STI571 prevents apoptosis, \textit{tau} phosphorylation and behavioural impairments induced by Alzheimer’s β-amyloid deposits

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There is evidence that amyloid β-protein (Aβ) deposits or Aβ intermediates trigger pathogenic factors in Alzheimer’s disease patients. We have previously reported that c-Abl kinase activation involved in cell signalling regulates the neuronal death response to Aβ fibrils (Aβf). In the present study we investigated the therapeutic potential of the selective c-Abl inhibitor STI571 on both the intrahippocampal injection of Aβf and APPsw/PSEN1ΔE9 transgenic mice Alzheimer’s disease models. Injection of Aβf induced an increase in the numbers of p73 and c-Abl immunoreactive cells in the hippocampal area near to the lesion. Chronic intraperitoneal administration of STI571 reduced the rat behavioural deficit induced by Aβf, as well as apoptosis and tau phosphorylation. Our in vitro studies suggest that inhibition of the c-Abl/p73 signalling pathway is the mechanism underlying the effects of STI571 on Aβ-induced apoptosis for the following reasons: (i) Aβf induces p73 phosphorylation, the TAp73 isoform levels increase so as to enhance its proapoptotic function, and all these effects where reduced by STI571; (ii) c-Abl kinase activity is required for neuronal apoptosis and (iii) STI571 prevents the Aβ-induced increase in the expression of apoptotic genes. Furthermore, in the Aβf-injected area there was a huge increase in phosphorylated p73 and a larger number of TAp73-positive cells, with these changes being prevented by STI571 coinjection. Moreover, the intraperitoneal administration of STI571 rescued the cognitive decline in APPsw/PSEN1ΔE9 mice, p73 phosphorylation, tau phosphorylation and caspase-3 activation in neurons around Aβ deposits. Besides, we observed a decrease in the number and size of Aβ deposits in the APPsw/PSEN1ΔE9—STI571-treated mice. These results are consistent with the role of the c-Abl/p73 signalling pathway in Aβ neurodegeneration, and suggest that STI571-like compounds would be effective in therapeutic treatments of Alzheimer disease.

Keywords: Alzheimer’s disease; p73; c-Abl; behavioural impairments; amyloid β-peptide

Abbreviations: aCSF = artificial cerebrospinal fluid; HRP = horseradish peroxidase; ThS = Thioflavin-S


Introduction

Amyloid β-protein (Aβ) accumulation has been causally implicated in the neuronal dysfunction and neuronal loss that underlies the clinical manifestations of Alzheimer’s disease (Walsh and Selkoe, 2004; Hardy, 2006). However, the signal transduction pathways involved in this neuronal death and the genesis of the cytoskeleton alterations are not understood. We have previously shown that the kinase c-Abl plays a central role in neurodegeneration induced \textit{in vitro} by Aβ fibrils (Aβf) (Alvarez et al., 2004). These fibrils induce increases in c-Abl levels, activity in rat hippocampal neurons, both total and nuclear p73 protein levels, and the p73/c-Abl complex.

The non-receptor tyrosine protein kinase c-Abl participates in neuronal development and morphogenesis (Moresco and Koleske, 2003), controlling the function...
and stabilization of p73 in response to genotoxic stress (Tsai and Yuan, 2003), and the choice between cell survival in an arrested state and apoptosis (Wang, 2005).

p73 is a structural and functional homolog of p53 (Irwin and Kaelin, 2001). The many different p73 protein isoforms—which result from C-terminal alternative splicing and the use of two different promoters—fall into two classes: (i) the full-length, or TAp73 proteins that have a N-terminal domain (TA) participate in DNA-damage-induced cell-cycle arrest or apoptosis (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999; Melino et al., 2004) and (ii) ΔNp73, or short-form proteins, which lack the TA domain. The ΔNp73 proteins cannot induce apoptosis, but instead appear to block the function of full-length forms showing anti-apoptotic properties.

ΔNp73 isoforms are the predominant isoforms expressed postnatally in nervous system, and their expression prevents neuronal apoptosis during development (Walsh et al., 2004) and is required for the long-term survival of neurons (Pozniak et al., 2002). Members of the p53 family members are known to interact function ally and ultimately determine neuronal survival versus neuronal death in the developing and mature nervous system (Jacobs et al., 2006), whereas the role of p73 in neurodegenerative disease has not been studied.

Consistent with the c-Abl/p73 participation in the pathogenic mechanism of Alzheimer’s disease, it was shown that p73 accumulates in the nucleus of neurons and localizes to neurofibrillar tangles in the Alzheimer’s disease brain (Pozniak et al., 2002). Members of the p53 family members are known to interact functionally and ultimately determine neuronal survival versus neuronal death in the developing and mature nervous system (Jacobs et al., 2006), whereas the role of p73 in neurodegenerative disease has not been studied.

Here we show that the Aβ-induced pathology in vivo is associated with p73 phosphorylation and changes in the levels of p73 and c-Abl. Moreover, the c-Abl kinase inhibitor STI571 (imatinib mesylate or Gleevac) is an approved drug used in patients with chronic myelogenous leukemia—in which the therapeutic target is the aberrant oncogenic fusion protein Bcr-Abl and binding to the ATP-binding site of c-Abl and other tyrosine kinases (Druker et al., 1996; Capdeville et al., 2002)—and protects against Aβ toxicity both at the behavioural and morphological levels. In addition, the increase in TAp73 induced by Aβ depends on c-Abl activity and is associated with the induction of apoptosis in neurons. Our results show that the c-Abl/p73 signal is activated by Aβ deposits in vivo and suggest that this signalling pathway has a pathogenic role in Alzheimer’s disease.

Materials and Methods

Animals

Animals were maintained in the Animal Care Facility of our faculty, which follows the Guide for the Care and Use of Laboratory Animals published by NIH, USA (Publication 86-23). The animal protocols used were reviewed and approved by the animal studies review board at our institution. Transgenic B6C3-Tg (APPSw/PSEN1ΔE9)85Dbo/J mice were purchased from Jackson Laboratory (Bar Harbor, ME, #004462).

Primary hippocampal cell culture

Hippocampi from Sprague–Dawley rats at embryonic day 18 were dissected, and primary rat hippocampal cultures were prepared as described by Alvarez et al. (2004). Hippocampal cells were seeded in polylysine-coated wells and maintained in Neurobasal medium supplemented with B27 (Invitrogen, Carlsbad, CA) plus 100 U/ml penicillin and 100 μg/ml streptomycin for 5–7 days before the cell treatments. To inhibit glial proliferation, 2 μM cytosine arabinoside was added on the third day.

