Ascorbic acid for Charcot–Marie–Tooth disease type 1A in children: a randomised, double-blind, placebo-controlled, safety and efficacy trial

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Summary

Background Charcot–Marie–Tooth disease type 1A (CMT1A) is the most common inherited nerve disorder. CMT1A is characterised by peripheral nerve demyelination, weakness, and impaired motor function and is caused by the duplication of *PMP22*, the gene that encodes peripheral myelin protein 22. High-dose ascorbic acid has been shown to have remyelinating potential and to correct the phenotype of a transgenic mouse model of CMT1A by decreasing expression of *PMP22*. We tested the efficacy and safety of ascorbic acid supplementation in children with CMT1A.

Methods This 12-month, randomised, double-blind, placebo-controlled trial undertaken between June, 2007, and December, 2008, assessed high-dose oral ascorbic acid (about 30 mg/kg/day) in 81 children with CMT1A (2–16 years). Randomisation was done on a 1:1 ratio by a computer-generated algorithm. All investigators and participants were blinded to treatment allocation with the exception of the trial pharmacist. The primary efficacy outcome was median nerve motor conduction velocity (m/s) at 12 months. Secondary outcomes were foot and hand strength, motor function, walking ability, and quality of life. Compliance was measured by plasma ascorbic acid concentration, pill count, and medication diary entries. Analysis was by intention to treat. This trial is registered with the Australian New Zealand Clinical Trials Registry, number 12606000481572.

Findings 81 children were randomly assigned to receive high-dose ascorbic acid (n=42) or placebo (n=39). 80 children completed 12 months of treatment. The ascorbic acid group had a small, non-significant increase in median nerve motor conduction velocity compared with the placebo group (adjusted mean difference 1·7 m/s, 95% CI –0·1 to 3·4; p=0·06). There was no measurable effect of ascorbic acid on neurophysiological, strength, function, or quality of life outcomes. Two children in the ascorbic acid group and four children in the placebo group reported gastrointestinal symptoms. There were no serious adverse events.

Interpretation 12 months of treatment with high-dose ascorbic acid was safe and well tolerated but none of the expected efficacy endpoints were reached.

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Introduction

Charcot–Marie–Tooth disease type 1A (CMT1A) is the most common inherited neuropathy. CMT1A is characterised by demyelination of the peripheral nerves, progressive weakness of the distal limb musculature, cavus foot deformity, impairment of motor function, and sensory loss. CMT1A is most commonly caused by the duplication of *PMP22*, the gene that encodes peripheral myelin protein 22, on chromosome 17p11.2. Duplication of *PMP22* is thought to cause over-expression, leading to a toxic gain of function. Natural history data in patients with CMT1A aged 4–76 years show slow progression with age, resulting in an annual average functional deterioration of 5% and slowing of motor conduction velocity of the median nerve, a sign of demyelination, of about 0·6 m/s per year.[1] There is currently no cure or effective treatment for CMT1A.

In 2004, Passage and co-workers[2] described the role of ascorbic acid in correcting the phenotype of a mouse model of CMT1A (C22). They showed that high-dose oral ascorbic acid, a known promoter of myelination, significantly ameliorated the CMT1A phenotype by reducing expression of *PMP22* in the Schwann cells of peripheral nerves in 63 transgenic mice force fed oral ascorbic acid (1·12 mg for a 20 g mouse) once a week for 3 months. The mice had improved strength, balance, agility and endurance, reactivated myelination in their sciatic nerves, and down-regulation of *PMP22* over-expression. Remyelination was thought to underlie these improvements.[2]

Subsequent studies have shown that high-dose ascorbic acid reduces *PMP22* mRNA in a dose-dependent manner by decreasing adenylate cyclase activity and intracellular cAMP concentrations.[3] As a promoter of myelination, ascorbic acid is therefore a potential treatment for CMT1A. The study by Passage and co-workers[2] has provided the foundation for several ongoing trials, and ascorbic acid is currently the most active area of research for patients with CMT1A.[4]

Ascorbic acid, an essential micronutrient, is safe, palatable, inexpensive to produce, and has regulatory approval by the Therapeutic Goods Administration (TGA) in Australia and the US Food and Drug Administration (FDA) for the treatment of deficiency-related disorders. Orphan designation has been granted by the European Commission for the treatment of CMT1A. Although several trials of the efficacy of ascorbic acid in adults with CMT1A are ongoing, starting treatment at a young age might have more benefit because the most active phase of demyelination and axonal loss in CMT1A occurs during childhood.[5]

Muscle weakness in CMT1A indicates axonal loss secondary to demyelination of peripheral nerve fibres during childhood.[6] Neurophysiological changes in patients with CMT1A parallel this nerve pathology and typically antedate the functional manifestations.[7] Early prevention of peripheral nerve demyelination and axon loss might therefore prevent the neurological deficits of CMT1A. The aim of this study was to measure the efficacy, safety, and tolerability of oral ascorbic acid supplementation on neurophysiological function, muscle strength, functional ability, and quality of life in children with CMT1A.

