Contents

Anti-infective and Anti-inflammatory Activities of Manuka Honey: A Review of the Current State

Background: Honey and Wound Care

Honey has been used since 2500 BC as a wound dressing, its physical properties allowing it to serve as a viscous ointment for topical treatments providing an effective barrier (Lu et al. 2014). But the eventual resolution of wound healing requires coordination across numerous physiological processes which benefit from the chemical makeup of honey that provides antibacterial and antioxidant activities. Recognition of Manuka honey as especially efficacious for wound treatment began in the mid-1980s when Peter Molan of Waikato University in New Zealand began reporting on its activity against a wide range of bacterial strains (Carter et al. 2016). An important indication of the recognition that *Leptospermum* based honey has achieved for efficacy, is registration under the medical authorities of Australia, Canada, the EU, Hong Kong, New Zealand and the US as a medical device for use in wound dressings (Jenkins and Cooper 2012; Cokcetin et al. 2016). There is considerable need for new and effective interventions to address chronic wounds; a survey conducted in 2012 of the US Wound Registry determined that chronic wounds, wounds that have not fully healed after a period of two months, affect 6.5 million people across the US incurring enormous costs in health care resources and lost time, pain and suffering. This trend is only expected to increase as the population ages further (Fife and Carter 2012). It is critically important then to explore new modalities for addressing the bacterial infections and inflammation that contribute to the failure to achieve wound resolution.

Sourcing and Variability

Honey is first, a food source, highly concentrated in the sugars fructose and glucose, but also including a wide range of additional components that make essential contributions to its nutritional and medicinal properties. Like all natural, biological products, its exact make up will vary significantly depending on seasonal and environmental factors and on the age and health of the bee colony (Ohashi, Natori, and Kubo 1999). But medicinal activity appears to depend especially on the raw material of the honey's manufacture, that is, the floral source of the nectar. Honey can be categorized as multi- or polyfloral when it has been gathered from a variety of plant species as will occur in a forest or meadow setting. If wholly derived from just one species of flower it is termed monofloral. The term "Manuka honey" indicates honey derived from the Manuka tree, *Leptospermum scoparium*, of the family *Myrtaceae*, that grows as a shrub or small tree throughout New Zealand and eastern Australia (Kato et al. 2012). The possibility of standardizing

a honey by composition and biological efficacy increases markedly with a monofloral honey (Alvarez-Suarez et al. 2014).

Antioxidant activity

The yellow-gold color of honey is imparted by flavonoids, those predominant in Manuka honey are pinobanksin, pinocembrin and chrysin. In addition to attracting pollinators, flavonoids are also effective antioxidants. An important aspect of the wound healing activity is honey's ability to quench reactive oxygen species (ROS) reducing further cell necrosis and tissue damage and helping to prevent fibrosis during healing (Molan 2001). While the levels of polyphenolics present in Manuka honey would be expected to contribute to the antioxidant capacity, its scavenging capacity against free radicals is further supported by methyl syringate (MSYR) and its glycoside, methyl syringate 4-O-beta-D-gentiobiose (known commonly as leptosin) which together serve as inhibitors of myeloperoxidase. Leptosin is unique to Oceanic honeys being particularly abundant in Manuka and has been proposed as a potentially useful chemical marker for purity. Levels of leptosin show a positive correlation with antimicrobial activity (Kato et al. 2012).

Antibacterial Activity

The search for the basis of the antibacterial properties of honey was a subject of Victorian science initiated by van Ketel in 1892 (Dustmann 1979). The bactericidal/bacteriostatic activity common to various honeys is provided by biochemical and physicochemical factors including high osmotic pressure and low pH. The osmotic gradient created by the elevated levels of solutes contributes to desiccation of microorganisms and acidity slows growth.