Surgical and Aβ injection protocol

Male Sprague–Dawley rats (280–320 g) were anaesthetized with 40 mg/kg ketamine and 12 mg/kg xylazine, with unilateral injections performed stereotaxically into the upper leaf of the dentate gyrus in the dorsal hippocampus (−3.5 mm anteroposterior, +2.0 mm mediolateral and −2.7 mm dorsoventral from the dura, according to the bregma) (Paxinos and Watson, 1986). The animals were injected at a rate of 0.25 μl/min with 4.5 μl of aCSF or Aβ, (20 μg in aCSF), or Aβ (20 μg) plus STI571 (10 μM) in aCSF or STI571 alone.

Treatment of hippocampal cells

The cultured hippocampal cells (1.3 × 10^5 cells/well) were usually treated with 5 μM Aβ and 5 μM Aβ plus 10 μM STI571. To obtain Aβ, the human Aβ1-40 peptide (Bachem, Torrance, CA) was subjected to a stirring aggregation assay (Alvarez et al., 1998). STI571 was a gift from Novartis (Basel, Switzerland). The expression vectors encoding for GFP-c-Abl(WT) (wild-type), GFP-c-Abl(KD) (kinase-dead) and GFP-SH2-c-Abl were kind gifts from Dr Zhi-Min Yuan (Department of Genetics and Complex Diseases, Harvard School of Public Health) (Tsai and Yuan, 2003), and pcDNA-HA-p73 and pcDNA-HA-ΔNp73 were kind gifts from Dr Guo-Jun Zhang (Dana-Farber, Cancer Institute, Boston, MA). All expression vectors were transfected using a Lipofectamine 2000 (Invitrogen) protocol 24 h before the challenge with Aβ, which yielded 10–15% transfected neurons.

Western blot analysis

Hippocampus extracts from rats injected with Aβ or artificial cerebrospinal fluid (aCSF), and extracts from hippocampal neurons treated with Aβ or Aβ plus STI571 were prepared in ice-cold RIPA buffer [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1 mM NaF and 1 mM NaVO₃], and 50-μg aliquots were analysed by Western blotting for c-Abl/p73 proteins. The following antibodies were used: anti-c-Abl (K12, 1:1000), anti-p73 (H-979 PAN-p73 antibody, 1:1000) and anti-β-tubulin (H-235, 1:500) polyclonal antibodies from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-c-Abl (Tyr412, 1:1000; Sigma, St Louis, MO); anti-phospho-p73 (Tyr99 1:800; Cell Signaling Technology, Danvers, MA) and anti-ΔNp73 (IMG-246, 1:500) and anti-ΔNp73 (IMG-313, 1:500) monoclonal antibodies from ImageX (San Diego, CA). The secondary horse-radish peroxidase (HRP)-conjugated antibodies (1:5000) were obtained from Pierce (Rockford, IL).
For the quantitative analysis of the Western blot we used Scion Image program (Beta 4.02).

**Immunofluorescence on coverslips**

Hippocampal cells were plated on polylysine-coated coverslips at 30,000 cells/coverslip. After 5–6 days in Neurobasal/B27 medium, neurons were transfected with the expression plasmid, and 24 h thereafter treated with Aβ or Aβ plus STI571 for 24 h. The cells were fixed in 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100. Immunostaining was performed using anti-HA (F-7, 1:100) or anti-MAP2 (1:500) (Chemicon International, Temecula, CA). Anti-mouse FITC (1:1000) and anti-rabbit TRITC (1:1000) (Sigma) were used as secondary antibodies.

**Immunohistochemical and immunofluorescence procedures**

The Aβ-injected rats and APPsw/PSEN1ΔE9 mice treated or not treated with STI571 were anaesthetized and perfused with 4% paraformaldehyde in PBS. Brains were removed and post-fixed overnight at 4°C, placed in 20% sucrose in PBS at 4°C overnight, and then cut in 25-μm-thick coronal sections using a cryostat (Leitz, 1900) at −20°C. Slices of the same brain were analysed by immunohistochemical and immunofluorescence procedures. Four or five slices were stained in each animal, and at least three animals were examined for each condition.

The specific antibodies used for immunohistochemistry were anti-c-Abl (K12, 1:500), anti-p73 (H-79, 1:100), anti-phospho-p73 (1:100), antiphosphorylated tau: AT8 (recognizing Ser-199- and Ser-202-phosphorylated tau), 1:500; Innogenetics, Gent, Belgium) and PHF1 (recognizing Ser-369- and Ser-404-phosphorylated tau, 1:500; a kind gift from Dr P Davies, Department of Pathology, Albert Einstein College of Medicine, Bronx, NY) antibodies. The sections were pretreated with 0.3% H2O2 to eliminate the endogenous peroxidase activity, and then incubated in 3% BSA in PBS. The washes and antibody dilutions were performed using 0.4% Triton X-100 in PBS. Immunohistochemistry was performed using the ABC (avidin biotin–HRP complex) method (Vector Laboratories, Burlingame, CA). Free-floating sections were mounted on gelatin-precoated slides, air-dried, dehydrated in graded ethanol and covered with Entellan solution (Merck, NJ, USA). The sections were fixed in 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100. Immunostaining was performed using the ABC (avidin biotin–HRP complex) method (Vector Laboratories, Burlingame, CA). Free-floating sections were mounted on gelatin-precoated slides, air-dried, dehydrated in graded ethanol and covered with Entellan solution (Merck, NJ, USA).

The antibodies used for immunofluorescence were anti-c-Abl (K12, 1:500), anti-c-Abl monoclonal (24-11, 1:200; Santa Cruz Biotechnology), anti-p73 (H-79, 1:250), AT8 (1:500), anti-caspase-3-active (1:200, Chemicon International), anti-GFAP (GA5, 1:200; Sigma), anti-TAp73 (IMG-246, 1:250) and anti-ΔNp73 (IMG-313, 1:250) antibodies. The slices were incubated with primary antibodies in 3% BSA in PBS and then with the secondary anti-mouse-Alexa Fluor-488 (1:1000) or anti-rabbit-Alexa Fluor-594 (1:1000) (Molecular Probes, OR, USA) antibody. Light and fluorescence images were captured under an Olympus BX51 microscope and analysed with Image-Pro Express. The images in the same experiment were always acquired using the same settings, and they were quantified by different persons blinded to the treatment conditions. AT8-positive neurons were analysed using 20 slices per animal (equivalent to a thickness of 500 μm around the injection site), counting the AT8-positive cells in the hippocampus in each slice. Thioflavin-S (ThS) staining was performed as described by Chacon et al. (2004). The number of plaques and area of each plaque was calculated in each image using Image J (National Institutes of Health, Bethesda, MD).

