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The contribution of catalase and other natural products to the antibacterial activity of honey: a review

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Abstract

Several natural products are collected or manufactured by bees to construct their hive and produce honey. These include beeswax, flower volatiles, nectar, pollen, propolis and honey itself. Some of the components of these materials possess antibacterial properties and are discussed briefly to ascertain their contribution to the antibacterial activity of honey. New Zealand's manuka honey is known to possess a high level of "non-peroxide" antibacterial activity and research to identify the origin of this activity is briefly reviewed. Finally a hypothesis is advanced to explain the phenomenon of "non-peroxide" antibacterial activity in honey. The author concludes that this activity should be interpreted as residual hydrogen peroxide activity, which is probably due to the absence of plant-derived catalase from honey, an idea first suggested by Dustman in 1971. [Dustman, J. H. (1971). Über die Katalaseaktivität in Bienenhonig aus der Tracht der Heidekrautgewächse (Ericaceae). *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 145, 292–295]

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1. Introduction

Along with the rapidly increasing interest and research into natural health remedies and supplements, is a resurgence of interest in the therapeutic use of honey. Foremost in this respect is its use to promote the healing of skin wounds (Effem, 1988). The healing effect of honey is due to both the physical property of osmosis and the antibacterial properties of hydrogen peroxide. White, Subers and Schepartz (1963) published a paper, which identified the major antibacterial substance in honey as hydrogen peroxide and also demonstrated that it is produced by the enzyme glucose oxidase, when honey is diluted. The oxidase originates from the hypopharyngeal glands of honey bees (Gauhe, 1941). An important finding by White et al., was that "far greater amounts of catalase were required to destroy hydrogen peroxide ...than indicated by the ...total amount of hydrogen peroxide produced". They also commented that "catalase is not highly effective at destroying physiological levels of hydrogen peroxide". The enzyme catalase, which destroys hydrogen peroxide, also occurs in honey but, unlike glucose oxidase, it originates from flower pollen. Dustman (1971) found very high catalase activity for pollen, but very little for nectar and found that very high peroxide values were found in honey samples that were devoid of catalase activity.

It is therefore, clear, that the absolute level of hydrogen peroxide in any honey is determined by the respective levels of glucose oxidase and catalase in that honey. The higher the glucose oxidase level, the higher the peroxide level and the lower the catalase level, the higher the peroxide level, as found by Dustman. As the glucose oxidase in honey originates in bees, one might expect a similar glucose oxidase level in most honeys world-wide, since bees control the ripening of honey within narrow limits. On the other hand, because catalase originates in plants, the level of catalase in honey will effectively determine the level of peroxide in a honey and this will depend on how much pollen is collected by bees, the floral source of the pollen and also on the catalase activity of that pollen.

The antibacterial properties of honey were reviewed in depth by Molan (1992a and b) and again briefly by Armstrong and Otis (1995), McCarthy (1995) and

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Molan (1995) so the theme for discussion in the present paper is first, that hydrogen peroxide is the only antibacterial substance of any consequence in honey and that other substances such as propolis-derived phenolics, are insignificant in comparison to hydrogen peroxide; second, the level of hydrogen peroxide in a honey is essentially determined by the amount of plant-derived catalase in a honey; third, based on the findings of White et al. (1963) and Dustman (1971) hydrogen peroxide is generated by glucose oxidase in samples of honey or fractions thereof, when they are diluted and prepared for antibacterial assays and the amount of catalase added to these samples, in present methods (Allen, Molan & Reid, 1991b; Molan & Russell, 1988), is insufficient to destroy all of the hydrogen peroxide produced in this way. This result gives rise to the belief that other factors of plant origin are responsible for the "non-peroxide" antibacterial activity of some honeys. This stance is reviewed and contested in this paper.

2. Discussion

The antibacterial activity of honey is attributed both to physical factors: acidity and osmolarity and chemical factors: hydrogen peroxide, volatiles, beeswax, nectar, pollen and propolis. Any discussion of the antibacterial activity of honey must be considered in relation to its application. In vivo application may be topical (skin wounds and infection) or internal (throat or stomach ailments) and in vitro uses include agar plate assays. The physical factors involved in the antibacterial action of honey were discussed in detail by Molan (1992a,b). Osmolarity is an important factor in the efficacy of honey when used as an antibacterial agent for skin wounds (Effem, 1988). A recent study by Osato, Reddy and Graham (1999) demonstrated that osmosis determined the bacteriocidal effects of honey towards *Helicobacter pylori,* in vitro, although their conclusions differed from those reached in two earlier studies with this bacterium, which causes stomach ulcers (Al-Somal, Coley, Molan & Hancock, 1994; Moosa & Kadri, 1994). The products which complement hydrogen peroxide to produce the observed antibacterial effects of honey all occur in plants and are collected by honey bees for specific purposes in the hive. Some of these products or their components possess antibacterial properties and these products will be discussed briefly here to ascertain their possible contribution to the antibacterial activity of honey.

