TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

Present invention is directed to a novel medicament for medical treatment of motor neuron disease (NMD) such as amyotrophic lateral sclerosis. More particularly, the present invention involves the use of ivermectin and analogues, to prevent, retard and ameliorate a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS) and the associated motor neuron degeneration.
TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

Background and Summary

BACKGROUND OF THE INVENTION

A. Field of the Invention

The present invention relates generally to the use of certain compounds for treatment, prevention or retardation of a Motor Neuron Disease (MND) and more particularly of amyotrophic lateral sclerosis (ALS). It in particularly relates to such use of an ivermectin or an analogue of ivermectin from the class of the macrocyclic lactones. The invention also involves an Ivermectin or analogues of ivermectin from the class of the macrocyclic lactones for the preparation of a medicament to treat amyotrophic lateral sclerosis.

Several documents are cited throughout the text of this specification. Each of the documents herein (including any manufacturer's specifications, instructions etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention.

B. Description of the Related Art

Amyotrophic lateral sclerosis (ALS) is a late onset neurodegenerative disease caused by the degeneration of motor neurons in the motor cortex, brainstem and spinal cord. This results in a progressive muscle weakness and atrophy followed by paralysis and leading to the death of the patient within 2 to 5 years after diagnosis. The disease exists in a sporadic (90%) and a familial form (10%) with very similar pathology. A mutation in the gene coding for superoxide dismutase 1 (SOD1) causes 15-20% of familial ALS. Transgenic mice and rats overexpressing various SOD1 mutants develop a progressive motor neuron disease with pathological features reminiscent of both sporadic and familial ALS.

The selective toxicity of the mutant SOD1 for motor neurons is not fully understood and most likely involves multiple pathways including formation of protein aggregates, proteasome dysfunction, axonal strangulation, oxidative damage, mitochondrial defects, caspase activation, changes in levels of Bcl-2 family members, changes in Ca^{2+} homeostasis and

Most people with ALS die from respiratory failure, usually within 3 to 5 years from the onset of symptoms. However, about 10 percent of ALS patients survive for 10 or more years. As many as 20,000 Americans have ALS, and an estimated 5,000 people in the United States are diagnosed with the disease each year. ALS is one of the most common neuromuscular diseases worldwide. Worlwide about 400 000 people are affected by this disease and people of all races and ethnic backgrounds are affected. In 90 to 95 percent of all ALS cases, the disease occurs apparently at random with no clearly associated risk factors. Patients do not have a family history of the disease, and their family members are not considered to be at increased risk for developing ALS. ALS most commonly strikes people between 40 and 60 years of age, but younger and older people also can develop the disease. Men are affected more often than women.

Despite the tremendous impact of this fatal disorder, an efficient treatment is currently non existing. There thus is a clear need in the art to find new treatment and medicaments for ALS. Present invention and the subject matter herein claimed and disclosed have solved some of the above mentioned long-felt needs in the art since it demonstrates that a medicament comprising an ivermectin or a functional derivative thereof that can be used to decrease discomfort caused by a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS) and moreover can be used to prevent, retard and ameliorate a motor neuron disease (MND) such as
amyotrophic lateral sclerosis (ALS). In particularly it has been demonstrated that an ivermectin can extend the lifespan of a subject who is affected by ALS.

**SUMMARY OF THE INVENTION**

In accordance with the purpose of the invention, as embodied and broadly described herein, the invention is broadly drawn to a novel medicament for medical treatment of Motor Neuron Disease (MND) such as Progressive Bulbar Palsy (PBP) or such as amyotrophic lateral sclerosis (ALS), a fatal neurological disorder, characterised by progressive degeneration of the motor cells in the spinal cord and the brain.

We surprisingly found that an ivermectin has such action to retardate or heal these disorders. Healing is the process by which the cells in the body regenerate and repair to reduce the size of a damaged.

Motor Neuron Disease (MND) is a disease affecting the motor neurons in the brain and spinal cord. Motor neurons are the nerve cells along which the brain sends instructions, in the form of electrical impulses, to the muscles. There are two types of motor neurons, the upper motor neurons, which have long, thin nerve trunks connecting the brain to the spine. Within the spine, they connect with the lower motor neurons which, in turn, have nerve trunks connecting to the muscles of the body. Degeneration of the motor neurons leads to weakness and wasting of muscles. This generally occurs in arms or legs initially, some groups of muscles being affected more than others. Some people may develop weakness and wasting in the muscles supplying the face and throat, causing problems with speech and difficulty chewing and swallowing. MND does generally not affect touch, taste, sight, smell or hearing, nor directly bladder, bowel, or sexual function. In the vast majority of cases, the intellect remains unaffected. MND is generally a steadily progressive disease, but the rate of progression varies greatly from one person to another, hi most cases of MND, degeneration of both the upper and lower motor neurons occurs. This condition is called amyotrophic lateral sclerosis (ALS), characterised by muscle weakness, stiffness and fasciculations (muscle twitching) or, when the muscles involved in speech and swallowing are solely affected, Progressive Bulbar Palsy (PBP). There are also less common forms in which a more selective degeneration of either the
upper motor neurons (such as Primary Lateral Sclerosis, PLS) or lower motor neurons (such as Progressive Muscular Atrophy, PMA) is observed. There is considerable overlap between these forms of MND. People with PMA in time develop upper motor neuron involvement and in both PMA and ALS some people may eventually experience speech and swallowing difficulties in varying degrees. So far Motor Neuron Disease is a dangerous incurable disease. It causes paralysis. Motor neuron disease often begins with weakness of the muscles of the hands or feet. It eventually leads to generalised paralysis. People with motor neuron disease need help with daily activities and have a life expectancy of three to five years after their diagnosis. ALS patients manifest symptoms associated with the loss of motor neurons, and/or the nerve cells in the spinal cord, brainstem, and motor cortex, which are normally in good control of the body's voluntary muscles. In ALS, as motor neurons die, muscles weaken and shrink, and the body manifests the early-stage symptoms of ALS. Such symptoms include, for example, unusual fatigue, clumsiness, muscle weakness, slurred speech, muscle atrophy, spasticity, spinal function disorders, and convulsions. As ALS progresses, patients gradually lose the use of their hands, arms, legs, and neck muscles, ultimately becoming paralyzed. Speaking and swallowing ability are greatly compromised. Psychiatric manifestations (e.g., depression) may also result. However, while cognitive impairment is generally not observed with ALS, some data suggest that as many as 15% of all ALS patients may experience some memory loss, behavioural changes, and problems with both judgment and simultaneously performing multiple tasks. The usual cause of death from ALS is failure of the diaphragm muscles that control breathing. ALS patients can prolong their lives by using a ventilator, especially since bladder and bowel function, sexual function, and all five senses are unaffected. But living on a ventilator is neither desirable nor free of complications such as pneumonia (resulting from pooling of secretions or aspiration).

An efficient treatment of ALS was currently non-existing. Present invention and the subject matter herein claimed and disclosed have thus solved the above mentioned long-felt needs in the art since it demonstrates that a medicament comprising an ivermectin or a functional derivative thereof in particular of the microcyclic lactones can be used to decrease discomfort caused by a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS) and moreover can be used to prevent, retard and ameliorate a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS).
The active compounds employed in the formulations according to the invention are known.

