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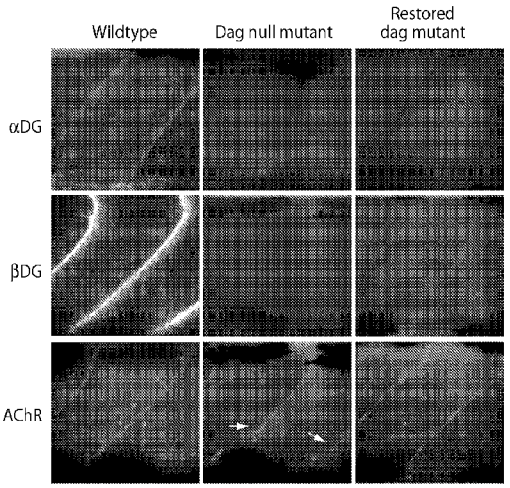


Fig. 4

(57) Abstract: Compounds have been identified and tested in a zebrafish model of dystroglycanopathy and found to be efficacious based on an increase in the survival rate of dystroglycan deficient fish. Several of the compounds affect the GABA and acetylcholine pathways. Accordingly, methods and compositions for the treatment of dystroglycanopathies are provided.

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METHODS AND COMPOUNDS FOR THE TREATMENT OF DYSTROGLYCANOPATHIES

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application filed March 15, 2013, entitled "METHODS AND COMPOUNDS FOR THE TREATMENT OF DYSTROGLYCANOPATHIES", Serial No.61/790,150, the contents of which are incorporated by reference herein in their entirety.

BACKGROUND OF INVENTION

Dystroglycanopathies are a group of muscular dystrophies characterized by the reduced or absent glycosylation of alpha-dystroglycan. The hypoglycosylation of alpha-dystroglycan leads to decreased binding of its ligands, including laminin, agrin and perlecan in skeletal muscle and neurexin in the brain. The clinical manifestations of dystroglycanopathies are variable, leading to a broad spectrum of phenotypes with limb-girdle muscular dystrophy (LGMD) without mental retardation delineating the milder end, and Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB) and Fukuyama type congenital muscular dystrophy (FCMD) the severe end.

Currently, no effective therapies and drugs exist for treating muscular dystrophies, including the dystroglycanopathies. Identification of drugs that have the capacity to mitigate symptoms of muscular dystrophy would thus be of significant therapeutic value.

SUMMARY OF INVENTION

In some aspects, methods and compositions for the treatment dystroglycanopathies are provided. In other aspects, methods and assays are provided for the identification of compounds effective in the treatment of dystroglycanopathies.

Thus according to one aspect of the disclosure, a method of treating a subject having or at an increased risk of having a dystroglycanopathy is provided. The method comprises administering to a subject in need thereof a pharmaceutical composition comprising a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

According to another aspect of the disclosure, a method of treating a subject having or at an increased risk of having a dystroglycanopathy is provided. The method comprises administering to a subject in need thereof a pharmaceutical composition comprising a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions of the subject, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

In yet another aspect of the disclosure, a method of treating a subject having or at an increased risk of having a dystroglycanopathy is provided. The method comprises administering to a subject in need thereof a pharmaceutical composition comprising a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

Another aspect of the disclosure provides a pharmaceutical composition for the treatment of a dystroglycanopathy. The composition comprises a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

In yet another aspect of the disclosure, a pharmaceutical composition for the treatment of a dystroglycanopathy is provided. The pharmaceutical composition comprises a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

According to another aspect of the disclosure, a pharmaceutical composition for the treatment of a dystroglycanopathy is provided. The composition comprises a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

In yet another aspect of the disclosure, a pharmaceutical composition for use in treating a dystroglycanopathy is provided. The composition comprises a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

According to another aspect of the disclosure, a pharmaceutical composition for use in treating a dystroglycanopathy is provided. The pharmaceutical composition comprises a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

According to another aspect of the disclosure, a pharmaceutical composition for use in treating a dystroglycanopathy is provided. The composition comprises a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

According to still another aspect of the disclosure, a method of treating a subject having or at an increased risk of having a dystroglycanopathy is provided. The method comprises administering to a subject in need thereof a pharmaceutical composition comprising a compound that increases the expression of an integrin, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound increases the expression of integrin alpha 7 (*ITGA7*). In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

According to yet another aspect of the disclosure, a pharmaceutical composition for the treatment of a dystroglycanopathy is provided. The composition comprises a compound that increases the expression of an integrin, wherein the compound is present in an amount effective to restore muscle function or phenotype. In some embodiments, the compound increases the expression of integrin alpha 7 (*ITGA7*). In some embodiments, the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 depicts photographs of muscular dystrophy model fish and Western blot analyses comparing proteins between wild type and *dag1* zebrafish. The muscular dystrophy model fish have a muscle phenotype that can be detected with the birefringence assay (e.g., at 4 dpf). A, B: Wild type, C, D: *sapje*, dystrophin null fish, E, F: *dag1*, dystroglycan null fish and G, H: Laminin alpha2 null fish. A, C, E, G: Brightfield image, B, D, F, H: Birefringence image. I: Western blot analysis of *dag1* fish showing a complete loss of alpha- and beta-dystroglycan (αDG, βDG, respectively).

Figure 2 depicts a graph demonstrating increased survival of ethosuximide treated *dag1* zebrafish, and photographs comparing the muscle phenotypes of wild type and ethosuximide treated *dag1* zebrafish. Analysis of chemically treated fish occurred at time points up to 20 dpf. A: long term treatment of affected *dag1* mutant picked at 4 dpf. Ethosuximide (middle line) increased survival of *dag1* mutants as compared to untreated mutants (bottom line); Top line indicates wild type survival. B: Immunostaining chemically treated dystroglycan null fish. Recovered fish have no βDG expression and normal muscle structure. BR: Birefringence analysis. βDG: beta-dystroglycan. MHC: Myosin Heavy Chain.

Figure 3 depicts a graph showing $GABA_A$ receptor alpha expression in wild type, mutant, and treated mutant zebrafish. $GABA_A$ receptor alpha expression level is restored in ethosuximide treated *dag1* zebrafish compared to those of non-treated mutants (p=0.0018: wildtype vs *dag1* mutant. p=0.029: Recovered mutant vs *dag1* mutant).

Figure 4 depicts photographs showing the acetylcholine receptor (AChR) phenotype in wild type, mutant, and treated mutant zebrafish. Immunostaining in chemically treated dystroglycan null fish at 4 dpf. Ethosuximide treated *dag1* mutants have no expression of alpha- and beta-dystroglycan. Treatment with ethosuximide rescued the abnormal AChR cluster distribution at myosepta area, which is found in *dag1* mutants (arrows). AChR: alphabungarotoxin staining (acetylcholine receptor maker). αDG: alpha-dystroglycan, βDG: beta-dystroglycan.

Figure 5 depicts graphs and a photograph showing integrin alpha 7 (integrin α7) expression in wild type, mutant, and ethosuximide treated mutant fish. qRT-PCR (A) of

integrin α7 in skeletal muscle of wild type (WT), untreated fish (NT) and ethosuximide chemically treated fish (CT) at 10 dpf and 20 dpf. In the ethosuximide treated dystroglycan null fish, expression of integrin α7 is upregulated at the mRNA level (A: *p= 0.0034, **p=0.038, n=3). Western blot analysis (B and C) of integrin α7 in skeletal muscle of wild type (WT), untreated fish (NT) and ethosuximide chemically treated fish (CT) at 20 dpf. In the ethosuximide treated dystroglycan null fish, expression of integrin α7 is upregulated at protein level (C: *p= 0.00026, n=3).

DETAILED DESCRIPTION OF INVENTION

Muscular dystrophy is a degenerative muscle disease in which the muscle forms normally at first, but then starts to degenerate faster than it can be repaired. Mutations in many parts of the dystrophin associated protein complex (DAPC), or dystrophin-glycoprotein complex (DGC), have been reported as the cause of other forms of muscular dystrophy (Hewitt, *Biochim Biophys Acta.* 2009;1792(9):853-61; Bozzi *et al., Matrix Biol.* 2009;28(4):179-87; Ervasti & Sonneman, *Int Rev Cytol.* 2008;265:191-225). One such grouping of muscular dystrophies, the dystroglycanopathies, involve deficiencies in the dystroglycan proteins. The alpha- and beta-dystroglycan proteins are central integral membrane components of the DGC. Beta-dystroglycan is a transmembrane protein in the sarcolemma with alpha-dystroglycan tightly associated with the extracellular matrix (ECM). Alpha-dystroglycan acts as a receptor of several ECM ligands such as laminins and agrin in muscle, and neurexin and pikachurin in the brain and retina, respectively (Ibraghimov-Beskrovnaya *et al., Nature.* 1992;355 (6362):696–702; Sugiyama *et al., Neuron.* 1994;13(1):103-115; Hohenester *et al., Mol Cell.* 1999;4(5):783-92; Sato *et al., Nat Neurosci.* 2008;11(8):923-31; Sugita *et al., J Cell Biol.* 2001;154(2):435-45). Alpha-dystroglycan and beta-dystroglycan are expressed in skeletal muscle, inhibitory synapses, and at the neuromuscular junction (NMJ) (Pilgram *et al., Mol Neurobiol.* 2010;41(1):1-21). Deficiency in alpha-dystroglycan glycosylation is a common feature of many of the muscular dystrophies, for example dystroglycanopathies (Hino-Fukuyo *et al., Neuromuscul Disord.* 2006;16(4): 274-6; Dobyns *et al., Am J Med Genet.* 1989;32(2): 195-210; Clement *et al., Ann Neurol.* 2008;64(5):573-82; Brown *et al., Am J Pathol.* 2004;164(2):727-37; Puckett *et al., Neuromuscul Disord.* 2009; 19(5):352-56). Mutations in glycosyltransferase genes associated with hypoglycosylation of alpha-dystroglycan are known to cause various dystroglycanopathies. Severe congenital onset of muscle weakness with brain and/or eye abnormalities is found in glycosyltransferase-associated dystroglycanopathies such as

Fukuyama-type congenital muscle dystrophy (FCMD), Muscle-Eye-Brain (MEB) disease, and Walker-Warburg Syndrome (WWS). FCMD, WWS and MEB patients have been reported with severe brain phenotypes such as epilepsy (Yoshioka *et al., Child Neurol.* 2005;20(4):385-91; Akiyama *et al., Brain Dev.* 2006;28(8):537-40; Messina *et al., Neurology.* 2009;73(19):1599-601). The muscle phenotype in dystroglycanopathies is characterized by muscle weakness that progressively gets worse, delayed development of muscle motor skills, difficulty using one or more muscle groups, loss of strength in a muscle or group of muscles, loss in muscle size, etc.

Zebrafish are an ideal organism for the study of muscle diseases such as dystroglycanopathies because they reproduce in large quantities, grow rapidly, and are easy to assay for muscle abnormalities. Additionally, like mammals, the zebrafish DAPC localizes to the muscle cell membrane in adult fish (Chambers *et al., Biochem Biophys Res Commun.* 2001;286(3):478-83.). Much evidence suggests that the proteins of the DAPC function similarly in zebrafish as in mammals, and that mis-expression of these proteins gives rise to a muscle-specific phenotype that can be scored early in zebrafish development. Further, various model fish for muscular dystrophies exhibit decreased birefringence and motility defects. Birefringence measures the rotation of polarized light through the transparent zebrafish embryo at the highly ordered sarcomeric structure of the somatic muscle. These "dystrophic" mutants all show similar phenotypes and develop a muscle phenotype at 4 days post-fertilization (dpf). The phenotype can be detected by the birefringence assay, which detects the disorganization of muscle structure without harming the fish (See Examples).

One model particularly suited to the study of dystroglycanopathies, is a dystroglycan null (DAG1) fish, which were shown to exhibit a muscle disease phenotype (Gupta *et al., Hum Mol Genet.* 2011;20(9):1712-25; Lin *et al., Hum Mol Genet.* 2011;20(9):1763-75 19, 20). This dystroglycan null fish lacks alpha- and beta-dystroglycan expression (Gupta *et al., Hum Mol Genet.* 2011;20(9):1712-25). The dystroglycan mutant fish produces no dystroglycan protein, has a phenotype with substantially reduced mobility, characteristic structural defects that manifest as reduced birefringence at 4-5 dpf, and markedly decreased survival (See Examples; Gupta *et al., Hum Mol Genet.* 2011;20(9):1712-25).

