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# Randomised masked trial of the clinical safety and tolerability of MGO Manuka Honey eye cream for the management of blepharitis

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## ABSTRACT

**Objective** To assess the clinical safety and tolerability of a novel MGO Manuka Honey microemulsion (MHME) eye cream for the management of blepharitis in human subjects.

**Methods and analysis** Twenty-five healthy subjects were enrolled in a prospective, randomised, paired-eye, investigator-masked trial. The MHME eye cream (Manuka Health New Zealand) was applied to the closed eyelids of one eye (randomised) overnight for 2 weeks. LogMAR visual acuity, eyelid irritation symptoms, ocular surface characteristics and tear film parameters were assessed at baseline, day 7 and day 14. Expression of markers of ocular surface inflammation (matrix metalloproteinase-9 and interleukin-6) and goblet cell function (MUC5AC) were quantified using impression cytology at baseline and day 14.

**Results** There were no significant changes in visual acuity, eyelid irritation symptoms, ocular surface characteristics, tear film parameters and inflammatory marker expression during the 2-week treatment period in treated and control eyes (all p>0.05), and measurements did not differ significantly between eyes (all p>0.05). No major adverse events were reported. Two subjects experienced transient ocular stinging, presumably due to migration of the product into the eye, which resolved following aqueous irrigation.

**Conclusion** The MHME eye cream application was found to be well tolerated in healthy human subjects and was not associated with changes in visual acuity, ocular surface characteristics, tear film parameters, expression of markers of inflammation or goblet cell function. The findings support future clinical efficacy trials in patients with blepharitis.

**Trial registration number** ACTRN12616000540415

## Key messages

What is already known about this subject?

- Blepharitis is a common eyelid inflammatory condition, often characterised by intermittent episodes of inflammatory exacerbations, associated with high bacterial load. Current management strategies involve the use of topical antibiotics and corticosteroids during exacerbations. However, concerns of antibiotic resistance and risks of side effects of corticosteroids highlight the need for alternative management strategies to be developed. *In vitro* and *in vivo* preclinical studies have shown promising results for a recently developed MGO Manuka Honey microemulsion.

What are the new findings?

- The Manuka Honey microemulsion was found to be well tolerated in healthy human subjects as an eye cream and was not associated with changes in visual acuity, ocular surface characteristics, tear film parameters, expression of markers of inflammation or goblet cell function.

How might these results change the focus of research or clinical practice?

- The findings of this tolerability trial support future clinical efficacy trials of the Manuka Honey microemulsion eye cream in patients with blepharitis.

## INTRODUCTION

Blepharitis is a common chronic inflammatory condition of the eyelids, associated with ocular surface irritation and dry eye development.[1–5] It can have a significant impact on ocular comfort, vision and quality of life.[1 6 7] Classified anatomically into anterior and posterior blepharitis, the former affects the anterior eyelid lamellae and eyelashes, and the latter affects the posterior lamellae, most commonly as a result of Meibomian gland dysfunction.[1–4] Although the pathophysiology of blepharitis is not fully understood, both anatomical subtypes are associated with abnormally high levels of bacterial colonisation, which may contribute towards the inflammatory process.[2] Lipases from

bacteria contribute to tear film destabilisation and exacerbate symptoms of ocular irritation and dry eye.[8 9]

The natural history of blepharitis is commonly characterised by intermittent episodes of inflammatory exacerbations, associated with high bacterial load.[1 2] Current management strategies involve a combination approach of topical antibiotics and anti-inflammatory agents during exacerbations and the long-term use of eyelid hygiene techniques and/or warm compresses.[2 3 5] However, symptoms often persist despite treatment,[1 2] negatively affecting perceived therapeutic efficacy. Furthermore, concerns of antibiotic resistance and risks of side effects of anti-inflammatory agents necessitate prudent clinical judgement surrounding their use[1 2] and highlight the need for alternative management strategies to be developed.

