

# Failure of a minocycline trial in patients with amyotrophic lateral sclerosis

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**TITLE** Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial

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**LIST OF ABBREVIATIONS** Amyotrophic lateral sclerosis (ALS), ALS functional rating scale (ALSFERS-R), central nervous system (CNS), forced vital capacity (FVC), National Institutes of Health (NIH), superoxide dismutase-1 (SOD1)

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## SUMMARY

Minocycline is thought to inhibit cell death in the central nervous system by reducing the activity of proapoptotic and proinflammatory enzymes, and has been shown to be neuroprotective in animal models of stroke, trauma and a variety of neurodegenerative disorders. In a transgenic mouse model of ALS (motor neurone disease), several independent studies have shown minocycline to prolong survival by 10–22% and to diminish the loss of motoneurons.

Minocycline was approved more than 35 years ago by the US Food and Drug Administration for treatment of bacterial infections, and importantly is known to cross the blood-brain barrier following a standard oral dose. Phase II trials suggested minocycline was safe when given to patients with ALS, and several phase II and III trials are planned or in progress for a variety of neurological diseases. This paper reports on a NIH-funded multi-centre randomised controlled trial in which ALS patients treated with an escalating dose of minocycline up to a total daily dose of 400 mg per day deteriorated 25% faster than the control group over a nine-month period.

The deterioration occurred over a number of scales including the revised ALSFERS-R. Treatment was associated with non-significant worsening of FVC and muscle strength and a greater mortality. Non-serious gastrointestinal upsets

and neurological adverse effects were more common in the treatment group. However, the quality-of-life scores did not differ between the two groups. The number of adverse events appeared to be independent of dose.

The trial was conducted in 31 centres. Patients were assessed monthly and only assigned to the minocycline or control group after a four-month assessment period. Randomisation was stratified by centre, riluzole use and site of onset (limb versus bulbar), in blocks of four. All drugs were distributed double blind. Two thirds of the patients in each arm of the study were on riluzole, and this additional treatment appeared to have no significant effect on ALSFERS-R scores. Forty-one of 206 patients on minocycline and 32 of the same number on placebo died during the trial. During a 42-month follow-up, the median time to reach a predefined failure (death, tracheostomy or noninvasive ventilation for >23 hours/day) was 17.8 months for the patients on minocycline and 20.1 months for placebo patients.

The trial used a lead-in design, strict enrolment criteria for breathing capacity and was powered to detect an 18% difference in the rate of change in the primary outcome measure, the ALSFERS-R, in 400 patients. The study design was based on a linear mixed effects model. However, the deterioration of both groups of patients was non-linear and appeared to increase after the fourth month of the drug-free period.

## OPINION

In spite of the negative outcome, this report underscores both the importance of publishing negative results and of conducting clinical trials in the assessment of new therapies. It also underlines how only carefully planned and executed two-sided studies will identify an adverse effect, as opposed to a benefit or no effect. Future studies in ALS will need to take on board the non-linear deterioration seen in this trial over a period of about a year, in spite of a linear deterioration having been reported in a previous trial designed to evaluate the ALSFRS-R questionnaire and the efficacy of a drug.<sup>1</sup>

Presumably the use of a different model based on a curvilinear effect, in which the rate of deterioration accelerated with time, would have highlighted the adverse effect sooner, perhaps before all 400 patients had completed the 42-week follow-up study.

Clearly, the justification for a number of other trials of minocycline in ALS and other neurological studies, including Huntington's disease and multiple sclerosis, is now questionable.<sup>2</sup> The problem is compounded by the possibility that the deterioration highlighted in this study could be due to an adverse interaction between minocycline and riluzole, in which minocycline negated the small beneficial effect shown in earlier studies.<sup>3-4</sup>

An interaction may well be a serious possibility, since a paper published by Milane *et al.*<sup>5</sup> after the *Lancet Neurology* paper and the commentary<sup>2</sup> were published has shown that in mice minocycline and riluzole interact at the level of the blood-brain barrier at a common efflux site, the p-glycoprotein (p-gp) efflux pump. This was confirmed by showing the uptake into the brain of both compounds to be greater in transgenic mice in which the pump had been deleted. Furthermore, in normal mice the uptake of riluzole into the brain was shown to be increased by the co-administration of minocycline. The interaction was confirmed in vitro using a transfected rat brain endothelial cell line.

