Phenytoin for neuroprotection in acute optic neuritis: a randomised, placebo-controlled, phase 2 trial

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Summary

Background: Acute optic neuritis (AON), a common feature of multiple sclerosis, can damage vision through neurodegeneration in the retina and optic nerve. Inhibition of voltage-gated sodium channels is neuroprotective in preclinical models. In this phase 2 trial we assessed whether sodium channel inhibition with phenytoin is also neuroprotective in AON.

Methods: We undertook a randomised, placebo-controlled, double-blind trial at two UK neuroscience centres. Patients with AON aged 18-60 years and presenting within two weeks of onset were randomly assigned 1:1 via a web-based service by minimisation to phenytoin (6mg/kg) or placebo for three months. Participants, treating and assessing physicians were all masked to group assignment. The primary outcome was affected eye retinal nerve fibre layer (RNFL) thickness at six months, adjusted for fellow eye RNFL thickness at baseline. The primary intention-to-treat analysis included all randomised participants assessed at six months. The trial is registered with ClinicalTrials.gov (NCT 01451593).

Findings: We recruited 86 participants between February 2012 and May 2014 (42 phenytoin, 44 placebo). Primary analysis included 81 participants (39 phenytoin, 42 placebo). Mean affected eye RNFL thickness at six months was significantly greater in the phenytoin group (81·46µm [SD 16·27] vs 74·29µm [SD 15·14] placebo; adjusted active-placebo difference 7·15µm [95% CI 1·08-13·22; p=0·021]), a 30% beneficial treatment effect when comparing the extent to which the RNFL thickness was lost in the two groups. Treatment was well tolerated, with five serious adverse events (13%) in the phenytoin group (but only one attributable to phenytoin) and two (5%) in the placebo group.

Interpretation: These findings support the concept of neuroprotection with phenytoin in AON. Inhibition of voltage-gated sodium channels should also be neuroprotective in other relapses of multiple sclerosis, and could thereby address a major unmet therapeutic need.

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system in which disability arises largely from neuroaxonal loss, which occurs in relapses and in progressive phases of the disease[1]. Corticosteroids hasten recovery from relapses without affecting the final prognosis for recovery[2,3,4], and immunomodulation has so far had limited effects on progressive disability[5]. Hence, neuroprotection for both processes contributing to disability remains a key unmet need in MS.

Different mechanisms are likely to contribute to neurodegeneration in relapses and in progressive disease. In acute relapses, there is growing evidence of a cascade arising from neuronal energy failure, leading in turn to reduced activity of the membrane Na^+-K^+ ATPase, accumulation of sodium ions entering mainly via $Na_V1{\cdot}6$ channels, reverse operation of the membrane Na^+:Ca^{2+} exchanger, and finally toxic accumulation of calcium ions[6]. $Na_V1{\cdot}6$ channels in microglia are also likely to play an important role in their activation and subsequent immune attack[7]. Consistent with this mechanism, voltage-gated sodium channels inhibitors are neuroprotective in several preclinical models of neuroinflammation[8-10], suggesting that they may also be neuroprotective in MS.

Phenytoin is a selective sodium channel inhibitor at concentrations therapeutic for epilepsy and is neuroprotective at these concentrations in experimental models[8-,9,11]. It can be loaded rapidly to achieve therapeutic serum concentrations within days. This property is important because experimental studies indicate that neuroprotection for relapses should be started as early as possible during the phase of acute inflammatory injury[10], an inflammatory penumbra which corresponds to approximately the first two weeks of a clinical episode, then potentially sustained until beyond the period of active inflammation, which may be detected for a median of two months after symptom onset[12].

The anterior visual system has many advantages for testing neuroprotection in MS[13]: acute demyelinating optic neuritis (AON) is a common and often presenting manifestation of MS; the inflammatory optic nerve lesion is comparable to plaques found elsewhere in the central nervous system; and the visual system can be studied using clinical, electrophysiological and imaging techniques. In addition, the optic nerve lesion leads to retrograde degeneration of the retinal nerve fibre layer (RNFL)[14], a relatively pure compartment of unmyelinated axons whose thickness can be measured sensitively and non-invasively using optical coherence tomography (OCT). Therefore, the RNFL thickness provides a plausible biomarker of axonal loss. Reduction of RNFL thickness also corresponds with visual loss in AON and with changes of disability in MS, suggesting that it may provide information on treatment response that is also clinically relevant [14].

From these considerations we undertook a phase 2 clinical trial to determine whether early and sustained sodium channel inhibition with phenytoin is neuroprotective in AON.

Methods

Study design

We carried out an investigator-led, randomised, parallel-group, double-blind, placebo-controlled phase 2 trial. There were two trial centres, in London and Sheffield UK. Participants were enrolled between February 2012 and May 2014. The study was approved by the London-South East UK Research and Ethics Committee. The full protocol is available online: https://www.ucl.ac.uk/ion/queen-square-multiple-sclerosis-centre/trial-protocol-neuroprotection-with-phenytoin-in-optic-neuritis

Participants

Patients who presented to the trial centres, or were referred there from a UK network of Patient Identification Centres, were eligible if they were 18-60 years old, had a clinical diagnosis of unilateral AON (confirmed by a neuro-ophthalmologist, and with no alternative pathology on OCT at presentation), visual acuity ≤6/9, and an interval of ≤14 days between onset of vision loss and randomization. Patients with a previous diagnosis of relapsing MS were eligible within 10 years of disease onset and with an Expanded Disability Status Scale score ≤3. Concurrent treatment with glatiramer acetate or beta-interferon was permitted and corticosteroids for AON could be given at the treating physician's discretion (all participants were offered equivalent regimens of methylprednisolone, either 1 g intravenously daily for three days, or 500 mg orally daily for five days[15]. Exclusions were: previous history of AON in either eye, co-morbid ocular disease, clinical or biochemical hepatic, renal or cardiac dysfunction (including abnormal electrocardiogram), contraindications to phenytoin (including pregnancy), disabling temperature dependent MS symptoms, use of sodium or calcium channel inhibitors in the preceding 2 weeks, corticosteroids (except for treatment of this episode of AON) or other immune therapies in the preceding 2 months, or seropositivity for aquaporin-4 antibodies, tested using a cell-based assay (Euroimmun UK). All participants gave written informed consent before entry.

