

Vidofludimus Calcium Requires Nurr1 to Promote Neuronal Survival *In Vitro* and is Associated with Neuroprotective Effects in Murine Models of Multiple Sclerosis



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Conclusions

- Vidofludimus calcium may provide neuroprotective effects in both *in vitro* and *in vivo* models.
- These neuroprotective effects may be mediated by activation of nuclear receptor-related factor 1 (Nurr1). Dihydropyridine dehydrogenase (DHODH) inhibition alone is insufficient to fully explain the pro-survival effects.
- These findings provide additional preclinical evidence of the effects of vidofludimus calcium on pathways associated with neuronal survival and neuroinflammation; the relevance to clinical outcomes has not been established.



Introduction/Aim

- Nurr1 regulates transcriptional programs relevant to neuronal survival, maintenance, and integrity. In microglia and astrocytes, it restrains pro-inflammatory gene expression that contributes to neuronal loss¹⁻³.
- Higher Nurr1 expression is associated with greater neuronal density. Additionally, nuclear Nurr1 localization in layer 3 neurons is correlated with older age-at-death and delayed time to wheelchair in progressive multiple sclerosis (PMS)⁴.
- Vidofludimus calcium, currently in clinical development for MS, directly activates Nurr1 and selectively inhibits DHODH.
- Here we present additional pre-clinical evidence suggestive of a Nurr1-dependent neuroprotective mechanism of vidofludimus calcium.



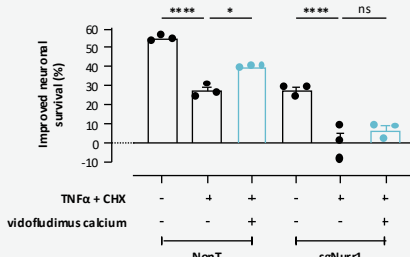
Methods

- In vitro* studies assessed the direct and indirect effects of vidofludimus calcium to promote neuronal survival under neurotoxic conditions and evaluated the role of Nurr1 in mediating these effects.
- In vivo*, murine MOG₃₅₋₅₅-induced experimental autoimmune encephalomyelitis (EAE) models were used to assess the neuroprotective potential of vidofludimus calcium.
- Statistical differences were determined by ANOVA with appropriate multiple-comparison post hoc tests or unpaired two-tailed Student's t-tests (see figure legends for details). Data are shown as mean ± SEM. P values: * < 0.05; ** < 0.01, *** < 0.001, **** < 0.0001.



Results

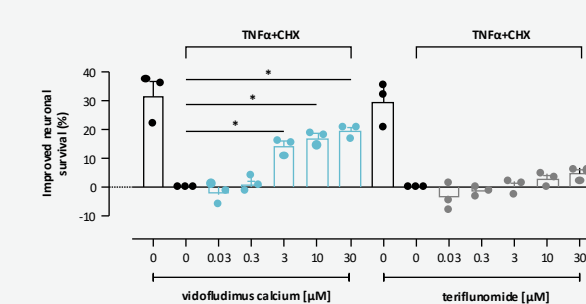
Figure 1: Vidofludimus calcium promotes Nurr1-dependent neuronal survival *in vitro*



Vidofludimus calcium promoted neuronal survival under apoptotic conditions in cells expressing Nurr1 (blue bar, NonT). When Nurr1 was knocked out, this effect was abolished (blue bar, sgNurr1), indicating that Nurr1 is required for vidofludimus calcium-mediated neuroprotection.

SH-SY5Y cells, a human neuroblastoma cell line, were treated with vidofludimus calcium (3 μM) for 1 hour, prior to treatment with TNFα + CHX. Cells were then treated with TNFα + CHX for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with TNFα + CHX for 1 hour, prior to treatment with vidofludimus calcium (3 μM) for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with TNFα + CHX for 1 hour, prior to treatment with vidofludimus calcium (3 μM) for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with TNFα + CHX for 1 hour, prior to treatment with vidofludimus calcium (3 μM) for 1 hour. Cell survival was assessed by flow cytometry.

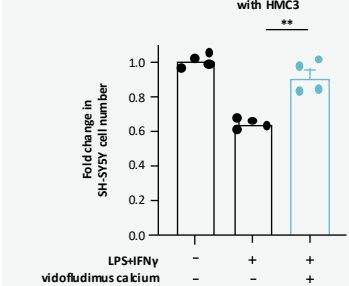
Figure 2: Vidofludimus calcium promotes neuronal survival under apoptotic stress *in vitro*



Vidofludimus calcium at 3, 10 and 30 μM promoted neuronal survival under apoptotic conditions. A similar neuroprotective effect was not observed with teriflunomide (a DHODH inhibitor), suggesting that the observed pro-survival effect involves mechanisms beyond DHODH inhibition.

Human SH-SY5Y cells were treated with increasing concentrations of vidofludimus calcium (3, 10, 30 μM) for 1 hour, followed by treatment with TNFα + CHX for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with TNFα + CHX for 1 hour, followed by treatment with vidofludimus calcium (3, 10, 30 μM) for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with TNFα + CHX for 1 hour, followed by treatment with vidofludimus calcium (3, 10, 30 μM) for 1 hour. Cell survival was assessed by flow cytometry.

