The Tyrosine Kinase Inhibitor STI571 Induces Cellular Clearance of PrPSc in Prion-infected Cells*

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The conversion of the cellular prion protein (PrPc) into pathologic PrPSc and the accumulation of aggregated PrPSc are hallmarks of prion diseases. A variety of experimental approaches to interfere with prion conversion have been reported. Our interest was whether interference with intracellular signaling events has an impact on this conversion process. We screened ~50 prototype inhibitors of specific signaling pathways in prion-infected cells for their capacity to affect prion conversion. The tyrosine kinase inhibitor STI571 was highly effective against PrPSc propagation, with an IC50 of ≤1 μM. STI571 cleared prion-infected cells in a time- and dose-dependent manner from PrPSc without influencing biogenesis, localization, or biochemical features of PrPc. Interestingly, this compound did not interfere with the de novo formation of PrPSc but activated the lysosomal degradation of pre-existing PrPSc, lowering the half-life of PrPSc from ≥24 h to <9 h. Our data indicate that among the kinases known to be inhibited by STI571, c-Abl is likely responsible for the observed anti-prion effect. Taken together, we demonstrate that treatment with STI571 strongly activates the lysosomal degradation of PrPSc and that substances specifically interfering with cellular signaling pathways might represent a novel class of anti-prion compounds.

Prion diseases are fatal neurodegenerative infectious disorders that can manifest as sporadic, inherited, or acquired forms. They are also known as transmissible spongiform encephalopathies, which include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, kuru, familial insomnia, and variant CJD in humans, scrapie in sheep, and bovine spongiform encephalopathy in cattle. Prion diseases originate from the accumulation of an abnormally folded isoform of the cellular prion protein (PrPc), denoted PrPSc, which is the main component of infectious prions (1–3). Newly synthesized PrPc is transported along the secretory pathway through the endoplasmic reticulum and the Golgi to the plasma membrane or in the early compartments of the endocytic pathway, e.g. caveolae or rafts, specialized regions enriched in sphingolipids, cholesterol, and glycosyl phosphatidylinositol-anchored proteins (7, 8). The conversion process comprises profound changes in the structure and the biochemical properties of PrP. The α-helical conformation of PrPc is converted into the mainly β-sheeted structure of PrPSc. In contrast to PrPc, the pathogenic PrPSc is highly insoluble and partially resistant to proteolytic digestion (1–3, 9, 10). The exact mechanism of the conversion is still enigmatic, although two models are currently under consideration. The first model favors a crystallization reaction, where PrPSc acts as the crystal seed. Newly converted PrPSc molecules are added to that seed, forming PrPSc aggregates (11). The second model postulates a template-assisted conversion with intermediates, possibly PrPc−PrPSc heterodimer complexes (1, 9). A variety of experimental approaches for interfering with prion conversion have been reported. Some of them target PrPc, as the conversion can be blocked by removing the substrate of the process. This can be achieved by preventing the expression of PrPc (1, 3, 12) or by inhibiting its transport to the plasma membrane (13). A different mechanism has been suggested for the archetypical anti-prion compound Congo Red. Like chemical chaperones (14), Congo Red is thought to overstabilize PrPc in its equilibrium with suggested folding intermediates (1, 9, 15) in the prion conversion process (16). Other substances are thought to interfere with the interaction of PrPSc with complexes consisting of PrPc and folding intermediates. Such substances include β-sheet breakers, anti-PrP aptamers, and anti-PrP antibodies (16–24). There is evidence that additional cellular components are involved in prion conversion (factor X) (1, 16). A soluble PrP dimer molecule has been postulated to prevent the binding of such components to folding intermediates, thereby inhibiting PrPSc propagation (25). Only very few compounds like branched polyamines directly target PrPSc, increasing its intracellular clearance (26–28).

The physiological function of PrPc is not known. PrP-null mice develop normally and show no gross behavioral abnormalities (29), albeit alterations in circadian activity and sleep were reported (30). Furthermore, it has been shown that copper binds to the octarepeat-containing N-terminal region of PrPc, resulting in a suggested antioxidant activity of the protein (31). Therefore, a functional role for PrPc in copper metabolism and cellular protection against oxidative stress has been proposed (32). Recent work points to a role for PrPc in signal transduction. Antibody-mediated cross-linking of PrPc on the surface of the murine neuronal 1C11-differentiated cell line promotes the dephosphorylation and activation of Fyn kinase (33). A further implication of PrPc in neuronal survival and differentiation is...
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supported by the finding that different signal transduction pathways involved in neurite outgrowth and neuronal survival are elicited by PrP<sup>C</sup> (34), and PrP<sup>1<sub>C</sub>-<subröH</sub></sup> mice expressing N-terminally truncated PrP show severe neurodegeneration soon after birth (35). We have shown previously that PrP<sup>C</sup> interacts in <i>vitro</i> and in cultured cells with p110, synapsin 1, and grb2, the latter two representing proteins involved in cellular signaling (36). In the present study we investigated the effect of not cell signaling interference in PrP<sup>Sc</sup> propagation. Multiple substances were screened that interfere with specific signaling pathways in prion-infected cells for their effect on PrP<sup>C</sup>.

