Omigapil Ameliorates the Pathology of Muscle Dystrophy Caused by Laminin-α2 Deficiency

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ABSTRACT

Laminin-α2-deficient congenital muscular dystrophy, called MDC1A, is a rare, devastating genetic disease characterized by severe neonatal hypotonia (“floppy infant syndrome”), peripheral neuropathy, inability to stand or walk, respiratory distress, and premature death in early life. Transgenic overexpression of the proapoptotic glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-Siah1-CBP/p300-p53 pathway is activated in a mouse model for MDC1A. Moreover, we show that omigapil, which inhibits GAPDH-Siah1-mediated apoptosis, ameliorates several pathological hallmarks in the MDC1A mouse model. Specifically, we demonstrate that treatment with omigapil inhibits apoptosis in muscle, reduces body weight loss and skeletal deformation, increases locomotive activity, and protects from early mortality. These data qualify omigapil, which is in late phase of clinical development for human use, as a drug candidate for the treatment of MDC1A.

Laminin-α2 is an extracellular matrix protein expressed in skeletal muscle and peripheral nerve that associates with the β1 and the γ1 subunit to form the heterotrimeric laminin-211. Laminin-211 is tightly attached to the basement membrane, interacts with α-dystroglycan and α7β1 integrin on the muscle fiber surface (Colognato and Yurchenco, 2000), and thus confers mechanical stability to the contracting muscle. Thus, mutations in the LAMA2 gene coding for laminin-α2 disrupt the linkage of the basement membrane to the plasma membrane. In humans, this results in a severe form of congenital muscular dystrophy, called laminin-α2-deficient congenital muscular dystrophy, or MDC1A, which is characterized by neonatal hypotonia (“floppy infant syndrome”), peripheral neuropathy, inability to stand or walk, respiratory distress, and premature death (Muntoni and Voit, 2004; Schessl et al., 2006). The dyW/dyW mouse model (Kuang et al., 1998) of MDC1A largely recapitulates the human disease. It is characterized by a severe degeneration and incomplete regeneration of damaged muscle fibers (Miyagoe et al., 1997; Kuang et al., 1999; Bentzinger et al., 2005; Meinen et al., 2007). Histology of affected muscles typically shows variation in muscle fiber size, extensive fibrosis, infiltration of adipose tissue, and high levels of creatine kinase (CK) in the blood. In addition, the hindlegs of laminin-α2-deficient mice are paralyzed within a few weeks from birth because of progressing demyelination of the peripheral nervous system (Kuang et al., 1998). Finally, like human patients, these mice die prematurely. Apoptosis has been reported as a pathological hallmark at the cellular level of affected muscle tissue both in dyW/dyW mice (Kuang et al., 1999; Girgenrath et al., 2004; Bentzinger et al., 2005; Dominov et al., 2005) and MDC1A patients (Hayashi et al., 2001).

Several transgenic approaches in dyW/dyW mice have shown that proteins sharing biochemical binding properties with laminin-α2 can ameliorate the pathology. These approaches include transgenic expression of laminin-α1 (Gaw-
lik et al., 2004), of a miniaturized form of the extracellular matrix molecule agrin (Moll et al., 2001; Bentzinger et al., 2005; Qiao et al., 2005; Meinen et al., 2007), or of perlecan (Meinen et al., 2007). All these attempts ameliorated the pathology of dyW/dyW mice in several aspects. However, because of largely technical obstacles, it is currently difficult to translate protein replacement strategies into a treatment of human patients.

Pharmacological intervention into the apoptotic pathways underlying the disease offers an alternative approach. This approach aims at preventing cell death of muscle fibers, which in the laminin-α2-deficient muscle is a consequence of muscle fiber detachment from the disrupted basement membrane (Miyagoe et al., 1997), possibly because of loss of intracellular signaling mediated by α7 integrin and/or dytaglycan (Langenbach and Rando, 2002; Laprise et al., 2003). Indeed, a first proof-of-concept for this treatment approach was accomplished by transgenic overexpression of the anti-apoptotic protein BCL-2, or deletion of the proapoptotic Bax gene (Girgenrath et al., 2004; Dominov et al., 2005). Both approaches prolong survival and mitigate disease-specific parameters in dyW/dyW mice, suggesting that pharmacological inhibition of apoptosis could be a valid strategy to overcome the pathological manifestations of laminin-α2 deficiency. Indeed, minocycline and doxycycline have recently been shown to alleviate some of the disease parameters in dyW/dyW mice (Girgenrath et al., 2009).