Hi the mid-1970's, a survey of natural products revealed that a fermentation broth of the soil actinomycete, Streptomyces avermitilis, ameliorated infection with Nematospiroides dubius in mice. Isolation of the anthelmintic components from cultures of this organism led to discovery of the avermectins, a novel class of 16-membered lactones. Ivermectin (MECTIZAN; 22,23 dihydroavermectin B1a) is a semisynthetic analog of avermectin B1a (abamectin), an insecticide developed for crop management. More specifically, it is a mixture in the ratio of approximately 80:20 of 22, 23-dihydro C-076 B1a and B1b. It is disclosed in U.S. Pat. No. 4,199,569, issued Apr. 22, 1980 to Chabala and Fisher. Avermectins were isolated from the microorganism Streptomyces avermitilis as microbial metabolites (U.S. Pat. No. 4,310,519) and can occur essentially as a mixture consisting of the eight components A 1a, Alb, A2a, A2b, B1a, B1b, B2a, and B2b (I. Putter et al., Experentia 37 (1981) p. 963, Birkhauser Verlag (Switzerland)). In addition, the synthetic derivatives, in particular 22, 23-dihydroavermectin B1 (ivermectin), are also of interest (U.S. Pat. No. 4,199,569). Milbemycin B-41 D was isolated from Streptomyces hygroscopicus by fermentation (cf. "Milbemycin: Discovery and Development", I. Junya et al., Annu. Rep. Sankyo Res. Lab. 45 (1993), pp. 1-98; JP Pat. 8,378,549; GB 1,390,336). The use of the avermectins, e.g. 22,23-dihydroavermectins B1, (ivermectin) and milbemycins as endoparasiticides is known and is the subject of numerous patent applications and review articles (e.g. biological actions in: "Ivermectin and Abamectin", W. C. Campbell, Ed., Springer Verlag, New York, N.Y., 1989; "Avermectins and Milbemycins Part IT" H. G. Davies et al., Chem. Soc. Rev. 20 (1991) pp. 271-339; chemical modifications in: G. Lukacs et al. (Eds.), Springer Verlag, N.Y., (1990), Chapter 3; Cydectin® [moxidectin and derivatives]: G. T. Carter et al., J. Chem. Soc. Chem. Commun. (1987), pp. 402-404); EP 423 445-A1) "Doramectin - a potent novel endectocide" A. C. Goudie et al., Vet. Parasitol. 49 (1993), pp. 5-15).

Hi one aspect of the invention, the treatment of amyotrophic lateral sclerosis said method comprise administering to an individual an effective amount of the group consisting of Ivermectin, Abamectin, Doramectin, Eprinomectin, Milbemycin oxime, Moxidectin and Selamectin. These compounds can also be used in the preparation of a medicament for the prevention or treatment of motor neuron disease such as amyotrophic lateral sclerosis.
It is well understood in by the skilled man that the dose of ivermectin or its functional derivatives will be different for different patients. The time allowed between doses and the length of time to take the medicine depend on several factors related to the condition of the patient and/or the medical problem for which he/she is taking ivermectin. These compounds may be administered in a dosage of 20 to 500 microgram per kilogram (kg) of body weight, preferably in a dosage of 50 to 250 microgram per kilogram (kg) of body weight and most preferably in a dosage of 80 to 150 microgram per kilogram (kg) of body weight.

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

Detailed Description

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The following detailed description of the invention refers to the accompanying drawings. The same reference numbers in different drawings identify the same or similar elements. Also, the following detailed description does not limit the invention. Instead, the scope of the invention is defined by the appended claims and equivalents thereof.

It will be apparent to those skilled in the art that various modifications and variations can be made in delivery form and dosage form and the functional ivermectin derivatives of the present invention and in construction of the system and method without departing from the scope or spirit of the invention. Examples of such modifications have been previously provided.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and
spirit of the invention being indicated by the following claims.

The mechanism of ALS is far from understood and most likely multiple genetic and environmental factors are implicated in the pathogenesis of this disease. This is at the basis of the fact that there is currently no efficient treatment.

While several autoimmune theories have been advanced in the past decade, immunosuppressive therapy, including drug (e.g., azathioprine) treatment, plasmapheresis, or intravenous immunoglobulin injection, has been ineffective in combating ALS. Even the very potent immunosuppression method of total lymph node irradiation proved unsuccessful. Annals of Neurology, Editorial, "Amyotrophic Lateral Sclerosis: Theories and Therapies," Vol. 35, 1994, pp. 129-130. Currently, one area of ongoing investigation is the use of growth factors, such as Insulin-like growth factor 1 (IGF-I or Myotrophin®), ciliary neurotrophic factor (CNTF), and, most recently, vascular epithelial growth factor (VEGF) that have shown protection of motor neurons in animal models and cell culture systems.

The effectiveness of growth factor therapy (e.g. IGF-I), is limited by the degradation of the protein in the liver, before it crosses the blood brain barrier (BBB). Another avenue of therapy is in the regulation of brain glutamate levels, based on abnormally high glutamate concentrations found in cerebrospinal fluid of some ALS patients. An abundance of the glutamate transporters in astrocytes (cells surrounding the neurons) known as EAAT2, involved in the removal of excess glutamate, is decreased in the cortex and spinal cord of patients with ALS and in mouse models. A high glutamate level leads to "excitotoxicity" (which is neuronal death due to the overactivation of glutamate receptors), a flooding of neurons with calcium, and a host of damaging downstream events. Other studies have linked excitotoxicity to high zinc levels as a cause of motor neuron death. From these findings, a promising area of investigation is in the use of glutamate antagonists, which inhibit the release of glutamate in the brain. The working mechanism of the FDA-approved drug riluzole (Rilutek®) is thought to be based on interference with excitotoxicity and is currently the only drug of which it has been proven using double blind and controlled studies that is increases the life expectancy of ALS patients. However, the average increase in life expectancy is limited to a few months.
Other glutamate antagonists of interest include dextromethorphan and lamotrigine. As with all such ALS drugs, their potential to degrade before crossing the BBB remains a significant concern. Finally, the identification of mutations in the gene encoding for the enzyme superoxide dismutase (SOD1) in FALS has been a landmark in ALS research. SOD1 mutations are present in 15-20% of FALS cases. SOD1 converts the superoxide free radical anion (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$), which is further detoxified by enzymes such as catalase and glutathione peroxidase. The importance of SOD1 in handling free radicals during oxidative stress has been recognized for some time, leading some scientists to believe that mutant SOD1 may result in motor neuron destruction through the action of an excessive amount of free radicals ('loss of function' hypothesis). However, treatments with antioxidants alone or in combination with other drugs such as riluzole of both sporadic and familial ALS failed to prolong survival. In contrast, mutations in SOD1 and, as a result, its protein product are thought to initiate motor neuron disease through the gain of one or more toxic properties ('gain of function' hypothesis). This is consistent with observations that the inactivation of SOD1 does not cause motor neuron disease in mice, while transgenic mice that overexpress ALS-associated, mutant SOD1 develop motor neuron disease despite having normal or elevated SOD1 activity. Finally, neither age of onset nor rapidity of disease progression correlates with SOD1 activity in ALS patients. A popular hypothesis proposes that the conformational instability of mutant SOD1 induces the formation of harmful aggregates. In transgenic rodents with SOD1-mediated ALS (including copper-binding-site-null mice) and in some human ALS cases, aggregates that are immunoreactive for SOD1 are detected in motor neurons, the neuropil and astrocytes, and these transgenic mice, these aggregates become evident by the time of disease onset and increase in abundance with disease progression (for a review see Pasinelli, P. and Brown R.H. 2006. Nat. Rev. Neurosci. 7, 710-723).

particular, the higher sensitivity of motor neurons to excitotoxicity mediated by Ca\textsuperscript{2+} entry upon stimulation of AMPA receptors is considered to be of crucial importance (Van Damme, P., Dewil, M., Robberecht, W., Van Den Bosch, L. 2005. Neurodegenerative Dis. 2, 147-159.).

We now found that an ivermectin can increase the life span in a relevant transgenic mouse model affected by ALS. The study demonstrated that ivermectin induces a mechanism in motor neurons that protects a subject affected by a motor neuron disease such as ALS.