**STI571 treatment and behavioural analysis of the Aβ-injected animals**

Male Sprague–Dawley rats (280–320 g) were bilaterally injected with Aβ or aCSF into the hippocampus (intracerebral, i.c.). Three days before the injection and during the training protocol, the animals began receiving an intraperitoneal (i.p.) injection of 30 mg/kg STI571 or saline every 2 days. Five days after the bilateral injection, the training of the animals commenced in a circular Morris water maze (Morris, 1984) pool (1.6 m in diameter, 75 cm deep and painted black) using a two-trial-per-day regime for five consecutive days, followed by 2 days off, and then for an additional 4 days. For descriptive data collection the pool was imaginary divided into three equidistant concentric annuli and four quadrants. The platform (9 cm in diameter) was located in the centre of the northwest quadrant (hidden quadrant), and trial data were gathered using a water maze video tracking system (HVS Imagem, Hampton, UK). The water temperature was maintained at 19–21°C.

**APPsw/PSEN1ΔE9 mice treatments and behavioural testing**

Three days before and during the training protocol the mice received an i.p. injection of 25 mg/kg STI571 or saline every 2 days. Mice were trained in a circular water maze (1.2 m in diameter and 30 cm deep) using a four-trial-per-day regimen for five consecutive days, followed by 2 days off, and then for an additional 3 days. Data were gathered as described earlier.

**TUNEL staining**

TUNEL staining was performed using an apoptosis detection kit (Roche Molecular Biochemicals, Indianapolis, USA). Briefly, the sections were first incubated in a blocking solution containing 3% H2O2 in methanol, and then in 0.1% Triton X-100 in 0.1% sodium citrate overnight at 4°C. Then the sections were immersed in the TUNEL reaction mixture for 60 min at 37°C and washed twice in PBS (pH 7.4).

**Apopptotic genes mRNA levels**

Total RNA was extracted from rat hippocampal cultures (1 × 10⁶ cells) using the TRIZOL method (Invitrogen). Reverse transcription was carried out with 3 μg of total RNA following the Superscript II protocol (Invitrogen). For PCR amplification, different amounts of the synthesized cDNA were analysed to evaluate the linearity of the reaction; we used Platinum Taq DNA polymerase according the company instructions (Invitrogen). The primers and annealing temperatures are described in Supplementary Table 1.

**Statistical analysis**

Mean and standard error (SE) values with the number of experiments are indicated in the figures. Probability values were obtained using no-pair Student’s t-tests or two-way ANOVA with SigmaPlot (version 9.0).
Results

Aβ<sub>f</sub> injection increases p73 and c-Abl signals in the rat hippocampus

We assessed the participation of the c-Abl/p73 signalling pathway in neurodegeneration in vivo by injecting Aβ<sub>f</sub> into the hippocampus as an in vivo model of Alzheimer’s disease neurodegeneration. Twenty micrograms of Aβ<sub>f</sub> or aCSF (as a control) was injected unilaterally into the upper leaf of the dentate gyrus, and 6 days later we evaluated the p73 expression. Figure 1A shows that there were more neurons positive for p73 protein in Aβ<sub>f</sub>-injected animals in hippocampal regions near to the injection site (region between the leaves of the dentate gyrus) than in the same area in control animals (Fig. 1A). As expected, in the Aβ<sub>f</sub>-injected area we observed the presence of amyloid, a loss of tissue organization, and Aβ<sub>f</sub>-induced tau phosphorylation (Supplementary Fig. 1). Supplementary Fig. 2A presents the results of an analysis of different hippocampal regions, which also revealed an increase in p73-positive cells. We evaluated the expression of p73 using the H-79 p73 antibody generated against an epitope shared by TAp73 and ΔNp73 forms (a PAN-p73 antibody). It has been described that this antibody shows a preference for TAp73 (Saifudeen et al., 2005), but we refer the H-79 antibody signal as the p73 signal, because we cannot discard the ΔNp73 signal contribution. GFAP coimmunolabeling showed that p73 signals were mainly neuronal, with no significant p73 signal observed in glial cells, although the population of activated glial cells was clearly increased by the Aβ<sub>f</sub>.

![Fig. 1](http://brain.oxfordjournals.org/) Aβ<sub>f</sub> injection induces c-Abl and p73 signals in rat hippocampus. aCSF and Aβ<sub>f</sub> were injected unilaterally into the rat hippocampus using a stereotaxic procedure. After 6 days the animals were perfused with fixing solution, and their brains were analysed by immunohistochemical or immunofluorescence procedures. (A–C) Coronal sections from aCSF- and Aβ<sub>f</sub>-injected brains stained using anti-p73 polyclonal (H-79) (A), anti-p73 polyclonal (H-79), anti-GFAP monoclonal G-A-5 (B) and anti-c-Abl polyclonal (K12) antibodies (C). p73 was expressed in the rat hippocampus, and p73 signals were increased when Aβ<sub>f</sub> was injected. The lower images (scale bar = 10 μm) show magnifications of the upper images (scale bar = 20 μm). Arrow and arrowheads indicate neurons with nuclear and cytoplasmic p73 signal, respectively. c-Abl was also expressed in the hippocampus, and the c-Abl signal was increased in Aβ<sub>f</sub>-injected hippocampi. (D) Western blot analysis of the levels of c-Abl and p73 proteins in hippocampi injected with Aβ<sub>f</sub> or aCSF (area next to injection site). 1, 2 and 3 are hippocampus extracts from different injected rats. The numbers below correspond to expression ratios for c-Abl and p73 were normalized to the β-tubulin signal used as a loading control. *P < 0.05. Aβ<sub>f</sub>-injected hippocampi showed higher c-Abl and p73 protein levels than aCSF-injected hippocampi. (E) Immunofluorescence of coronal sections from aCSF- and Aβ<sub>f</sub>-injected brains analyzed for c-Abl, monoclonal antibody (24-11), and p73 polyclonal antibody (H-79). c-Abl and p73 signals were colocalized in the same neuron. Scale bar = 15 μm. (F) c-Abl and p73 colocalized in neurons with apoptotic morphology in hippocampi injected with Aβ<sub>f</sub>. Lower image is a magnified view of the boxed area in the merged image, and shows the apoptotic morphology of a neuron. Scale bar = 15 μm.
injection (Fig. 1B). In control hippocampi we observed both neurons with cytoplasmic p73 staining (Fig. 1A and B, arrowheads) and nuclear p73 staining (Fig. 1A and B, arrows). However, in addition to the greater number of p73-positive neurons in Aβf-injected animals, we observed an increase in p73 predominantly in the nucleus (Fig. 1A and B, arrows), with only some neurons showing high cytoplasmic p73 signals (Fig. 1E and F).

We also observed an increase in c-Abl-positive cells in different regions close to the site of Aβf injection (Fig. 1C and Supplementary Fig. 2B). In agreement with the results of immunohistochemical analysis, Western blot analysis showed higher p73 and c-Abl protein levels in the hippocampi of Aβf-injected animals (Fig. 1D).