2.1. Beeswax

This substance, which forms the honey comb in a hive, consists largely of triacontanyl hexadecanoate (Tomás-Barberán, Ferreres, Garcia-Viguera & Tomás-Lorente, 1993). Triacontanol from this wax has been considered for use as a preservative for butter (Farag, Hassan & Ali, 1993) and also for the treatment of ulcers (Carbajal, Molina & Mas, 1995). Tomás-Barberán, Ferreres and Tomás-Lorente (1993) examined the phenolic components of beeswax and found that it contained a family of flavonoids, the composition of which was identical with that of honey and propolis. These authors concluded that the flavonoids were not characteristic of beeswax but, like propolis, were derived primarily from *Poplar* resin and from the honey. Consequently, beeswax does not contribute any unique product to honey and thereby to the antibacterial activity of honey, but rather the reverse is true.

2.2. Volatiles

Several research groups have examined extracts of honey by GC–MS in order to identify the volatile constituents and to use the profile of components as a means of fingerprinting a unifloral honey (Bicchi, Belliardo & Frattini, 1983; Bonaga & Giumanini, 1986; Bonseta, Collin & Dufour, 1992; Graddon, Morrison & Smith, 1979; Rowland, Blackman, D'Arcy & Rintoul, 1995; Tan, Holland, Wilkins & Molan, 1988; Tan, Wilkins, Holland & McGhie, 1989, 1990; Tan, Wilkins, Molan, Holland & Reid, 1989; Wilkins, Lu & Molan, 1993; Wilkins, Lu & Tan, 1993). In general, the volatile compounds found in honey are components of nectar and contribute to the aroma of flowers. Consequently, the number and variety of components is large, but the quantities in which they occur in honey are very small. Some of these volatile components are unique to a particular flower source and honey, but none appear to have any antibacterial properties, at their level of occurrence in honey.

2.3. Nectar

The HPLC profile of phenolic components of nectar has also been used to relate nectar to a unifloral honey (Gil, Ferreres, Ortiz, Subra & Tomás-Barberán, 1995; Ferreres, Andrade, Gil & Tomás-Barberán, 1996). These phenolic components are generally the same as those found in propolis, but some are characteristic of a unifloral honey. These phenolics are usually flavonoids which occur in the nectar as glycosides and are hydrolysed in the bee stomach to aglycones that are subsequently transferred to the honey. While the common flavonoids have antioxidant properties, none have been identified as having noteworthy antibacterial activity. A recent study by Ares et al. (1996) has suggested that flavonoids might be useful as gastroprotective agents in the treatment of stomach ulcers. This application is interesting in view of the work quoted above (see physical factors) on the use of honey to treat infections of *H. pylori*.

2.4. Pollen

Pollen is a good source of flavonoid glycosides (Dauguet, Bert, Dolley, Bekart & Lewin, 1993; Zerback, Bokel, Geiger & Hess, 1989) and HPLC profiles of these have been used to characterise bee pollens (Campos, Markham, Mitchell & da Cunha, 1997). The flavonoids almost exclusively occur in pollen as glycosides which are hydrolysed in the bee stomach (see above for nectar) and these flavonoids may also characterise a unifloral honey. Since the original work of Dustman (1971), no work appears to have been carried out to determine the level of the enzyme catalase in pollens. Such data would indicate whether a honey, which was manufactured by bees from that pollen, was likely to have elevated levels of hydrogen peroxide. Information of this nature is critical to the hypothesis proposed in this paper.

2.5. Propolis

Propolis is a resinous material collected by bees from the gum exudates of trees, mainly *Poplar* and used by them as an antibacterial agent within their hives (Marcucci, 1995). The antibacterial activity of propolis is due to several classes of components including substituted benzoic and cinnamic acids and flavonoids (Metzner, Bekemeier, Paintz & Schneidewind, 1979). Interestingly, the flavonoids in propolis, to which some of its antibacterial activity is attributed, all lack substituents in the cinnamic acid portion of the flavonoid nucleus (B-ring). Scheller, Szaflarski, Tustanowski, Nolewajka and Stojko (1977) demonstrated that individual components of propolis lacked antimicrobial activity but that bioactivity was observed only for whole propolis. This conclusion indicated that propolis and its constituents possessed weak antibacterial activity.

2.6. Honey

The phenolic composition of honey (Amiot, Aubert, Gonnet & Tacchini, 1989; Andrade, Ferreres & Amaral, 1997; Tomás-Barberán, Ferreres, Garcia-Viguera et al., 1993) is essentially similar to that of propolis and the Spanish group also showed that honey flavonoids are derived from propolis (Ferreres, Ortiz, Silva, Garcia-Viguera, Tomás-Barberán & Tomás-Lorente, 1992). Since the flavonoids in propolis have only weak antibacterial activity (see above) and because they are 1000 times less abundant in honey than in propolis (Ferreres et al., 1992), one might conclude that flavonoids, benzoic and cinnamic acids contribute to the antibacterial activity of honey but that the contribution of these components in reality is small compared to the contribution from hydrogen peroxide.

