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Attenuation of AMPA receptor activity improves motor skills in a mouse model of juvenile Batten disease

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Abstract

Juvenile Batten disease, caused by mutations in the *CLN3* gene, is a fatal, incurable neurodegenerative disorder in children. The *Cln3*-loss-of-function ($Cln3^{\Delta ex1\text{-}6}$) mouse model of the disease exhibits many characteristic pathological features of the human disorder including a deficit in motor skills. Our recent findings (Kovács et al., 2006) suggested that the neurological deficit in the $Cln3^{\Delta ex1\text{-}6}$ mouse model of the disease might result from an abnormally increased AMPA receptor activity in the cerebellum. Therefore, we tested if administration of low doses of an AMPA receptor antagonist, that attenuate AMPA receptor function but avoid a toxic, complete blockade of the receptor, have beneficial effects in $Cln3^{\Delta ex1\text{-}6}$ mice. Here we show that attenuation of AMPA receptor activity by a single intraperitoneal injection of the non-competitive AMPA antagonist, EGIS-8332 (1 mg/kg), significantly improves the motor skills of $Cln3^{\Delta ex1\text{-}6}$ mice. Our results provide a new, promising therapeutic approach for juvenile Batten disease.

Keywords

juvenile Batten disease; *Cln3*; $Cln3^{\Delta ex1\text{-}6}$; mouse model; motor skills; AMPA receptor antagonist; EGIS-8332

Batten disease (also known as Neuronal Ceroid Lipofuscinosis), is a group of recessively inherited lysosomal storage disorders characterized by progressive neurodegeneration (Goebel, 1995). Batten disease is the most common degenerative brain disease in children, with a global incidence of 1 to 8 in 100,000 births (Zhong, 2000). Mutations in the *CLN3*

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gene are responsible for the development of the most prevalent, juvenile onset form of Batten disease (The International Batten Disease Consortium, 1995). The disease begins between five and eight years of age, and the typical clinical symptoms are progressive vision loss, frequent occurrence of seizures, loss of motor skills and progressive cognitive decline, cumulatively leading to premature death in the late teens or early 20s. As yet, no specific treatment is known that can halt or slow the progress of the disease. Progressive deterioration of motor skills is one of the primary clinical features in juvenile Batten disease (Goebel and Wisniewski, 2004).

The *Cln3*-loss-of-function ($Cln3^{\Delta ex1\text{-}6}$) mouse model of the disease exhibits many characteristic pathological features of the human disorder including a deficit in motor skills (Mitchison et al., 1999; Kovács et al., 2006). We have recently found that $Cln3^{\Delta ex1\text{-}6}$ cerebellar granule cells in dissociated cultures and in organotypic cerebellar slice cultures have a selectively increased sensitivity to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptor overactivation, indicating an abnormally enhanced AMPA receptor activity, and suggesting that AMPA receptor dysregulation may be a major contributor to the cerebellar dysfunction and progressive neurological decline associated to juvenile Batten disease (Kovács et al., 2006). Therefore, we tested if attenuation of AMPA receptor activity by a single intraperitoneal injection of the selective, non-competitive AMPA receptor antagonist, EGIS-8332 (Matucz et al., 2004; Gressens et al., 2005; Végh et al., 2007; Gigler et al., 2007), have a beneficial effect on the motor skills of $Cln3^{\Delta ex1\text{-}6}$ mice.

An accelerating rotarod (AccuScan Instruments, Inc., Columbus, OH) was used to measure the motor skills of one-month-old 129S6/SvEv wild type (WT) and homozygous *Cln3*-loss-of-function mice ($(Cln3^{\Delta ex1\text{-}6})^5$ inbred on a 129S6/SvEv background. (All procedures were carried out according to the guidelines of the Animal Welfare Act, NIH policies and the University of Rochester Animal Care and Use Committee.) The rotarod measures the ability of the mouse to maintain balance on a motor-driven, rotating rod. Thus, the fore- and hind limb motor coordination and balance can be analyzed (Karl et al., 2003). In our rotarod test, due to the repeated, multiple test trials, motor learning also contributed to the rotarod performance of mice. During the training period, mice were placed on the rotarod starting at zero rpm and accelerating to 30 rpm over a period of 240 seconds. Animals were trained for three consecutive runs. Following training, animals rested for 1 h and then were tested for three Pre-treatment test trials consisting of three consecutive runs with 15 min of rest between each test trial. Two hours and thirty minutes after the end of the Pre-treatment test, animals were intraperitoneally injected with the selective, non-competitive AMPA antagonist, EGIS-8332 (a generous gift of EGIS Pharmaceuticals PLC, Budapest, Hungary). Three different doses, 1, 3 and 10 mg/kg, were used (injection volume: 10 ml/kg). Control mice were injected with the vehicle of the drug (20 mM HCl containing 10% DMSO). Thirty minutes after the injection, animals were tested for three Post-treatment test trials consisting of three consecutive runs with 15 min of rest between each test trial. The latencies to fall from the rotating rod during the Pre- and Post-treatment testing periods were calculated for each mouse. All data sets passed the normality test (alpha level 0.05), and

therefore, two-tailed t-tests and one-way ANOVA were applied in the statistical analysis (alpha level in all cases was 0.05) using GraphPad Prism 4 and SigmaStat 3.5 programs.