Randomisation and masking

Participants were randomly assigned (1:1) to phenytoin or placebo via a website (www.sealedenvelope.com) by minimisation at 0·75 probability, with time from onset (≤7 days, >7 days), Centre (London, Sheffield), prior MS diagnosis (yes/no), disease modifying treatment (yes/no), and corticosteroids for AON (yes/no), as binary minimisation variables. The minimising allocation to active vs placebo was assigned with 0.75 probability, to reduce predictability. Participants were allocated a randomization code by the treating physician, which was matched to a confidential treatment list by the study pharmacist to assign participants either to phenytoin or placebo (which were identical in appearance). Only the pharmacist was aware of treatment allocation. Treating and assessing physicians as well as participants remained masked to treatment allocation.

Procedures

Participants were loaded orally with a total medication dose of 15mg/kg divided into three equal doses, each rounded up to the nearest 50mg, over three days, to achieve serum concentrations which are therapeutic for epilepsy, and which, as noted earlier, are neuroprotective in experimental models . A daily maintenance dose of

4mg/kg (rounded up to the nearest 50mg, with a maximum of 350mg) was given for 3 months, and was increased to 6 mg/kg from August 2013 at the recommendation of the Data Monitoring and Ethics Committee to achieve higher serum drug concentrations, as concentrations with the lower dose were considered to be subtherapeutic; the protocol was amended accordingly. 58 participants were given the lower dose, and 28 the higher dose. Participants were assessed by a treating physician after one and three months, and blood samples obtained to measure phenytoin concentration.

Outcomes

The primary endpoint was mean RNFL thickness in the affected eye at six months, measured with OCT.

Secondary structural endpoints were macular volume (MV), measured with OCT, and optic nerve cross-sectional area and lesion length, measured with magnetic resonance imaging (MRI). Secondary clinical endpoints were monocular high and low contrast letter visual acuities and colour perception. Latencies and amplitudes of the visual evoked potential (VEP) were also measured. Brain MRI was obtained at baseline for participants without a prior diagnosis of MS.

Primary and secondary endpoints were measured at baseline and six months by trained staff blinded to treatment allocation. The three-month gap between cessation of treatment and the final assessment was designed to allow any artefactual effects of sodium channel inhibition (eg pseudoatrophy)[16] to reverse before the final readout.

Optical coherence tomography: High resolution spectral domain OCT images (Spectralis, Heidelberg Engineering, Germany, Software V 5·4B) were acquired using identical protocols at both sites. Appropriate quality assurance was undertaken to ensure comparability, with acceptable inter-rater coefficients of variation for measurements of the RNFL (0.51%) and MV (0.45%) . RNFL measurements used a 3·45 mm diameter circle scan. A fast MV scan (20 x20° field, 25 horizontal B scans, ART 9) was also performed. Scans were excluded if they had a signal strength of <25 or violated international consensus quality control criteria[17].

Magnetic resonance imaging: MR images were obtained on two 3T scanners with identical scanning protocols at both sites. Each optic nerve was imaged separately and for all acquisitions the imaging plane for the optic nerves was set orthogonal to the longitudinal axis of the nerve.

The following sequences were performed: 1) A multi-dynamic fat-suppressed heavily T2-weighted multi-slice “single-shot” two-dimensional (2D) turbo spin echo (TSE)[18]; 2) a conventional fat-suppressed T2-weighted 2D-TSE; 3) a T1-weighted fluid attenuated inversion recovery (FLAIR) 2D-TSE. Lesion length and position were measured by three independent assessors (RR, AT, MY) masked to treatment allocation and participant identity, using a combination of the conventional and multidynamic T2 weighted sequences, and rare discrepancies were resolved by consensus, still based on the blinded data. Mean optic nerve cross-sectional area was measured by a blinded assessor using a semi-automated contouring technique on the baseline and six-month T1 weighted images. Mean lesional baseline and six-month cross-sectional areas were calculated by registering a baseline T2 lesion mask to the six-month T1 scan. Measurements were corrected for the corresponding

baseline mean 'non–lesional' cross-sectional area in the unaffected eye by applying the T2 lesion mask to baseline unaffected eye T1 images.

Vision: Low contrast letter scores were measured using retro-illuminated 1·25% and 2·5% Sloan charts (Precision Vision, La Salle, IL) using best refractive correction for each eye at two metres. Best corrected high contrast logMAR visual acuity was measured using retro-illuminated Early Treatment Diabetic Retinopathy Study charts at 4m. When no letters could be correctly identified a score of 1·7 was assigned. Colour vision was assessed using the Farnsworth Munsell 100 Hue test and recorded as the total error score. This was assessed under standard daylight conditions using daylight linear full spectrum bulbs with a colour temperature of 6,500K in participants with a logMAR visual acuity better than 1·0.

Visual evoked potential: VEPs to reversal achromatic checks (subtending 15 mins of arc visual angle) were recorded at both sites according to International Federation of Neurophysiology guidelines on a Synergy system in standard background office lighting. Responses were recorded from Oz using Fz as reference and Cz as ground. Latency and amplitude of the P100 component were measured to one decimal place in the replicates. Participants with absent VEP latencies or amplitudes were assigned a value of 200 and 0 respectively. At baseline 20 affected eyes in each group had their absent VEP latencies and amplitudes replaced this way and none in the baseline unaffected eye. At 6 months this replacement was made in three participants, all in the active group. Although the 200 value is arbitrary, it is higher than the highest measured value in the study – 188. It is therefore conservative to include these values at 6 months rather than to exclude them which would have reduced the mean latency of the active group.

Adverse events were recorded, and blood samples taken at each study visit to measure full blood count, liver and renal function.