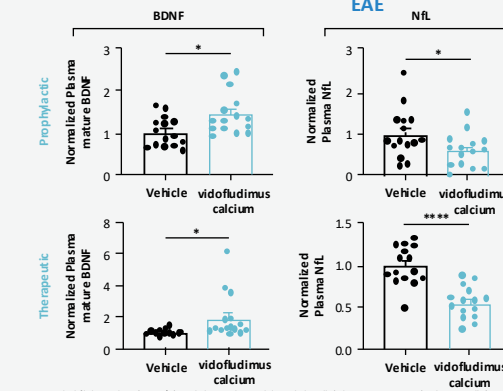
Figure 3: Vidofludimus calcium attenuates microglia-induced neuronal cell loss



Vidofludimus calcium attenuated microglia-induced neuronal loss in a human microglia-neuronal co-culture, suggesting indirect neuroprotective effects (above and Supplemental Figure 2).

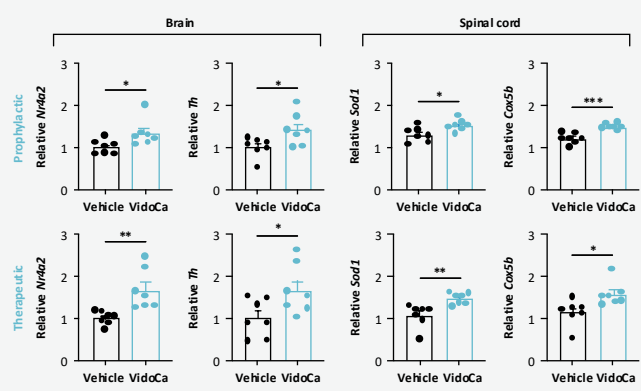
Human microglia and SH-SY5Y cells were co-cultured at a 1:1 ratio. Cells were treated with LPS + IFNγ for 1 hour, followed by treatment with vidofludimus calcium (3 μM) for 1 hour. Cell survival was assessed by flow cytometry. SH-SY5Y cells were treated with LPS + IFNγ for 1 hour, followed by treatment with vidofludimus calcium (3 μM) for 1 hour. Cell survival was assessed by flow cytometry.

Figure 4: Vidofludimus calcium treatment suggests neuroprotective potential in EAE



EAE mice received vidofludimus calcium (30 mg/kg) or vehicle (saline) daily or on alternate days (5 days post-immunization) for the specified time period. Plasma BDNF and NfL were measured using commercial ELISA kits. Data are shown as mean ± SEM. P values: * < 0.05; ** < 0.01, *** < 0.001, **** < 0.0001.

Figure 5: Nurr1 target gene expression after vidofludimus calcium treatment in EAE models



EAE mice received vidofludimus calcium (30 mg/kg) or vehicle (saline) daily or on alternate days (5 days post-immunization) for the specified time period. Relative expression of Nurr1 target genes (Sod1, Th, Cox5b) in brain and spinal cord was assessed by quantitative real-time PCR. Statistical differences were determined by unpaired two-tailed Student's t-test.

- Prophylactic and therapeutic treatment of EAE mice with vidofludimus calcium upregulated Nurr1 target genes, tyrosine hydroxylase (Th), superoxide dismutase 1 (Sod1), and cytochrome c oxidase subunit 5b (Cox5b) in the brain and spinal cord.
- These genes are implicated in neuronal function (Th), protection against oxidative stress (Sod1), and support of cellular energy production (Cox5b). Their upregulation suggests activation of Nurr1-associated pathways linked to neuroprotection.

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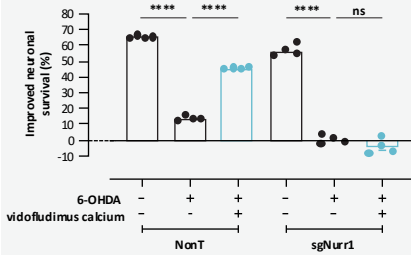
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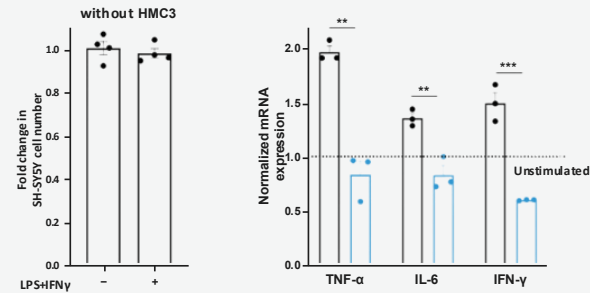
Supplemental Figure 1: Vidofludimus calcium promotes neuronal survival under neurotoxic conditions *in vitro*



- Vidofludimus calcium promoted neuronal survival under neurotoxic conditions at 1 μ M, this effect was lost following Nurr1 knockout.

Human neuronal neuroblastoma SH-SY5Y cells were treated with vidofludimus calcium for 4 hours, followed by incubation with 6-hydroxydopamine (6-OHDA, 10 μ M/ml) for 16 hours to induce apoptosis. Cell survival was quantified by Annexin V/Live-Dead flow cytometry.