Some of the same physical/chemical properties which inhibit microbial growth also promote tissue regeneration. The osmotic gradient draws fluid from circulation into the wound. The resulting layer of diluted honey that is continually created helps to prevent adherence of dressings to fragile healing tissues avoiding tearing and helping to reduce the need for surgical debridement (Subrahmanyam 1993; Efem 1993). Acidification of the wound bed by honey's low pH promotes the release of oxygen from hemoglobin helping to alleviate the low oxygen tension under dressings (Kaufman et al. 1985). Another potential aid to healing is the observation that honey stimulates the growth both of epithelial cells and fibroblasts speeding the closing of wounds without the need for skin grafts (Subrahmanyam 1998; Efem 1993).

However, even if the honey is diluted to reduce osmolarity to negligible levels and buffered to raise the pH to neutrality, antimicrobial activity is still observed. Although the same phenolic compounds providing the antioxidant activity in honey are known to also possess antibacterial activity, the concentrations present in honey are insufficient to significantly inhibit bacterial growth (Mavric et al. 2008). In many honeys the antimicrobial activity is provided by hydrogen peroxide (Carter et al. 2016). The H_2O_2 is derived from glucose oxidase, secreted from the bees, reacting with glucose, but peroxide levels are low in Manuka relative to other honeys. The antibacterial activity that remains following the elimination of incidental H_2O_2 is termed non-peroxide activity (NPA) which is equivalent to the trademarked designation Unique Manuka Factor (UMF) registered by the UMF™ Honey Association and available under license for Manuka producers in New Zealand (Cokcetin et al. 2016). The UMF is particularly valuable because unlike hydrogen peroxide it is not destroyed by endogenous human catalase activity and while it is heat labile and cannot be autoclaved, it will withstand sterilization,

required for wound dressings, by gamma irradiation used to kill clostridial spores which can occur in honey (Molan and Allen 1996).

The antibacterial activity used to grade the UMF value is typically determined by an agar well diffusion assay of *S. aureus*. A reference curve is generated by assaying titrations of phenol. A UMF equivalent in activity to 10% phenol is generally considered therapeutically active (Cokcetin et al. 2016). Electron microscopic analysis of Manuka treated *S. aureus* cultures revealed cell morphology suggesting that cell division was disrupted by failure to complete the cycle, leading to an accumulation of cells with finished septa failing to separate, but which were otherwise normal in appearance suggesting a bacteriostatic mechanism (Henriques et al. 2010).

The factor most commonly cited as the source of NPA in Manuka honey is a simple dicarbonyl compound: methylglyoxal (MGO). MGO is found in other food sources, for example it is formed during coffee roasting in amounts of 23–47 ppm (Hayashi and Shibamoto 1985). Dihydroxy acetone (DHA) is derived from Manuka nectar and as honey ripens, it is converted to MGO (Mavric et al. 2008; Adams, Manley-Harris, and Molan 2009). In New Zealand Manuka honey MGO levels have been shown to be closely correlated to NPA across MGO concentrations from 200 - 800 mg/kg. MGO was unambiguously identified by Mavric et al in 2008 in various New Zealand Manuka honey samples when the structure was confirmed by LC-TOF-Mass Spectrometry. A survey of the antibacterial activities of honeys from various sources showed that only the Manuka honeys were able to significantly inhibit bacterial growth at concentrations from 15-30% (w/v) whereas honey from other sources failed at concentrations lower than 80%. It was also noted that the antibacterial activities of the Manuka samples were directly related to the concentrations of MGO in the honey. Various dicarbonyl compounds displayed antibacterial activity against *E. coli* and *S. aureus*., but the potency of MGO was several-fold greater than glyoxal or 3-deoxyglucosulose showing a minimally inhibitory concentration of 1.1 mM. The dilutions of Manuka, 15-30%, that possessed antibacterial activity were determined to contain MGO at concentrations of 1.1 to 1.8 mM; sufficient to account for the observed activity. Finally, when a 20% solution of otherwise inactive honey was spiked with neat MGO to a final concentration of 1.9 mM, inhibition of bacterial growth equivalent to native Manuka honey was observed (Mavric et al. 2008). It can be predicted then that a honey with MGO concentrations greater than 260 mg/kg can be expected to have antibacterial activity meeting the therapeutic threshold of >10% phenol equivalents (Adams et al. 2008; Atrott and Henle 2009).

