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RESEARCH

Randomised controlled trial of topical antibacterial Manuka (*Leptospermum* species) honey for evaporative dry eye due to meibomian gland dysfunction

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Julie M Albietz PhD BAppSc (Hons) GradCertOcTher
Katrina L Schmid PhD BAppSc (Hons) GradCertOcTher
School of Optometry and Vision Science, Faculty of Health, Queensland University of Technology, Brisbane, Queensland, Australia
E-mail: k.schmid@qut.edu.au

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Background: The aim was to evaluate the efficacy of standardised Manuka (*Leptospermum* species) antibacterial honey as adjunctive twice daily treatment to conventional therapy (warm compresses, lid massage and preservative-free lubricant), in participants with evaporative dry eye due to moderate to advanced meibomian gland dysfunction.

Methods: This prospective, open-label study involved 114 participants. After two weeks of conventional therapy participants were randomised to one of three treatment groups: Optimel Antibacterial Manuka Eye Gel (98 per cent *Leptospermum* species honey) plus conventional therapy (n = 37), Optimel Manuka plus Lubricant Eye Drops (16 per cent *Leptospermum* species honey) plus conventional therapy (n = 37) and a control (conventional therapy) (n = 40). Clinical evaluations performed at baseline and Week 8 included: symptom scores (Ocular Surface Disease Index, Ocular Comfort Index), daily lubricant use, tear assessments (break-up time, secretion, osmolarity and InflammaDry), corneal sensation, ocular surface staining, meibomian gland secretion quality and expressibility, bulbar conjunctival, limbal and lid marginal redness and eyelid marginal bacterial cultures and colony counts.

Results: Significant improvements ($p \leq 0.05$) occurred at Week 8 in symptoms, tear break-up time, staining, tear osmolarity, meibum quality and bulbar, limbal and lid margin redness for all treatments. Improvement in staining was significantly greater with Optimel 16 per cent drops ($p = 0.035$). Significant improvements ($p < 0.05$) in meibomian gland expressibility and InflammaDry occurred for both Optimel treatments. Optimel 98 per cent gel was significantly more effective in improving meibum quality ($p = 0.005$) and gland expressibility ($p = 0.042$). Total eyelid marginal bacterial colony counts reduced significantly with Optimel 16 per cent drops ($p = 0.03$) but not the other treatments. *Staphylococcus epidermidis* counts reduced significantly with Optimel 16 per cent drops ($p = 0.041$) and Optimel 98 per cent gel ($p = 0.027$). Both Optimel treatments significantly reduced the need for lubricants, with Optimel 16 per cent drops decreasing lubricant use most ($p = 0.001$). Temporary redness and stinging were the only adverse effects of Optimel use.

Conclusions: Optimel antibacterial honey treatments are effective as adjunctive therapies for meibomian gland dysfunction.

Key words: antibacterial honey, dry eye, Manuka honey, *Leptospermum* species honey, meibomian gland dysfunction

Meibomian gland dysfunction (MGD) is characterised by chronic, diffuse functional abnormalities of the meibomian glands and altered secretion quality and expressibility.[1] MGD leads to increased tear evaporation, increased tear osmolarity and an increased susceptibility to ocular surface inflammation, ocular surface epithelial damage and discomfort.[1] MGD is recognised as the leading cause of evaporative dry eye disease[1,2] and affects between four per cent and 20 per cent of Caucasians and more than 60 per cent of Asians.[3] Conventional evidence-based therapy for obstructive MGD (warm compresses and eyelid massage and lipid-containing lubricants) is limited in efficacy in moderate to advanced disease.[4,5] Adherence to prolonged, time-consuming home-based therapies is traditionally poor.[4,5] Prescription medications (topical steroids, topical and oral antibiotics and topical immunomodulatory agents) and oral omega 3 essential fatty acids have demonstrated efficacy in reducing symptoms and signs of MGD;[2,4–7] however, side effects, adverse effects, development of antibiotic resistance, pregnancy and lactation contraindications, cost, lack of reimbursement, availability of commercial versions, off-label use and need for ongoing treatment are issues that can limit their long-term use.[1,2,4–6]

Clinic-based treatments for MGD including LipiFlow vectored thermal eyelid

pulsation[8] and intense pulsed light therapy[9] potentially offer sustained (three to six months) improvements in symptoms and signs. Currently, limitations to these treatments include expense of the LipiFlow device and consumables,[10] lack of efficiency of LipiFlow in advanced disease,[9] multiple treatments are required before clinical improvements are achieved with intense pulsed light[11] and individuals with dark African skin phototype, those using photosensitising medications and having photosensitising conditions, are excluded from treatment.[11]

Honey has a long history in eye care and wound care.[12,13] Honey is a supersaturated solution of sugars with an acidic pH, high osmolarity and low water content and can inhibit the growth of microorganisms, reduce oedema and promote epithelialisation.[12,13] Honey from a variety of floral sources and geographic locations and in a range of concentrations, has been used in the chronic management of ocular surface diseases, including post-operative corneal oedema and bullous keratopathy,[14,15] Sjögren's and non-Sjögren's aqueous deficient dry eye,[14,16] evaporative dry eye due to MGD,[14,16] neurotrophic keratitis,[17] vernal keratoconjunctivitis,[18] contact lens-related microbial keratitis[19] and as antimicrobial prophylaxis for eye surgery.[20]

Two standardised *Leptospermum* species (spp.) antibacterial medical honey products are regulatory approved as medical devices to treat dry eye disease associated with MGD in Australia, New Zealand and Europe: Optimel Antibacterial Manuka Eye Gel (98 per cent *Leptospermum* spp. honey, Melcare Biomedical Pty Ltd, Brisbane, Australia) and Optimel Manuka + Dry Eye Drops (16 per cent *Leptospermum* spp. honey, Melcare Biomedical Pty Ltd). These medically regulated *Leptospermum* spp. antibacterial honey products have numerous practical advantages in the chronic care of dry eye and MGD: low cost, over-the-counter, sterile, non-preserved (gel product) and non-benzalkonium chloride preserved (eye drop product), non-cytotoxic with frequent dosing and long-term dosing, multidose, broad spectrum, unaffected by standard room temperature (being most active at 25°C) and unaffected by UV and extended shelf life.[12–14,17,21] *Leptospermum* spp. honeys can also be used concurrently with topical lubricant, antimicrobial, antiglaucoma and anti-inflammatory therapies.[14,16,17]

Currently, there are few published clinical studies on the efficacy of antibacterial honeys in eye care and none involving use of regulatory-approved honey products for dry eye due to MGD. This prospective randomised controlled clinical trial assessed the efficacy of standardised *Leptospermum* spp. antibacterial honey eye gel and eye drop products as adjunctive therapy to conventional treatment for MGD (warm compresses, lid massage and lubricants) in participants with evaporative dry eye due to moderate to advanced MGD.

METHODS

This single-centre, prospective study was conducted at the Queensland University of Technology Optometry Clinic from September 2014 to June 2015. The study was performed in accordance with the 1975 Declaration of Helsinki (revised in Tokyo in 2004) and the requirements of the Queensland University of Technology Human Research Ethics Committee. All participants provided written informed consent.

Participants

Adult participants (n = 114, aged 20 to 92 years, 83 female, 102 Caucasian) with a clinical diagnosis of moderate to severe evaporative dry eye due to MGD were recruited (Table 1).