Statistical analysis

The target sample size of 45 per arm was chosen to give 80% power to detect a treatment effect (reduction of the extent of loss of RNFL thickness) of 50% at 5% significance level, whilst allowing for a 20% combined rate of loss to follow-up and non-adherence. This was based on 35 per arm calculated from longitudinal OCT data on participants with acute demyelinating optic neuritis, as detailed in sample size calculations published previously[18]. This sample size calculation maximized power by assuming an active vs placebo comparison of follow-up affected eye RNFL thickness, adjusted for baseline fellow eye RNFL thickness. The fellow eye was chosen because acute swelling in the affected eye makes this eye a poor predictor of follow-up thickness, and makes affected eye change uninterpretable. Normal individuals have very similar RNFL thickness in both eyes, so the baseline fellow eye thickness provides a reliable estimate of affected eye RNFL thickness prior to AON. Henderson et al[19] found a correlation of r=0.63 (p=0.007) between the baseline fellow eye RNFL and the six-month affected RNFL.

Accordingly, an ANCOVA analysis method was used, using multiple linear regression of the follow-up affected eye RNFL on a trial arm indicator with the following pre-specified covariates: baseline fellow eye value, centre (binary), days between onset and baseline assessment, and whether the participant was prescribed corticosteroids at the time of baseline assessment (three categories: no/1-5 days prior to assessment/6-30 days prior). Two planned binary covariates were not used

because of a pre-specified minimum of 10 for their smallest category: “Prior MS” (4 yes, 82 no) and “Prescribed disease-modifying treatment” (1 yes, 85 no). Secondary outcomes were analyzed similarly, with the corresponding baseline fellow-eye value and the same pre-specified covariates. An exception was lesion length, for which the baseline fellow eye was not specified as covariate; also, for imaging outcomes only, centre was not used as a covariate due to only three participants undergoing MRI at one of the sites (Sheffield).

The primary intention-to-treat (ITT) analyses included all randomized participants who were followed up. Secondary per protocol (PP) analyses, after excluding participants with a subsequent further episode of optic neuritis, compared all placebo participants with just adherent active participants, defined as having phenytoin present in their one-month blood; however, this PP comparison has the potential for bias since there is no placebo subset corresponding to the adherent active subset.

Where regression residuals showed signs of non-normality and/or heteroscedasticity, p-values were checked using a permutation test, but none of the reported p-values required correction. Statistical significance, where referred to, indicates $p<0{\cdot}05$ and all p-values refer to two-tailed tests. Analyses were conducted in Stata 13.1 (Stata Corporation, College Station, Texas, USA).

The study was overseen by a Data Monitoring and Ethics Committee independent of the study group (Dr Zoe Fox, Prof Richard Hughes, Dr Brennan Kahan, and Prof Christopher Kennard), and is registered with ClinicalTrials.gov (NCT 01451593).

Role of Funding Source

Neither the funders of the study, nor the Sponsor (University College London), had a role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Participants were recruited between February 2012 and May 2014, and final assessments were performed in December 2014. None of the eligible participants had antibodies to aquaporin-4; exclusions for other reasons are detailed in figure 1. 86 participants (70 London, 16 Sheffield) were randomly assigned to receive phenytoin (n=42) or placebo (n=44, Figure 1). The two groups had similar baseline characteristics (Table 1). 28 participants (33%) either had a prior diagnosis of MS, or were diagnosed with MS upon presentation, while 68 (79%) had brain lesions on MRI.

Five participants were lost to follow up, leaving 81 who attended for assessment of the primary outcome at six months (39 phenytoin, 42 placebo). Of these, 10 in the phenytoin group were withdrawn from treatment due to skin rash after a mean of 18·4 days from starting treatment, but continued to be followed up. The remaining 29 in the phenytoin group were serum adherent (mean serum phenytoin concentration 7 mg/L). The combined overall rate of loss to follow-up, withdrawal from treatment and non-adherence was 19%.

RNFL thickness and macular volume remained stable in the unaffected eye, with insignificant change between baseline and six months (Table 2). Six-month affected RNFL was significantly correlated with baseline unaffected RNFL ($r=0{\cdot}50$, $p<0{\cdot}001$) but not with baseline affected RNFL ($r=0{\cdot}13$, $p=0{\cdot}253$).

The mean RNFL thickness in the affected eye fell after 6 months compared with the baseline unaffected eye by 23·79 µm (24%) in the placebo group, and 16·69 µm (17%) in the phenytoin group, giving a significantly higher mean 6-month affected eye RNFL thickness in the phenytoin group compared to placebo (Figure 2). The ITT adjusted phenytoin-placebo mean 6-month affected eye RNFL difference was 7·15 µm (95% CI 1·08, 13·22; $p=0{\cdot}021$), indicating a 30% beneficial treatment effect; the corresponding PP adjusted difference was similar, 7·40 µm (95% CI 0·76, 14·04; $p=0{\cdot}029$).

The mean macular volume in the affected eye fell after six months compared with the baseline unaffected eye by 0·59mm^3 (7%) and 0·39 mm^3 (4%) in placebo and phenytoin groups respectively, giving a significantly higher mean 6-month affected eye MV in the phenytoin group compared to placebo (Figure 2). The ITT adjusted phenytoin-placebo mean 6-month affected eye RNFL difference was 0·20 mm^3 (95% CI 0·06, 0·34; $p=0{\cdot}005$), a 34% beneficial treatment effect; the corresponding PP adjusted difference was again similar, 0·21 mm^3 (95% CI 0·05, 0·36; $p=0{\cdot}010$)

ITT comparison showed no significant difference in optic nerve lesion length between the groups at 6 months (mean adjusted active-placebo difference -2·45mm, CI 95% -6·97, 2·08, $p=0{\cdot}285$). However, there was a borderline significant treatment effect on lesional optic nerve cross-sectional area, with an adjusted active-placebo difference of 0·40 mm^2 (95% CI -0·02, 0·83, $p=0{\cdot}061$) a 38% treatment effect. The corresponding adjusted difference in cross-sectional area in the PP comparison was 0·47mm^2 (95% CI -0·04, 0·97, $p=0{\cdot}070$).

There were no significant ITT or PP active-placebo differences in visual function, VEP latency or amplitude at 6 months (Table 3).

