ARTICLE

The safety and tolerability of levodopa eye drops for the treatment of ocular disorders: A randomized first-in-human study

Kate Thomson[1] | Cindy Karouta[1] | Faran Sabeti[2,3] | Nicola Anstice[4] | Myra Leung[2] | Tina Jong[2] | Ted Maddess[3] | Ian G. Morgan[5] | Jeremy Game[1] | Regan Ashby[1]

[1]Faculty of Science and Technology, University of Canberra, Canberra, Australian Capital Territory, Australia

[2]Discipline of Optometry, Faculty of Health, University of Canberra, Canberra, Australian Capital Territory, Australia

[3]John Curtin School of Medical Research (JCSMR), The Australian National University, Canberra, Australian Capital Territory, Australia

[4]Optometry & Vision Science, College of Nursing and Health Sciences, Flinders University, Adelaide, South Australia, Australia

[5]Research School of Biology, The Australian National University, Canberra, Australian Capital Territory, Australia

Correspondence
Kate Thomson, Faculty of Science and Technology, University of Canberra, University Drive, Bruce, Canberra, ACT, Australia.
Email: kate.thomson@canberra.edu.au

Abstract

Myopia is the leading cause of low vision worldwide and can lead to significant pathological complications. Therefore, to improve patient outcomes, the field continues to develop novel interventions for this visual disorder. Accordingly, this first-in-human study reports on the safety profile of a novel dopamine-based ophthalmic treatment for myopia, levodopa/carbidopa eye drops. This phase I, first-in-human, monocenter, placebo-controlled, double-blind, paired-eye, multi-dose, randomized clinical trial was undertaken in healthy adult males aged 18–30 years (mean age 24.9 ± 2.7) at the University of Canberra Eye Clinic, Australia. Participants were randomly assigned to receive either a low (1.4 levodopa:0.34 carbidopa [μmoles/day], $n = 14$) or standard dose (2.7 levodopa:0.68 carbidopa [μmoles/day], $n = 15$) of levodopa/carbidopa eye drops in one eye and placebo in the fellow eye once daily for 4 weeks (28 days). Over this 4-week trial, and after a 4-month follow-up visit, levodopa/carbidopa treatment had no significant effect on ocular tolerability and anterior surface integrity, visual function, ocular health, refraction/ocular biometry, and did not induce any non-ocular adverse events. These results indicate that topical levodopa/carbidopa is safe and tolerable to the eye, paving the way for future studies on the efficacy of this novel ophthalmic formulation in the treatment of human myopia. The findings of this study have implications not only for the treatment of myopia, but in a number of other visual disorders (i.e., amblyopia, diabetic retinopathy, and age-related macular degeneration) in which levodopa has been identified as a potential clinical intervention.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Preclinical data indicate that levodopa/carbidopa eye drops can safely inhibit the development of experimental myopia in animal models.

WHAT QUESTION DID THIS STUDY ADDRESS?
This study investigated whether levodopa/carbidopa eye drops are safe and tolerable to the human eye.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
In this phase I, first-in-human, monocenter, randomized, double-blind, placebo-controlled, multidose trial, levodopa/carbidopa eye drops were found to be safe and well tolerated in healthy adult males over a 4-week period. Furthermore, no non-ocular adverse events were reported.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
As topical administration of levodopa/carbidopa eye drops is well tolerated in humans, the efficacy of this novel ophthalmic treatment at inhibiting the development of myopia in patient populations can now be assessed. If found to be effective, this will provide a powerful new method for inhibiting the onset and development of the leading cause of low vision worldwide. This topical formulation may also provide a valuable tool in the treatment of other visual disorders shown to be responsive to levodopa therapy (i.e., amblyopia, diabetic retinopathy, and age-related macular degeneration).

INTRODUCTION

The refractive disorder myopia (short-sightedness) is the leading cause of low vision worldwide,[1] with some estimates predicting that half of the world's population may be affected by 2050.[2] Although the refractive error associated with myopia can be optically corrected, such corrections do not address the underlying pathology of myopia – that is, excessive ocular growth. Such growth places myopes, particularly high myopes (refractive error < -6 diopters [D]), at a significantly higher risk of developing sight-threatening complications later in life. This can include degenerative retinal changes (e.g., tears and detachments, myopic macular degeneration, peripheral lattice changes, myopic choroidal neovascularisation, peripapillary atrophy, and myopic macular schisis and holes), early onset cataracts, open angle glaucoma, and optic disc tilt and pits.[3] There are a number of pharmacological, optical, and behavioral interventions currently in use to prevent the onset or slow the progression of human myopia (e.g., increased time outdoors, atropine, orthokeratology, and contact and spectacle lenses that impose myopic defocus; for review see Jonas et al.[4]). In an attempt to further improve treatment outcomes, the field continues to investigate and develop novel interventions for myopia. One promising pharmacological intervention is the subject of this study.

One of the earliest and most consistent biochemical changes implicated in animal models of myopia is a reduction in retinal dopamine release (for review see Troilo et al.[5]). Accordingly, pharmacological administration of dopamine agonists, which mimic the effects of dopamine, are able to inhibit the development of experimental myopia in all species studied (for review see Troilo et al.[5]). Most of the evidence on a role for dopamine comes from studies on experimental myopia in animals, including non-human primates, but, as expected, dopaminergic pathways also seem to be involved in humans. Specifically, systemic administration of the dopamine reuptake inhibitor methylphenidate has recently been reported to inhibit myopia development over 12 months in a small, placebo-controlled study following 19 children (aged 8–18 years old) with attention deficit hyperactivity disorder.[6] If this dysregulation of the dopaminergic system is a universal requirement for excessive growth, restoring dopamine levels may form an effective clinical treatment for myopia in humans.