STI571 (Gleevec, imatinib mesylate), an inhibitor of the tyrosine kinase c-Abl, was found to be highly effective in inducing the clearance of PrP<sup>Sc</sup> in prion-infected cells without interfering with biogenesis, localization, and biochemical features of PrP<sup>C</sup>. Our results demonstrate that, although STI571 has no effect on the de novo formation of PrP<sup>Sc</sup>, it accelerates the lysosomal degradation of pre-existing PrP<sup>Sc</sup>.

**EXPERIMENTAL PROCEDURES**

Reagents—STI571 was purchased from Novartis Pharmaceuticals Corp. (Basel, Switzerland). Proteinase K, Pefabloc proteinase inhibitor, and pronase A were obtained from Boehringer Mannheim (Mannheim, Germany). Solvent A was prepared from Bio-Rad (Richmond, CA). 35S] Met/Cys (Promix; 1000 Ci/mmol) and [35S] Insulin (ICN, Costa Mesa, CA) were purchased from Amersham Biosciences. c-Abl substrate was from New England Biolabs (Beverly, MA). Cell culture media and solutions were obtained from Invitrogen (Karlsruhe, Germany). All other chemicals were from Roche Applied Science.

**Cell Culture and Mode of Drug Application**—The mouse neuroblastoma cell lines N2a (ATCC CCL 131), 3F4-PrP overexpressing N2a and ScN2a, the mouse hypothalamic cell line ScGT1, and the non-neuronal cell lines N2a (ATCC CCL 131), 3F4-PrP overexpressing N2a and ScN2a, the mouse hypothalamic cell line ScGT1, and the non-neuronal cell line SM2f15 have been described (13, 38, 39). Cells were maintained in Opti-MEM medium containing 10% fetal calf serum, antibiotics, and glutamine. STI571 was dissolved in DMSO to a stock solution of 10 mM (storage at −20 °C) and was added to the medium to a final concentration of 10 μM (if not otherwise stated). Suramin was dissolved in NaCl (0.9%) at a stock solution of 200 mg/ml (light protected at 4 °C) and was added to the medium to a final concentration of 10 μM (if not otherwise stated). A stock solution of 200 mg/ml (light protected at 4 °C) and was added to the medium to a final concentration of 10 μM (if not otherwise stated).

In all experiments mock-control cells were used that were treated with solvent in an identical fashion to drug-treated cells. Immu blot Analysis and Detergent Solubility Assay—Immunoblot analyses were performed as previously described (13). Confluent cell cultures were lysed in cold lysis buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM EDTA, 0.5% Triton X-100, 0.5% deoxycholate) for 10 min. For proteinase K (PK) treatment post-nuclear lysates were divided into two halves. One-half was incubated with PK (20 μg/ml) for 30 min at 37 °C, and digestion was stopped by the addition of protease inhibitor (5 mM phenylmethylsulfonyl fluoride, 0.5 mM Pefabloc, and aprotinin) and directly precipitated with ethanol. The sample without PK treatment was directly supplemented with proteinase inhibitors and precipitated with ethanol. After centrifugation for 30 min at 3500 rpm (4 °C), the pellets were re-disolved in TNE buffer, and gel-loading buffer was added. After boiling for 5 min, an aliquot was analyzed on 12.5% SDS-polyacrylamide electrophoresis gels. For solubility assay, post-nuclear cell lysates were supplemented with proteinase inhibitors and N-lauryl sarcosine to 1% and ultracentrifuged in a Beckman TL-100 table ultracentrifuge for 1 h at 40,000 rpm (100,000 × g; TLA-45 rotor; 4 °C). Supernatant fractions were precipitated with ethanol, and pellet fractions were resuspended in 50 μl of TNE (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA) and analyzed in immunoblot or radioimmunoprecipitation assay.

**PIPLC Treatment**—For PIPLC treatment, ~80% confluent cells were washed twice with PBS. 200 milliliters of PIPLC (Sigma) and STI571 were added in 4 ml of serum-free Opti-MEM medium and incubated for 4 h at 37 °C. The medium was collected, and the cells were washed extensively with PBS and lysed as described for immunoblotting. After a methanol precipitation of the medium and the cell lysate, both fractions were analyzed in immunoblot.