Thus, aspects of the present disclosure relate to the surprising discovery of compounds, provided herein, that are able to rescue the muscle phenotypes and in some cases increase survival of dystroglycan null fish. Using a chemical library screen (*e.g.,* the Prestwick Chemical Library (Illkirch, France); comprising 1,100 compounds, many of which

are Food and Drug Administration (FDA)-approved drugs), compounds were identified that demonstrate therapeutic benefit in the zebrafish model of dystroglycanopathy. Surprisingly, many of the compounds provided herein affect γ-aminobutyric acid (GABA) related pathways. GABA is the major inhibitory neurotransmitter in the central nervous system, and is considered to be a multifunctional molecule that has different situational functions in the central nervous system (CNS), the peripheral nervous system (PNS), and in some nonneuronal tissues. For example, components of the GABA pathway are expressed in muscle (Watanabe *et al., Int Rev Cytol.* 2002;213:1-47).

Interestingly, the compounds were also shown, as described herein, to affect the distribution of acetylcholine receptors (AChRs) in the neuromuscular junction (NMJ) of *dag1* zebrafish. Acetylcholine is a neurotransmitter in the autonomic nervous system (ANS) and is a neurotransmitter used in the motor division of the somatic nervous system (SoNS). In cardiac tissue, acetylcholine neurotransmission has an inhibitory effect, which lowers heart rate. However, acetylcholine also behaves as an excitatory neurotransmitter at NMJs in skeletal muscle.

Accordingly, in some aspects, compounds provided herein are able to restore muscle function or muscle phenotype in subjects having or at risk of having a dystroglycanopathy (e.g., by affecting GABA related pathways and/or acetylcholine signaling). For example, the muscle phenotype of subjects suffering from (or in some aspects, subjects at risk of having) a dystroglycanopathy is characterized by muscle weakness that progressively gets worse, delayed development of muscle motor skills, difficulty using one or more muscle groups, loss of strength in a muscle or group of muscles, loss in muscle size, etc. Thus, restoring muscle function or phenotype, in some aspects, means improving one or more characteristics of the dystroglycanopathy muscle phenotype, e.g., by preventing muscle weakness, promoting development of muscle motor skills, increasing muscle strength and size, etc. Methods for monitoring such improvements are known, and include, for example, those described herein.

In some embodiments and without being bound by any particular mechanism, the compounds provided herein are able to restore or improve muscle function or phenotype observed in dystroglycanopathy by affecting the GABA pathway and/or AChR distribution. For example, the dystroglycan null (*dag1*) zebrafish, as demonstrated herein, displays abnormal overexpression of a GABA pathway component, the $GABA_A$ receptor alpha. Compounds identified in the screen, for example ethosuximide, were able to restore the expression level of $GABA_A$ receptor alpha to the normal, or wild type level, which coincided with increased motility, survival, and improvement in the muscle phenotype. Similarly, as

demonstrated herein, the *dag1* mutants display abnormal AChR distribution in the NMJ. Compounds, *e.g.*, ehthosuximide, were shown to restore normal, or wild type distribution of AChR, which also coincided with increased motility, survival, and improvement in the muscle phenotype. While these drugs were not shown to restore dystroglycan expression, there are many other ways muscle can be stabilized, including stabilization of other structural components of the membrane such as the integrins. In addition, data indicates that GABA and acetylcholine signaling plays an important role in the disease, and restoration of normal signaling may influence membrane stability in the absence of dystroglycan.

In some embodiments and without being bound by any particular mechanism, the compound(s) provided herein are able to restore or improve muscle function or phenotype observed in dystroglycanopathy by affecting the expression and/or distribution of structural components of the cell membrane, e.g., integrins. For example, in some embodiments, the provided compound(s) increase the expression of an integrin, which leads to restoration of muscle function or phenotype. Integrins are transmembrane receptors that mediate the attachment between a cell and its surroundings, such as other cells or the extracellular matrix (ECM). Integrins work alongside other proteins such as cadherins, immunoglobulin superfamily cell adhesion molecules, selectins and syndecans to mediate cell–cell and cell–matrix interaction and cell-cell communication. Integrins bind cell surface and ECM components such as fibronectin, vitronectin, collagen, and laminin. Integrins are well known to those of ordinary skill in the art, and include, without limitation, integrin alpha 1 (*ITGA1*), integrin alpha 2 (*ITGA2*), integrin alpha 3 (*ITGA3*), integrin alpha 4 (*ITGA4*), integrin alpha 5 (*ITGA5*), integrin alpha 6 (*ITGA6*), integrin alpha 7 (*ITGA7*), integrin alpha 8 (*ITGA8*), integrin alpha 9 (*ITGA9*), integrin alpha 10 (*ITGA10*), integrin alpha 11 (*ITGA1*), integrin alpha D (*ITGAD*), integrin alpha E (*ITGAE*), integrin alpha L (*ITGAL*), integrin alpha M (*ITGAM*), integrin alpha V (*ITGAV*), integrin alpha 2b (*ITGA2B*), integrin alpha X (*ITGAX*), integrin beta 1 (*ITGB1*), integrin beta 2 (*ITGB2*), integrin beta 3 (*ITGB3*), integrin beta 4 (*ITGB4*), integrin beta 5 (*ITGB5*), integrin beta 6 (*ITGB6*), integrin beta 7 (*ITGB7*), and integrin beta 8 (*ITGB8*). In some embodiments, the provided compound(s) increase the expression of one or more integrins, e.g., those described herein. In some embodiments, the provided compound(s) increase the expression of an integrin to a level at or above a wild type level. In some embodiments, the provided compound(s) increase the expression of an integrin by 10%, 20%, 30,%, 40%, 50%, 75%, 100%, 125%, 150%, 175%, 200%, 250%, 300%, 350%, 400%, 450%, or 500% or more, as compared to wild type or mutant levels. In

some embodiments, the provided compound(s) increases the expression (e.g., as described herein) of integrin alpha 7 (*ITGA7*).

Thus, in certain aspects, methods and pharmaceutical compositions are provided for the treatment of dystroglycanopathies (e.g., to restore muscle function or muscle phenotype). To "treat" a dystroglycanopathy, means to reduce or eliminate a sign or symptom of the disease, to stabilize the disease, and/or to reduce or slow further progression of the disease. In some embodiments, "treat", "treatment" or "treating" is intended to include prophylaxis, amelioration, prevention or cure from the disease. For example, treatment of dystroglycanopathies may result in e.g., preventing or slowing of muscle degeneration, preventing or decreasing fatigue, increasing muscle strength, reducing blood levels of creatine kinase (CK), preventing or decreasing difficulty with motor skills, preventing or decreasing muscle fiber deformities, increasing cognition, preventing or improving epileptic symptoms (e.g., preventing or decreasing seizure activity; decreasing frequency of convulsions), improving eye function, restoring or preventing of eye abnormalities (e.g., retinal detachment), preventing or improving dystrophic abnormalities (e.g., as determined by muscle biopsy), reversing, reducing, or preventing cardiac dysfunction (resulting from, e.g., cardiomyopathy) manifested by e.g., congestive heart failure and arrhythmias, etc.

An "effective amount," or an "amount effective," as used herein, refers to an amount of a compound and/or an additional therapeutic agent, or a composition thereof that is effective in producing the desired molecular, therapeutic, ameliorative, inhibitory or preventative (prophylactic) effect, and/or results in a desired clinical effect. The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration, and like factors are within the knowledge and expertise of the health practitioner. For example, an effective amount can depend upon the duration the subject has had the disease. In some aspects, an effective amount of a composition described herein when administered to a subject results in e.g., increased muscle strength, increased motility, restoration of muscle function or phenotype, decreased fatigue, decreased difficulty with motor skills, decreased epileptic symptoms, etc. In some aspects, the desired therapeutic or clinical effect resulting from administration of an effective amount of a composition described herein, may be measured or monitored by methods known to those of ordinary skill in the art e.g., by monitoring the creatine kinase (CK) levels in a subject's blood, by electromyography, by electroencephalography (EEG), and/or by histological examination of a muscle biopsy.

In the combination therapies of the present invention, an effective amount can refer to each individual agent or to the combination as a whole, wherein the amounts of all agents administered are together effective, but wherein the component agent of the combination may not be present individually in an effective amount.

In some embodiments, compositions for the treatment of dystroglycanopathies provided herein comprise a compound (e.g., a small molecule, a protein, a peptide, an antibody, an antibody fragment, a ligand, a receptor, etc.) that targets the GABA pathway. By "targets," it is meant that the compound affects one or more components of the GABA pathway. For example, a compound that targets the GABA pathway, in some aspects, is a compound that restores normal expression of one or more GABA pathway components, e.g., GABA, GABA receptors (e.g., $GABA_A$, $GABA_B$), GABA receptor subunits (e.g., $GABA_A$ receptor alpha), glutamic acid decarboxylase (GAD), vesicular GABA transporters (VGATs), GABA transaminase (GABA-T), $GABA_A$-receptor-associated protein (GABARAP), glutamate, etc. Methods for monitoring expression of GABA pathway components are known in the art, and include for example, PCR based strategies (e.g., RT-PCR), electrophysiology, and immunohistochemistry. For example, in some aspects, a provided compound that is beneficial in the treatment of dystroglycanopathies is a compound that restores normal expression levels of $GABA_A$ receptor alpha. In some aspects, restoration of normal expression levels means an expression level that is within 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the normal or wild type expression level. In some aspects, a compound that targets the GABA pathway is a compound that restores normal GABA signaling, for example, by increasing or decreasing the expression or function of one or more GABA pathway components (e.g., those described herein). Methods for monitoring GABA signaling in the context of disease (e.g., dystroglycanopathy) treatment are known in the art, and include for example, electrophysiological methods, immunohistochemistry, and magnetic resonance imaging (MRI), e.g., as described by Levy & Degnan, GABA-Based Evaluation of Neurologic Conditions: MR Spectroscopy, *AJNR Am J Neuroradiol.* 2013; 34(2):259-65.

In some embodiments, the compound selectively targets the GABA pathway. By "selectively targets," it is meant that the compound binds a target (e.g., a biomolecule or component in the GABA pathway), with greater affinity than it binds to a non-target (e.g., a component that is not part of the GABA pathway). In some embodiments, the compound binds its target with a dissociation constant (KD) of less than 10^{-6} M, of less than 10^{-7} M, of less than 10^{-8} M, of less than 10^{-9} M, or of less than 10^{-10} M. For example, a compound may

be a small molecule, a chemical, a protein, a peptide, an antibody, an antibody fragment, a ligand, or a receptor, that binds to a target molecule with a KD as specified above. In some embodiments, "selectively targets" refers to binding of a compound to a target with high affinity, e.g. with a KD of less than 10^{-8}, of less than 10^{-9} M, of less than 10^{-10} M, of less than 10^{-11} M, or of less than 10^{-12} M. In some embodiments, the compound binds to the target molecule with high selectivity or specificity, e.g., in that it does not bind to molecules other than the target molecule with a KD of less than 10^{-6} M, of less than 10^{-7} M, or of less than 10^{-8} M.

In some embodiments, the "GABA pathway" refers to the collection of biomolecules or components (e.g., proteins, peptides, amino acids, receptors, ligands, enzymes, etc.) that mediate or are involved in a cellular signaling cascade effectuated by GABA and its receptors. In some aspects, genes expressing GABA components are considered part of the GABA pathway.

Components of the GABA pathway are expressed in muscle, e.g., GABA, GAD, GABA-T, GABARAP, etc. (Watanabe *et al., Int Rev Cytol.* 2002;213:1-47). Thus, without being bound by any particular mechanism, it is contemplated that compounds that target the GABA pathway in muscle, such as those described herein, are beneficial in the treatment of dystroglycanopathies, for example by restoring muscle function or phenotype. In some embodiments, again without being bound by any particular mechanism, because GABA is the primary inhibitory neurotransmitter, it is contemplated that compounds that target the GABA pathway (e.g., as provided herein) are beneficial in treating neurological symptoms of dystroglycanopathies, such as mental retardation and epilepsy.