However, the underlying signaling pathways leading to apoptosis in laminin-α2-deficient muscle are still poorly understood. One of the newly described apoptotic pathways involves the glycolytic housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its S-nitrosylation via inducible or neuronal nitric-oxide synthase (Hara et al., 2005). S-Nitrosylation abolishes the catalytic activity of GAPDH and, upon the binding of the E3 ubiquitin ligase Siah1, GAPDH is translocated into the nucleus. Nuclear GAPDH binds to the acetyltransferase CBP/p300 and enhances acetylation and transcription of various targets, including proapoptotic genes, such as p53, p53-up-regulated modifier of apoptosis (PUMA), and p21 (Sen et al., 2008). This proapoptotic signaling cascade can be interrupted by omigapil, an orally available chemical derivative of (-)-deprenyl, which exhibits virtually no monoamine oxidase type B- or type A-inhibiting properties and is not metabolized to amphetamines (Tatton et al., 2003; Hara et al., 2006; Waldmeier et al., 2006).

We provide evidence that the GAPDH-Siah1-CBP/p300 signaling pathway (Chuang et al., 2005) is dysregulated in muscle tissue of dyW/dyW mice and that application of omigapil once per day results in reduced apoptosis in muscle. Omigapil also reduces body weight loss and skeletal deformation, increases locomotive activity, and protects from early mortality in dyW/dyW mice. Based on these encouraging results, combined with the advanced nonclinical and clinical development status, omigapil holds the potential to offer a valid treatment option for MDC1A.

Materials and Methods

Gene Expression Analysis. Muscle biopsies were snap frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted by use of the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hombrechtikon, Switzerland). The initial homogenization step was carried out by use of the gentleMACS M Tubes (Miltenyi Biotec, Bergisch Gladbach, Germany). One microgram of total RNA was reverse transcribed into cDNA by use of the QuantiTect Rev. Transcription Kit (Qiagen). Quantitative polymerase chain reaction (qPCR) was carried out on the LightCycler 480 System (Roche Diagnostics, Rotkreuz, Switzerland) using the LightCycler 480 SYBR Green I Master (Roche Diagnostics). The housekeeping genes were amplified by use of the primer pairs: β-actin forward GTGTTACACTGGGACGAC and reverse GGCGGTGGTGAAGGCTCTGAAA; β-2 microglobulin forward ATGGG-GAACGGCAACATCTG; and reverse GGGGTTGAACTTCACTGAG; GAPDH forward ATTTGCTCAATGCTGCTT and reverse ATGGACTGTGGTCATGAGCC; the genes of interest were amplified by use of the primer pairs: GOSPEL forward AGAGGGTCATCGACCAACAC and reverse TCCAGGCTCTTCTTGTGCTT; p53 forward AGGGCTACTCAGGACTTAC and reverse CCCCCCTTCTTGAGCACTT; PUMA forward CCAGAAATGGAGGCGCAACTA and reverse AAGGGCTGGCAGCTCAGTATG; p21 forward GTAATGGAGACAGGACCC and reverse CGAAGTCAAAGGTGTCACCTG. The primers were designed by use of the NCBI/Primer-BLAST software. Relative levels of mRNA are expressed after normalization to all three housekeeping genes.

Preparation and Analysis of Nuclear Extracts. The cytosolic and nuclear fractions were prepared from freshly isolated diaphragm muscles from 4-week-old dyW/dyW mice using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce, Lausanne, Switzerland) according to the manufacturer’s protocol. The initial homogenization of the fresh muscles was carried out at 4°C using the gentleMACS M Tubes (Miltenyi Biotec, Bergisch Gladbach, Germany). The protein concentrations of the cytosolic and nuclear fractions were determined by the BCA Protein Assay Kit (Pierce, Ludwigsburg, Germany). The initial homogenization step was carried out by use of the Odyssey Infrared Imaging System (LI-COR Biosciences, Bad Homburg, Germany). The relative amount of nuclear GAPDH protein was calculated as the ratio of the densitometric values from the nuclear and the cytosolic fractions.