**Metabolic Radiolabeling and Immunoprecipitation Assay**—Metabolic radiolabeling and immunoprecipitation assays were done as described previously (15). In brief, confluent cells were washed twice with PBS and incubated for 1 h in RPMI without methionine/cysteine containing 10% fetal calf serum. Due to the fact that 10% fetal calf serum is more convenient for the cells and had no negative influence on the labeling efficiency in our hands, the medium was supplemented with 800 μCi/ml l-[<sup>35</sup>S]Met/Cys (Amersham Biosciences) for 16 h. For analysis of the de novo generation of PrP<sup>Sc</sup>, ScN2a cells were simultaneously treated with STI571 (10 μM) or with suramin (200 μg/ml) (no chase). For determination of PrP<sup>Sc</sup>-half-life, STI571 (30 μM) was added only during the chase period. For both experiments, cells were washed twice in ice-cold PBS and lysed in cold lysis buffer (100 mM NaCl, 10 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.5% Triton X-100, 0.5% deoxycholate). Insoluble material was removed by centrifugation at 14,000 rpm for 1 min. Then lysates with and without PK treatment were subjected to radioimmunoprecipitation assay as described above. Lysates were re-suspended in 100 μl of radioimmunoprecipitation assay buffer (0.5% Triton X-100, 0.5% deoxycholate in PBS) with 1% SDS and boiled for 10 min. The pellet fraction was diluted with 900 μl of radioimmunoprecipitation assay buffer (supplemented with 1% sarkosyl), and the primary antibody (1:100) was incubated overnight at 4 °C. Protein A-Sepharose beads were then added for 60 min at 4 °C. The immunoadsorbed proteins were washed in cold radioactive precipitation assay buffer supplemented with 1% SDS, subjected to a deglycosylation step with peptide N-glycosidase F at 37 °C, and analyzed on 12.5% SDS-PAGE followed by autoradiography.

**FACS Analysis**—For surface protein analysis, STI571-treated (10 μM) confluent or mock-treated cells were suspended with PBS containing 1 mM EDTA, centrifuged, resuspended in FACS buffer (PBS with 2.5% fetal calf serum and 0.05% sodium azide), and incubated for 5 min on ice. Primary antibody (1:100) was incubated in a 1:100 dilution in FACS buffer for 45 min on ice and washed 3 times in FACS buffer, and the secondary antibody (fluorescein isothiocyanate-labeled, 1:100) was incubated for another 45 min. After the last wash cells were resuspended in FACS buffer with propidium iodide (2 μg/ml). Cells incubated only with the secondary antibody served as a control. The FACS analysis was performed in a BD Biosciences FACS Calibur apparatus. In total, from each sample 10,000 living cells, as determined by the propidium iodide staining, were analyzed.

**Laser-scanning Microscopy**—Cells were plated on poly-L-lysine-coated glass coverslips (Marienfeld, Bad Mergentheim, Germany) at low density 1–3 days before staining. Polyclonal antibody A7 was applied for 1 h on ice. Then cells were fixed in 3% paraformaldehyde for 30 min at room temperature. After sequential treatment with NH<sub>4</sub>Cl (50 mM in 20 mM glycine), Triton X-100 (0.5%), and gelatin (0.2%) for 10 min each at room temperature, a fluorescent isothiocyanate-labeled secondary antibody (1:100 in PBS) was added and incubated for 30 min at room temperature. Slides were mounted in anti-fading solution (Histogel; Linaris, Wertheim-Bettingen, Germany) and kept dry at −20 °C. Confocal laser-scanning microscopy was done using a Leica TCS NT/DIMMB Confocal System (Heerbrugg, Switzerland).

**Transient Expression of Trans-dominant Negative c-Abl Mutant**—For generation of a trans-dominant negative mutant of c-Abl tyrosine kinase, the lysine residue at position 271 was mutated to a methionine. This mutation is located in the ATP binding domain of the protein, leading to a complete inactivation of the kinase. As vector we used...
of ~50 prototype compounds in ScN2a cells.

RESULTS

STI571 Reduces PrP<sup>Sc</sup> in a Time- and Dose-dependent Manner in Prion-infected Cells—Because various studies suggest a possible role of PrP<sup>C</sup> in cellular signaling, we screened about 50 prototype inhibitors known to interfere with specific intracellular signal transduction pathways in prion-infected neuronal cells for their effect on PrP<sup>Sc</sup> propagation (by proteinase K digestion and immunoblotting). The initial screening was done by adding the compounds for 3 days at a concentration of 10 μM to the culture medium (Fig. 1). Compounds that had no detectable effect on PrP<sup>Sc</sup> were excluded from further analysis. Compounds that were obviously toxic for the cells and those that had an effect on both PrP<sup>Sc</sup> and PrP<sup>C</sup> were tested again at a concentration of 2 μM for 3 days. Most remaining compounds either had no effect on PrP<sup>Sc</sup> or also profoundly affected PrP<sup>C</sup>. Several substances were toxic to the cells even at low concentrations. Among the compounds tested, only one substance (STI571, signal transduction inhibitor 571, also known as imatinib mesylate, Gleevec®) that had a highly significant effect on PrP<sup>Sc</sup> propagation without cytotoxicity (as revealed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and trypan blue staining, data not shown) was identified. Treatment of prion-infected ScN2a, ScGT1, and SMBs.15 cells with STI571 for 3 days (ScN2a and ScGT1) or 10 days (SMBs.15), respectively, reduced PrP<sup>Sc</sup> to undetectable levels (Fig. 2A, lanes 1–4, 9, and 12). In contrast, mock-treated cells showed significant amounts of PrP<sup>Sc</sup> (Fig. 2A, lanes 2, 6, and 10, respectively). The reduction of PrP<sup>Sc</sup> was found to be time- and dose-dependent (Fig. 2, B and C). ScN2a cells treated for 1 day with 10 μM STI571 harbored the same amount of PrP<sup>Sc</sup> as mock-treated control cells. After the second day, the signal for PrP<sup>Sc</sup> was significantly reduced and was undetectable after 3 days of treatment (Fig. 2B, lanes 1–8). ScN2a cells treated for 6 days with different concentrations of STI571, varying from 0.25 to 10 μM (procedure according to East et al. 41), demonstrated a slight reduction of PrP<sup>Sc</sup> with 0.25 μM STI571. Treatment with 1 μM STI571 led to a significant reduction of PrP<sup>Sc</sup> to less than 10% of the amount present in the mock-treated control (Fig. 2C, lanes 1–7). Under these experimental conditions the IC<sub>50</sub>, which is defined here as the concentration with a 50% inhibitory effect on PrP<sup>Sc</sup> propagation, was measured to be between 0.75 and 1 μM. Because the optimal results for the clearance of PrP<sup>Sc</sup> in ScN2a cells were obtained with treatment for 3 days with 10 μM STI571, we decided to use a concentration of 10 μM for treatments longer than 1 day and 20 μM for 1-day treatments. In a longer term experiment, the efficiency of PrP<sup>Sc</sup> clearance by STI571 was investigated. To test whether the observed effect on PrP<sup>Sc</sup> is reversible or whether the cells remain free of PrP<sup>Sc</sup> even after the removal of the drug (Fig. 2D), ScN2a cells were treated for 10 days with 10 μM STI571. In immunoblotting assays, the signal for PrP<sup>Sc</sup> entirely disappeared in treated cells (lanes 1–4). The cells were then cultured for further 30 days without the drug. Every 10 days, an aliquot of cells was tested for the presence of PrP<sup>Sc</sup> by immunoblotting. Even after 30 days without STI571 treatment, PrP<sup>Sc</sup> could not be detected (lanes 5–16). Taken together, our studies show that STI571 is able to reduce PrP<sup>Sc</sup> in a time- and dose-dependent manner in various prion-infected cell lines and that this clearance is irreversible.

