DINACICLIB, A POTENT CYCLIN-DEPENDENT KINASE INHIBITOR, AND ITS NOVEL FUNCTION IN NEURONAL DEVELOPMENT

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BACKGROUND

Cyclin-dependent kinase 5 (Cdk5) is a multifaceted serine/threonine kinase protein playing an essential role in neuronal development. Over the last decade, Cdk5 activity has been demonstrated to regulate such pivotal cellular processes as neuronal migration, neurogenesis 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 has been associated with diverse neurological disorders including Alzheimer's disease, amyotrophic lateral sclerosis or Niemann-Pick type C disease. Recent evidence also suggests a pivotal role for Cdk5 in synaptic plasticity, behavior, and cognition.

Thus, we were interested if the novel Cdk5 inhibitor Dinaciclib with its known function 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.

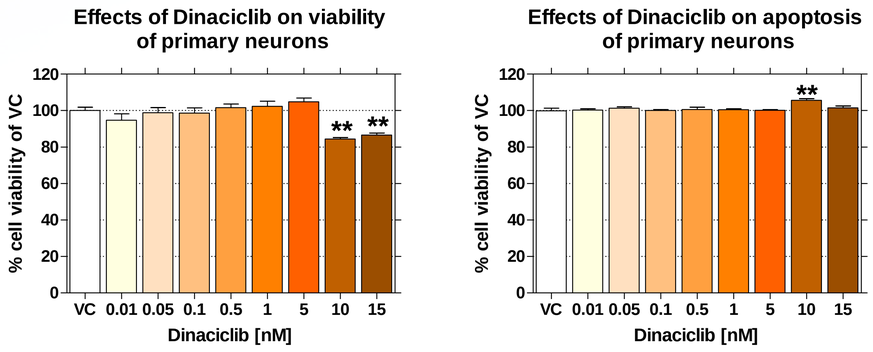


Figure 1. Effects of Dinaciclib on cell viability and apoptosis of primary rat hippocampal neurons. Dinaciclib (0.01 to 15 nM) was applied on DIV8 for 24h to primary rat hippocampal neurons. Effects on cell viability and apoptosis were determined by MTT and YOPRO assay, respectively. Data are shown as % 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.

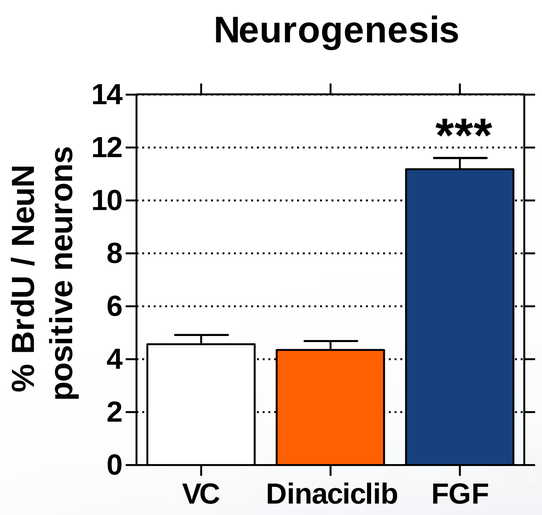


Figure 2. Effects of Dinaciclib on neurogenesis of primary rat hippocampal neurons. Dinaciclib (10 nM) and FGF (20 ng/ml) were applied on DIV0 for 48h to primary rat hippocampal neurons. After 24h, BrdU [10 µM] was added. Next, cells were fixed and subjected to indirect immunofluorescences analysis. 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.