New Zealand native Mānuka (*Leptospermum scoparium*) honey has received interest for its reported antimicrobial and anti-inflammatory properties.[10–13] A topical formulation of MGO Manuka Honey microemulsion (MHME) was recently developed as an eye cream for periocular use.[14] *In vitro* and *in vivo* preclinical studies of the product have shown promising results, suggesting potential for clinical efficacy and safety in the management of blepharitis.[14] This trial is the first to evaluate the safety and tolerability of the MHME eye cream, in human subjects, through the clinical assessment of the ocular surface and tear film and laboratory analysis of inflammation and goblet cell function marker expression.

## MATERIAL AND METHODS

### Subjects

This prospective, 2-week, randomised, paired eye, investigator-masked trial, followed the tenets of the Declaration of Helsinki, was approved by the institutional ethics committee and was registered as a clinical trial (ACTRN12616000540415). Participants were required to be 18 years or older and non-contact lens wearers, with no history of major systemic, dermatological or ocular conditions, non-pregnant, no previous ocular surgery, no use of topical or systemic medications known to affect the eye and no allergies or hypersensitivity to topical medications or bee products. Eligible participants were enrolled after providing written informed consent and were required to attend three visits over the 2-week period at baseline, day 7 and day 14.

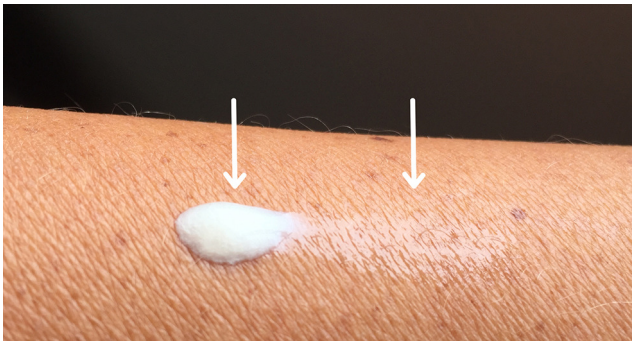

**Figure 1** The Manuka honey microemulsion cream as it appears on extrusion from the tube (left arrow) and as it would following application thinly to the periocular area (right arrow).

A total of 25 eligible participants were recruited, exceeding the sample size requirements for the desired study power. The designated outcome measure for determining sample size was tear film lipid layer grade. Power calculations showed that a minimum of 15 subjects was required to detect a clinically significant difference of one lipid layer grade in any of the pairwise comparisons, with 80% power ($\beta$=0.2), at a two-sided statistical significance level of 5% ($\alpha$=0.05). The standard deviation (SD) of normal values was estimated at one lipid layer grade.[15] Sample size estimates were determined using a uniform non-parametric adjustment, with PASS 2002 (NCSS Statistical Software, Kaysville, Utah, USA).

### Treatments

Participants were randomly assigned to apply the MHME eye cream (figure 1), which was manufactured according to Good Manufacturing Practice (GMP) standards (Manuka Health New Zealand) to the left or right eye (treated eye) once a day, at night, for a period of 14 days. The fellow eye received no intervention (control eye). Two 20 mL tubes of eye cream were provided to each participant, and product application was demonstrated during the enrolment visit. Instructions were given to participants to squeeze approximately a 0.5–1 cm strip of the product (equivalent to a minimum of approximately 0.034 g±0.001 g) onto clean fingertips and to spread the cream thinly over the periocular skin of the closed upper and lower eyelids of one eye, in order to avoid direct contact with the ocular surface. Participants were then instructed to wash their hands immediately to prevent cross-contamination of the fellow eye. Participants were also advised against facial cleansing until the following morning and to exercise care to avoid the transfer of residual products to the fellow eye during facial cleansing and drying. Unused product was returned to the investigators at the end of the 14-day trial period and weighed as a measure of patient compliance.

### Clinical measurements

McMonnies Dry Eye Questionnaire was administered to grade the severity of dry eye symptomatology at baseline. A telephone interview was conducted following the first day of eye cream application to check for immediate tolerability issues or adverse events. Participants were advised to contact the investigators during the treatment period to report adverse events at any other time.

