Published in WCS News, dec 2006

Sweet after acid

Revamil Honey gel, a successful remedy for wounds

W. van Eijk en O. Groenhart*

Introduction

In recent years, the number of patients with difficult to heal wounds has increased significantly. Several developments effect the increase of patients with chronic wounds:

- On average 4,4% of the total population in the UK suffers from Diabetus Mellitus, equating to 2.2 million (Forouhi, 2006). The estimated number of Americans with diagnosed diabetes is projected to increase 165%, from 11 million in 2000 (prevalence of 4.0%) to 29 million in 2050 (prevalence of 7.2%) (Boyle, 2001). About 25% of all diabetes patients will need specialised medical care during the course of there illness, while 10% will develop diabetic foot ulcers.
- The proportional increase of the ageing population causes an increase of patients with venous leg ulcers. The prevalence of venous leg ulcers is 1% over the total population, but equates to 4 to 5% for people aged >80 in The Netherlands.

Furthermore, several developments are causing a serious draw back on the successfulness of current wound healing treatments. For example, by the advance of antibiotics resistant bacteria it is more difficult to fight wound infections. The estimated prevalence of wound infections caused by MRSA bacteria in The Netherlands is 1%, while in Japan and the United States 50% of Staphylococcus aureus isolates are methicillin resistant. (Landelijke coördinatiestructuur Infectiebestrijding, www.infectieziekten.info). In conclusion, there will be an increasing need for antibacterial wound dressings that do not build up resistance and that stimulate the wound healing process. Based on pre-clinical and clinical evidence this paper discusses the role of Revamil® honey gel in the healing of difficult to heal wounds.

What is honey?

In a beehive hundreds of honeybees work together to process sugar containing nectar into honey. By enrolling nectar repeatedly over their tongues, water evaporates from the nectar. When the sugar concentration has decreased to ±17% and the sugar concentration amounts to ±83% the ripened honey is stored in closed honey cells. During the process of honey production honeybees add several important enzymes to the honey. The most important enzyme is glucose oxidase (GOX). In diluted honey honey this enzyme converts glucose into gluconic acid and hydrogen peroxide ((Figure 1) (Inés et al, 1997). In ripened honing the enzyme GOX is preserved but inactive. Once honey is diluted by water, e.g. in a wound, the enzyme is activated and releases small quantities of hydrogen peroxide and gluconic acid. The slow release of hydrogen peroxide disinfects the wound, while gluconic acid creates an acid wound environment that is inhibitory to many pathogenic bacteria. However, a high enzyme concentration is not at all obvious, as the majority of honey types contain low amounts of enzyme. Furthermore, high enzyme concentrations do not always guarantee a good antibacterial activity, as the enzyme activity as well as the production of hydrogen peroxide can be inhibited by other honey components.

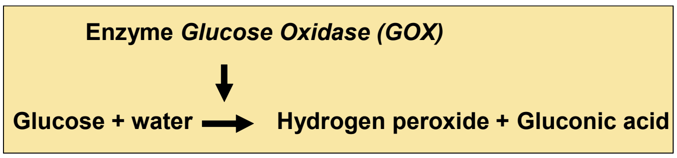


Figure 1. In diluted honey the enzyme glucose oxidase (GOX) catalysis the conversion of glucose into gluconic acid and hydrogen peroxide.

Revamil® honey gel

For medical applications it is a prerequisite that honey is of constant quality and reproducible effectiveness. The antibacterial activity of honey largely depends on the amount of active GOX enzyme. Several investigations reveal that the amount of active GOX varies importantly between different honey types (Kerkvliet, 1996). Underlying factors that cause this variation are:

1. *Nectar composition.* Some plants produce nectar that inhibit the activity of GOX. One example is thyme honey that naturally contains high levels of vitamin C. Vitamin C immediately inactivates hydrogen peroxide, thereby destroying the antibacterial activity of the honey. In addition, metal ions can be present in honey (e.g. iron and zinc) and these ions inhibit GOX activity.
2. *Honey harvest and storage.* Heating of honey during harvest, storage or processing leads to inactivation of honey enzymes. In addition, exposure to light affects a gradual decrease of enzyme activity.
3. *Climatic conditions.* Variable weather conditions affect the activity of the honey bees and the amount of enzymes they add to honey.
4. *Condition of bee colonies.* Pests, plagues and food shortage weaken a honeybee colony, leading to low enzyme production levels in honey.