In the middle of the 1980s, ivermectin was presented as a broad-spectrum anti-parasitic medicinal product for veterinary use (W. C. CAMPBELL, et al., (1983). Ivermectin: a potent new anti-parasitic agent, Science, 221, 823-828). It is effective against most common intestinal worms (except tapeworms), most acarids and some lice. Ivermectin is a macrocyclic lacton that is efficient as a veterinary antiparasitic drug and as a successful medicine against river blindness in humans (Fisher, M.H., Mrozik, H. 1992. Annu. Rev. Pharmacol. Toxicol. 32, 537-553).

Ivermectin is generally a mixture of two compounds belonging to the avermectin class, 5-O-demethyl-22,23-dihydroavermectin Ala and 5-O-demethyl-22,23-dihydroavermectin Alb. They are also known as 22,23-dihydroavermectin B1a and 22-23-dihydroavermectin B1b. Ivermectin contains at least 80% of 22,23- dihydroavermectin B1a and less than 20% of 22,23- dihydroavermectin B1b. This active agent is part of the avermectin class, a group of macrocyclic lactones produced by the bacterium Streptomyces avermitilis (Reynolds J E F (Ed) (1993) Martindale). The extra pharmacopoeia, 29th Edition, Pharmaceutical Press, London). They consist of two sub groups, the avermectins and the milbemycins. Their basic chemical structure consists of a cyclic lactone and a spiroketal addition constructed of two 6-membered rings. The avermectins also include a sugar (disaccharide oxy) linked at position 13.
Its binding to glutamate-dependent chloride channels promotes an increase in membrane
permeability to chloride ions, resulting in hyperpolarization of cell. But several other ligand-
gated ion channels are activated and/or modulated by IVM. These include a crayfish
multiagonist-gated chloride-selective channel (Zufall, F., C. Franke, and H. Hatt. 1989. The
insecticide avermectin B1a activates a chloride channel in crayfish muscle membrane. J. Exp.
Biol. 142:191-2), human glycine receptor, the histamine receptor from fly (Shan, Q., J.L.
Haddrill, and J.W. Lynch. 2001. Ivermectin, an unconventional agonist of the glycine receptor
chloride channel. J. Biol. Chem. 276:12556-12564) and is an allosterical modulator of the
nicotinic acetylcholine receptor (Krause, R.M., Buisson, B., Bertrand, S., Corringer, P.-J.,
mammalian P2X_4 receptor (Khakh, B.S., Proctor, W.R., Dunwiddie, T.V., Labarca, C., Lester,

We have previously shown that Cl^-influx during AMPA receptor stimulation aggravated
excitotoxic motor neuron death (Van Damme, P., Callewaert, G., Eggermont, J., Robberecht, W., Van Den Bosch, L. 2003aJ. Neurosc. 23, 4942-4950.). This Cl⁻-influx was enhanced by coadministration of GABA. However GABA antagonists, in the absence of added GABA, did not have an effect on excitotoxicity in our motor neuron cultures, indicating that no endogenous GABA is present in our culture system. Therefore, it is unlikely that the ivermectin effect is mediated by allosteric modulation of the activity of GABA on the GABA<sub>A</sub> receptor. We now also found that the electrophysiological characteristics of the AMPA receptor did not change after pretreatment with ivermectin.

To investigate the relevance of the ivermectin effect on AMPA receptor-mediated excitotoxicity in the pathology of ALS, we treated SOD1(G93A)-mice with ivermectin and found a significant increase in survival of almost 10%. The protective effect of ivermectin was confirmed by histological examination of the lumbar spinal cord and lumbar ventral roots. These pathological studies clearly showed more motor neurons in the spinal cord sections and more axons in the lumbar ventral roots in 120-day-old ivermectin-treated SOD1(G93A)-mice compared to controls. Ivermectin had no effect on the characteristics of the AMPA receptor and induces a protective post-receptor mechanism. The absence of a real dose-response effect on the survival of the SOD1(G93A)-mice indicates an indirect effect of ivermectin.

The findings of present invention has now been developed into a compositions and methods of treating, preventing or retarding amyotrophic lateral sclerosis. The invention concerns a pharmaceutical compositions and methods of use thereof for the acute, chronic and prophylactic treatment of amyotrophic lateral sclerosis, and prophylaxis of amyotrophic lateral sclerosis. In spite of the seriousness of the problem, the pharmacological arsenal to fight, prevent, and/or decrease its symptoms and progress, is surprisingly limited.

**Motor Neuron Disease (MND) & amyotrophic lateral sclerosis (ALS)**

Amyotrophic lateral sclerosis (ALS) is one of several, clinically defined, motor neuron diseases (MNDs). Progressive loss of lower and upper motor neurons occurs in several diseases (e.g., primary lateral sclerosis, spinal-bulbar muscular atrophy (SBMA, or Kennedy's disease) benign focal amyotrophy). However, ALS is the most common form of motor neuron disease. Loss of both lower and upper motor neurons occurs in ALS. Symptoms include
progressive skeletal muscle wasting, weakness, fasciculations, and cramping. Some cases have predominant involvement of brainstem motor neurons (progressive bulbar palsy). Unfortunately, treatment of motor neuron and related diseases is largely supportive at this time. ALS afflicts 1.5 times more men than women. In about two thirds of cases, the onset of the disease occurs between ages 50 and 70. James T. Caroscio, et al., "Amyotrophic Lateral Sclerosis: Its Natural History," Neurologic Clinics, Vol. 5, No. 1, February 1987, pp. 1-8. Overall, ALS afflicts 5 to 10 people out of every 100,000 people. The progression of the disease is rapid. Most patients die within 5 years of onset. About 5-10% of ALS cases, known as familial ALS (FALS), are inherited. Although FALS is clinically indistinguishable from the "sporadic" form of ALS, there is no predominance of FALS in men as with sporadic ALS. However, the mean age of ALS onset is comparatively earlier. J. de Belleroche, et al., "Amyotrophic Lateral Sclerosis: Recent Advances in Understanding Disease Mechanisms," J. Neuropathol. and Exp. Neurol., Vol. 55, No. 7, July 1996, pp. 747-757. No single test can diagnose ALS. Because of the slow onset of the disease, diagnosis of ALS is usually difficult in its early stages. By the time of positive diagnosis, the disease has generally progressed for 1-2 years. The invention thus involves a novel medicament and treatment of a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS).

Between 25,000 and 30,000 Americans are thought to have amyotrophic lateral sclerosis (ALS), with an estimated prevalence of up to six cases per 100,000 of the adult population and an annual incidence of one to two cases per 100,000. The peak age of onset is between 55 and 75 years, with a male preponderance of 1.5 to 2:1. There is no cure for ALS. Instead, the management of ALS involves the continual adaptation of therapy as the disease progresses and different muscles are affected. The only drug that is currently approved by the FDA for the treatment of ALS is Aventis' Rilutek, although various other products are also used to control the symptoms of the disease.

In a preferred embodiment treatment of treating, preventing or retarding amyotrophic lateral sclerosis is carried out by administration of ivermectins, or their derivatives thereof of the Avermectins class or which are microcyclic lactones may administered orally in a regime of 5 to 1000 mg/patient/day, more preferably 20 to 500 mg/patient/day, and yet more preferably 50 to 300 mg/patient/day, and most preferably 80 to 150 mg/patient/day. A possible daily dose can for instance be 7 to 10 mg/kg body weight. The active compound may be delivered as a solid medicine in pill or tablet form and alternatively as liquid, semi-solid. The parenteral
administration form may be an isotonic injection solution. The daily dose could be 3 to 6 mg/kg body weight.

Administration of these ivermectins may be carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitory or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes.

