocycline showed no therapeutic effect ($P>0.05$) and, as mentioned above, the result of ibuprofen treatment is unclear because of the occurrence of adverse effects. Prolonged survival times upon dapsone treatment have been observed in a CJD rat model, in which treatment was initiated immediately after the infection (Manuelidis et al., 1998). In contrast, our experiments were designed to address treatment of established prion infections of the CNS and not to simulate situations of post-exposure prophylaxis. Whether these differences are due to different prion models and/or are due to different study designs remains to be determined. Of note, the dapsone results reported here are in agreement with the described lack of therapeutic efficacy of dapsone therapy in a C57BL/6-mouse/scrapie strain ME7 setting (Guenther et al., 2001).

The therapeutic failure of the tetracycline antibiotic minocycline was unexpected. Minocycline treatment at a dosage comparable to the one used in our study was previously reported to significantly delay onset of motor neuron degeneration, to reduce microglial activation and to increase survival times in a transgenic mouse model of ALS (Kriz et al., 2002). However, the effectiveness of minocycline treatment in murine ALS models was specifically attributed to anti-apoptotic inhibition of mitochondrial cytochrome c release (Zhu et al., 2002). Hence, failure of the drug in our prion model might indicate that this pathway towards apoptotic cell death is more relevant in ALS than in prion diseases.

Furthermore, tetracycline and doxycycline have been shown to interact directly with misfolded prion protein PrPSc and to reduce prion infectivity (Forloni et al., 2002). In vitro tests of tetracyclines for inhibition of amyloid formation by prion protein-derived peptides demonstrated an even lower 50% inhibitory concentration (IC50) for minocycline than for tetracycline or doxycycline (Boshuizen et al., 2004). Moreover, amongst tetracycline antibiotics, minocycline diffuses most efficiently through the BBB (Jonas & Cunha, 1982). Still, it is possible that direct interference with prion infectivity would require higher drug concentrations in brain tissue than those obtained in the present study. Alternatively or in addition, it could be that tetracyclines display strain specificity in

### Table 1. Survival times of drug-treated mice and untreated controls

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg kg⁻¹)</th>
<th>Mean ± SD survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infectious dose 10⁻² i.c.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen*</td>
<td>100</td>
<td>172 ± 8.9 (n=11)</td>
</tr>
<tr>
<td>Dapsone†</td>
<td>16.75</td>
<td>189 ± 11.4 (n=9)</td>
</tr>
<tr>
<td>Minocycline†</td>
<td>200</td>
<td>178 ± 5.7 (n=17)</td>
</tr>
<tr>
<td>Curcumin†</td>
<td>500</td>
<td>196 ± 11.7 (n=8)</td>
</tr>
<tr>
<td>Memantine‡</td>
<td>30</td>
<td>174 ± 11.7 (n=8)</td>
</tr>
<tr>
<td><strong>Infectious dose 10⁻¹ i.c.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen*</td>
<td>100</td>
<td>191 ± 17.3 (n=12)</td>
</tr>
<tr>
<td>Curcumin†</td>
<td>50</td>
<td>208 ± 8.4 (n=12)</td>
</tr>
</tbody>
</table>

*Scrapie-infected ibuprofen-treated mice developed severe adverse side effects (see main text).

†$P>0.05$ versus untreated control group.

‡$P<0.01$ versus untreated control group.
regard of prion infectivity reduction. In other words, tetracyclines may be less effective against scrapie strains like 139A used here than against the hamster-adapted scrapie strain 263K.

The curry spice curcumin (diferuloylmethane) has anti-inflammatory as well as anti-oxidative properties (Menon & Sudheer, 2007). Moreover, curcumin inhibits aggregation and promotes disaggregation of beta-amyloid (Garcia-Alloza et al., 2007; Yang et al., 2005). It is also a potent inhibitor of prion replication in cell culture assays, with an IC₅₀ of 10 nM (Caughey et al., 2003). In this study, curcumin was administered at a low and high dosage, because in a murine Alzheimer’s disease model low-dose treatment was described to be more effective in reducing beta-amyloid deposition and in attenuating astrocyte activation (Lim et al., 2001). Furthermore, high-dose curcumin treatment of prion-infected hamsters had no significant effect on disease duration (Caughey et al., 2006). As shown in Table 1 and Fig. 1, low-dose curcumin prolonged survival times by an average of 12 days ($P<0.01$), whereas the high-drug dosage was ineffective. Inhibition of multiple kinases and other drug effects at high curcumin concentrations may provide an explanation for this observation, as this spectrum of activities could potentially offset beneficial drug effects (Gautam et al., 2007; Menon & Sudheer, 2007). Whether the drug effect observed is due to the anti-oxidative/anti-inflammatory properties of curcumin, direct inhibition of prion replication, or both is currently unknown. Overall, the low toxicity of curcumin, the inhibition of prion replication in vitro and the prolongation of survival times in vivo shown here encourage further studies with this compound or derivatives thereof.

As one pathway towards neuronal cell death, excitotoxic overstimulation of the N-methyl-D-aspartate (NMDA) receptor by glutamate was implicated in neurodegenerative disorders like AD and similarly in prion diseases (Reisberg et al., 2003; Scallet & Ye, 1997). Memantine is a low-affinity noncompetitive NMDA receptor antagonist, which is used to slow down clinical deterioration in patients with moderate to severe AD (Reisberg et al., 2003). In vitro, neurotoxicity of a prion protein-derived peptide PrP₁₀₆₋₁₂₆ was shown to be mediated by glutamate (Sassoon et al., 2004). Likewise, memantine protected cultured neurons against cytotoxic effects of PrP<sup>Sc</sup> (Muller et al., 1993). Memantine-treated mice survived the prion infection on average 15 days longer than the similarly infected untreated control group ($P<0.01$; Table 1, Fig. 1). This result is in support of the idea that excitotoxicity could be part of the mechanisms leading to neuronal loss in prion diseases. Accordingly, NMDA receptor antagonists like memantine would provide some degree of neuroprotection in infected brain tissue.

Taken together, we report here results obtained on the treatment of established prion infections of the CNS with five different drugs, which are all known to pass efficiently through the BBB. Only low-dose curcumin and memantine treatment showed a small, but statistically significant, therapeutical benefit (Fig. 1). One approach towards a more substantial therapeutic efficacy could be to combine for example curcumin and/or memantine therapy with administration of 3-hydroxymethyl-3-glutaryl coenzyme A reductase inhibitor simvastatin, which was recently shown to prolong survival times in established prion infections of the CNS (Kempster et al., 2007; Mok et al., 2006). Ultimately, more research into underlying pathomechanisms in prion diseases is needed to aid in the development of improved therapeutic concepts.

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