The investigators conducting clinical measurements were masked to treatment randomisation. Participants

**Table 1** Repeated-measures analysis of variance of measurements for treatment, time and interaction (treatment-by-time) effects. Ordinal data were converted to rank-values prior to assessment. Data are presented as p values

| | p Value | | |
|---|---|---|---|
| | **Treatment** | **Time** | **Interaction** |
| General evaluation | | | |
| Best corrected visual acuity (logMAR) | 0.96 | 0.15 | 0.33 |
| Eyelid itching grade | 0.16 | 0.95 | 0.62 |
| Eyelid pain grade | 0.33 | 0.77 | 0.38 |
| Ocular surface evaluation | | | |
| Bulbar conjunctival hyperaemia | 0.15 | 0.18 | 0.92 |
| Palpebral erythema | 0.94 | 0.14 | 0.35 |
| Sodium fluorescein staining score (out of 55) | 0.12 | 0.62 | 0.16 |
| Lissamine green staining score (out of 55) | 0.54 | 0.35 | 0.15 |
| Lid wiper epitheliopathy grade | 0.27 | 0.79 | 0.24 |
| Tear film evaluation | | | |
| Tear film lipid layer grade | 0.32 | 0.13 | 0.25 |
| Tear evaporation rate ($g/m^2/h$) | 0.31 | 0.15 | 0.47 |
| Non-invasive tear film break-up time (s) | 0.67 | 0.74 | 0.51 |
| Tear film osmolarity (mOsmol/kg) | 0.66 | 0.58 | 0.25 |
| Tear meniscus height (mm) | 0.83 | 0.67 | 0.26 |
| Impression cytology | | | |
| MMP-9 expression | 0.75 | 0.69 | 0.61 |
| IL-6 expression | 0.23 | 0.13 | 0.10 |
| MUC5AC expression | 0.39 | 0.55 | 0.31 |

IL-6, interleukin-6; MMP-9, matrix metalloproteinase-9; MUC5AC, mucin-5AC; SD standard deviation.

were assessed at a single site, with a mean±SD room temperature of 20.2°C±1.4°C and relative humidity of 69.1%±4.6%. Clinical assessments were performed at baseline, day 7 and day 14 of the treatment period. Six-metre best spectacle-corrected LogMAR visual acuity (VA) was recorded. Participants were asked to rate symptoms of eyelid itching and pain on a four-point grading scale: grade 0, absent; grade 1, mild; grade 2, moderate; grade 3, severe. Bulbar conjunctival hyperaemia and palpebral erythema was assessed using the Oculus Keratograph 5M, on a grading scale from 0 to 4, using automated objective evaluation and estimation to the nearest 0.5 grade from high magnification digital imaging, respectively.

Tear film lipid layer grade and non-invasive tear film break-up time were assessed using the Oculus Keratograph 5M. Tear film lipid layer grading was based on the Guillon-Keeler grading system: grade 1, open meshwork; grade 2, closed meshwork; grade 3, wave or flow; grade 4, amorphous; grade 5, coloured fringes; grade 0, non-continuous layer (non-visible or abnormal coloured fringes).[16] Break-up time was recorded as the time taken following a blink for the grid reflection to first show distortion, while the subject maintained fixation and was requested to refrain from blinking. Three measurements were averaged in each case.

The lower tear meniscus height was assessed using high magnification digital imaging captured by the Oculus Keratograph 5M. Three measurements near the centre of the lower meniscus were averaged. Tear evaporation rate was measured using a Vapometer (Delfin, Kuopio, Finland) within a swimming goggle housing.[17] Differences between the evaporation rates in the open and closed eye state were recorded to factor out skin evaporation and allow quantification of evaporation from the tear film of the exposed ocular surface only. Tear film osmolarity was evaluated with a clinical osmometer (TearLab, San Diego, California, USA), using 50 nL tear samples collected from the lower lid tear meniscus. Two measurements were taken from each eye, and the higher reading for each eye was recorded.

