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A medicinal herb *Scutellaria lateriflora* inhibits PrP replication *in vitro* and delays the onset of prion disease in mice

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Transmissible spongiform encephalopathies (TSE) are characterized by the misfolding of the host encoded prion protein (PrP^C) into a pathogenic isoform (PrP^{Sc}) which leads to the accumulation of β-sheet-rich fibrils and subsequent loss of neurons and synaptic functions. Although many compounds have been identified which inhibit accumulation or dissolve fibrils and aggregates *in vitro* there is no therapeutic treatment to stop these progressive neurodegenerative diseases. Here we describe the effects of the traditional medicinal herb *Scutellaria lateriflora* (*S. lateriflora*) and its natural compounds, the flavonoids baicalein and baicalin, on the development of prion disease using *in vitro* and *in vivo* models. *S. lateriflora* extract as well as both constituents reduced the PrP^{res} accumulation in scrapie-infected cell cultures and cell-free conversion assays and lead to the destabilization of pre-existing PrP^{Sc} fibrils. Moreover, tea prepared from *S. lateriflora*, prolonged significantly the incubation time of scrapie-infected mice upon oral treatment. Therefore *S. lateriflora* extracts as well as the individual compounds can be considered as promising candidates for the development of new therapeutic drugs against TSEs and other neurodegenerative diseases like Alzheimer's and Parkinson's disease.

Keywords: prion protein, inhibitor, *Scutellaria lateriflora*, baicalein, baicalin

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases which are characterized by the accumulation and deposition of a pathogenic isoform (PrP^{Sc}) of the host encoded cellular prion protein (PrP^C) designated PrP^{Sc}. Both isoforms share the same amino acid sequence but differ in conformation, resistance to proteinase K (PK), and pathogenicity. PrP^{Sc} tends to oligomerize by a seeded polymerization mechanism followed by the formation of multimers and eventually of fibril structures. This aggregation can be reproduced in an analogous manner *in vitro*, using cell-based as well as cell-free assays. The evolved PrP^{Sc} like isoforms – termed PrP^{res} – harbor similar biochemical characteristics like resistance to Proteinase K and detection by same antibodies.

Accumulation of PrP^{Sc} in the central nervous system (CNS) is accompanied by neurological dysfunctions, neuronal vacuolation, and astrocytic gliosis. Although the exact disease causing mechanism is unknown to date, there is evidence for a general neurotoxicity of these aggregates, which deteriorate synaptic function and induce oxidative stress and membrane disruption (Soto and Estrada, 2008). Prion diseases belong to the group of protein misfolding diseases like Alzheimer's (AD), Parkinson's (PD), and Huntington's disease (HD), which are generally characterized by an incorrect folding process of a host encoded protein with a conformation different from its native structure. The misfolding is followed by a self-aggregation and polymerization of the protein according to a "seeding-nucleation" process (Jarrett and Lansbury, 1993).

As PrP^{Sc} formation and aggregation is the central event in prion diseases, the inhibition of oligomer formation and fibril extension as well as the enhancement of fibril degradation are major targets for the development of therapeutic strategies against TSEs. Several substances have been identified which inhibit PrP^{res} formation and accumulation *in vitro* and prolonged survival in scrapie-infected animals: Congo red (Caughey and Race, 1992), branched polyamines (Supattapone et al., 2001), porphyrins and phthalocyanines (Priola et al., 2000; Caughey et al., 2007), heparan sulfate mimetics (Adjou et al., 2003), amphotericin (Mange et al., 2000), curcumine (Caughey et al., 2003; Yang et al., 2009), and tetracyclines (De Luigi et al., 2008). However, for various reasons none of these compounds has been included in prevention and treatment regimes for humans yet. Most recently two new substance classes, benzothiazoles (Geissen et al., 2011) and diphenylpyrazoles (Leidel et al., 2011), were identified by high-throughput screening approaches that inhibit PrP^{res} accumulation in cell culture models and prolong incubation times in scrapie-infected mice.

Other therapeutic strategies rely on passive immunization (White et al., 2003), RNA interference (Pfeifer et al., 2006), RNA aptamers (Proske et al., 2002), copper chelating antibiotics (Murakami-Kubo et al., 2004), or on the induction of autophagy by Lithium (Heiseke et al., 2009).

Recent studies on AD suggest that phenolic compounds like green tea epigallocatechin gallate (Rezai-Zadeh et al., 2005), herb extracts like grape seed polyphenolic extract (Wang et al., 2009; Liu et al., 2011), or medicinal herbs like *Paeonia suffruticosa* (Fujiwara

et al., 2009) can be used for the treatment of neurodegenerative diseases.