Oral dosage forms are preferred for those therapeutic agents that are orally active, and include tablets, capsules, caplets, solutions, suspensions and/or syrups, and may also comprise a plurality of granules, beads, powders or pellets that may or may not be encapsulated. Such dosage forms are prepared using conventional methods known to those in the field of pharmaceutical formulation and described in the pertinent texts, e.g., in Remington: The Science and Practice of Pharmacy, 20th Edition, Gennaro, A.R., Ed. (Lippincott, Williams and Wilkins, 2000). Tablets and capsules represent the most convenient oral dosage forms, in which case solid pharmaceutical carriers are employed.

Tablets may be manufactured using standard tablet processing procedures and equipment. One method for forming tablets is by direct compression of a powdered, crystalline or granular composition containing the active agent(s), alone or in combination with one or more carriers, additives, or the like. As an alternative to direct compression, tablets can be prepared using wet-granulation or dry-granulation processes. Tablets may also be moulded rather than compressed, starting with a moist or otherwise tractable material; however, compression and granulation techniques are preferred.

hi addition to the active agent(s), then, tablets prepared for oral administration using the method of the invention will generally contain other materials such as binders, diluents, lubricants, disintegrants, fillers, stabilisers, surfactants, colouring agents, and the like. Binders are used to impart cohesive qualities to a tablet, and thus ensure that the tablet remains intact after compression. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinised starch), gelatine, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose,
hydroxypropyl methylcellulose, methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, and
the like), and Veegum. Diluents are typically necessary to increase bulk so that a practical size
tablet is ultimately provided.

Suitable diluents include lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch and
powdered sugar. Lubricants are used to facilitate tablet manufacture; examples of suitable
lubricants include, for example, magnesium stearate and stearic acid. Stearates, if present,
preferably represent at no more than approximately 2 wt. % of the drug-containing core.

Disintegrants are used to facilitate disintegration of the tablet, and are generally starches,
clays, celluloses, algins, gums or crosslinked polymers. Fillers include, for example, materials
such as silicon dioxide, titanium dioxide, alumina, talc, kaolin, powdered cellulose and
microcrystalline cellulose, as well as soluble materials such as mannitol, urea, sucrose,
lactose, dextrose, sodium chloride and sorbitol. Stabilisers are used to inhibit or retard drug
decomposition reactions that include, by way of example, oxidative reactions. Surfactants may
be anionic, cationic, amphoteric or non-ionic surface active agents.

The dosage form may also be a capsule, in which case the active agent-containing
composition may be encapsulated in the form of a liquid or solid (including particulates such
as granules, beads, powders or pellets). Suitable capsules may be either hard or soft, and are
generally made of gelatine, starch, or a cellulosic material, with gelatin capsules preferred.
Two-piece hard gelatine capsules are preferably sealed, such as with gelatine bands or the like.
See, for example, Remington: The Science and Practice of Pharmacy, which describes
materials and methods for preparing encapsulated pharmaceuticals. If the active agent-
containing composition is present within the capsule in liquid form, a liquid carrier is
necessary to dissolve the active agent(s). The carrier must be compatible with the capsule
material and all components of the pharmaceutical composition, and must be suitable for
ingestion.

Solid dosage forms, whether tablets, capsules, caplets, or particulates, may, if desired, be
coated so as to provide for delayed release. Dosage forms with delayed release coatings may
be manufactured using standard coating procedures and equipment. Such procedures are
known to those skilled in the art and described in the pertinent texts, e.g., in Remington,
supra. Generally, after preparation of the solid dosage form, a delayed release coating composition is applied using a coating pan, an airless spray technique, fluidised bed coating equipment, or the like. Delayed release coating compositions comprise a polymeric material, e.g., cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose- propionate phthalate, polyvinyl acetate phthalate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose, hydroxypropyl methylcellulose acetate succinate, polymers and copolymers formed from acrylic acid, methacrylic acid, and/or esters thereof.

Sustained release dosage forms provide for drug release over an extended time period, and may or may not be delayed release. Generally, as will be appreciated by those of ordinary skill in the art, sustained release dosage forms are formulated by dispersing a drug within a matrix of a gradually bioerodible (hydrolysable) material such as an, insoluble plastic, a hydrophilic polymer, or a fatty compound, or by coating a solid, drug containing dosage form with such a material. Insoluble plastic matrices may be comprised of, for example, polyvinyl chloride or polyethylene. Hydrophilic polymers useful for providing a sustained release coating or matrix cellullosic polymers include, without limitation: cellullosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropylmethyl cellulose phthalate, hydroxypropylcellulose phthalate, cellulose hexahydrophthalate, cellulose acetate hexahydrophthalate, and carboxymethylcellulose sodium; acrylic acid polymers and copolymers, preferably formed from acrylic acid, methacrylic acid, acrylic acid alkyl esters, methacrylic acid alkyl esters, and the like, e.g. copolymers of acrylic acid, methacrylic acid, methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate, with a terpolymer of ethyl acrylate, methyl methacrylate and trimethylammonioethyl methacrylate chloride (sold under the tradename Eudragit RS) preferred; vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylenevinyl acetate copolymers; zein; and shellac, ammoniated shellac, shellac-acetyl alcohol, and shellac n-butyl stearate. Fatty compounds for use as a sustained release matrix material include, but are not limited to, waxes generally (e.g., camauba wax) and glycercyl tristearate.
Alternatively transepidermal effective amounts of Ivermectins, their derivatives thereof may be administered topically on the respective areas of motor neuron loss. The transdermal administration of Ivermectins or derivatives thereof can be transdermal electromotive administration, the transdermal absorption being accelerated by use of an electrode-drug receptacle attached to the patients. For such topical treatment the pharmaceutical product can be used as liquid, semi-solid or solid medicine. Liquid medicines are solutions, suspensions, emulsions or dispersions of the above-cited active ingredients or combinations of active ingredients as drops, tinctures and sprays. As semi-solid medicines, for example, gels, ointments, creams and foams are used while, for example, powders, toilet powders, granulates, pellets and microcapsules are used as solid medicines.

If the pharmaceutical product containing as active ingredient Ivermectins or their derivatives thereof, is used as a liquid, it is recommended to use as far as possible irritation-free diluting agents, as for example water, monovalent alcohols, especially ethanol, polyvalent alcohols, especially glycerine and/or propanediol, polyglycols, especially polyethylene glycols and/or miglyols, glycerine formal, dimethylisosorbide, natural and synthetic oils and/or esters.

For the production of semi-solid products, as for example gels, ointments, creams and foams, in addition to the above-cited diluting agents basic materials, as for example bentonite, veegum, guar flour and/or cellulose derivatives, especially methylcellulose and/or carboxymethylcellulose, are suitable. The ivermectin may be in the form of a physico-chemical complex with a phospholipid selected from the group consisting of lecithin, cephalin, phosphatidylinerine, phosphoinositide, and phosphatidic acid, or mixtures thereof in the form of a cream, an ointment, a pomade, a gel, or an emulsion to the area to be treated. The process of manufacture of such complexes has been described by Bertini Curri in US5,280,020.

Furthermore, instead of the above-cited basic materials or in addition to these materials polymers of vinylalcohol and vinylpyrrolidone, alginates, pectines, polyacrylates, solid and/or liquid polyethylenglycols, paraffins, fatty alcohols, vaseline and/or waxes, fatty acids and/or fatty acid esters are used. It is possible to use the above-cited active ingredients without filler for the production of solid products, as for example powders, toilet powder, granulates, pellets and microcapsules. The pharmaceutical product described here is especially suited for the
attention of such of the above-described diseases which are in a very progressed stage so that at first an increased concentration of active ingredients is necessary. With less serious disease conditions or with progressive healing of the disease such embodiments of the solid pharmaceutical product are used which contain fillers, as for example Colloidal silicic acid, powdered soapstone, milk sugar, starch powder, sugar, cellulose derivatives, gelatin, metal oxides and/or metal salts, wherein the concentration of the active ingredient or of the combination of active ingredients varies between 0.001% by weight and 50% by weight.