The Biogenesis and Localization of PrP<sup>P</sup> Are Not Affected by STI571—Because PrP<sup>Sc</sup> propagation is dependent on a preceding PrP<sup>P</sup> localization at the plasma membrane, we tested whether the clearance of PrP<sup>Sc</sup> by STI571 is related to changes in the expression, biochemical features, or localization of PrP<sup>P</sup>. Previous experiments demonstrated that there is no obvious difference in the expression level of PrP<sup>P</sup> between treated and mock-treated cells (Fig. 2, A–D). To address the subcellular localization of PrP<sup>P</sup> in more detail, we first performed a diges-
FIG. 2. STI571 inhibits PrPSc-propagation in prion-infected cells. A, the chemical structure of STI571 (left). PrPSc propagation is abolished by STI571. ScN2a (lanes 1–4), ScGT1 (lanes 5–8), and SMBs.15 cells (lanes 9–12) were mock-treated (− STI571) or treated (+ STI571) for 3 days with 10 μM (ScN2a) or 5 μM (ScGT1) or for 10 days with 10 μM STI571 (SMBs.15), respectively. Cell lysates were either left untreated (− PK) or digested with PK (+ PK) and analyzed in immunoblotting using mAb 3F4 (lanes 1–4) or mAb 4H11 (lanes 5–12). Bars on the right indicate the three bands specific for non-, mono-, and diglycosylated PrP. B, the effect of STI571 on PrPSc propagation is time-dependent. ScN2a cells were mock-treated or treated for 1, 2, or 3 days with 10 μM STI571. Cell lysates were either subjected to PK digestion (+ PK) or left untreated (− PK) and analyzed in immunoblotting using mAb 3F4. Bars on the right indicate PrP-specific bands. C, the IC50 of STI571 is 1 μM. ScN2a cells were mock-treated (0/0) or treated for 6 days with rising concentrations of STI571 (lanes 2–7). The medium was changed every day. Cell lysates were digested with PK and analyzed in immunoblotting using mAb 4H11. D, STI571 irreversibly removes PrPSc from prion-infected cells. ScN2a cells were mock-treated (0/0) or treated for 10 days with 10 μM STI571 (10/0). After removal of STI571, the untreated and treated cells were cultured for further 10 (0/10, 10/10), 20 (0/20, 10/20), or 30 (0/30, 10/30) days. Cell lysates were left untreated (− PK) or digested with PK (+ PK) and analyzed in immunoblotting using mAb 3F4. The figure shows the composition of individual comparable immunoblots.
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**Fig. 3.** STI571 does not influence PrP\textsuperscript{Sc}. A, PrP\textsuperscript{Sc} can be released by PIPLC from the plasma membrane. ScN2a and N2a cells were treated for 3 days with 10 \mu M STI571 (lanes 5–8 and 11–16, respectively) or left untreated (lanes 1–4, and 9, 10, 13, and 14, respectively). Subsequently, a PIPLC digestion of all cells was performed. Cell lysates (L) and media (M) fractions were analyzed in immunoblot analysis using the mAb 3F4 (ScN2a) and 4H11 (N2a). Bars on the left indicate PrP-specific bands. B, STI571 treatment does not affect surface localization of PrP. Indirect immunofluorescence and confocal microscopy were performed with 3F4-N2a cells cultivated for 3 days in the absence (left panel) or presence (right panel) of STI571 (10 \mu M). C, amount of surface PrP\textsuperscript{Sc} is not reduced by STI571. 3F4-N2a cells were treated for 3 days with 10 \mu M STI571 or mock-treated. Levels of surface PrP\textsuperscript{Sc} were analyzed by surface FACS analysis. As a control, the primary antibody was omitted (left and middle panel). In the right panel an overlay of the signals of mock-treated and STI571-treated cells (10 \mu M for 3 days) is shown. From each sample, 10,000 living cells were analyzed. D, no induction of insoluble PrP. Lysates of treated (10 \mu M for 3 days) (lanes 3 and 4) or mock-treated (lanes 1 and 2) ScN2a cells were subjected to a solubility assay. Supernatant (S) and pellet (P) fractions were analyzed in immunoblot. For detection of PrP-specific signals, mAb 3F4 was used. The molecular weight marker is shown on the left. Bars on the right indicate PrP-specific bands.