Thus, in some aspects, the present disclosure contemplates methods and compositions for the treatment of dystroglycanopathies, by affecting components of the GABA pathway in such a way as to restore normal muscle phenotype and/or improve neurological symptoms. In some embodiments, provided compounds that affect the GABA pathway in such a way as to restore normal muscle phenotype (e.g., by affecting GABA pathway components or restoring normal GABA signaling) and/or improve neurological symptoms include, but are not limited to, ethosuximide, vitamin B_{12} (e.g., cyanocobalamin), remoxipride, memantine, risperidone, and/or salts and/or derivative of any of the foregoing. In some embodiments, compounds that affect the GABA pathway include, but are not limited to, gaboxadol, ibotenic acid, muscimol, progabide, bicuculline, gabazine, barbiturates, benzodiazepines (e.g., alprazolam, bretazenil, bromazepam, brotizolam, chlordiazepoxide, cinolazepam, clorazepate, cloxazolam, delorazepam, diazepam, estazolam, etizolam, ethyl loflazepate,

flunitrazepam, flurazepam, halazepam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, nordazepam, oxazepam, phenazepam, pinazepam, prazepam, premazepam, pyrazolam, quazepam, temazepam, tetrazepam, triazolam, clobazam, flumazenil, eszopiclone, zaleplon, zolpidem, zopiclone), carisoprodol, etomidate, glutethimide, kavalactones, cicutoxin, oenanthotoxin, pentylenetetrazol, picrotoxin, thujone, lindane, zolpidem, adipiplon, imidazenil, Dilantin, phenobarbital, phenytoin, trileptal, levetiracetam, valproic acid, oxcarbazepine, primidone, lacosimide, zonisamide, pyridoxine, zonisamide, vitamin B_6, primadone, tiagabine, lacosamide, valproate, mercaptopropionic acid, aminooxyacetic acid, baclofen, and/or salts and/or derivatives of any of the foregoing.

In some aspects, compositions described herein comprise compounds that restore normal acetylcholine receptor (AChR) distribution in neuromuscular junctions (NMJs) in subjects having a dystroglycanopathy. In some embodiments, the compounds affect AChR signaling, e.g., restore normal AChR signaling by altering the function or expression of AChRs. As described herein, acetylcholine is a neurotransmitter used in the motor division of the somatic nervous system (SoNS). Acetylcholine acts to stimulate muscles when it binds to AChRs on skeletal muscle fibers, opening ligand-gated sodium channels in the cell membrane. Sodium ions then enter the muscle cell, initiating a sequence of steps that finally produce muscle contraction. Interestingly, as described herein, *dag1* mutant zebrafish display abnormal AChR distribution in the NMJ (See Examples). These fish have abnormal muscle structure, and decreased motility and survival. When treated with compounds described herein, *dag1* zebrafish have restored, or wild type NMJ AChR distribution, restored muscle phenotype, increased motility, and increased survival. Thus, without being bound by any particular mechanism, compounds which restore normal, or wild type AChR distribution in NMJs are beneficial in the treatment of dystroglycanopathies, e.g., by restoring muscle function or phenotype (e.g., as described herein). Methods for assessing normal AChR distribution in NMJs are known in the art, and include for example, immunocytochemistry analysis of biopsied muscle tissue using antibodies against AChR or AChR markers (e.g., alpha-bungarotoxin). In some embodiments, because restoration of AChR distribution in NMJs results in a desired therapeutic effect, such restoration may be monitored by e.g., the monitoring of certain clinical parameters (e.g., increased muscle strength, motility, restoration of muscle function or phenotype, CK blood levels, electromyography, muscle biopsy, etc.). In some embodiments, provided compounds that restore normal AChR distribution in NMJs include, but are not limited to, ethosuximide, vitamin B_{12} (e.g., cyanocobalamin), remoxipride, memantine, risperidone, and/or salts and/or derivative of any

of the foregoing. In some embodiments, compounds that restore normal AChR distribution or signaling in NMJs include, but are not limited to acetyl l-carnitine, acetylcholine, bethanechol, carbachol, cevimeline, muscarine, nicotine, pilocarpine, suberylcholine, suxamethonium, physostigmine, galantamine, neostigmine, pyridostigmine, varenicline, succinylcholine, atracurium, vecuronium, tubocurarine, pancuronium, epibatidine, trimethaphan, mecamylamine, bupropion, dextromethorphan, hexamethonium, methacholine, oxotremorine, atropine, tolterodine, oxybutynin, vedaclidine, talsaclidine, xanomeline, ipatropium, pirenzepine, telenzepine, methoctramin, darifenacin, solifenacin, donepezil, rivastigmine, tacrine, edrophonium, neostigmine, physostigmine, pyridostigmine, and/or salts and/or derivatives of any of the foregoing.

The term "subject," as used herein, refers to an individual organism. In some embodiments, a subject is a mammal, for example, a human, a non-human primate, a mouse, a rat, a cat, a dog, a cattle, a goat, a pig, or a sheep. In some embodiments, the subject is a human having or at in increased risk of having a dystroglycanopathy. By "at in increased risk" of having a dystroglycanopathy, it is meant that in some aspects, preventative or prophylactic treatment is contemplated. For example, a subject may be at an increased risk of having a dystroglycanopathy due to family history of the disease (e.g., the subject has a genetic predisposition). Dystroglycanopathies are primarily if not entirely genetic based diseases. For example, mutations in six genes, *POMT1, POMT2, POMGnT1, FKTN, FKRP and LARGE*, encoding proteins involved in the post-translational modification of alpha-dystroglycan, have been implicated in dystroglycanopathies (Roscioli *et al., Nat Genet.* 2012;44(5):581-5). Thus, in some aspects, a subject having one or more mutations in genes associated with dystroglycanopathies (but not yet diagnosed with having a dystroglycanopathy), is a subject at increased risk of having a dystroglycanopathy. In some aspects, such subjects benefit from prophylactic treatment (e.g., by preventing the onset of progressive muscle weakness).

In some embodiments, the subject is free of indications that would otherwise call for treatment using a compound provided herein. For example, the compound ehthosuximide is sometimes prescribed as an anti-convulsant, thus in some embodiments a subject receiving ehthosuximide as an anti-convulsant is not a subject contemplated by the present disclosure. In some instances, compounds that affect the GABA pathway include benzodiazepines, which are often prescribed for anxiety related disorders. Thus, in some embodiments, a subject receiving benzodiazepines for anxiety related disorders is not a subject contemplated by the present disclosure. In some instances, compounds that affect acetylcholine signaling

or AChRs are prescribed for myasthenia gravis. Thus, in some embodiments, a subject receiving compounds that affect acetylcholine signaling or AChRs for myasthenia gravis is not a subject contemplated by the present disclosure.

The term “administering" or "administration" means providing a compound to a subject in a manner that is pharmacologically useful. Compositions as described herein may be administered by a variety of routes of administration, including but not limited to subcutaneous, intramuscular, intradermal, oral, intranasal, transmucosal, intramucosal, intravenous, sublingual, rectal, ophthalmic, pulmonary, transdermal, transcutaneous or by a combination of these routes.

The pharmacological agents or compounds used in the methods of the invention are preferably sterile and contain an effective amount of a provided compound for producing the desired response in a unit of weight or volume suitable for administration to a subject. The doses of pharmacological agents administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that subject tolerance permits. The dosage of a pharmacological agent may be adjusted by the individual physician or veterinarian, particularly in the event of any complication. A therapeutically effective amount typically varies from 0.01 mg/kg to about 1000 mg/kg, preferably from about 0.1 mg/kg to about 500 mg/kg, and most preferably from about 0.2 mg/kg to about 250 mg/kg, in one or more dose administrations daily, for one or more days.

In some embodiments, compounds of the present invention may be administered alone, in a pharmaceutical composition or formulation (e.g., as described herein), or combined with other therapeutic regimens (e.g., including other compounds provided herein). Provided compounds and optionally other therapeutic agent(s) may be administered simultaneously or sequentially. When the other therapeutic agents are administered simultaneously they can be administered in the same or separate formulations, but are administered at the same time. The other therapeutic agents may be administered sequentially with one another and with a provided compound when the administration of the other therapeutic agents and the compound is temporally separated. The separation in time between the administration of these compounds may be a matter of minutes or it may be longer.

Formulations

Parenteral Formulations

The compounds described herein can be formulated for parenteral administration. "Parenteral administration", as used herein, means administration by any method other than through the digestive tract or non-invasive topical or regional routes. For example, parenteral administration may include administration to a subject intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intravitreally, intratumorally, intramuscularly, subcutaneously, subconjunctivally, intraocularly, intravesicularly, intrapericardially, intraumbilically, by injection, and by infusion.

Parenteral formulations can be prepared as aqueous compositions using techniques is known in the art. Typically, such compositions can be prepared as injectable formulations, for example, solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a reconstitution medium prior to injection; emulsions, such as water-in-oil (w/o) emulsions, oil-in-water (o/w) emulsions, and microemulsions thereof, liposomes, or emulsomes.

The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, one or more polyols (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), oils, such as vegetable oils (e.g., peanut oil, corn oil, sesame oil, etc.), and a combination thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Solutions and dispersions of the active compounds as the free acid or base or pharmacologically acceptable salts thereof can be prepared in water or another solvent or dispersing medium suitably mixed with one or more pharmaceutically acceptable excipients including, but not limited to, surfactants, dispersants, emulsifiers, pH modifying agents, viscosity modifying agents, and combination thereof.

Suitable surfactants may be anionic, cationic, amphoteric or nonionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions. Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxyl)-sulfosuccinate;

and alkyl sulfates such as sodium lauryl sulfate. Cationic surfactants include, but are not limited to, quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetrimonium bromide, stearyl dimethylbenzyl ammonium chloride, polyoxyethylene and coconut amine. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glyceryl monostearate, glyceryl stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000 cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer® 401, stearoyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl-.beta.-alanine, sodium N-lauryl-.beta.-iminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

The formulation can contain a preservative to prevent the growth of microorganisms. Suitable preservatives include, but are not limited to, parabens, chlorobutanol, phenol, sorbic acid, and thimerosal. The formulation may also contain an antioxidant to prevent degradation of the active agent(s).

The formulation is typically buffered to a pH of 3-8 for parenteral administration upon reconstitution. Suitable buffers include, but are not limited to, phosphate buffers, acetate buffers, and citrate buffers.

Water soluble polymers are often used in formulations for parenteral administration. Suitable water-soluble polymers include, but are not limited to, polyvinylpyrrolidone, dextran, carboxymethylcellulose, and polyethylene glycol.

Sterile injectable solutions can be prepared by incorporating the active compounds in the required amount in the appropriate solvent or dispersion medium with one or more of the excipients listed above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those listed above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The powders can be prepared in such a manner that the particles are porous in nature, which can increase dissolution of the particles. Methods for making porous particles are well known in the art.

Controlled release formulations

The parenteral formulations described herein can be formulated for controlled release including immediate release, delayed release, extended release, pulsatile release, and a combination thereof.

Nano- and microparticles

For parenteral administration, the one or more compounds, and optional one or more additional active agents, can be incorporated into microparticles, nanoparticles, or combinations thereof that provide controlled release of the compounds and/or one or more additional active agents. In embodiments wherein the formulations contains two or more drugs, the drugs can be formulated for the same type of controlled release (e.g., delayed, extended, immediate, or pulsatile) or the drugs can be independently formulated for different types of release (e.g., immediate and delayed, immediate and extended, delayed and extended, delayed and pulsatile, etc.).

For example, the compounds and/or one or more additional active agents can be incorporated into polymeric microparticles which provide controlled release of the drug(s). Release of the drug(s) is controlled by diffusion of the drug(s) out of the microparticles and/or degradation of the polymeric particles by hydrolysis and/or enzymatic degradation. Suitable polymers include ethylcellulose and other natural or synthetic cellulose derivatives.

Polymers which are slowly soluble and form a gel in an aqueous environment, such as hydroxypropyl methylcellulose or polyethylene oxide may also be suitable as materials for drug containing microparticles. Other polymers include, but are not limited to, polyanhydrides, poly(ester anhydrides), polyhydroxy acids, such as polylactide (PLA), polyglycolide (PGA), poly(lactide-co-glycolide) (PLGA), poly-3-hydroxybutyrate (PHB) and copolymers thereof, poly-4-hydroxybutyrate (P4HB) and copolymers thereof, polycaprolactone and copolymers thereof, and a combination thereof.

Alternatively, the drug(s) can be incorporated into microparticles prepared from materials which are insoluble in aqueous solution or slowly soluble in aqueous solution, but are capable of degrading within the GI tract by means including enzymatic degradation, surfactant action of bile acids, and/or mechanical erosion. As used herein, the term "slowly soluble in water" refers to materials that are not dissolved in water within a period of 30 minutes. Preferred examples include fats, fatty substances, waxes, wax-like substances and mixtures thereof. Suitable fats and fatty substances include fatty alcohols (such as lauryl, myristyl stearyl, cetyl or cetostearyl alcohol), fatty acids and derivatives, including but not limited to fatty acid esters, fatty acid glycerides (mono-, di- and tri-glycerides), and

hydrogenated fats. Specific examples include, but are not limited to hydrogenated vegetable oil, hydrogenated cottonseed oil, hydrogenated castor oil, hydrogenated oils available under the trade name Sterotex®, stearic acid, cocoa butter, and stearyl alcohol. Suitable waxes and wax-like materials include natural or synthetic waxes, hydrocarbons, and normal waxes. Specific examples of waxes include beeswax, glycowax, castor wax, carnauba wax, paraffins and candelilla wax. As used herein, a wax-like material is defined as any material which is normally solid at room temperature and has a melting point of from about 30 to 300°C.