The p73 level increases in a damaged cell, depending on the presence of c-Abl in the cell. We observed the colocalization of c-Abl and p73 signals in Aβf-injected hippocampi (Fig. 1E). Neurons positive for p73 levels were also positive for c-Abl. In addition, some cells at the Aβf injection site showed a strong p73 signal in condensed nuclei (considered a common apoptotic morphology) (Fig. 1F). This increase in the p73 levels in Aβf-injected animals—associated with the presence of c-Abl—suggests in vivo activation of the c-Abl/p73 signalling pathway.

The tau hyperphosphorylation signal is associated with nuclear c-Abl and p73

Tau phosphorylation in specific phosphorylated epitopes is a sign of Aβ-induced neuronal damage. As expected, the intrahippocampal injection of Aβf induced an increase in the number of AT8-positive cells (Fig. 2A and Supplementary Fig. 1D and E), and we observed that c-Abl and p73 signals were colocalized with AT8 signals in the Aβf-damaged area (Fig. 2A). The correlation between c-Abl and AT8 signals was not strong, since even though almost all AT8-positive neurons were c-Abl positive, not all c-Abl-positive neurons showed AT8 signals. However, we observed a strong correlation between high p73 levels and tau phosphorylation, with almost all p73-positive neurons being positive for AT8.

It also appeared in Aβf-injected brain regions that in some AT8-positive neurons the c-Abl and p73 signals in the nucleus were stronger (Fig. 2B), suggesting the c-Abl and p73 nuclear translocation results from activation of the c-Abl/p73 signalling pathway. Those neurons with higher AT8 signals (Fig. 2B, arrows) contained c-Abl in their cytoplasm, but also showed c-Abl signal that appears to be in the nucleus, whereas those neurons in which c-Abl appeared only in the cytoplasm did not show AT8 signals (Fig. 2B, arrowheads). A similar trend was seen when we analysed the relationship between p73 and AT8 signals (Fig. 2B, lower images). This result suggests that the increased AT8 signal is correlated with an increase in nuclear p73 levels and activation of the c-Abl/p73 signalling pathway.

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Fig. 2  

**Fig. 2** tau hyperphosphorylation in Aβ1-injected hippocampus is associated with high c-Abl and p73 signals. aCSF and Aβ1 were injected into rat hippocampi, and 6 days later the animals were killed. The brains were fixed, and then analysed by immunofluorescence. Coronal sections from control and injected animal brains were analysed for tau phosphorylation (AT8 antibody), c-Abl (K12 antibody) and p73 (H-79 antibody).  

(A) c-Abl and p73 signals were increased and colocalized with AT8 signals in Aβ1-injected brains. Right images are c-Abl or p73 images merged with AT8 images. Scale bar = 15 μm.  

(B) The AT8 signal was highest in cells that exhibited higher c-Abl or p73 signals and an increased nuclear localization of these proteins. Arrows indicate neurons with c-Abl and p73 signals in the nucleus and high AT8 signals. Arrowheads indicate neurons with mainly cytoplasmic c-Abl and p73 signals, with no AT8 signals. Scale bar = 10 μm.
Fig. 3 Intraperitoneal STI571 administration prevents behavioural and spatial impairments induced by Aβ42. (A and B) Water maze escape latencies of rats whose hippocampi were bilaterally injected with Aβ (A) or aCSF (B) and also received i.p. injections of saline (closed circles) or STI571 (open circles) over 12 days. Graphs show average escape latencies per day for each condition. (A) Lower escape latencies of the Aβ-STI group show that the i.p. injection of STI571 strongly prevented spatial impairment induced by Aβ injection (two-way ANOVA: F = 9.064; P = 0.0026; n = 4 for each condition). (B) Control groups injected with aCSF showing that the i.p. injection of STI571 did not induce spatial learning impairments in a control condition. (C) Representative swimming paths at day 12 of training. (D) Spatial acuity parameter versus escape latency for the different animal groups. The decrease in spatial acuity induced by Aβ injection was prevented by STI571. Data are averages in the second week training for each condition. (E) Average swimming speed in the different groups showed no motor alteration with Aβ or STI571 treatments. (F) Graph shows the average path length to reach the platform. "**"P = 0.0014, Student's t-test. (G) Graph shows the percentage of the total time spent in the four quadrants. The Aβ-STI group spent more time in quadrant four, similar to the aCSF-Saline group. "*"P = 0.0036, Student's t-test. (H) The Aβ-STI group spent more time in quadrant four than in the other quadrants. "*"F = 35.05; P = 0.0001, two-way ANOVA. Plots in panels E–H show the averages for the second week training for each condition. The values are in agreement with a protective effect of STI571 against the cognitive impairments induced by intrahippocampal injection of Aβ. All graphs show mean values, with error bars indicating SE values.
STI571 prevents the neurodegenerative changes induced by Aβf

We also examined the effect of STI571 on the neurodegenerative changes induced by Aβf. We coinjected Aβf and STI571 or Aβf alone and evaluated apoptosis and tau hyperphosphorylation (Fig. 4). At 14 days post-injection Aβf had induced a clear increase in TUNEL-positive neurons in the hippocampal area (Fig. 4A). The higher magnification images in the figure show representative areas where the number of TUNEL-positive cells is clearly higher in the Aβf-injected hippocampus than in controls. The coinjection of STI571 and Aβf resulted in fewer TUNEL-positive neurons, similar to the control condition (Fig. 4A, bottom images). For tau phosphorylation, Fig. 4B shows that the number of AT8-positive neurons at 3, 6 and 14 days post-injection was higher in the Aβf-injected hippocampus than in the saline-injected hippocampus (Fig. 4B and C). However, coinjection of Aβf and STI571 resulted in a clear decrease in the number of AT8-positive neurons. The number of AT8-positive neurons in the hippocampus coinjected with Aβf and STI571 was significantly lower than that in the hippocampus injected with Aβf alone at 3 days (P<0.0001), 6 days (P<0.01) and 14 days (P<0.01) post-injection, and similar to the number of AT8-positive neurons in controls. These results suggest that C.A. activity is required in the pathogenic signalling pathway activated by Aβf that leads to apoptosis and tau pathology in vivo, and the ability of STI571 to prevent the behavioural deficit appeared to be associated with a decrease in the neurodegenerative changes induced by Aβf.

