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Treatment of amyotrophic lateral sclerosis – What is the next step?

Abstract Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which was thought to be untreatable. However, recent evidence in both experimental animals and men indicates that antiglutamatergic strategies are the first to have an influence on its pathogenesis and slow down the disease process. Since the effect of drugs is still small, this progress cannot only be seen as a success of the present but must also be acknowledged as a basis for future developments. How will future studies be designed? They will have to take into account that the disease presumably has a long preclinical period and they will use a number of novel compounds and treatment strategies which have been shown to be effective in transgenic animal models. This also implies that we are likely to use a combination of therapies and we will try to treat patients early. The latter will be associated with the demand for a novel clinical attitude toward the diagnosis of the disease and the development of novel markers for both the preclinical period and the longitudinal course of the disease.

Key words Amyotrophic lateral sclerosis · neurodegeneration · riluzole

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease which had not evoked any therapeutic hopes since its earliest descriptions [12]. A characteristic pattern of vulnerability which primarily involves the Betz cells of the motor cortex and the spinal and bulbar motor neurons is the hallmark of the disease. An improved understanding of this pattern might result in rational therapeutic approaches. Charcot – who first suggested to name the disease amyotrophic lateral sclerosis – predicted that therapy of ALS would be a matter of the far future. Now, in the beginning of the 21st century, we are finally entering the era of pharmacological treatment of ALS. In 1994, the initial step was taken when Gilbert Bensimon and Vincent Meininger published their paper on the positive effect of riluzole on the course of ALS in the New England Journal of Medicine [7]. These results were confirmed by these two authors and their collaborators in the 301 study as published in the Lancet in 1996 [34]. The group showed in this large phase III study that the antiglutamatergic drug riluzole increases life expectancy of ALS patients dose-dependently. Then, because of its effect/side effect profile riluzole was marketed all over the world in a dosage of 100 mg and this finally initiated the first period of improved pharmacological and also non-pharmacological treatment of ALS [17]. However, treatment effects are not in the range which are commonly seen in acute and treatable diseases since the neuroprotective effect of riluzole is in the range of the achievements obtained in early chemotherapy of cancer – in an individual with a life expectancy of 18 months four months are gained. But, in sharp contrast to early and contemporary chemotherapy of cancers this is achieved without major side effects.

Because of these limitations of treatment, it is necessary to develop the concepts of future ALS therapy. Based on the experience with the effect of riluzole and the results of recent experimental animal studies, in this article the following aspects will be discussed in more detail: 1) the evidence for a need of earlier treatment, 2) the need for a development of biological markers, 3) the likelihood of the introduction of combination therapies, and 4) and finally, thoughts on preventive measures will be developed.

The experimental model

Today, experimental ALS research has benefited from the development of an important and widely acknowledged model for its pathogenesis which is based on the one and only etiological factor known to play a role in human ALS unequivocally. For five years now, transgenic Cu/Zn SOD mice serve the ALS research community to study the pathogenesis of selective motor neuron death and screen potential therapeutic approaches. The development of this model is based on the discovery that about 15–20% of patients suffering from the familial form of ALS (fALS) carry mutations in the gene for the cytosolic form of the Cu/Zn SOD (SOD1) [50]. Introduction of several copies of the mutated gene into the mouse genome leads to an animal disease characterized by the development of a flaccid tetraparesis late in life which is associated with prominent anterior horn cell loss in the spinal cord [14, 24, 54]. Consistent with the autosomal-dominant transmission in 5–10% of the human patients, a number of convincing experiments showed that the pathogenesis of the animal disease is governed by a gain-of-function mechanism [11, 46, 54]. Studies of the temporal pattern of the pathogenesis in transgenic mice revealed that the early phase of the disease is ultrastructurally characterized by mitochondrial damage resulting in extensive vacuolation of the soma, the proximal axon and the dendritic tree of anterior horn cells [14, 32, 54]. Interestingly, these morphological features resemble excitotoxic damage which may be explained by the mechanism of slow or weak excitotoxicity entertained by slowly evolving chemical hypoxia [6, 29, 39, 47, 48].

