CDK5 INHIBITION AND ITS NOVEL FUNCTION IN NEURONAL DEVELOPMENT AND TREATMENT OF NEURODEGENERATION

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BACKGROUND

Cyclin-dependent kinase 5 (Cdk5) is a multifaceted serine/threonine kinase protein playing an essential role in neuronal development. Cdk5 activity regulates such pivotal cellular processes as neuronal migration, neurogenesis, synaptic plasticity, behavior, cognition or dendritic outgrowth. The aberrant activation of Cdk5 results in hyperphosphorylation of its various substrates, like Amyloid Precursor Protein, Tau protein and neurofilaments and subsequently to neuronal death. Dysfunction of Cdk5 is associated with diverse neurological disorders including Alzheimer's disease or amyotrophic lateral sclerosis.

Thus, we were interested if Dinaciclib, a novel Cdk5 inhibitor successfully used as therapeutic agent in advanced malignancies, is able to promote neurite outgrowth and neurogenesis in primary rat hippocampal neurons.

MATERIALS AND METHODS

For this purpose, primary embryonic rat hippocampal neurons were treated with Dinaciclib for 48 h on DIV1. The following parameters were determined: the number of neurites, the total length of neurites and the length of the longest neurite. To address the effects of Dinaciclib on neurogenesis of primary rat hippocampal neurons we determined the percentage of BrdU-NeuN double positive cells within the total number of neurons. For these analyses a software supported automatic quantification method was used (Axio.Imager Z1 microscope, ImageProPlus).

RESULTS

Dinaciclib is not detrimental to primary neurons

Dinaciclib (SCH 727965) inhibits Cdk2, Cdk5, Cdk1, and Cdk9 activity *in vitro* with IC(50) values of 1, 1, 3, and 4 nmol/L, respectively.

In vitro and *in vivo* analyses demonstrated that Dinaciclib inhibits the growth of a broad spectrum of human cancers most probably based on apoptosis. Thus we were interested in the effects of Dinaciclib on cell viability and apoptosis of primary rat hippocampal neurons.

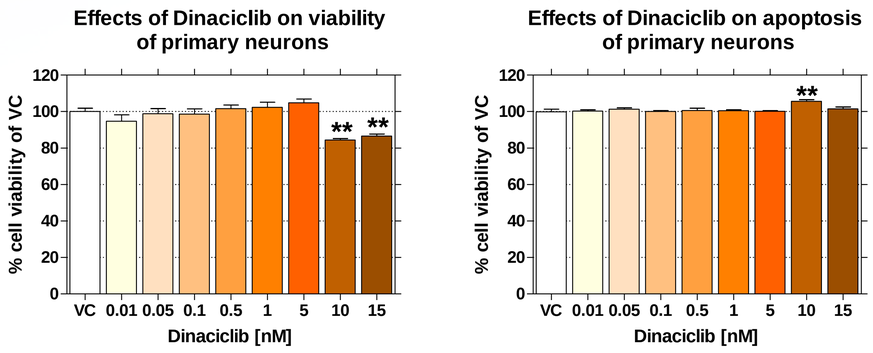
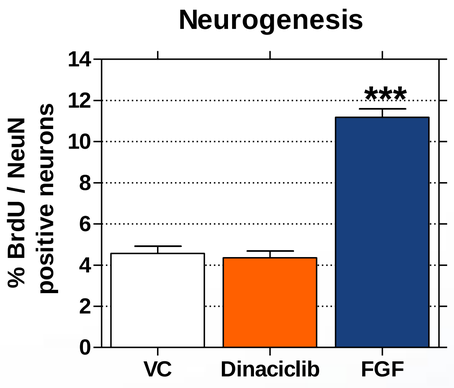


Figure 1. Effects of Dinaciclib on cell viability and apoptosis of primary rat hippocampal neurons. Dinaciclib (0.01 to 15 nM) was applied on DIV8 for 24h to primary rat hippocampal neurons. Effects on cell viability and apoptosis were determined by MTT and YOPRO assay, respectively. Data are shown as % of vehicle control (VC). Statistical significance is indicated by **<0.01 as determined by One-Way ANOVA (Dunnett's Multiple Comparison Test). Data are shown as group mean + SEM (n=6).