A survey of Australian honeys derived from various species of *Leptosporum* showed that under the right storage conditions (in the dark under refrigeration at 4C) the NPA is remarkably stable showing little change over a period of seven years (Cokcetin et al. 2016). Samples were derivatized with PFBHA reagent and quantified by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) as described by Windsor (Sarah Windsor 2012). Prior to conducting dose response experiments, honey samples were treated with catalase to remove any potential H_2O_2 activity, then assessed for their ability to inhibit the growth of *S. aureus* ATCC 25923. An exceptionally strong correlation ($r^2 = 0.95$, Pearson's rho = 0.97, $p<0.05$) was established between the antibacterial activity and MGO concentrations. The highest levels of activity (>20% phenol equivalence from >800 mg/kg MGO) were associated with monofloral samples. When grouped by source species, the honey samples did not show a signficant reduction in mean antibiotic activity following the seven years of storage. Only two samples lost activity completely and 23 of the *L. polygalifolium* samples showed increases in NPA possibly due to further conversion of DHA to MGO (Cokcetin et al. 2016).

Concentrations of MGO measured in Manuka are up to 100-fold greater than that found in conventional honeys (Mavric et al. 2008). But not all honeys derived from *Leptospermum* sources show antibacterial activity and even when restricted to the species *L. scoparium* and *L. polygalifolium*, the MGO levels can vary by more than an order of magnitude (Sarah Windsor 2012). Another important chemical marker, also quantifiable by HPLC, is hydroxymethylfurfural (HMF). It can be found in all types of honey and serves as an indicator of spoilage; if levels exceed 40 mg/kg the product cannot be exported or sold. No relation between HMF and MGO levels has been observed (Cokcetin et al. 2016).

The formation of biofilms is especially problematic in bacterial infections of wounds and in oral care. The films are a gel-like matrix of polysaccharides and other components in which pathogens embed. The biofilm matrix limits the access of antibiotics to the pathogens to the extent that the dose effectiveness can be reduced by as much as three orders of magnitude (Hoyle and Costerton 1991). The continuing release of pathogenic cells from the matrix can contribute to sustaining chronic inflammation in the wound (Alvarez-Suarez et al. 2014; Ngo, Vickery, and Deva 2012). Biofilms are common to chronic wounds contributing to the difficulty in achieving satisfactory healing (Woodward 2019).

Four honeys were compared for their ability both to prevent the initial formation of biofilms and degrade biofilms that had already been established. The most effective honey, both in terms of preventing and degrading *S. aureus* biofilms was a monofloral (*Leptospermum scoparium* var. *incanum*) Manuka honey with 958 ppm MGO that caused a 95% reduction in biofilm mass accumulation, at a concentration of 8% (w\v) *in vitro*. Slightly less active was a commercial medical grade Manuka/Kanuka combination, Medihoney®, with 776 ppm MGO. Interestingly MGO, at concentrations equivalent to those present in the Manuka honeys and in combination with an artificial solution of sugars found in honey (45% glucose, 48% fructose, 1% sucrose) was also able to inhibit formation of biofilms, but was far less potent than the complete, natural honeys. Further, the MGO-sugar solutions were wholly ineffective against established biofilms. This suggests either that other components in complete and natural Manuka honey besides MGO contribute significantly to the efficacy against biofilms or that MGO in the context of complete honey is better able to access and act on the embedded pathogens (Lu et al. 2014).

