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The Selective Glucocorticoid Receptor Modulator Cort 113176 Reduces Neurodegeneration and Neuroinflammation in Wobbler Mice Spinal Cord

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Abstract—Wobbler mice are experimental models for amyotrophic lateral sclerosis. As such they show motoneuron degeneration, motor deficits, and astrogliosis and microgliosis of the spinal cord. Additionally, Wobbler mice show increased plasma, spinal cord and brain corticosterone levels and focal adrenocortical hyperplasia, suggesting a pathogenic role for glucocorticoids in this disorder. Considering this endocrine background, we examined whether the glucocorticoid receptor (GR) modulator CORT 113176 prevents spinal cord neuropathology of Wobblers. CORT 113176 shows high affinity for the GR, with low or null affinity for other steroid receptors. We employed five-month-old genotyped Wobbler mice that received s.c. vehicle or 30 mg/kg/day for 4 days of CORT 113176 dissolved in sesame oil. The mice were used on the 4th day, 2 h after the last dose of CORT 113176. Vehicle-treated Wobbler mice presented vacuolated motoneurons, increased glial fibrillary acidic protein (GFAP)+ astrocytes and decreased glutamine synthase (GS)+ cells. There was strong neuroinflammation, shown by increased staining for IBA1+ microglia and CD11b mRNA, enhanced expression of tumor necrosis factor-α, its cognate receptor TNFR1, toll-like receptor 4, the inducible nitric oxide synthase, NFkB and the high-mobility group box 1 protein (HMGB1). Treatment of Wobbler mice with CORT 113176 reversed the abnormalities of motoneurons and down-regulated proinflammatory mediators and glial reactivity. Expression of glutamate transporters GLT1 and GLAST mRNAs and GLT1 protein was significantly enhanced over untreated Wobblers. In summary, antagonism of GR with CORT 113176 prevented neuropathology and showed anti-inflammatory and anti-glutamatergic effects in the spinal cord of Wobbler mice.

Key words: neurodegeneration, neuroinflammation, glucocorticoid receptor antagonism, wobbler mice.

INTRODUCTION

Increasing evidence supports that hyperadrenocorticism is an associated comorbidity of several neurodegenerative diseases. This relationship applies to amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder characterized by damage to upper and lower motoneurons and progressive paralysis (Mitchell and Borasio, 2007). Early reports have shown in ALS patients an elevated and flattened cortisol circadian rhythm due to higher basal evening trough of saliva cortisol. This rise in circulating cortisol is accompanied by an impaired response to a psychological stressor (Patacchioli et al., 2003). Our group has shown a rise in morning plasma cortisol in the sporadic form of ALS, expressed as the dehydroepiandrosterone/cortisol ratio (Gargiulo-Monachelli et al., 2014). In the patient cohort

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Abbreviations: ALS, amyotrophic lateral sclerosis; CD11b, a CD antigen that contains a conserved domain and serves as a marker of microglia; GFAP, glial fibrillary acidic protein; GLAST, glutamate aspartate transporter; GLT-1, glutamate transporter 1; GR, glucocorticoid receptor; GS, glutamine synthase; HMGB1, high-mobility group box 1 protein; HPA, hypothalamicpituitaryadrenal axis; IBA1, Ionized calcium-binding adaptor molecule 1; IL1β, interleukin 1 beta; iNOS, inducible form of nitric oxide synthase; MR, mineralocorticoid receptor; NFkB, nuclear factor kappa-light-chain enhancer of activated B cells; NFR, background strain of Wobbler mice; NO, nitric oxide; SOD1, superoxide dismutase I; TDP43, transactive response DNA-binding protein 43 kDa; TLR4, toll-like receptor 4; TNFα, tumor necrosis factor alpha; TNFαR1, TNFα receptor type I.

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studied by Roozendaal et al. (2012), disease severity correlates with a blunted cortisol awakening response, whereas Spataro et al. (2015) have reported increased plasma cortisol in spinal-onset ALS patients and in those with intermediate or rapidly progressive disease.

In harmony with human findings, rodent models of ALS show several abnormalities of the adrenal steroid system. Thus, glucocorticoids potentiate the neurotoxic effects of transactive response DNA-binding protein (TDP) 43, a molecule linked to ALS (Caccamo et al., 2013). In the SOD1 transgenic mouse model of familiar ALS, higher plasma corticosterone correlates with early onset of paralysis and shorter survival (Fidler et al., 2011). In the Wobbler mouse model of ALS, basal and stress levels of plasma corticosterone are elevated, whereas binding to glucocorticoid receptors (GR) is altered in the spinal cord (Gonzalez Deniselle et al., 1997, 2016). Steroid measurements by gas chromatography / mass spectrometry have shown in Wobbler mice a substantial rise of corticosterone and deoxycorticosterone levels in adrenal glands, plasma and spinal cord, coupled to focal adrenal hyperplasia (Gonzalez Deniselle et al., 2016). These findings supported that adrenal hyperfunction could be a signature of neurodegeneration. Further proof for the role of hyperadrenocorticism in the Wobbler mouse is provided by blockade of GR with the selective modulator CORT 108297. Treatment of Wobblers with this GR modulator enhances neurogenesis, decreases astrogliosis and changes the phenotype of Iba1+ microglia from an activated to a quiescent form in the hippocampus (Meyer et al., 2014).