Sodium fluorescein and lissamine green dyes were applied, in turn, to the bulbar conjunctiva in order to evaluate localised corneal and conjunctival epithelial desiccation. Staining was recorded using the modified

**Table 2** Clinical and impression cytology measurements of the eyes of subjects randomised to treatment and control groups at baseline, day 7 and day 14. Data are presented as mean±SD or median (IQR). Impression cytology measurements are reported as calibrated normalised relative quantity

| | Treated eye (n=25) | Control eye (n=25) | p Value |
|---|---|---|---|
| Best corrected visual acuity (logMAR) | | | |
| Baseline | −0.05±0.07 | −0.06±0.07 | 0.54 |
| Day 7 | −0.06±0.07 | −0.06±0.06 | 0.88 |
| Day 14 | −0.07±0.06 | −0.07±0.06 | 0.92 |
| p | 0.12 | 0.52 | |
| Eyelid itching grade | | | |
| Baseline | 0 (0–0) | 0 (0–0) | >0.99 |
| Day 7 | 0 (0–0) | 0 (0–0) | 0.55 |
| Day 14 | 0 (0–0) | 0 (0–0) | 0.55 |
| p | 0.82 | 0.38 | |
| Eyelid pain grade | | | |
| Baseline | 0 (0–0) | 0 (0–0) | >0.99 |
| Day 7 | 0 (0–0) | 0 (0–0) | 0.25 |
| Day 14 | 0 (0–0) | 0 (0–0) | >0.99 |
| p | 0.81 | 0.77 | |
| Bulbar conjunctival hyperaemia score (out of 4) | | | |
| Baseline | 0.6±0.3 | 0.6±0.3 | 0.48 |
| Day 7 | 0.6±0.3 | 0.6±0.3 | 0.19 |
| Day 14 | 0.6±0.2 | 0.5±0.2 | 0.27 |
| p | 0.18 | 0.68 | |
| Palpebral erythema score (out of 4) | | | |
| Baseline | 0.3±0.3 | 0.3±0.3 | 0.60 |
| Day 7 | 0.2±0.2 | 0.2±0.2 | 0.72 |
| Day 14 | 0.2±0.2 | 0.2±0.2 | 0.97 |
| p | 0.83 | 0.21 | |
| Sodium fluorescein staining score (out of 55) | | | |
| Baseline | 2.3±2.1 | 2.4±2.3 | 0.96 |
| Day 7 | 2.6±1.9 | 2.0±1.8 | 0.09 |
| Day 14 | 2.0±1.8 | 1.8±1.6 | 0.83 |
| p | 0.22 | 0.48 | |
| Lissamine green staining score (out of 55) | | | |
| Baseline | 1.1±0.9 | 1.2±0.9 | 0.98 |
| Day 7 | 0.9±0.8 | 1.0±0.8 | 0.99 |
| Day 14 | 1.2±0.9 | 0.6±0.4 | 0.10 |
| p | 0.51 | 0.21 | |
| Lid wiper epitheliopathy grade | | | |
| Baseline | 0 (0–0) | 0 (0–0) | 0.91 |