Treatment of dyW/dyW Mice with Omigapil. Homozygous dyW/dyW mice (Kuang et al., 1998) were treated with 0.1 or 1 mg/kg omigapil dissolved in 0.5% ethanol as vehicle. For all experiments, treatment started at the age of 15 days, except for the 1 week of treatment shown in Fig. 2b. For the first week of drug treatment, omigapil was administered once daily by intraperitoneal injection. Thereafter, omigapil was given orally by gavage once daily. Age-matched animals treated with vehicle only were used as controls. To ensure optimal access to water and food, cages were supplied with long-necked water bottles and wet food was placed inside the cage, which resulted in substantially prolonged survival of dyW/dyW mice in our colony compared with survival data presented by others (e.g., Kuang et al., 1998; Girgenrath et al., 2004; Dominov et al., 2005; Girgenrath et al., 2009). At the end of the treatment period, mice were sacrificed by CO2 asphyxiation. Genotyping of dyW/dyW mice was performed as described previously (Kuang et al., 1998). All procedures were performed in accordance with the Swiss regulations for animal experimentation and under the required licenses.

Body Weight and Survival. Body weight and death events were recorded daily. Kaplan-Meier survival curves were generated and compared by use of the Peto-Peto Wilcoxon test. The body weight was recorded for each animal from day 15 (onset of the experiment) onwards. For each animal, the average weight gain per week was calculated. Two sets of body weight curves were recorded: a curve only for animals that survived the entire observation period of 18 weeks, and a separate curve including all animals irrespective of when they died during the observation period. Both sets of data produced similar results.

Histological Analysis and CR Assay. Muscle cross-sections were prepared and analyzed for fiber size distribution as described.
previously (Briguet et al., 2004). The triceps brachii muscle was chosen for histological analysis to exclude muscles that are affected by the secondary atrophic effect due to hindlimb paralysis. In brief, frozen muscle cross-sections were stained by use of Alexa-488-conjugated wheat-germ agglutinin (WGA-Alexa; Molecular Probes, Eugene OR) to stain membrane-bound and extracellular epitopes and 1 μg/ml 4’,6-diamidino-2-phenylindole (DAPI; Molecular Probes) to stain nuclei. Apoptotic myonuclei were detected by TdT-mediated dUTP-biotin nick end labeling (TUNEL) with use of the In Situ Cell Death Detection Kit, Fluorescein according to the manufacturer’s protocol (Roche Diagnostics). Pictures of TUNEL/wheat-germ agglutinin/DAPI-stained cross-sections were collected by use of a fluorescence microscope (DM5000B; Leica, Heerbrugg, Switzerland), a digital camera (F-View; Soft Imaging System; Olympus, Hamburg, Germany), and analySIS software (Soft Imaging System, Olympus). Only TUNEL- and DAPI-positive nuclei that were located within muscle fibers were counted as apoptotic myonuclei. Values are expressed relative to the total number of myonuclei. General histology was performed by use of hematoxylin and eosin (Merck, Rayway, NJ) according to standard histological techniques. Blood levels of creatine kinase (EC 2.7.3.2) were determined with 2 μl of serum by using the CK-NAC Liqui-UV kit (Rolf Greiner Biochemica, Flacht, Germany).

**Locomotion and Examination of Spine Deformation.** Locomotive behavior was determined as described previously (Moll et al., 2001). In brief, mice were placed into a new cage and motor activity (walking, digging, and standing upright) was measured during 10 min. To determine spine deformation, X-ray pictures were taken of anesthetized mice. The extent of kyphosis was quantified for mice at the age of 11 weeks by visual inspection at the same day by an investigator blinded to the treatment status of the animals. The degree of kyphosis was scored and allocated to one of the following categories: 1, barely detectable; 2, mild; 3, moderate; 4, severe.