Exclusion criteria were: hypersensitivity or allergy to honey or bee products; active infection of the eye or adnexae; ocular surgery, contact lens wear or punctal plug insertion within the previous six months; current or recent (within three months) use of topical eye drops other than ocular lubricants; planning a pregnancy, pregnant or lactating; and initiation of or alteration to the dose of a systemic medication known to affect tear production within 30 days of the initial assessment.

Dry eye due to MGD was diagnosed and staged on the severity scale (1 to 4) recommended by the Diagnosis Subcommittee of the International Workshop on Meibomian Gland Dysfunction,[22] presence of one or more Ocular Comfort Index (OCI) symptoms of ocular surface irritation ('dry', 'gritty', 'stingy', 'tired', 'painful', 'itching'),[23] tear film instability as measured by fluorescein break-up time less than 10 seconds, interpalpebral ocular surface fluorescein staining score 1 or more (Oxford Grading Scale)[24] and slitlamp biomicroscopic evidence of MGD, diffuse abnormalities of the meibomian glands, including qualitative and/or quantitative changes in meibum quality and gland expressibility.[22]

Prior to recruitment, an instillation trial of each Optimel treatment product was performed to assess for a hypersensitive reaction (right eye received Optimel 16 per cent drops and left eye received 98 per cent gel). It was expected that the topical ocular use of honey would produce transient stinging and conjunctival inflammation.[13–16,25] If protracted inflammation and/or stinging (more than five minutes after instillation) was experienced or any late stage reactions were reported, the participant was excluded from further participation.

Treatments

Following recruitment, all participants commenced a wash-out period of two weeks involving the commencement of conventional MGD treatment. This treatment involved twice daily warm compresses (using a warm wet face cloth applied to the eyes, reheated if necessary to maintain warmth, for a total of five minutes) followed by gentle lid massage to both eyes. Preservative free lubricant (Systane Ultra [polyethylene glycol 400 0.4 per cent; propylene glycol 0.3 per cent], Alcon, Fort Worth, Texas, USA) was permitted to be used as required in both eyes. No other treatments were permitted.

Following these initial two weeks of conventional treatment, participants were advised to continue the conventional therapy for MGD and randomised to one of three treatment groups:

1. Optimel Antibacterial Manuka Eye Gel (*Leptospermum* spp. honey 980 mg/g, gum [*Acacia senegal*]) (Optimel 98 per cent) (n = 37)
2. Optimel Manuka + Lubricant Eye Drops (*Leptospermum* spp. honey 160 mg/g, water, sodium chloride, benzoic acid 0.2 per cent) (Optimel 16 per cent) (n = 37)
3. no additional treatment (n = 40).

Optimel products were applied twice daily to both eyes. Participants were required to keep a daily log of their adherence to treatment and topical lubricant use.

Variable	Total	Optimel gel 98 per cent	Optimel drops 16 per cent	Control	p
Participants (number)	114	37	37	40	
Age (years)	60.9 ± 16.3	58.9 ± 16.8	62.2 ± 12.7	61.4 ± 18.8	0.66
Gender (number female/ male)	83/114	27/36	28/38	28/40	0.88
Ethnicity (number Caucasian/Asian)	102/12	34/2	33/5	35/5	0.58
MGD severity stage	3.7 ± 0.6	3.8 ± 0.5	3.7 ± 0.7	3.8 ± 0.6	0.42
Lubricant use (dose frequency)	4.0 ± 2.1	4.6 ± 1.6	4.0 ± 2.3	3.4 ± 2.3	0.07

Data are mean and standard deviation except where indicated.
MGD: meibomian gland dysfunction.

Table 1. Participant characteristics at baseline

Six additional participants were recruited but did not complete the trial for the following reasons: unrelated illness requiring commencement of oral antibiotic medication (n = 1); non-adherence to conventional treatment (n = 2); intolerance to temporary stinging (without protracted redness) with repeated use of Optimel 16 per cent drops (n = 2) and Optimel 98 per cent gel (n = 1). The five per cent drop out rate (6/120) is less than that reported for other longitudinal studies of topical treatment for MGD.[7,16] Only the data of the 114 participants that completed both the washout and treatment phases of the trial were included in the analyses (Table 1).

Ocular surface assessments

The following subjective parameters were assessed at baseline (Week 0, following two weeks of conventional treatment) and at Week 8 (after commencing Optimel treatment).

Dry eye symptoms on a score of zero to 100 using validated dry eye symptoms surveys (Ocular Surface Disease Index [OSDI][26] and OCI).[23] The scores of these questionnaires exhibit a positive correlation with each other with a high validity, reliability, specificity and sensitivity.[27] The OSDI assesses both the frequency of dry eye symptoms and their impact on activities of daily living and correlates moderately with clinical signs of moderate aqueous tear-deficient dry eye.[26] In the absence of a specific and validated questionnaire for symptoms of MGD,[1] the OCI, which assesses frequency and severity of non-subtype specific symptoms of ocular surface irritation,[23] was also administered.

Daily lubricant use was assessed via participant log books.

The Schirmer I test of aqueous tear secretion (without anaesthetic, over five minutes) was performed. Values of less than 7.0 mm are considered diagnostic of aqueous tear deficiency.[28] Tear osmolarity was measured using the Tearlab Osmolarity System (Tearlab, San Diego, California, USA) (normal value less than 308 mOsmol/l).[29,30] Central corneal sensation was measured with a 0.12 mm nylon monofilament (Cochet-Bonnet aesthesiometer, Luneau Ophthalmlogie, Chartres, France) (normal reference value 5.5 ± 0.8 cm).[31] InflammaDry (Rapid Pathogen Screening, Inc., Sarasota, Florida, USA) point-of-care immunoassay was used to detect elevated matrix metalloproteinase 9 (MMP-9 tear levels) (40 or more ng/mL). MMP-9 is an inflammatory biomarker that is elevated in the tears of patients with dry eyes.[32]

All participants also underwent an anterior eye slitlamp examination. Ocular surface one per cent sodium fluorescein staining enhanced by a yellow Wratten filter (No. 12, Kodak) and cobalt light was graded using the Oxford Score (zero to 15 for the total exposed inter-palpebral conjunctiva and cornea).[24]

Conjunctival bubar and limbal redness (vascular injection) were graded zero (normal), 1 (trace), 2 (mild), 3 (moderate), 4 (severe) according to the Efron Grading Scales.[33] Eyelid margin redness (vascularity) was graded zero (normal), 1 (mild engorgement), 2 (moderate engorgement), 3 (severe engorgement) with a score of 2 or more considered diagnostic of MGD.[34] Meibum quality was assessed in each of eight glands of the central third of the lower lid on a scale of zero to 3 for each gland: zero, clear; 1, cloudy; 2, cloudy with debris (granular); and 3, thick, like toothpaste (total score range, zero to 24).[22] Meibomian gland expressibility was assessed on a scale of zero to 3 in five glands on the central lower lid, according to the number of glands expressible: zero, all glands; 1, three to four glands; 2, one to two glands; and 3, no glands.[22] The overall severity of the MGD was assessed as Stage 1 (minimal) to Stage 4 (advanced) according to the guidelines of the International Workshop on Meibomian Gland Dysfunction based on symptoms, corneal staining and meibomian gland secretion quality and expressibility.[1]

A swab of the lower eyelid margin in the most symptomatic eye or, if symptoms were equal, the eye with the greatest Oxford staining score, was taken for bacterial cultures and colony counts of the most dominant organisms using previously described methods.[16] With the exception of the lid margin swab, assessments were performed on both eyes of each participant at baseline: safety outcomes were assessed via ophthalmic examinations and the recording of any adverse events that occurred throughout the study.