At Wageningen University and Research Centre a unique honey type has been developed for application on wounds. This honey type (Revamil®) is produced under controlled conditions in greenhouses. The procedure ensures that honeybees only feed on selected nectar sources that do not interfere with enzyme activity. The use of healthy and strong honeybee colonies excludes the need to use pesticides or other chemicals, guaranteeing honey that is absolutely free of any residues. The controlled production system ensures a medical grade honey with a reproducible high enzyme activity and a low pH. Each honey batch is routinely checked for enzyme activity, pH, water content and residues. Only those batches that meet all quality requirements are used for Revamil®.

Antibacterial activity

From many case studies and several clinical studies it can be concluded that the antibacterial activity is considered the most important role honey has in the wound healing process. (Molan 2006, Hoeksema et al, 2005). The antibacterial activity of Revamil® was assessed by:

- Measurement of the enzyme activity of glucose oxidase (GOX)
- The antibacterial activity against the wound pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aerigunosa*
- The antibacterial activity against antibiotic resistant (MRSA) and antibiotic sensitive (MSSA) *Staphylococcus aureus*

Enzyme activity

The enzyme activity of glucose oxidase (GOX) can be determined by measuring the amount of peroxide produced in diluted honey at specific time intervals by dipping a test strip in de solution (Kerkvliet, 1996). Honey was diluted 5 times with water and after 1, 2, 3, 5, 24, 48 and 50 hrs the hydrogenperoxide value was determined. The hydrogen peroxide value represents the quantity (μg) of hydrogen peroxide produced per gram honey per hr.

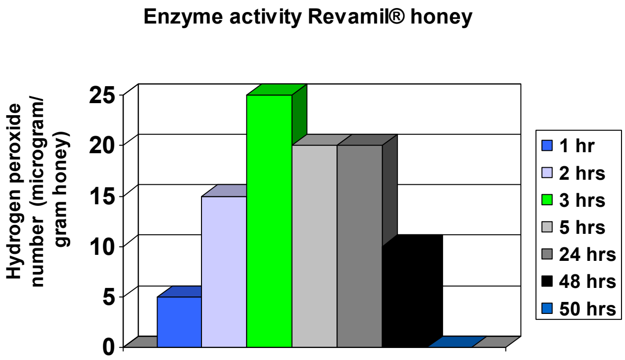
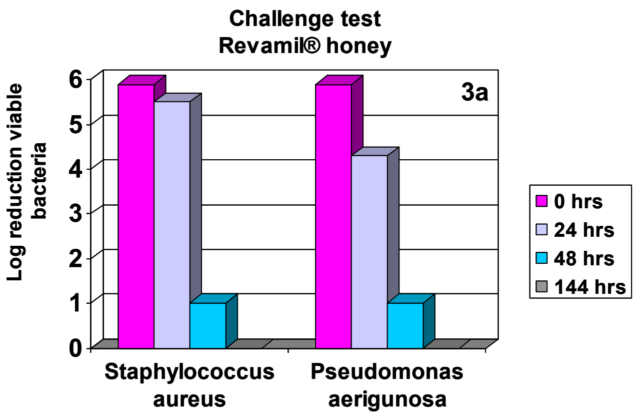


Figure 2. The enzyme activity of Revamil® honey, represented as the amount of hydrogen peroxide produced per gram honey.

Figure 2 shows that the enzyme activity of Revamil® honey comes to a maximum after three hours. Next the production of hydrogen peroxide gradually decreases, but remains at a relatively high level for 48 hrs (≥10 µg per gram honey).

Antibacterial activity

A Challenge Test* was carried out with Revamil® honey in order to determine the bactericidal effect of Revamil® honey against the two wound pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aerigunosa* (Figure 3a). This Challenge Test as carried out according to the guidelines of the European Pharmacopoeia, comprises that 1-10 million bacteria are added per gram honey. Samples taken at different time intervals are then analysed to determine the number of surviving bacteria.