STI571 Decreases the Half-life of PrP\textsuperscript{Sc} by Inducing Its Lysosomal Clearance—Our previous studies showed that STI571 clears prion-infected cultured cells from PrP\textsuperscript{Sc}. To characterize the effect of STI571 on PrP\textsuperscript{Sc} propagation in more detail, we analyzed the de novo formation of PrP\textsuperscript{Sc} in cells treated with STI571. Immunoprecipitation assays of radioactively labeled PrP\textsuperscript{Sc} were performed. ScN2a cells were metabolically labeled overnight in the presence of STI571. As a control, ScN2a cells were treated with the compound suramin, a drug that inhibits the de novo formation of PrP\textsuperscript{Sc}. Cells were lysed, half of each lysate was subjected to digestion with PK, and a solubility assay was performed. After immunoprecipitation of the insoluble pellet fraction with an anti-PrP antibody, the samples were deglycosylated and analyzed by autoradiography (Fig. 4A). Whereas in cells treated with suramin PrP\textsuperscript{Sc} was not detectable (lane 5), there was a signal for newly synthesized PrP\textsuperscript{Sc} (19 kDa) in the control cells and in cells treated with STI571 (lanes 4 and 6). In suramin-treated cells aggregated and, therefore, insoluble PrP\textsuperscript{Sc} migrated at a higher molecular weight (lane 2). Under STI571 treatment, full-length, deglycosylated PrP\textsuperscript{Sc} remained soluble and could not be detected in the pellet fraction (lane 3). These data show that STI571 does not significantly affect the de novo formation of PrP\textsuperscript{Sc} but induces the degradation of pre-existing PrP\textsuperscript{Sc}. To confirm this observation and to analyze the half-life of PrP\textsuperscript{Sc} under STI571 treatment, ScN2a cells were metabolically labeled overnight. Cells were either harvested directly or cultured further in the presence or absence of 20 \mu M STI571. At different time points, the cells were lysed, digested with proteinase K, and subjected to a solubility assay. Subsequently, the insoluble PrP was immuno-

A biochemical hallmark of PrP\textsuperscript{Sc} is its solubility in non-ionic detergents, whereas PrP\textsuperscript{Sc} forms insoluble aggregates under such conditions. Alterations of the folding and aggregation behavior of PrP\textsuperscript{Sc} is often followed by changes in its solubility. Therefore, we examined the solubility of PrP\textsuperscript{Sc} after 3 days of treatment with STI571 in a standard solubility assay (Fig. 3D). In mock-treated ScN2a cells, soluble PrP\textsuperscript{Sc} could be detected in the supernatant, and insoluble PrP\textsuperscript{Sc} could be detected in the pellet fraction. The same behavior was observed in cells treated with STI571. PrP\textsuperscript{Sc} remained soluble and was present in the supernatant, and insoluble PrP was not detectable in the pellet fraction. In summary, STI571 clears prion-infected cells of PrP\textsuperscript{Sc} without interfering with the expression level, the localization, or biochemical properties of PrP\textsuperscript{Sc}.
precipitated and deglycosylated (Fig. 4B). In mock-treated control cells, the signal for PrPSc did not change during the chase points, corresponding to the predicted 24-h half-life of PrPSc in these cells (lanes 1–6) (42). However, there was a rapid decrease in the signal from cells treated with STI571, indicating that PrPSc was degraded more rapidly (lanes 7–12). Fig. 4C shows the densitometric evaluation of three independent experiments. Taken together, the treatment with STI571 induced the degradation of pre-existing PrPSc and lowered the half-life of PrPSc from more than 24 h to less than 9 h.

Because STI571 obviously activates the intracellular degradation of PrPSc, we asked whether the simultaneous treatment with an inhibitor of proteolytic degradation could counteract this effect. Therefore, we analyzed the effect of STI571 on ScN2a cells in the presence of ammonium chloride (NH4Cl) by immunoblot analysis. NH4Cl is known to inhibit lysosomal degradation by raising the lysosomal pH. The cells were mock-treated or treated for 3 days with 10 μM STI571. Then the medium was changed, and the cells were kept for one further day in medium containing either 10 μM STI571 or 10 mM NH4Cl or both substances. Mock-control cells were treated with Me2SO. Then the cells were harvested, and half of each lysate was PK-digested (+PK) or left untreated (−PK) and, after methanol precipitation, analyzed in immunoblot using the mAb 4H11.