Objective parameters were assessed by a single investigator to reduce inter-observer variability. In an attempt to avoid more invasive tests influencing the outcome of subsequent tests, the following testing order was used: symptom surveys, slitlamp examination to grade lid margin, bulbar and limbal redness, TearLab osmolarity, InflammaDry, Schirmer I, lid margin swab, fluorescein ocular surface staining, fluorescein break-up time, meibum quality and meibomian gland expressibility and corneal sensation.

Data analysis

The data on the participant's most symptomatic eye at baseline (or if symptoms were

equal, the eye with the greatest baseline Oxford staining score), was selected for data analysis. All values are presented as mean and standard deviation unless indicated otherwise. Statistical Package for the Social Sciences (IBM SPSS Statistics 22.0, Armonk, New York, USA) was used for analysis. One-way factorial analyses of variance were used for assessment of continuous normally distributed data for comparisons of the three treatment groups, with post-hoc Least Significant Difference subsequently applied. Normality was assessed using the Shapiro-Wilk test for assessment of normality in small data sets (less than 2,000 elements), if p is greater than 0.05, the data were normally distributed. Non-parametric independent samples Kruskal–Wallis test was used for group comparisons of scaled data (MGD severity stage, meibomian gland expressibility score, meibum secretion quality score, ocular surface staining score, all of the ocular redness scores, MMP-9, daily lubricant dose). Baseline and Week 8 data for each treatment were compared separately using paired t-tests or related-samples Wilcoxin signed rank test (dependent on whether a parametric or non-parametric test was required). The difference between the Week 8 and baseline data was used to quantify the treatment effect. Difference data and baseline measures were used in two-tailed Pearson correlation analyses to assess relationships between the baseline measures and treatment effects. A p-value of ≤ 0.05 was considered significant.

Power calculation

If the baseline OSDI is 40 ± 15 and a decrease of 10 in OSDI[27] is considered clinically relevant, then the power calculation gives n = 20, for a power 1 − β = 0.8 and α = 5 per cent. If a clinically relevant difference in treatment effect between groups is 10 on the OSDI, then the power calculation gives n = 36 in each group, for the same power.

RESULTS

Participant characteristics at baseline

The participants in the three treatment groups were of similar age, gender distribution and ethnicity. The participant cohort was predominantly middle aged, predominantly female and predominantly Caucasian (Table 1). MGD severity stage and the need to use ocular lubricants also were similar. On average, participants had moderate to advanced staged MGD and used lubricants four times daily (Table 1).

At baseline, the three groups were similar for all assessments of dry eye and MGD (Table 2), except for the limbal and bulbar redness scores, which varied between the groups (p = 0.05). Limbal and bulbar redness scores were slightly higher for Optimel 98 per cent gel group.

Participants were highly symptomatic (OSDI score greater than 33 indicates severe dry eye)[26,27] had normal aqueous tear production (Schirmer I test seven or more mm per five minutes),[28] normal tear osmolarity and very poor tear film stability.[22] Corneal sensitivity was normal[31] and moderate interpalpebral ocular surface staining was present.[1] Mild to moderate limbal,[33] bulbar[33] and lid margin redness[34] were present and 33 per cent of participants' tears had elevated levels of MMP-9.[32]

The most dominant organisms cultured from the lid margins is listed in Table 3. The most common cultured organism was the ubiquitous coagulase *Staphylococcus epidermidis*, which was present on the lid margins of 42 per cent of the participants and the next most common was *Staphylococcus aureus* with positive cultures in 20 per cent (Table 3). Lid margin bacterial colony counts were high and variable (Table 4).

Variable	Total	Optimel gel 98 per cent	Optimel gel 16 per cent	Control	p
OSDI score	39.8 ± 19.4	45.4 ± 17.3	38.2 ± 15.6	36.2 ± 23.3	0.10
OCI score	40.1 ± 12.0	41.9 ± 8.7	37.1 ± 9.9	41.4 ± 15.6	0.16
MG expressibility score	1.2 ± 2.4	1.7 ± 3.9	1.1 ± 1.0	0.8 ± 0.8	0.29
Meibum quality score	15.1 ± 5.3	15.6 ± 5.2	15.2 ± 5.7	14.6 ± 5.2	0.71
Schirmer I (mm/5 minutes)	15.2 ± 9.9	15.9 ± 11.0	14.3 ± 9.2	15.4 ± 9.8	0.78
Osmolarity (mOsmol/L)	299 ± 17	304 ± 16	297 ± 19	297 ± 14	0.14
FBUT (seconds)	1.7 ± 1.6	1.8 ± 1.8	1.8 ± 1.8	1.5 ± 1.2	0.74
Corneal sensitivity (mm)	5.1 ± 1.5	5.1 ± 1.6	5.2 ± 1.4	5.1 ± 1.5	0.84
Staining score	5.5 ± 3.2	6.0 ± 4.0	5.4 ± 2.8	5.2 ± 2.7	0.58
Limbal redness score	2.1 ± 0.9	2.3 ± 0.9	2.2 ± 0.9	1.9 ± 0.7	0.05*
Bulbar redness score	2.4 ± 0.7	2.6 ± 0.7	2.2 ± 0.7	2.3 ± 0.7	0.05*
Lid margin redness score	1.3 ± 0.7	1.4 ± 0.6	1.3 ± 0.8	1.1 ± 0.8	0.21
MMP-9 (number ≥40 ng/ml)	38	15	12	11	0.41
Lid margin colony count	344 ± 661	305 ± 626	349 ± 712	373 ± 658	0.91

*Baseline data significantly different across treatment groups at p ≤ 0.05. Data are mean and standard deviation except for MMP-9 data.
FBUT: fluorescein break-up time, Lid margin colony count: number of colony-forming units cultured from the eyelid margin, MG: meibomian gland, MMP-9: tear matrix metalloproteinase 9, OCI: Ocular Comfort Index, OSDI: Ocular Surface Disease Index, Staining score: Oxford interpalpebral staining score.

Table 2. Participant symptoms, tear film and ocular surface characteristics at baseline

Organism	Optimel gel 98 per cent		Optimel drops 16 per cent		Control	
	BL	W8	BL	W8	BL	W8
Staphylococcus epidermidis	13	15	17	13	18	16
Staphylococcus aureus	7	4	7	7	9	8
Corynebacterium spp.	0	1	1	0	2	2
Seratia marcescens	0	1	1	0	0	0
Enterococcus spp.	0	0	0	1	1	1
Streptococcus spp.	3	0	0	0	0	0
Acintobacter spp.	0	0	1	0	1	0
None	13	16	11	17	9	13

BL: baseline assessment, None: no organisms cultured, W8: Week 8 assessment.

Table 3. Comparison of the number of participants culturing different bacterial species from the lid margin before (BL) and after (W8) treatment

Treatment effects

SYMPTOMS

For all three treatment groups, treatment resulted in statistically significant improvement ($p \leq 0.05$) in symptoms based on both the OSDI and OCI surveys (Table 5 and Figure 1). Improvements on the OSDI were clinically significant[27] for all three treatment groups (16.4 ± 20.3, 12.7 ± 17.6 and 10.9 ± 26.0 for Optimel 98 per cent gel, Optimel 16 per cent drops and control groups, respectively; Table 6); the improvements across the three groups were not significantly different (Figure 1A and Table 6). Improvements on the OCI were also not significantly different for the three treatment groups (Figure 1B and Table 6). For the OSDI, 86, 81 and 60 per cent of participants reported a subjective improvement in the Optimel 98 per cent gel, Optimel 16 per cent drops and control groups, respectively; related numbers were 86, 84 and 82 per cent for the OCI. Only one participant in the Optimel 16 per cent drop group and two participants in the control group reported no improvement on either survey.