A number of exploratory analyses were undertaken. In 10 active participants who discontinued treatment due to skin rash, the adjusted mean active-placebo

difference in affected eye RNFL thickness was 8·29 µm, similar to the difference observed in the whole ITT sample. Primary analysis treatment effects for participants with normal and abnormal brain MRI brain scans at baseline were 7·90 (p=0·029) and 4·34 (p=0·496) respectively, but the difference was not significant (p=0·629); however, the study was not powered to explore such subgroup analyses. There were no significant associations in the active group between 1-month phenytoin concentrations and any of the primary or secondary outcomes, apart from a borderline significant positive correlation with optic nerve cross-sectional area (r=0·39, p=0·063). There were no significant correlations between any of the primary or secondary outcome measurements and the time from onset of visual loss to initiation of treatment.

Similar proportions of participants in the phenytoin and placebo groups experienced adverse events (Table 4). A higher, but non-significant, proportion of participants in the active group discontinued the study medication due to an adverse event (in all cases this was a maculopapular rash typical of a drug reaction). With the exception of a severe rash in one participant, serious adverse events were not attributable to phenytoin (Table 5). 63% of participants guessed their treatment allocation (p=0.033), due to all patients with skin rashes guessing correctly that they were in the active group. When these numbers were excluded, correct guesses lost significance (58%, p=0.228).

There was no impairment of vision or other neurological function with initial loading or following withdrawal of phenytoin. None of the participants developed steroid dependent visual failure, evidence of neuromyelitis optica, or evidence of other causes of optic neuritis, during the follow up period. Demyelinating relapses occurred in 17% of participants in the phenytoin group, and 21% in the placebo group. Of these, 6 participants had another episode of AON (4 placebo, 2 phenytoin). Of these, three (one phenytoin, 2 placebo) affected the contralateral eye. These participants were excluded from the per protocol analysis. In total, 13 participants experienced an MS defining relapse during the study.

Discussion

In this phase 2 clinical trial, use of phenytoin was associated with a 30% reduction of the loss of the RNFL after AON compared to placebo, and of loss of MV by 34%. Together with a near significant 38% reduction in loss of optic nerve cross-sectional area, these results are consistent with the suggestion that phenytoin protected the compartment comprising the retinal ganglion cells (which make up 34% of macular volume) and their axons in the RNFL and optic nerve, and they support the concept of neuroprotection with partial inhibition of voltage-gated sodium channels in an episode of inflammatory demyelination.

In contrast to the beneficial effects of treatment on structural outcomes, we observed no significant treatment effects on visual outcomes or on the VEP. While we cannot exclude the possibility that treatment protected neurons and their axons which nevertheless remained non-functional, the findings are perhaps more consistent with the floor effect we observed on high contrast visual acuity and VEP amplitude, which recovered well in both phenytoin and placebo groups, and with the fact that the trial was not powered to detect treatment effects on these and on low contrast acuity and colour vision. In addition, we relied on central and whole field VEPs to measure optic nerve function, whereas more sensitive determination of treatment effects may be possible using multifocal VEPs in future trial designs.

Translation of tissue protection into improved visual function is also limited by redundancy in the anterior visual system[20] and neuroplasticity in higher visual areas, so that better neuroprotection may be required for improved clinical outcomes in reasonably sized trials. For this, trials might consider: 1) more potent and specific inhibitors of sodium channels; 2) higher drug concentrations (mean phenytoin concentration in this trial were possibly sub-therapeutic); and 3) an earlier window of treatment in the evolution of relapse. The last two suggestions are consistent with the lack of correlation between structural outcomes and the concentration of phenytoin and time to initiation of treatment in the present study. Conversely, our results do not place a lower limit on the duration for which treatment is required for successful neuroprotection. We treated participants for 3 months, which is beyond the interval when gadolinium enhancement indicates inflammation in the optic nerve[12], yet an exploratory analysis showed improved OCT outcomes in those who withdrew after receiving phenytoin for only a mean of 18·4 days.

Concerning potential sources of bias, it is worth noting that the characteristics of the placebo and phenytoin groups at baseline were generally comparable, typical of AON, and that the loss of RNFL thickness in the placebo group after 6 months was consistent with previous natural history studies of AON[19,21]. Care was taken to exclude patients with atypical AON, and none of the participants developed features of conditions such as neuromyelitis optica (for which antibodies were also tested at presentation), or chronic relapsing inflammatory optic neuropathy (CRION). After the study started, further immunological subtypes of optic neuritis have been suggested (for example those with antibodies to myelin oligodendrocyte glycoprotein[22]) and it would be important to include appropriate testing for these subtypes in future studies for any differences in their response to neuroprotective therapies. In the present study, 41 out of 86 participants had (or later developed) MS, or had a clinically isolated syndrome. In keeping with a demyelinating aetiology,

three quarters of participants had brain lesions on MRI at baseline[23]. Those with and without brain lesions could not be distinguished clinically or with other measurements at baseline, nor during follow up, suggesting that the study involved a largely homogeneous AON population. In addition, there was no statistically significant difference in the primary treatment effect between the groups with and without brain lesions.

Baseline low contrast letter acuity, a predictor of RNFL thickness at six months[19], was noted to be a little worse in the active group. This would bias the active group towards a lower six month RNFL thickness. The opposite finding, of a higher six month RNFL thickness in the active group, is therefore all the more consistent with a neuroprotective treatment effect.

As expected, there was swelling of the RNFL in the affected eye at baseline, supporting the use of the fellow eye RNFL at baseline for comparisons of change in the affected eye at follow up. This method is prone to error if the fellow eye is not actually normal because of previous and possibly subclinical episodes of AON, but this is unlikely to have affected the present study: none of the participants had experienced visual symptoms previously, and measurements of the fellow eye revealed no significant subclinical abnormalities. Future trial designs may avoid this issue by using OCT segmentation methods to compare the retinal ganglion cell layer at baseline and follow up in the affected eye alone, since this structure does not swell acutely in AON[24].