Methods

Patients

Children aged 2–16 years with a confirmed diagnosis of CMT1A (ie, a duplication in the 17p11.2 locus that includes *PMP22* or confirmed duplication in a first-degree or second-degree relative, with a consistent clinical phenotype and confirmatory neurophysiological testing [median nerve motor conduction velocity <35 m/s] in the child)[1] were eligible for inclusion. Exclusion criteria were: the use of drugs that predispose the patient to neuropathy; pregnancy; clinical signs of nephrolithiasis; glucose-6-phosphate dehydrogenase deficiency; a diagnosis of hereditary haemochromatosis, thalassaemia, or polycythaemia; leukaemia, sideroblastic anaemia, or sickle cell anaemia; acute foot injuries (eg, ankle sprain) or recent foot surgery; or a diagnosis of inflammatory arthritis, diabetes, or peripheral neurological disorders other than CMT1A.

The protocol was approved and monitored by the institutional ethics review boards at each hospital, in accordance with the Australian TGA. Ethics approval was obtained from the Children's Hospital at Westmead (ethics approval reference number 2006/056), the University of Sydney (ethics approval reference number 9733), and the Royal Children's Hospital, Melbourne (ethics approval reference number 26144A). One parent or guardian provided written informed consent for each child.

Procedures

Patients were randomly assigned to oral ascorbic acid (250 mg) or oral placebo for 12 months. All tablets had identical appearance and orange flavour and could be chewed or mixed with food or drink. A twice-daily dose was prescribed to reduce crystal formation, to reduce the risk of adverse events, and to ensure near-maximum tissue concentrations and absorption with minimum urinary excretion.[8] Supplements were prepared and packaged in identical containers, stored in the central pharmacy, and given to the parents after randomisation. The doses used in this trial were based on the tolerable upper safe limit by age group recommended by the Food and Nutrition Board of the US Institute of Medicine.[8] After adjusting for the recommended dietary allowance and tablet size (multiples of 250 mg tablets or 125 mg half tablets divided with a pill cutter), children aged 2–3 years received 375 mg/day (11 times the recommended daily intake [RDI]), children aged 4–8 years received 625 mg/day (18 times the RDI), children aged 9–13 years received 1125 mg/day (28 times the RDI), and children aged 14–16 years received 1625 mg/day (41 times the RDI). These doses approximated to 30 mg/kg/day.

All primary and secondary outcomes were measured at baseline and after 12 months. The primary outcome was median nerve motor conduction velocity (m/s), which is a measure of myelin integrity. This variable was selected as the primary outcome because it is clearly abnormal in all children with CMT1A aged between 2 and 16 years.[9] Furthermore, with strict adherence to a standardised protocol (ie, temperature, side of testing, stimulation technique, and placement of electrodes), motor conduction velocities have a coefficient of variation of only 3% in healthy participants and in patients with peripheral neuropathy.[10] Secondary neurophysiological outcomes were compound muscle action-potential amplitude (mV) and distal motor latency (ms) of the median nerve, which are measures of axon integrity and nerve depolarisation velocity, respectively. Antidromic sensory responses at the wrist and over the second digit were also recorded.[9] Nerve-conduction studies are well-validated measures of peripheral neuropathy that provide reproducible measures of disease severity and are sensitive to small, often subclinical, changes that are related to disease progression and treatment response.[11] All nerve conduction studies were done by the same paediatric neurologist at each site (RAO in Sydney and MMR in Melbourne). A two-channel Viking Quest Electromyography instrument (Nicolet Biomedical, Madison, WI, USA) was used in Sydney and a four-channel Keypoint Electromyography instrument (Medtronic, Minneapolis, MN, USA) was used in Melbourne, according to a standardised protocol that has been described previously.[9] All children received conscious sedation with inhaled nitrous oxide given by a qualified nurse according to hospital protocol, and skin temperature was monitored with an infrared skin thermometer (Dermatemp, Exergen, Watertown, MA, USA). For the comfort of the patient and to satisfy the requirement for independence for statistical analysis, only one arm was tested with surface-stimulating and recording electrodes, and the same arm was tested at follow-up. The median nerve motor response was recorded over the abductor pollicis brevis muscle, with as close as possible to a standard distance of 5 cm between

the distal stimulator and the recording electrode. The median nerve was chosen owing to the ease of testing, the rarity of median nerve neuropathies at the wrist in children,[12] and the frequent absence of lower-limb responses in patients with CMT1A.[13] If required, distal motor latency was corrected to a standard distance of 5 cm using Slomic's formula.[9]