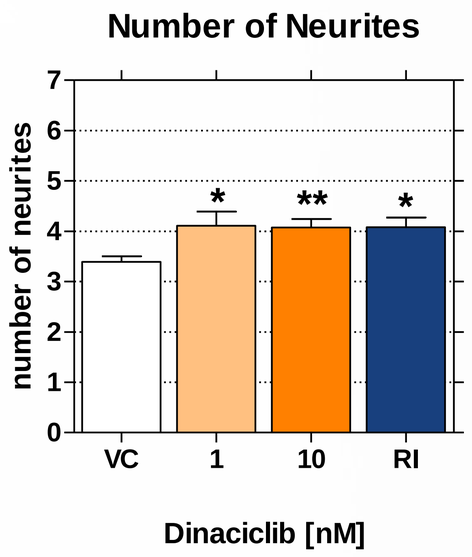
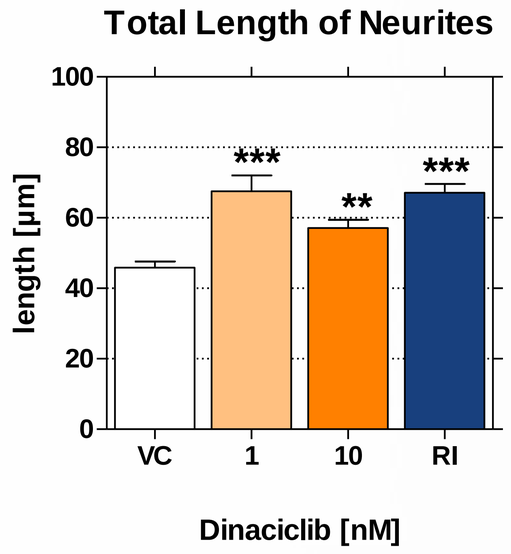
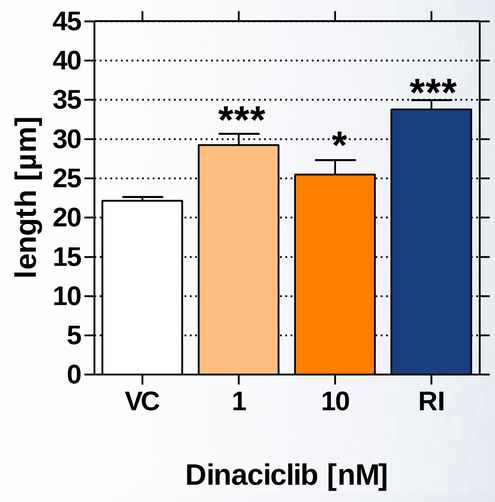


Figure 3. Effects of Dinaciclib on neurite outgrowth of primary rat hippocampal neurons. Primary rat hippocampal neurons were treated with Dinaciclib (1 and 10 nM) or reference item (RI, beta-estradiol, 0.1 nM) on DIV0 for 48h. Cells were fixed and subjected to indirect immunofluorescence analysis. Digital 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 favours 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.

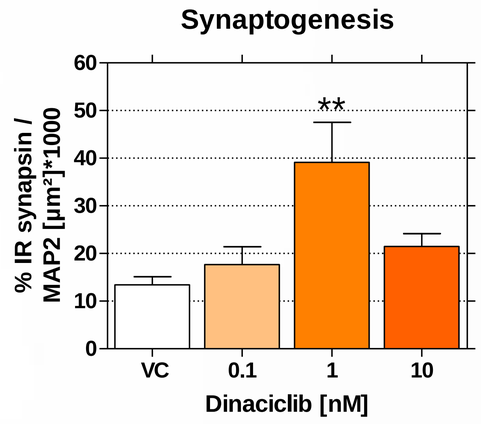


Figure 4. Effects of Dinaciclib on synaptogenesis of primary rat hippocampal neurons. Graph represents effects of Dinaciclib (0.1, 1, 10 nM) on synaptogenesis of primary embryonic rat hippocampal neurons. Substances were applied on DIV6 for 24h. Cells were fixed and subjected to indirect immunofluorescence 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).

CONCLUSIONS

Interestingly, Dinaciclib did not affect neurogenesis. However, for the first time, we were able to demonstrate beneficial effects of the novel Cdk inhibitor Dinaciclib on neurite outgrowth and synaptogenesis of primary rat hippocampal neurons and identified a promising new drug candidate in neurodegenerative diseases.
Thus, targeting Cdk activity by a new and potent inhibitor represents a promising strategy targeting neurodegenerative diseases by promoting neurite outgrowth.

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