OBJECTIVE SIGNS

All three treatments significantly improved ($p \leq 0.05$) the following objective signs of dry eye and MGD (Table 5): meibum quality, tear osmolarity, fluorescein tear break-up time, corneal staining, limbal redness, bulbar redness, lid margin redness and InflammaDry. Meibomian gland expressibility score and InflammaDry were improved by both Optimel treatments ($p \leq 0.05$) but not by the control treatment. None of the treatments improved tear production (based on the Schirmer I) or corneal sensitivity, which were normal at baseline and remained unchanged (Table 5).

OSMOLARITY

All three treatments significantly ($p \leq 0.05$) lowered tear osmolarity (Table 5). The improvements across the three groups were not significantly different (Table 6 and Figure 2A). For all treatments the decrease in tear osmolarity was correlated to the baseline tear osmolarity (Optimel 98 per cent gel, $R = 0.975$, $p < 0.001$; Optimel 16 per cent drop, $R = 0.773$, $p < 0.001$; control, $R = 0.605$, $p < 0.001$).

FLUORESCEIN BREAK-UP TIME

All three treatments significantly increased ($p \leq 0.05$) the tear break-up time (Table 5). The improvements across the three groups were not significantly different (Table 6 and Figure 2B).

MEIBUM QUALITY AND MEIBOMIAN GLAND EXPRESSIBILITY

All three treatments significantly improved ($p \leq 0.05$) meibum quality (Table 5 and Figure 3B). Both the Optimel treatments significantly improved meibomian gland expressibility but the control did not (Table 5 and Figure 3A). There were significant differences in the abilities of the three treatments on both measures (Table 6). Optimel 98 per cent gel had the greater effect on both gland expressibility (post-hoc Optimel 98 per cent gel versus control $p = 0.04$) and meibum quality (post-hoc Optimel 98 per cent gel versus control $p = 0.001$ and Optimel 16 per cent

Organism	Optimel gel 98 per cent		Optimel drops 16 per cent		Control	
	BL	W8	BL	W8	BL	W8
Staphylococcus epidermidis	696 ± 911	226 ± 442	421 ± 763	288 ± 817	248 ± 391	155 ± 123
Staphylococcus aureus	240 ± 248	168 ± 145	263 ± 133	71 ± 79	669 ± 1,055	855 ± 1,124
Corynebacterium spp.	0	20	50	0	1,245 ± 502	170 ± 212
Enterococcus spp.	1,500	0	0	990	1,500	1,300
Serratia marcescens	0	1,200	0	0	0	0
Acinetobacter spp.	0	0	3,000	0	430	0
Streptococcus spp.	87 ± 115	0	0	0	0	0

Data are mean and standard deviation. No standard deviation means that only one participant cultured the indicated organism.
BL: baseline assessment, W8: Week 8 assessment.

Table 4. Comparison of colony counts of each bacterial species cultured from the lid margin before (BL) and after (W8) treatment

Variable	Optimel gel 98 per cent	p	Optimel drops 16 per cent	p	Control	p
OSDI score	29.1 ± 18.7	0.0005*	24.6 ± 13.6	0.0005*	25.3 ± 16.8	0.01*
OCI score	35.3 ± 12.3	0.0005*	27.8 ± 11.2	0.0005*	34.3 ± 11.6	0.003*
MG expressibility score	0.4 ± 0.6	0.001*	0.7 ± 1.4	0.008*	0.6 ± 0.8	0.11
Meibum quality score	8.1 ± 4.4	0.0005*	9.5 ± 6.7	0.0005*	11.2 ± 6.0	0.003*
Schirmer I (mm/5 minutes)	18.6 ± 19.7	0.42	16.1 ± 9.4	0.07	15.5 ± 9.8	0.84
Osmolarity (mOsmol/L)	292 ± 14	0.001*	291 ± 12	0.04*	288 ± 14	0.003*
FBUT (seconds)	3.0 ± 1.7	0.005*	3.9 ± 2.6	0.0005*	3.2 ± 3.8	0.01*
Corneal sensitivity (mm)	5.2 ± 1.7	0.48	5.7 ± 0.9	0.06	5.2 ± 1.4	0.48
Staining score	2.7 ± 3.1	0.0005*	1.6 ± 2.2	0.0005*	3.1 ± 3.0	0.0005*
Limbal redness score	1.5 ± 0.9	0.001*	1.2 ± 0.7	0.0005*	1.3 ± 0.8	0.003*
Bulbar redness score	1.6 ± 0.9	0.0005*	1.5 ± 0.6	0.0005*	1.5 ± 0.8	0.0005*
Lid margin redness	0.9 ± 0.7	0.0005*	0.9 ± 0.6	0.004*	0.8 ± 0.8	0.01*
MMP-9 (number ≥40 ng/ml)	7	0.005*	2	0.003*	8	0.26
Lid colony count	113 ± 302	0.10	141 ± 512	0.03*	274 ± 601	0.41
Daily lubricant use	2.6 ± 2.2	0.0005*	1.0 ± 1.4	0.0005*	2.8 ± 2.2	0.12

Statistics represent comparison of baseline and Week 8 data for each treatment group separately. Data are mean and standard deviation except for MMP-9 data.
FBUT: fluorescein break-up time, Lid margin colony count: number of colony-forming units cultured from the eyelid margin, MG: meibomian gland, MMP-9: tear matrix metalloproteinase 9, OCI: Ocular Comfort Index, OSDI: Ocular Surface Disease Index, Staining score: Oxford interpalpebral staining score.

Table 5. Participant symptoms, tear film and ocular surface characteristics at Week 8

drops versus control p = 0.026). For Optimel 98 per cent gel the improvement in gland expressibility was correlated with baseline gland expressibility (R = 0.988, p < 0.005) and to the improvement in corneal staining (R = 0.35, p = 0.036) and improvement in meibum quality (R = 0.417, p = 0.011).

INFLAMMATION

All three treatments significantly (p ≤ 0.05) improved lid margin redness, bulbar redness and limbal redness (Table 5 and Figure 4). There were no significant differences in the three treatments' abilities to impact either measure (Table 6). Tear MMP-9 expression was above threshold in significantly fewer participants with both Optimel 98 per cent gel and Optimel 16 per cent drops without any significant difference noted between the Optimel treatment groups in the ability to reduce MMP-9 expression.

CORNEAL STAINING

All three treatments significantly (p ≤ 0.05) improved interpalpebral staining (Table 5). There were significant differences in the three treatments' abilities to do this, with Optimel 16 per cent having the greater effect (post-hoc Optimel 16 per cent versus control p = 0.012) (Table 6 and Figure 5). Observed reductions in staining occurred in 83, 89 and 75 per cent of participants in the Optimel 98 per cent, Optimel 16 per cent and control groups, respectively (Figure 5). The improvement in corneal staining was correlated with baseline severity for all treatment groups (Optimel 98 per cent R = 0.646, p < 0.001; Optimel 16 per cent R = 0.358, p = 0.023; and control R = 0.659, p < 0.001).

