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Title: Valnoctamide inhibits cytomegalovirus infection in the developing brain and attenuates virally induced brain defects and neurological dysfunctions.

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Background: Cytomegalovirus (CMV) is the leading infectious cause of brain abnormalities and neurological deficits in developing babies. Therapeutic interventions during critical moments of brain ontogeny are pivotal to prevent or ameliorate the virally induced sequelae. Yet, no anti-CMV drugs are approved for use during gestation and marked limitations affect therapy in infected neonates due to the teratogenicity and toxicity of the licensed CMV antivirals. We reported that valnoctamide (VCD), a mood stabilizer safely used for many years in clinics and with no known teratogenicity, can inhibit CMV. However, whether this anti-CMV activity could translate into therapeutic effects on the CMV-infected developing brain is not known.
Objective: To investigate whether low-dose VCD could safely suppress CMV inside the brain of infected developing mice and exert beneficial effects on the virally induced neurological sequelae and brain defects.
Methods: Pups inoculated i.p. with murine CMV (mCMV, 750 PFU) on the day of birth received VCD or vehicle (1.4mg/mL) subcutaneously from postnatal day (P)1 to P21. Uninfected animals served as controls. Newborn mice were chosen because the development of their brain parallels the human brain in the early second trimester of pregnancy (Clancy et al., 2001), critical moment of brain ontogeny and for CMV infection. Viral load in the brain was determined by qPCR. Neurobehavioral phenotyping was performed during the neonatal period (P2-P14) and in juvenile mice (P30) by assessing the acquisition of neurological milestones, according to a modified Fox battery, and the motor performance, using the clasping test, vertical pole test, and challenging beam traversal test. At completion of testing, brain size and histology were analyzed.

Results: VCD treatment decreased the amount of virus detected in the brain of mCMV-infected mice by approximately 100- to 1000-fold at all the time-points tested (Fig. 1). The anti-CMV effect showed a quick onset and suppressed viral load was identified after only 3 days of therapy. The delayed acquisition of neurological milestones observed in infected pups was rescued by drug administration, with VCD-treated neonates displaying a timely acquisition of all the behaviors measured. Long-lasting beneficial effects of VCD treatment were observed in juvenile mice, in which drug restored normal motor function and substantially ameliorated CMV-induced deficient brain growth (Fig. 2) and neuronal loss. No adverse effects on neurodevelopment of uninfected controls receiving VCD were identified.
Conclusions: Subcutaneous low-dose VCD can effectively and safely control CMV replication in the developing brain and rescue virally induced brain defects and adverse neurological outcomes. Given that VCD is already available and has been proved safe in multiple models of early development, it may represent a valid and safer therapeutic option in CMV-infected human fetuses and neonates.

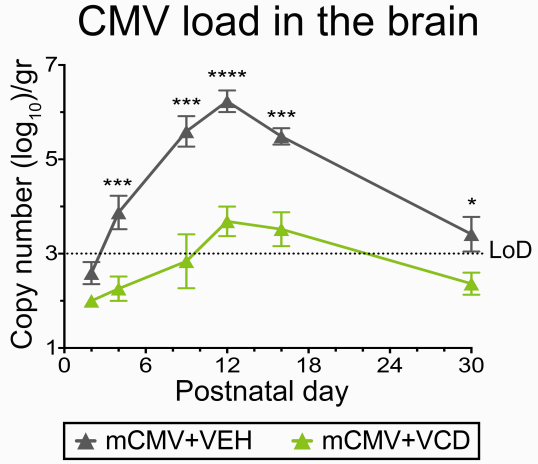

Fig. 1. Valnoctamide suppresses mCMV load in the brain of infected mice. Newborn mice were infected at P0 with mCMV i.p. and treated with either vehicle (mCMV+VEH) or VCD (mCMV+VCD) from P1 till P21. Viral load was quantified in the brain by qPCR at the specified time-points and expressed as $\log_{10}$ genome copies per gram of harvested tissue. Mean±SEM; n=7-10 mice/time-point. Viral titers below the limit of detection (LoD, dotted line) were plotted as 2 $\log_{10}$ genome copies. * $p < 0.05$, *** < 0.001, **** < 0.0001; two-way ANOVA with postnatal day as repeated measures.

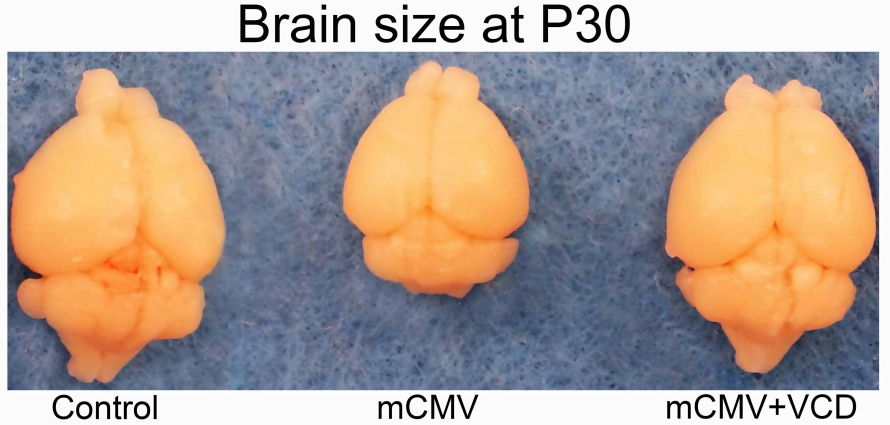

Fig. 2. Valnoctamide reverses deficient brain growth induced by mCMV infection. Photograph shows decreased brain size in an infected, untreated mouse (mCMV, middle), as compared to an uninfected control (left). VCD treatment restores normal brain growth (mCMV+VCD, right).