The most obvious mechanism by which this can be achieved is through the direct pharmacological administration of dopamine to the eye. Due to its polarity, however, dopamine does not easily cross epithelial barriers, limiting the delivery avenues available. In contrast, the dopamine precursor levodopa is readily transported across such barriers[7] and has therefore been in clinical use for

over five decades as the primary treatment of neurological disorders characterized by diminished dopamine release (i.e., Parkinson's disease).[8,9] In a clinical setting, levodopa is commonly coadministered with carbidopa to prevent its premature conversion to dopamine before reaching the target tissue (i.e., the brain), enhancing treatment outcomes.[9]

Systemic administration of levodopa/carbidopa is not appropriate for the treatment of myopia due to unwanted distribution to the brain. Therefore, our group has reformulated levodopa/carbidopa into a topical ophthalmic solution for direct application to the eye. Preclinical data have shown that, in animal models, topically applied levodopa/carbidopa can inhibit experimental (form deprivation and optically induced) myopia in a dose-dependent manner, providing full protection at higher concentrations.[10,11] These preclinical data also demonstrate a significant increase in efficacy during topical levodopa/carbidopa treatment relative to levodopa alone.[10,11] Furthermore, in animals this topical levodopa/carbidopa treatment shows minimal systemic distribution and does not alter normal ocular development or ocular health over long-term treatment periods.[10,11] To examine the safety profile of this topical formulation further, this study reports on the findings of a phase I, first-in-human, placebo-controlled, double-blind, randomized, paired-eye, safety and tolerability trial in young healthy adults (18–30 years old) treated once a day for a period of 4 weeks (28 days).

Various clinical measures were undertaken to understand the effects of levodopa/carbidopa treatment on ocular surface tolerability and integrity, visual function, ocular health, as well as refraction and biometry. As will be detailed, no adverse events or complications were observed during the study. The efficacy of levodopa/carbidopa at inhibiting the development of myopia in patient populations can now be assessed. If effective, this would provide a novel therapeutic intervention for myopia that may show significant benefits over, or complement, current treatment options. This ophthalmic solution may also have significant implications for several other visual disorders in which levodopa has been identified as a potential treatment (i.e., amblyopia,[12] age-related macular degeneration [AMD],[13] and diabetic retinopathy[14]).

METHODS

Study design

This first-in-human, placebo-controlled, double-blind, paired-eye, randomized clinical trial of topical levodopa/

carbidopa was conducted at the University of Canberra (UC) Eye Clinic, Australia. Over a 4-week (28-day) period (from June to July 2021), this study assessed the safety and tolerability of daily treatment with a topical ophthalmic levodopa/carbidopa solution in healthy young adult males. A post-treatment follow-up was completed in November 2021, 4 months following treatment cessation. The complete trial protocol is detailed in Supplement S1 – Protocol Document.

Based on preclinical safety and efficacy data,[10,11] participants were randomly assigned to receive either a low (1.4 levodopa:0.34 carbidopa [μmoles/day]) or standard dose (2.7 levodopa:0.68 carbidopa [μmoles/day]) of levodopa/carbidopa eye drops in one eye (two drops, once daily) and placebo (vehicle solution consisting of 0.1% w/v ascorbic acid and 0.001% w/v benzalkonium chloride dissolved in 1× phosphate-buffered saline) in the fellow eye (two drops, once daily) (manufactured by PCI Pharma Services Pty Ltd; Table S1).

This trial was designed in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline for Good Clinical Practice (GCP) and guideline M3 adopted by the Australian Clinical Trial Handbook (ACTH), was undertaken under the Clinical Trial Notification scheme (CT2020-CTN-04134-1-v1), and was registered with the Australian New Zealand Clinical Trial Register (ANZCTR; ACTRN12620001259932). This study adhered to the CONSORT statement, tenets of the Declaration of Helsinki, and was approved by the University of Canberra Human Ethics Committee (HREC-0406).