In some cases, it may be desirable to alter the rate of water penetration into the microparticles. To this end, rate-controlling (wicking) agents may be formulated along with the fats or waxes listed above. Examples of rate-controlling materials include certain starch derivatives (e.g., waxy maltodextrin and drum dried corn starch), cellulose derivatives (e.g., hydroxypropylmethyl-cellulose, hydroxypropylcellulose, methylcellulose, and carboxymethyl-cellulose), alginic acid, lactose and talc. Additionally, a pharmaceutically acceptable surfactant (for example, lecithin) may be added to facilitate the degradation of such microparticles.

Proteins which are water insoluble, such as zein, can also be used as materials for the formation of drug containing microparticles. Additionally, proteins, polysaccharides and combinations thereof which are water soluble can be formulated with drug into microparticles and subsequently cross-linked to form an insoluble network. For example, cyclodextrins can be complexed with individual drug molecules and subsequently cross-linked.

Encapsulation or incorporation of drug into carrier materials to produce drug containing microparticles can be achieved through known pharmaceutical formulation techniques. In the case of formulation in fats, waxes or wax-like materials, the carrier material is typically heated above its melting temperature and the drug is added to form a mixture comprising drug particles suspended in the carrier material, drug dissolved in the carrier material, or a mixture thereof. Microparticles can be subsequently formulated through several methods including, but not limited to, the processes of congealing, extrusion, spray chilling or aqueous dispersion. In a preferred process, wax is heated above its melting temperature, drug is added, and the molten wax-drug mixture is congealed under constant stirring as the mixture cools. Alternatively, the molten wax-drug mixture can be extruded and spheronized to form pellets or beads. These processes are known in the art.

For some carrier materials it may be desirable to use a solvent evaporation technique to produce drug containing microparticles. In this case drug and carrier material are co-dissolved in a mutual solvent and microparticles can subsequently be produced by several

techniques including, but not limited to, forming an emulsion in water or other appropriate media, spray drying or by evaporating off the solvent from the bulk solution and milling the resulting material.

In some embodiments, drug in a particulate form is homogeneously dispersed in a water-insoluble or slowly water soluble material. To minimize the size of the drug particles within the composition, the drug powder itself may be milled to generate fine particles prior to formulation. The process of jet milling, known in the pharmaceutical art, can be used for this purpose. In some embodiments drug in a particulate form is homogeneously dispersed in a wax or wax like substance by heating the wax or wax like substance above its melting point and adding the drug particles while stirring the mixture. In this case a pharmaceutically acceptable surfactant may be added to the mixture to facilitate the dispersion of the drug particles.

The particles can also be coated with one or more modified release coatings. Solid esters of fatty acids, which are hydrolyzed by lipases, can be spray coated onto microparticles or drug particles. Zein is an example of a naturally water-insoluble protein. It can be coated onto drug containing microparticles or drug particles by spray coating or by wet granulation techniques. In addition to naturally water-insoluble materials, some substrates of digestive enzymes can be treated with cross-linking procedures, resulting in the formation of non-soluble networks. Many methods of cross-linking proteins, initiated by both chemical and physical means, have been reported. One of the most common methods to obtain cross-linking is the use of chemical cross-linking agents. Examples of chemical cross-linking agents include aldehydes (gluteraldehyde and formaldehyde), epoxy compounds, carbodiimides, and genipin. In addition to these cross-linking agents, oxidized and native sugars have been used to cross-link gelatin. Cross-linking can also be accomplished using enzymatic means; for example, transglutaminase has been approved as a GRAS substance for cross-linking seafood products. Finally, cross-linking can be initiated by physical means such as thermal treatment, UV irradiation and gamma irradiation.

To produce a coating layer of cross-linked protein surrounding drug containing microparticles or drug particles, a water soluble protein can be spray coated onto the microparticles and subsequently cross-linked by the one of the methods described above. Alternatively, drug containing microparticles can be microencapsulated within protein by coacervation-phase separation (for example, by the addition of salts) and subsequently cross-linked. Some suitable proteins for this purpose include gelatin, albumin, casein, and gluten.

Polysaccharides can also be cross-linked to form a water-insoluble network. For many polysaccharides, this can be accomplished by reaction with calcium salts or multivalent cations which cross-link the main polymer chains. Pectin, alginate, dextran, amylose and guar gum are subject to cross-linking in the presence of multivalent cations. Complexes between oppositely charged polysaccharides can also be formed; pectin and chitosan, for example, can be complexed via electrostatic interactions.

In certain embodiments, it may be desirable to provide continuous delivery of one or more compounds to a subject in need thereof. For intravenous or intraarterial routes, this can be accomplished using drip systems, such as by intravenous administration. For topical applications, repeated application can be done or a patch can be used to provide continuous administration of the compounds over an extended period of time.

Injectable/Implantable Solid Implants

The compounds described herein can be incorporated into injectable/implantable solid or semi-solid implants, such as polymeric implants. In one embodiment, the compounds are incorporated into a polymer that is a liquid or paste at room temperature, but upon contact with aqueous medium, such as physiological fluids, exhibits an increase in viscosity to form a semi-solid or solid material. Exemplary polymers include, but are not limited to, hydroxyalkanoic acid polyesters derived from the copolymerization of at least one unsaturated hydroxy fatty acid copolymerized with hydroxyalkanoic acids. The polymer can be melted, mixed with the active substance and cast or injection molded into a device. Such melt fabrication require polymers having a melting point that is below the temperature at which the substance to be delivered and polymer degrade or become reactive. The device can also be prepared by solvent casting where the polymer is dissolved in a solvent and the drug dissolved or dispersed in the polymer solution and the solvent is then evaporated. Solvent processes require that the polymer be soluble in organic solvents. Another method is compression molding of a mixed powder of the polymer and the drug or polymer particles loaded with the active agent.

Alternatively, the compounds can be incorporated into a polymer matrix and molded, compressed, or extruded into a device that is a solid at room temperature. For example, the compounds can be incorporated into a biodegradable polymer, such as polyanhydrides, polyhydroalkanoic acids (PHAs), PLA, PGA, PLGA, polycaprolactone, polyesters, polyamides, polyorthoesters, polyphosphazenes, proteins and polysaccharides such as

collagen, hyaluronic acid, albumin and gelatin, and combinations thereof and compressed into solid device, such as disks, or extruded into a device, such as rods.

The release of the one or more compounds from the implant can be varied by selection of the polymer, the molecular weight of the polymer, and/or modification of the polymer to increase degradation, such as the formation of pores and/or incorporation of hydrolyzable linkages. Methods for modifying the properties of biodegradable polymers to vary the release profile of the compounds from the implant are well known in the art.

Enteral Formulations

Suitable oral dosage forms include tablets, capsules, solutions, suspensions, syrups, and lozenges. Tablets can be made using compression or molding techniques well known in the art. Gelatin or non-gelatin capsules can prepared as hard or soft capsule shells, which can encapsulate liquid, solid, and semi-solid fill materials, using techniques well known in the art.

Formulations may be prepared using a pharmaceutically acceptable carrier. As generally used herein "carrier" includes, but is not limited to, diluents, preservatives, binders, lubricants, disintegrators, swelling agents, fillers, stabilizers, and combinations thereof.

Carrier also includes all components of the coating composition which may include plasticizers, pigments, colorants, stabilizing agents, and glidants. Delayed release dosage formulations may be prepared as described in standard references. These references provide information on carriers, materials, equipment and process for preparing tablets and capsules and delayed release dosage forms of tablets, capsules, and granules.

Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate and hydroxypropyl methylcellulose acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

Additionally, the coating material may contain conventional carriers such as plasticizers, pigments, colorants, glidants, stabilization agents, pore formers and surfactants.

Optional pharmaceutically acceptable excipients include, but are not limited to, diluents, binders, lubricants, disintegrants, colorants, stabilizers, and surfactants. Diluents, also referred to as "fillers," are typically necessary to increase the bulk of a solid dosage form so that a practical size is provided for compression of tablets or formation of beads and granules. Suitable diluents include, but are not limited to, dicalcium phosphate dihydrate,

calcium sulfate, lactose, sucrose, mannitol, sorbitol, cellulose, microcrystalline cellulose, kaolin, sodium chloride, dry starch, hydrolyzed starches, pregelatinized starch, silicone dioxide, titanium oxide, magnesium aluminum silicate and powdered sugar.

Binders are used to impart cohesive qualities to a solid dosage formulation, and thus ensure that a tablet or bead or granule remains intact after the formation of the dosage forms. Suitable binder materials include, but are not limited to, starch, pregelatinized starch, gelatin, sugars (including sucrose, glucose, dextrose, lactose and sorbitol), polyethylene glycol, waxes, natural and synthetic gums such as acacia, tragacanth, sodium alginate, cellulose, including hydroxypropylmethylcellulose, hydroxypropylcellulose, ethylcellulose, and veegum, and synthetic polymers such as acrylic acid and methacrylic acid copolymers, methacrylic acid copolymers, methyl methacrylate copolymers, aminoalkyl methacrylate copolymers, polyacrylic acid/polymethacrylic acid and polyvinylpyrrolidone.

Lubricants are used to facilitate tablet manufacture. Examples of suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, glycerol behenate, polyethylene glycol, talc, and mineral oil.

Disintegrants are used to facilitate dosage form disintegration or "breakup" after administration, and generally include, but are not limited to, starch, sodium starch glycolate, sodium carboxymethyl starch, sodium carboxymethylcellulose, hydroxypropyl cellulose, pregelatinized starch, clays, cellulose, alginine, gums or cross linked polymers, such as cross-linked PVP (Polyplasdone® XL from GAF Chemical Corp).

Stabilizers are used to inhibit or retard drug decomposition reactions which include, by way of example, oxidative reactions. Suitable stabilizers include, but are not limited to, antioxidants, butylated hydroxytoluene (BHT); ascorbic acid, its salts and esters; Vitamin E, tocopherol and its salts; sulfites such as sodium metabisulphite; cysteine and its derivatives; citric acid; propyl gallate, and butylated hydroxyanisole (BHA).

Oral dosage forms, such as capsules, tablets, solutions, and suspensions, can for formulated for controlled release. For example, the one or more compounds and optional one or more additional active agents can be formulated into nanoparticles, microparticles, and combinations thereof, and encapsulated in a soft or hard gelatin or non-gelatin capsule or dispersed in a dispersing medium to form an oral suspension or syrup. The particles can be formed of the drug and a controlled release polymer or matrix. Alternatively, the drug particles can be coated with one or more controlled release coatings prior to incorporation in to the finished dosage form.

In another embodiment, the one or more compounds and optional one or more additional active agents are dispersed in a matrix material, which gels or emulsifies upon contact with an aqueous medium, such as physiological fluids. In the case of gels, the matrix swells entrapping the active agents, which are released slowly over time by diffusion and/or degradation of the matrix material. Such matrices can be formulated as tablets or as fill materials for hard and soft capsules.

In still another embodiment, the one or more compounds, and optional one or more additional active agents are formulated into a sold oral dosage form, such as a tablet or capsule, and the solid dosage form is coated with one or more controlled release coatings, such as a delayed release coatings or extended release coatings. The coating or coatings may also contain the compounds and/or additional active agents.

Extended release dosage forms

The extended release formulations are generally prepared as diffusion or osmotic systems, which are known in the art. A diffusion system typically consists of two types of devices, a reservoir and a matrix, and is well known and described in the art. The matrix devices are generally prepared by compressing the drug with a slowly dissolving polymer carrier into a tablet form. The three major types of materials used in the preparation of matrix devices are insoluble plastics, hydrophilic polymers, and fatty compounds. Plastic matrices include, but are not limited to, methyl acrylate-methyl methacrylate, polyvinyl chloride, and polyethylene. Hydrophilic polymers include, but are not limited to, cellulosic polymers such as methyl and ethyl cellulose, hydroxyalkylcelluloses such as hydroxypropyl-cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and Carbopol® 934, polyethylene oxides and mixtures thereof. Fatty compounds include, but are not limited to, various waxes such as carnauba wax and glyceryl tristearate and wax-type substances including hydrogenated castor oil or hydrogenated vegetable oil, or mixtures thereof.