Corticosteroid treatment at presentation is unlikely to have influenced the main neuroprotective findings because care was taken to adjust the analysis for the use and timing of corticosteroids. Also, measurements of the RNFL and of MV remained stable in the fellow eye in both the phenytoin and placebo groups, and previous studies show that corticosteroids do not prevent atrophy of the RNFL[25] or optic nerve[26], nor visual recovery after optic neuritis[2].

A final consideration is the extent to which the study excluded people with AON whose contribution might reflect the response to treatment in real world settings. The details of participation (Fig 1) suggest that the inclusion criteria excluded mainly those with characteristics of AON (eg prolonged symptom duration), or alternative causes of visual failure, which would be unlikely to benefit from sodium channel blockade: only the 25 out of 188 at screening for inclusion, who declined participation, were not able to contribute to the study. If successful, the treatment might be offered in addition to a small group with AON currently excluded from the study, ie those with typical AON and outside the current age range, or else with more severe or longstanding MS. It is unlikely that such a group would be vulnerable to any additional risk from the treatment.

Treatment with phenytoin was generally well tolerated, and was not associated with significant abnormalities in the blood count or liver function. We did not observe any acute deterioration of vision that might be attributed to conduction block from inhibition of sodium currents, nor any rebound deterioration upon withdrawal of treatment, effects which had previously been considered to potentially limit the use of drugs acting on this target in demyelinating disorders. Only one participant experienced a severe adverse reaction, a skin rash, attributable to phenytoin, but nine further participants developed minor, self-limiting skin rashes and were withdrawn from treatment by the investigators according to protocol. As noted

above (Results) blinding of participants to treatment allocation was unmasked in some cases due to skin rashes. While this might have an effect on patient based clinical assessment, the primary outcome, which was assessed fully blinded, should not be affected.

As a result of patient withdrawal from treatment, 10 of the 39 active participants available at follow-up were classified as non-adherent. While this may affect the power of the ITT analysis, the robustness of the results is supported by the agreement between the ITT and PP analyses, and the consistency of treatment effects in the macula, RNFL and optic nerve; and though there were more missing values in the optic nerve analysis, these were due to MRI acquisition problems, which are very plausibly missing at random and unlikely to cause bias.

Our results support the utility of OCT for measuring outcome in future trials of neuroprotection in AON, and consolidate the experience of OCT in previous small trials of other agents[27-29]. In comparison to OCT, MRI of the optic nerve is limited by the effects of myelin and other supporting tissue, and by lower resolution. OCT is also easier to use and costs less. In the previous trials, memantine reduced the loss of the RNFL[27], while erythropoietin was effective in one trial[28] but not in another[29]. Our study addressed limitations of these studies by correcting measurements in the affected eye for baseline measurements in the unaffected eye, and reporting MV data as well as more detailed MRI data.

At the average concentration achieved in this trial, phenytoin is an almost pure activity-dependent inhibitor of voltage-gated sodium channels[30]. By analogy, other sodium channel inhibitors could also be neuroprotective in AON and, given its similarities to other relapses of MS, in those relapses as well. In turn, the present trial design should enable proof of concept of neuroprotection after relapse for treatments with other modes of action. Implications for treating progressive MS are harder to define because of possible differences in pathophysiology: microglial activation is likely to remain important in progressive disease, whereas sodium channel expression may change[31]. Previously, we reported a trial of neuroprotection using lamotrigine to inhibit sodium channels in secondary progressive MS[16]. Treatment did not affect the rate of cerebral atrophy, although interpretation was hampered by a high rate of non-adherence, and there were positive treatment signals, including significant slowing of the rate of deterioration of the timed walk and lower serum neurofilament concentrations in the adherent group of participants[32].

In conclusion, the results of this clinical trial support the concept of neuroprotection using phenytoin to inhibit voltage-gated sodium channels in AON. These results should encourage larger, phase 3 trials of sodium channel inhibitors in optic neuritis and other relapses of MS. Future studies should also establish more precisely the optimal therapeutic window for neuroprotection in relapses.

Contributors

RK was the principal investigator and RS the project manager. DRA, KS, GG, DHM, and RK contributed to the concept and design of the study. DRA did the statistical plan and statistical analysis. RR, SH, AT, and BS recruited the participants and followed them up. SM and DP did the six-month masked vision and OCT measurements. RR, SH, AT, MY, NH, CGW-K, and DHM were responsible for MRI acquisition and analysis, and RR, PM, PS, and MK for VEP acquisition and analysis. RR, DRA, and RK wrote the first draft, and all the authors contributed to and approved the final version.

Declaration of interests

RR, RS, DRA, DHM and RK report grants from The MS National MS Society and grants from MS Society of Great Britain and Northen Ireland, during the conduct of the study; RK also reports grants from Novartis, during the conduct of the study; in addition, RK has a patent pending. Outside the submitted work: AT reports personal fees from from Biomedia, EXCEMED (formerly SSIF) and Bayer and meeting expenses from Biogen Idec; DP reports personal fees from Teva pharmaceuticals; DRA reports personal fees from Merck & Co., Inc.; MK reports grants and personal fees from Pfizer, personal fees from GSK, Merck, Neuromerics and Calchan; CGW-K reports clinical trials work from Biogen Idec and Novartis; KS reports clinical trials work from Roche, Teva and Novartis; personal fees from Biogen Teva, and Novartis; GG reports clinical trial steering committees from AbbVie, Biogen, Novartis Teva, and Roche; personal fees from Biogen, GSK, Merck-Serono, Novartis, Genzyme-Sanofi, and Synthon BV; and Co-chief editor Multiple Sclerosis and Related disorders (Elsevier); DHM reports grants from UCL/UCLH Biomedical Research Centre, during the conduct of the study; grants from Biogen, GSK, NIHR, Novartis, and Apitope, and Board membership from Biogen Idec, GSK, Bayer Schering Pharma, and Mitsubishi Pharma Europe; consultancies from Merck and Chugai; personal fees from McAlpine's Multiple Sclerosis, 4th edition; RK reports clinical trial steering committees with Biogen; personal fees and travel support from Biogen, Genzyme, grants, Novartis, and Teva; personal fees from Roche, and KaroBio; SH, BS, SM, MY, PM, PS, NH have nothing to disclose.