**Statistical Analysis.** Quantitative data are expressed as mean ± S.E.M. Significance between two groups was determined by use of the unpaired Student’s t test. Significance for differences in the fiber size distribution was determined by use of cumulative distribution curves on which the Kolmogorov-Smirnov goodness-of-fit test (Chakravarti et al., 1967) was applied. Comparison of survival curves between treatment groups and vehicle controls was performed by the Peto-Peto Wilcoxon test. The Wilcoxon rank sum test was used to compare the kyphosis score of omigapil- and vehicle-treated animals.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** The GAPDH-Siah1-CBP/p300 pathway is activated in dy/w/dy/w mice. a, b, qPCR analysis of proapoptotic genes in triceps brachii (a) and diaphragm (b) muscle in 4-week-old wild-type (WT) and dy/w/dy/w mice. The transcriptional levels of p53, PUMA, and p21 are increased in both muscles with a higher relative increase in the diaphragm compared with the triceps muscle (WT, n = 5; dy/w/dy/w, n = 6). c, qPCR analysis of proapoptotic genes in triceps brachii muscle of 7-week-old mice demonstrating sustained activation of proapoptotic genes (WT, n = 5; dy/w/dy/w, n = 6). d, qPCR analysis of GOSPEL in the diaphragm muscle of 4-week-old mice. GOSPEL levels are significantly reduced in dy/w/dy/w mice to less than 50% of the WT levels (WT, n = 5; dy/w/dy/w, n = 6). e, accumulation of GAPDH in nuclear preparations. Quantitative assessment of GAPDH protein by Western blot analysis in nuclear preparations isolated from 4-week-old WT and dy/w/dy/w diaphragm. In muscle from dy/w/dy/w mice a ~40% increase in GAPDH protein in nuclear preparations is detected (WT, n = 3; dy/w/dy/w, n = 3). a–e. Data are mean ± S.E.M.; **, p < 0.01, Student’s t test.
Results

The GAPDH-Siah1-CBP/p300 proapoptotic Signaling Pathway Is Activated in dy/dy Mice. As a consequence of the laminin-α2 deficiency, muscle fibers in dy/dy mice are prone to undergoing apoptosis (Miyagoe et al., 1997; Girgenrath et al., 2004). Because the underlying signaling cascade(s) causing muscle cell apoptosis are still poorly understood, we investigated whether the GAPDH-Siah1-CBP/p300 proapoptotic pathway could be involved in this marked loss of muscle mass. We first tested whether the expression of the downstream proapoptotic genes of this pathway was increased, which was to be expected if the GAPDH-Siah1-CBP/p300 pathway was activated in dy/dy mice. Indeed, expression of p53 and its downstream genes PUMA and p21 was markedly elevated in triceps brachii and diaphragm muscle of the dy/dy mice compared with wild-type mice (Fig. 1, a–c). The increased expression of these proapoptotic genes was already seen at a young age, indicating that the activation of the GAPDH-Siah1-CBP/p300 pathway precedes tissue apoptosis. Specifically, in 4-week-old dy/dy mice the increase in all three genes was already evident in diaphragm and triceps brachii muscle (Fig. 1, a and b). At this young age, the expression of p53, PUMA, and p21 was generally higher in the diaphragm than in the triceps brachii. Expression of p53, PUMA, and p21 continued to increase over time as seen in their expression levels in triceps brachii at 7 weeks of age (Fig. 1c). Because the proapoptotic GAPDH-Siah1-CBP/p300 pathway has recently been shown to be antagonized by GAPDH’s competitor pathway has recently been shown to be antagonized by GAPDH’s competitor (Fig. 1c). Because the proapoptotic GAPDH-Siah1-CBP/p300 pathway by its binding to GAPDH and inhibiting the interaction of GAPDH and Siah1 (Hara et al., 2006). Consistent with this activity, omigapil, when given to dy/dy mice (designated here dy/dy-omigapil) for 1 week at a dose of 1 mg/kg starting at 21 days of age, consistently reduced the levels of p53, PUMA, and p21 compared with vehicle-treated dy/dy littermates (dy/dy-veh) (Fig. 2b). In addition, treatment of dy/dy mice with omigapil for 4 weeks starting at 15 days of age significantly reduced the number of apoptotic myonuclei (Fig. 2, c and d). Thus, omigapil by its well described binding to GAPDH inhibits nuclear accumulation of GAPDH, thereby reducing the expression of proapoptotic genes p53, PUMA, and p21, and decreasing the number of apoptotic myonuclei in dy/dy mice.