BACTERIAL COLONY COUNTS

Total lid margin bacterial colony counts for all bacterial species were significantly improved with Optimel 16 per cent drops but not Optimel 98 per cent gel or the control (Table 5). The range of organisms cultured from the lid margins of participants both before and after treatment is shown in Table 5; the most commonly cultured organism was *Staphylococcus epidermidis*. The number of participants culturing no organisms increased slightly in all groups from 36 to 44 per cent, 30 to 46 per cent and 23 to 33 per cent for Optimel 98 per cent gel, Optimel 16 per cent and the control, respectively (Table 3). For those participants culturing *Staphylococcus epidermidis* on the lid margins at baseline, a significant reduction in colony forming units was achieved at Week 8 compared with baseline for Optimel 16 per cent drops (p = 0.041) and Optimel 98 per cent gel (p = 0.027) but not the control group (p = 0.062); however, these reductions in colony counts were not significantly different between the treatment groups (p = 0.055) (Table 3).

LUBRICANT USE

Both Optimel treatments but not the control resulted in participants being able to reduce their daily lubricant use (Table 5). There were significant differences in the three treatments' abilities to reduce the need for lubricants, with Optimel 16 per cent having the greater effect (Table 6 and Figure 6). The control treatment did significantly reduce the need for lubricants (Table 5). Reductions in daily lubricant use averaged 2.1 ± 2.4, 2.7 ± 2.1, 0.7 ± 2.8 drops per day for Optimel 98 per cent gel, Optimel 16 per cent drops and the control, respectively. Seventy-three per cent, 95 and 48 per cent of participants reported decreased use of lubricants in the Optimel 16 per cent drops, Optimel 98 per cent gel and control groups, respectively.

SAFETY

No adverse effects of treatment were reported other than temporary stinging and redness

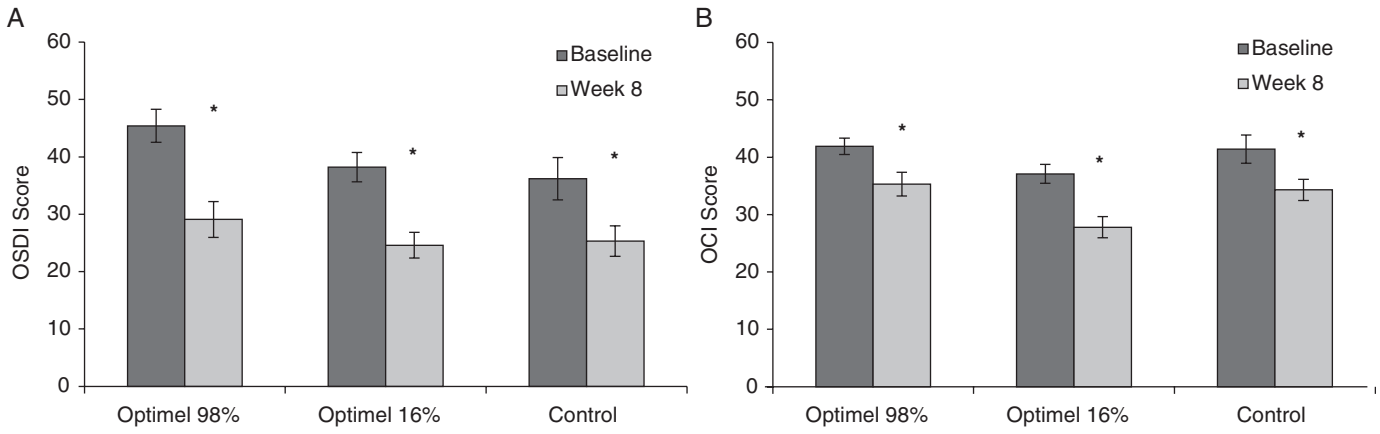

Figure 1. Ocular Surface Disease Index (OSDI) (A) and Ocular Comfort Index (OCI) (B) symptom scores for the three treatment groups at baseline and Week 8 (after treatment). All three treatments significantly improved the subjective measures of dry eye disease. There were no significant differences for the subjective improvements of the three treatments. Data are mean and standard error. *p < 0.05.

for less than five minutes after instillation of honey drops and gel.

DISCUSSION

This is the first prospective randomised controlled study to report the clinical improvements in moderate to advanced MGD with the topical use of medically certified antibacterial *Leptospermum* spp. honey. MGD is associated with multiple pathological mechanisms including eyelid inflammation, microbial proliferation and tear lipid layer deficiencies.[35] The stasis of the meibum in MGD can promote the growth of bacteria and mites (predominantly *Staphylococcus* spp. and *Demodex folliculorum)* and increase release of esterases and lipases from commensal lid margin bacteria.[36] As a consequence of this increased enzyme activity, bacteria can change the viscosity of the meibum, leading to further stasis of the meibum within the meibomian glands and generate free

Variable	Optimel gel 98 per cent	Optimel drops 16 per cent	Lid hygiene	p
OSDI score	16.4 ± 20.3	12.7 ± 17.6	10.9 ± 26.0	0.54
OCI score	6.6 ± 9.1	9.1 ± 10.5	7.1 ± 14.2	0.62
MG expressibility score	1.3 ± 3.8	0.5 ± 1.6	0.2 ± 0.7	*0.042**
Meibum quality score	7.5 ± 6.0	5.8 ± 6.6	2.7 ± 5.3	*0.005**
Schirmer I (mm/5 minutes)	2.8 ± 20.1	2.1 ± 6.8	0.2 ± 6.4	0.65
Tear osmolarity (mOsmol/L)	11.5 ± 18.5	5.5 ± 15.6	8.2 ± 16.3	0.31
FBUT (seconds)	1.2 ± 1.6	2.1 ± 3.0	1.7 ± 3.9	0.47
Corneal sensitivity (mm)	0.1 ± 1.1	0.4 ± 1.3	0.2 ± 1.3	0.50
Staining score	3.3 ± 3.3	3.8 ± 2.6	2.2 ± 2.5	*0.035**
Limbal redness score	0.8 ± 1.2	1.0 ± 1.0	0.5 ± 1.0	*0.13*
Bulbar redness score	1.0 ± 1.0	0.7 ± 0.9	0.8 ± 0.8	*0.69*
Lid margin redness score	0.5 ± 0.7	0.4 ± 0.9	0.3 ± 0.7	*0.38*
MMP-9 (number improved)	8	10	3	*0.15*
Lid margin colony count	192 ± 690	210 ± 560	99 ± 748	0.73
Daily lubricant use	2.1 ± 2.4	2.7 ± 2.1	0.7 ± 2.8	*0.001**

*Improvements across treatment groups significantly different at p ≤ 0.05. Italicised p-values represent non-parametric test outcomes. Data are mean and standard deviation except for MMP-9 data.
FBUT: fluorescein break-up time, Lid margin colony count: number of colony forming units cultured from the eyelid margin, MG: meibomian gland, MMP-9: tear matrix metalloproteinase 9, OCI: Ocular Comfort Index, OSDI: Ocular Surface Disease Index, Staining score: Oxford interpalpebral staining score.