It is conceivable that the reaction of hydrogen peroxide with the benzoic acids can create peroxyacids, which are more stable than hydrogen peroxide. These acids will escape destruction when catalase is added to a solution of honey prior to an antibacterial assay, due to the selectivity of the catalase, which is specific for hydrogen peroxide and does not destroy alkyl peroxides or peroxycarboxylic acids. Peroxycarboxylic acids are more powerful antimicrobial agents than hydrogen peroxide and this fact might compensate for the low abundance of the carboxylic acids in honey. Furthermore, peroxycarboxylic acids are particularly effective as antimicrobial agents in media of low pH (honey has an average pH of 3.9).The existence of peroxycarboxylic acids in honey has not been established.

2.7. Manuka honey

In a survey of unifloral New Zealand honeys, Allen et al. (1991a,b) found several which exhibited non-peroxide antibacterial activity. The greatest activity was observed for manuka honey which is produced from the nectar of the manuka tree (*Leptospermum scoparium:* Myrtaceae) a species endemic to New Zealand. Not all manuka honey exhibits non-peroxide antibacterial activity but instead, the bioactivity is recorded in manuka honey only from specific localities, particularly the East Cape region of the North Island of New Zealand (Molan, 1995). Recently, an Australian honey from a very similar source (*Leptospermum polygalifolium*) has also been found to possess a high level of non-peroxide antibacterial activity (Mossel, D'Arcy, Davis & Wallace, 1999).

To explain these observations, Molan proposed that a unique plant-derived product was responsible for the non-peroxide antibacterial activity of this honey and that this product originated from the nectar of manuka flowers (Allen et al., 1991a,b; Molan, Smith & Reid, 1988). Since then, a large body of work has been carried out by the Waikato group (Molan, 1999) as well as by our own group but to date no substance has been identified which can be demonstrated to account for the above observations.

Our earlier work showed that the antibacterial activities of phenolic fractions from both manuka honey which had high "non-peroxide" activity and from manuka honey which lacked "non-peroxide" antibacterial activity were identical (Weston, Mitchell & Allen, 1999). Our current work shows that the absolute levels of flavonoids and aromatic acids in active and non-active manuka honey are similar and are also similar to the levels of these products in European honeys which do not possess non-peroxide antibacterial activity (Weston, Brocklebank & Lu, 2000). No other known antibacterial substances have been detected in manuka honey. This work appears, so far, to discount the possibility of the existence of a unique manuka product and leans more towards the views expressed by Schepartz and Subers

(1966), White (1966) and Dustman (1979). In reality, plant-derived antibacterial substances are uncommon, except for benzoic and cinnamic acid derivatives and flavonoids (Bycroft, 1988).

Antibacterial assays are conducted with 25% solutions of honey and Molan and Russell (1988) pointed out that the amount of catalase added was sufficient to destroy the antibacterial effect of hydrogen peroxide at a concentration equivalent to a 50% solution of honey. Molan showed that a neat (undiluted) manuka honey had a concentration of hydrogen peroxide equivalent to 4.5 mM and that sufficient catalase solution was used in all subsequent assays to destroy hydrogen peroxide at a concentration (450 mM) that was 100 times greater than that in honey. However, the concentration of hydrogen peroxide in honey was not assessed by the Waikato group but, instead, was estimated by comparison of antibacterial assays of honey with those of external standard solutions of hydrogen peroxide. The amount of catalase that should be added in an assay of this nature is crucial to the conclusions that can be reached regarding the presence and level of non-peroxide activity. Ideally, the level of hydrogen peroxide in a honey needs to be determined, prior to catalase addition, but the hydrogen peroxide levels in honey vary widely along with factors such as metal ion content and antioxidant levels (which include phenolic acids) and, ultimately only the net level of peroxide can be determined (White et al., 1963). Most importantly, if manuka honey contains a much higher level of glucose oxidase and a lower level of natural catalase than other honeys, then hydrogen peroxide will be generated at a higher level than is allowed for in the above assay, when that honey is diluted for analysis.

It is the thesis of this paper that no unique manuka product exists but, instead, the observed activity might be due to the presence of unusually high levels of hydrogen peroxide in the honey, which accumulate in the proposed absence of a plant catalase and which are not completely destroyed on addition of catalase prior to an assay, i.e. the ''non-peroxide'' activity might be more accurately described as residual hydrogen peroxide activity. It is conceivable that the presence or absence of catalase in manuka honey might be the ''unique factor'' which differentiates ''active'' from ''inactive'' manuka honeys. To prove this view, a survey is needed to determine the levels of both glucose oxidase and catalase in manuka and other honeys, with and without non-peroxide antibacterial activity. The greater the difference in the molar concentration of these two enzymes in honey then the greater will be the potential to generate hydrogen peroxide and the greater will be the amount of catalase required to add to that sample for an antibacterial assay of non-peroxide activity. To date, data of this nature have not been published for any type of honey. The practicalities and problems of such a survey have not been considered; e.g. is the structure (and therefore chromatographic mobility) of catalase in European heather pollen (and honey) the same or different from the catalase of New Zealand manuka pollen (and honey)? Is it possible to detect catalase and glucose oxidase in honey directly and specifically?

It is hoped that the views expressed in this paper will stimulate further research to clarify the role which catalase might play in determining the levels of antibacterial activity of honey.

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