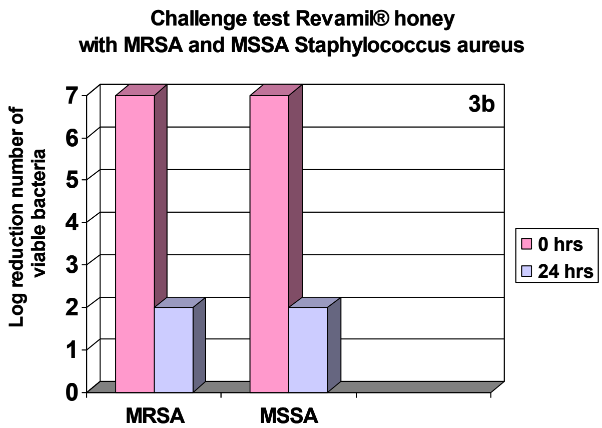


Figure 3. Challenge test of Revamil® honey gel with Pseudomonas aeriginosa and Staphylococcus aureus (3a), and for methicillin resistant (MRSA) and methicillin sensitive (MSSA) (3b).

Figure 3a shows that Revamil® honey affects a rapid decline of the number of viable bacteria. After 48 hrs only 10 bacteria per gram honey have survived (log reduction 5), after 6 days no viable bacteria have survived. To test whether Revamil® honey is also effective against the antibiotics resistant *Staphylococcus aureus* (MRSA), a comparable Challenge Test was carried out by the Department of Medical Microbiology of the Amsterdam Medical Centre. Figure 3b shows that Revamil® honey is equally effective against MR SA and MSSA Staphylococcus aureus, as a reduction of 100 bacteria per gram honey is achieved for both bacterial strains within 24 hrs.

* Tests were carried out by Regilabs Certified Laboratories Figure 3a.

Slow release antibacterial activity

The antibacterial activity of honey in wounds is mainly ascribed to the production of hydrogen peroxide. However, the production of hydrogen peroxide by honey at the wound site is in no way comparable to the old-fashioned treatment of infected wounds with 3% hydrogen peroxide. Since it was acknowledged that hydrogen peroxide at high concentrations can damage the fragile wound tissue by the production of free radicals (Saïssy et al, 1995), this method has fallen into disuse. Interestingly, the concentration of hydrogen peroxide produced by honey at the wound site is very low, reaching a maximum at around 0.003% (Molan, 1992). Furthermore, Pruitt et al (1985) showed that continual presence of low concentrations of hydrogen peroxide kills bacteria more effectively than a single high dosage treatment, while fibroblasts are not damaged (Hyslop et al., 1995). Revamil® honey therefore very effectively disinfects a wound by slow release of small amounts of hydrogen peroxide, without damaging the regenerating wound tissue.

Anti-inflammatory effect of honey

The anti-inflammatory action of honey can be partly explained by the presence of flavonoids, originating from floral nectar and pollen (Siess et al., 1996). Flavonoids exert a strong anti-oxidant action by scavenging oxygen radicals. Chronic wounds contain increased amounts of inflammatory cells (Loots et al., 1998). The continuous presence of such inflammatory cells is responsible for overproduction of oxygen radicals that keep the wound in a painful, inflammatory state. By application of Revamil® honey chronic wounds calm down and transfer to the healing proliferation stage. Furthermore, Tonks et al. (2003) showed that honey stimulates the production of anti-inflammatory cytokines in wounds.

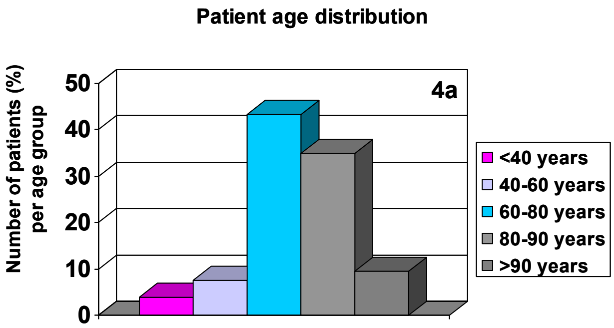
The anti-inflammatory action of Revamil® honey was tested in a bioluminescence assay with human leucocytes that were activated to produce oxygen radicals. A concentration of only 2% Revamil® honey was already sufficient to affect a 50% inhibition of oxygen radical production under the test conditions used.

Clinical investigations

Specialised wound department at Bronovo hospital

Starting from 2004 a specialised wound department was initiated at Bronovo hospital at The Hague. Patients from all surrounding regions are referred to this department to be treated for difficult to heal wounds, such as chronic leg ulcers. In addition, many of the patients show extensive comorbidity like diabetes mellitus, vein disorders, high blood pressure, low resistance etc. In short, this department is specialised to treat wounds that appeared very difficult to treat in regular dermatological and surgical departments. From an early stage the Bronovo wound department experimented with honey gel. At first honey was incidentally applied, however, due to positive results honey gel was soon incorporated in the regular wound treatment protocol. From February 2005 until September 2005 eighty patients were treated with Revamil® liquid honey gel. Typical wounds existed for 3 to 6 months and showed no tendency for healing before treatment with Revamil® was initiated.