Table 6. Comparison of measured improvements in symptoms, tear film and ocular surface characteristics (difference between baseline and Week 8 data) for the three treatments groups

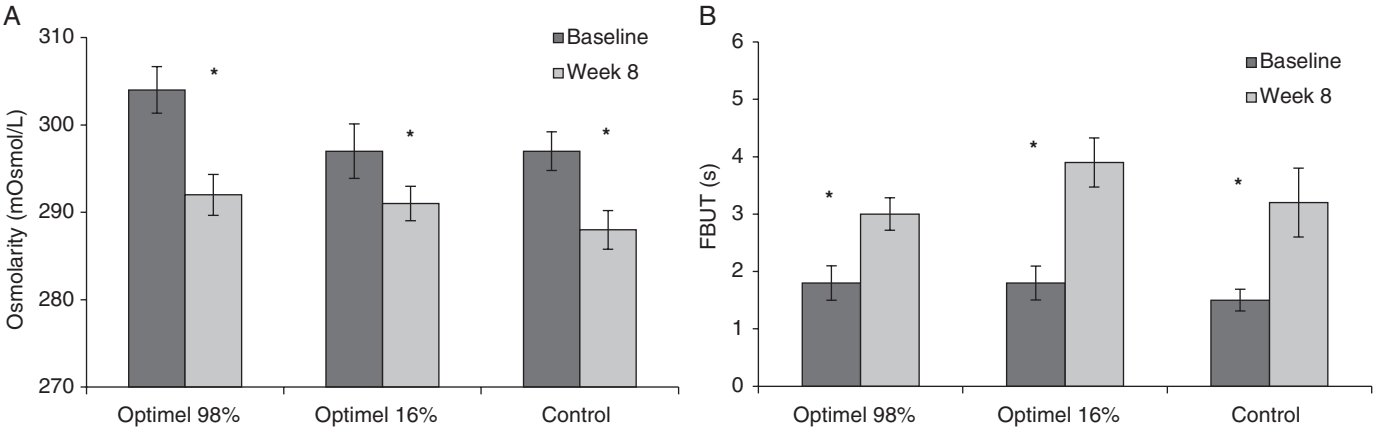

Figure 2. Tear osmolarity (A) and tear fluorescein break-up time (B) for the three treatment groups at baseline and Week 8 (after treatment). All three treatments significantly improved both the osmolarity (lowered) and the break-up time (increased). There were no significant differences among the measured improvements of the three treatments. Data are mean and standard error. *p < 0.05.

fatty acids, causing inflammation and hyperkeratinisation and leading to further gland obstruction.[36] Optimel *Leptospermum* spp. honey products used twice daily over two months were demonstrated to improve multiple factors involved in pathogenesis of MGD and evaporative dry eye and improve related dry symptoms and clinical signs. Compared with conventional therapy, significant improvements in meibum quality and gland expressibility, reduced lid margin *Staphylococcus* spp. bacterial isolates and reduced ocular surface expression of the inflammatory cytokine MMP-9 were achieved with both Optimel 98 per cent gel and Optimel 16 per cent drops. Reduced ocular surface epithelial damage (staining) occurred with use of Optimel 16 per cent drops.

Symptomatic improvement

While subjective improvements with Optimel products over conventional therapy were not achieved according to the validated symptoms survey scores (OSDI, OCI) (Figure 1), over 20 per cent more participants in each Optimel treatment group reported symptomatic improvement in the OSDI compared with the control group. Additionally, twice daily use of Optimel honey 16 per cent significantly reduced the need for lubricants by approximately three instillations per day.

One of the major limitations of topical ophthalmic honey is the temporary redness

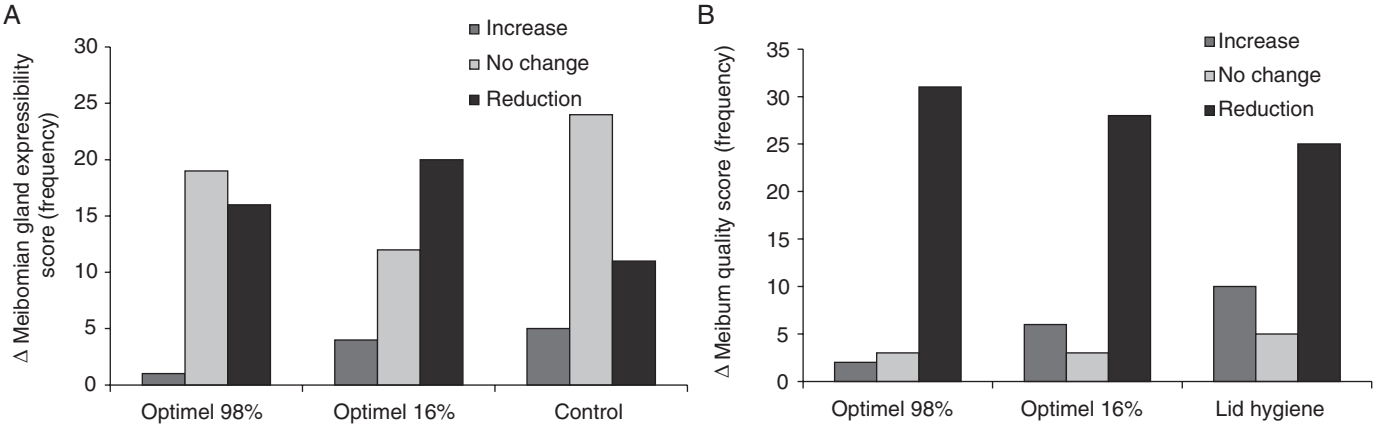

Figure 3. Frequency histograms of change in meibomian gland expressibility score (A) and change in meibum quality score (B) after the eight weeks of treatment. For both scores a reduction represents an improvement. All three treatments significantly improved meibum quality. Both Optimel treatment groups significantly improved meibum gland expressibility. The control group did not give a significant improvement for meibomian gland expressibility. There were significant differences in the three treatments on both measures. Optimel 98 per cent had a significantly greater effect on both meibomian gland expressibility and meibum quality.

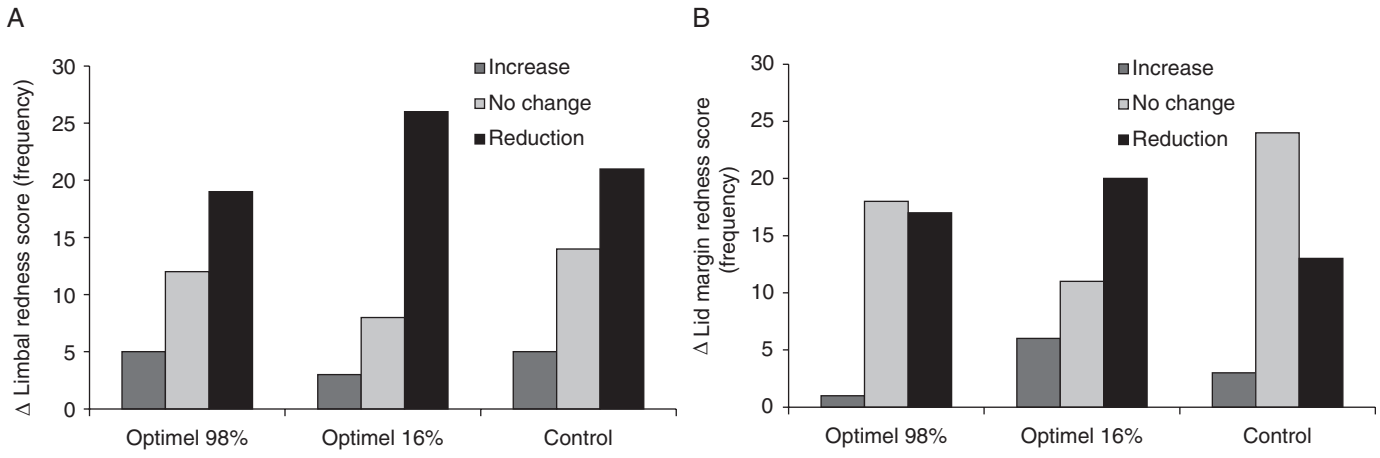

Figure 4. Frequency histograms of change in limbal redness score (A) and change in lid margin redness score (B) over the eight weeks of treatment. All three treatments significantly improved limbal and lid margin redness. There were no significant differences among the measured improvements of the three treatments.

and sting on instillation, which limit uniform acceptance and reduce long-term adherence to treatment;[13–16,25] however, our dropout rate due to stinging and redness in this study (five per cent) was much lower than in our pilot study.[16] This may be due to our improved participant education associated with the use of Optimel and increased participant acceptance associated with use of regulatory approved ophthalmic products.