Another antibacterial factor not related to hydrogen peroxide is the amphipathic protein, bee defensin-1 (previously royalisin). The mature protein contains 51 residues (5.5 kDa) with potent activity against gram negative, but not gram positive bacteria (Fujiwara et al. 1990). This protein was readily detected in samples of the medical grade honey Revamil® which is produced under controlled greenhouse conditions in The Netherlands. Following electrophoretic separation of Revamil sourced (RS) samples on native polyacrylamide gels, the location of defensin-1 can be visualized by overlay bioassays with *B. subtilis*, similar analyses of a Manuka honey with UMF™ of 16+ yielded no defensin-1 related response. The two honey samples also differed in hydrogen peroxide levels: RS samples diluted to 40% (v/v) accumulated 3.5 mM H_2O_2 after 24 h, Manuka treated in the same manner showed no detectable H_2O_2. In contrast the Manuka samples contained over 40-fold higher concentrations of MGO than the RS samples. The bactericidal activity of the two honeys was compared directly by conducting dose response assays against a battery of strains including *Bacillus subtilis* ATCC6633, *Escherichia coli* ML-35p, *Pseudomonas aeruginosa* PAO-1 (ATCC 15692) and against methicillin-resistant *Staphylococcus aureus* (MRSA) strain AMC201. The RS honey displayed a more rapid bactericidal activity showing greater potency

when assessed after a two-hour exposure; however, after 24 h the Manuka sample proved to be significantly more inhibitory than the RS sample showing 8-fold greater activity against MRSA. Against *B. subtilis* and *E. coli* Manuka was twice as potent while the activity of both the Manuka and RS samples were equivalent on *P. aeruginosa* after 24 h. The multivalent nature of the Manuka sample was demonstrated in follow-up assays where the MGO in the honey was neutralized by chemical conversion to the non-bactericidal S-lactoylglutathione. This step eliminated the activity against MRSA, but the activity against *E. coli* was unaffected and though toxicity towards *B. subtilis* and *P. aeruginosa* was significantly reduced, it was not eliminated (Kwakman et al. 2011).

The variety of antiinfective agents identified in honey points to an important aspect of its utility for future use, this diversity of activities greatly reduces the potential for bacterial strains to develop resistance relative to pharmaceuticals that target a single metabolic locus. There have not yet been any reports of clinical instances of acquired resistance to honey. Attempts have been made to generate such strains from *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*, in a laboratory setting, by culturing under sub-lethal concentrations, but honey resistant mutants were not generated (Cooper et al. 2010; Blair et al. 2009). A practical demonstration of this aspect of honey's antibacterial activity is given in the results of a screen conducted against MRSA and *P. aeruginosa*. Sublethal concentrations of Manuka honey were tested in combination with fifteen antibiotics, yielding five combination therapies of honey and antibiotics that significantly improved *in vitro* efficacy against these two important wound pathogens (Jenkins and Cooper 2012). An especially attractive combined treatment might be the combination of honey as a topical dressing used with a systemic antibiotic that could attack circulating pathogens (Carter et al. 2016). Synergistic activity observed *in vitro* by combinations of Manuka and antibiotics against a MRSA strain have included oxacillin, tetracycline, imipenem and mupirocin (Jenkins and Cooper 2012).

Case Studies and Clinical Trials

Honey as a dressing was compared directly to silver sulfadiazine in a trial with 52 patients in each treatment group. Under the honey treatments healthy granulation tissue appeared in almost half the time required for the silver sulfadiazine treatments. The great majority of honey patients (91%) achieved sterility within a week where only a few of those on the silver sulfadiazine treatment (7%) managed control in the same time period. After two weeks, 87% of the honey treated wounds had healed whereas only 10% of the silver sulfadiazine dressed wounds were assessed as healed. The frequency of hypertrophic scarring and postburn contracture was also lower in the honey treatment group (Subrahmanyam 1991).

