

The Effects of CDK Inhibitors in a Mouse Model For Alzheimer's Disease

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Alzheimer's disease (AD) is an irreversible neurological disorder that progressively attenuates the cognitive abilities of those afflicted and ultimately leads to death. Amyloid plaques, neurofibrillary tangles and neuronal loss are the three invariant features of AD. Cyclin dependent kinase 5 (cdk5) is a neuron specific kinase essential for several functions of the nervous system. Cdk5 activity depends on binding to one of its two activators, p35 or p39. Hyperactivation of Cdk5 occurs when p25, the C-terminal fragment of p35, is liberated by proteolytic calpain cleavage. p25 levels accumulate during aging and in the early stages of Alzheimer's brains. A forebrain specific inducible p25 mouse model (p25Tg) develops all the pathological features of AD including neuronal loss/brain atrophy, neurofibrillary tangles and Aβ pathology. In addition, p25 mice exhibit learning deficits and an impairment in synaptic plasticity. The potential neuroprotective effects of small molecule inhibitors against the Cdks in the inducible p25 transgenic model render Cdk5 a promising therapeutic target.

To determine whether overactivation of cdk5 directly leads to the neuropathology observed in the p25Tg animals, we examined the effect of cdk inhibitors on the neuronal loss and amyloid and neurofibrillary pathology in the p25Tg mice. The first inhibitor that we tested was roscovitine, which binds competitively and reversibly to the ATP binding pocket. Roscovitine is a selective inhibitor of cdk1, cdk2 and cdk5, with the greatest potency against cdk5 (IC₅₀ = 0.16 μM). Due to the relative impermeability of roscovitine across the blood brain barrier, we administered the inhibitor intracerebroventricularly using a cannula implanted into the lateral

ventricle of mice. To maintain a continuous infusion, the cannula is attached by tubing to an Alzet osmotic pump inserted subcutaneously in the interscapular region of the animals. We have previously shown that deregulation of cdk5 contributes to neurodegeneration by hyperphosphorylation of the cytoskeletal protein tau and the transmembrane protein amyloid precursor protein (APP). Thus, we examined the phosphorylation state of tau and APP on cdk5 phosphorylation sites in p25Tg mice after administering roscovitine (dissolved in aCSF containing 50% DMSO) at a dose of 120 nmols/day, a similar concentration to that which has previously been shown to prevent the neuropathology in Niemann-Pick Type C mice. Following a two week intraventricular (ICV) infusion of roscovitine concomitant with p25 induction in the p25Tg mice, hyperphosphorylation of tau (AT-8) was attenuated in contrast to vehicle-treated p25Tg animals. In addition, phosphorylation of APP at Thr668 in p25Tg mice was also significantly reduced with roscovitine treatment compared to vehicle-treated controls. Phosphorylation of other cdk5 substrates such as GSK3-beta (Ser9), Rb (S780 and S795) and FAK (S732) were increased in vehicle-treated p25Tg mice. Following roscovitine treatment, phosphorylation of these substrates were similarly reduced.

We have previously reported that the p25Tg mice exhibit forebrain atrophy accompanied by astrogliosis, hippocampal and cortical cell loss and impaired learning and memory following five weeks of p25 induction. We tested whether roscovitine could prevent or attenuate these histological and behavioral phenotypes by implanting a 4-week pump and cannula ICV one week following induction. We tested two concentrations, 60 and 120 nmols/day, to determine if there was a dose-dependent effect by the inhibitor. The GFAP immunoreactivity, a marker that often accompanies neurodegeneration, was slightly reduced and nearly absent in the low and high dose roscovitine-treated mice, respectively, compared to vehicle-treated p25Tg animals. In order

to determine the effect of roscovitine treatment on the neuronal loss in the p25Tg mice, we performed NeuN staining in vehicle and roscovitine-treated wild-type and p25Tg animals. After quantifying neurons in the CA region of the hippocampus, we found a significant dose-dependent protective effect of roscovitine as compared the neuronal loss in vehicle-treated p25Tg mice. Moreover, the learning deficits in vehicle-treated p25Tg mice in the context-dependent fear conditioning test were prevented with roscovitine treatment. Importantly, administration of roscovitine in wild-type mice did not affect learning and memory or any of the histological parameters assessed.

We next tested a novel cdk inhibitor, CP681301 (CP), in collaboration with Pfizer. The CP compound is ~3- and 40-fold more potent towards cdk5 than cdk2 and GSK3-beta, respectively. Moreover, the CP compound is nearly 10-fold more potent ($IC_{50} = 13.7$ nM) than roscovitine. Importantly, CP readily crosses the blood-brain barrier, which facilitates testing in the p25Tg mouse model as this eliminates the requirement for ICV administration. We implanted 2-week Alzet pumps subcutaneously containing vehicle or 10mg/kg/day of CP vehicle concomitant with p25 induction. After 2 weeks, pumps were replaced with a 4-week pump containing the same concentration of vehicle or CP. Following a total of six weeks p25 induction and six weeks of continuous CP administration, we analyzed the neuroprotective potential of this compound. Hippocampal GFAP immunoreactivity was markedly attenuated in the p25Tg animals that received CP as compared to vehicle-treated controls. In addition, there was minimal cleaved caspase-3 immunoreactivity in the hippocampus of CP-treated animals in contrast to the robust caspase-3 staining in vehicle-treated p25Tg controls. CP-treated p25Tg animals also exhibited no learning impairments in the fear conditioning paradigm compared to vehicle-treated controls.

Taken together, these findings provide compelling evidence that deregulation of Cdk5 by p25 directly leads to neuronal cell loss, offer the first proof of concept that Cdk5 inhibitors are effective in a mouse model of neurodegeneration and establish the therapeutic potential for Cdk5 inhibitors in human diseases.