Continued

**Table 2** Continued

| | Treated eye (n=25) | Control eye (n=25) | p Value |
|---|---|---|---|
| Day 7 | 0 (0–0) | 0 (0–0) | 0.83 |
| Day 14 | 0 (0–0) | 0 (0–0) | 0.20 |
| p | 0.90 | 0.28 | |
| Tear film lipid layer grade | | | |
| Baseline | 3 (2–4) | 3 (2–4) | 0.86 |
| Day 7 | 3 (2–4) | 3 (2–4) | 0.98 |
| Day 14 | 3 (2–5) | 3 (2–4) | 0.29 |
| p | 0.71 | 0.21 | |
| Tear evaporation rate ($g/m^2/h$) | | | |
| Baseline | 60±35 | 54±27 | 0.22 |
| Day 7 | 60±32 | 59±31 | 0.99 |
| Day 14 | 47±24 | 46±25 | 0.98 |
| p | 0.14 | 0.23 | |
| Non-invasive tear film break-up time (s) | | | |
| Baseline | 11.9±8.1 | 13.7±10.8 | 0.73 |
| Day 7 | 13.7±11.6 | 12.0±8.8 | 0.68 |
| Day 14 | 14.3±11.5 | 12.7±9.0 | 0.86 |
| p | 0.76 | 0.53 | |
| Tear film osmolarity (mOsmol/L) | | | |
| Baseline | 301±12 | 307±21 | 0.28 |
| Day 7 | 307±18 | 304±10 | 0.90 |
| Day 14 | 303±19 | 303±17 | >0.99 |
| p | 0.50 | 0.26 | |
| Tear meniscus height (mm) | | | |
| Baseline | 0.26±0.13 | 0.27±0.12 | 0.78 |
| Day 7 | 0.27±0.12 | 0.26±0.08 | 0.87 |
| Day 14 | 0.27±0.12 | 0.25±0.08 | 0.49 |
| p | 0.93 | 0.24 | |
| MMP-9 expression | | | |
| Baseline | 6.54±13.53 | 13.42±42.23 | 0.37 |
| Day 14 | 9.27±22.46 | 8.52±19.09 | 0.80 |
| p | 0.93 | 0.58 | |
| IL-6 expression | | | |
| Baseline | 5.18±6.94 | 2.91±4.16 | 0.85 |
| Day 14 | 3.89±5.93 | 10.68±22.75 | 0.12 |
| p | 0.89 | 0.11 | |
| MUC5AC expression | | | |

Continued

Table 2 Continued

| | Treated eye (n=25) | Control eye (n=25) | p Value |
|---|---|---|---|
| Baseline | 906.3±857.4 | 940.3±745.5 | 0.65 |
| Day 14 | 725.7±738.3 | 1196.7±1455.2 | 0.43 |
| p | 0.32 | 0.75 | |

IL-6, interleukin-6; MMP-9, matrix metalloproteinase-9; MUC5AC, mucin-5AC.

Oxford grading scheme, where the nasal and temporal conjunctiva were divided into three areas each, and the cornea into five areas.[18] Staining was graded from 0 to 5 with increasing confluence in each area and summed to provide a maximum score of 55. Lid wiper epitheliopathy (LWE) grade was assessed using lissamine green dye.[19]

### Quantitative real-time PCR

Conjunctival impression cytology was conducted at baseline and day 14 of the treatment period, following topical anaesthetic application of one drop of Oxybuprocaine hydrochloride 0.4% (minims; Bausch & Lomb (NZ), Auckland, New Zealand) at the ocular surface. Bulbar conjunctival cells from the superior temporal ocular surface were collected using the EYEPRIM conjunctival impression device (OPIA Technologies, Paris, France). RNA extraction and purification from the conjunctival cell samples was conducted using the PureLink RNA Mini Kit (Thermo Fisher Scientific). Extracted RNA samples were tested for the presence of inhibitors before undergoing complementary DNA (cDNA) synthesis. Synthesis was performed using the SuperScript VILO cDNA Synthesis Kit and Master Mix (Invitrogen by Life Technologies). A standard beta-actin PCR and subsequent gel electrophoresis was run on a representative selection of the synthesised cDNA samples to ensure cDNA synthesis was successful. Six reference genes were tested among the sample population for stability with beta-actin and B2M being established as the most stable genes according to the NormFinder algorithm (MOMA, Aarhus, Denmark). A normalisation factor was calculated as the geometric mean of the expression of these two most stable genes and, in accordance with currently accepted practice,[20] the genes of interest were quantified relatively against this factor. Quantitative PCR (qPCR) runs for quantifying the three genes of interest; matrix metalloproteinase-9 (MMP-9), interleukin-6 (IL-6) and mucin-5AC (MUC5AC) were set up using the QiAgility PCR robot (Qiagen, Valencia, California, USA) with PrimeTime Assays (Integrated DNA Technologies). Internal calibrators were used in all qPCR to compensate for inter-run variations. Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were followed to ensure validity of the qPCR experiments.[21]

### Statistics

Statistical analyses were performed with GraphPad Prism V.6.02 (http://www.graphpad.com). Repeated measures two-way analysis of variance (ANOVA) testing was performed to test the significance of treatment, time and interaction (treatment-by-time) effects on measurements over the 2-week period, where continuous variables with a normal distribution had been confirmed by the Kolmogorov-Smirnov test ($p>0.05$). Non-normally distributed continuous measures (non-invasive tear break-up time (NIBUT) and impression cytology measurements) were logarithmically transformed before being assessed. Ordinal data were converted to rank-values prior to undergoing analysis. Post hoc analysis for the significance of treatment effects at each time point was conducted using multiplicity adjusted Sidak's test. All tests were two tailed, and $p<0.05$ was considered significant. Data are presented as mean±SD, or median (IQR) unless otherwise stated.