A suitable kind of pharmaceutical form may be a topical deliver form of the above-described active ingredient, which is made by the application of the solid, liquid or semi-solid pharmaceutical product onto a gauze strip, a compress or a plaster so that such a gauze strip, such a compress or such a plaster then is only locally applied onto the spot which is to be treated. The pharmaceutical product can be filled into the known receptacles, as for example bottles, tubes, toilet powder boxes and baby powder boxes as well as seal edge bags, which are possibly provided with metering means, as for example droplet forming means, metering valves or metering chambers.

Ivermectin (CAS-7-288-86-7) is synonym of a mixture of 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin Bla and 22,23-dihydroavermectin Blb. In particular the ivermectin a mixture of at least 80% of 22,23-dihydroavermectin Bla and less than 20% of 22,23-dihydroavermectin Blb.
Avermectins and their derivatives which may be particularly emphasized are those of the general formula (above) in which the radicals $R_1$ to $R_4$ have the meaning indicated in Table 2 which follows and $X$ can be a single or double bond between the $C_{22}$ - and $C_{23}$ - positions ($\sim C_{22} R_1 - X - C_{23} R_2$).

If there is a double bond, there are no substituents ($R_1$, $R_2$) in the $C_{22}$ - and $C_{23}$ - positions.
## TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Macroyclic lactone</th>
<th>Avermectin A_{1a}</th>
<th>Avermectin A_{2a}</th>
<th>Avermectin A_{2b}</th>
<th>Avermectin B_{1a}</th>
<th>Avermectin B_{ib}</th>
<th>Avermectin B_{2a}</th>
<th>Avermectin B_{2b}</th>
<th>22,23-dihydroavermectin B_{ia}</th>
<th>22,23-dihydroavermectin B_{ib}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-C_{22} R_i -X-C_{23} R_2 -</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
<td>-CH=CH-</td>
</tr>
<tr>
<td></td>
<td>R_3 R_4</td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-Me</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
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</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-Me</td>
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<td>-iso-Pr</td>
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<tr>
<td></td>
<td></td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-Me</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-Me</td>
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</tr>
<tr>
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<td>-iso-Pr</td>
<td>-H</td>
<td>-iso-Pr</td>
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<tr>
<td></td>
<td></td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-H</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
<td>-sec-Bu</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-H</td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
<td>-iso-Pr</td>
</tr>
<tr>
<td>20</td>
<td>22,23-dihydroavermectin B_{ia}</td>
<td>-CH_2-CH_2-</td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
<td>-H</td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
<td>-H</td>
<td>sec-Bu</td>
<td>-sec-Bu</td>
</tr>
</tbody>
</table>
As a rule, the avermectins and 22,23-dihydroavermetcins Bi (ivermectin) of the general formula (I) are employed as mixtures. Of particular interest in this connection is the product abamectin, which essentially contains the avermectins Bi, and their hydrogenation products, the 22,23-dihydroavermetcins Bi (ivermectin).

The compounds of the macrocyclic lactones marked with "b" which in the C_{25} -position have an iso-propyl radical, do not necessarily have to be separated from the "a" compounds, which have a sec-butyl group in the C_{25} -position. Generally the mixture of both substances, consisting of > 80% sec-butyl derivative (B_{Ia}) and < 20% iso-propyl derivative (B_{Ib}), is isolated, and can be used according to the invention. Additionally, in the stereoisomers the substituents in the C_{13} - and C_{23} -positions can be arranged on the ring system both in the α- and β-positions, i.e., are located above or below the plane of the molecule. In each case, all stereoisomers are taken into account according to the invention.

The Ivermectins which are particularly suitable for the purpose of present invention are compound with the formula

![Diagram of Ivermectin structure]

\[ R = \text{CH}_3 \text{ or C}_2\text{H}_5 \]
or functional derivatives thereof from the class of the macrocyclic lactones.

The milbemycins may be mentioned particularly. The milbemycins have the same macrolide ring structure as the avermectins or 22,23- dihydroavermectins B₁ (ivermectin), but carry no substituents (i.e. missing oleandrose disaccharide fragment) in position 13 (R₅ = hydrogen).

As examples of milbemycins from the class of macrocyclic lactones, the compounds having the general formula

```
in which the radicals R₁ to R₄ have the meaning indicated in Table 3 which follows:
```
The active compounds which may be very particularly emphasized are avermectin B\textsubscript{i\textsubscript{a}}/B\textsubscript{i\textsubscript{b}} (Abamectin), 22,23-dihydroavermectin B\textsubscript{i\textsubscript{a}}/B\textsubscript{i\textsubscript{b}} (ivermectin), doramectin, moxidectin.

The active compounds are present in the formulations according to the invention in concentrations from 0.1 to 10\% by weight, preferably from 0.5 to 5\% by weight, particularly preferably 1-2\% by weight.
Ivermectin can also have the following formula:

\[
R_i \text{ is an alkyl group and in particular wherein } R \text{ is CH3 or C2H5.}
\]

Compounds that are particularly suitable for present invention may be a compounds of the group consisting of

\[
\text{Formula: C48 H74 O15, CA Index Name: Avermectin Ala, 5-O-demethyl-22,23-dihydro-}
\]

Formula: C51 H80 015, CA Index Name: Avermectin Ala, 5-O-demethyl-25-de(l-methylpropyl)-22,23-dihydro-4'-O-(methoxymethyl)-25-(l-methylbutyl)- (9CI)

CAS-7-288-86-7, Ivermectin is generally known as a mixture of 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin BLa and 22,23-dihydroavermectin BLb. In particular the ivermectin is a mixture of at least 80% of 22,23-dihydroavermectin BLa and less than 20% of 22,23-dihydroavermectin BLb.

In a particular embodiment the present invention thus involves a method for the treatment of amyotrophic lateral sclerosis (a fatal neurological disorder, characterised by progressive degeneration of the motor cells in the spinal cord and the brain), or a related motor neuron disease (MMD) said method comprising administering to an individual an effective amount of ivermectin, or an effective amount of 5-0-demethyl-22,23-dihydroavermectin Ala or of 5-0-
demethyl-22,23-dihydroavermectin Albo a mixture thereof, or an effective amount of 22,23-
dihydroavermectin Bla, or 22,23-dihydroavermectin Blb or a mixture thereof. In particular a
mixture of at least 80% of 22,23-dihydroavermectin Bla and less than 20% of 22,23-
dihydroavermectin Blb is suitable for the present invention.

Ivermectin derivatives suitable for the treatment of medicament of present invention are
preferably Ivermectin derivatives of pentacyclic sixteen-membered lactones or
pharmacologically acceptable salts and/or functional derivatives thereof. Some are
methoxylated at the carbon atom in position five (A series) or compounds having an
underivatized hydroxyl-group at this position (B group). Some of these compounds may have
an olefinic bond between the two carbon atoms C22 and C23 (1-subset) or this double bond
can be hydrated (2-subset) resulting in a hydroxyl group at position 23.

The medicament to prevent, retard and ameliorate CNS degenerative disease and the
associated loss of neurons can furthermore comprise magnesium (Mg), its pharmacologically
acceptable salt or its derivatives, such as magnesium oxide, magnesium aspartate, magnesium
sulphate, magnesium citrate chelated magnesium, magnesium EAP in a amount sufficient to
treat, to prevent, to retard, to ameliorate or to cure amyotrophic lateral sclerosis (ALS).