Aβf activates the c-Abl/p73 signalling pathway, induces TAp73 stabilization and increases its proapoptotic function

To elucidate the mechanism responsible for the reduction in apoptosis associated with STI571 treatment, we examined the c-Abl/p73 signalling pathway in hippocampal neurons exposed to Aβf. Exposing neurons to Aβf (Fig. 5A) increased p73 phosphorylation on Tyr99 (a c-Abl phosphorylation site that is shared by TAp73 and ΔNp73 isoforms) and p73 levels. Moreover, Tyr99 phosphorylation was prevented by cotreating the neurons with STI571. c-Abl Tyr412 phosphorylation and c-Abl expression levels also increased. These observations indicate that the c-Abl/p73 signalling pathway is activated by Aβf in neurons.

p73 is expressed as distinct isoforms that play antagonistic roles in cell survival and apoptosis. Using antibodies that recognize the TAp73 or ΔNp73 isoforms, we found that Aβf induced a significant increase in the TAp73 isoform levels in hippocampal neurons (Fig. 5B), whereas ΔNp73 were moderately decreased (Fig. 5B). The TAp73 increase induced by Aβf was significantly blocked by STI571 (Fig. 5C and D). We transfected neurons with expression plasmids for TAp73 or ΔNp73 isoforms and then challenged the cells with Aβf (Fig. 5E). Consistent with the roles of p73 isoforms in other cell types, the expression of TAp73 or ΔNp73 isoforms in neurons regulated apoptosis in an antagonistic manner: the TAp73 expression enhanced Aβf-induced apoptosis (Fig. 5E), while the truncated ΔNp73 isoform prevented apoptosis (Fig. 5E). Therefore, Aβf activates the c-Abl/TAp73 proapoptotic signalling pathway in neurons.

c-Abl activity is required for the apoptosis induced by Aβf

Even though our data are consistent with STI571 regulating the c-Abl/TAp73 signalling pathway in neurons, this inhibitor has targets other than the c-Abl protein. Therefore, to determine the link between apoptosis and c-Abl/p73 signalling, we expressed an inactive mutant c-Abl kinase or an SH2-c-Abl peptide (a peptide that prevents c-Abl–p73 interactions) in neurons exposed to Aβf to block the downstream signalling associated with c-Abl activation. The expression of c-Abl(KD) in neurons prevents the apoptosis induced by Aβf, whereas the expression of c-Abl(WT) kinase potentiates it (Fig. 6A and Supplementary Fig. 3). Moreover, the expression of an SH2-c-Abl peptide also prevents neuronal apoptosis (Fig. 6A), suggesting a role of the c-Abl–p73 interaction in the apoptotic pathway.

Furthermore, the apoptosis induced by Aβf in neurons expressing TAp73 was prevented by STI571, indicating that the proapoptotic effects of TAp73 induced by Aβf depended on c-Abl activity (Fig. 6B). We also evaluated how the TAp73 increase was linked to apoptosis induced by Aβf by determining the expression of apoptotic genes that respond to TAp73. Figure 6C shows that Aβf induced increases in the expressions of Bcl-XL, Bcl-XS, Bax, Mdm2, Apaf1 and Bcix genes in neurons, with these increases being partially prevented by STI571. Together these in vitro results are consistent with a role of the c-Abl/p73 signalling pathway in the apoptosis induced by Aβf, and support the role of TAp73 isoforms.

Aβf induces p73 phosphorylation and TAp73 increase in the hippocampus

Our in vitro studies indicate that the effect of STI571 on apoptosis induced by Aβf is due to inhibition of the c-Abl/p73 signalling pathway activation, and so we used our in vivo model to evaluate the phosphorylation of p73 in Tyr99, which is related to the activation of this pathway. The phosphorylated Tyr99 signal was not detected in the control (aCSF) hippocampus (Fig. 7A), while it was significantly increased in the Aβf-injected hippocampus (Fig. 7A). Coinjection of Aβf and STI571 substantially prevented the p73 phosphorylation induced by Aβf.

We also analysed the expressions of TAp73 and ΔNp73 (Fig. 7B). In control animals there were a few TAp73-positive neurons, while ΔNp73 expression in the
c-Abl inhibition prevents neurodegenerative hallmarks induced by Aβ42 injection in the rat hippocampus. Rat hippocampi were injected with aCSF, Aβ42 or Aβ42 plus STI571. Animals were perfused with fixing solution 3, 6 or 14 days later, and the brains were analysed for apoptosis by TUNEL and for tau phosphorylation by AT8 immunofluorescence. (A) Coronal slices from injected hippocampus analysed by TUNEL at day 14. STI571 strongly prevented apoptosis induced by Aβ42 in the hippocampus. Arrows indicate TUNEL-positive cells. Scale bars = 250, 100 and 50 μm in left, middle and right images, respectively. (B) Representative images of AT8 signals in the rat brain at day 6 post-injection. STI571 prevented tau phosphorylation in Aβ42-injected brains. Scale bar = 15 μm. (C) Graphs showing the mean numbers of AT8-positive cells per slide (25 μm) in the hippocampal area at 3, 6 and 14 days post-injection. Eight slices for each condition were quantified in four independent experiments. The graphs show that STI571 significantly inhibited tau hyperphosphorylation in Aβ42-injected brains. **P < 0.01; ***P < 0.001 (t-test). Error bars, SE.
hippocampus was not detected above the low background signal, and Aβ injection induced an increase in TAp73-positive neurons in the hippocampus, which was prevented by STI571 coinjection (Fig. 7B). Moreover, neurons that expressed TAp73 in the nucleus frequently showed active caspase-3 expression and nuclei with apoptotic morphology (Fig. 7C, arrows), whereas cells that did not express TAp73 did not show active caspase-3-positive signals.

Fig. 5 Aβ induces p73 phosphorylation and enhances proapoptotic TAp73 function. (A) Western blot analysis of the hippocampal neurons treated with Aβ (5 μM) or Aβ plus STI571 (10 μM) for 6 h. The increases in Tyr99 p73 phosphorylation, p73 protein levels and Tyr412 c-Abl phosphorylation induced by Aβ were prevented by STI571. (B) Temporal changes in TAp73 levels (increased) and ΔNp73 (decreased) in hippocampal neurons exposed to Aβ (decreased) in hippocampal neurons exposed to Aβ (decreased) in hippocampal neurons exposed to Aβ (decreased). (C) TAp73 expression potentiated Aβ-induced apoptosis, which was prevented by ΔNp73. Hippocampal neurons were transfected with 1 μg of HA-TAp73 or HA-ΔNp73 expression plasmids. After 20 h, the neurons were treated (or not, for controls) with Aβ for 8 h. The graph quantifies apoptotic nuclei in transfected neurons (followed by anti-HA and Hoechst staining) in 10 fields (300 neurons) in three independent preparations. **P < 0.01; ***P < 0.001 (t-test) between treatment with control. (D) STI571 treatment prevented TAp73-selective stabilization. Hippocampal neurons were treated with Aβ (5 μM) or Aβ plus STI571 (10 μM), or left untreated (control). (E) Results of densitometric analysis of four experiments corresponding to the experiment in panel D. *P < 0.05; **P < 0.01 (t-test). All graphs show mean values, with error bars indicating SE values.
In APPsw/PSEN1ΔE9 transgenic mice, STI571 ameliorates spatial learning, memory impairments and neuronal damage