Characteristics patients and wound types



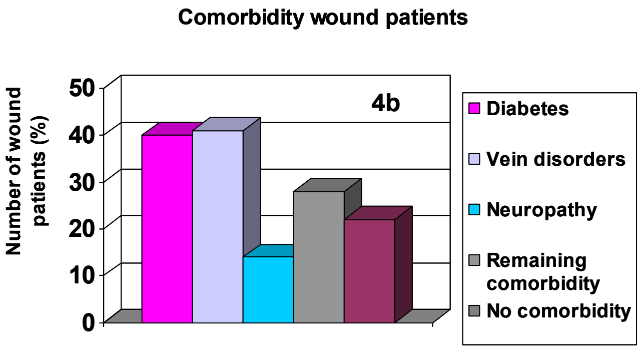


Figure 4. Characteristics of patients: Age groups of patients (4a) and Comorbidity (4b).

The average age of patients in this study is very high. Almost 90% of the patients is older than 60 years while 45% of the patients is even older than 80 years (Figure 4a). Furthermore, 40% of the patients suffers from Diabetes mellitus, 41% suffers from vein disorders, while 14% suffered from neuropathy (Figure 4b). Wounds are mainly localised at the lower leg region and feet (Figure 5).

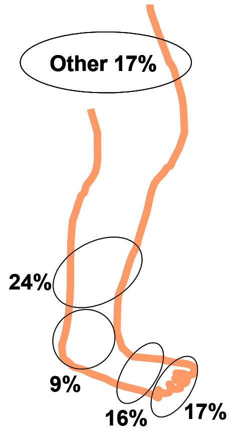


Figure 5. Localisation of wounds treated with Revamil®

Results of treatment with Revamil® honey gel

In the majority of case studies treatment with Revamil® honey gel resulted in rapid clearing of the wound site. Within 30 days 45% of the wounds were clean. In deep wounds Revamil® was combined with a paraffin gauze that was put into the wound. This treatment appears especially effective to prevent pocket formation. Theoretically, the gauze affects aeration of the wound because the temperature difference between the outer and inner wound surface causes a so-called funnel activity. Aeration of the wound thus prevents lodging of anaerobic bacteria that can cause wound infections. In addition, Revamil® stimulated the formation of granulation tissue. The majority of wounds (57%) healed to full closure within 3 months, the remaining wounds (28%) closed after 3 to 6 months and after 6 months (14%). Revamil® honey gel can be applied up to wound closure, however, in some cases additional products were applied to achieve full closure more rapidly. Around eight percent of the patients complained of pain, immediately following treatment with Revamil®. These patients tolerated honey better when the wound surface was wetted with clean water or physiological salt solution prior to applying honey.

Case study 1: Diabetic foot ulcer

This case study concerns an 84 year old patient with insulin independent diabetes mellitus, vein disorders and neuropathy. The patient has a toe wound of 5mm deep and a surface of 20x30 mm^2. Due to wound infection the wound spreads a strong putrefying smell. The toe is due to be amputated, unless treatment with Revamil® will be successful. Treatment with Revamil® starts at March 7th 2005. Every two days wound dressings are changed and the wound is treated with Revamil®. After 45 days the wound is totally clean and islands of granulation tissue have appeared. After 75 days the wound has progressed to full closure and the toe has escaped the fate of amputation.

7/3 20/4 14/5 25/6

Figure 6. Treatment of a diabetic foot ulcer to full closure withe Revamil®

Case study 2: Infected wound

The second case study concerns a child with an infected wound. The initial wound is due to an accident. Initially the wound even closed, but due to infection at a later stage the wound burst open. The wound is then 3mm deep and has a surface of 50x10 mm^2. Treatment with Revamil® starts at March 18th. After two weeks the wound is clean and shows the onset of granulation tissue. After eight weeks the wound has closed.