Omigapil Ameliorates Muscle Histology, Improves Locomotion and Overall Health in dy/dy Mice. A hallmark of laminin-α2 deficiency is a shift of the myofiber size distribution to small-caliber fibers. Treatment of dy/dy mice with omigapil for 4 weeks at a dose of 0.1 mg/kg starting at 15 days of age normalized the size distribution in triceps brachii muscle by reducing the proportion of small-caliber and increasing that of large-caliber fibers (Fig. 3, a and b). Furthermore, omigapil treatment reduced the rela-

![Diagram](https://via.placeholder.com/150)

**Fig. 2.** Omigapil reduces apoptosis in dy/dy mice. a, chemical structure of omigapil, N-(dibenzo(b,f)oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine maleate (also referred to as TCH346 or CGP3466). b, qPCR analysis of proapoptotic genes in diaphragm of 4-week-old dy/dy mice. The transcriptional levels of p53, PUMA, and p21 are decreased in dy/dy mice treated for 1 week with 1 mg/kg omigapil compared with vehicle-treated dy/dy mice (dy/dy-vehicle, n = 12; dy/dy-omigapil, n = 13). c, quantitative assessment of apoptotic myonuclei in triceps brachii muscle. The percentage of apoptotic myonuclei is significantly lower in dy/dy mice treated for 4 weeks with 0.1 mg/kg omigapil compared with animals treated with vehicle (WT, n = 6; dy/dy-vehicle, n = 12; dy/dy-omigapil, n = 13). d, TUNEL staining of triceps brachii muscle of 6-week-old mice. Arrows, TUNEL-positive myonuclei. b and c, data are mean ± S.E.M., **, p < 0.01, Student’s t test. d, Scale bar, 50 μm.
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Fig. 3. Omigapil ameliorates muscle histology and reduces blood CK levels in dyw/dyw mice. a, as detected by hematoxylin and eosin staining, treatment of mice with 0.1 mg/kg/day omigapil reduces fibrosis in triceps brachii muscle of 6-week-old dyw/dyw mice compared with muscle from vehicle-treated mice. b, muscle fiber size distribution in triceps brachii muscle of dyw/dyw and WT animals. Values represent relative numbers of fibers in a given diameter class determined as minimum Feret’s diameter (Briguet et al., 2004). In vehicle-treated dyw/dyw mice a higher proportion of muscle fibers have small diameters. Treatment with 0.1 mg/kg omigapil shifted the fiber size distribution toward a higher percentage of fibers with larger diameters (WT, n = 6; dyw-vehicle, n = 12; dyw-omigapil, n = 13). There is a significant difference between dyw-vehicle and dyw-omigapil mice by comparison of the cumulative distribution curves of muscle fiber diameters (p < 0.001; Kolmogorov-Smirnov analysis). c, number of muscle fibers in triceps brachii muscle per cross-sectional area. The increased number of muscle fibers per mm² in vehicle-treated dyw/dyw mice is normalized to wild-type muscle histology and body weight, but also affected locomotor behavior, an open field test was performed with 5- to 6-week-old and 10-week-old mice. Omigapil treatment indeed improved locomotion in the younger mice (Fig. 4c, left) with a similar efficacy for the two doses of omigapil (see also Supplemental Data; Movie). Although there was still a slight improvement in locomotion in omigapil-treated mice at 10 weeks of age, the effect did not reach statistical significance (Fig. 4c, right).

Effect of Omigapil on Skeletal Deformation and Overall Survival. Muscle weakness and degeneration results in the deformation of the spine, which is a major complication in MDC1A patients because it causes respiratory distress. Spine deformation, identified as hunchback, is also a very obvious phenotype in laminin-α2-deficient dyw/dyw mice and became apparent in our mouse colony at approximately 11 weeks of age. The degree of scoliosis and kyphosis was clearly visible by X-ray imaging in a lateral view (Fig. 5a, b). Finally, omigapil lowered CK levels in the blood compared with age-matched vehicle-treated dyw/dyw animals (Fig. 3d), indicative of reduced muscle cell damage. In summary, these data demonstrate that omigapil markedly reduces apoptotic events and protects muscle fibers from degeneration caused by laminin-α2 deficiency.