Other secondary outcomes were foot and hand strength, motor function, walking ability, and health-related quality of life. The principal functional outcome was ankle dorsiflexion strength, which was recorded in triplicate by JB in all children by hand-held dynamometry (Citec, CIT Technics, Haren, Netherlands), which is a highly reliable and validated portable digital device incorporating a load cell to measure isometric muscle strength in children and adults.[14] Weakness of ankle dorsiflexion is the most common sign of CMT1A and leads to foot deformity, ankle contracture, poor motor function, and difficulty in walking.[15] Secondary strength outcomes were ankle plantarflexion, foot inversion and eversion, hand grip, and finger-tip pinch, which were also measured with hand-held dynamometry according to standardised procedures.[15,16] To satisfy the independence requirement for statistical analysis, the strength of only the dominant limb was tested. Gross motor function was assessed barefoot with the most widely validated paediatric measures of balance, agility, power, and endurance.[15] Data on timed balance and agility performance were assessed according to subtests of the second edition of the Bruininks–Oseretsky test of motor proficiency (Pearson Education, Upper Saddle River, NJ, USA) in children older than 3 years. Power was evaluated by standing long jump, and endurance was assessed in a gait laboratory by the 6-min walk test along a 10 m level walkway. Fine motor function of the hand was assessed with the 9-hole peg test, according to a validated protocol.[16] Walking ability was assessed in a subset of 53 children (aged 2–16 years) with the GAITRite electronic instrumented walkway (CIR Systems, Haverton, PA, USA). The temporospatial walking gait parameters recorded included speed (cm/s), cadence (steps/min), step time (s), step length (cm), stride length (cm), and base of support (cm).[15] Health-related quality of life was assessed with the parent-reported Child Health Questionnaire for children aged 5–16 years, to quantify the everyday function and wellbeing of the children and their families before and after treatment.[17]

Plasma concentration of ascorbic acid, pill counts, and medication diaries were used to assist and document compliance. To measure plasma ascorbic acid concentration, a 3 mL venous blood sample was collected into lithium heparin tubes, separated within 20 min of collection, and stored at –80°C before analysis by high-performance liquid chromatography with electrochemical detection. Adverse events were reported during phone calls between the parents and the principal investigator (JB) once every 2 months and monitored in accordance with the TGA of Australia. An independent data and safety monitoring board comprising medical, statistical, and research experts reviewed the adverse events that occurred during the study.

Randomisation and masking

Patients were randomly assigned in a 1:1 ratio by a computer-generated algorithm. Randomisation data were retained by central pharmacy, stratified according to age (2–8 years or 9–16 years) and study centre (Sydney or Melbourne). The principal investigator (JB) and the other investigators, study coordinators, assessors, children, and their parents were unaware of the treatment assignment during the study. The trial pharmacist (PDJ) was the only unblinded investigator. The data and safety monitoring board could request unblinding of trial participants if necessary.

Statistical analysis

Sample size was estimated a priori, with a power of 80% (α=0·05). On the basis of historical data, which reported a deterioration of 2·5 m/s per year in median nerve motor conduction velocity in very young children with CMT1A,[18] the sample size was calculated to detect a difference between groups at 12 months' follow-up of 2·5 m/s (SD 3·2). With a total dropout and non-compliance rate of 5%, a minimum sample size of 30 participants in each group (total n=60) was estimated. Shortly after the trial had started and in response to further data on the natural history of median nerve motor conduction velocity in older children and adults,[1] additional funding was secured to increase the total number of participants to 80 and the power of the trial to 90% (α=0·05), according to the deterioration rate of 2·5 m/s per year (registry amendment on Sept 28, 2007).

Descriptive statistics were used to characterise the study sample (SPSS version 15.0 [SPSS, Chicago, IL, USA]). The