Cross-breeding of mice carrying copies of the G93A mutation with mice deficient in manganese superoxide dismutase (SOD2) reduced motor performance and shortened the life span of the offspring by 10%, further indicating that mitochondrial damage and oxidative damage could play a role in the pathogenesis of motor neuron death [1]. The concept of a pathogenetic role of free radicals is also underlined by the demonstration of increased levels of 3-nitrotyrosine and evidence of "hydroxyl-radical-like" activity by using microdialysis in the transgenic SOD1 mice carrying human ALS mutations [9, 10, 20]. The evidence for a role of free radicals in this concept is further supported by the finding of increased protein oxidation and enhanced oxygen radical formation in the same model [3, 36]. However, it remains presently unclear whether these changes reflect clinically and therapeutically relevant specific aspects of the concept of selective vulnerability or are entirely non-specific. On the other hand, the concept of a role for indirect excitotoxicity and free radicals in the pathogenesis of the rodent disease is supported by the proven potential of anti-excitotoxic drugs and antioxidants, such as gabapentin and riluzole, vitamin E and carboxyfullerenes, to increase the life expectancy of these animals by up to 10% [18, 25, 28].

Taken in concert, studies of the pathogenesis of anterior horn cell loss in our currently best animal model of motor neuron disease has not revealed the entire sequence of events (cascade) leading to the death of motor neurons, but has shown parts of a mosaic which might be also relevant for human sporadic ALS (sALS).

Evidence for a preclinical period and the need for earlier treatment

In other neurodegenerative diseases such as Parkinson's or Huntington's disease, the existence of a preclinical period for the disease is increasingly likely [2, 4, 40, 42, 43]. Studies of the transgenic Cu/Zn SOD animals also indicate that the existence of a preclinical period in human sporadic ALS needs to be seriously considered. For example, animals carrying the human G37A mutation die on average on day 135, they develop frank paralysis on day 125, but they start to lose their motor neurons at day 90, and even show the first ultrastructural changes of mitochondria as early as in late childhood [13, 25, 54]. Azzouz and collaborators [5] have studied the amplitude of the muscle compound action potential recorded from foot muscles after stimulation of the sciatic nerve in these transgenic animals. If compared with age-matched controls, the amplitude decreased already at day 60–70, indicating that hypotheses raised in studies done in the past might be of more relevance than previously thought [16]. If a preclinical phase exists also in human ALS patients, this has an impact on our expectations from effects of neuroprotective drugs: riluzole has a reproducible effect on the life expectancy of the transgenic Cu/Zn SOD animals [25, 25a] and this effect is comparatively large since life is prolonged by 13 days in animals which survive on average 136 days resulting in a gain of life expectancy approaching 10%. If compared with this percentage, the neuroprotective effect in men is comparatively small – an increase of less than 1% [34]. This difference between mice and men is partly explained by the treatment protocols used: whereas treatment in mice was initiated early in the preclinical period (at day 50), in men we can only treat after the clinical diagnosis has been made – during the clinical disease. In summary, the existence of a long preclinical phase might partly explain the difference of the effect of riluzole in mice and men.

Therefore, if a significant preclinical period exists, we have to treat as early as possible – and this implies that there must be a larger therapeutical effect if we begin to treat in earlier rather than in later disease states. This assumption is indeed supported by a retrospective analysis of the riluzole 301 study data done by Riviere and collaborators [34, 49]. Their study compared treatment effects in late stages – stages 3 and 4 – and early disease stages of amyotrophic lateral sclerosis – stages 1 and 2 (Table 1). The patients taking placebo survived 242 (stage 1) + 304 days (stage 2) = 546 days, those taking riluzole 317 (stage 1) + 347 days (stage 2) = 664 days. This plus of 118 (664 minus 546) days is pre-

Table 1 Riluzole has a lerger affect on mild and moderate stages of ALS than on late stages (adapted from 49).