The effect of omigapil on the overall health and behavior of dyw/dyw mice was studied next. In these experiments, mice were treated with two different doses of omigapil (0.1 and 1 mg/kg/day) starting at 15 days of age. Body weight was measured daily up to an age of 18 weeks. In an analysis that included only animals that did not die before the age of 18 weeks, vehicle-treated dyw/dyw mice gained weight until −7 weeks of age, after which no further increase was measured (Fig. 4a). In contrast, animals treated with either dose of omigapil continued to gain weight up to −9 weeks of age and thus reached a higher weight gain than vehicle-treated animals. After 12 weeks of age, animals treated with omigapil at 0.1 mg/kg/day slowly lost weight and eventually reached body weights equivalent to vehicle-treated dyw/dyw mice. In contrast, dyw/dyw mice treated with 1 mg/kg omigapil ended the study period with a higher body weight gain than vehicle-treated animals. A comparable pattern of weight gain was seen when all animals were included in the analysis, irrespective of when they died (data not shown). In this latter group, the peak body weight reached by dyw/dyw mice allocated to either one of the omigapil treatment groups differed significantly from the peak body weight reached by animals in the vehicle group (dyw-vehicle: 8.00 ± 0.50 g, mean ± S.E.M., n = 33; dyw-omigapil 0.1 mg/kg: 9.91 ± 0.48 g, n = 33, p = 0.008 versus vehicle; dyw-omigapil 1.0 mg/kg: 9.75 ± 0.56 g, n = 28, p = 0.020 versus vehicle). As body weight is known to be a confounding factor for survival, we also determined the percentage of animals in each treatment group reaching a threshold of 12 g over time (Fig. 4b). In all age groups, a higher percentage of omigapil-treated animals were above this threshold body weight compared with vehicle-treated animals. In contrast to omigapil-treated animals, none of the vehicle-treated animals maintained this threshold body weight from 15 weeks of age onward. Again, the effect of omigapil was dose-dependent favoring the higher dose, which becomes particularly evident at 12 weeks of age and older.
Significant loss of lean tissue in dyW/dyW animals, which causes the rib cage to collapse, was seen in dorsoventral X-ray images (Fig. 5a, right, white arrows). Treatment with 1 mg/kg/day omigapil starting at 15 days of age conspicuously reduced tissue loss, scoliosis, kyphosis, and the collapse of the rib cage. A visual assessment of the severity of spine deformation with use of a kyphosis score confirmed that omigapil-treated dyW/dyW mice were less affected than vehicle-treated animals. A total of 77% of vehicle-treated dyW/dyW mice had moderate to severe kyphosis, whereas a comparable percentage of omigapil-treated animals had barely detectable or mild kyphosis (Fig. 5b; p < 0.05, Wilcoxon rank sum test).

Loss of muscle tissue, locomotive immobility, spinal deformation, and rib cage collapse as a consequence of laminα2 deficiency causes early death in dyW/dyW mice. The administration of omigapil starting at 15 days of age exhibited a dose-dependent and significant effect on the survival of dyW/dyW mice (Fig. 5c). Overall survival of vehicle-treated dyW/dyW mice showed a biphasic pattern with approximately half of the population dying by ~35 days. Both doses of omigapil largely prevented the death of dyW/dyW mice in this early phase and resulted in a 50% survival time of ~85 days (0.1 mg/kg omigapil) and ~105 days (1 mg/kg omigapil), respectively. Although the 0.1 mg/kg dose of omigapil had only a small effect on maximum survival time, the 1 mg/kg dose of omigapil prolonged survival for a higher proportion of animals, with more than 25% of animals surviving beyond the age of 28 weeks.

**Discussion**

Neuromuscular diseases are rare genetic disorders that are characterized by the loss of muscle control and subsequent loss of muscle mass. Whereas the primary cause for this group of diseases can originate in different cell types (e.g., motor neurons or skeletal muscle) and can be triggered by mutations in many different genes, there are certain commonalities to the diseases that may open new avenues for treatment. For example, apoptosis and necrosis of muscle fibers or the invasion of fibrotic cells are hallmarks of several muscular dystrophies. Thus, drugs that affect such secondary consequences of a disease might be useful for the treatment of several neuromuscular disorders irrespective of their primary cause.