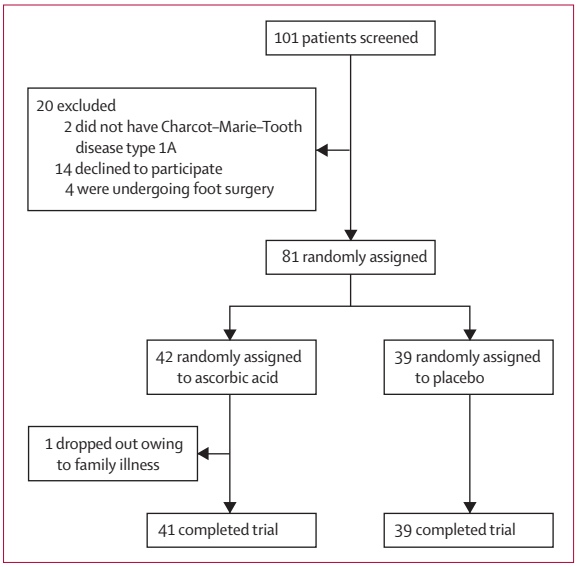

Figure 1: **Study design, enrolment, and follow-up of participants**

	Ascorbic acid (n=42)	Placebo (n=39)	All (n=81)
Age (years)	8·5 (3·3)	8·1 (3·5)	8·3 (3·5)
Age group			
2–8 years	22 (52%)	23 (59%)	45 (56%)
9–16 years	20 (48%)	16 (41%)	36 (44%)
Boys	26 (62%)	21 (54%)	47 (58%)
Body weight (kg)	33·1 (16·8)	30·1 (12·8)	31·6 (15·0)
Height (m)	1·32 (0·21)	1·31 (0·21)	1·32 (0·21)
Body-mass index (kg/m²)	17·8 (3·9)	16·8 (2·8)	17·3 (3·5)
Socioeconomic status*	1004 (51)	1004 (54)	1004 (52)
Number with co-existing disorders	6 (14%)	7 (18%)	13 (16%)
Clinical marker of disease severity			
Foot pain	11 (26%)	11 (28%)	22 (27%)
Hand pain	10 (24%)	7 (18%)	17 (21%)
Pes cavus (highly arched foot deformity)	17 (40%)	11 (28%)	28 (35%)
Ankle contracture	33 (79%)	29 (74%)	62 (77%)
Muscle cramps	10 (24%)	7 (18%)	17 (21%)
Regular trips and falls	33 (79%)	32 (82%)	65 (80%)
Study centre			
Children's Hospital at Westmead (Sydney)	30 (71%)	28 (72%)	58 (72%)
Royal Children's Hospital (Melbourne)	12 (29%)	11 (28%)	23 (28%)

Data are mean (SD) or number (%). *Based on the Australian Bureau of Statistics residential postcode method for the general Australian population (mean index=1000).

Table 1: **Physical, demographic, and disease-related characteristics at baseline**

normality of data distribution was assessed, and appropriate parametric or non-parametric tests were subsequently applied. Analysis of treatment effect between groups was by intention to treat at 12 months with a linear regression approach to analysis of covariance (ANCOVA) to adjust for baseline differences of the respective covariates.[19] The precision of the treatment effect was based on the 95% CI and a p value of less than 0·05. To enable the reader to make their own interpretation of the findings, no adjustment was made for multiple comparisons.

This trial is registered with the Australian New Zealand Clinical Trials Registry, number 12606000481572.

Role of the funding source

The funding source had no role in the study design, data collection, data analysis, data interpretation, or in the preparation of the manuscript or the decision to submit for publication. The corresponding author and all co-authors had access to all data and had final responsibility for the decision to submit for publication.

Results

Of the 101 children we screened, 81 (age 2–16 years) from 50 families affected by CMT1A were randomly assigned to high-dose oral ascorbic acid (n=42) or to placebo (n=39). Figure 1 shows the trial profile. 53 patients (30 who received ascorbic acid and 23 who received placebo) had a confirmed duplication of *PMP22* and 28 patients (16 who received ascorbic acid and 12 who received placebo) had a first-degree or second-degree relative with confirmed 17p11.2 duplication and a phenotype or neurophysiology consistent with CMT1A. The ascorbic acid and placebo groups did not differ at baseline with respect to physical, demographic, or disease-related characteristics (table 1). Coexisting disorders were reported in 13 children (four had asthma, two had attention-deficit hyperactivity disorder (ADHD) and one had each of the following: Asperger's syndrome with ADHD and oppositional defiant disorder; Asperger's syndrome and epilepsy; Asperger's syndrome with bladder dysfunction; eczema; cardiac arrhythmia; migraine; and nystagmus of the palate). 80 children completed 12 months of treatment. One child dropped out owing to parent illness. One child could not tolerate nerve-conduction studies at baseline, and two other children could not tolerate nerve-conduction studies at the follow-up visit. Data related to growth were not scaled because there were no significant differences between groups for age, height, or weight between baseline and follow-up (p>0·35).

Children in the ascorbic acid group did not reach any of the expected efficacy endpoints (tables 2 and 3). At 12 months, there was a small increase in median nerve motor conduction velocity in the ascorbic acid group compared with the placebo group (adjusted mean difference 1·7 m/s, 95% CI –0·1 to 3·4; p=0·06 [figure 2]). Three children on ascorbic acid had motor conduction velocities that were faster than 35 m/s at follow-up, and in one patient, motor conduction velocity increased from 24·6 m/s at baseline to 41·7 m/s at follow-up. The greatest decrease in motor conduction velocity at follow-up was recorded in one patient in the placebo group (–12·5 m/s). Neither age nor body size (height, weight, and body-mass index) were related to changes in motor conduction velocity in either the ascorbic acid group (r≤0·146; p≥0·37) or the placebo group (r≤0·209; p≥0·21) at follow-up. Skin temperature measured 30–35°C at baseline and was within 1°C of the baseline measurement at follow-up for each child. Skin temperature did not differ between the groups at baseline (ascorbic acid 32·7°C [SD 0·9] and placebo 32·8°C [1·0]) or at follow-up (ascorbic acid 32·6°C [1·0] and placebo 32·6°C [0·9]).