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Table 1

Baseline clinical, structural and electrophysiological characteristics of the affected eye, and baseline retinal nerve fibre layer thickness of the unaffected eye

	Phenytoin (n=42)	Placebo (n=44)
Age (yrs)	33 (8·2)	35 (9·1)
Women	31 (74%)	32 (73%)
Days from onset to randomization	8·2 (3·1)	8·1 (3·3)
Prescribed corticosteroids	35 (83%)	33 (75%)
Clinical Diagnosis: Prior diagnosis of multiple sclerosis Multiple sclerosis diagnosed at screening[a] Clinically isolated syndrome	 1 (2%) 13 (31%) 28 (67%)	 3 (7%) 11 (25%) 30 (68%)
≥ 1 T2 hyperintense MRI brain lesion	32(73%)	32(76%)
RNFL thickness affected eye (µm)	130·62 (46·4)	125·20 (43·4)
RNFL thickness unaffected eye (µm)	98·02 (11·08)	98·36 (10·99)
Macular volume (mm^3)	8·71 (0·46)	8·63 (0·43)
LogMAR visual acuity	1·11 (0·54)	1·07 (0·60)
1·25% low contrast letter score	0·07 (0·46)	0·45 (3·02)
2·5% low contrast letter score	0·21 (1·24)	0·77 (3·83)
FM 100-Hue total error score	1066 (764·6)	1139 (775·5)
VEP latency (ms)[b]	167·9 (35·2)	167·6 (35·8)
VEP amplitude (µV)[b]	2·8 (3·8)	3·0 (3·8)
Optic nerve cross-sectional area (mm^2)	7·60 (1·55)	7·48 (1·43)
Lesion length (mm)	17·2 (8·1)	18·0 (7·1)

Data are mean (SD), or number (%)

[a]Multiple sclerosis diagnosed using the 2010 McDonald criteria[33]

[b]Twenty subjects in each group had no VEP response in the affected eye, but the baseline affected eye VEP was not used in analyses.

Table 2: Stability of measurements in the unaffected eye in the active and placebo groups

	Phenytoin		Placebo	
	Baseline	6 months	Baseline	6 months
RNFL thickness (*u*m)	98·02 (11·08)	98·69 (11·62)	98·36 (10·99)	97·37 (13·18)
Macular volume (mm^3)	8·67 (0·41)	8·66 (0·40)	8·67 (0·39)	8·63 (0·43)
LogMAR visual acuity	0·03 (0·10)	-0·05 (0·13)	-0·08 (0·08)	-0·08 (0·10)
Low contrast letter score (1·25%)	26·33 (9·90)	27·79 (10·47)	29·48 (10·35)	27·76 (9·75)
Low contrast letter score (2·5%)	32·86 (9·55)	33·62 (9·65)	34·52 (9·96)	34·14 (10·77)
FM 100-Hue total error score	88·19 (49·20)	84·62 (54·56)	90·88 (56·49)	95·33 (106·56)
VEP latency (ms)	104·0 (6·3)	108·3 (19·8)	104·8 (5·9)	106·1 (6·2)
VEP amplitude (µV)	10·2 (5·0)	9·5 (5·0)	10·8 (5·9)	9·8 (6·1)
Optic nerve cross-sectional area (mm^2)	5·51 (0·90)	5·15 (0·82)	5·32 (0·73)	5·41 (0·72)

Data are mean (standard deviation)

Table 3: Baseline and 6 month measurements in the affected eye by treatment group, and intention to treat comparison of primary and secondary outcomes at six months

	Baseline		6 months			
	Phenytoin Mean (SD)	Placebo Mean (SD)	Phenytoin Mean (SD)	Placebo Mean (SD)	Adjusted¶ six month active-placebo difference (95% CI)	p value
RNFL thickness (*u*m)	130·62 (46·4) n=42	125·20 (43·4) n=44	81·46 (16·27) n=39	74·29 (15·14) n=42	7·15 (1·08, 13·22)	0·021
Macular volume (mm^3)	8·71 (0·46) n=42	8·63 (0·43) n=44	8·25 (0·45) n=39	8·07 (0·42) n= 41	0·20 (0·06, 0·34)	0·005
LogMar visual acuity	1·08 (0·56) n=42	1·04 (0·62) n=44	0·09 (0·40) n=39	0·04 (0·18) n=42	0·02 (-0·11, 0·16)	0·728
1·25% low contrast letter score	0·07 (0·46) n=42	0·45 (3·02) n=44	13·38 (12·14) n=39	12·33 (12·13) n=42	1·19 (-4·16, 6·53)	0·660
2·5% low contrast letter score	0·21 (1·24) n=42	0·77 (3·83) n=44	19·69 (13·80) n=39	17·55 (14·19) n=42	2·07 (-4·10, 8·25)	0·506
FM 100-Hue total error score	1066 (764·6) n=42	1139 (775·5) n=43	181·28 (223·79) n=39	195·24 (212·61) n=42	-18·46 (-116·44,79·51)	0·708
VEP latency (ms)[a]	167·9 (35·2) n=39	167·6 (35·8) n=43	133·0 (24·8) n=35	127·4 (19·3) n=40	5·71 (-4·56, 15·99)	0·271
VEP amplitude (*u*V)[a]	2·7 (3·8) n=39	3·0 (3·8) n=43	7·1(4·6) n=35	7·3 (4·6) n=40	-0·18 (-1·83, 1·46)	0·827
Lesion length (mm)	17·2 (8·1) n=39	18·0 (7·1) n=42	15·15 (7·62) n=34	17·17 (10·11) n=36	-2·45 (-6·97, 2·08)	0·285
Lesional optic nerve cross-sectional area (mm^2)	7.60 (1.55) n=34	7.48 (1.43) n=39	4·58 (0·88) n=31	4·48 (1·01) n=34	0·40 (-0·02-0·83)	0·061

Data are mean (SD) and number of participants

¶ Pre-specified adjustment for baseline unaffected value, centre, days between onset and baseline, days between steroid and baseline; centre was dropped for optic nerve area, and centre and baseline unaffected value were dropped for lesion length

[a] The six month comparison includes three participants on active drug, and none on placebo,with vision too poor to obtain VEP response, for which amplitudes of 0µV and latencies of 200ms were used. The baseline summaries include 20 active and 20 placebo patients with vision too poor, for which these imputations were used. All the unaffected eye VEPs were recordable. Excluding the three subjects gives active – placebo differences of -0.22, p=0.955 (latency) and 0.36, p=0.647 (amplitude).