# of patients	Placebo	Riluzole	Gain of life expextancy
mild and moderate stages (days until patient left this stage)	242	317	75
severe stages (days until patient left this stage)	304	347	43
Sum (days)	546	664	118

dominantly due to a treatment effect on earlier disease stages – 317 minus 242 = 75 days compared with late stages (347 minus 304 = 43 days). In sum, these results *might* indicate that treatment in early disease stages is more efficient than in late ones, but they *certainly show* that treatment initiation in early disease stages results in an improved life expectancy – as predicted from the animal data.

What does this mean for future clinical approaches to the ALS patient? We have to overcome therapeutic *and diagnostic* nihilism and use modern tools such as imaging techniques for the early differential diagnosis of ALS [38]. The diagnosis of ALS is not without clinical consequences anymore; it has some – and possibly more in the future – therapeutic impact to differentiate ALS from classical disease states such as multiple disk protrusions.

The need for biological markers

Trials of neuroprotective drugs in ALS and other neurodegenerative diseases are time-consuming and expensive [52]. The preclinical period of a disease process is a particular challenge. These problems can be overcome if both objective longitudinal and objective activity biological markers for the disease process can be established. Longitudinal markers of a disease process are markers which indicate the stage of the process by measuring loss of biological activity and loss of neurons; activity markers measure the activity of the disease process.

In ALS research, the goal to establish longitudinal and activity markers is far from being accomplished, but initial steps are being taken by a number of groups in the world. As a longitudinal marker, single volume proton spectroscopy of the primary motor cortex has been explored in ALS patients. At the present time, short echo spectra from individual ALS patients can hardly be distinguished from those of controls, but after quantitation individual longitudinal time courses of the disease process can be defined and group comparisons can be made by appropriate statistical means [8, 45]. For this purpose, N-acetylaspartate (NAA) is used as a marker for neuronal integrity, whereas choline levels are thought to reflect morphological and biochemical integrity of membranes or to mirror gliosis.

Fluordesoxy-glucose positron emission tomography (FDG PET) studies are also a candidate method for longitudinal studies [27], whereas ligands for the GABA receptor show preclinical damage to interneurons (Nigel Leigh, personal communication), but their usefulness for longitudinal studies is presently unknown.

Not much is known about activity markers. Based on the results in transgenic Cu/Zn SOD animals [26, 31], microglial activation is one of the earliest morphological changes of the disease process; therefore, labeling of these cells with the help of the peripheral benzodiazepine receptor ligand pk11195 is a promising means to demonstrate microglial activation in motor neuron disease, as has been already demonstrated in stroke, multiple sclerosis and multisystem atrophy [19, 22, 23]. However, this costly and time-consuming methodology will presumably only be in transitory use to establish biochemical markers in blood and cerebrospinal fluid (CSF) for objective observations which do not only define course and activity of the disease process, but also the selective damage of distinct cell populations which is the major characteristic feature of the disease. Presumably, studies in animal models will pave the way for these future steps.

Neuroprotective compounds

The transgenic Cu/Zn SOD animals are increasingly used as a tool to screen neuroprotective compounds. During recent years, an astonishingly large number of compounds have been shown to be useful in these animals – although quantitative studies or dose response curves are rare. Therefore, the effects of the individual drugs cannot be compared in a quantitative manner but are better seen as showing proof of principle. The following strategies and principles have been shown to be effective:

- Glutamate antagonists, such as riluzole and gabapentin [25]
- The use of antiapoptotic principles, such as co- and overexpression of bcl-2 [33] and dominant negative inhibition of the apoptosis-associated protease interleukin-1B converting enzyme (ICE) [21]. Recently, a neuroprotective effect has been demonstrated by intraventricular administration of the broad-spectrum caspase inhibitor zVAD-fmk [35].
- The therapeutic usefulness of antioxidants has been documented by the administration of vitamin E and selene, d-penicillamin, and carboxyfullerenes [18, 25, 28]; also, the recent demonstration of a therapeutic effect of trientine and ascorbate [44] is consistent with the view of a neuroprotective effect of antioxidants.
- The moderate neuroprotective effect of d-penicillaminium might also be related to the metal chelating agents of this compound [28].