As expected, the brains of 11-month-old APPsw/PSEN1ΔE9 mice—which is a well-accepted Alzheimer’s disease model—showed widespread Aβ accumulation and ThS-positive amyloid plaques (Fig. 8A) throughout the hippocampus. In these animals we observed many p73-positive neurons around the amyloid plaques, supporting that the Aβ deposits induce p73 accumulation and that c-Abl/p73 signalling is activated in this Alzheimer’s disease model. Treating 11-month-old transgenic mice with STI571 showed significantly lower escape latencies and covered significantly shorter distances to find the hidden platform than in saline-injected animals (Fig. 8B and C). At day 10 of training, control APPsw/PSEN1ΔE9 mice displayed a navigation pattern typical of spatial learning acquisition in impaired animals, while the animals treated with STI571 showed a much better navigation pattern (Fig. 8D). These results indicate that STI571 treatment is able to reduce the cognitive impairment of spatial memory associated with the accumulation of Aβ peptide. Moreover, the brain of the APPsw/PSEN1ΔE9 mice treated with STI571 showed a decrease in Aβ accumulation (Fig. 8E), with a significant reduction in the number (Fig. 8F) and size (Fig. 8G) of ThS positive amyloid plaques. Furthermore, transgenic mice brain showed high Tyr99/phospho-p73 signals, especially in cells next to the amyloid deposits (Fig. 9A), whereas brains treated with STI571 exhibited lower signals (Fig. 9A and B). Moreover, the STI571-treated brains presented fewer neurodegeneration markers (PHF1 signal for tau phosphorylation and active caspase-3 for apoptosis) than saline-treated animals (Fig. 9C and E). Analysis of tissue in close proximity to amyloid deposits of similar size clearly revealed that, phospho-p73, tau-phosphorylated and active caspase-3-positive cells were reduced in STI571-treated brains (Fig. 9B, D and F).

Fig. 6  c-Abl kinase activity and c-Abl/p73 are required for Aβf-induced neurodegeneration. (A) c-Abl kinase activity and c-Abl–p73 interaction are necessary for the induction of apoptosis by Aβf. We induced apoptosis in neurons that express GFP, GFP-c-Abl, GFP-c-Abl(KD) or GFP-SH2-c-Abl in a control situation or exposed to Aβf, and analysed which cells showed apoptotic nuclei (Supplementary Fig. 3). The graph indicates the mean number of apoptotic nuclei in 10 fields (300 neurons) of four independent experiments. ***P < 0.001 (t-test). Error bars, SE. c-Abl-WT-expressing neurons treated with Aβf showed increased apoptosis compared to control-vector-expressing neurons, whereas the neurons that express the c-Abl kinase death form showed fewer apoptotic nuclei, similar to that in controls. Also, the GFP-SH2-c-Abl neurons showed significantly lower apoptosis values. (B) The effect of the TAp73 isoform on apoptosis depends on the c-Abl kinase activity. The graph shows the mean number of apoptotic nuclei in HA-TAp73-transfected neurons that were untreated (control), treated with Aβf (5 μM), and treated with Aβf and STI571 (10 μM) in four independent experiments. ***P < 0.001, Student’s t-test. Error bars, SE. (C) The expression of p73-dependent apoptotic genes was inhibited by STI571. Hippocampal neurons were treated with Aβf (5 μM) or Aβf plus STI571 (10 μM) for 6 h. mRNA levels for Bcl-Xs, Bcl-Xl, Mdm2, Bax and Apaf 1 were evaluated by RT-PCR, with a β-actin transcript used as a control.
Fig. 7 STI571 prevents the p73 phosphorylation and TAp73 increase induced by Aβ_{1-42} in vivo. Rat hippocampi were injected with aCSF, Aβ_{1-42} or Aβ_{1-42} plus STI571, and 6 days later the animals were perfused with fixing solution. Coronal brain sections were analysed for phospho-p73, TAp73, ΔNp73 and active caspase-3. (A) Results of immunohistochemistry for p73 phosphorylation (Tyr99). There was a strong phospho-p73 signal in Aβ_{1-42}-injected hippocampi, which was inhibited by STI571. Middle and bottom images show magnified views of the boxed areas in the corresponding top and middle images, respectively. Arrows indicate neurons positive for phosphorylated p73. Scale bar = 15 μm. (B) Immunofluorescence for TAp73 and ΔNp73. TAp73 was increased and the TAp73 stabilization was inhibited by STI571 in Aβ_{1-42}-injected hippocampi. Arrows indicate TAp73-positive neurons. Scale bar = 15 μm. (C) Strong TAp73 signals were colocalized with activated caspase-3 and apoptotic nuclei (indicated by Hoechst stain) in Aβ_{1-42}-injected hippocampi. Arrows indicate TAp73, activated caspase-3 and apoptosis-positive neurons. Arrowheads indicate non-apoptotic neurons. Scale bars = 250, 50 and 15 μm in top, middle and bottom images, respectively.
Fig. 8 Intraperitoneal STI571 administration prevents behavioural, spatial impairments and reduces Aβ deposits in APPsw/PSEN1 ΔE9 mice. 
(A) Eleven-month-old APPsw/PSEN1 ΔE9 transgenic mice were perfused with fixing solution, and their brains analysed by immunohistochemical procedures. Coronal sections from brains were stained using anti-p73 polyclonal (H-79) and ThS. p73-positive neurons (black arrowheads) were evident around the amyloid deposits (white arrowheads scale bars = 50 μm). The bottom-right image shows a magnified view of the boxed area in the merged image (scale bar = 10 μm). 
(B) Water maze escape latencies of mice that were treated with saline (Tg-saline, open circles) or STI571 (Tg-STI, closed circles) by i.p. injection over 13 days. STI571 was chronically administered to 11-month-old APPsw/PSEN1 ΔE9 transgenic mice (n = 3 per group). The mice were given i.p. injections of STI571 (25 mg/kg/day) every 2 days, starting 3 days before the water maze training protocol. Graphs show the average escape latency per day for each condition. The lower escape latencies of the STI group indicate that i.p. injection of STI571 strongly prevented spatial impairment (two-way ANOVA between days 4 and 10, F=3 0 . 7 3 ; P<0.001; n=3 for each condition). 
(C) Graph showing path length average to reach the platform (F=64.88; P<0.001, two-way ANOVA between days 4 and 10). 
(D) Representative swimming paths at day 10 of training. In all graphs error bars show SE values. 
(E) The brains of STI571 treated APPsw/PSEN1 ΔE9 transgenic mice stained with ThS showed an evident decrease of the amyloid deposits respect the control (white arrowheads scale bars = 100 μm). 
(F) Graphs show the number and average size of amyloid deposit for each condition. ***P<0.001, **P<0.005 Mann–Whitney U’s test. Error bars, SE.
Fig. 9 Intraperitoneal ST571 administration prevents neurodegenerative changes in APPsw/PSEN1ΔE9 mice. The transgenic APPsw/PSEN1ΔE9 mice (control and chronically treated with ST571) used for the water maze study, were perfused with fixing solution, and their brains analysed by immunohistochemical procedures. (A) Immunofluorescence for Tyr99 p73 phosphorylation (in red) and amyloid (with ThS, in green). Strong phospho-p73 signals were evident in APPsw/PSEN1Δ9 mice brains, and the p73 phosphorylation was inhibited by ST571. Arrows indicate phospho-p73-positive neurons (scale bars = 50 μm). (B) The graph indicates the number of phospho-p73 positive cells in a circular area (r = 155 μm) around amyloid plaques of similar size. Error bars, SE. (C) Representative images of PHF1 signals in saline- and ST571-treated brains. The bottom images show magnified views of the boxed areas in the middle images around the amyloid plates (arrowhead), with many PHF1-positive cells (arrows) being observed in saline-treated brains. Scale bars = 250, 20 and 10 μm in top, middle and bottom images, respectively. (D) The graph indicates the number of PHF1 positive cells per area as described in B. Error bars, SE. (E) Immunofluorescence for active caspase-3 (in red) and amyloid (in green). Several active caspase-3-positive cells (arrows) were observed next to amyloid staining in saline-treated brains, whereas there were fewer in ST571-treated brains (scale bars = 20 μm). The right image shows a magnified view of the boxed area in the top-left image (scale bars = 10 μm). (F) The graph indicates the number of caspase-3 positive cells per area as described in B. Error bars, SE.
Discussion