Thus, treatment with STI571 decreases the half-life of PrPSc in prion-infected cells by inducing its cellular clearance. The finding that this degradation can be counteracted by the lysosomal inhibitor NH4Cl suggests that STI571 activates the lysosomal degradation of PrPSc.

The Effect of STI571 on PrPSc Is Presumably Caused by Inhibition of the Tyrosine Kinase c-Abl—The studies above have shown that STI571 is able to reduce PrPSc in prion-infected cells by inducing its cellular degradation. We were then interested in further characterizing the cellular signaling pathways that are affected by STI571, thus leading to the degradation of PrPSc. The known targets of STI571 are the tyrosine kinases c-Abl, the
PDGF receptor, and c-Kit (43, 44). To specify the signal transduction pathways that are involved in the induction of the degradation of PrPSc, the specific tyrosine kinase responsible for the observed anti-prion effect needed to be determined. Therefore, ScN2a cells were treated for 3 days with CT52923, a substance that specifically inhibits PDGF receptor and c-Kit kinase without having an effect on the activity of c-Abl (40). In a cell-based assay the autophosphorylation of PDGF receptor and c-Kit is inhibited by CT52923, with an IC50 of about 100 and 200 nM, respectively (40). The IC50 for CT52923 in inhibition of cellular responses to PDGF-like proliferation and migration has been reported to be 280 and 64 nM, respectively (40). Therefore, cells were treated accordingly with concentrations up to 30 μM, the highest concentration that was non-toxic. However, even at this concentration of CT52923 no effect on PrPSc could be detected, in contrast to STI571-treated cells (Fig. 5A, lane 6 and 8). A further experiment to confirm the specificity of c-Ab was to mimic the effect of STI571 in the cells by overexpressing a trans-dominant negative mutant of the kinase that causes an abrogation of the activity of endogenous c-Abl. ScN2a cells were transiently transfected with a trans-dominant negative mutant of c-Abl and after 4 days were assayed for PrPSc by PK digestion and immunoblottting (Fig. 5B). In cells transiently expressing the c-Abl mutant there was a significant reduction of PrPSc (lane 6) as compared with untransfected (lane 2) and mock-transfected cells (lane 4). To investigate the effects of wild type c-Abl overexpression on PrPSc, ScN2a cells were transfected with a wild type c-Abl construct. Unfortunately, 2 days after transfection the cells stopped growing and began to die (data not shown). The observed effects are consistent with reports of cytostatic and cytotoxic effects of overexpressed c-Abl (45–47). To analyze the inhibitory effect of STI571 on endogenous c-Abl in N2a and prion-infected N2a cells, we performed in vitro kinase assays. C-Abl was immunoprecipitated from equal amounts N2a and ScN2a cell lysates and subjected to an in vitro kinase assay using a specific c-Abl substrate peptide and [γ-32P]ATP. The kinase activity of the reaction with three different concentrations of STI571 was compared with the activity in the reaction without STI571 (7). Negative controls were a reaction in which the immunoprecipitation was performed without anti-c-Abl-antibody (−Ab) and a reaction without c-Abl substrate (−substrate). Samples were spotted on a P81 filter, and the kinase activity was measured by scintillation counting.

**DISCUSSION**

This study aimed to investigate whether interfering with cellular signal transduction can interfere with PrPSc accumulation. We performed a screen of well characterized compounds known to interfere with specific signaling pathways to examine their effect on PrPSc propagation in prion-infected cells. The tyrosine kinase inhibitor STI571 was, thus, identified as the compound with the most pronounced effect on PrPSc.

**STI571, a Compound with Applications in Modern Cancer Therapy**—With STI571 we describe a new class of compounds with a significant effect on PrPSc in prion-infected cultured cells. STI571 (signal transduction inhibitor 571), also known as Gleevec® or imatinib mesylate, is a derivative of 2-phenylaminopyrimidine and inhibits the tyrosine kinase c-Abl by blocking its ATP binding site (43). It has been developed against chronic myeloid leukemia. Chronic myeloid leukemia is...
caused by Bcr-Abl, the product of the t(9;22) Philadelphia chromosome translocation. In this fusion protein, the kinase activity of c-Abl is constitutively active, causing an unregulated proliferation of blood cells. STI571 also inhibits the PDGF receptor and the c-Kit kinase (43, 44). Dereglulation of c-Kit activity in gastrointestinal stromal tumors was shown to be efficiently inhibited by the compound (48). Although STI571 inhibits the wild type kinases in healthy cells, there are only minor side effects, probably due to redundancy in signal transduction pathways. Since May 2001 STI571 has been available to patients as an oral treatment against chronic myeloid leukemia, and since February 2002, it has also been prescribed against gastrointestinal stromal tumors. In addition, STI571 may be of potential therapeutic use against the neurodegenerative disorder Alzheimer’s disease. In a recent report it has been shown that STI571 inhibits the β-amyloid production without interfering with the cleavage of Notch-1 by the γ-secretase complex. The authors suggested STI571 to be a useful tool for the development of novel therapies for Alzheimer’s disease (49).