The here presented study shows that the American skullcap *Scutellaria lateriflora*, a traditional medical herb in North America, exhibits strong anti-prion activity. Extracts of this plant have been traditionally used as sedative and for the treatment of insomnia, anxiety, or neuralgia (Foster, 1996). Moreover, we can show here that the crude aqueous extract inhibits prion propagation and dissolves prion aggregates *in vitro*. Continuous oral administration of *S. lateriflora* tea (water extract) significantly prolonged incubation times in scrapie-infected mice. Notably, the natural constituents, the flavonoids baicalein and baicalin-hydrate, exhibit the same inhibitory effects on prion propagation and dissolve existing fibrils.

MATERIALS AND METHODS

FLAVONOIDS AND TEA OF *SCUTELLARIA LATERIFLORA*

Baicalein and baicalein-hydrate were purchased from Sigma-Aldrich and solubilized in DMSO. The dried herb *S. lateriflora* was purchased from Goldener Zweig (Buchholz, Germany). Water extracts (tea) were prepared by mixing 30 g of sliced dry herbs in 1.0 l boiling water for 30 min. After cooling the tea was administered directly to mice. For *in vitro* experiments tea was lyophilized to yield dried extracts. In cell-based assays the dried material was resuspended in H_2O, concentrated by a factor of 10 (termed tea concentrate) and added to the cell culture medium. In cell-free conversion assay the dried extract was directly resuspended in the conversion buffer in a final volume of 20 µl.

PROPAGATION OF SCRAPIE STRAINS

C57Bl/6 mice were inoculated with cloned mouse scrapie strains RML intracerebrally (i.c.; 30 µl of 1% mouse brain homogenate) or intraperitoneally (i.p.; 50 µl of 1% brain homogenate). The health status of the mice was inspected daily, and their body weights were recorded weekly. After the onset of TSE-associated clinical symptoms (and weight loss, abnormal tail tonus, hind limb paralysis), the animals were euthanized. The incubation times were calculated as the time between inoculation and death. The brains were removed and kept as following: one half of each brain was stored at −20°C, and the other half was fixed in 4% neutral buffered formalin.

Pathogenic isoform for PrP conversion reactions was purified from brains of C57/Bl6 mice i.c. inoculated with mouse scrapie strain Me7. Brains were taken at the beginning of clinical symptoms in the animals and stored at −20°C. PrP^{Sc} was purified according to Eiden et al. (2006).

HEMATOXYLIN AND EOSIN STAINING AND IMMUNOHISTOCHEMISTRY

Before embedding in paraffin the formalin fixed brain was cut at five different levels to reveal caudal medulla, rostral medulla, midbrain, thalamic, and frontal slices. Lesion profile scoring was carried out on Hematoxylin and Eosin (H&E) stained sections according to standard methodology (Fraser and Dickinson, 1968). For immunohistochemistry samples were processed (with minor modifications) as described previously (Hardt et al., 2000). Three micrometers section were de-paraffinized and rehydrated. Pretreatment included an incubation step with 98% formic acid (15 min) and blocking of the endogenous peroxidase activity with 3% H_2O_2/methanol (30 min) followed by autoclaving in citrate buffer (pH 6.0, 20 min). The monoclonal antibody SAF 84 was applied at a dilution of 1:2000 for 2 h at room temperature and detected by avidin–biotin–horseradish peroxidase (Vector Elite). Color reactions were finally developed using diaminobenzidine (DAB) substrate.

PROTEINASE K DIGESTION AND SODIUM PHOSPHOTUNGSTIC ACID PRECIPITATION

Mouse brain samples 10% (w/v) were prepared in lysis buffer (0.42 mM sucrose solution containing 0.5% deoxycholic acid sodium salt (DOC) and 0.5% Non-idet P40) and incubated with PK (50 µg/ml, final concentration) for 60 min at 55°C. Reactions were stopped with pefabloc (Roche) followed by incubation with 0.3% (w/v) phosphotungstic acid precipitation (PTA) and selective precipitation of PrP^{Sc} according to Gretzschel et al. (2005).

PRP CONVERSION REACTION

Cell-free conversion studies were performed as described before (Eiden et al., 2006; Kupfer et al., 2007). For the conversion reaction 400 ng of recombinant ovine PrP^{C}, expressed in *E. coli*, was incubated with 200–400 ng of purified PrP^{Sc} in a conversion buffer [50 mM citrate buffer (pH 6.0), 200 mM KCl, 5 mM $MgCl_2$, and 1.25% sarkosyl; Horiuchi et al., 2000]. Standard conversion reactions were carried out for 3 days at 37°C. Afterward, samples were incubated with PK (final concentration of 30 µg/ml) for 1 h at 37°C. PK was diluted in TN-buffer (0.15 M NaCl, 0.05 M Tris/HCl pH 7.4). The reaction was stopped with PMSF (phenyl methansulfonyl fluoride; 10 mM). Twenty micrograms of a carrier protein (thyroglobulin) was added and the samples were incubated with a fourfold volume of methanol at –20°C to precipitate the proteins. After centrifugation at 12000 *g* for 15 min, the proteins were pelleted and resolubilized by heating to 95°C for 5 min in SDS-PAGE loading buffer [1% (w/v) SDS, 25 mM Tris/HCl pH 7.4, 0.5% mercaptoethanol, and 0.001% bromophenol blue].