Conduction block at baseline had no effect on the between-group changes in motor conduction velocity at follow-up. Four of the ten children with conduction block at baseline[9] were in the placebo group (change at 12 months=1·0 to 4·0 m/s) and six were in the ascorbic acid group (change at 12 months=–1·0 to 1·0 m/s). None of the children in the ascorbic acid group with large increases in motor conduction velocity at follow-up had conduction block at baseline. Sensory nerve responses were present in only seven children (age ≤8 years) at baseline. Two of these (aged 3 and 7 years at enrolment) retained sensory responses at follow-up (one received placebo and one received ascorbic acid, respectively). No significant differences were seen between the two groups in compound muscle action-potential amplitude or distal motor latency at baseline or at follow-up (table 2).

Ankle dorsiflexion strength increased in 26 (67%) children in the placebo group and 30 (71%) children in the ascorbic acid group, but there was no significant difference between the groups at follow-up (adjusted mean difference –4·9 N, 95% CI –12·5 to 2·7; p=0·20 [table 2]). Despite mean strength gains of 10–19% in other muscle groups (except for finger pinch) after 12 months, there were no significant differences between groups at follow-up (table 2). For the other secondary outcomes of motor function and walking ability, only small and clinically non-significant differences were seen between groups at follow-up (table 3), and changes in health-related quality of life scores between groups after 12 months were not statistically significant (webappendix).

Plasma ascorbic acid concentration increased between baseline and follow-up in the ascorbic acid group (48·9 µmol/L [SD 30·7] *vs* 111·1 µmol/L [43·6]) and in the placebo group (52·2 µmol/L [29·1] *vs* 69·7 µmol/L [31·3]). After 12 months, the ascorbic acid group had a significantly higher plasma ascorbic acid concentrations than the placebo group (adjusted mean difference 42·4 µmol/L, 95% CI 23·2–61·5; p<0·0005). Mean percentage of compliance according to pill count (ascorbic acid 84% and placebo 88%) and medication diaries (ascorbic acid 85% and placebo 85%) were similar between groups.

Minor and occasional adverse events were reported by only six patients. Fewer adverse events were reported by the patients in the ascorbic acid group than by those in the placebo group (2 [5%] *vs* 4 [10%]). Adverse events included nausea (1 [2%] *vs* 3 [8%]), gastrointestinal discomfort (0 [0%] *vs* 1 [3%]), and constipation (1 [2%] *vs* 0 [0%]). No adverse events were deemed to be serious. At the end of the trial, all parents were asked to guess which intervention their child had been assigned to. Only 32 (40%) parents felt able to guess, and only 16 (50%) of these guessed correctly, which was consistent with chance. All but one parent said their child would continue to take ascorbic acid if it was effective.

Not all children in the ascorbic acid group had increased motor conduction velocity after 12 months. To identify which factors were associated with a large increase in motor conduction velocity, the ascorbic acid group was split into two groups for exploratory analyses: children with a large increase in motor conduction velocity identified post-hoc (figure 3) and the remainder of the group. In the post-hoc analysis, there were five outliers (aged 6–16 years) with large increases in motor conduction velocity between baseline and follow-up (increases of 6·0–17·2 m/s). The five outliers were all in the ascorbic acid group and none were in the placebo group (Fisher's exact probability χ^2=3·102, p=0·03). The mean change in motor conduction velocity of these five children (11·0 m/s [SD 4·8]) was significantly greater than the change in the rest of the sample (0·3 m/s [2·7]; t=–4·907, p=0·007). In addition, there was a significant difference in motor conduction velocity between the five largest improvers in the ascorbic acid group and the five largest improvers in the placebo group (11·0 [4·8] *vs* 4·4 [0·6]; t=–3·062, p=0·04). The five children with the largest increases in motor conduction velocity had higher mean motor conduction velocities at baseline (24·6 m/s [SD 3·0]) *vs* 18·0 m/s [5·3]; t=–2·685, p=0·01), and also had higher mean compound muscle action-potential amplitudes at baseline than the remainder of the group (9·6 mV [2·4] *vs* 7·1 mV [2·5]; t=–2·127, p=0·04). The children with the largest increases in motor conduction velocity had greater functional power at baseline than the remainder of the group (mean long jump distance 103·2 cm [38·4] *vs* 77·0 cm [22·3]; t=–2·230, p=0·03) and had greater endurance (mean 6-min walk distance 590 m, [86] *vs* 507 m [84]; t=–2·050, p=0·05). Finally, the children with the largest increases in motor conduction velocity were more compliant than the

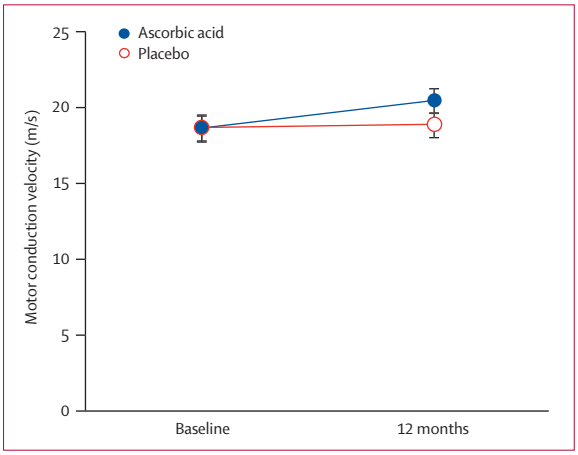

Figure 2: **Median nerve motor conduction velocity at baseline and 12 months**
Data are mean (SE).