Here we show that the apoptosis inhibitor omigapil ameliorates many of the pathological symptoms in the dyW/dyW mouse, a well accepted animal model for MDC1A. Specifically, we show that omigapil inhibits apoptosis and thus protects muscle tissue, reduces body weight loss and skeletal deformation, and increases locomotive activity. It is noteworthy that loss of muscle mass, impaired mobility, and skeletal deformation are also disease hallmarks seen in MDC1A patients, and any improvement in such parameters is of ther-
apeutic relevance. We also find that omigapil protects dyW/dyW mice from early mortality and prolongs overall survival, although the latter effect was less strong. However, comparison of our data with published survival curves of dyW/dyW mice indicates that our vehicle-treated dyW/dyW mice seem to survive much longer in general, especially after an early phase of mortality. For example, in a recent study reporting on the use of doxycycline for the treatment of dyW/dyW mice (Girgenrath et al., 2009), all the vehicle-treated dyW/dyW mice had died after 56 days, and those treated with doxycycline died after 140 days. In our experiments, approximately 47% of vehicle-treated mice were still alive after 56 days and approximately 18% of the mice were still alive after 140 days. It is noteworthy that our current study and the study by Girgenrath et al. (2009) show an almost identical early phase of mortality that lasts until approximately day 40. Although almost all mice died in the Grigenrath study at about this age, many mice in our colony survived this crisis period. One of the reasons for the prolonged survival in vehicle-treated animals in our cohort might be the use of wet food and long-necked drinking bottles to ascertain adequate food and water intake in this fragile mouse strain. In summary, the markedly different survival times in untreated or vehicle-treated dyW/dyW mice reported in studies from different laboratories will have to be taken into consideration when interpreting treatment effects of experimental drugs for this indication. Nevertheless, the 50% survival time in animals treated with omigapil at a dose of 1 mg/kg/day was 3 times longer than for vehicle-treated animals, and more than 25% of the omigapil-treated animals survived past 28 weeks.

Omigapil was originally developed for the treatment of neurodegenerative diseases (Waldmeier et al., 2000; Waldmeier et al., 2006), and the therapeutic efficacy of omigapil has been tested in models of several neurological diseases both in vitro and in vivo. For example, omigapil has neuroprotective effects in cellular and animal models of Parkinson’s disease (Andringa et al., 2000) and enhances survival in an animal model of motoneuron disease (Sagot et al., 2000). Based on these positive preclinical results, omigapil was tested in clinical trials for Parkinson’s disease and amyotro-

Fig. 5. Effect of omigapil on skeletal deformation and survival. a, X-ray examination of 11-week-old WT and dyW/dyW mice in lateral (left) and dorsoventral (right) view. The angle of spine curvature (kyphosis) is indicated in the lateral view (dotted line). Although the curvature of the spine in a wild-type mouse was >120° between the costal and abdominal region, vehicle-treated dyW/dyW mice often show very pronounced dorsoventral curvature (hunchback) with angles between the costal and abdominal spinal region being as small as 66°. Kyphosis and scoliosis is ameliorated upon treatment with omigapil, resulting in reduced dorsoventral spine curvature reaching ~90°. The body dimension is indicated by the vertical arrows depicting the loss of lean and fat mass in vehicle-treated animals and the preservation thereof in omigapil-treated dyW/dyW mice. Horizontal arrows indicate the anteroposterior dimension of the rib cage, which is enlarged after omigapil treatment compared with vehicle-treated dyW/dyW mice. Note: paralysis of hindlimbs is seen in both the vehicle-treated and omigapil-treated animals. b, quantification of kyphosis by visual inspection performed at the same day using a kyphosis score (1, barely detectable; 2, mild; 3, moderate; 4, severe). Dots: scoring of individual 11-week-old dyW/dyW mice. c, Kaplan-Meier cumulative survival plot shows that omigapil-treated dyW/dyW mice survive longer than vehicle-treated animals (0.1 mg/kg omigapil versus vehicle, p < 0.05; 1 mg/kg omigapil versus vehicle, p < 0.01; Peto-Peto Wilcoxon test). Omigapil treatment particularly protects from early death (dyW-vehicle, n = 36; dyW-omigapil 0.1 mg/kg, n = 33; dyW-omigapil 1.0 mg/kg, n = 28).
phic lateral sclerosis. Although omigapil was shown to be safe, no significant difference in key outcome measures could be demonstrated between placebo- and omigapil-treated patients (Olanow et al., 2006; Miller et al., 2007), and the possible reasons for this failure were explored and discussed (Waldmeier et al., 2006).