Recruitment and eligibility

As summarized in Figure 1, 36 males enrolled to participate in this safety and tolerability trial (between February and June 2021). Following the baseline/screening visit (inclusion/exclusion criteria are detailed in Table S2), seven subjects were excluded from this study due to retinal pathology, significant ametropia, or systemic medications, leaving 29 subjects (mean age 24.9 ± 2.7 years) eligible to commence treatment (low dose group: $n = 14$, standard dose group: $n = 15$). Over the course of the study (June–July 2021), two subjects withdrew due to inability to attend the UC Eye Clinic (high dose: one participant withdrew after the week 1 measures; low dose: one participant withdrew after the week 2 measures). Therefore, 27 subjects completed the main study. Three subjects (all from the low dose group) were lost to follow-up (November 2021), while one further subject (from the standard dose group) did not
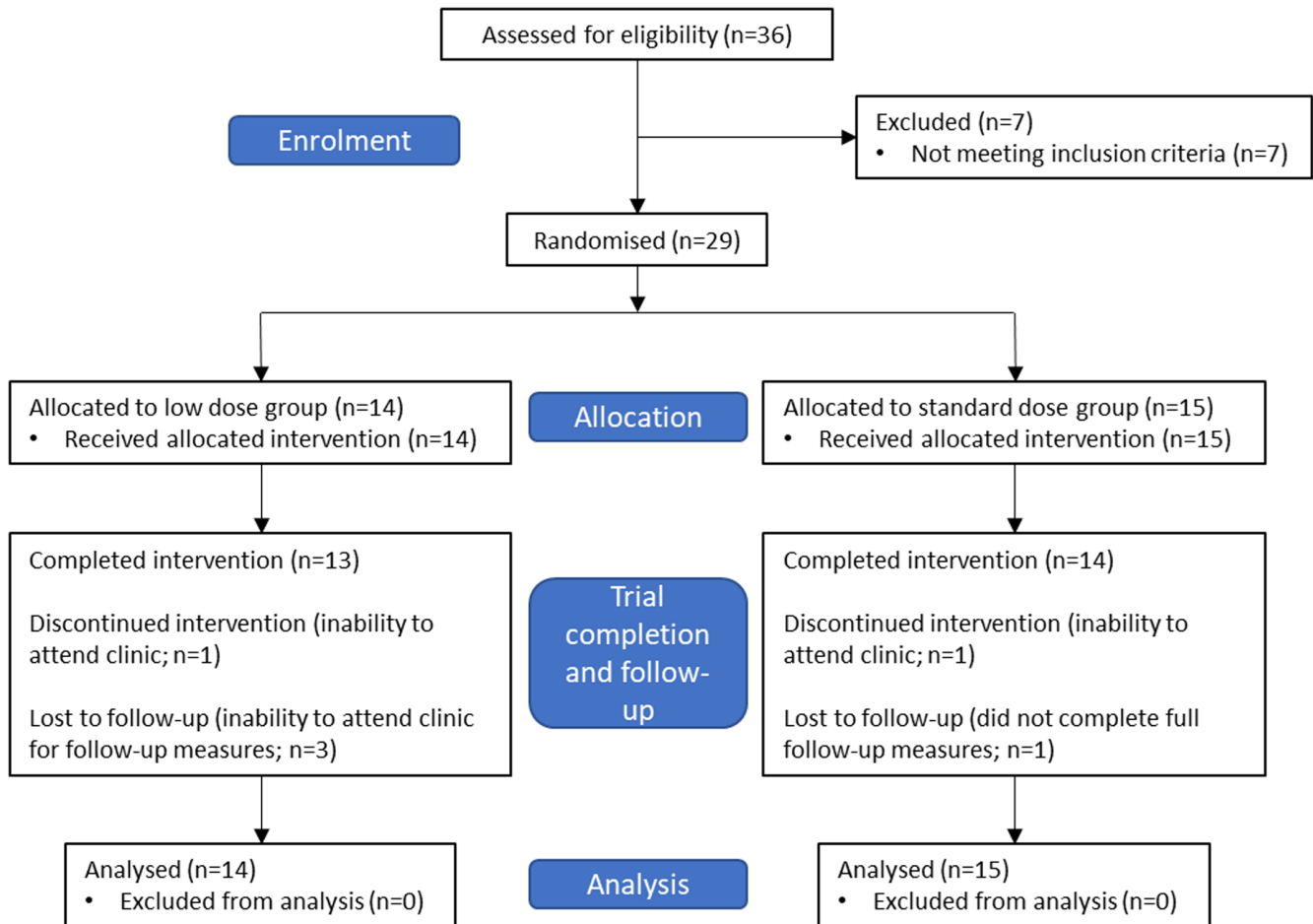
FIGURE 1 Participant enrollment summary. Trial enrollment and participant retention summarized using the CONSORT enrollment flowchart.

participate in the measures requiring cycloplegia during the follow-up visit.

Study measurements and outcomes

The safety and tolerability of levodopa/carbidopa eye drops was assessed by evaluating whether treatment induced any clinically significant changes in ocular surface tolerability and integrity, visual function, ocular health, and refraction and biometry, as well as the incidence of adverse events (clinical measures are summarized in Table S3). All eligible participants underwent the full examination procedure at baseline, end of treatment (4 weeks), and at follow-up (4 months) to determine the safety of levodopa/carbidopa (these will be referred to as the primary measurement points). A subset of these measures was also collected each week during the 4-week treatment period (referred to as the secondary measurement points). A summary of baseline demographics and clinical characteristics is detailed in Table S4.

Measures of ocular tolerability and anterior surface integrity

Dry eye symptomology was assessed using the validated Ocular Surface Disease Index (OSDI) questionnaire (Allergan Inc., Dublin, Ireland). Corneal and conjunctival epithelial integrity were also evaluated via the 0–3 National Eye Institute (NEI) sodium fluorescein grading scale.[15] Following conjunctival and corneal epithelial grading, fluorescein breakup time was evaluated at each visit. Participants had their tear film osmolarity assessed using the TearLab Osmolarity System (TearLab).

Measures of visual function

Multifocal electroretinograms (mfERGs) were assessed using a Visual Evoked Response Imaging System (VERIS 5.1.5× refractor/camera system, Electro-Diagnostic Imaging Inc.). Additionally, prior to cycloplegia at the primary measurement points, all participants undertook a 10–2 threshold strategy (central 20°) visual field test

using Frequency Doubling Technology (FDT) perimetry (Matrix; Carl Zeiss Meditech) to subjectively measure the overall function of the visual pathway.