In certain preferred embodiments, the plastic material is a pharmaceutically acceptable acrylic polymer, including but not limited to, acrylic acid and methacrylic acid copolymers, methyl methacrylate, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), methacrylic acid alkylamine copolymer poly(methyl methacrylate), poly(methacrylic acid)(anhydride), polymethacrylate, polyacrylamide, poly(methacrylic acid anhydride), and glycidyl methacrylate copolymers.

In certain preferred embodiments, the acrylic polymer is comprised of one or more ammonio methacrylate copolymers. Ammonio methacrylate copolymers are well known in the art, and are described in NF XVII as fully polymerized copolymers of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups.

In one preferred embodiment, the acrylic polymer is an acrylic resin lacquer such as that which is commercially available from Rohm Pharma under the tradename Eudragit®. In further preferred embodiments, the acrylic polymer comprises a mixture of two acrylic resin lacquers commercially available from Rohm Pharma under the tradenames Eudragit® RL30D and Eudragit ® RS30D, respectively. Eudragit® RL30D and Eudragit® RS30D are copolymers of acrylic and methacrylic esters with a low content of quaternary ammonium groups, the molar ratio of ammonium groups to the remaining neutral (meth)acrylic esters being 1:20 in Eudragit® RL30D and 1:40 in Eudragit® RS30D. The mean molecular weight is about 150,000. Edragit® S-100 and Eudragit® L-100 are also preferred. The code designations RL (high permeability) and RS (low permeability) refer to the permeability properties of these agents. Eudragit® RL/RS mixtures are insoluble in water and in digestive fluids. However, multiparticulate systems formed to include the same are swellable and permeable in aqueous solutions and digestive fluids.

The polymers described above such as Eudragit® RL/RS may be mixed together in any desired ratio in order to ultimately obtain a sustained-release formulation having a desirable dissolution profile. Desirable sustained-release multiparticulate systems may be obtained, for instance, from 100% Eudragit® RL, 50% Eudragit® RL and 50% Eudragit® RS, and 10% Eudragit® RL and 90% Eudragit® RS. One skilled in the art will recognize that other acrylic polymers may also be used, such as, for example, Eudragit® L.

Alternatively, extended release formulations can be prepared using osmotic systems or by applying a semi-permeable coating to the dosage form. In the latter case, the desired drug release profile can be achieved by combining low permeable and high permeable coating materials in suitable proportion.

The devices with different drug release mechanisms described above can be combined in a final dosage form comprising single or multiple units. Examples of multiple units include, but are not limited to, multilayer tablets and capsules containing tablets, beads, or granules. An immediate release portion can be added to the extended release system by means of either applying an immediate release layer on top of the extended release core using a coating or compression process or in a multiple unit system such as a capsule containing extended and immediate release beads.

Extended release tablets containing hydrophilic polymers are prepared by techniques commonly known in the art such as direct compression, wet granulation, or dry granulation. Their formulations usually incorporate polymers, diluents, binders, and lubricants as well as the active pharmaceutical ingredient. The usual diluents include inert powdered substances such as starches, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders. Typical diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Typical tablet binders include substances such as starch, gelatin and sugars such as lactose, fructose, and glucose. Natural and synthetic gums, including acacia, alginates, methylcellulose, and polyvinylpyrrolidone can also be used. Polyethylene glycol, hydrophilic polymers, ethylcellulose and waxes can also serve as binders. A lubricant is necessary in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant is chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

Extended release tablets containing wax materials are generally prepared using methods known in the art such as a direct blend method, a congealing method, and an aqueous dispersion method. In the congealing method, the drug is mixed with a wax material and either spray- congealed or congealed and screened and processed.

Delayed release dosage forms

Delayed release formulations can be created by coating a solid dosage form with a polymer film, which is insoluble in the acidic environment of the stomach, and soluble in the neutral environment of the small intestine.

The delayed release dosage units can be prepared, for example, by coating a drug or a drug-containing composition with a selected coating material. The drug-containing composition may be, e.g., a tablet for incorporation into a capsule, a tablet for use as an inner core in a "coated core" dosage form, or a plurality of drug-containing beads, particles or granules, for incorporation into either a tablet or capsule. Preferred coating materials include bioerodible, gradually hydrolyzable, gradually water-soluble, and/or enzymatically degradable polymers, and may be conventional "enteric" polymers. Enteric polymers, as will be appreciated by those skilled in the art, become soluble in the higher pH environment of the lower gastrointestinal tract or slowly erode as the dosage form passes through the gastrointestinal tract, while enzymatically degradable polymers are degraded by bacterial

enzymes present in the lower gastrointestinal tract, particularly in the colon. Suitable coating materials for effecting delayed release include, but are not limited to, cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxymethyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl methyl cellulose acetate succinate, hydroxypropylmethyl cellulose phthalate, methylcellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate and carboxymethylcellulose sodium; acrylic acid polymers and copolymers, preferably formed from acrylic acid, methacrylic acid, methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate, and other methacrylic resins that are commercially available under the tradename Eudragit® (Rohm Pharma; Westerstadt, Germany), including Eudragit® L30D-55 and L100-55 (soluble at pH 5.5 and above), Eudragit® L-100 (soluble at pH 6.0 and above), Eudragit® S (soluble at pH 7.0 and above, as a result of a higher degree of esterification), and Eudragits® NE, RL and RS (water-insoluble polymers having different degrees of permeability and expandability); vinyl polymers and copolymers such as polyvinyl pyrrolidone, vinyl acetate, vinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymer; enzymatically degradable polymers such as azo polymers, pectin, chitosan, amylose and guar gum; zein and shellac. Combinations of different coating materials may also be used. Multi-layer coatings using different polymers may also be applied.

The preferred coating weights for particular coating materials may be readily determined by those skilled in the art by evaluating individual release profiles for tablets, beads and granules prepared with different quantities of various coating materials. It is the combination of materials, method and form of application that produce the desired release characteristics, which one can determine only from the clinical studies.

The coating composition may include conventional additives, such as plasticizers, pigments, colorants, stabilizing agents, glidants, etc. A plasticizer is normally present to reduce the fragility of the coating, and will generally represent about 10 wt. % to 50 wt. % relative to the dry weight of the polymer. Examples of typical plasticizers include polyethylene glycol, propylene glycol, triacetin, dimethyl phthalate, diethyl phthalate, dibutyl phthalate, dibutyl sebacate, triethyl citrate, tributyl citrate, triethyl acetyl citrate, castor oil and acetylated monoglycerides. A stabilizing agent is preferably used to stabilize particles in the dispersion. Typical stabilizing agents are nonionic emulsifiers such as sorbitan esters, polysorbates and polyvinylpyrrolidone. Glidants are recommended to reduce sticking effects during film formation and drying, and will generally represent approximately 25 wt. % to 100 wt. % of the polymer weight in the coating solution. One effective glidant is talc. Other

glidants such as magnesium stearate and glycerol monostearates may also be used. Pigments such as titanium dioxide may also be used. Small quantities of an anti-foaming agent, such as a silicone (e.g., simethicone), may also be added to the coating composition.

Topical Formulations

Suitable dosage forms for topical administration include creams, ointments, salves, sprays, gels, lotions, emulsions, and transdermal patches. The formulation may be formulated for transmucosal, transepithelial, transendothelial, or transdermal administration. The compositions may further contain one or more chemical penetration enhancers, membrane permeability agents, membrane transport agents, emollients, surfactants, stabilizers, and combination thereof.

"Emollients" are an externally applied agent that softens or soothes skin and are generally known in the art and listed in compendia, such as the "Handbook of Pharmaceutical Excipients", 4th Ed., Pharmaceutical Press, 2003. These include, without limitation, almond oil, castor oil, ceratonia extract, cetostearoyl alcohol, cetyl alcohol, cetyl esters wax, cholesterol, cottonseed oil, cyclomethicone, ethylene glycol palmitostearate, glycerin, glycerin monostearate, glyceryl monooleate, isopropyl myristate, isopropyl palmitate, lanolin, lecithin, light mineral oil, medium-chain triglycerides, mineral oil and lanolin alcohols, petrolatum, petrolatum and lanolin alcohols, soybean oil, starch, stearyl alcohol, sunflower oil, xylitol and combinations thereof. In one embodiment, the emollients are ethylhexylstearate and ethylhexyl palmitate.

"Surfactants" are surface-active agents that lower surface tension and thereby increase the emulsifying, foaming, dispersing, spreading and wetting properties of a product. Suitable non-ionic surfactants include emulsifying wax, glyceryl monooleate, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polysorbate, sorbitan esters, benzyl alcohol, benzyl benzoate, cyclodextrins, glycerin monostearate, poloxamer, povidone and combinations thereof. In one embodiment, the non-ionic surfactant is stearyl alcohol.

"Emulsifiers" are surface active substances which promote the suspension of one liquid in another and promote the formation of a stable mixture, or emulsion, of oil and water. Common emulsifiers are: metallic soaps, certain animal and vegetable oils, and various polar compounds. Suitable emulsifiers include acacia, anionic emulsifying wax, calcium stearate, carbomers, cetostearyl alcohol, cetyl alcohol, cholesterol, diethanolamine, ethylene glycol palmitostearate, glycerin monostearate, glyceryl monooleate, hydroxpropyl cellulose, hypromellose, lanolin, hydrous, lanolin alcohols, lecithin, medium-chain triglycerides,

methylcellulose, mineral oil and lanolin alcohols, monobasic sodium phosphate, monoethanolamine, nonionic emulsifying wax, oleic acid, poloxamer, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene stearates, propylene glycol alginate, self-emulsifying glyceryl monostearate, sodium citrate dehydrate, sodium lauryl sulfate, sorbitan esters, stearic acid, sunflower oil, tragacanth, triethanolamine, xanthan gum and combinations thereof. In one embodiment, the emulsifier is glycerol stearate.

Suitable classes of penetration enhancers are known in the art and include, but are not limited to, fatty alcohols, fatty acid esters, fatty acids, fatty alcohol ethers, amino acids, phospholipids, lecithins, cholate salts, enzymes, amines and amides, complexing agents (liposomes, cyclodextrins, modified celluloses, and diimides), macrocyclics, such as macrocylic lactones, ketones, and anhydrides and cyclic ureas, surfactants, N-methyl pyrrolidones and derivatives thereof, DMSO and related compounds, ionic compounds, azone and related compounds, and solvents, such as alcohols, ketones, amides, polyols (e.g., glycols). Examples of these classes are known in the art.

Lotions, creams, gels, ointments, emulsions, and foams

"Hydrophilic" as used herein refers to substances that have strongly polar groups that readily interact with water.

"Lipophilic" refers to compounds having an affinity for lipids.

"Amphiphilic" refers to a molecule combining hydrophilic and lipophilic (hydrophobic) properties

"Hydrophobic" as used herein refers to substances that lack an affinity for water; tending to repel and not absorb water as well as not dissolve in or mix with water.

A "gel" is a colloid in which the dispersed phase has combined with the continuous phase to produce a semisolid material, such as jelly.

An "oil" is a composition containing at least 95% wt of a lipophilic substance. Examples of lipophilic substances include but are not limited to naturally occurring and synthetic oils, fats, fatty acids, lecithins, triglycerides and combinations thereof.

A "continuous phase" refers to the liquid in which solids are suspended or droplets of another liquid are dispersed, and is sometimes called the external phase. This also refers to the fluid phase of a colloid within which solid or fluid particles are distributed. If the continuous phase is water (or another hydrophilic solvent), water-soluble or hydrophilic drugs will dissolve in the continuous phase (as opposed to being dispersed). In a multiphase

formulation (e.g., an emulsion), the discreet phase is suspended or dispersed in the continuous phase.

An "emulsion" is a composition containing a mixture of non-miscible components homogenously blended together. In particular embodiments, the non-miscible components include a lipophilic component and an aqueous component. An emulsion is a preparation of one liquid distributed in small globules throughout the body of a second liquid. The dispersed liquid is the discontinuous phase, and the dispersion medium is the continuous phase. When oil is the dispersed liquid and an aqueous solution is the continuous phase, it is known as an oil-in-water emulsion, whereas when water or aqueous solution is the dispersed phase and oil or oleaginous substance is the continuous phase, it is known as a water-in-oil emulsion. Either or both of the oil phase and the aqueous phase may contain one or more surfactants, emulsifiers, emulsion stabilizers, buffers, and other excipients. Preferred excipients include surfactants, especially non-ionic surfactants; emulsifying agents, especially emulsifying waxes; and liquid non-volatile non-aqueous materials, particularly glycols such as propylene glycol. The oil phase may contain other oily pharmaceutically approved excipients. For example, materials such as hydroxylated castor oil or sesame oil may be used in the oil phase as surfactants or emulsifiers.