Antimicrobial effects

In this study, use of Optimel 16 per cent drops significantly reduced total bacterial lid margin isolates (Table 5) and both Optimel products significantly reduced *Staphylococcus epidermidis* isolates (Table 4). These results confirm those obtained in an earlier pilot study assessing the effect of thrice daily application of an unapproved pure medical grade *Leptospermum* spp. honey (Antibacterial Medical Honey, Medihoney, Comvita Pty Ltd, Paengaroa, New Zealand) on the ocular flora in patients with aqueous tear-deficient dry eye and/or MGD.[16]

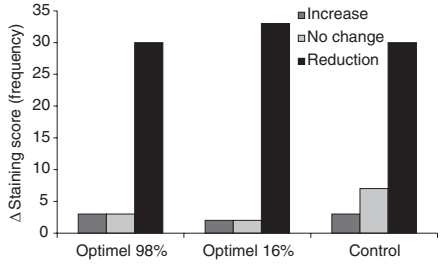

Figure 5. Frequency histograms of change in Oxford interpalpebral ocular surface staining score over the eight weeks of treatment. A reduction represents a decrease in the staining. All three treatments significantly improved ocular surface staining. There were significant differences in the three treatments' abilities to do this, with Optimel 16 per cent having the greatest effect.

The acidic pH (mean 4.4), high osmotic concentration and low water content of pure raw honeys inhibit bacterial colonisation.[12,13] Additional antimicrobial activity in some honeys, including *Leptospermum* spp. honey, is generated on dilution of honey by the activation of bee-derived glucose oxidase to produce low levels of hydrogen peroxide.[37,38] On the ocular surface, this dilution is likely to occur with reflex tearing produced in response to the stinging from the low pH of the honey. Methylglyoxal[38–40] and cationic antimicrobial peptide bee defensin-1[38] also act as antibacterial substances in some honeys, including some *Leptospermum* spp. honeys.

Optimel products are prepared for medical use to a rigorous set of systems and standards from a unique proprietary mix of Australian and New Zealand *Leptospermum* spp. honeys. These honeys are selected for their highest and most consistent level of antibacterial activity, including activity against antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[41–45] Regulated medical grade honeys, such as Optimel are sterilised by gamma irradiation to destroy spore-forming organisms that may be present in the honey, without loss of antibacterial activity.[21]

In vivo, *Leptospermum scoparium* (manuka) can inhibit a diverse range of bacterial pathogens, including multi-drug-resistant bacteria,[43,45–47] prevent biofilm formation and disrupt pre-formed biofilms;[48–50] however, in our clinical study, there were single isolated cases of significant growth of *Serratia Marcescens* and *Enterococcus* spp. after eight weeks of Optimel gel and drop treatment, respectively (Table 4).

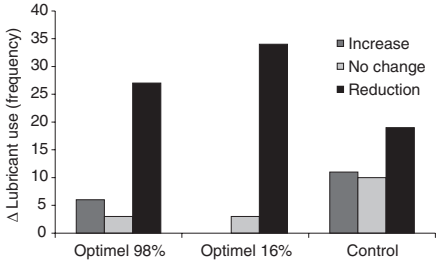

Figure 6. Frequency histograms of change in daily lubricant use over the eight weeks of treatment. A reduction represents a decrease in the number of times per day an ocular lubricant was used. There were significant differences in the three treatments' abilities to reduce the need for lubricants, with Optimel 16 per cent having the greater effect. The control group did not significantly reduce the need for lubricants.

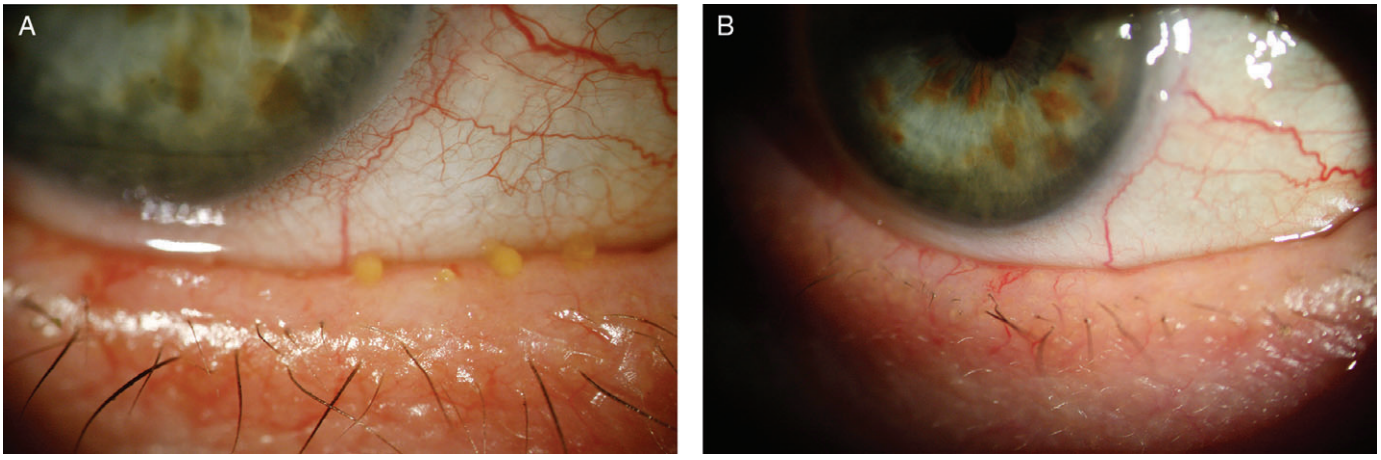

Figure 7. Inferior lid margin of a participant before (A) and after (B) eight weeks of Optimel 16 per cent honey eye drops used twice daily, as an adjunctive treatment to warm compress therapy and eyelid massage. Reduced conjunctival and lid margin redness and improved meibomian gland secretion quality were observed with the honey treatment.