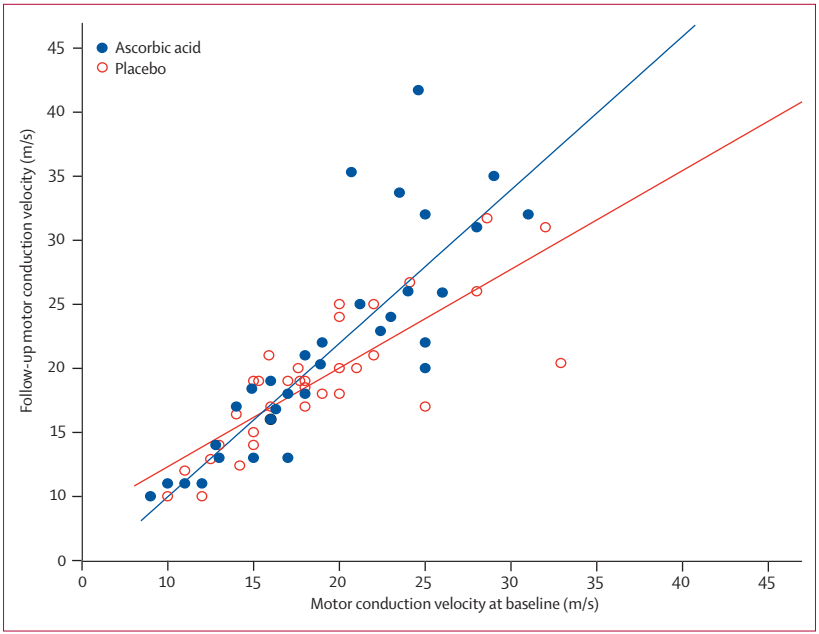

Figure 3: **Median nerve motor conduction velocities for all participants at baseline and 12 months**
Fitted linear regression lines and the five children with the largest improvement from baseline are shown.

See **Online** for webappendix

	Baseline		Follow-up (12 months)			
	Ascorbic acid (n=42)	Placebo (n=39)	Ascorbic acid (n=41)	Placebo (n=39)	Difference*	p*
Neurophysiological						
MCV (m/s)	18·7 (5·4)	18·8 (5·5)	20·5 (7·8)	18·9 (5·2)	1·7 (–0·1 to 3·4)	0·06
CMAP (mV)	7·4 (2·5)	6·8 (2·1)	7·6 (2·4)	7·3 (2·4)	0·1 (–1·0 to 1·1)	0·93
DML (ms)	7·5 (1·4)	7·2 (1·4)	7·5 (1·3)	7·5 (1·5)	0·2 (–0·2 to 0·6)	0·39
Strength						
Ankle dorsiflexion (N)	56·9 (22·0)	52·6 (16·3)	63·7 (23·4)	64·7 (26·2)	–4·9 (–12·5 to 2·7)	0·20
Ankle plantar flexion (N)	178·1 (48·4)	171·1 (50·3)	195·2 (57·0)	205·4 (61·8)	–16·0 (–35·5 to 3·6)	0·11
Foot inversion (N)	77·5 (29·5)	75·5 (30·2)	92·4 (32·7)	95·1 (33·5)	–4·2 (–14·3 to 5·8)	0·41
Foot eversion (N)	62·8 (23·3)	62·1 (24·8)	71·5 (27·0)	71·6 (29·8)	–0·7 (–9·8 to 8·5)	0·89
Hand grip (N)	78·7 (64·6)	61·7 (38·9)	88·8 (65·3)	79·0 (62·0)	–8·6 (–20·4 to 3·2)	0·15
Finger pinch (N)	17·7 (8·5)	15·1 (7·4)	17·6 (8·4)	16·0 (7·3)	–0·5 (–2·5 to 1·5)	0·60

Data are mean (SD) or difference (95% CI). MCV=median nerve motor conduction velocity (primary outcome). CMAP=compound muscle action-potential amplitude. DML=distal motor latency. *Between-group mean differences at follow-up were ANCOVA adjusted for the baseline score of each outcome; positive values favour ascorbic acid.