Compounds exhibiting anti-prion activity in prion-infected cells include Congo Red (50), dextran sulfate, pentosan polysulfate (51), suramin (13), branched polyamines like poly(pro-lyleineimine) (PPI) dendrimers and poly(ami)doamine) (PAMAM) dendrimers (26–28), acridine derivatives like quinacrine and chlorpromazine (41), and lovastatin (52). We have demonstrated that STI571 significantly reduces PrPSc without influencing PrPc. This makes the drug a potential candidate for prophylaxis and therapy, if some important requirements are met. (i) Results from cell culture studies usually cannot be transferred directly to the in vivo situation. For example, quinacrine has been shown to interfere with the de novo formation of PrPSc in vivo (41). Unfortunately, when tested therapeutically it did not prolong the survival of prion-infected mice (53). Nevertheless, quinacrine might be a candidate for a post-exposure prophylaxis, as is the case for suramin (13). (ii) Similarly, the human situation may not be reflected by a murine in vivo system. The metabolism of STI571, as observed in mice, is completely different from that in humans. The biological half-life of the drug upon oral application is about 12–16 h in humans, whereas in mice it is less than 4 h (54). Therefore, it is difficult to achieve and maintain the necessary concentration of STI571 in the blood of mice. (iii) A therapeutic anti-prion drug has to reach the CNS and, therefore, must cross the blood-brain barrier (55). It has been reported that only about 2.8% of orally administered STI571 is found in the cerebrospinal fluid (56). This amount might not be sufficient to elicit a therapeutic anti-prion effect. If STI571 or derivatives could overcome the discussed problems, they could be considered as candidates for in vivo studies in animals or eventually in humans. The results with STI571 in vitro demonstrate a proof of principle that this signal transduction inhibitor is able to interfere with PrPSc propagation. Therefore, signal transduction pathways should be considered as potential targets for prophylaxis and therapy of prion diseases.

**Anti-prion Effect by Inhibition of c-Abl?**—STI571 specifically inhibits the tyrosine kinases c-Abl, c-Kit, and the PDGF receptor (43, 44). Because each tyrosine kinase plays a role in specific signal transduction pathways, we tried to specify the kinase pathway that is responsible for the observed anti-prion effect in prion-infected cells. Our experiments revealed that specific inhibition of the c-Kit kinase and the PDGF receptor had no effect on PrPSc propagation. In contrast, abrogation of endogenous c-Abl activity by overexpression of a trans-dominant negative c-Abl mutant led to a decrease of PrPSc in ScN2a cells. The reported IC50 of STI571 for c-Abl activity ranges between 0.1 and 0.3 μM for the inhibition of autophosphorylation (in vitro) and <1 μM for the inhibition of cellular proliferation (as determined by cell culture experiments) (57). We observed a comparable difference between the IC50 of STI571 in vitro (~0.1 μM) and that determined for the anti-prion effect in cell cultures (~1 μM). The wide range is likely due to the diverse effects that are measured. The in vitro assays show the direct effect of STI571 on the phosphorylation of a synthetic substrate of c-Abl. The anti-prion effect, calculated in cell culture experiments, is an indirect effect lying downstream of a signaling cascade. Therefore, it is difficult to compare IC50 values determined by the two different assays. A concentration of STI571 leading to a 50% inhibition of the phosphorylation of a synthetic substrate in an in vitro kinase assay most probably is not sufficient to activate the degradation of PrPSc, resulting in a 50% reduction of PrPSc in prion-infected cells. Based on our studies we conclude that among the known targets of STI571, the tyrosine kinase c-Abl is responsible for the enhanced lysoosomal degradation observed in our experiments. Interestingly, c-Abl seems not to be involved in the observed reduction of β-amyloid production. STI571 inhibited the production of β-amyloid in wild type as well as in c-Abl−/− mouse fibroblasts (49). This would suggest that different mechanisms underlie the anti-prion effect and the effect of STI571 on the β-amyloid production.

C-Abl is a ubiquitously expressed protein that has a variety of functions, but little is known about its activators and targets. In neurons and hematopoetic cells, c-Abl is primarily located in the cytosol, where it is involved in cytoskeletal rearrangement. In most other cell lines, c-Abl is found in the nucleus and has various functions. Under normal conditions, c-Abl is inactive or is strictly regulated. Constitutively active c-Abl has transforming properties (58), and overexpression of c-Abl in cell culture induces cell cycle arrest in G1 with subsequent apoptosis (45–47). This is consistent with the failure of overexpressing c-Abl in our cell culture experiments. Studies to further characterize the underlying anti-prion mechanism, the affected downstream signaling cascade that leads to the activation of the degradation, and the involved proteins are in progress. The results might help define the normal cellular PrPSc degradation mechanism and indicate potential targets for the therapy of prion diseases.