Table 4: Adverse events

Adverse Events	Phenytoin	Placebo
At least one adverse event	34 (81)	40 (91)
At least one adverse event leading to discontinuation of study medication	10 (24)	3 (7)
Any serious adverse event	5 (12)	2 (5)
Any event leading to death	0	0
Mean number (range) of all adverse events per participant	3·17 (0, 10)	3·64 (0, 14)

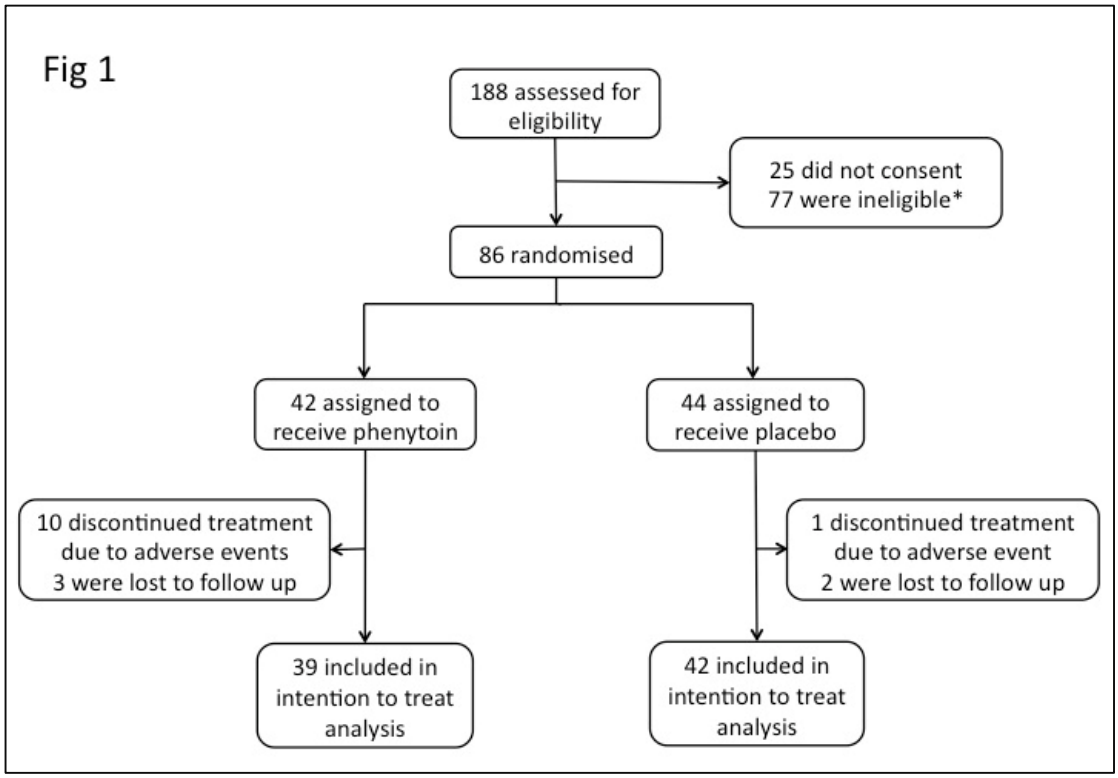
Data are number (%), except for last row

Table 5: Serious Adverse Events

	Phenytoin	Placebo
Breast Malignancy	1	0
Dilated superior ophthalmic vein seen on MRI (requiring catheter angiogram)	1	0
Appendicitis	2	0
Cellulitis	0	1
Severe rash	1	0
Congenital malformation	0	1

Data are number of each adverse event.

Figure 1: Trial profile



* Reasons for ineligibility were as follows:

- 28 visual acuity better than allowed
- 11 time to presentation longer than allowed
- 5 had bilateral optic neuritis
- 3 had previous optic neuritis
- 1 treated with long term immunosupression
- 9 had an uncertain diagnosis
- 20 had alternative diagnoses (4 functional visual loss; 3 sarcoidosis; 2 each: migraine with aura, posterior scleritis, Leber's hereditary optic neuropathy, compressive optic nerve lesions; 1 each: uveitis, toxic optic neuropathy, neuroretinitis, central serous retinopathy, optic nerve drusen)

Figure 2: Retinal nerve fibre layer thickness and macular volume, by trial group. Retinal nerve fibre layer thickness in the unaffected and affected eyes at baseline (a) and at six months (b); macular volume in the unaffected and affected eyes at baseline (c) and at six months (d). Active-placebo comparisons were based on the difference between the six month active eye and baseline unaffected eye. Bars are standard errors around unadjusted group means.