Other suitable compounds are the ivermectin like derivatives of the pentacyclic sixteen-
membered lactones for instance the avermectins. Within the family of the avermectins, there
exist two series, A and B, within which are two structural subsets, designated 1 and 2,
consisting of two homologs a and b. Members of the A-series

For the manufacture of the pharmaceutical product for treatment of treating, preventing or
ameliorating a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS), 5-
0-demethyl-22,23-dihydroavermectin Ala or of 5-0-demethyl-22,23-dihydroavermectin Alb,
can be used. The pharmaceutical products which can be used for the treatment of a motor
neuron disease such as ALS or the associated motor neuron loss, in addition to the above-cited
active ingredients or instead of the above-cited ingredients, can also contain derivatives,
preferably pharmacologically active metabolic products (metabolites), such as the compounds
selected from the group consisting of avermectin, avermectin derivatives, milbemycin,
milbemycin derivatives, ivermectin, ivermectin derivatives, milbemycin oxime, milbemycin oxime derivatives, moxidectin, and moxidectin derivatives

The active ingredient of present invention may preferably be orally or parenterally administered.

Pharmaceutical compositions according to the invention can be periodically administered to a mammalian patient (e.g., a human patient), in need of such treatment, to promote neuron regeneration and functional recovery and thereby to treat damage to the neurons caused by a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS). The compounds of present invention are thus useful to enhance regeneration of the motor neurons. The present invention thus involves a method of promoting motor neuron regeneration in a mammal having a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS), the method comprising: administering to the mammal a pharmaceutical composition comprising a motor neuron regeneration stimulating amount of the compounds of present invention. The compounds of present invention can also be used to enhance motor recovery following a motor neuron damage by a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS).

To further enhance brain and/or motor neuron repair the compounds of present invention may be combined with known neurotrophic factors, which have been shown to play an essential trophic role in the development, maintenance and regulation of neuronal function such as for instance ciliary neurotrophic factor (CNTF), glial growth factors (glial mitogenic factors), Schwann cell mitogenic factors, the nerve growth factors (NGF) and other members of the NGF family, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) or platelet-derived growth factor (PDGF). Progressive CNS degeneration or degeneration of the motor cells in the spinal cord and the brain can be treated or retarded by combining compounds selected from the group consisting of the ivermectins of present invention with Idebenone or with CDP choline (known as cytidine 5-diphosphocholine).

To further enhance brain and/or motor neuron repair the compounds of present invention may also be combined with known neurotrophic factors, which have been shown to play an essential trophic role in the development, maintenance and regulation of neuronal function
such as for instance ciliary neurotrophic factor (CNTF), glial growth factors (glial mitogenic factors), Schwann cell mitogenic factors, the nerve growth factors (NGF) and other members of the NGF family, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) or platelet-derived growth factor (PDGF). Progressive CNS degeneration or degeneration of the motor cells in the spinal cord and the brain can be treated or retarded by combining compounds selected from the group consisting of Ivermectins of present invention with compounds selected from the group consisting of Free radical scavenger, alpha-phenyl-tert-butyl nitrone (PBN), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), platelet-derived growth factor (PDGF), non-competitive antagonists of glutamate such as for instance l-(l-(2-thienyl)cyclohexyl)piperidine (TCP), superoxide dismutases, adenosine agonists such as cyclohexyladenosine (CHA), Gamma-amino butyric acid (GABA) and GABA-mimetic drugs, non-NMDA receptor antagonist such as 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), green tea polyphenol (-)-epigallocatechin-3-gallate, NAALADase inhibitors such 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), small molecule inhibitors of cyclin-dependent kinases (CDKs), cyclooxygenase 2 (COX-2) inhibitors alone or in combination with creatine, (IR)-l-benzo [b] thiophen-5-yl-2-[2-(diethylamino) ethoxy] ethan-1-ol hydrochloride (T-588), riluzole (an inhibitor of glutamate transmission), phosphodiesterase (PDE) inhibitors such as the selective PDE5 inhibitors (dipyridamole, T-1032, and zaprinast) and the nonselective PDE inhibitor aminophylline.

To further enhance brain and/or motor neuron repair the compounds of present invention may be combined with known neuroprotective compounds such as the compounds of the group consisting of free radical scavenger, alpha-phenyl-tert-butyl nitrone (PBN); non-competitive antagonists of glutamate such as for instance l-(l-(2-thienyl)cyclohexyl)piperidine (TCP) or topiramate, peroxide dismutases, adenosine agonists such as cyclohexyladenosine (CHA), Gamma-amino butyric acid (GABA) and GABA-mimetic drugs, non-NMDA receptor antagonist such as 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), the green tea polyphenol (-)-epigallocatechin-3-gallate, NAALADase inhibitors such 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), small molecule inhibitors of cyclin-dependent kinases (CDKs), cyclooxygenase 2 (COX-2) inhibitors alone or in combination with creatine, (IR)-l-benzo [b] thiophen-5-yl-2-[2-(diethylamino) ethoxy] ethan-1-ol hydrochloride (T-588), riluzole, an inhibitor of glutamate transmission and drug used in in amyotrophic lateral sclerosis (ALS), Cardiotrophin-1 (CT-I), nocycline, cyclin-dependent
kinase CDK selective inhibitors (CDKIs) and phosphodiesterase (PDE) inhibitors such as the selective PDE5 inhibitors (dipyridamole, T-1032, and zaprinast) as well as the nonselective PDE inhibitor (aminophylline).

Particularly to enhance recovery of a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS) the ivermectins or functional analogues of present may in particular be combined with VEGF homologous, which have been shown to play a role in mediated survival signals to motor neurons or motor neuron protection. The compounds of present invention may for instance be combined with for instance VEGFA, VEGFB, PLGF or compounds selected from the group consisting of 4-(pyrrolidinyl)-l (2,4,6-trimethoxyphenyl) -1-butane, 2', 4', 6'-trimethoxy-4-(r-pyrrolidinyl) butyrophenone; (2,4,6-trimethoxyphenyl) (3-pyrrolidinopropyl) ketone, (2,4,6-Trimethoxyphenyl) (3-piperidinopropyl) ketone, (2,4,6- triethoxyphenyl) (3-diethylaminopropyl) ketone, (2,4,6-trimethoxyphenyl) [4-β-hydroxyethyl-piperazino] methyl] ketone dihydrochloride and (2,4,6-triethoxyphenyl) (3-pyrrolidinopropyl) ketone.

Particularly to enhance recovery of a motor neuron disease (MND) such as amyotrophic lateral sclerosis (ALS) the ivermectins or functional derivatives of present invention may also be combined with a compound selected of the group consisting of minocycline, Neurontin (gabapentin), Neurodex (dextromethorphan hydrobromide + quinidine sulfate), tamoxifen, Topamax (opiramate), CoQ10, PC-Ol, Celebrex (celecoxib), Indinavir, buspirone, oxandrolone, creatine, NAALADase, neotrophin and Rilutek (riluzole).

Examples of the application

Materials and Methods

Animals

Transgenic mice with overexpression of the human mutant SOD1(G93A) gene were obtained from Jackson Laboratories (Bar Habor, Maine). They were crossbred for more than 5 generations in the C57/B16 background before the experiment was started. Treatment was started at the age of 50 days and continued until time of death. There was a random distribution of littermates and sexes between the control group and the treatment groups. The treatment groups were given 3, 6 or 15 ml of Oramec®/ of drinking water (Oramec® is a
trademark of Merial SAS, Lyon, France), which corresponds to a concentration of the active substance ivermectin of 2.4, 4.8 and 12 mg/l, respectively. The control group received the same amount of solvent in their drinking water without the active substance. When mice could no longer roll over within 10 sec when pushed on their side, they were killed and this age was considered as the time of death. At all times the animals were kept in a strictly controlled environment and were free of parasites. The ethical committee of the University of Leuven approved all experiments.