Gross changes in foveal visual function were evaluated via standard clinical measures of high (>90%) and low (10%) contrast visual acuity (VA; Hi-Low contrast logMAR chart, Australian College of Optometry).[16]

Assessment of ocular health

Retinal structure, total retinal thickness, retinal nerve fiber layer thickness, and subfoveal choroidal thickness were assessed in the central 32 degrees of the retina by enhanced depth imaging (EDI) ocular coherence tomography (OCT) imaging (Spectralis, HRA + OCT; Heidelberg Engineering, Heidelberg, Germany, software version 6.9.5.0). Autofluorescence of images from the Spectralis were visually analyzed by clinicians at each primary measurement point for the occurrence of hyper- or hypofluorescent lesions as discussed by Yung et al.[17] Digital retinal imaging was also conducted on all subjects using wide-field fundus color photography (Clarus 500; Carl Zeiss Meditec) and was assessed for signs of pathology using the early treatment of diabetic retinopathy study (ETDRS) grading system.[18,19] Intraocular pressure (IOP) was assessed using rebound tonometry (iCare ic100) as described by Fernandes et al.[20]

Assessment of refractive error and ocular biometry

Changes in spherical equivalent refraction were measured following cycloplegia (achieved by administering one drop each of 1.0% cyclopentolate and 2.5% phenylephrine to each eye) via autorefraction (Nidek Tonoref III).

Ocular biometry (axial length, corneal thickness, anterior chamber depth, and corneal curvature) was measured using low coherence interferometry (Lenstar; Haag Streit). Monocular amplitude of accommodation was measured at each clinical visit using a Royal Air Force (RAF) rule.

Statistical analyses

Changes in measures of ocular tolerability and anterior surface integrity, visual function, ocular health, as well as refraction and ocular biometry, were analyzed via a linear mixed-effects model adjusted for repeated measures. Using the restricted maximum likelihood method, this mixed-effects model studied the effects of time, treatment (placebo vs. low-dose levodopa/carbidopa vs. standard-dose levodopa/carbidopa) and the interactions between time and treatment on an intention-to-treat principle. Random effects in the model included the subject effects (within-subject variability and errors). Data were analyzed using GraphPad Prism v9.3.1 and Matlab (2020b Mathworks Inc.).

RESULTS

Summary of results

Levodopa/carbidopa treatment had no significant effect on measures of ocular tolerability and anterior surface integrity, visual function, ocular health, as well as refraction and ocular biometry. For some measures, a significant change was observed between measurement points; however, this was observed as a cohort-wide change, with no significant difference between drug- and placebo-treated eyes. No adverse events occurred over the course of the trial. Participants also did not report any systemic symptoms associated with levodopa/carbidopa treatment during the trial.

Measures of ocular tolerability and anterior surface integrity

Drug treatment had no significant effect on the participants' responses to the OSDI questionnaire ($p = 0.629$; Tables 1 and S5). However, there was a significant decrease in the overall OSDI score as the trial progressed, indicating a lower level of dry eye symptomology ($p < 0.05$, Tables S5 and S6). Similarly, drug treatment had no significant effect on tear film breakup time ($p = 0.895$) or tear film osmolarity ($p = 0.204$), although both underwent a statistically significant change over the different measurement points ($p < 0.001$ and $p < 0.05$, respectively; Tables 1, S5, and S6). This was seen as a decrease in tear breakup time during weeks 2 and 3 and an increase in osmolarity at week 4 (Table S6).

Anterior surface integrity, as measured by corneal ($p = 0.181$) and conjunctival staining ($p = 0.999$), was unaffected by drug treatment (Tables 1 and S5). While a significant difference in both corneal ($p < 0.05$, decreasing at follow-up) and conjunctival staining ($p < 0.001$, decreasing during week 2 of the trial) was observed between some timepoints in both treated and control eyes (Tables S5 and S6), there was no significant difference between treated and control eyes.

TABLE 1 Measures of ocular tolerability/anterior surface integrity and visual function. Data presented for baseline, end of treatment, and follow-up visits. Data from intermediate visits are detailed in Tables S5 and S7, while mixed-effects model results for time are detailed in Tables S6 and S8. All data presented as means ± SD of the means

Measure	Dosage	Baseline		End of treatment		Follow-up		Mixed-effects model (treatment)
		Treatment	Placebo	Treatment	Placebo	Treatment	Placebo	
Participants measured	Low dose	14	14	13	13	10	10	
	Standard dose	15	15	14	14	13	13	
OSDI	Low dose	4.06±5.22	4.06±5.22	2.09±2.41	2.09±2.41	3.12±2.64	3.12±2.64	p = 0.629
	Standard dose	7.33±10.28	7.33±10.28	1.79±2.69	1.79±2.69	4.17±5.03	4.17±5.03	
Tear film osmolarity (mOsm/L)	Low dose	301.43±10.91	306.00±11.94	307.92±13.08	300.08±7.74	300.00±11.89	297.90±12.94	p = 0.204
	Standard dose	308.93±13.75	304.80±8.75	311.21±13.50	313.50±20.57	300.86±7.73	299.29±9.56	
Tear film breakup time (s)	Low dose	16.29±7.01	16.14±7.09	14.31±9.89	13.31±11.60	9.30±5.74	9.40±4.14	p = 0.894
	Standard dose	13.00±7.77	11.60±5.42	15.07±14.34	14.29±10.28	15.07±8.11	14.71±10.51	
Corneal staining (total score)	Low dose	0.93±2.16	1.14±1.83	1.08±2.14	0.92±1.38	0.20±0.63	0.00±0.00	p = 0.181
	Standard dose	0.40±0.74	0.20±0.41	0.71±1.20	0.50±1.16	0.21±0.80	0.21±0.80	
Conjunctival staining (total score)	Low dose	3.50±2.62	3.93±2.46	5.23±4.10	5.31±4.11	5.40±5.17	5.50±5.15	p = 0.999
	Standard dose	4.67±4.24	5.13±4.12	5.14±2.66	5.00±2.60	5.93±4.80	5.40±4.54	
Perimetry main defect (dB)	Low dose	0.33±2.04	0.64±2.09	0.17±3.30	1.00±2.95	0.38±2.88	0.90±2.59	p = 0.930
	Standard dose	0.32±2.68	0.01±2.23	0.40±1.57	0.40±2.20	−0.46±2.48	−0.34±2.62	
Perimetry pattern SD (dB)	Low dose	2.68±0.46	2.74±0.37	2.82±0.75	2.58±0.51	2.57±0.48	2.86±0.71	p = 0.993
	Standard dose	2.71±1.43	2.56±0.44	2.64±0.36	2.67±0.37	2.86±0.34	2.91±0.43	