An emulsion is a preparation of one liquid distributed in small globules throughout the body of a second liquid. The dispersed liquid is the discontinuous phase, and the dispersion medium is the continuous phase. When oil is the dispersed liquid and an aqueous solution is the continuous phase, it is known as an oil-in-water emulsion, whereas when water or aqueous solution is the dispersed phase and oil or oleaginous substance is the continuous phase, it is known as a water-in-oil emulsion. The oil phase may consist at least in part of a propellant, such as an HFA propellant. Either or both of the oil phase and the aqueous phase may contain one or more surfactants, emulsifiers, emulsion stabilizers, buffers, and other excipients. Preferred excipients include surfactants, especially non-ionic surfactants; emulsifying agents, especially emulsifying waxes; and liquid non-volatile non-aqueous materials, particularly glycols such as propylene glycol. The oil phase may contain other oily pharmaceutically approved excipients. For example, materials such as hydroxylated castor oil or sesame oil may be used in the oil phase as surfactants or emulsifiers.

A sub-set of emulsions are the self-emulsifying systems. These drug delivery systems are typically capsules (hard shell or soft shell) comprised of the drug dispersed or dissolved in a mixture of surfactant(s) and lipophilic liquids such as oils or other water immiscible liquids. When the capsule is exposed to an aqueous environment and the outer gelatin shell

dissolves, contact between the aqueous medium and the capsule contents instantly generates very small emulsion droplets. These typically are in the size range of micelles or nanoparticles. No mixing force is required to generate the emulsion as is typically the case in emulsion formulation processes.

A "lotion" is a low- to medium-viscosity liquid formulation. A lotion can contain finely powdered substances that are in soluble in the dispersion medium through the use of suspending agents and dispersing agents. Alternatively, lotions can have as the dispersed phase liquid substances that are immiscible with the vehicle and are usually dispersed by means of emulsifying agents or other suitable stabilizers. In one embodiment, the lotion is in the form of an emulsion having a viscosity of between 100 and 1000 centistokes. The fluidity of lotions permits rapid and uniform application over a wide surface area. Lotions are typically intended to dry on the skin leaving a thin coat of their medicinal components on the skin's surface.

A "cream" is a viscous liquid or semi-solid emulsion of either the "oil-in-water" or "water-in-oil type". Creams may contain emulsifying agents and/or other stabilizing agents. In one embodiment, the formulation is in the form of a cream having a viscosity of greater than 1000 centistokes, typically in the range of 20,000-50,000 centistokes. Creams are often time preferred over ointments as they are generally easier to spread and easier to remove.

The difference between a cream and a lotion is the viscosity, which is dependent on the amount/use of various oils and the percentage of water used to prepare the formulations. Creams are typically thicker than lotions, may have various uses and often one uses more varied oils/butters, depending upon the desired effect upon the skin. In a cream formulation, the water-base percentage is about 60-75 % and the oil-base is about 20-30 % of the total, with the other percentages being the emulsifier agent, preservatives and additives for a total of 100 %.

An "ointment" is a semisolid preparation containing an ointment base and optionally one or more active agents. Examples of suitable ointment bases include hydrocarbon bases (e.g., petrolatum, white petrolatum, yellow ointment, and mineral oil); absorption bases (hydrophilic petrolatum, anhydrous lanolin, lanolin, and cold cream); water-removable bases (e.g., hydrophilic ointment), and water-soluble bases (e.g., polyethylene glycol ointments). Pastes typically differ from ointments in that they contain a larger percentage of solids. Pastes are typically more absorptive and less greasy that ointments prepared with the same components.

A "gel" is a semisolid system containing dispersions of small or large molecules in a liquid vehicle that is rendered semisolid by the action of a thickening agent or polymeric material dissolved or suspended in the liquid vehicle. The liquid may include a lipophilic component, an aqueous component or both. Some emulsions may be gels or otherwise include a gel component. Some gels, however, are not emulsions because they do not contain a homogenized blend of immiscible components. Suitable gelling agents include, but are not limited to, modified celluloses, such as hydroxypropyl cellulose and hydroxyethyl cellulose; Carbopol homopolymers and copolymers; and combinations thereof. Suitable solvents in the liquid vehicle include, but are not limited to, diglycol monoethyl ether; alklene glycols, such as propylene glycol; dimethyl isosorbide; alcohols, such as isopropyl alcohol and ethanol. The solvents are typically selected for their ability to dissolve the drug. Other additives, which improve the skin feel and/or emolliency of the formulation, may also be incorporated. Examples of such additives include, but are not limited, isopropyl myristate, ethyl acetate, C12-C15 alkyl benzoates, mineral oil, squalane, cyclomethicone, capric/caprylic triglycerides, and combinations thereof.

Foams consist of an emulsion in combination with a gaseous propellant. The gaseous propellant consists primarily of hydrofluoroalkanes (HFAs). Suitable propellants include HFAs such as 1,1,1,2-tetrafluoroethane (HFA 134a) and 1,1,1,2,3,3,3-heptafluoropropane (HFA 227), but mixtures and admixtures of these and other HFAs that are currently approved or may become approved for medical use are suitable. The propellants preferably are not hydrocarbon propellant gases which can produce flammable or explosive vapors during spraying. Furthermore, the compositions preferably contain no volatile alcohols, which can produce flammable or explosive vapors during use.

Buffers are used to control pH of a composition. Preferably, the buffers buffer the composition from a pH of about 4 to a pH of about 7.5, more preferably from a pH of about 4 to a pH of about 7, and most preferably from a pH of about 5 to a pH of about 7. In a preferred embodiment, the buffer is triethanolamine.

Preservatives can be used to prevent the growth of fungi and microorganisms. Suitable antifungal and antimicrobial agents include, but are not limited to, benzoic acid, butylparaben, ethyl paraben, methyl paraben, propylparaben, sodium benzoate, sodium propionate, benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, and thimerosal.

In certain embodiments, it may be desirable to provide continuous delivery of one or more compounds to a subject in need thereof. For topical applications, repeated application

can be done or a patch can be used to provide continuous administration of the compounds over an extended period of time.

Pulmonary Formulations

In one embodiment, the compounds are formulated for pulmonary delivery, such as intranasal administration or oral inhalation. The respiratory tract is the structure involved in the exchange of gases between the atmosphere and the blood stream. The lungs are branching structures ultimately ending with the alveoli where the exchange of gases occurs. The alveolar surface area is the largest in the respiratory system and is where drug absorbtion occurs. The alveoli are covered by a thin epithelium without cilia or a mucus blanket and secrete surfactant phospholipids.

The respiratory tract encompasses the upper airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by bifurcations into the bronchi and bronchioli. The upper and lower airways are called the conducting airways. The terminal bronchioli then divide into respiratory bronchioli which then lead to the ultimate respiratory zone, the alveoli, or deep lung. The deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic drug delivery.

Pulmonary administration of therapeutic compositions comprised of low molecular weight drugs has been observed, for example, beta-androgenic antagonists to treat asthma. Other therapeutic agents that are active in the lungs have been administered systemically and targeted via pulmonary absorption. Nasal delivery is considered to be a promising technique for administration of therapeutics for the following reasons: the nose has a large surface area available for drug absorption due to the coverage of the epithelial surface by numerous microvilli, the subepithelial layer is highly vascularized, the venous blood from the nose passes directly into the systemic circulation and therefore avoids the loss of drug by first-pass metabolism in the liver, it offers lower doses, more rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, fewer side effects, high total blood flow per cm3, porous endothelial basement membrane, and it is easily accessible.

The term aerosol as used herein refers to any preparation of a fine mist of particles, which can be in solution or a suspension, whether or not it is produced using a propellant. Aerosols can be produced using standard techniques, such as ultrasonication or high pressure treatment.

Carriers for pulmonary formulations can be divided into those for dry powder formulations and for administration as solutions. Aerosols for the delivery of therapeutic

agents to the respiratory tract are known in the art. For administration via the upper respiratory tract, the formulation can be formulated into a solution, e.g., water or isotonic saline, buffered or unbuffered, or as a suspension, for intranasal administration as drops or as a spray. Preferably, such solutions or suspensions are isotonic relative to nasal secretions and of about the same pH, ranging e.g., from about pH 4.0 to about pH 7.4 or, from pH 6.0 to pH 7.0. Buffers should be physiologically compatible and include, simply by way of example, phosphate buffers. For example, a representative nasal decongestant is described as being buffered to a pH of about 6.2. One skilled in the art can readily determine a suitable saline content and pH for an innocuous aqueous solution for nasal and/or upper respiratory administration.

Preferably, the aqueous solutions is water, physiologically acceptable aqueous solutions containing salts and/or buffers, such as phosphate buffered saline (PBS), or any other aqueous solution acceptable for administration to a animal or human. Such solutions are well known to a person skilled in the art and include, but are not limited to, distilled water, de-ionized water, pure or ultrapure water, saline, phosphate-buffered saline (PBS). Other suitable aqueous vehicles include, but are not limited to, Ringer's solution and isotonic sodium chloride. Aqueous suspensions may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

In another embodiment, solvents that are low toxicity organic (i.e. nonaqueous) class 3 residual solvents, such as ethanol, acetone, ethyl acetate, tetrahydofuran, ethyl ether, and propanol may be used for the formulations. The solvent is selected based on its ability to readily aerosolize the formulation. The solvent should not detrimentally react with the compounds. An appropriate solvent should be used that dissolves the compounds or forms a suspension of the compounds. The solvent should be sufficiently volatile to enable formation of an aerosol of the solution or suspension. Additional solvents or aerosolizing agents, such as freons, can be added as desired to increase the volatility of the solution or suspension.

In one embodiment, compositions may contain minor amounts of polymers, surfactants, or other excipients well known to those of the art. In this context, "minor amounts" means no excipients are present that might affect or mediate uptake of the compounds in the lungs and that the excipients that are present are present in amount that do not adversely affect uptake of compounds in the lungs.

Dry lipid powders can be directly dispersed in ethanol because of their hydrophobic character. For lipids stored in organic solvents such as chloroform, the desired quantity of solution is placed in a vial, and the chloroform is evaporated under a stream of nitrogen to form a dry thin film on the surface of a glass vial. The film swells easily when reconstituted with ethanol. To fully disperse the lipid molecules in the organic solvent, the suspension is sonicated. Nonaqueous suspensions of lipids can also be prepared in absolute ethanol using a reusable PARI LC Jet+ nebulizer (PARI Respiratory Equipment, Monterey, CA).

Dry powder formulations ("DPFs") with large particle size have improved flowability characteristics, such as less aggregation, easier aerosolization, and potentially less phagocytosis. Dry powder aerosols for inhalation therapy are generally produced with mean diameters primarily in the range of less than 5 microns, although a preferred range is between one and ten microns in aerodynamic diameter. Large "carrier" particles (containing no drug) have been co-delivered with therapeutic aerosols to aid in achieving efficient aerosolization among other possible benefits.

Polymeric particles may be prepared using single and double emulsion solvent evaporation, spray drying, solvent extraction, solvent evaporation, phase separation, simple and complex coacervation, interfacial polymerization, and other methods well known to those of ordinary skill in the art. Particles may be made using methods for making microspheres or microcapsules known in the art. The preferred methods of manufacture are by spray drying and freeze drying, which entails using a solution containing the surfactant, spraying to form droplets of the desired size, and removing the solvent.

The particles may be fabricated with the appropriate material, surface roughness, diameter and tap density for localized delivery to selected regions of the respiratory tract such as the deep lung or upper airways. For example, higher density or larger particles may be used for upper airway delivery. Similarly, a mixture of different sized particles, provided with the same or different EGS may be administered to target different regions of the lung in one administration.

Formulations for pulmonary delivery include unilamellar phospholipid vesicles, liposomes, or lipoprotein particles. Formulations and methods of making such formulations containing drugs are well known to one of ordinary skill in the art. Liposomes are formed from commercially available phospholipids supplied by a variety of vendors including Avanti Polar Lipids, Inc. (Birmingham, Ala.). In one embodiment, the liposome can include a ligand molecule specific for a receptor on the surface of the target cell to direct the liposome to the target cell.

EXAMPLES

The present invention will be more specifically illustrated by the following Examples. However, it should be understood that the present invention is not limited by these examples in any manner.

Example 1: *dag1* zebrafish: a model organism for dystroglycanopathies that displays a muscle phenotype.