Table 2: **Primary and secondary neurophysiological and strength outcomes at baseline and 12-month follow-up**

	Baseline		Follow-up (12 months)			
	Ascorbic acid	Placebo	Ascorbic acid	Placebo	Difference*	p*
Motor function						
Balance (points)†	24·4 (6·3)	25·6 (8·2)	25·6 (7·3)	26·9 (7·2)	–0·3 (–2·1 to 1·5)	0·73
Agility (points)†	29·1 (8·2)	31·4 (9·1)	32·9 (7·6)	34·6 (8·4)	0·2 (–1·6 to 2·0)	0·85
Long jump (cm)‡	79·9 (25·3)	76·4 (29·8)	84·5 (27·7)	82·3 (29·5)	–1·1 (–6·9 to 4·6)	0·70
6-min walk (m)§	519 (86)	521 (98)	538 (71)	541 (49)	–0·7 (–23·4 to 21·9)	0·95
9-hole peg test (s)¶	26·5 (6·0)	27·3 (7·3)	24·4 (3·7)	25·2 (5·3)	–0·4 (–1·9 to 1·1)	0·61
Walking ability**						
Speed (cm/s)	118·6 (16·3)	124·1 (18·8)	117·5 (21·4)	117·7 (11·9)	1·8 (–7·9 to 11·4)	0·71
Cadence (steps/min)	133·0 (15·9)	133·3 (18·9)	125·0 (12·9)	124·2 (14·1)	0·8 (–6·3 to 8·0)	0·82
Step time (s)	0·5 (0·1)	0·5 (0·1)	0·5 (0·1)	0·5 (0·1)	0·0 (–0·1 to 0·0)	0·70
Step length (cm)	54·6 (8·3)	56·9 (9·3)	56·4 (7·9)	57·3 (7·2)	0·6 (–1·9 to 3·2)	0·62
Stride length (cm)	108·9 (16·5)	113·2 (19·4)	112·9 (15·7)	114·7 (13·2)	0·9 (–4·1 to 6·0)	0·71
Base of support (cm)	9·1 (4·4)	8·8 (3·5)	8·1 (4·1)	7·4 (5·0)	–0·5 (–2·8 to 1·8)	0·68

Data are mean (SD) or difference (95% CI). *Between-group mean differences at follow-up were ANCOVA adjusted for the baseline score of each outcome; positive values favour ascorbic acid. †n=39 for ascorbic acid; n=33 for placebo. ‡n=41 for ascorbic acid; n=36 for placebo. §n=35 for ascorbic acid; n=30 for placebo. ¶n=41 for ascorbic acid; n=37 for placebo. **n=29 for ascorbic acid; n=24 for placebo.

Table 3: **Motor function and walking ability secondary outcomes for ascorbic acid and placebo groups at baseline and 12-month follow-up**

remainder of the group (mean proportion 99% [0·7] *vs* 83% [15·9]; t=–5·927, p<0·0005). No other physical, demographic, or disease-related factors were associated with a large increase in median nerve motor conduction velocity. Taken together, preserved myelin and axon integrity at baseline, higher functional ability at baseline, and better compliance were associated with the largest response in motor conduction velocity to ascorbic acid therapy.

Discussion

In this study, we assessed the most promising potential treatment for CMT1A. We found no measurable effect of high-dose ascorbic acid on neurophysiological, strength, function, or quality of life outcomes. The median nerve motor conduction velocities were, on average, 1·7 m/s faster at follow-up in the ascorbic acid-treated group than they were in the placebo group (difference 0·1 m/s). This difference is a 9% increase in motor conduction velocity, but the clinical significance of such a small increase is questionable. Five children in the ascorbic acid group had a large increase in motor conduction velocity (6·0–17·2 m/s). Post-hoc analysis shows that these children had a milder neurophysiological and functional phenotype at baseline and were more compliant than were the remainder of the sample. The finding that these children had significantly higher baseline values of motor conduction velocity and compound muscle action-potential amplitude than the remainder of the group was unexpected because it was not consistent with a regression to the mean response of an otherwise ineffective treatment. A plausible biological explanation for this finding is that the children who were least affected, or those with preserved myelin and axon

integrity, might have the greatest response to ascorbic acid. This explanation suggests that it might be more efficacious to start ascorbic acid therapy in those children who are less affected or at the earliest stages of CMT1A. Because ascorbic acid supplementation is cheap (AUD$0·03/tablet) and is associated with low inconvenience and harm, such a large increase in motor conduction velocity in some children warrants further pharmacogenomic investigation.

Ascorbic acid promotes myelin formation in cultures of axons with Schwann cells.[20–22] Ascorbic acid is thought to improve the CMT1A phenotype by inducing remyelination, possibly by inhibition of cAMP-stimulated overexpression of *PMP22*,[3] an effect that is not seen with other antioxidants or ascorbic acid in combination with other vitamins.[23] An increase in the conduction velocities of patients treated with ascorbic acid might indicate restoration of peripheral nerve myelin. Future studies of ascorbic acid therapy for CMT1A should ideally include serial studies of peripheral or cutaneous nerve pathology. However, because these studies are invasive and difficult to carry out in young children, they were not deemed appropriate in this trial.