Supplemental Figure 2: Microglia-dependent neuronal cell loss and anti-inflammatory effects of vidofludimus calcium *in vitro*

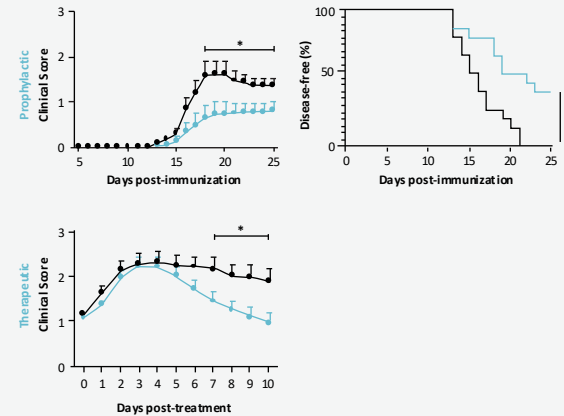


- In this assay, the absence of neuronal loss in cultures lacking microglia suggests that LPS- and IFN- γ -induced neurotoxicity is mediated by microglia rather than a direct effect on neurons.
- Vidofludimus calcium (blue bars) reduced mRNA expression of the pro-inflammatory cytokines in LPS-stimulated HMC3 microglia, suggesting attenuation of microglial inflammatory activity.

Left: SH-SY5Y cells cultured without HMC3 microglia were stimulated with LPS (1 μ g/mL) and IFN- γ (50 ng/mL) for 24 hours. The number of live SH-SY5Y-GFP cells was quantified by flow cytometry.

Right: HMC3 microglia were pretreated with vidofludimus calcium (0.1 μ M, blue bars) for 4 hours, followed by 24 hours of stimulation with LPS (1 μ g/mL). The expression of cytokines was quantified by qRT-PCR.

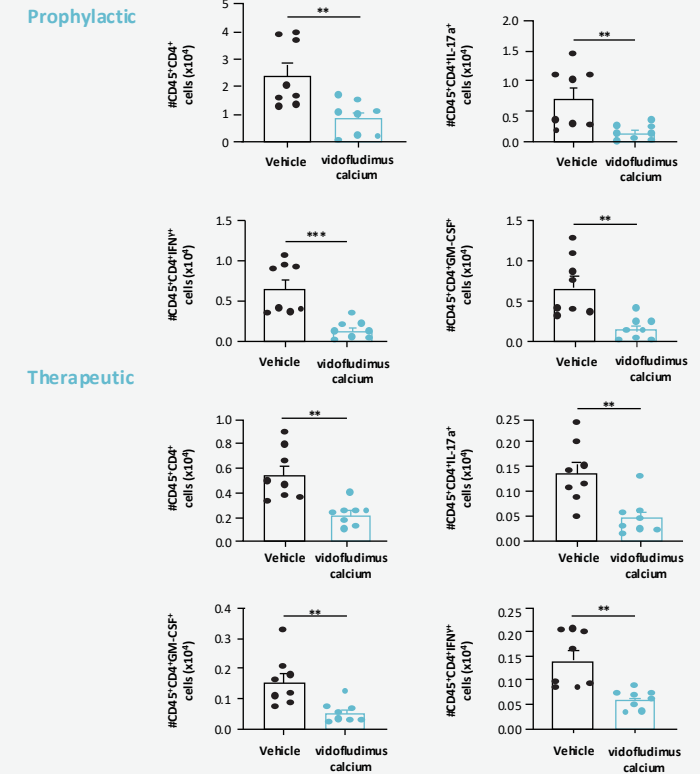
Supplemental Figure 3: Vidofludimus calcium attenuates disease severity in EAE models



- Vidofludimus calcium attenuated disease severity in both prophylactic and therapeutic EAE models (blue lines). In the prophylactic EAE model, vidofludimus calcium-treated mice showed lower disease severity and a greater percentage of disease-free animals (clinical score of 0) compared with vehicle-treated mice.

EAE mice received 150 mg/kg of vidofludimus calcium as described in Figure 4. Severity of EAE was monitored by an investigator and a clinical score ranging from 0 to 5 was assigned: 0=no disease; 0.5=partially limp tail; 1=paralyzed tail; 2=hind limb weakness; 3=hind limb paralysis; 4=hind limb and forelimb paralysis; 5=morbidity and death.

Supplemental Figure 4: Vidofludimus calcium decreases CNS immune cell infiltrates in EAE models



- Lower absolute numbers of proinflammatory CD4⁺ T helper (Th) cells, including Th17 (IL-17A⁺), GM-CSF⁺ Th and Th1 subsets (IFN- γ ⁺) were observed in the CNS of EAE mice after vidofludimus calcium treatment, suggesting reduced CNS infiltration and inflammation.

Absolute number of IL-17A⁺, GM-CSF⁺, and IFN- γ ⁺ cells among CD4⁺ T cells recovered from the CNS of EAE mice were quantified using flow cytometry. EAE mice were treated with 150 mg/kg of vidofludimus calcium as described in Figure 4.