## RESULTS

The mean±SD age of the 25 participants (eight male, 17 female) was 29±8 years. The mean±SD McMonnies score was 7.2±3.5, with only one participant (4%) displaying a score of ≥15. The mean±SD amount of product used during the 14-day trial period was 1.8±1.3 g, exceeding, in 96% of cases, the expected minimum total application of approximately 0.5 g of product over 14 days, according to the participant instructions, and reflecting good levels of compliance.

Tables 1 and 2 illustrate the summary statistics of clinical and impression cytology measurements made during the 2-week treatment period. There were no statistically significant differences in baseline clinical or impression cytology measurements between treated and control eyes (all $p>0.05$).

### Adverse events

Twenty-three (92%) of 25 participants did not report any tolerability issues or adverse events following the first day of product application. In two individuals, application too close to the eyelash margin and the use of an excessive amount of eye cream, respectively, was presumed to result in migration of product onto the ocular surface, causing a transient

stinging sensation. Aqueous irrigation to remove excess product and careful reapplication of a more modest quantity of eye cream external to the lash line resolved the issue in both cases. No further adverse events were reported during the remainder of the treatment period, and no participants withdrew early from the trial.

### General clinical evaluation

Repeated measures ANOVA demonstrated no significant treatment, time or treatment-by-time interaction effects on best-corrected VA, eyelid itching and pain grades (all p>0.05, table 1). Post hoc analysis showed that measurements did not change significantly during the 2-week period in either treated or control eyes (all p>0.05, table 2), and there were no significant differences between groups at day 7 or day 14 (all p>0.05).

### Ocular surface evaluation

The effects of treatment, time and treatment-by-time interaction on bulbar conjunctival hyperaemia, palpebral erythema, ocular surface staining and LWE were not found to be significant (all p>0.05, table 1). There were no changes in ocular surface characteristics in either treated or control eyes during the treatment period (all p>0.05, table 2), and measurements at day 7 and day 14 were not found to differ significantly between groups (all p>0.05).

### Tear film evaluation

There were no significant treatment, time or treatment-by-time interaction effects on lipid layer grade, tear evaporation rate, non-invasive tear film break-up time, tear film osmolarity or tear meniscus height (all p>0.05, table 1). During the 14-day period, tear film parameters did not change significantly in either treated or control eyes (all p>0.05, table 2), and no differences were observed between groups at day 7 or day 14 (all p>0.05).

### Quantitative real-time PCR

Conjunctival impression cytology samples were inadequate for two participants, and thus samples from only 23 participants were analysed. Expression levels of MMP-9, IL-6 and MUC5AC did not change from baseline to day 14 in either treated or control eyes (all p>0.05, table 2). On day 14, levels of all impression cytology markers did not differ between treated and control eyes (all p>0.05).

## DISCUSSION

Current management strategies for blepharitis aim to prevent and manage inflammatory exacerbations, which can be associated with high bacterial loads.[1–3 5] However, concerns surrounding commonly used antibacterial and anti-inflammatory therapeutic agents, including antibiotic resistance and the long-term effects of corticosteroid use, suggest the need for alternative management strategies. The natural antibacterial and anti-inflammatory properties of New Zealand's native Mānuka honey have been reported, previously.[10–13] An ophthalmic formulation of MGO MHME was recently developed for periocular topical application.[14] Preclinical studies have confirmed the *in vitro* antimicrobial efficacy of cyclodextrin-complexed Manuka honey on bacteria commonly associated with blepharitis,[22] the *in vitro* safety on human corneal cells and *in vivo* tolerability on rabbit eyes following the instillation of the MHME product diluted to 10%.[14]