Several of the difficulties encountered in this study might be of relevance to improve future trials of ascorbic acid in children. In addition to an absence of efficacy, the reasons for there being no significant effect between groups might relate to limitations in dose, length of follow-up, sample size, the chosen outcome measures, or changes in dietary behaviour.

First, the ascorbic acid dose was based on established upper safe levels[8] and would have ensured tissue saturation in all children (>200 mg/day) but plasma saturation (>1000 mg/day) in only some children.[24] Many studies have shown that only dehydroascorbic acid, the oxidised form of ascorbic acid, passes through the blood–brain barrier and is found at high concentrations in the brain and spinal cord;[25] however, whether plasma saturation of ascorbic acid is needed for added physiological benefits in children with CMT1A beyond those achieved by tissue saturation is not known. Because ongoing trials[4] of CMT1A are assessing doses up to 4000 mg/day, and owing to the few adverse events encountered in our study, further trials to assess a dose-dependent response are warranted. However, high doses can be harmful. In a 2-year pilot study of 12 adults with CMT1A, ingestion of 5000 mg/day of ascorbic acid resulted in half the participants reporting intolerable gastrointestinal events, and the study had a 42% drop-out rate.[26]

Ascorbic acid might have a significant effect on the CMT1A phenotype after longer treatment and follow-up, and 12 months might not have been long enough to detect a significant change for this slowly progressive disorder. Whether ascorbic acid would have a deleterious effect over longer periods is unclear, and phase III and IV trials are needed.

Furthermore, our trial might have been underpowered to detect a significant difference between groups. We based our original sample-size estimation on annual change in median nerve motor conduction velocity over 6 years in young children with CMT1A.[18] However, recent natural history data of the change in median nerve motor conduction velocity over 8 years in older children and adults with CMT1A (mean age 39·8 years)[1] indicates progression is much slower and variability much greater in older children than it is in younger children. Sample size recalculation on the basis of these data suggest that the power of this study might not have been large enough to detect a treatment effect. Further neurophysiological natural history data in children with CMT1A are needed to estimate more accurately the power needed in clinical trials of interventions for paediatric CMT1A.

Outcomes of disease progression in childhood CMT1A are poorly defined. Many researchers acknowledge that reduced amplitudes of compound muscle action-potential are an indicator of weakness and disability in CMT1A.[27–30] However, in our study compound muscle action-potential amplitudes and many other potential primary outcomes, such as ankle dorsiflexion strength, were not abnormal in all children and would not have been sensitive enough to detect a treatment effect after 12 months in those children who are only mildly affected. Motor conduction velocity slowing as an indicator of myelination status and a determinant of axonal dysfunction later in life[30] is one of the few neurophysiological abnormalities that are apparent in all children with CMT1A.[9] Indeed, the effect of motor conduction slowing on disability in children and adults with CMT1A has been reported as stronger than the effect of compound muscle action-potential attenuation.[31] Slowing of motor conduction velocity is also thought to be related to clinical severity,[30,32] and has been identified as an early marker of neurological abnormality later in life.[13] However, the functional significance of the small change seen with ascorbic acid in motor conduction velocity in our trial is uncertain. Our failure to see a between-group benefit for strength and function measures might reflect the findings of some studies that show changes in motor conduction velocity might not predict functional disease progression,[27,28] particularly in older children and adults, in whom conduction velocities correlate poorly with age.[33,34] Longitudinal assessment of potential neurophysiological and clinical outcomes in children with CMT1A, possibly as part of a paediatric version of the well-validated Charcot–Marie–Tooth disease neuropathy score,[1] might resolve some of these apparent contradictions and limitations.

The significantly higher plasma ascorbic acid concentrations at follow-up compared with baseline in the placebo group might explain why there was no deterioration in these children. All parents denied giving off-study supplements to their children during the trial, but a deliberate or subconscious change in dietary behaviour might have occurred. Because many parents commented anecdotally that they commenced their own ascorbic acid supplementation during the trial, heightened expectations might have led to an increase in the intake of vitamin C-rich fruit and vegetables by the children. Although participants were advised against vitamin supplementation,

future trials could more stringently limit additional dietary ascorbic acid intake; if such behaviour is inevitable, a more pragmatic approach to trial design with a larger sample to account for associated statistical noise might be necessary.

We provide evidence that 12 months' dietary supplementation of high-dose ascorbic acid is safe and well-tolerated in children with CMT1A. However, ascorbic acid treatment did not reach any of the expected efficacy endpoints. An increase in the median nerve motor conduction velocity with ascorbic acid was seen in some patients, but the clinical significance of this finding is yet to be determined.

Contributors

JB, RAO, PDJ, and MMR contributed to the design of the study. JB, RAO, EMY, AJK, MCF, and MMR participated in the collection and assessment of data, and JB did the statistical analysis. All authors participated in the writing and editing of the manuscript.

Conflicts of interest

We have no conflicts of interest.

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