Demodex folliculorum is thought to have a key role in the pathogenesis of chronic MGD and the associated chronic inflammatory dermatological condition, acne rosacea.[35,36,51,52] While the efficacy of honey on *Demodex* spp. was not assessed in this study, a recent randomised controlled trial found that a 90 per cent medical-grade New Zealand kanuka honey (floral source *Kunzea ericoides* bush) effectively treated rosacea.[53] In rosacea, antigenic proteins related to the bacterium *Bacillus oleronius* isolated from *Demodex folliculorum* are thought to exacerbate the inflammatory response.[51] The effect of medical-grade honey on *B. oleronius* and the *Demodex folliculum* mite in MGD would be a potential avenue for further investigation.[53]

Anti-inflammatory effects

Some *Leptospermum* spp. honeys have immunomodulatory activity additional to their antimicrobial effects.[54–56] In this study, topical honey did not significantly reduce clinically observed signs of ocular surface inflammation (lid margin, bulbar or conjunctival redness) compared with the control; however, a significant reduction in the number of participants with the elevated tear cytokine MMP-9 occurred with use of each honey product (Table 5). MMP-9 levels on the ocular surface are elevated in MGD.[57] MMP-9 is a non-specific biomarker for inflammation and is intimately associated with the other mediators of the inflammatory pathway on the ocular surface.[58] Hence, the reduced expression of MMP-9 with honey use suggests a potential anti-inflammatory role of *Leptospermum* spp. honey on the ocular surface.

Honeys from floral sources other than *Leptospermum* spp. have exhibited anti-inflammatory effects in ocular surface disease (decreased conjunctival hyperaemia,[18,59] decreased neutrophilic infiltration[59] and decreased corneal expression of vascular endothelial growth factor, transforming growth factor beta, interferon gamma, interleukin 12, chemokines and tumour necrosis factor alpha).[60] Further investigation is warranted to identify the active component(s) and mechanisms responsible for these anti-inflammatory and wound-healing activities of honeys[59–61] on the ocular surface.

Effects on meibomian gland expressibility and secretion quality

Both Optimel treatments improved meibum quality and expressibility more effectively than control (Table 5 and Figures 3A and 3B). The more concentrated Optimel 98 per cent gel product was statistically more effective than Optimel 16 per cent or the control, improving these measures of meibomian gland function (Table 6), with clinical improvements from Stage 1 to 2 (mildly altered expressibility) to Stage zero to 1 (normal expressibility) and from Stage 4 (severely altered secretions) to Stage 3 (moderately altered secretions) for meibum secretion quality.[1] The increased viscosity of the more concentrated honey product (which also contains a small amount of a naturally occurring gum to increase viscosity) and prolonged retention time on the ocular surface and lid margins is likely responsible for its increased clinical efficacy in this regard. The exact mechanism by which honey improves meibomian gland expressibility and secretion quality requires further investigation involving the use of confocal microscopy and lipid chemistry. A possible explanation for this is the hyperosmolar effect of honey reducing inflammation, oedema and obstruction at the meibomian gland orifices. Optimel 98 per cent gel has been demonstrated previously to temporarily but significantly reduce corneal epithelial oedema,[14] so it may be having a similar effect on the lid margin.

The stasis of the meibum in MGD is thought to promote increased meibum viscosity and bacterial growth on the lid margins, which generate free fatty acids, leading to inflammation and hyperkeratinisation of the gland orifices and a vicious cycle of further meibum stasis.[36] To fit with this current thinking regarding the pathogenesis of MGD, the improvement in meibomian gland expressibility with the Optimel 98 per cent gel correlated with the improvement in meibum quality and with the improvement in the ocular surface staining (epithelial damage) in our study.

Effects on ocular surface staining

Both honey products were superior to the control in reducing interpalpebral ocular

surface epitheliopathy with Optimel 16 per cent drops being significantly more effective (Figure 5 and Table 6). This improvement in superficial epitheliopathy adds to the growing body of evidence from clinical trials,[16,25] retrospective reviews,[14,15] case reports,[17,19] animal models of wound healing[59,60] and *in vitro* studies[63,64] that honeys from a variety or floral sources and geographic locations and in varying concentrations can reduce corneal epitheliopathy,[14,25] and promote corneal epithelialisation.[19,59,60] The immunomodulatory activity of honey is highly complex because of the involvement of multiple quantitatively variable compounds among honeys of different origins.[61]

The high total sugar content of honey has been suggested to provide additional energy resources promoting epithelial wound closure[62] and the antimicrobial effects of honey may assist corneal epithelial wound healing by reducing bacterial colonisation.[62] The greater antibacterial effect of the more dilute Optimel 16 per cent drop in this study is a possible explanation for the greater efficacy of this product in reducing ocular surface staining. The identification of key compounds in honeys and their contributions to wound healing are ongoing[56,57] and are crucial for a better understanding of the mechanisms underlying honey-mediated ocular surface wound healing and the role of honeys in restoring epithelial integrity.

Effects on tear osmolarity

Tear hyperosmolarity due to increased tear electrolytes, is a key pathogenic factor inducing inflammation in dry eye disease and MGD.[1,35] Our participant cohort had normal baseline tear osmolarity (less than 308 mOsmol/L)[29,30] possibly because they were using and continued to use, warm compresses and topical lubricants at baseline and during the study. These conventional treatments have been demonstrated to lower tear osmolarity.[30,65]

Honey is powerfully hyperosmotic due to its high concentration of sugars and low moisture content.[12,13] When applied undiluted to the ocular surface in oedematous corneas, honey can rapidly (within 10 to 15 minutes) draw fluid from the corneal epithelium and anterior stroma, to temporarily clear an oedematous anterior cornea, resolve microcystic oedema and collapse epithelial bullae.[14,15] Therefore, one concern with use of honey was that it may elevate tear osmolarity and induce ocular surface epithelial osmotic stress. To the contrary, both honey treatments and control in this study significantly lowered tear osmolarity (Table 5), with the greatest decrease in measured tear electrolyte concentration across the three treatment arms occurring with the more concentrated Optimel 98 per cent product (Table 6). Osmotic concentration in this study was measured using the point of care TearLab test which uses a microelectrode to rapidly measure the number of charged particles in a tear sample[29] and therefore, not measure uncharged sugars. Hence, we are unable to determine the true effect of honey on tear osmolarity using this method. As measures of tear osmolarity were taken at least two hours after any eye drops (honey or lubricants) were instilled, the results of this study indicate that topical use of honey does not cause any long-term elevation of tear electrolyte concentration.

Limitations

The limitations of this study include the single site and lack of investigator and participant masking. Ophthalmic honey products have a distinctive colour, taste (via nasolacrimal drainage) and sting on instillation and hence, masking participants to treatment is problematic.

Additionally, the use of a wet warm face cloth versus an external lid warming device that may more consistently regulate the elevation of temperature to the external lid[64,65] is a further limitation. Similarly, we acknowledge the lack of regulation in technique, pressure and duration of the home-based 'lid massage' component of treatment compared with the use of an automated thermodynamic clinic-based treatment.[8] Regarding our choice of symptom tool, the Standard Patient Evaluation of Eye Dryness (SPEED survey) may correlate more with clinical parameters of evaporative dry eye[66] than the OSDI and may have been a more suitable instrument. Compliance was assessed only via patient logs versus strict compliance monitoring by measuring the volume of treatment of eye drops or gel used. Given the battery of tear film and ocular surface assessments performed in a single visit, we acknowledge that prior assessments may have influenced the results obtained for subsequent tests.

As data collection only occurred at baseline and Week 8, the initial time point where clinically significant improvements in symptoms and signs with treatment first occurred was not well defined, nor was it determined if clinical improvements were maintained after treatment cessation. Following on from this study, well-designed, masked multicentre clinical trials are required to confirm the therapeutic effects of *Leptospermum* spp. honey products in the management of evaporative dry eye due to MGD.

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