An evaluation of honey impregnated gauze vs a polyurethane film (OpSite®) was conducted in a randomized trial of 46 patients per treatment group. The results showed that used as a cover for fresh partial thickness burns, honey treated wounds healed significantly faster requiring on average only about two-thirds the time needed for healing under the polyurethane film and reducing the frequency of infection by over 50% relative to the film treatment (Subrahmanyam 1993). In a second trial again evaluating treatment of partial thickness burns, a similar honey impregnated gauze dressing was compared to dressings made with amniotic membrane, obtained from separations of the chorion and placenta shorty following delivery. Patients varied widely in age from 3 to 62 years of age. Burn injuries treated with honey required about half as many days on average for healing as compared to the membrane dressed patients ($P < 0.001$). In the honey treatment

group of those who tested initially as positive for bacterial infection, 82% achieved sterility after one week whereas 58% of the patients who had presented with infections were sterile by the same time under the membrane dressing (Subrahmanyam 1994).

In a prospective trial of 15 patients with abdominal wound disruptions following Cesarean sections, honey was applied rather than conventional dressing. Excellent results were obtained for all cases after two weeks. Comparing their responses retrospectively to 19 patients who had been treated with hydrogen peroxide, hypochlorite solution and saline soaked gauze, the honey treatment group benefitted in requiring on average, only half as many days of hospitalization. The honey treatment combined with the use of micropore tape for approximation avoided the need for general anesthesia and resuturing (Phuapradit and Saropala 1992).

The overhydration of skin, can initiate inflammation as the penetration of irritants through the dermis can be increased. In the specific case of overhydration due to incontinence this can give rise to incontinence-associated dermatitis (IAD). The overly hydrated, inflamed skin then also becomes more susceptible to tears and mechanical injury. If the condition becomes chronic, superations can further reduce skin integrity as exudates contain proteases that weaken the dermal matrix. This is especially a risk in older subjects with reduced epidermal thickness (Woodward 2019).

Medihoney® Barrier Cream (MBC) is a commercial product, silicone based, including 30% (w/v) Medihoney® Antibacterial Honey. A pilot scale, singly blinded, multicenter trial examined MBC as an intervention for intertrigo (moisture associated damage in large skin folds). The subjects randomized to this study presented with bilateral intertrigo and so served as their own study controls. Subjects reported a statistically significant reduction in pain and less than half as many pruritis complaints with the MBC treatment as compared to a zinc oxide ointment although no significant differences in the course of wound healing was observed (Nijhuis et al. 2012).

The high degree of safety associated with MBC was demonstrated in a recent case study where a 2 month old infant who had developed a persistent rash that resisted treatment at home and in the hospital with several skin treatments and protectant gels. Applications of MBC finally initiated significant improvements showing epithelialization after one week and a cessation of apparent pain and discomfort (Woodward 2019).

Mupirocin (Bactroban®) is often used for treatment of nasal colonization by methicillin resistant *S. aureus*, but the appearance of resistance limits continuous use. In a trial evaluating Manuka honey for nasal localized MRSA, patients older than 18 years received medical grade Manuka honey or 2% mupirocin 3 times per day over a 5 day period. Although the incidence of decolonization achieved by the drug (57%) was numerically greater than that observed for honey (43%) the difference between the two treatments was not statistically significant. Importantly, the rate of acquisition of new resistance to mupirocin during the trial was almost 10% and the study sponsors concluded that the honey provided a potential strategy for achieving significant treatment while addressing resistance (Poovelikunnel et al. 2018).