- The quantitatively surprising effect of creatine administration might be either related to its anabolic effect on muscle or due to its function as a substrate for energy supply and thereby permit those neurons to survive longer which synthesize less chemical energy because of damage to mitochondria [30].

Recently, gene therapy with myoblasts expressing growth factors, the administration of potassium channel openers [Link, J. & Ludolph, A. C., unpublished] and the administration of inhibitors of nitrous oxide have also been shown to be therapeutically successful in animals carrying human SOD1 mutations.

Although each of these studies has shown the principle success of these drugs, currently major drawbacks exist. Presently, nothing is known on dose-response effects of the majority of these drugs; translation of the results from mice to men has been successful for one drug (riluzole) but not for a presumably functionally related one (gabapentin) [25, 34, 41] and awaits studies for the others. Translation may be difficult since the only drug effective in mice and men – riluzole – was given to experimental animals in much higher doses than employed in human ALS patients. Therefore, combination therapy may be the future of ALS and other neurodegenerative diseases.

Preventive measures

Recent advances in ALS research were based on discoveries of the etiology of genetically caused subforms of the disease. However, sporadic ALS is apparently not a monogenic disease and convincing examples for motor neuron diseases exist which apparently are predominantly linked to environmental causes. The most prominent examples are

- The ALS syndromes once most prevalent on the Marianas and among the Auyu and Jakai of Irian Jaya (Western Pacific ALS) and
- A motor neuron disease observed in horses, equine motor neuron disease (EMND).

The endemic form of ALS on the Marianas and in the South West of Irian Jaya has disappeared or is disappearing suggesting that an unknown environmental factor has played a role in its etiology [37, 53]. EMND appeared as an epidemic in the late 1980s and early 1990s in the northeastern U. S. and was described by Jeff Cummings and colleagues [15]. The Cornell group could convincingly show that these horses had a motor neuron disease which did not result from inflammation, but they did not find its cause and finally the disease disappeared. Although major progress in ALS research was clearly based on discoveries made in the genetics of the disease, Western Pacific ALS and EMND must remind us that ALS can also – apparently and to unknown degree – be caused by environmental factors.

Since the best therapy of any disease is prevention, one of the next steps in ALS research should be the identification of risk factors which can be best done in experimental animals. As an example, the recent demonstration that cross-breeding of SOD2 deficient animals with animals carrying the SOD1 mutation accelerates the disease implies that oxidative stress from any source might influence disease onset and death of an individual predisposed to develop ALS by genetic background (1). In a recent study, we depleted animals carrying the human Cu/Zn SOD mutations from the potent endogenous antioxidant glutathione for up to ten days in early life [51]. For this purpose, we used L-buthionine sulfoxime (L-BSO), an inhibitor of glutathione synthase. This depletion was neither associated with acute neuropathological nor behavioral or motor deficits, but accelerated dose-dependently the deterioration of motor performance late in life, onset of pareses, and death of the animals [51]. Further studies will elucidate other genetic and non-genetic risk factors which will lead to preventive measures to delay disease onset or to slow down the disease process.

Summary and conclusions

In summary, after the initial introduction of a neuroprotective compound in the experimental animal model and human ALS, this article suggests four steps to be taken in the future:

- The introduction of earlier treatment, and this implies earlier clinical diagnosis,
- The development of both, biological markers for the longitudinal course of the disease and its activity,
- The introduction of combination therapies, and
- Finally, the development of preventive measures which are also based on results in reliable animal models.

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