Samples were separated on SDS-polyacrylamide gels containing 16% (w/v) acrylamide/bisacrylamide, 0.375 M Tris/HCl (pH 8.8), 0.5% (w/v) SDS (sodium dodecyl sulfate), 0.06% (w/v) APS (ammonium peroxydisulfate), and 0.06% (v/v) TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine) with a 4% stacking gel [4% (w/v) acrylamide/bisacrylamide, 0.12 M Tris/HCl (pH 6.8), 0.5% (w/v) SDS, 0.2% APS, 0.2% (v/v) TEMED].

For immunoblotting proteins were transferred in a semi-dry chamber to a polyvinylidene difluoride (PVDF) membrane. Membranes were then incubated in blocking buffer (PBS-0.1% Tween 20, 5% non-fat dry milk powder) followed by incubation for 60 min with the monoclonal antibody (mab) P4 (R-Biopharm), mab SAF-70 (SPIbio), or polyclonal antibody (pab) Ra 10 (Groschup et al., 1994, 1997). In contrast to mab P4, which detects ovine $PrP^{C/res}$, pab Ra10 detects only murine $PrP^{C/Sc}$. Membranes were washed three times for 10 min with PBS containing 0.1% Tween 20 and then incubated with a secondary antibody bound to alkaline phosphatase (Goat-anti-mouse-alkaline phosphatase or goat anti-rabbit-peroxidase). After washing, the chemiluminescence substrate CDP-Star (Tropix) was applied and membranes were incubated for 5 min before the light signals were

recorded on a Versadoc Imaging System (Biorad). Visualization was carried out with the Biorad VersaDoc™quantification software Quantity One. The percentage of converted substrate was calculated as: signal volume of PrP^{res} digested with PK/signal volume of PrP^{C} without PK*10. Conversion rates were calculated for each time point as a mean value from four independent reactions. For stripping, membranes were incubated twice for 15 min with a buffer containing 0.2 M glycine (pH 2.0) and 1% SDS.

PRP^{RES} DETECTION FROM CELL CULTURE

Two scrapie-infected mouse cell lines were used: ScN_2A-cells [RML infected neuroblastoma-(N_2A)-cells] and SMB-cells ("scrapie-mouse-brain," infected with strain 22F, a gift of TSE Resource Centre at the Institute for Animal Health, UK). ScN_2A-cells were cultured in Opti-MEM, 10% fetal calf serum (FCS), and 5% CO_2 at 37°C. SMB-cells were cultured in MEM+ [MEM199, 10% newborn calf serum (NCS), 5% FCS] and 5% CO_2 at 35°C.

The cell-based assay was carried out according to Leidel et al. (2011) with two scrapie-infected mouse cell lines: ScN_2A-cells and SMB-cells. These cells were adapted for dot blot procedure in 96-well plate format to identify potential inhibitors of PrP^{Sc} formation. Compounds were resolved in DMSO and added to cells in eight replicates in concentrations of 10, 100, and 1000 µM (final concentration). After 3 days of incubation in a CO_2-incubator, the supernatants were removed and cells lysed in 100 µl lysis buffer (50 mM Tris/HCl, 150 mM NaCl, 0.5% Na-deoxycholate, 0.5% TX-100, pH 8.0). Lysates were or electrophoresed and immunoblotted as described in the previous section or dot blotted on PVDF membranes using a 96-well dot blot apparatus (Transblotter, Roth). Dot blotting membranes were dried for 1 h at 37°C and fixed sample incubated with DNAse (100 µg/ml) for 1 h at 37°C. After rinsing the membranes twice with distilled water, the membrane was incubated with PK (25 µg/ml) for 90 min at 37°C. The digestion was terminated using PMSF (2 mM) and membranes were rinsed in 3 M guanidium thiocyanate for 10 min followed by washing them five times in distilled water.

The incubation of the membrane with blocking buffer (PBS-0.1% Tween 20, 5% non-fat dry milk powder) was followed by an incubation for 60 min with polyclonal antibody (pab) Ra 10. Membranes were washed three times for 10 min with PBS containing 0.1% Tween 20 and then incubated with a secondary antibody bound to peroxidase (Goat anti-rabbit-PO). Afterward, the chemiluminescence substrate ECL (Roche) was applied and incubated on the membrane for 1 min before the light signals on the membrane were detected directly in a camera. The intensity of the PrP^{Sc} signal of each well was quantified by using the Versadoc imaging system. The half maximal (50%) inhibitory concentration (IC_{50}) of each compound was calculated from eight independent reactions.

STATISTICAL ANALYSIS

Survival times were analyzed by Kaplan–Meier Survival analysis using the log-rank test to compare the curves. The Statistical analysis was done using SigmaBlot statistical software (San Jose, CA, USA). Survival times are expressed as mean ± SD.