Dinaciclib does not affect neurogenesis in primary neurons

CDKs are key regulators of cell cycle progression. Thus we were interested if Dinaciclib induces any effects on neurogenesis of primary embryonic rat hippocampal neurons.

Figure 2. Effects of Dinaciclib on neurogenesis of primary rat hippocampal neurons. Dinaciclib (10 nM) and FGF (20 ng/ml) were applied on DIV0 for 48h to primary rat hippocampal neurons. After 24h, BrdU [10 µM] was added. Next, cells were fixed and subjected to indirect immunofluorescences analysis. Digital images were analyzed for the % of BrdU positive cells compared to the total number of neurons (NeuN positive) using a software-supported automatic quantification method. Total number of counted neurons was between 14,000 and 42,000 cells. Analysis was performed using ImagePro Plus software. Statistical significance is indicated by ***<0.001 as determined by One-Way ANOVA (Newman-Keuls Multiple Comparison Test). Data are shown as group mean + SEM from two independent experiments (n= 18-20).



Dinaciclib promotes neurite outgrowth

Cdk5 is a multifaceted kinase in neurodegenerative diseases and is involved in the regulation of such critical processes as neurite outgrowth or synaptogenesis. Therefore, we investigated effects of Dinaciclib treatment on neurite formation in primary rat embryonic hippocampal neurons.

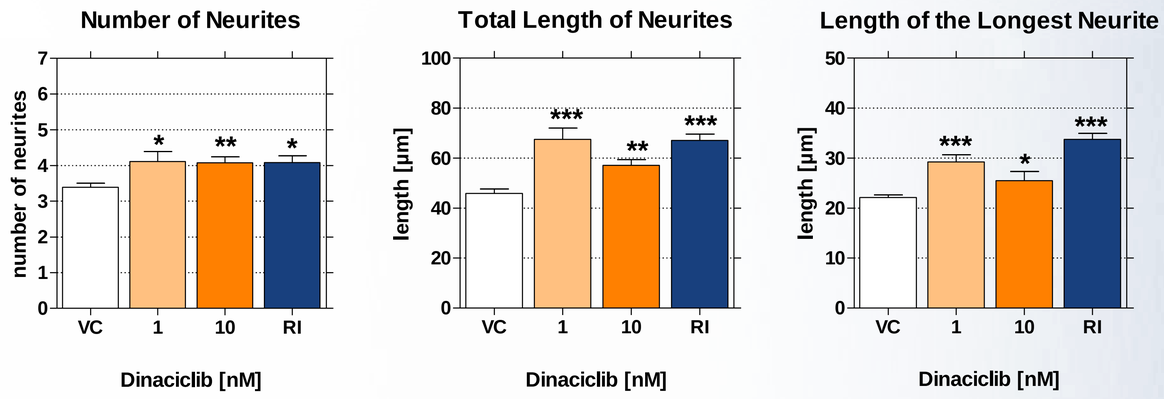


Figure 3. Effects of Dinaciclib on neurite outgrowth of primary rat hippocampal neurons. Primary rat hippocampal neurons were treated with Dinaciclib (1 and 10 nM) or reference item (RI, beta-estradiol, 0.1 nM) on DIV1 for 48h. Cells were fixed and subjected to indirect immunofluorescence analysis. Digital images were analyzed for the following parameters: number of neurites, total length of neurites and length of the longest neurite using a software-supported automatic quantification method. Total number of counted neurons was between 160 and 240 cells. Analysis was performed using ImagePro Plus software. Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by One-Way ANOVA (Newman-Keuls Multiple Comparison Test). Data are shown as group mean + SEM from two independent experiments (n= 18-20).

Dinaciclib favors synaptogenesis

Synaptic pathology has been implicated in many neurodegenerative disorders beyond Alzheimer's disease, like Huntington's disease, Tauopathies or Niemann Pick disease. Synapse formation and especially the synaptic maintenance are critical for neuronal health. Therefore, we examined effects of Dinaciclib treatment on synaptogenesis in primary rat embryonic hippocampal neurons.