Clearance of PrPSc without Interference within Biogenesis and Localization of PrP—The treatment of prion-infected cells with STI571 leads to a time- and dose-dependent decrease of PrPSc. The clearance of PrPSc from treated cells was irreversible, and even 30 days after the removal of STI571, PrPSc did not reappear. Bioassays in mice are in progress to confirm that STI571-treated cells are also cleared of specific prion infectivity. Cell specific differences were observed when ScGT1 and SMBs.15 cells were treated with STI571. ScGT1 and ScN2a cells were both almost equally sensitive to STI571. After a treatment for 3 days, PrPSc was undetectable in both cell types. In the non-neuronal SMB cell line, the anti-prion effect could only be seen after treatment for 10 days. Cell type-specific effects might be due to signaling differences and could also be explained by differences in the protein expression of the cells. Of note, both neuronal cell lines were highly sensitive to STI571, whereas the more resistant SMBs.15 cells are of non-neuronal origin (39). A compound can inhibit PrPSc propagation in prion-infected cells by various mechanisms. For example, inhibition can be caused by overstabilizing the conformation of PrP molecules as shown for Me3SO and Congo Red (14, 59) or by down-regulating the amount of PrP in the cell. Suramin induced the formation of insoluble PrP aggregates and the intracellular re-routing of PrP (13). Thus, PrP was removed from the cell surface and was no longer available
for the conversion into PrPSc. Therefore, it was mandatory to analyze possible effects of STI571 on PrP. In contrast, confocal microscopy and surface FACS analyses showed no difference in the subcellular localization of PrP between mock-treated cells and cells treated with STI571. This was biochemically confirmed by PIPLC digestion. Solubility assays demonstrated that the biochemical features of PrPw were not changed by STI571 treatment. We conclude that the anti-prion effect of STI571 is not due to an altered localization or biochemical behavior of PrPw, as has been reported for other drugs, suggesting the cellular isof orm of the prion protein is not affected by STI571.

Inducing the Lysosomal Degradation of Pre-existing PrPSc—Many substances have been identified that inhibit PrP Sc propagation in experimental systems. Interestingly, almost all of the anti-prion compounds interfere with the de novo formation of PrPSc. For branched polyamines, however, it has been reported that they affect pre-existing PrPSc and that they act in lysosomes. These studies also raised the possibility that lysosomal proteases normally degrade PrPSc in cultured cells. The authors claimed that branched polyamines could render PrPSc molecules protease-sensitive by dissociating PrP Sc aggregates or that they could facilitate the transport of PrP Sc from the membrane into secondary lysosomes (26, 27). Similarly, the two anti-prion compounds quinacrine and chloroquine were reported to act in lysosomes, eventually by altering the lysosomal pH (60). STI571 has no major effect on the de novo conversion of PrPw to PrPSc but induces the degradation of pre-existing PrPSc by decreasing its half-life from more than 24 h to less than 9 h. We were able to inhibit this effect with NH₄Cl, or that they could facilitate the transport of PrP Sc from the membrane into secondary lysosomes (26, 27). Similarly, the two anti-prion compounds quinacrine and chloroquine were reported to act in lysosomes, eventually by altering the lysosomal pH (60). STI571 has no major effect on the de novo conversion of PrPw to PrPSc but induces the degradation of pre-existing PrPSc by decreasing its half-life from more than 24 h to less than 9 h. We were able to inhibit this effect with NH₄Cl, which blocks the degradation in lysosomes by raising the lysosomal pH. Therefore, we suggest that STI571 activates the lysosomal degradation of pre-existing PrPSc. Notably, treatment with NH₄Cl alone seemed to lead to a slightly increased PrPSc accumulation. This confirms the observation that lysosomal proteases are involved in the normal degradation of PrPSc in prion-infected cells and that STI571 activates this process. Interestingly, the effect of branched polyamines on PrPSc accumulation could not be inhibited by NH₄Cl (26, 27). Therefore, STI571 and these compounds employ different pathways. It is important to note that the amount of PrPw was not influenced by STI571 treatment, indicating that PrPw and PrPSc are degraded by different proteolytic activities. This is, therefore, not contradictory to studies with proteasomal inhibitors that induce the accumulation of self-perpetuating “PrPSc-like” aggregates in the cytosol (61, 62). The authors claim that defective proteasomal degradation might have an important role in the origin of PrPSc. This might be of relevance for the spontaneous initiation in the sporadic or familial forms of prion diseases. Nevertheless, further propagation of PrPSc and the spread from cell to cell primarily occurs on the cell surface and/or in early compartments of the endocytic pathway rather than in the cytoplasm. Therefore, the cellular clearance of PrPSc is most probably carried out by lysosomal proteases in secondary lysosomes.

In summary, our data show that STI571 activates the cellular clearance of PrPSc in prion-infected cells, most probably by inhibition of the tyrosine kinase c-Abl. Clearance occurs in lysosomes, as lysosomal inhibition can abolish the effect. Moreover, our finding that inhibition of lysosomes alone led to an increase in PrPSc is consistent with the model in which lysosomes play an important role in the degradation of PrPSc in prion-infected cells.

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