Clinical safety of the MHME eye cream was demonstrated by the results of this study. During the treatment period, no significant change in VA was observed in treated eyes, and measurements did not differ between treated and control eyes. All ocular surface and tear film measurements remained within normal limits throughout the study period. Bulbar hyperaemia, palpebral erythema and ocular surface staining did not change during the 2-week period in treated eyes, and no differences were detected between groups. This suggests that periocular application of the eye cream was not associated with signs of ocular surface irritation, inflammation or epithelial damage. There were no significant changes in tear film parameters in treated eyes during the 14-day period, and measurements were not significantly different between treated and control eyes. This suggests that the ophthalmic formulation does not exhibit tear film destabilising effects.

The safety profile of the formulation was further supported by the quantification of markers of ocular surface inflammation and goblet cell function, from conjunctival impression cytology. MMP-9 is a matrix degrading enzyme,[23] which is thought to play a pathological role in inflammatory disease, through cleaving tight junction proteins and resulting in epithelial cell layer disruption.[24] Dry eye induced tear film hyperosmolarity can trigger the stress-activated protein kinase signalling cascade, leading to the release of MMP-9 from corneal epithelial cells,[25] initiating a cycle of progressive inflammation.[26] IL-6 is a pro-inflammatory cytokine released by conjunctival and corneal epithelium[27] and is an early biomarker of dry eye disease.[28] The upregulation of IL-6 has been shown to correlate significantly with corneal desiccation, staining and symptom severity.[29] MUC5AC is a goblet cell-specific mucin, which is an indication of conjunctival goblet cell density and integrity.[30] Inflammatory assault at the ocular surface can result in reduced protein expression, which can be associated with tear film instability and leave the eye susceptible to pathogenic invasion.[31–33] No significant changes were observed in the expression levels of the inflammatory and goblet cell function markers investigated in the treated eyes over the 2-week period, and measurements did not differ between treated and control eyes. This indicates that there is

unlikely to be any pro-inflammatory agents present within the emulsion and suggests the safety profile of the treatment to be satisfactory.

The findings of this study also showed that application of the formulation was generally well tolerated in healthy human subjects. No major ocular or systemic adverse events were reported during the 2-week treatment period, and 23 (92%) of 25 subjects did not report any tolerability issues or adverse events during the study period. Two subjects reported a transient stinging sensation, presumably related to product migration onto the ocular surface, following application in close proximity to the eyelash margin and the use of an excessive amount of eye cream, respectively. However, in both cases, symptoms resolved following aqueous irrigation of the ocular surface. The subjects were then instructed to carefully reapply smaller quantities of the product anterior to the eyelash margin, and no further adverse events were reported during the remainder of the trial period.

The tolerability of product application was also reflected in self-reported symptoms of eyelid itching and pain. No changes in eyelid symptomatology grading were observed in treated eyes during the 2-week period, and there were no differences between treated eyes and control eyes. As a patient-applied treatment, the therapeutic potential of the formulation may potentially be limited by compliance levels, which can be adversely affected by intolerability of treatment side effects. The lack of major adverse events or changes in eyelid symptoms in healthy subjects is encouraging and suggests potential for treatment tolerability in patients with blepharitis.

Of note, the MGO MHME formulation was found to be well tolerated as an externally applied eye cream in healthy human subjects. The 2-week treatment application was not associated with any changes in VA, ocular surface characteristics, tear film parameters, inflammatory and goblet cell function marker expression, and no major adverse events were reported. The results of the current study support future trials, of longer duration, investigating the clinical efficacy of the ophthalmic formulation in blepharitis patients.

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**Competing interests** None declared.

**Ethics approval** The University of Auckland Human Participants Ethics Committee.

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**Data sharing statement** All data relating to the study is published. Any requests for data can be made to the corresponding author. Any queries relating to the MGO Manuka Honey microemulsion can be made to Manuka Health New Zealand Ltd.



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