One-month-old *Cln3*$^{\Delta ex1\text{-}6}$ mice, as compared to WT mice, had a reduced ability to remain on the rotating rod as it accelerated (**Fig. 1A**). Although, older, 2, 6 and 12 months old animals have similar motor deficit, the difference between WT and *Cln3*$^{\Delta ex1\text{-}6}$ mice is the largest at the age of one month, mainly because the motor skills of WT mice as compared to *Cln3*$^{\Delta ex1\text{-}6}$ mice drop more significantly with the age (Kovács et al., 2006; and our unpublished results).

In a pilot experiment (n=4-6), the performance of one-month-old *Cln3*$^{\Delta ex1\text{-}6}$ mice on the rotarod was tested before and after a single intraperitoneal injection of the selective, non-competitive AMPA receptor antagonist, EGIS-8332, applying three different doses (1, 3 and 10 mg/kg). Post-treatment testing began 30 min after the injection. The lowest dose (1 mg/kg) significantly improved the performance of *Cln3*$^{\Delta ex1\text{-}6}$ mice. They were able to stay on the rotating rod 30 s longer than before the treatment (**Fig. 1B**). The middle and the highest doses (3 and 10 mg/kg) did not have any effects (**Fig. 1B**), indicating that there is a threshold level of beneficial AMPA receptor inhibition.

To confirm the beneficial effect of 1 mg/kg EGIS-8332, we tested more mice. **Fig. 1C** demonstrates that EGIS-8332 significantly improved the motor skills of *Cln3*$^{\Delta ex1\text{-}6}$ mice as compared to their own Pre-treatment performance or to Control, vehicle-injected mice (Control: n=11; EGIS-8332: n=17).

Next we examined whether a single intraperitoneal injection of 1 mg/kg EGIS-8332 has any effect on the motor skills of one-month-old WT mice. The Post-treatment motor performance of Control and EGIS-8332 treated mice was similar, statistically not different (**Fig. 1D**). Interestingly, the motor skills of Control WT mice remarkably improved in the Post-treatment test (**Fig. 1D**). It indicates that WT mice effectively learned during the Pre-treatment test and could recall and apply the newly acquired motor skills. In contrast, Control *Cln3*$^{\Delta ex1\text{-}6}$ mice were not able either to learn during the Pre-treatment test or to recall and apply the learned skills in the Post-treatment test (see **Fig. 1C**). The cumulative result is a very large difference (97 s) between the Post-treatment performances of Control WT and *Cln3*$^{\Delta ex1\text{-}6}$ mice. Although EGIS-8332 (1 mg/kg) significantly improved the motor skills of *Cln3*$^{\Delta ex1\text{-}6}$ mice, the improvement did not reach the WT level (EGIS-8332 post-treatment, WT vs. *Cln3*$^{\Delta ex1\text{-}6}$: 2-way ANOVA, p=0.00305).

Our results demonstrate that a single intraperitoneal injection of a selective AMPA receptor antagonist significantly improves the motor skills in a mouse model of the incurable juvenile Batten disease. The rotarod test we applied measured motor skills as a combination of motor coordination and motor learning. Therefore, the improved motor skills of EGIS-8332-treated *Cln3*$^{\Delta ex1\text{-}6}$ mice can be the result of an improvement in motor coordination and/or motor learning. The similar level of Post-treatment improvement in WT mice (**Fig 1D**), which is presumably all due to motor learning, suggests that EGIS-8332 mainly corrected a motor learning deficit in *Cln3*$^{\Delta ex1\text{-}6}$ mice. EGIS-8332-treated *Cln3*$^{\Delta ex1\text{-}6}$ mice were able either to recall and apply the skills learned in the Pre-treatment test or to learn and improve their

motor skills during the Post-treatment test trials. The cerebellum, basal ganglia and motor cortex are critical for motor learning. Our previous results (Kovács et al., 2006) indicated an abnormal gain in AMPA receptor function in the cerebellum of *Cln3*$^{\Delta ex1\text{-}6}$ mice. Future experiments will determine if AMPA receptor dysregulation in the basal ganglia and/or in the motor cortex also contributes to the motor deficit of *Cln3*$^{\Delta ex1\text{-}6}$ mice.

Taken together, our data suggest that administration of low doses of AMPA receptor antagonists that attenuate AMPA receptor activity but avoid a toxic, complete blockade of the receptor is a promising therapeutic approach for juvenile Batten disease, especially at an early stage of the disease that was modeled by one-month-old *Cln3*$^{\Delta ex1\text{-}6}$ mice in our study. Further studies will evaluate whether attenuation of AMPA receptor activity has a beneficial effect in older (6-7-month-old) *Cln3*$^{\Delta ex1\text{-}6}$ mice that model a later stage of the disease, and if chronic treatment with an AMPA receptor antagonist can restore motor skills to the WT level, and prevent the late onset neurodegeneration (Pontikis et la., 2004).

