In Preclinical Models, Vidofludimus Calcium Exhibits Potential Neuroprotective Effects in Multiple Sclerosis by Modulating Nurr1

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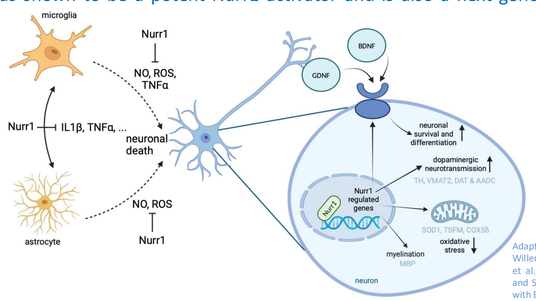
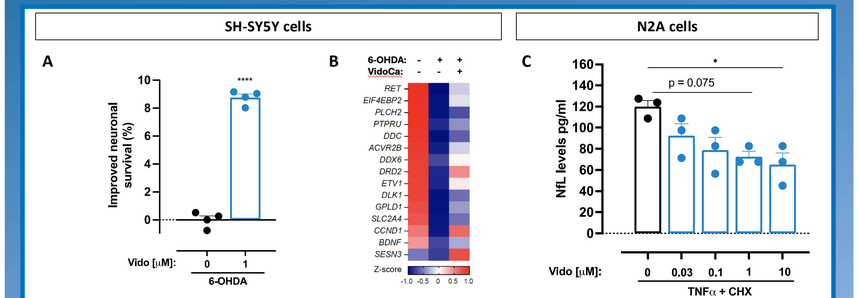
1. Background

Nuclear receptor-related 1 (Nurr1), a transcription factor, regulates genes that enhance neuronal survival and reduce neurotoxic mediators produced by microglia and astrocytes and growing evidence supports its protective role in neurodegenerative diseases. In persons with multiple sclerosis (PwMS), Nurr1 gene expression levels in blood were shown to be reduced. However, these levels revert to normal in pregnant PwMS, which coincides with a reduction in disease activity[1]. Also, findings by Pansieri (2023)[2] highlighted a potential protective role of Nurr1 in the post-mortem motor cortex of progressive PwMS. Vidofludimus calcium (VidoCa) is currently in phase 2 and 3 clinical trials for progressive and relapsing MS, respectively. It has a dual mode of action. VidoCa was shown to be a potent Nurr1 activator and is also a next generation DHODH inhibitor.



Adapted and modified from Willems, Merk et al., 2022, Vietor et al., 2023, Schiro et al., 2022 and Saijo et al., 2009[3-6]. Created with BioRender.com

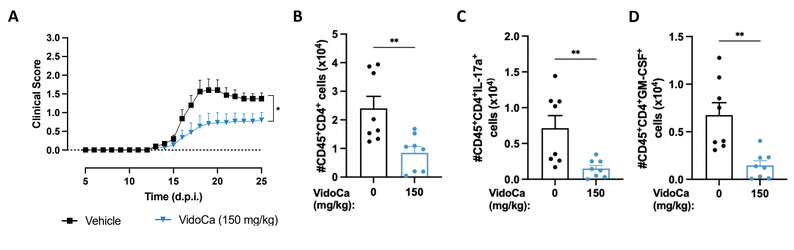
2. VidoCa enhances neuronal survival



VidoCa displays neuroprotective properties in human (SH-SY5Y) and murine (N2A) neuronal cells. (A, B) Four-hour pretreatment with VidoCa **(A)** significantly enhanced SH-SY5Y cell survival and **(B)** rescued Nurr1 target gene expression levels after apoptosis induction by the neurotoxic agent 6-OHDA (10 µg/ml) for 16 hours. **(C)** One-hour pretreatment with VidoCa dose-dependently reduced NfL levels, a marker for neuronal damage, in the supernatant after 6 hours of apoptosis induction by TNFα (50 ng/ml) and CHX (20 µg/ml). These data indicate that VidoCa protects neurons from apoptosis.

6-OHDA = 6-hydroxydopamine; CHX = cycloheximide; NfL = neurofilament light chain; TNFα = tumor necrosis factor alpha; Vido = vidofludimus calcium (VidoCa); .*p<0.05; ****p<0.0001. Cell viability was assessed by flow cytometry, Nurr1 target gene expression by RNA-seq and NfL by EUSA. Data are shown as mean + SEM. Statistics: (**A**) two-tailed t-test; (**C**) Kruskal-Wallis test with Dunn's multiple comparisons.