Aβ accumulation has been causally implicated in the neuronal dysfunction and neuronal loss that underlies the clinical manifestations of Alzheimer’s disease (Walsh and Selkoe, 2004; Hardy, 2006). However, the mechanisms involved in the pathogenic changes triggered by Aβ are not clearly understood. Neuronal dysfunction and cytoskeletal alterations are early manifestations that lead to aberrant remodelling of dendrites and axons, synaptic loss (Tsai et al., 2004), and eventually progressive loss of neuronal populations (LeBlanc, 2005; Bredesen et al., 2006; Hardy, 2006), which is associated with the appearance of dystrophic neurites and abnormal phosphorylation of cytoskeletal proteins (Dickson, 2004). Apoptosis, the general neuronal death pathway in neurodegenerative diseases, results from exposure to Aβ.

We previously described the activation of c-Abl as a mechanism underlying neuronal apoptosis in hippocampal cultures exposed to Aβf (Alvarez et al., 2004). In the present study we extended our observations to in vivo Alzheimer’s disease models assessing the therapeutic potential of the c-Abl inhibitor STI571. The results show that peripheral administration of this low-molecular-weight selective c-Abl inhibitor reduced the neuropathology associated with Aβ deposits and the cognitive deficits in animal Alzheimer’s disease models. We previously showed that c-Abl inhibition prevents Aβ-induced apoptosis in vitro, and we found that the same effect occurs in the Aβf intrahippocampal injection model. The intrahippocampal delivery of Aβf is a useful model for Alzheimer’s disease that allows several features of the disease to be studied, including the induction of tau phosphorylation and apoptosis. Furthermore, this animal model exhibits the development of behavioural and spatial learning deficits in a relatively short time.

We found that STI571 protects against Aβf toxicity both at the behavioural and morphological levels. Our in vitro and in vivo results suggest that the effects of STI571 are mainly related to the c-Abl/p73 signalling evident in Aβf-induced pathology. Moreover, though temporal studies of neurodegenerative disease models suggest that programmed cell death is a relatively late event, and that death is preceded by early functional alterations, neurite retraction, and synapse loss (Bredesen et al., 2006), we found that treatment with STI571 leads to surprisingly improved outcomes, indicating that the c-Abl/p73 signalling pathway could play important roles both in apoptosis and in the early steps of neurodegeneration. It is noteworthy that we also observed a surprisingly improved cognitive performance in APPsw/PSEN1ΔE9 transgenic mice treated with STI571, since the progression of Alzheimer’s disease pathology is faster in these transgenic mice than in other Alzheimer’s disease models (Borchelt et al., 1997), these transgenic mice have proven highly valuable for evaluating putative Alzheimer’s disease therapies (Riekkinen et al., 1998). We evaluated the therapeutic efficacy of STI571 in 11-month-old APPsw/PSEN1ΔE9 transgenic mice that show impaired spatial and contextual memory (Garcia-Alloza et al., 2006) and neuropathologically present Aβ plaques. The STI571-treated animals not only showed a better cognitive performance but also lower phospho-p73 signals around Aβ plaques. Furthermore, although cell loss (Takeuchi et al., 2000) or tau pathology have not been described in these animals, we observed PHF1 signals and activated caspase-3-positive cells near to amyloid deposits in our 11-month-old mice, and both effects were clearly reduced by STI571 treatment. Besides, in the STI571 APPsw/PSEN1ΔE9 brains we observed a lower amyloid plaque burden respect to untreated animals. It has been shown that STI571 reduces Aβ production in neuronal cultures and in guinea pig brains in vivo (Netzer et al., 2003). We do not know the relative contribution of the reduction in Aβ deposits in the cognitive improvement observed with STI571 treatment in this model; however when we compared the markers of neurodegeneration around the amyloid deposits of similar size the protective ability of STI571 was clear. These results indicate that STI571 is able to reduce the neuronal damage induced by Aβ deposits in both Alzheimer’s disease models. Furthermore, STI571 treatment decreases the Aβ deposit burden, an important effect that deserves further investigation.

STI571 is available commercially as Gleevec, a drug developed and registered by Novartis, and approved by the FDA for use in patients with chronic myelogenous leukaemia and gastrointestinal stromal tumours. Gleevec is not associated with major adverse symptoms (Druker et al., 2001; Capdeville et al., 2002), although mild cardiotoxicity has been described (Kerkela et al., 2006). One of the limitations of STI571 as a treatment for Alzheimer’s disease is the limited penetration of the drug into the CNS (Wolff et al., 2003). We overcame this by applying high-dose i.p. injections of STI571, which allowed it to cross the blood–brain barrier. Although expulsion by glycoprotein-P prevents it from reaching high levels in the brain, STI571 brain levels that are around 10% of the plasma levels have been reported when STI571 is administered directly by injection at 12.5 mg/kg (Dai et al., 2003).

Whilst we cannot eliminate the possibility that other targets of STI571 such as c-Kit and PDGFR (Buchdunger et al., 2000; Heinrich et al., 2000) could modulate its effects in vivo, both of these involve prosurvival pathways. The activation of PDGFR and c-Kit receptors has been associated with PI3K, AKT activation, and Bad inhibition, and inhibition of these pathways results in an increase in apoptosis and decrease in cell survival. Therefore, the inhibition of these effects by STI571 would probably enhance rather than prevent apoptosis induced by Aβ as we observed (Blume-Jensen et al., 1998; Simakajornboon et al., 2001; Zhang et al., 2003).