The finding of highly reactive microglia in Wobbler mice in the face of hyperadrenocorticism seems contradictory, because of the accepted anti-inflammatory effect of glucocorticoids (Vandevyver et al., 2013). However, Sapolsky and co-workers have demonstrated that excess levels of glucocorticoids exert proinflammatory effects in the brain, owing to a dysfunctional glutamatergic neurotransmission (Sorrells et al., 2014). In this regard, Diana et al. (2010) have found disturbances of the glutamate transporters in neurons and glial cells of the Wobbler mice, which probably accounts for the increased excitatory synaptic transmission exhibited by this mutant (Thielsen et al., 2013). In ALS patients, the anti-glutamatergic drug Riluzole moderately delays disease progression (Mitchell and Borasio, 2007). However, a relationship between glutamate pathways, excess glucocorticoids and neurodegeneration has not been reported for the spinal cord of Wobbler mice.

We have hypothesized that GR antagonism may be therapeutically useful to oppose the potential contribution of hyperadrenocorticism to the neurodegeneration, neuroinflammation and excitotoxicity of Wobbler mice. Among available tools, mifepristone is a classical GR antagonist used experimentally and clinically (Revsin et al., 2009; Moraitis et al., 2017). However, mifepristone antagonizes GR as well as the progesterone receptor and *in vitro* exerts agonist-like properties (Ghoumari et al., 2003). Recently, Zalachoras et al. (2013) have used the more selective GR modulator CORT 108297, which increases cell proliferation in the dentate gyrus of corticosterone-receiving rats. This finding is in agreement with previous results obtained by us in the Wobbler mouse (Meyer et al., 2014). The selective GR modulators labeled CORT 113176 and CORT 108297 have been used by Pineau et al. (2016) to reverse β-amyloid neurotoxicity, neuroinflammation and apoptosis in the hippocampus of an Alzheimer's disease model. In another study, the mixed mineralocorticoid receptor (MR)-(GR) ligand CORT 118335 opposes the deleterious effects of endogenous glucocorticoids on memory processes (Atucha et al., 2015). This compound also prevents unwanted effects of corticosterone resulting from binding to both MR and GR in the hippocampus.

The present study was focused on the spinal cord, a major target of the Wobbler disease (Gonzalez Deniselle et al., 1997, 2004). We examined the effects of CORT 113176 on motoneuron morphology, glial cells, neuroinflammatory mediators and glutamate-related molecules. Similarly to mifepristone, CORT 113176 exerts GR antagonistic effects. However, it shows higher receptor selectivity, with a Ki value for GR < 1 nM *in vitro*, without significant binding to mineralocorticoid, androgen or estrogen receptors (Beaudry et al., 2014). We here demonstrated that short-term treatment of Wobbler mice with CORT 113176 decreased spinal cord neuropathology, attenuated pro-inflammatory mediators and modulated glutamate homeostasis. Thus selective GR antagonism exerts beneficial effects in a preclinical model of ALS.

EXPERIMENTAL PROCEDURES

Experimental animals

Wobbler and control mice were obtained from the Instituto de Biología y Medicina Experimental animal facility. The Wobbler mutation (wr/wr) was determined immediately after birth by genotyping, according to published procedures (Rathke-Hartlieb et al., 1999; Meyer et al., 2014). Mice from both strains (control NFR/NFR and Wobbler mice) were maintained in a 12-h light–dark cycle, controlled temperature (22 °C) and offered water and standard mice chow *ad libitum* with vitamin supplementation (Ensure, Abbott, Zwolle, Holland).

Animals were housed in group cages containing 2–3 Wobblers and a control mouse. This social interaction plus nutritional supplementation substantially prolonged life span, improved health status and delayed pathology of Wobbler mice (M-P. Junier, personal communication) (Meyer et al., 2014). The present experiments used five-month-old Wobbler mice showing tremor, ambulatory difficulty (wobbling), flexion of proximal limbs and weight loss (av. body weight 15.5 g; av. control weight: 24.5 g). Wobbler mice received vehicle or the GR antagonist CORT 113176, a 1H-pyrazolo [3.4-g] hexahydroisoquinoline sulfonamide developed by Corcept Therapeutics (Menlo Park, CA, USA) (Clark et al., 2008). CORT 113176 was dissolved in vegetable oil and given s.c. for 4 days at the dose of 30 mg/kg; mice were used 2 h after the last injection. Before experimental use, mice were anesthetized with a mixture of xylazine (6 mg/kg) and ketamine (75 mg/kg). The total number of mice used in

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