Atopic dermatitis (AD) is characterized by pruritus and skin rash, it is more common in childhood and can affect as many as a fifth of children, but can persist into 1-3% of adults (Schneider et al. 2013). In a small, open-label, pilot-scale trial, 14 adult

(mean age 33) sufferers with AD affecting bilaterally similar areas were issued Medihoney® and sterile gauze. At baseline, the affected areas were graded by the Three Item Severity score (TIS), which accounts for erythema, edema/papulation, and excoriation (Wolkerstorfer et al. 1999). For seven days, the subjects applied honey and gauze to the treatment site in the evening, and removed the dressing and washed the site each morning. The other lateral site was left untreated and served as the control. At the end of the week of treatment, both sites were again scored on the TIS scale. The severity of the dermatitis, was significantly reduced by the honey treatment as assessed by the TIS score ($p<0.001$). This trial was then followed up with *in vitro* experiments conducted in an attempt to identify a possible mechanism of action for the activity that was observed. The inflammatory chemokine CCL26 released by keratinocytes plays an important role in the development of AD (Owczarek et al. 2010). The ability of cultured HaCaT cells, a model of keratinocytes, to secrete CCL26 following IL4 induction, was significantly inhibited by *in vitro* honey treatments to ~50% of maximum signal in the presence of 1% w/v honey. The itching and edema associated with AD is further exacerbated by the release of histamine by Mast cells, *in vitro* honey treatment also showed significant inhibition of histamine release from cultured LAD2 cells. As swabs which had been taken from the subjects in the AD trial had indicated that the Medihoney® treatments had not changed the skin bacterial flora, these results suggest that the relative relief that had been achieved was due to anti-inflammatory effects by the honey rather than anti-bacterial activity (Alangari et al. 2017).

A compelling indication of the wide safety margins that Manuka honey provides for use on delicate tissues is its application in ophthalmic treatments. Corneal edema is a common complication of cataract surgery leading to a thickening of the cornea and affecting vision. A retrospective studied reviewed the outcomes of 18 cases of persistent corneal edema following ocular surgery treated with Optimel™ Antibacterial Manuka Eye Drops 2 to 3 times daily. With continual use reductions in corneal thickness and improvements in visual acuity were achieved. No significant adverse events were reported (Albietz and Lenton 2015).

Manuka honey has also been assessed for potential protection against tooth decay. In *in vitro* experiments evaluating antibacterial activity against cariogenic *S. mutans* and *Lactobacillus* strains, a 25% w/v solution of Manuka honey was significantly inhibitory towards both strains and was statistically significantly more active than equal concentrations of Dabur (Indian) honey (Beena et al., n.d.). Recently, a relatively large (n=135), double-blinded, randomized controlled trial was conducted with 12 - 15 year-old school children evaluating mouth washes for both gingival and plaque control. Dental plaque is an example of a biofilm supporting multiple strain of bacteria. One of three mouthwash treatments, based either on Manuka honey, a raw honey or 0.2% chlorhexidine (CHX), was administered twice daily for 3 weeks. Gingival and plaque indices were scored at baseline, and one day and one week after the end of the trial. Both honeys caused statistically significant reductions in scoring for plaque of greater than 50% while CHX treatments scored reductions of two-thirds. CHX also reduced the score for gingivitis by about two-thirds whereas the honey mouthwashes led to a reduction of about 35% (Singhal et al. 2018). There were no adverse events reported among the child patients and no staining of subject's teeth by the honey mouthwashes which is commonly observed with CHX (Jones 1997).

Conclusions:

A considerable body of evidence has accumulated, both mechanistic and clinical to elevate consideration of Manuka honey from a folk remedy to an intervention for a variety of conditions. The advantages Manuka honey provides to the modern pharmacopeia is a combination of antibacterial and anti-inflammatory activities providing promise for problematic chronic conditions while delivering those activities in a context sufficiently gentle that it can be used in direct applications to the eye and for use in children. But just as importantly, given concerns about antibacterial resistance is the evidence from the laboratory and from clinical experience that interventions with medicinal honey have shown no potential, to date, for generating microbial resistance. It is hoped that the examples provided here, far from exhaustive, might give rise to further research in the indications cited or provide ideas for applications for new, currently unforeseen health benefits.

For a source of research grade Manuka honey with a consistently high UMF factor, contact NZ-Ruzio Llc at http://www.honeyforhealing.com. Samples can be made available for supporting studies following a review of protocols.

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