RESULTS

The effect of *S. lateriflora* tea on the PrP^{res} formation was first analyzed *in vitro* using two different scrapie-infected cell lines (SMB and ScN_2A-cells), which permanently accumulate PrP^{res}. When tea concentrate was added to the culture medium, a dose dependent reduction of PrP^{res} accumulation was observed in SMB as well as ScN_2A-cells (**Figure 1**). Cells were cultivated in T25 flasks and incubated with increasing amounts of *S. lateriflora* tea for 3 days. After this time, cells were harvested and the level of PK-resistant PrP^{res} was assessed by Western blotting. A dilution of 1:500 induced a reduction of PrP^{res} to 58% for ScN_2A (**Figure 1A**, lane 2) and to 64% for SMB (**Figure 1B**, lane 2) cells compared to the control (**Figures 1A,B**, lane 1). Dilutions of 1:50 resulted in a reduction to 33% for ScN_2A-cells (**Figure 1A**, lane 3) and 57% for SMB-cells (**Figure 1B**, lane 3). About 1:20 dilutions displayed reduction to 28% for ScN_2A (**Figure 1A**, lane 4) and 47% for SMB-cells (**Figure 1B**, lane 4) and reached values of 28.7% for ScN_2A-cells (**Figure 1A**, lane 5) and 42.3% for SMB-cells (**Figure 1B**, lane 5). Relative inhibition by tea is depicted in **Figure 1C**.

To test the direct effects of tea on the conversion of PrP^{C} to PrP^{res}, a cell-free conversion assay was used in which a purified murine PrP^{Sc} template induced the conversion of recombinant cellular PrP^{C} into a PK-resistant PrP^{res} fragment (Eiden et al., 2006; Kupfer et al., 2007). Newly converted PrP^{res}-fragments were detected by mab P4 (**Figure 2A**, lanes 1–2), while PrP^{Sc} aggregates were visualized by the polyclonal antibody Ra10 (**Figure 2B**, lanes 1–2). Dried tea extract (3, 0.3, and 0.03 mg respectively) was added to the conversion assay, inhibited PrP^{res} formation and led to clearance of PrP^{res}-fragments (**Figure 2A**, lanes 3–6). Even 0.03 mg caused a reduced PrP^{res} formation compared to the control (**Figure 2A**, lanes 7–8) by 23%.

Similar results were seen in the case of dissolution of PrP^{Sc} aggregates after PK digestion (**Figure 2B**). In contrast to the control (**Figure 2B**, lanes 1–2), PrP^{Sc} aggregates were completely digested if co-incubated with 3.0 mg dried tea extract (**Figure 2B**, lanes 3–4), as well as with 0.3 mg (**Figure 2B**, lanes 5–6). About 0.03 mg dried tea extract induced a partial disaggregation of PrP^{Sc} aggregates (**Figure 2B**, lanes 7–8) by 40% compared to the control.

To further characterize the underlying inhibitory mechanisms, two main polyphenolic compounds of this herbal extract, the flavonoids Baicalin (baicalein 7-*O*-glucuronide) and its aglycone baicalein (5,6,7-trihydroxyflavone), were studied with regard to their inhibitory properties. Both components have been isolated from this herb previously (Awad et al., 2003) und were also identified in this study as ingredients in the tea by LCMS–IT–TOF hybrid mass spectrometry (data not shown). In addition, a structural analog to baicalein, the flavonoid epicatechin and quercetin were analyzed.

The potentially inhibitory effects on the PrP^{res} accumulation and conversion were assayed by a cell-based dot blot assay system (Geissen et al., 2011) and the cell-free conversion assay. Structures, assay results, and half maximal (50%) inhibitory concentrations (IC_{50}) of both compounds are summarized in **Table 1**. The cell-based dot blot assay was carried out with two different scrapie-infected SMB and ScN_2A-cells again. Cells were seeded