An allele of dystroglycan deficiency in zebrafish that is associated with a DAG1 point mutation (c.1700T>A) was identified, resulting in a missense change p.V567D. Using Western blot analysis, this dystroglycan null fish was shown to lack alpha- and beta-dystroglycan expression, thus producing no dystroglycan protein (Fig. 1I).

The *dag1* mutant had a phenotype with substantially reduced mobility, and decreased survival (Figure 2A). Abnormal birefringence of muscle was observed and analyzed by placing anesthetized embryos on a glass-polarizing filter and subsequently covering them with a second polarizing filter. This analysis revealed structural defects that manifest as reduced birefringence at 4-5 dpf, and markedly decreased survival (Figure 1E,F; Figure 2A).

These results demonstrated that *dag1* zebrafish are an excellent model organism for the study and development of dystroglycanopathy treatments.

Example 2: Identification of compounds that rescue the *dag1* phenotype.

The Prestwick Library, a commercially available collection of 1120 compounds (many of which are FDA-approved drugs approved for human use), was used to screen for compounds capable of rescuing *dag1* phenotypes (e.g., by restoring the muscle phenotype as determined by birefringence). The screen revealed 11 compounds that rescued the *dag1* mutant phenotype as determined by birefringence, 5 of which affect the GABA pathway: ethosuximide, cyanocobalamin, remoxipride, memantine, and risperidone (Figures 2-4; data not shown). These findings suggest that the absence of dystroglycan affects GABA related pathways. In inhibitory GABAergic synapses, the GABA molecules have important roles as transmitter factors that adjust the inhibitory synapse signal. GABA synthesis is regulated by glutamic acid decarboxylase (GAD), an enzyme that converts glutamic acid to GABA. These findings indicate that GABA related pathways are novel therapeutic targets. Though most

dystroglycan null fish cannot survive over 20 days, ethosuximide treatment significantly increased the survival of dystroglycan null fish in long term culture. For example, as shown in Figure 2A, ehthosuximide treatment was able to increase survival of the mutants to nearly wild type levels. The surviving fish exhibited no dystroglycan expression, but normal muscle structure (Figure 2B). Analysis of $GABA_A$ receptor alpha indicated that untreated mutant fish have abnormally high $GABA_A$ receptor alpha expression, while treatment with ethosuximide restored it to normal levels (Figure 3A). Moreover treatment with ethosuximide restored normal distribution of acetylcholine receptors in NMJ, which was abnormal in *dag1* mutants (Figure 4). Ethosuximide is an effective drug for epilepsy treatment, and is known as a T-type calcium channel blocker (Greenhill et al., Neuropharmacology. 2012;62(2):807-14; Lévi *et al., J Neurosci.* 2002;22(11):4274-85). These drugs thus represent important therapeutics for dystroglycanopathies such as FCMD, WWS and MEB, whose patients have been reported with muscle degeneration and severe brain phenotypes such as epilepsy (Yoshioka *et al., Child Neurol.* 2005;20(4):385-91; Akiyama *et al., Brain Dev.* 2006;28(8):537-40; Messina *et al., Neurology.* 2009;73(19):1599-601).

Example 3: Up-regulation of integrin expression in *dag1* null fish by ethosuximide.

The RNA expression level of integrin α7, which is the binding partner of laminin α2, was significantly up-regulated in ethosuximide treated *dag1* mutant fish at 10 dpf and 20 dpf, as determined by qRT-PCR (Figure 5A). To confirm the results of integrin α7 expression at the protein level, integrin α7 protein was examined with western blot using skeletal muscle samples of wildtype, untreated, and ethosuximide treated *dag1* fish. In ethosuximide treated fish, the protein expression level of integrin α7 was significantly up-regulated compared to wildtype and untreated dystroglycan null fish (Figure 5, B and C). These results demonstrate that integrin α7 up-regulation by ethosuximide may compensate for lack of dystroglycan expression to improve the muscle structure/phenotype of the *dag1* mutant.

EQUIVALENTS AND SCOPE

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

In the claims articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should it be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, *etc.*, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, *etc.* For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of

the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

All references cited herein, including patents, published patent applications, and publications, are incorporated by reference in their entirety.

What is claimed is:

CLAIMS

1. A method of treating a subject having or at an increased risk of having a dystroglycanopathy, the method comprising administering to a subject in need thereof a pharmaceutical composition comprising a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype.

2. The method of claim 1, wherein the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha.

3. The method of claim 1 or 2, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

4. A method of treating a subject having or at an increased risk of having a dystroglycanopathy, the method comprising administering to a subject in need thereof a pharmaceutical composition comprising a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions of the subject, wherein the compound is present in an amount effective to restore muscle function or phenotype.

5. The method of claim 4, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

6. A method of treating a subject having or at an increased risk of having a dystroglycanopathy, comprising administering to a subject in need thereof a pharmaceutical composition comprising a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

7. A pharmaceutical composition for the treatment of a dystroglycanopathy, the composition comprising a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype.

8. The composition of claim 7, wherein the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha.

9. The composition of claim 7 or 8, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

10. A pharmaceutical composition for the treatment of a dystroglycanopathy, the composition comprising a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions, wherein the compound is present in an amount effective to restore muscle function or phenotype.

11. The composition of claim 10, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

12. A pharmaceutical composition for the treatment of a dystroglycanopathy, the composition comprising a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

13. A pharmaceutical composition for use in treating a dystroglycanopathy, the composition comprising a compound that targets the GABA pathway, wherein the compound is present in an amount effective to restore muscle function or phenotype.

14. The pharmaceutical composition for use in treating a dystroglycanopathy of claim 12, wherein the compound that targets the GABA pathway restores normal expression of $GABA_A$ receptor alpha.

15. The pharmaceutical composition for use in treating a dystroglycanopathy of claim 13 or 14, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

16. A pharmaceutical composition for use in treating a dystroglycanopathy, the composition comprising a compound that restores normal acetylcholine receptor distribution in neuromuscular junctions, wherein the compound is present in an amount effective to restore muscle function or phenotype.

17. The pharmaceutical composition for use in treating a dystroglycanopathy of claim 16, wherein the compound is selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof.

18. A pharmaceutical composition for use in treating a dystroglycanopathy, the composition comprising a compound selected from the group consisting of ethosuximide, cyanocobalamin, remoxipride, memantine, risperidone, and salts and derivatives thereof, in an amount effective to treat the dystroglycanopathy.

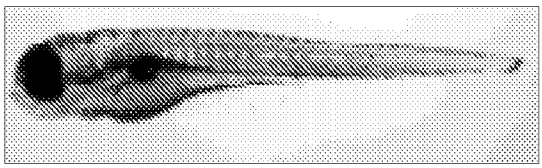


Fig. 1A

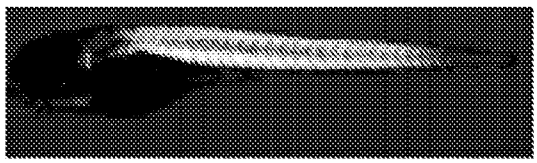


Fig. 1B

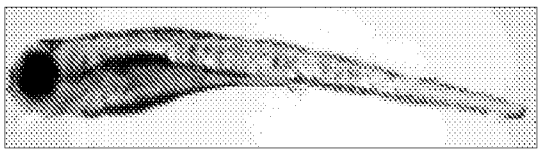


Fig. 1C

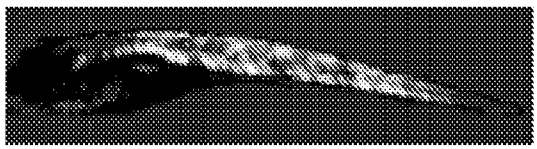


Fig. 1D

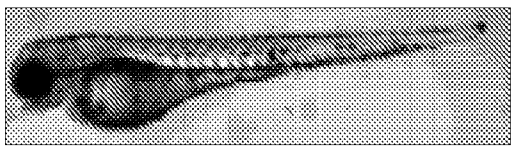


Fig. 1E

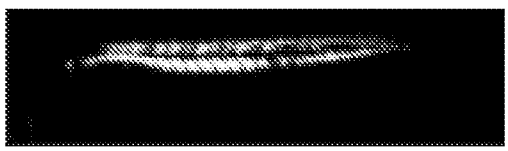


Fig. 1F

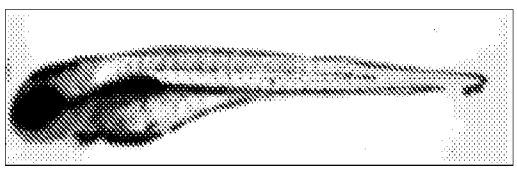


Fig. 1G

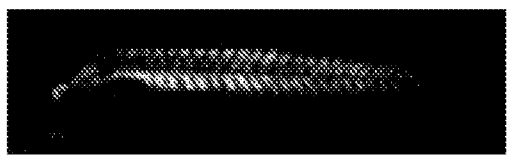


Fig. 1H

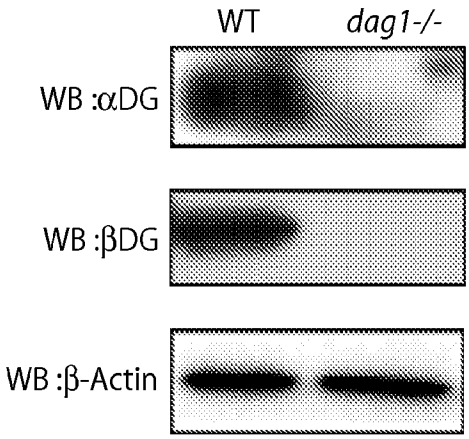
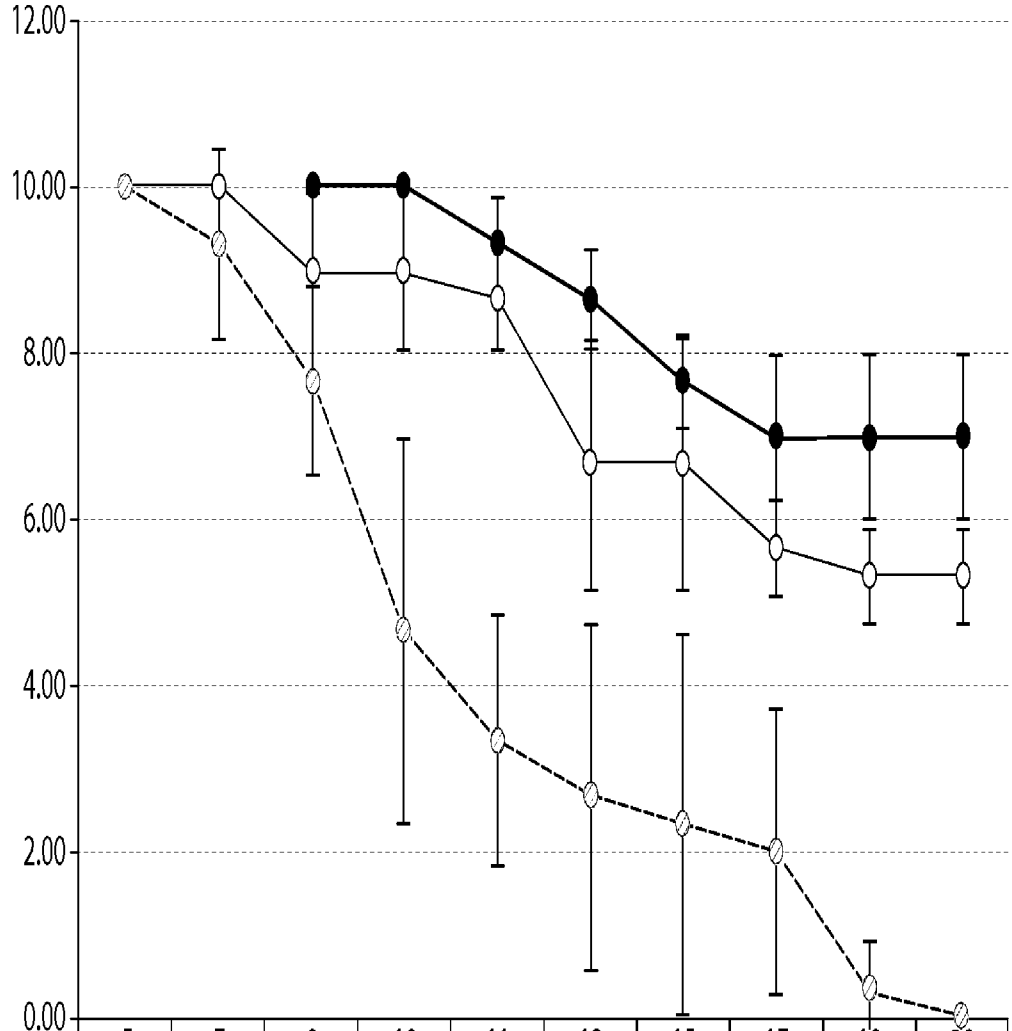