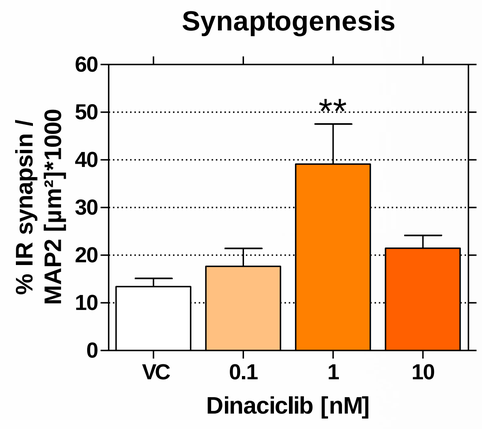
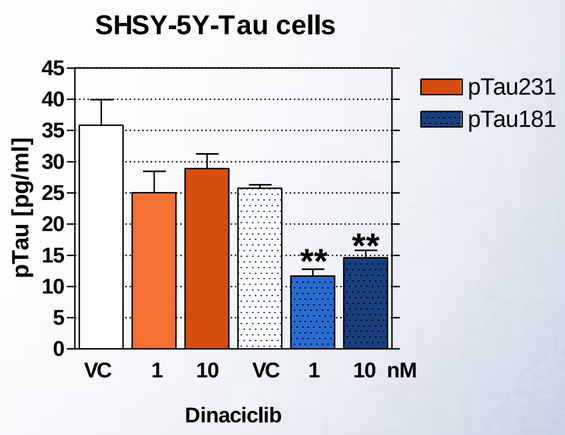


Figure 4. Effects of Dinaciclib on synaptogenesis of primary rat hippocampal neurons. Graph represents effects of Dinaciclib (0.1, 1, 10 nM) on synaptogenesis of primary embryonic rat hippocampal neurons. Substances were applied on DIV6 for 24h. Cells were fixed and subjected to indirect immunofluorescence analysis. Data are shown as % of immunoreactive area (IR) of synapsin colocalization to MAP2*1000. Digital images were analyzed by using a software-supported automatic quantification method. Total number of counted neurons was around 50 cells. Analysis was performed using ImagePro Plus software. Statistical significance is indicated by **<0.01, as determined by One-Way ANOVA (Dunnett's Multiple Comparison Test). Data are shown as group mean + SEM (n= 6).

Dinaciclib attenuates Tau hyperphosphorylation

Many protein kinases are involved in tau hyperphosphorylation. GSK-3 and cdk5 can phosphorylate tau at most of the known AD sites including pTau181 and pTau231. Thus these two kinases have drawn much attention as drug targets in attenuating Tau hyperphosphorylation. Thus we were interested in the effects of Dinaciclib on Tau hyperphosphorylation in SHSY-5Y-Tau441V337M/R406W.

Figure 5. Effects of Dinaciclib on Tau hyper-phosphorylation. Graph represents effects of Dinaciclib (1, 10 nM) on Tau hyperphosphorylation in SHSY-5Y-Tau441 cells. SHSY-5Y-Tau441 cells were treated Dinaciclib for 6h. pThr231 and pThr181 Tau levels were measured by immunosorbent assay. In SHSY-Tau cells, hyperphosphorylation at site Thr231 was reduced and at site Thr181 significantly downregulated by Dinaciclib. Statistical significance is indicated by *<0.05, **<0.01 as determined by One-Way ANOVA (Dunnetts Multiple Comparison Test). Data are shown as group mean + SEM (n= 4).



CONCLUSIONS

Our data indicate inhibition of Cdk5 activity to be a promising strategy to treat neurodegenerative diseases by promoting neurite outgrowth and synaptogenesis. Furthermore Dinaciclib attenuates tau hyperhosphorylation.Thus, these data identify Dinaciclib as a promising new drug candidate in neurodegenerative diseases.

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