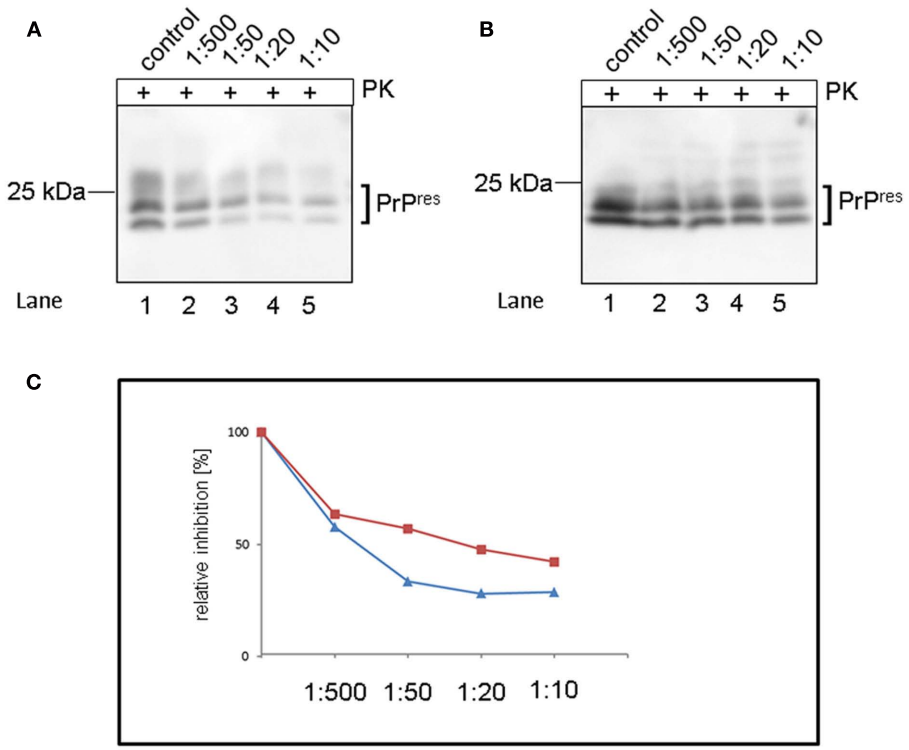

FIGURE 1 | Western blot analysis of inhibition of PrP^{res} formation in scrapie-infected cells by tea concentrate of *S. lateriflora*. (A) Tea concentrate of *S. lateriflora* inhibits PrP^{res} formation in ScN_2A-cells. Lane 1 shows PK-resistant fragments of the untreated control, lanes 2–5 a sample incubated with increasing concentrations of tea concentrate of *S. lateriflora*: 1:500 (lane 2), 1:50 (lane 3), 1:20 (lane 4), and 1:10 (lane 5). **(B)** Inhibition of PrP^{res} formation in SMB-cells by tea concentrate of *S. lateriflora*. PK-resistant fragments were detected in untreated control (lane 1), after incubation with tea in a 1:500 dilution (lane 2), 1:50 (lane 3), 1:20 and 1:10 (lane 5). Detection was carried out with pab Ra10 and analyzed by standard chemiluminescence method. **(C)** Relative inhibition (%) of PrP^{res} formation by tea concentrate for SMB (red line) and ScN_2A (blue line) cells. +, incubation with proteinase K (PK); u.d., undiluted.

in 96 wells and incubated with decreasing concentrations of the corresponding flavonoids (1 mM, 100 and 10 μM). IC_{50} values of Baicalein ranged from 138 μM (SMB-cells) to 63.7 μM (ScN_2A-cells). Baicalin showed significantly lower inhibitory effects compared to baicalein in SMB-cells (IC_{50}: >1000 μM) as well as in ScN_2A-cells (IC_{50}: 239.8 μM). Two additional flavonoids with similar structure, epicatechin, and quercetin, showed also strong inhibitory effects in both cell-types: epicatechin IC_{50} values were 96.6 μM (ScN_2A-cells) and 106.8 μM (SMB-cells) and quercetin IC_{50} values were 36.8 μM (ScN_2A-cells) and 274.3 μM (SMB-cells; **Table 1**). Baicalein displayed a strong dose dependent inhibitory effects to PrP^{res} formation (IC_{50}: 18.48 μM) and dissolution of PrP^{Sc} aggregates (IC_{50}: 41.56 μM) in the cell-free conversion assay, in contrast to baicalin which yielded IC_{50} values of >1000 μM for PrP^{res} inhibition and 440 μM for PrP^{Sc} dissolution. Epicatechin and quercetin, although highly similar in structural to baicalein, showed no inhibitory effects in cell-free conversion with regards to PrP^{res} formation and PrP^{Sc} disintegration.

In summary, these data demonstrate that tea of *S. lateriflora* contains two active compounds, baicalein and baicalin, which are specific inhibitors of PrP^{C} conversion and PrP^{res} amplification *in vitro* as well as enhance the degradation of PrP^{Sc} fibrils.

In another set of experiments *in vivo* effects of *S. lateriflora* tea were assessed. Wild-type mice were infected either by intracerebrally (i.c.) or intraperitoneally (i.p.) inoculation with mouse scrapie strain RML and the drinking water was replaced by freshly brewed tea starting 2 weeks prior to their inoculation and for the whole lifetime of the mice. Survival times are depicted in **Figure 3**. Six Bl6/C57 mice were used for the treatment and compared to six intracerebrally inoculated mice which received normal drinking water. The mean life spans of the control group (**Figure 5A**, bold line) was 147 ± 3.1 dpi (**Figure 3B**) whereas the tea treated mice (**Figure 5A** dashed line) survived significantly longer (184 ± 26.7 dpi; **Figure 3B**). Three of the treated mice survived up to 60 days compared to the control mice. In the case of i.p. inoculation of RML strain, a mean prolongation of 15 days was achieved (**Figures 3C,D**). The mean incubation time of the control group (**Figure 3C**, bold line) was 191 ± 8.6 dpi (**Figure 3D**) whereas the tea treated mice (**Figure 3C**, dashed line) survived significantly longer (206 ± 8.6 dpi; **Figure 3D**).