We found that c-Abl activation by Aβf in vitro is linked to p73 phosphorylation and increases in the proapoptotic TAp73 isoform, connecting Aβf with c-Abl/p73 apoptotic
signalling in neurons. The phosphorylation of p73 in Tyr99—coupled to the increase in Tyr412 c-Abl phosphorylation and the ability of STI571 to prevent p73 phosphorylation induced by Aββ—supports the activation of the c-Abl/p73 signalling pathway downstream of Aββ. Moreover, stabilization of the proapoptotic TAp73 links c-Abl/p73 activation to the induction of apoptosis, and the increase in TAp73 levels and its proapoptotic function were prevented by c-Abl inhibition. In addition, the ability of c-Abl(KD) and the SH2-c-Abl peptide to prevent the apoptosis induced by Aββ (similar to the effect seen with STI571) suggests that inhibition of the downstream c-Abl/p73 signalling pathway underlies the effect of STI571 on apoptosis induced by Aββ.

The function of c-Abl and p73 on apoptosis regulation has been related mainly to oxidative stress and DNA damage, but other stimuli can also activate the c-Abl/p73 signalling pathway. For example, the apoptosis induced by TNF-α requires p73 and c-Abl activation (Chau et al., 2004), and stress stimuli including sorbitol, nocardazole and taxol induce p73β accumulation (Lin et al., 2004). c-Abl activation has also been linked to apoptosis via endoplasmic reticulum stress (Ito et al., 2001) or proteasome function deregulation (Holcomb et al., 2006).

Moreover, our results showing that Aββ activates c-Abl in agreement with recent work showing that c-Abl activation by the Aβ peptide is linked to neuronal death in Drosophila (Lin et al., 2007). It is also possible that c-Abl activation is connected to oxidative stress. It has been proposed that the neurotoxicity of Aβ peptide is mediated by H2O2 toxicity (Atwood et al., 2003).

Our in vivo results of c-Abl/p73 signalling pathway activation by Aββ increase in phospho-p73 and TAp73 colocalization with caspase-3 are all consistent with a p73 proapoptotic function dependent on c-Abl activity (Tsai and Yuan, 2003). This function has not been previously described for p73 in the adult brain. Although there is evidence that TAp73 and ΔNp73 regulate cell differentiation and cell survival in neuronal tissues (Jacobs et al., 2004, 2006), the focus has been on the antiapoptotic roles of the ΔNp73 isoforms that are the most abundant in the nervous system. p73 is a member of the p53 family and participates in apoptosis induced by DNA damage by agents such as cisplatin and ionizing radiation (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999), p73 knockout mice, which are functionally deficient for all p73 isoforms, exhibit profound developmental defects, including hippocampal dysgenesis and hydrocephaly (Pozniak et al., 2000; Yang et al., 2000), and the progressive loss of cortical and peripheral sympathetic neurons in the first few weeks after birth (Pozniak et al., 2002). The ΔNp73 isoforms are the predominant isoforms expressed postnatally in the brain, and overexpression of ΔNp73 inhibits sympathetic neuronal apoptosis caused by NGF withdrawal and the apoptosis of cortical neurons induced by campothecin (Pozniak et al., 2000, 2002; Walsh et al., 2004).

In sympathetic neurons, p63 (another p53 homolog) is essential for developmental neuronal death (Jacobs et al., 2005).

Thus, p73 expression is considered to prevent neuronal death during development, with its expression being required for the long-term survival of neurons in the CNS (Pozniak et al., 2002), while the role of p73 in the induction of neuronal death has been largely ignored. Our data indicating that the proapoptotic isoform TAp73 is increased in the hippocampus after Aββ injection support a role for p73 in neurodegeneration. The p73 phosphorylation and TAp73 increase induced by Aββ in the hippocampus were prevented by STI571, suggesting that these effects depend on c-Abl activity. Moreover, TAp73 potentiates the apoptosis induced by Aββ, and this activity is also prevented by STI571 treatment. In agreement with a role for TAp73 in Alzheimer’s disease, the level of p73 is reportedly increased in the nuclei of Alzheimer’s disease pyramidal neurons, and p73 is present in dystrophic neurites with cytoskeletal pathology (Wilson et al., 2004). Furthermore, TAp73 modulates tau phosphorylation (Hooper et al., 2006). Whilst ΔNp73 is the most abundant isoform in the brain, TAp73 is also expressed in some areas, including the hippocampus (Cabrera-Soccoro et al., 2006). These regions might exhibit differential sensitivity to damage. Moreover, some p73 gene polymorphisms—probably associated with the relative expression of p73 isoforms—have been linked to Alzheimer’s disease (Li et al., 2004).

Whilst c-Abl has been described as the kinase that controls the function and stabilization of p73 in response to genotoxic stress (Tsai and Yuan, 2003), cytoplasmic c-Abl in neurons participates in neuronal development and is involved in neuronal morphogenesis and synaptic function (Moresco and Koleske, 2003; Moresco et al., 2003). In neurons, Abl tyrosine kinases relay axon guidance cues and growth factor receptor activation, and promote cytoskeletal rearrangements (Lu et al., 2002), neurite outgrowth (Woodring et al., 2002), and dendrogenesis (Jones et al., 2004). Our in vitro and in vivo data also suggest a role for c-Abl in Aββ-induced apoptosis.

Our in vivo data also suggest a role for c-Abl in Aββ-induced tau phosphorylation. Interestingly, c-Abl directly phosphorylates tau in Tyr394 (Derkinderen et al., 2005). In addition, c-Abl activation during neurodegeneration could be related to tau phosphorylation levels via the activation of cdk5 (Alvarez et al., 1999, 2001; Zukerberg et al., 2000; Lin et al., 2007). c-Abl activation might therefore contribute to neuronal dysfunction and the subsequent cytoskeletal pathology at the earliest steps of neurodegeneration.

Our results show that the c-Abl/p73 signalling pathway is involved in Aββ-induced neurodegeneration and that treatment with c-Abl inhibitors can delay the progression of neurodegeneration, supporting the use of STI571-like compounds in clinical treatments of Alzheimer’s disease patients.
Supplementary material
Supplementary material is available at Brain online.

Acknowledgements
This work was supported by FONDECYT 1040782, 1080221 and Ara Parsegian Medical Research Foundation. We thank Novartis for the gift of Gleevec (STI571), Dr Zhi-Min Yuan for the GFP-c-Abl(WT) and GFP-c-Abl(KD) plasmids, and Dr Guo-Jun Zhang for pcDNA-HA-p73α and pcDNA-HA-ΔNp73α.

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