Fig. 1I



	5	7	9	10	11	13	15	17	19	20
Wildtype	10.00	10.00	10.00	10.00	9.33	8.67	7.67	7.00	7.00	7.00
Non treated Dag1 mutant	10.00	9.33	7.67	4.67	3.33	2.67	2.33	2.00	0.33	0.00
Ethosuximide-treated Dag1 mutant	10.00	10.00	9.00	9.00	8.67	6.67	6.67	5.67	5.33	5.33

Fig. 2A

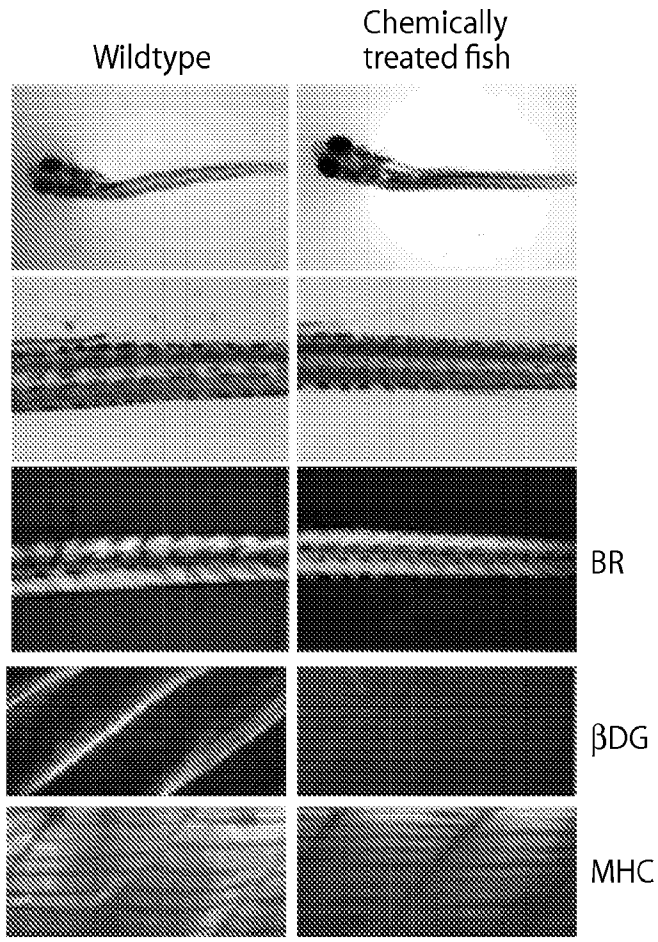


Fig. 2B

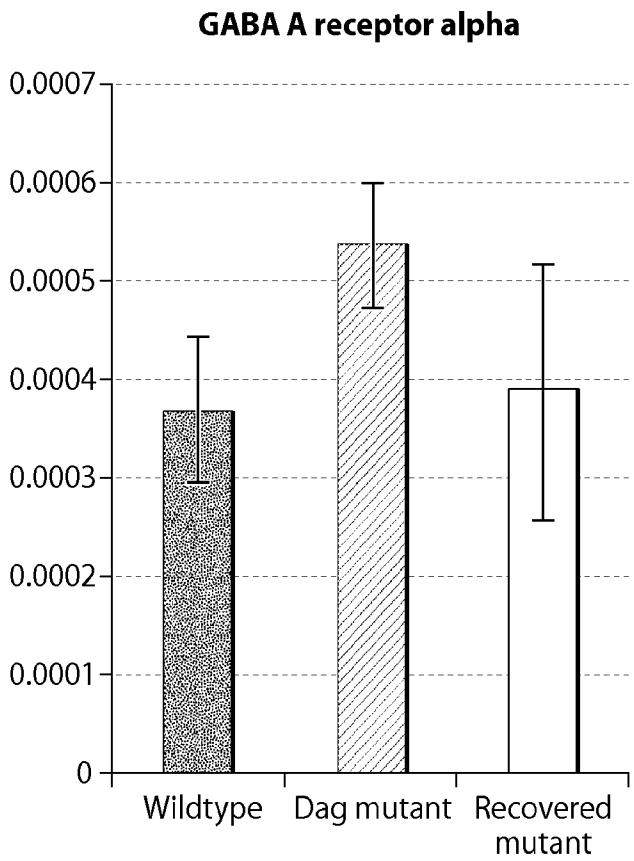

GABA A receptor alpha
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
Wildtype
Dag mutant
Recovered mutant

Fig. 3

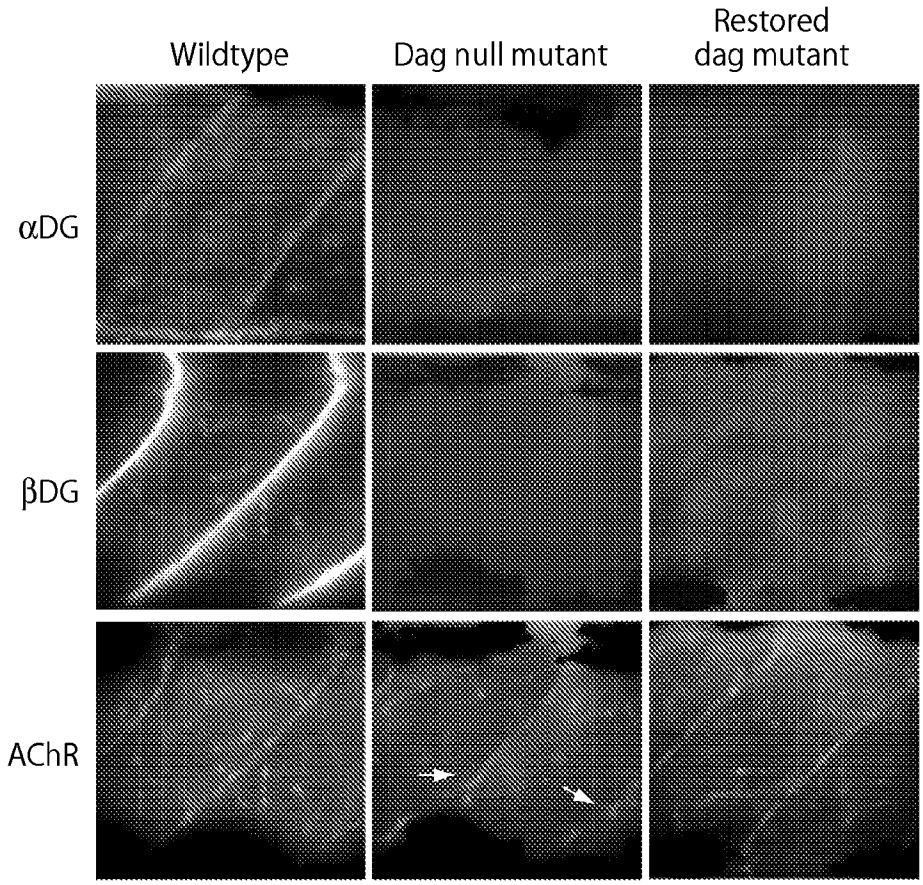


Fig. 4

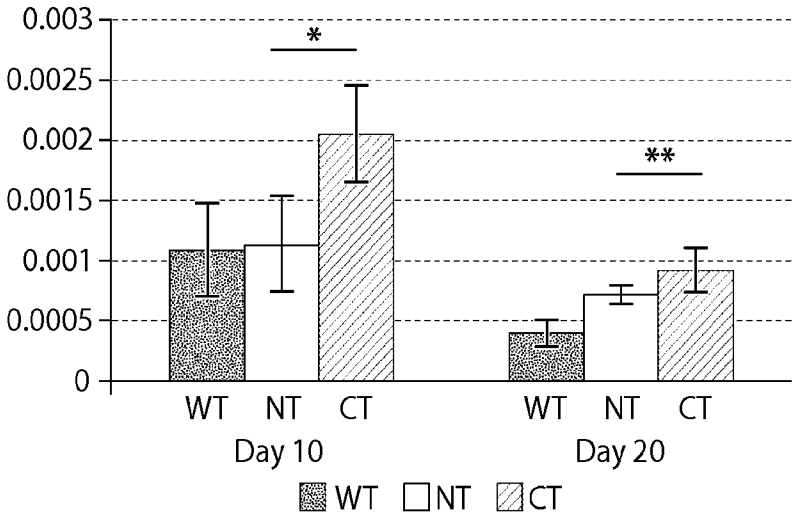


Fig. 5A

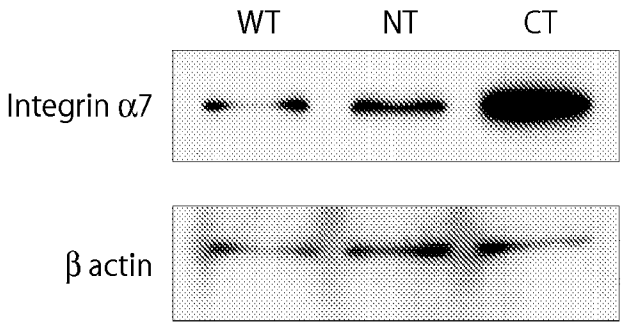


Fig. 5B

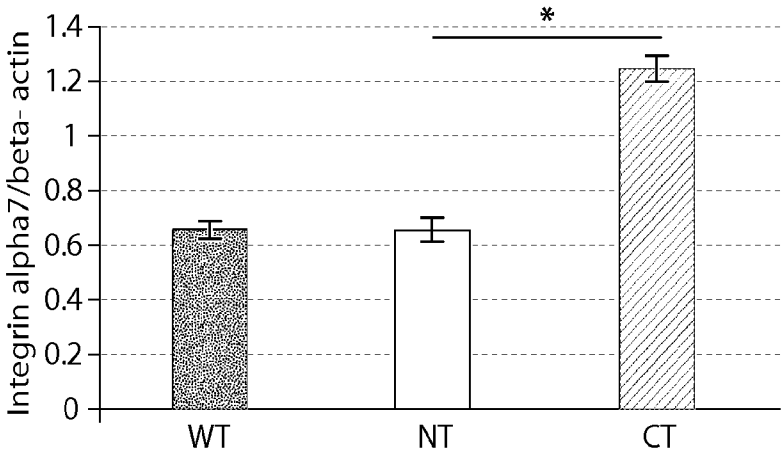


Fig. 5C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/29507

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 23/00 (2014.01)
USPC - 536/26.4; 514/52; 435/86
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 536/26.4; 514/52; 435/86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWest, ProQuest Dialog, Google Scholar, Google
Search Terms: dystroglycanopathy, muscular dystrophy, ethosuximide, Vitamin B12, cyanocobalamin, remoxipride, memantine, risperidone, muscle, phenotype, GABA pathway, GABAA receptor alpha, acetylcholine receptor distribution, neuromuscular junctions

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2008/0076829 A1 (Dalton et al.) 27 March 2008 (27.03.2008), para [0040], [0061], [0240]-[0241],	1, 3/1, 6-7, 9/7, 12-13, 15/13, 18 ---------- 2, 3/2, 4-5, 8, 9/8, 10-11, 14,15/14,16-17
Y	Kunesel et al. Short communication: altered synaptic clustering of GABAA receptors in mice lacking dystrophin (mdx mice). Eur J Neurosci. December 1999;11(12):4457-62, abstract	2, 3/2, 8, 9/8, 14, 15/14
Y	Jacobson et al. The Dystroglycan Complex Is Necessary for Stabilization of Acetylcholine Receptor Clusters at Neuromuscular Junctions and Formation of the Synaptic Basement Membrane" Jacobson et al. (hereinafter 'Jacobson'). The Journal of Cell Biology. February 5, 2001; Vol.152, Nov.3; pp435?450; pg444, col1, ln7 from bottom	4-5, 10-11, 16-17
Y	US 2006/0286167 A1 (Staunton et al.) 21 December 2006 (21.12.2006) para [0048], [0086]	1, 4, 6-7, 10, 12-13, 16
Y	WO 2012/149472 A2 (Dtephan) 1 November 2012 (01.11.2012) para [0025], [0138], [0156], [0160]	1, 4, 6-7, 10, 12-13, 16

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2014 (11.07.2014)	31 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)