Brains of the i.c. infected mice were subjected to comparative biochemical and immunohistochemical investigations. PrP immunoblot analysis revealed no differences of PrP^{Sc} banding patterns and total amounts of PrP^{Sc} in both groups. The PrP^{Sc} content

in all brains was analyzed after PK digestion and selective precipitation of PrP^{Sc} from corresponding mouse brain homogenates with sodium phosphotungstic acid (**Figure 4**). No differences in PrP^{Sc} banding patterns or PK resistances was detected in mice which eventually developed scrapie in the tea treated and control group (**Figure 4**, lanes 2–13). The brain lesion profiles and PrP^{Sc} deposition patterns in the different mouse brain regions were similar for the treated and the untreated mice (**Figures 5A–D**).

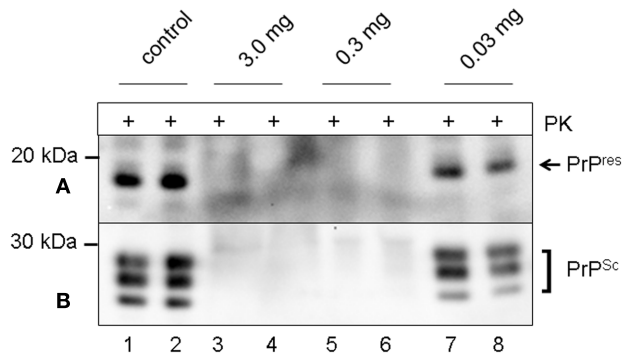

FIGURE 2 | Western blot analysis of inhibition of PrP^{res} formation and disaggregation of pre-existing PrP^{Sc} fibrils in a cell-free conversion assay. (A) Tea of *S. lateriflora* inhibits formation of PrP^{res}. Lanes 1–2 show PK-resistant PrP^{res}-fragments in samples without tea, lanes 3–4 samples after co-incubation with 3.0 mg dried tea extract, lanes 5–6 with 0.3 mg dried tea extract, and lanes 7–8 co-incubation with 0.03 mg dried tea extract. Detection was carried out with mab P4. **(B)** Tea of *S. lateriflora* disaggregates pre-existing fibrils after digestion with PK. The membrane from **(A)** was stripped and incubated another time with pab Ra10. Lanes 1–2 show PK-resistant PrP^{res}-fragments in samples without tea, lanes 3–4 samples after co-incubation with 3.0 mg dried tea extract, lanes 5–6 with 0.3 mg dried tea extract, and lanes 7–8 co-incubation with 0.03 mg dried tea extract. Western blot was analyzed by standard chemiluminescence method. +: incubation with proteinase K (PK).

DISCUSSION

The here presented study demonstrates that solubilized ingredients of *S. lateriflora* not only inhibit the scrapie fibril formation but also destabilize pre-formed PrP^{Sc} fibrils in a concentration dependent manner. Main constituents of the herb were the polyphenolic flavonoids baicalin and baicalein, which were found at high concentrations in corresponding extracts. Both components in purified form showed dose dependent inhibitory properties and thus were the active constituents responsible for the herbs effects on scrapie fibril regulation.

The strongest effects displayed baicalein, the main metabolite of baicalin, which inhibited PrP^{res} accumulation in both cell-based and cell-free assays and promoted the destabilization of PrP^{Sc} fibrils at low concentrations. A possible molecular mechanism is based on direct binding to the PrP^{C} and the formation of a stable PrP–baicalein/baicalin complex, which blocks PrP conversion and/or accumulation. In the same way the destabilization of pre-existing fibrils would be caused by a specific intercalation of baicalein/baicalin into β-sheet structures of the PrP^{Sc} aggregates which may lead to a forced PrP^{Sc} degradation by lysosomal proteases. Similar effects by Baicalein have been described for the inhibition of α-synuclein (Zhu et al., 2004) and amyloid-β peptide (Aβ) fibrillization (Lu et al., 2011).

Table 1 | Chemical structures of flavonoids and *in vitro* analyses.

Compound	Dot blot		IC_{50} (μM)		Cell-free conversion assay		IC_{50} (μM)	
	ScN_2A	SMB	ScN_2A	SMB	Prp^{res}	Prp^{Sc}	Prp^{res}	Prp^{Sc}
Baicalein	1.0 mM 0.1 mM 0.01 mM control	1.0 mM 0.1 mM 0.01 mM control	63.7	139	control 10 mM 1.0 mM 0.1 mM PK, 20 kDa	control 10 mM 1.0 mM 0.1 mM PK, 30 kDa	18.48	41.56
$\cdot xH_2O$	1.0 mM 0.1 mM 0.01 mM control	1.0 mM 0.1 mM 0.01 mM control	239.8	>1000	control 10 mM 1.0 mM 0.1 mM PK, 20 kDa	control 10 mM 1.0 mM 0.1 mM PK, 30 kDa	>1000	440
Epicatechin	1.0 mM 0.1 mM 0.01 mM control	1.0 mM 0.1 mM 0.01 mM control	96.9	106.8	control 10 mM 1.0 mM 0.1 mM PK, 20 kDa	control 10 mM 1.0 mM 0.1 mM PK, 30 kDa	n.e.	n.e.
Quercetin	1.0 mM 0.1 mM 0.01 mM control	1.0 mM 0.1 mM 0.01 mM control	36.8	274.3	control 10 mM 1.0 mM 0.1 mM PK, 20 kDa	control 10 mM 1.0 mM 0.1 mM PK, 30 kDa	n.e.	n.e.