**Histological evaluation**

At the age of 120 days, mice of the control group and of the 12 mg/l ivermectin group were anesthetized with i.p. Nembutal and a transcardiac perfusion with PBS and with 4% paraformaldehyde was performed. After dissection, the lumbar part of the spinal cords was fixed in 4% paraformaldehyde and embedded in paraffin. Sections of 7µm were deparaffinated and stained with hematoxylin and eosin. Lumbar ventral roots were dissected, fixed in 2.5% glutaraldehyde and embedded in epoxy resin. Ultra thin sections were stained with toluidine blue. Spinal cords and ventral roots were analyzed by using the Lucia Image program (version 4.60, Laboratory Imaging, Prague, Czech Republic). hi spinal cord sections, the area of neurons with nucleoli in the ventral horn was measured and per section, the number of neurons with an area larger than 200µm² was determined, hi roots, the area occupied by axons was expressed as percentage of the total area of the root section.

**Statistical analysis**

The in vitro data were analysed using Students' t-test or one or two-way ANOVA (NCSS) followed by the Fishers LSD test. Survival data were analysed using a log-rank test (NCSS).
Results

We treated SODI(G93A)-mice, a transgenic animal model for familial ALS, with ivermectin. This resulted in an extension of the life span of these mice with almost 10%.

Effect of ivermectin on motor neuron degeneration and survival in SODI(G93A)-mice.

To test the relevance of the ivermectin effect in ALS, we treated a transgenic mouse model for ALS with ivermectin. At the age of 50 days, SODI(G93A)-mice were randomly distributed in different treatment groups, receiving 3, 6 or 15 ml Oramec® (2.4, 4.8 or 12 mg ivermectin)/l or the same amount of solvent in their drinking water. For unknown reasons both the control and the treated mice did not learn to walk on the Rotarod and as a consequence we could not use this parameter to determine the effect of ivermectine on the onset of the disease. However, as is shown in Table 1 and Fig. 5, ivermectin significantly increased the survival of SODI(G93A)-mice in the treatment groups receiving 4.8 and 12 mg/l ivermectin from 140.6 ± 1.6 days for the control group to 149.3 ± 2.8 days for the 4.8 mg/l ivermectin group (log-rank test, p = 0.01) and 153.4 ± 2.8 days for the 12 mg/l ivermectin group (log-rank test, p = 0.0003). No significant difference was found between these two higher concentrations (log-rank test, p = 0.52).

At the age of 120 days randomly selected mice of the control and 12 mg/l ivermectin group were sacrificed for histological examination of the lumbar spinal cord and lumbar ventral roots. As shown in Fig. 6A-C the number of large neurons (> 200 μm²) in the ventral horn of the lumbar spinal cord was significantly higher (Students’ t-test; p < 0.005) in the ivermectin group (18.0 ± 0.4 large motor neurons/section) compared to the control group (10.9 ± 0.6 large motor neurons/section). In ventral roots (Fig. 6D-F) the area occupied by axons was 14.6 ± 1.3 % in the control group and 23.3 ± 1.5 % in the ivermectin group (p < 0.005).

Table 1: Effect of ivermectin on the survival of SODI(G93A)-mice.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>2.4 mg/l ivermectin</th>
<th>4.8 mg/l ivermectin</th>
<th>12 mg/l ivermectin</th>
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<tbody>
<tr>
<td>number of mice</td>
<td>32</td>
<td>7</td>
<td>21</td>
<td>11</td>
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<tr>
<td></td>
<td>mean ± s.e.m.</td>
<td>140.6 ± 1.6 days</td>
<td>139.0 ± 4.1 days</td>
<td>149.3 ± 2.8 days</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Difference with</td>
<td>- 1.6 days</td>
<td>8.7 days</td>
<td>12.8 days</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>- 1.1 %</td>
<td>6.2 %</td>
<td>9.1 %</td>
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<tr>
<td>expressed as %</td>
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<td>Log-rank test</td>
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<td>control)</td>
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</tr>
<tr>
<td></td>
<td>p = 0.61</td>
<td>p = 0.01</td>
<td>p = 0.0003</td>
<td></td>
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</table>

Treatment was started at 50 days of age and continued till time of death. The control group received solvent in their drinking water.

5 Drawings and figures of the application

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will become more fully understood from the detailed description given herein below and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, and wherein:

10 FIG 1 shows a Kaplan-Meier plots of the cumulative probability of survival of SOD1(G93A)-mice.

The ivermectin groups received 3, 6 or 15 ml/1 Oramec® (2.4, 4.8 and 12 mg/1 ivermectin) in their drinking water, the control group received the same amount of solvent. Treatment was started at 50 days of age and was continued till time of death. Number of animals in each group and statistical analysis of the probability of survival using a log-rank test is given in Table 1. The groups receiving 4.8 mg/1 and 12 mg/1 ivermectin in their drinking water were statistically different from the control group. No significant difference was found between these two concentrations (log-rank test, p = 0.52).
FIG 1 are graphs and histological pictures that demonstrate the effect of ivermectin on neuronal loss in the lumbar spinal cord and on the axon loss in the ventral roots of 120-days-old SODI(G93A)-mice.

Spinal cord sections of control (A) or ivermectin (B) treated SODI(G93A)-mice were stained with hematoxilin and eosin. Bar = 50 µm. The number of large neurons (> 200 µm²) in the ventral part of lumbar spinal cord sections was determined (C). Numbers are the mean ± S.E.M. of 4 mice per treatment. For each mouse 5 sections were analyzed.

Ventral root sections of control (D) or ivermectin (E) treated SODI(G93A)-mice were stained with toluidine blue. Bar = 50 µm. The inside area of axons is measured and expressed as percentage of total area of the ventral root section (F). Numbers are the mean ± S.E.M. of 4 different ventral roots per treatment. For each root 2 sections were analyzed.

*: p < 0.005 significantly different from the control group (Students' t-test).
TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

CLAIMS

What is claimed is:

1. The use of an ivermectin or a macrocyclic lacton analogue of an ivermectin or a combination thereof for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

2. The use of an Ivermectin analogue of claim 1 with the formula

![Chemical Structure Image]
in which the radicals \( R_1 \) to \( R_4 \) have the meaning indicated in table 2 which follows and \( X \) can be a single or double bond between the \( C_{22} \) and \( C_{23} \) positions (\( \sim C \, 22 \, R_1 \, X \, C \, 23 \, R_2 \sim \)) and if there is a double bond, there are no substituents \((R_1, R_2)\) in the \( C_{22} \) and \( C_{23} \)-positions or a combination thereof for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

3. The use of claim 1 of an ivermectin analogue with the formula

![Chemical Structure](image)

in which the radicals \( R_1 \) to \( R_4 \) have the meaning indicated in table 3 or a combination thereof for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

4. The use of claim of an ivermectin analogue 1, characterised in that it has the formula
or a combination thereof for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

5. The use of claim 1 of a mixture of the compounds, wherein the ivermectin analogues are 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin Bla and 22,23-dihydroavermectin Blb for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

6. The use of a mixture of claim 5, wherein at least 80% of 22,23-dihydroavermectin Bla and less than 20% of 22,23-dihydroavermectin Blb for the preparation of a pharmaceutical composition to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

7. The use of claim 1, wherein the Ivermectin analogue is selected from the groups consisting of Ivermectin, Abamectin, Doramectin, Eprinomectin, Milbemycin oxime, Moxidectin and Selamectin.

8. The use of claim 1, wherein the Ivermectin analogue is pharmaceutical composition is to administered orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes.

9. The use of claim 1 of an ivermectin analogue, characterised in that it has the formula
10. The use of claim 1 of an ivermectin analogue, characterised in that it has the formula

11. The use of claim 1 of an ivermectin analogue, characterised in that it has the formula
12. The use according to claim 1 of an ivermectin analogue, characterised in that it has the formula but selected from the group consisting of avermectin, avermectin derivatives, milbemycin, milbemycin derivatives, ivermectin, ivermectin derivatives, milbemycin oxime, milbemycin oxime derivatives, moxidectin, and moxidectin derivatives, or mixtures thereof.