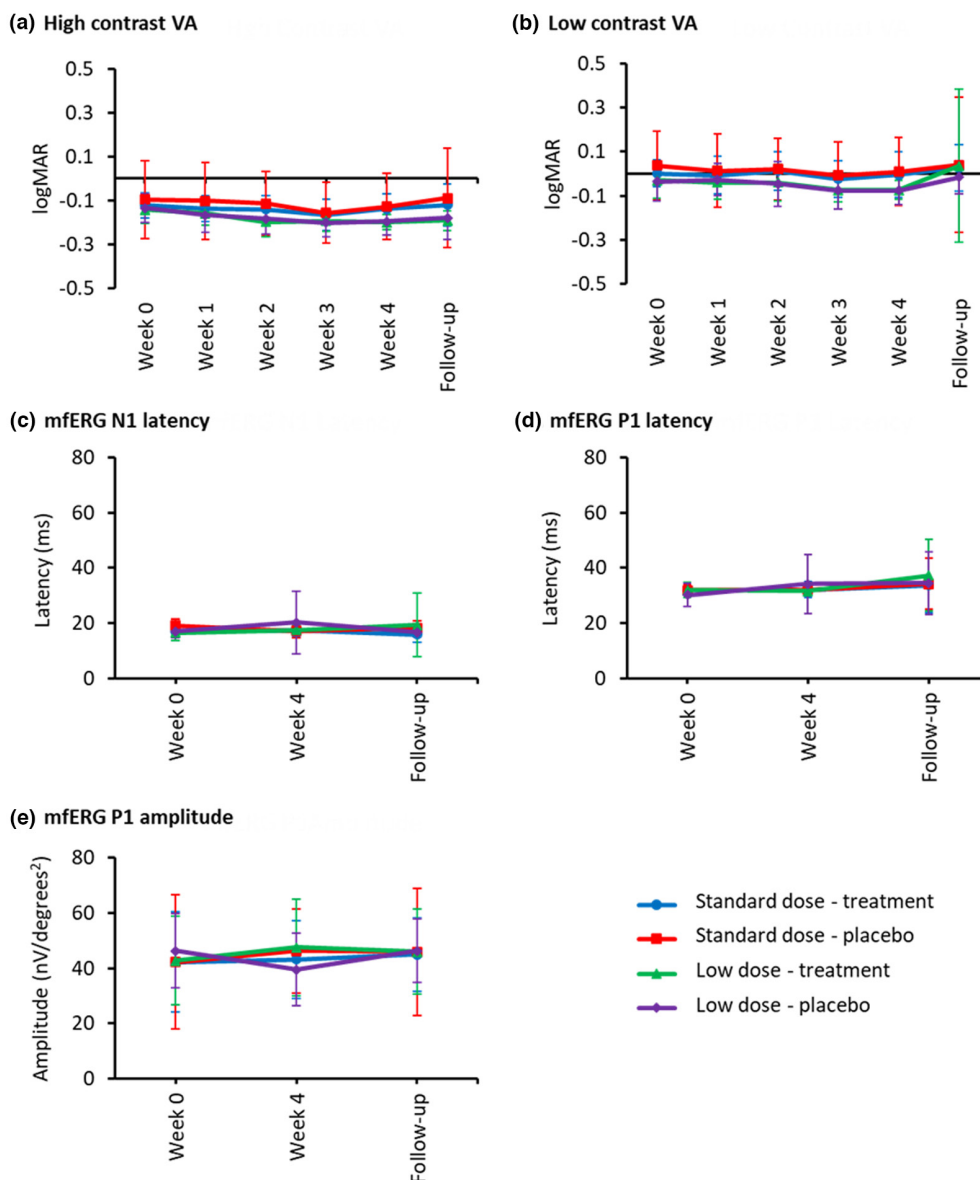Abbreviation: OSDI, ocular surface disease index.

FIGURE 2 Effects of levodopa/carbidopa treatment on visual function. Topical application of levodopa/carbidopa did not induce any significant changes in (a) high- and (b) low-contrast visual acuity (VA), or in multifocal electroretinogram (mfERG) responses as represented by (c) N1 (a-wave) latency, (d) P1 (b-wave) latency, and (e) P1 (b-wave) amplitude recordings in the innermost ring (Ring 1) which represents ~5° eccentricity from the fovea. Data presented as means ± SD of the means.