IC_{50}, Half maximal (50%) inhibitory concentration; n.e., not effective.

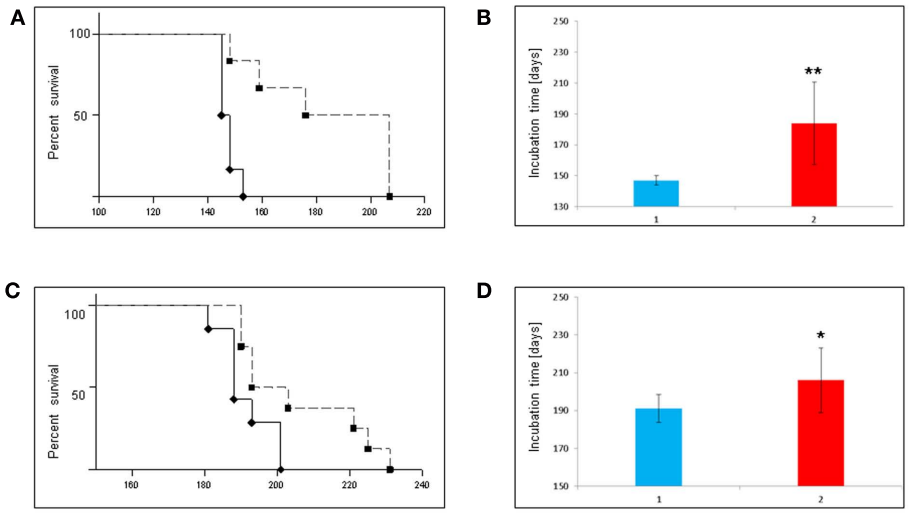

FIGURE 3 | Tea of *S. lateriflora* prolongs incubation time in scrapie-infected mice after oral treatment. (A) Oral treatment of intracerebrally infected mice: Kaplan–Meier survival analysis of intracerebrally scrapie (RML) infected C57/Bl6 mice after oral treatment with tea of *S. lateriflora*. Treatment groups consisted of untreated controls (♦), and tea treated mice (■). **(B)** Mean survival times in primary efficiency test ± SD. Comparison of tea versus untreated controls was carried out by log-rank test, $p < 0.01$ (**). **(C)** Oral treatment of intraperitoneally infected mice: Kaplan–Meier survival analysis of intraperitoneally scrapie (RML) infected C57/Bl6 mice after oral treatment tea of *S. lateriflora*. Treatment groups consisted of untreated controls (♦), and tea treated mice (■). **(D)** Mean survival times in primary efficiency test ± SD. Comparison of tea versus untreated controls, was carried out by log-rank test, $p < 0.05$ (*).

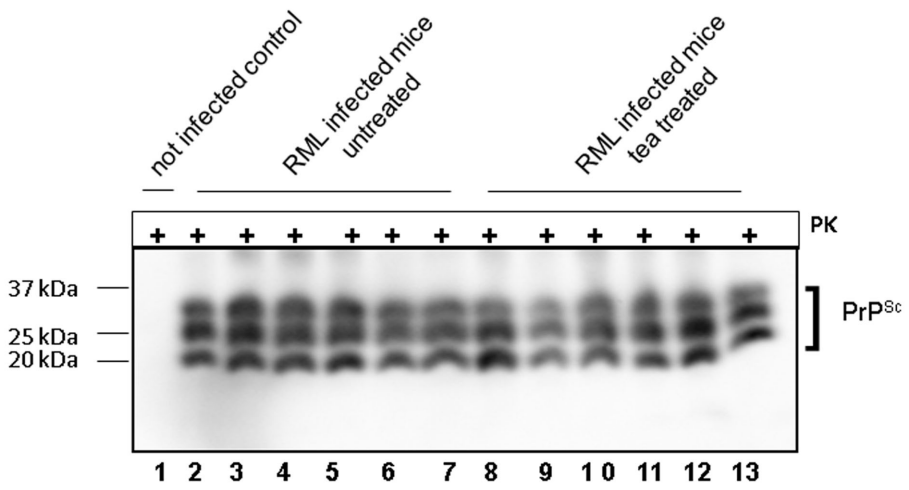

FIGURE 4 | Western blot analysis of sodium phosphotungstic acid precipitation of PrP^{Sc} from brain homogenate of RML infected mice. Proteinase K digested and sodium phosphotungstic acid precipitated PrP^{Sc} was detected by mab SAF-70. Lane 1 shows an uninfected control, lanes 2–7 PrP^{Sc} from RML infected mouse brains and lanes 8–13 PrP^{Sc} from RML infected mouse brains that were treated with tea from *S. lateriflora*. Western blot was carried out with pab Ra10 and analyzed by chemiluminescence method.