AMPA receptor antagonists are very effective anticonvulsants in animal seizure models (Lees, 2000; Szabados et al., 2001). Since children with juvenile Batten disease also suffer from frequent occurrence of seizures, therapeutic application of an AMPA receptor antagonist could improve their motor skills and prevent seizure development, as well. EGIS-8332 belongs to the family of the selective AMPA receptor antagonist 2,3-benzodiazepines, and is a very close analogue of GYKI 53405 (Gressens et al., 2005). The active enantiomer of GYKI 53405, called Talampanel, effectively decreased the occurrence of epileptic seizures in a phase II clinical trial (Bialer et al., 2007). Thus, this orally active new drug would be an obvious choice for clinical trials with juvenile Batten disease patients. The *Cln3*$^{\Delta ex1\text{-}6}$ mouse may also serve as a model for efficacy of compounds targeted as AMPA receptor antagonists for other neurological disorders.

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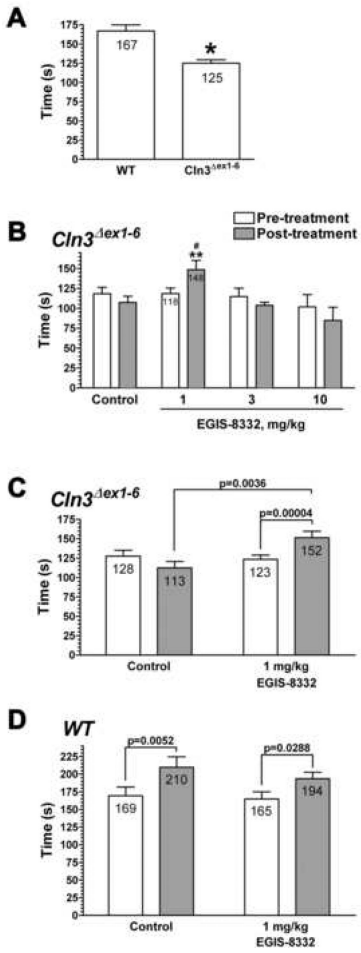

Fig. 1. A single intraperitoneal injection of a selective, non-competitive AMPA receptor antagonist significantly improves the motor skills in the *Cln3*-loss-of function (*Cln3*$^{\Delta ex1-6}$) mouse model of juvenile Batten disease

An accelerating rotarod was used to measure motor skills of one-month-old *Cln3*$^{\Delta ex1-6}$ and wild type (WT) mice. Two hours and thirty minutes after the end of the Pre-treatment test, animals were intraperitoneally injected with the selective, non-competitive AMPA antagonist, EGIS-8332, in the indicated doses. Control mice were injected with the vehicle of the drug (20 mM HCl containing 10% DMSO). Thirty minutes after the injection, the Post-treatment test was performed. The latencies to fall from the rotating rod during the Pre- and Post-treatment testing periods were calculated for each mouse. Columns and bars represent mean ± S.E.M. of the time (s) mice were able to stay on the rotating rod. All data sets passed the normality test (alpha level 0.05), and therefore, two-tailed t-tests and one-way ANOVA were applied in the statistical analysis. (**A**) Impaired motor skills in one-month-old *Cln3*$^{\Delta ex1-6}$ mice. Combined Pre-treatment test results of 19 WT and 28 *Cln3*$^{\Delta ex1-6}$ mice are shown. *p=0.0000071, unpaired t-test (**B**) The selective, non-competitive AMPA receptor antagonist, EGIS-8332, dose-dependently affects the motor skills of one-month-old *Cln3*$^{\Delta ex1-6}$ mice (n=4-6). The lowest, 1 mg/kg, dose significantly improved motor skills: **p=0.0066, paired t-test: Post-treatment vs. Pre-treatment; $^{\#}$p=0.0272, oneway ANOVA followed by Bonferroni's test for comparison of

Post-treatment times (Control vs. 1, 3 and 10 mg/kg). (**C**) A single intraperitoneal injection of EGIS-8332 at a low dose (1 mg/kg) significantly improves the motor skills of *Cln3*$^{\Delta ex1\text{-}6}$ mice (Control: n=11; 1 mg/kg EGIS-8332: n=17). Empty columns: Pre-treatment test results; grey columns: Post-treatment test results. Pre- vs. Post-treatment: paired t-test, p=0.00004; Comparison of Post-treatment results: unpaired t-test, p=0.0036. (**D**) A single intraperitoneal injection of EGIS-8332 at a low dose (1 mg/kg) does not affect the motor skills of WT mice (Control: n=9; 1 mg/kg EGIS-8332: n=10). Empty columns: Pre-treatment test results; grey columns: Post-treatment test results. Pre- vs. Post-treatment: paired t-test, p=0.0052 for Control and p=0.0288 for 1 mg/kg EGIS-8332; Comparison of Post-treatment results: unpaired t-test, p=0.3567, not significant.