Measures of visual function

Treatment had no significant effect on mfERG amplitude or latency across all rings measured (Figure 2, Table S7). Similarly, Matrix perimetry found that drug treatment had no significant effects on the mean defect ($p = 0.930$) or pattern standard deviation ($p = 0.993$) over all regions measured (Tables 1 and S7). Levodopa/carbidopa treatment also had no significant effect on either high- ($p = 0.079$) or low-contrast ($p = 0.093$) visual acuity (Figure 2, Table S7), although a statistically significant improvement (of about 1.5 letters) in high-contrast visual acuity was observed for all treatment groups over the course of the trial ($p < 0.001$; Table S8).

Assessment of ocular health

As shown in Figure 3, drug treatment did not induce any significant changes in regional retinal thickness over an 8×8 macular grid during the trial ($p = 0.963$). Similarly, levodopa/carbidopa eye drops did not induce any significant changes in retinal nerve fiber layer thickness ($p = 0.401$) or choroidal thickness ($p = 0.111$; Tables 2 and S9). Although a small overall change in retinal nerve fiber layer thickness was observed between timepoints for all groups ($p < 0.05$; Tables S9 and S10), there was no significant difference between drug- and placebo-treated eyes. No changes in retinal
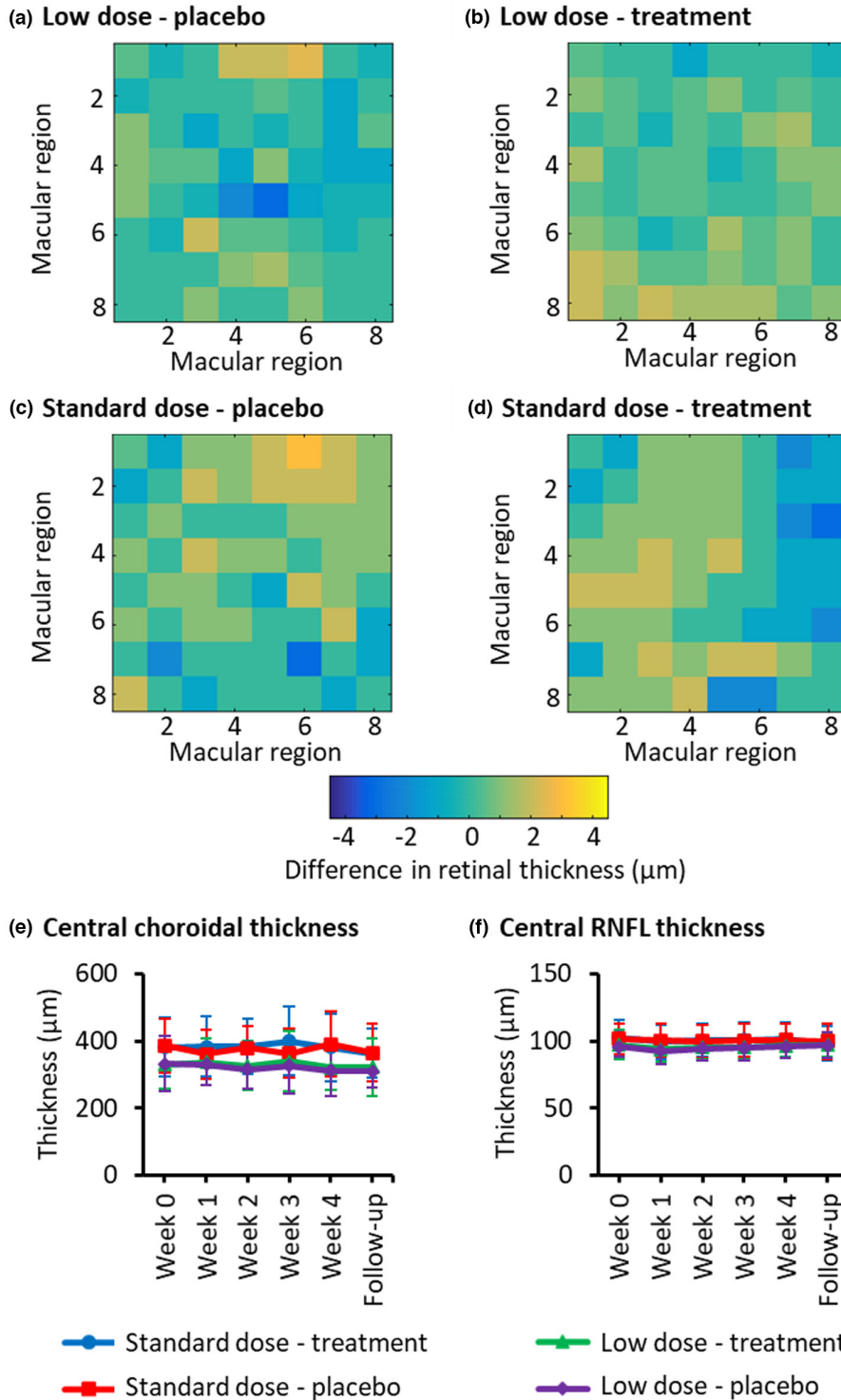
FIGURE 3 Effects of levodopa/carbidopa treatment on ocular health. Median differences across eyes in regional retinal thickness between end of treatment and baseline for placebo (a, b) and treated eyes (b, d) over the OCT posterior pole 8×8 macular grid. Right eyes were flipped to match left eyes before medians were computed. Also shown are the central (e) choroidal and (f) retinal nerve fiber layer (RNFL) thickness measures over the course of the study (data presented as means $\pm$ SD of the means).

autofluorescence were observed at each primary measurement point (baseline, end of treatment, or at the follow-up visit). Likewise, ETDRS grading of fundus photographs found no incidence of disease or damage at each primary measurement point.