Fig 2a

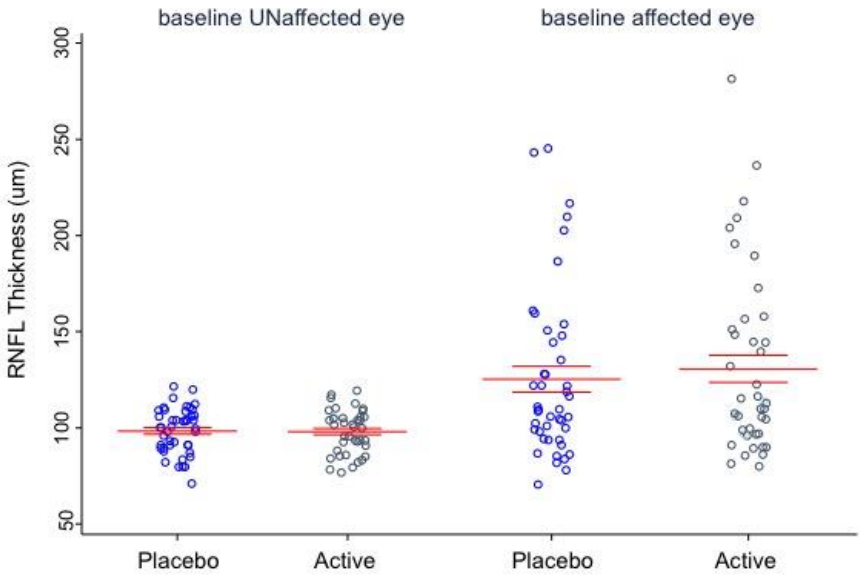


Fig 2b

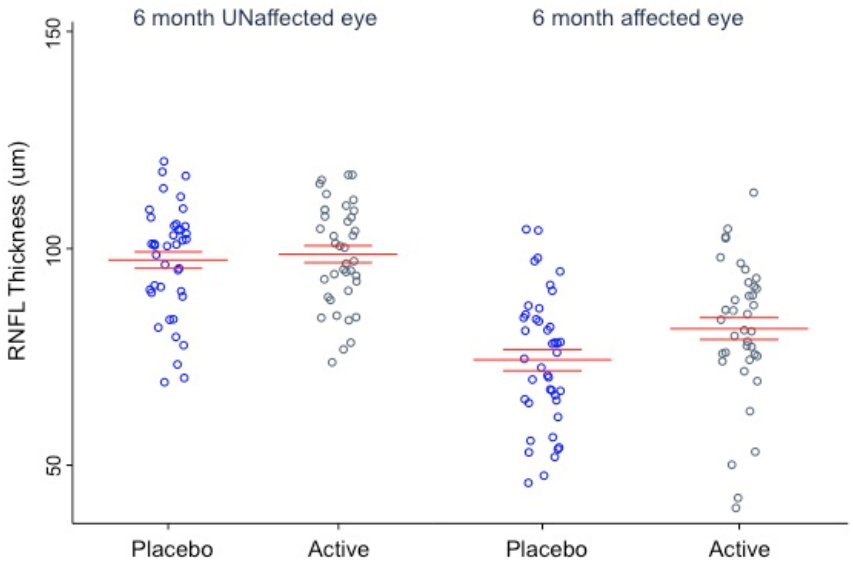


Fig 2c

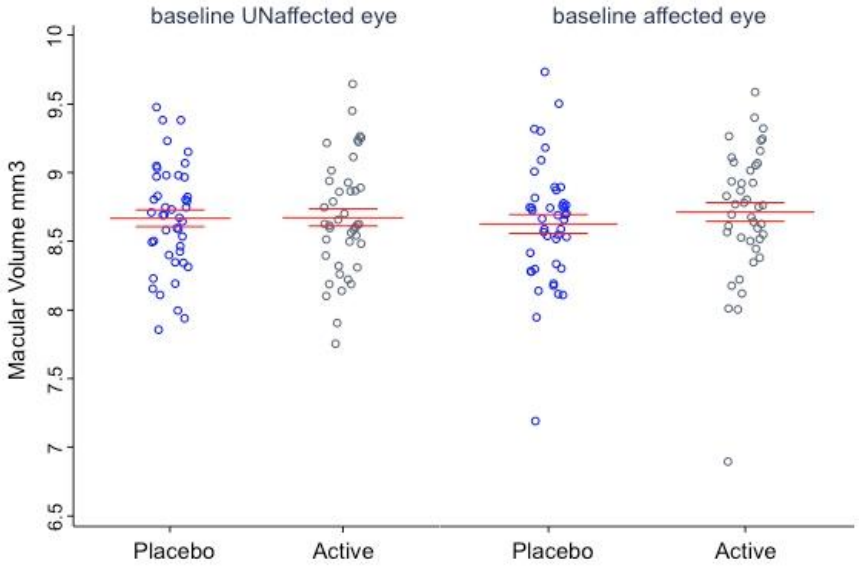
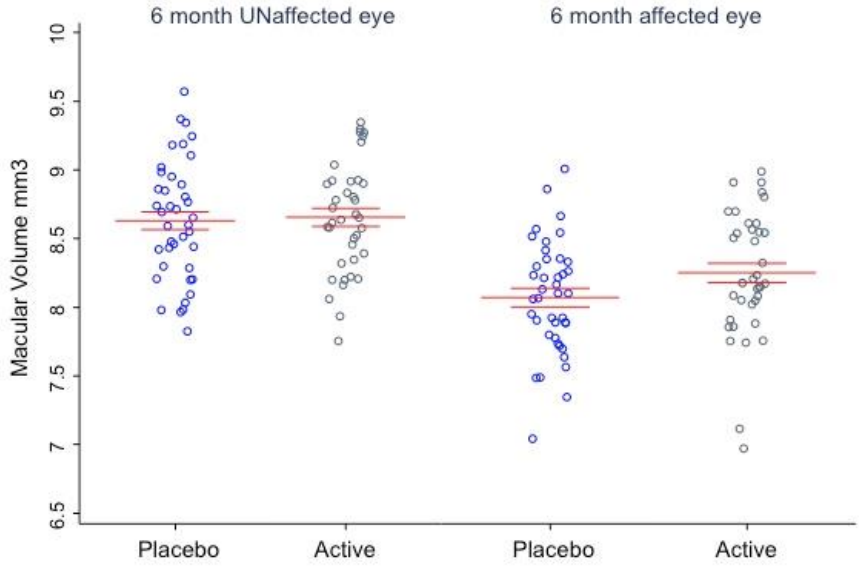

baseline UNaffected eye
baseline affected eye
Macular Volume mm3
10
9.5
9
8.5
8
7.5
7
6.5
Placebo
Active
Placebo
Active

Fig 2d


6 month UNaffected eye
6 month affected eye
Macular Volume mm3
10
9.5
9
8.5
8
7.5
7
6.5
Placebo
Active
Placebo
Active