The antiapoptotic effect of omigapil has been proposed to be caused by its binding to GAPDH (Kragten et al., 1998). Independent of its enzymatic function in glycolysis, several lines of evidence strongly indicate that GAPDH plays an important role in apoptosis and that this activity requires its translocation into the nucleus (Dastoor and Dreyer, 2001; reviewed in Chuang et al., 2005). The nuclear translocation of GAPDH is mediated by Siah1, an E3 ubiquitin ligase with a nuclear localization signal (Hara et al., 2005). Specifically, it has been demonstrated that the GAPDH-Siah1 complex forms upon 5-nitrosylation of GAPDH, and then the complex translocates into the nucleus (Hara et al., 2005, 2006). Once in the nucleus, GAPDH becomes acetylated through the acetyltransferase CBP/p300, which in turn further stimulates CBP/p300 activity. Consequently, downstream targets of CBP/p300 such as p53 are induced, which triggers apopto-
sis through the activation of the proapoptotic genes PUMA and p21 (Sen et al., 2008). The protein GOSPEL can act as a modifier of this proapoptotic pathway by preventing the init-
ial formation of the GAPDH-Siah1 complex (Sen et al., 2009). Omigapil has been shown to directly bind to GAPDH (Carlile et al., 2000). This omigapil-GAPDH interaction prevents the formation of the GAPDH-Siah1 complex in a man-
er similar to GOSPEL (Hara et al., 2006; Sen et al., 2009). In muscle from dyW/dyW mice we found that the antiapoptotic protein GOSPEL is significantly reduced, whereas we found GAPDH to be accumulated in the nuclear fraction. Further-
more, the proapoptotic genes that are activated downstream in the GAPDH-Siah1-CBP/p300 pathway (p53, PUMA, p21) are up-regulated. This provides evidence that apoptosis in lamin-deficient muscle may be caused by activation of the GAPDH-Siah1-CBP/p300 signaling pathway. Omigapil may thus compensate for the loss of GOSPEL and block the activation of this proapoptotic GAPDH-Siah1-CBP/p300 pathway, thereby preventing apoptosis.

In addition, omigapil could also interfere with an alterna-
tive pathway that causes apoptosis. This pathway is based on the “permeability transition” of mitochondria as a response to cellular insults that cause a strong increase in intracellular calcium. This process results in the regulated formation of a large pore complex that spans the outer and inner mito-
chondrial membranes, leading to the swelling and long-last-
ing opening of this permeability transition pore complex (PTPC) and causing necrotic and/or apoptotic cell death.

GAPDH has recently been shown to accumulate in mitochondria during apoptosis and to induce PTPC-dependent pro-
apoptotic mitochondrial membrane permeabilization via its association with voltage-dependent anion channel, one of the key components of the PTPC (Tarze et al., 2007). A role of the PTPC in muscle dystrophies has recently been suggested by the finding that deletion of the gene encoding cyclophilin D, another key component of the PTPC, reduces muscle fiber necrosis in mouse models for several muscle dystrophies including MDC1A (Milay et al., 2008; Palma et al., 2009). Taken together, our study demonstrates for the first time the activation of the GAPDH-Siah1-CBP/p300 proapoptotic signaling pathway in muscle of dyW/dyW mice. Furthermore, we show that omigapil inhibits apoptosis in muscle, protects from body weight loss, reduces skeletal deformation, in-
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ty. Because omigapil is well advanced in nonclinical and clinical development, and has proven to be safe in large clinical trials with patients who have Parkinson’s disease and amyotrophic lateral sclerosis, this orally bioavailable drug candidate is well suited to be tested as a potential therapy for MDC1A.

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References


Bentzinger CF, Burqaghi MA, and Ruangs MA (2005) Overexpression of mini-
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