Levodopa/carbidopa treatment also had no significant effect on IOP over the course of the trial ($p = 0.823$; Tables 2 and S9). There was a significant difference in IOP between timepoints ($p < 0.001$), which was primarily

observed as a decrease in IOP at follow-up for both drug- and placebo-treated groups (Tables S9 and S10).

Assessment of refractive error and ocular biometry

As demonstrated in Table 2, neither time nor levodopa/carbidopa treatment had a significant effect on cycloplegic

TABLE 2 Measures of ocular health, refraction, and ocular biometry. Data presented for baseline, end of treatment, and follow-up visits. Data from intermediate visits are detailed in Tables S9 and S11, while mixed-effects model results for time are detailed in Tables S10 and S12. All data presented as means ± SD of the means

| Measure | Dosage | Baseline | | End of treatment | | Follow-up | | Mixed-effects model |
		treatment	Placebo	treatment	Placebo	treatment	Placebo	(treatment)
Participants measured	Low dose	14	14	13	13	10	10	
	Standard dose	15	15	14	14	13	13	
Subfoveal choroidal thickness (μm)	Low dose	329.08±71.02	332.38±81.40	320.15±67.64	311.84±74.99	321.71±85.82	310.00±47.33	$p = 0.111$
	Standard dose	381.64±88.12	385.85±80.50	381.00±100.30	390.23±96.88	362.61±72.75	364.23±86.12	
Retinal nerve fiber layer thickness (μm)	Low dose	97.07±10.89	95.79±7.94	96.46±8.49	95.85±8.90	97.10±8.85	96.7±9.51	$p = 0.401$
	Standard dose	102.47±12.93	101.53±11.71	101.14±12.64	100.79±12.21	98.15±12.57	100±13.20	
Intraocular pressure (mmHg)	Low dose	15.43±4.80	17.36±4.27	16.00±3.83	15.77±3.81	11.60±3.13	13.00±3.16	$p = 0.823$
	Standard dose	12.73±3.73	15.20±3.17	14.29±3.00	15.43±3.94	12.43±3.11	12.86±2.71	
Refraction (spherical equivalent; D)	Low dose	0.77±0.45	0.81±0.52	0.77±0.36	0.75±0.34	0.84±0.52	0.90±0.59	$p = 0.063$
	Standard dose	−0.25±2.21	−0.42±2.01	0.40±1.51	0.06±1.83	0.03±1.82	−0.11±1.68	
Axial length (mm)	Low dose	23.74±0.88	23.75±0.93	23.69±0.88	23.71±0.92	23.71±0.99	23.69±1.05	$p = 0.940$
	Standard dose	23.77±0.63	23.92±0.99	23.76±0.63	23.76±0.81	23.77±0.63	23.77±0.82	
Keratometry (flat meridian; D)	Low dose	42.31±1.24	42.37±1.25	42.46±1.14	42.47±1.22	42.61±1.24	42.61±1.32	$p = 0.994$
	Standard dose	42.28±1.28	42.46±1.21	42.53±1.25	42.55±1.18	42.55±1.25	42.54±1.15	
Corneal thickness (μm)	Low dose	529.86±44.42	534.36±44.52	530.77±46.77	531.85±45.63	534.60±40.10	537.5±42.11	$p = 0.784$
	Standard dose	539.43±30.77	540.87±29.60	541.14±31.50	540.21±29.76	541.50±35.30	541.86±34.13	
Anterior chamber depth (mm)	Low dose	3.20±0.20	3.17±0.18	3.22±0.19	3.25±0.23	3.20±0.18	3.20±0.17	$p = 0.371$
	Standard dose	3.05±0.25	3.05±0.20	3.09±0.28	3.06±0.24	3.07±0.26	3.06±0.25	
Lens thickness (mm)	Low dose	3.48±0.18	3.51±0.15	3.44±0.15	3.49±0.15	3.51±0.17	3.52±0.17	$p = 0.194$
	Standard dose	3.61±0.14	3.56±0.14	3.58±0.19	3.60±0.15	3.60±0.14	3.61±0.15	
Amplitude of accommodation (D)	Low dose	11.29±1.38	11.21±2.19	12.46±2.03	12.38±2.10	12.20±2.25	12.15±2.21	$p = 0.219$
	Standard dose	10.56±2.72	10.83±2.42	11.21±3.37	11.57±2.62	11.29±2.94	11.07±2.23	

autorefraction ($p = 0.063$; Table S11). Similarly, levo-dopa/carbidopa treatment did not induce any significant changes in ocular biometry measurements (keratometry [$p = 0.994$], axial length [$p = 0.940$], corneal thickness [$p = 0.784$], anterior chamber depth [$p = 0.371$], and lens thickness [$p = 0.194$], Tables 2 and S11]). Drug treatment also had no significant effects on the participants' amplitude of accommodation ($p = 0.219$; Tables 2 and S11).

Of the above measures, time (but not treatment) was found to have a significant effect on anterior chamber depth ($p < 0.001$), lens thickness ($p < 0.001$) and amplitude of accommodation ($p < 0.001$, Table S12). This was observed in all groups as a decrease in anterior chamber depth and increase in lens thickness in those weeks where participants did not undergo cycloplegia (Table S12). Accommodation showed a significant increase in amplitude at week 1 (Tables 2, S11, and S12).