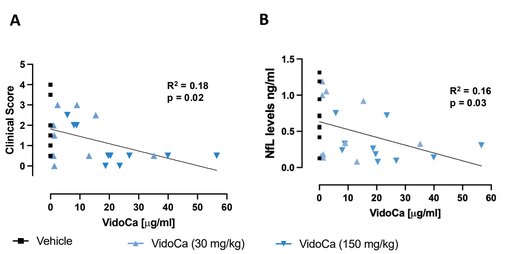
3. VidoCa attenuates disease severity in an EAE model



VidoCa attenuates disease severity in a prophylactic MOG_{35-55} murine EAE model. EAE was induced in 11-week-old female C57BL/6 mice by immunization with MOG_{35-55}. Daily treatment per oral gavage with VidoCa or vehicle was started at 5 d.p.i. **(A)** VidoCa reduced disease severity (clinical score) up to 25 d.p.i. **(B, C, D)** Immune cell infiltration in the CNS was assessed at 25 d.p.i. by FACS. 150 mg/kg VidoCa significantly reduced the number of (B) T helper (Th) cells, (C) Th17 cells, and (D) GM-CSF-producing Th cells, with statistical significance confirmed.

d.p.i. = days post immunization; EAE = experimental autoimmune encephalomyelitis; FACS = fluorescence-activated cell sorting; MOG = myelin oligodendrocyte glycoprotein; vehicle = PEG400; VidoCa = vidofludimus calcium; *p<0.05; **p<0.01. Data in the graphs are shown as mean + SEM. Statistics: (**A**) two-way repeated measures ANOVA and (**C**) two-tailed t-test

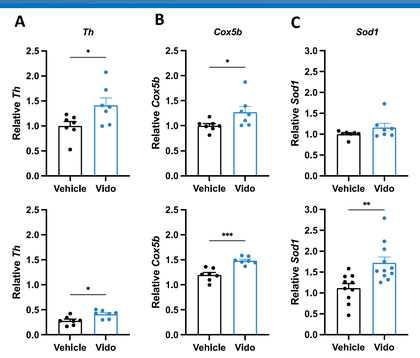
4. Potential neuroprotective activity of VidoCa *in vivo*



VidoCa displays neuroprotective activity *in vivo* in EAE model. The EAE model was performed as described before but with an additional treatment group (30 mg/kg). Weak but significant negative correlations between plasma VidoCa concentrations and clinical score **(A)** as well as plasma NfL levels **(B)** at end-of-study (19 or 25 d.p.i.) were observed. **(C)** The potential peripheral Nurr1 activation biomarker, BDNF, is increased and **(D)** the biomarker for axonal damage and neurodegeneration, NfL, is reduced in the plasma of mice treated with 150 mg/kg VidoCa (Vido) at study end (25 d.p.i.).

BDNF = brain derived neurotrophic factor; d.p.i. = days post immunization, EAE = experimental autoimmune encephalomyelitis; NfL = neurofilament light chain; Nurr1 = nuclear receptor-related 1; Vido = vidofludimus calcium (VidoCa); *p<0.05. Data in the graphs are shown as mean + SEM. Statistics: (A, B) simple linear regression; (C, D) two-tailed t-test.

5. VidoCa enhances Nurr1 target gene expression in the CNS



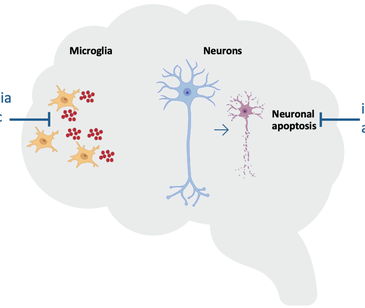
VidoCa treatment augments Nurr1-regulated gene expression in the CNS in EAE. Brain and spinal cord samples were obtained from the same mice shown in the previous figure (box 3). The study revealed significantly higher (**A**) *Th*, and (**B**) *Cox5b* levels in brain and spinal cord as well as (**C**) higher *Sod1* levels in the spinal cord of mice treated with 150 mg/kg VidoCa. These data support the potential activation of Nurr1 by VidoCa treatment *in vivo*.

Cox5b = cytochrome c oxidase subunit 5B; d.p.i. = days post immunization, EAE = experimental autoimmune encephalomyelitis; Nurr1 = nuclear receptor-related 1; Sod1 = superoxide dismutase 1; Th = tyrosine hydroxylase; Vido = vidofludimus calcium (VidoCa); . *p<0.05; **p<0.01; ***p<0.001. Data in the graphs are shown as mean + SEM. Statistics: two-tailed t-test.

6. Summary

Target Nurr1

reduces activation of microglia and production of neurotoxic mediators[6,7]



improves survival of neurons in a neurotoxic environment[7, 8, 9]

VidoCa

- **activates Nurr1 *in vitro* and possibly *in vivo*.**
- **reduces neuronal loss and disease manifestations, potentially through both direct and indirect mechanisms involving reduced microglial activation.**
- **displays neuroprotective properties *in vitro* and possibly *in vivo*, most likely mediated by Nurr1-driven regulation of survival signals.**

Nurr1 = nuclear receptor-related 1

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Disclosures

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