In our present study oral administration of *S. lateriflora* tea significantly prolonged incubation of mice either infected intracerebral or intraperitoneal. These effects can be explained by direct interference of baicalein to evolving PrP aggregates in the CNS. Baicalein is able to cross the blood brain barrier and ultimately reach the CNS (Tsai et al., 2002). Baicalin, on the other hand, may act as pro-drug: After oral uptake, the sugar residue is cleaved by enzymes in the intestinal tract to generate baicalein. The more lipophilic baicalein is better absorbed in the gastrointestinal tract and can then enter the brain via the blood system (Tarragó et al., 2008).

Within the CNS, the inhibition and dissolution of prion aggregates by Baicalein could protect neuronal cells from membrane disruption and subsequent neuronal cell death. Additional antioxidant properties of baicalein on reactive oxygen species (ROS), which evolve during protein aggregation, may reinforce this

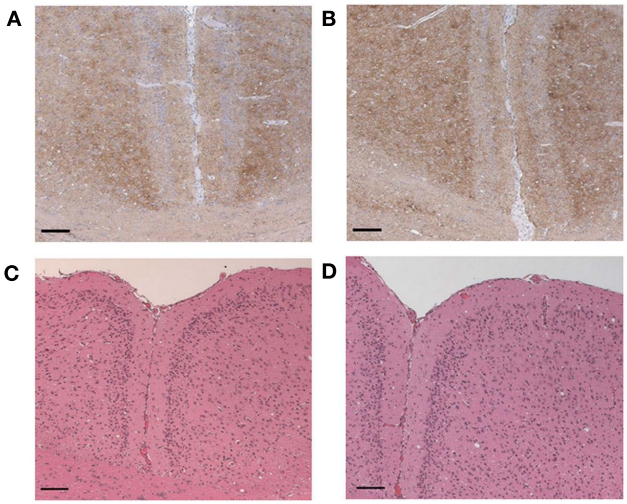

FIGURE 5 | PrP^{Sc} accumulation in the brains of mice challenged with RML. Immunohistochemistry: moderate diffuse PrP^{Sc} accumulation in the cerebral cortex and multifocally in the corpus callosum of both **(A)** untreated and **(B)** with tea treated mice. Detection was carried out with mab SAF 84. Bar 200 μm. H&E staining: mild vacuolation in the cerebral cortex of both **(C)** untreated and **(D)** with tea treated mice, H&E staining, Bar 200 μm.

neuroprotective effect (Li et al., 2010). Baicalein is able to minimize ROS by directly quenching free radicals or indirectly inducing antioxidant enzymes like superoxide dismutase and catalase (Shieh et al., 2000; Kang et al., 2011).

Direct effects on the prion conversion were also seen in other plant derived polyphenols like curcumin which is a strong inhibitor of prion conversion replication *in vitro* (Caughey et al., 2003). It is *in vivo* efficacy, however, remains controversial: the oral administration of curcumin had no significant effect on the onset of scrapie in hamsters (Caughey et al., 2003), whereas scrapie-infected mice significantly survived longer after oral treatment (Riemer et al., 2008). Effects of other polyphenols (e.g., tannic acid, katacine, and 2′-2″-*bis* epigallocatechin gallate) that interfere with PrP conversion *in vitro* were also studied *in vivo* (Kocisko et al., 2003). However, neither these substances nor mixtures of natural compounds including polyphenolic tea extract and pine bark extracts displayed any effects on incubation periods in scrapie-infected animals (Kocisko et al., 2004).

Up to now only few compounds have been reported that delay onset on prion diseases after oral treatment. This includes pravastatin (Vetrugno et al., 2009), which is involved in cholesterol biosynthesis and influences membrane structure and function, fucoidan (Doh-Ura et al., 2007), as well as amyloid dye derivatives (Kawasaki et al., 2007). However, possible drug associated side-effects have to be further evaluated. Recently, diphenyl-pyrazoles (Leidel et al., 2011) were identified as a new class of anti-prion compounds, which prolong incubation time of scrapie-infected mice even after oral application.

In conclusion, beneficial effects of *S. lateriflora* tea can be explained by the anti-aggregatory and potential anti-oxidative effects of its natural constituents, baicalein and baicalin. Only few studies were conducted with herbal extracts of *S. lateriflora* for prevention and therapy of protein misfolding diseases to date. Results shown here for prion diseases are promising also in the context of other diseases in this group.

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