DISCUSSION

Similar to the preclinical safety findings reported by our group,[10] this study found that daily topical application of an ophthalmic levodopa/carbidopa solution over a 1-month period was tolerable and did not induce any changes in anterior surface integrity, visual function, ocular health, or refraction/ocular biometry in healthy adult males. These findings concur with previous interventional and longitudinal studies in humans showing that systemic levodopa treatment has no adverse effects on visual function as assessed by visual acuity or electroretinogram responses.[21–26]

With regards to non-ocular side effects, previous studies involving oral (systemic) administration of levodopa for the treatment of Parkinson's disease or amblyopia have reported a number of adverse events, which include: low blood pressure, headaches, nausea, confusion, fatigue, mood changes, hallucinations, nightmares, emesis, dyskinesia, dizziness, dry mouth, and a decreased appetite.[27,28] Over the course of this study, participants did not report any of the above non-ocular symptoms associated with levodopa/carbidopa treatment, although this was not extensively assessed and relied on questionnaire responses. This is not unexpected, as such adverse events are normally observed at doses well above (30-fold) those tested presently and are associated with changes in dopamine levels within the brain rather than retina. Furthermore, they commonly manifest over longer treatment durations (decades) and are often interlinked with disease progression.[27,28]

Our results show that irrespective of treatment (i.e., drug or placebo), time had a significant effect on several ocular measures. For example, a decrease in tear film breakup time was observed during weeks 2 and 3 and an increase in tear film osmolarity was observed at week 4. However, these values did not reach clinical significance for a dry eye diagnosis[29,30] and appear to be attributable to external variables (e.g., studying, sleep patterns, and weather) as noted on participants' weekly OSDI questionnaires. The improvement in high-contrast visual acuity observed in all groups over the course of the study appears to be associated with a "learning effect" as reported in previous studies.[31] The drop in IOP at the follow-up visit compared to all other clinic visits appears to be due to seasonal variation, with previous studies observing a drop in IOP during summer months[32] (when the follow-up visit took place). Finally, the decrease in anterior chamber depth and increase in lens thickness observed in the secondary measurement points can be attributed to the absence of cycloplegia[33] in these clinic visits (as participants were able to accommodate during ocular biometry measures).

Based on the current findings, this ophthalmic solution appears to be safe and tolerable to the eye and may be a viable tool for the treatment of human myopia. Although its anti-myopia effects are yet to be assessed in humans, topical levodopa/carbidopa shows significant potential as a myopia treatment through work in animal models, eliciting a high degree of protection against experimental myopia.[10,11,34] Importantly, in animal models, topical levodopa/carbidopa elicits greater protection against myopia than atropine,[10] the primary pharmacological treatment for human myopia.

The applicability of topical levodopa may extend beyond the treatment of myopia, with systemic levodopa treatment proving to be beneficial in amblyopia,[12] diabetic retinopathy,[14] and AMD.[13] For example, levodopa enhances visual function in amblyopia and diabetic retinopathy, although the mechanism is not yet fully elucidated.[12,14] In AMD, levodopa appears to inhibit the release of vascular endothelial growth factor (VEGF) in the retinal pigment epithelium, thereby decreasing the frequency at which anti-VEGF injections are required to slow disease progression.[13,35]

LIMITATIONS

Due to the duration of this trial, we are unable to comment on the long-term safety of topically administered levodopa/carbidopa in humans although chronic treatment was not associated with adverse events in animal models.[10] This study was also undertaken in young adults, rather than the target population (pediatric/adolescents) for myopia intervention, and was limited to male participants. Phase II studies assessing the efficacy of this formulation at inhibiting myopia progression will address

each of these limitations (i.e., will be undertaken in a pediatric population over a significantly longer treatment period [12–24 months] and will involve male and female participants).

This study employed a paired-eye comparison design, naturally matching control and experimental eyes for age, ethnicity, environmental, and genetic factors. The validity of such a comparison relies on the assumption that the two eyes remain independent for the duration of the trial. This assumption is supported by preclinical studies in which no detectable change in levodopa levels were observed in the blood or contralateral control eyes of monocularly treated animals.[10,11] As this was an early safety and tolerability trial, it was important to ensure that the individual factors that may influence ocular tolerability, such as tear volume and tear film breakup time, could be accounted for.

CONCLUSIONS

This is the first study investigating the effects of topical administration of levodopa/carbidopa on the human eye. Together, these results show no evidence of adverse side effects of high or low concentrations of levodopa/carbidopa on ocular tissues, indicating that topical levodopa/carbidopa administration is safe to the eye. This paves the way for future studies on the efficacy of levodopa/carbidopa eye drops in the treatment of human myopia.

AUTHOR CONTRIBUTIONS

K.T., C.K., F.S., N.A., and R.A. wrote the manuscript. K.T., C.K., N.A., I.G.M., and R.A. designed the research. F.S., M.L., T.J. K.T., C.K., and J.G. performed the research. K.T., F.S., N.A., T.M., J.G., and R.A. analyzed the data. R.A. contributed new reagents/analytical tools.

CONFLICT OF INTEREST

R.A. holds a patent for this ophthalmic solution (PCT/AU2017/050310). All other authors declared no competing interests for this work.

ORCID

Kate Thomson https://orcid.org/0000-0002-2272-2800

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.