18/3 1/4 30/4 21/5

Figure 7. Treatment to full closure of infected wound with Revamil®

Conclusions

Honey broadly applicable

From the pre-clinical and clinical evidence as discussed is this paper, we can conclude that much is to be gained when controlled Revamil® honey is applied for wound treatment. Summarized, Revamil® has a number of features beneficial to patients with moderate to severe wounds:

- *Infected wounds*. Infected wounds become clean and no residues are left in the wound.
- *Chronic wounds*. Chronic wounds proceed to the regenerative phase, because inflammation decreases and the formation of granulation tissue is stimulated.
- *Acute wounds*. Revamil® honey is unique in creating a moist wound environment and simultaneously protecting the wound for infection.
- *MRSA*. MRSA and MSSA bacteria are equally sensitive to killing by Revamil® honey.

- *Diabetic patients.* Honey applied to a wound does not affect a raise in blood sugar level. Honey can thus be applied safely to wounds of patients suffering from diabetes mellitus. (Molan, 2001).

Controlled honey production
Although the wound healing properties of honey have already been described in numerous publications (see also a review by Molan, 2006), doctors still appear skeptical about the effectiveness of honey for wound healing. The skepticism often refers to the question whether honey types used in different studies are comparable with respect to effectiveness and composition. Indeed, the variation between honey types is quite high and unpredictable. Therefore, the University of Wageningen has developed a honey type that is of constant, reproducible composition, by producing this honey under controlled conditions in greenhouses. Based on this controlled honey the product line Revamil® has been developed, which comprises a pure honey gel with reproducible high enzyme level, low pH and constant composition.

Literature

Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, et al. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the U.S. Diabetes Care 2001; 24(11): 1936-40

Hoeksema H., Pirayesh S., Monstery S. Honing en wondgenezing. WCS Nieuws 2005; 3: 38-42

Hyslop PA, Hinshaw DB, Scraufstatter IU, Cochrane CG, Kunz S and Vosbeck K. Hydrogen peroxide as a potent bacteriostatic antibiotic: implications for host defence. Free radical Biology and Medicin 1995; 19(1): 31-7

Forouhi NG, Merrick D, Goyder E, Ferguson BA, Abbas J, Lachowycz K, et al. Diabetes prevalence in England, 2001--estimates from an epidemiological model. Diabet Med 2006; 23(2): 189-97.

Inés Mato, José F. Huidobro,* M. Pilar Sánchez, Soledad Muniategui, Miguel A. Fernández-Muiño, and M. Teresa Sancho. Enzymatic Determination of Total D-Gluconic Acid in Honey. J. Agric. Food Chem.1997; 45 (9): 3550 -3553.

Kerkvliet JD. Screening method for the determination of peroxide accumulation in honey and relation with HMF content. J. Apicult Res 1996; 35: 110-117

Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. J. Invest Dermatol 1998; 111(5): 850-857

Molan PC. The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. Bee World 1992; 73(2): 59-76.

Molan PC. Why honey is effective as a medicine. 2. The scientific explanation of its effects. In: Honey and Healing, 2001, Munn P and Jones (eds), International Bee Research Association (IBRA), pp 14-26.

Molan PC. The evidence supporting the use of honey as a wound dressing. Lower Extremity Wounds 2006; 5(1): 40-54.

Pruitt KM, Reiter B. Biochemistry of peroxidase system: antimicrobial effects. In: K.M. Pruitt, J.O. Tenovo (eds) The lactoperoxidase system: chemistry and biological significance. Marel Dekker; New York, 1985; pp 144-178

Saïssy JM, Guignard B, Pats B, Guiavarch M, Rouvier B. Pulmonary endema after hydrogen peroxide irrigation of a war wound. Intensive Care Medicine 1995; 21(3): 287-288.

Siess MH, Le Bon AM, Canivenc-Lavier MC, Amiot MJ, Sabatier S, Aubert SY, Suschetet M. Flavonoids of Honey and Propolis: Characterization and Effects on Hepatic Drug-Metabolizing Enzymes and Benzo[a]pyrene-DNA Binding in Rats. J. Agric. Food Chem. 1996; 44 (8): 2297 -2301

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J, Tonks A. Honey stimulates inflammatory cytokine production from monocytes. Cytokine 2003; 21: 242-247
www.infectieziekten.info

The authors would like to thank Paul Kwakman, PhD student at the Department of Medical Microbiology of AMC Academic Hospital in Amsterdam for carrying out Challenge Tests with MRSA bacterial strains. Furthermore we acknowledge the expertise of Wageningen University Research Centre (WURC) for carrying out several analysis on Revamil® honey.

*W. van Eijk
Medical Scientist, Director Scientific Advisory Office Medi

O. Groenhart
Wound specialist, Bronovo Hospital, Den Haag, The Netherlands