13. The use according to claim 1 of an ivermectin analogue, characterised in that the Motor Neuron Disease is amyotrophic lateral sclerosis (ALS).

14. The use of an ivermectin or a macrocyclic lacton analogue of an ivermectin or a combination thereof for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease.

15. The use of an Ivermectin analogue of claim 14 with the formula
in which the radicals R₁ to R₄ have the meaning indicated in table 2 which follows and X can be a single or double bond between the C₂₂ - and C₂₃ - positions (→C₂₂ R₁-X-C₂₃ R₂←) and if there is a double bond, there are no substituents (R₁, R₂) in the C₂₂ - and C₂₃ -positions or a combination thereof for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affected by a Motor Neuron Disease.

16. The use of claim 14 of an ivermectin analogue with the formula
in which the radicals R₁ to R₄ have the meaning indicated in table 3 or a combination thereof for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affected by a Motor Neuron Disease.

17. The use of claim 14 of an ivermectin analogue, characterised in that it has the formula

or a combination thereof for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient
affect by a Motor Neuron Disease.

18. The use of claim 14 of a mixture of the compounds, wherein the ivermectin analogues are 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin BLa and 22,23-dihydroavermectin BLib for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease..

19. The use of a mixture of claim 18, wherein at least 80% of 22,23-dihydroavermectin BLa and less than 20% of 22,23-dihydroavermectin BLib for the preparation of a pharmaceutical composition to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease..

20. The use of claim 14, wherein the Ivermectin analogue is selected from the groups consisting of Ivermectin, Abamectin, Doramectin, Eprinomectin, Milbemycin oxime, Moxidectin and Selamectin.

21. The use of claim 14, wherein the Ivermectin analogue is pharmaceutical composition is to administered orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes

22. The use of claim 14 of an ivermectin analogue, characterised in that it has the formula
23. The use of claim 14 of an ivermectin analogue, characterised in that it has the formula

24. The use of claim 14 of an ivermectin analogue, characterised in that it has the formula
25. The use according to claim 14 of an ivermectin analogue, characterised in that it has the formula but selected from the group consisting of avermectin, avermectin derivatives, milbemycin, milbemycin derivatives, ivermectin, ivermectin derivatives, milbemycin oxime, milbemycin oxime derivatives, moxidectin, and moxidectin derivatives, or mixtures thereof.

26. The use according to claim 14 of an ivermectin analogue, characterised in that the Motor Neuron Disease is amyotrophic lateral sclerosis (ALS).

27. An ivermectin or a macrocyclic lactone analogue of an ivermectin or a combination thereof for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patients affected by a Motor Neuron Disease.

28. An Ivermectin analogue of claim 27 with the formula
in which the radicals R₁ to R₄ have the meaning indicated in table 2 which follows and X can be a single or double bond between the C₂₂- and C₂₃-positions (—C₂₂ R₁ -X-C₂₃ R₂—) and if there is a double bond, there are no substituents (R₁, R₂) in the C₂₂- and C₂₃-positions or a combination thereof for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease..

29. An ivermectin analogue of claim 27 with the formula
in which the radicals $R_1$ to $R_4$ have the meaning indicated in table 3 or a combination thereof for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease.

30. An ivermectin analogue of claim 27, characterised in that it has the formula

or a combination thereof for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron
Disease.

31. A mixture of the compounds of claim 27, herein the ivermectin analogues are 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin BLa and 22,23-dihydroavermectin BLb for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease.

32. A mixture of claim 31, wherein at least 80% of 22,23-dihydroavermectin BLa and less than 20% of 22,23-dihydroavermectin BLb for use in a treatment to heal motor neuron degeneration or to retard the progress of the degeneration of motor neurons in patient affect by a Motor Neuron Disease.

33. An Ivermectin analogue for use in the treatment of claim 27, wherein the Ivermectin analogue is selected from the groups consisting of Ivermectin, Abamectin, Doramectin, Eprinomectin, Milbemycin oxime, Moxidectin and Selamectin.

34. An Ivermectin analogue for use in the treatment of claim 27, wherein the Ivermectin analogue is in a pharmaceutical composition to be administered orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes

35. An Ivermectin analogue for use in the treatment of claim 27, characterised in that it has the formula
36. An Ivermectin analogue for use in the treatment of claim 27, characterised in that it has the formula

37. An Ivermectin analogue for use in the treatment of claim 27, characterised in that it has the formula
38. An Ivermectin analogue for use in the treatment of claim 27, characterised in that it has the formula but selected from the group consisting of avermectin, avermectin derivatives, milbemycin, milbemycin derivatives, ivermectin, ivermectin derivatives, milbemycin oxime, milbemycin oxime derivatives, moxidectin, and moxidectin derivatives, or mixtures thereof.

39. An Ivermectin analogue for use in the treatment of claim 27, characterised in that the Motor Neuron Disease is amyotrophic lateral sclerosis (ALS).

40. An ivermectin or a macrocyclic lacton analogue of an ivermectin or a combination thereof for use in a treatment to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

41. An Ivermectin analogue of claim 40 with the formula
in which the radicals R_i to R_4 have the meaning indicated in table 2 which follows and X can
be a single or double bond between the C_{22} and C_{23} positions (\sim C_{22} R_i \sim X \sim C_{23} R_2 ) and
if there is a double bond, there are no substituents (R_1, R_2) in the C_{22} and C_{23} positions or a
combination thereof for use in a treatment to increase the lifespan or the life expectancy of a
patient affected by a Motor Neuron Disease.

42. An ivermectin analogue of claim 40 with the formula
in which the radicals \( R_1 \) to \( R_4 \) have the meaning indicated in table 3 or a combination thereof for use in a treatment to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

43. An ivermectin analogue of claim 40, characterised in that it has the formula

or a combination thereof for use in a treatment to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.
44. A mixture of the compounds of claim 40, wherein the ivermectin analogues are 5-0-demethyl-22,23-dihydroavermectin Ala and 5-0-demethyl-22,23-dihydroavermectin Alb or a mixture of 22,23-dihydroavermectin BLa and 22,23-dihydroavermectin BLb for use in a treatment to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

45. A mixture of claim 44, wherein at least 80% of 22,23-dihydroavermectin BLa and less than 20% of 22,23-dihydroavermectin BLb for use in a treatment to increase the lifespan or the life expectancy of a patient affected by a Motor Neuron Disease.

46. An Ivermectin analogue for use in the treatment of claim 40, wherein the Ivermectin analogue is selected from the groups consisting of Ivermectin, Abamectin, Doramectin, Eprinomectin, Milbemycin oxime, Moxidectin and Selamectin.

47. An Ivermectin analogue for use in the treatment of claim 40, wherein the Ivermectin analogue is in a pharmaceutical composition to be administered orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, or by application to mucous membranes

48. An Ivermectin analogue for use in the treatment of claim 40, characterised in that it has the formula

\[
\text{\includegraphics{formula.png}}
\]

49. An Ivermectin analogue for use in the treatment of claim 40, characterised in that it has
50. An Ivermectin analogue for use in the treatment of claim 40, characterised in that it has the formula

![Chemical structure](image)

51. An Ivermectin analogue for use in the treatment of claim 40, characterised in that it has the formula but selected from the group consisting of avermectin, avermectin derivatives, milbemycin, milbemycin derivatives, ivermectin, ivermectin derivatives, milbemycin oxime, milbemycin oxime derivatives, moxidectin, and moxidectin derivatives, or mixtures thereof.

52. An Ivermectin analogue for use in the treatment of claim 40, characterised in that the Motor Neuron Disease is amyotrophic lateral sclerosis (ALS).
Fig 1

- control
- 2.4 mg/l ivermectin
- 4.8 mg/l ivermectin
- 12 mg/l ivermectin

probability of survival
age (days)
Fig. 2