Other experiments showed minocycline to interfere with [<sup>3</sup>H] digoxin transport across the blood-brain barrier, suggesting that minocycline is a p-gp inhibitor. The authors also suggest that riluzole and minocycline may interact at other efflux sites on the blood-brain barrier.<sup>5</sup> These results contradict the results of an earlier study<sup>6</sup> of riluzole pharmacokinetics in SOD1-G93A transgenic mice. The use of transgenic mice dominates a new review highlighting possible new treatments for ALS.<sup>7</sup>

How this interaction might negate the action of riluzole is difficult to imagine since the exact pharmacological action of riluzole is unknown, although several neuroprotective properties have been ascribed to it, such as inhibition of presynaptic glutamate release, modulation of voltage-

activated sodium channels, enhancement of glutamate uptake, inhibition of protein kinase C or the upregulation of growth factors. Furthermore, a reduction or increase in the concentration of riluzole itself at a critical site is unlikely to be the cause, since a recent study<sup>8</sup> in which the mean survival at 18 months after the start of use of riluzole was 74% and measurements of trough and peak serum concentrations of riluzole in 160 ALS patients showed there to be no association between riluzole levels and survival or rate of deterioration over a five-year period. Clearly, one possibility is that that one of the metabolites of riluzole, N-hydroxyriluzole,<sup>9</sup> is the active therapeutic agent.

Thus it is possible that the adverse effect of the interaction between riluzole and minocycline occurs outside the CNS at a site unrelated to neuroprotection. For instance, the interaction may occur at the level of, say, the liver microsomes and thus prevent riluzole degradation to an active metabolite. This may not be too far-fetched since minocycline was developed from tetracycline – a known blocker of P450 – and it has recently been suggested that the substrates of P450 and p-gp overlap.<sup>10</sup> This explanation offers a number of possible alternative approaches, including the use of a riluzole metabolite or the replacement of minocycline with a derivative that is neuroprotective and does not interfere with microsomal activity. The explanation would also be comforting for those planning trials of minocycline in multiple sclerosis and Huntington's, where the use of riluzole is unwarranted.

Not only will the negative outcome of this study have an adverse effect on other planned trials of minocycline, but there may also be a need to track patients who have been prescribed minocycline off-label by their physicians. Although the use of off-label prescribing is understandable in relentless neurological diseases such as ALS with a poor prognosis and devastating outcome, there is a clear need to identify compounds with an adverse effect that only becomes obvious when large numbers of patients are exposed. Nevertheless, for people with ALS and their families, the prospect of entering a randomised controlled trial is exhausting and emotionally daunting, and this study will not help recruitment.

In this study, an attempt was made to overcome the variable rates of progression seen across patients by using a four-month lead-in period, which allowed within-patient comparisons. However, this method not only delays initiation of what might prove to be a life-saving therapy, but may allow the disease to progress to a stage where the measurement of the deterioration is no longer linear. The desire for earlier treatment is highly dependent on the development of techniques that would allow diagnosis and trial enrolment to be made at a stage where function is less impaired. Indeed, it is possible that the use of drugs thought to block apoptosis will only be beneficial when

used during a relatively benign stage of the disease. It is noteworthy that in animal studies the drug treatment is often initiated before the symptoms are apparent.

Perhaps more significantly, this study follows closely on the heels of the failure of a stroke trial<sup>11</sup> involving 1,588 patients in the treatment arm of the randomised trial in which the treatment was again based on the outcome of experiments on animal models, which showed NXY-059 to be efficacious. As in ALS, this trial followed many other

studies in humans that have failed to show a benefit from a drug proved to be potentially neuroprotective in animal models. The way forward is hard to see. However, there is clearly a need to design informative and sensitive studies based on small numbers of patients before large phase III studies are contemplated. Clearly, earlier diagnosis is crucial to such studies. Meanwhile, reviews and commentaries highlighting the possible weaknesses and defects in the execution of pre-clinical studies on animal models would be less than helpful.<sup>12-13</sup>

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