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ANTIBACTERIAL ACTION OF A BEE VENOM FRACTION (MELITTIN) AGAINST A PENICILLIN-RESISTANT STAPHYLOCOCCUS AND OTHER MICROORGANISMS

by

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ABSTRACT

Bee venom and its melittin fraction were shown to have antibacterial activity against a penicillin-resistant strain of Staph aureus (strain 80). This activity of bee venom and melittin was demonstrated by a method similar to that used for plate sensitivity tests. Both whole bee venom and its melittin fraction were also able to inhibit the growth of 20 of the 30 different bacterial organisms tested. More Gram positive organisms (86%) were sensitive to bee venom and to melittin than Gram negatives (46%). The antibacterial activity of bee venom and melittin were of the same magnitude. The zones of inhibition created by bee venom and melittin were compared with those caused by penicillin, and the equivalent units of penicillin were computed. The antibiotic potency of a single bee sting was also determined. Among the Gram positives, the antibacterial effect of a 1:10 dilution of whole liquid bee venom was equal to that of penicillin at a concentration of 0.093 to 17.0 units/ml. The same dilution of bee venom when tested against Gram negative organisms compared to a higher range of penicillin values-93 to 1,700 units/ml.

SUMMARY

The Problem Previous studies from this Laboratory have shown that bee venom is radioprotective for mice. It is also known that whole bee venom exhibits antibacterial properties. The objectives of this present work were to define the chemical fraction in bee venom responsible for the antibacterial action, to determine its activity against a variety of Gram positive and Gram negative bacteria and to compare its antibacterial potency with a standard antibiotic, i.e., penicillin.

The Findings The antibacterial activity of bee venom and its principle component, melittin, was measured. Whole bee venom and its melittin fraction (obtained by column chromatography) showed similar activity levels; they inhibited the growth of 86% of the Gram positive organisms and 46% of the Gram negatives. A comparison with penicillin was made in order to estimate the potency of the bee venom and melittin. Among the Gram positives, the antibacterial effect of a 1:10 dilution of whole liquid bee venom was equal to that of penicillin at a concentration of 0.093 to 17.0 units/ml. The same dilution of bee venom when tested against Gram negative organisms compared to a higher range of penicillin values-93 to 1,700 units/ml.

INTRODUCTION

The ability of bee venom to increase the radiation resistance of mice was recently reported from this Laboratory (1). Studies are now underway to determine the fraction(s) of bee venom responsible for this effect. Pursuant to this study, it was noted that one of the fractions, melittin, failed to culture microorganisms when all of the fractions were accidently contaminated with bacteria. This observation led to an evaluation of the potential bacteriostatic and/or bacteriocidal characteristics of bee venom and of melittin. Melittin is the largest single component (by weight) of bee venom; it is a polypeptide of molecular weight 2850, and evidence suggests that in bee venom it exists mostly as a tetramer (2).

In 1941 Schmidt-Lange discovered that bee venom was antibacterial (3). This observation was extended by Ortel and Markwardt in 1955 (4,5). They measured the effect of bee venom against thirteen Gram positive and nine Gram negative bacteria and showed that the Gram positives were the most sensitive.

In the present work, we were interested in determining whether melittin itself was antibacterial, and the range of its activity. In the course of these studies it was found that a penicillin-resistant bacterial species, Staph. aureus strain 80, was sensitive to the antibacterial action of melittin.

MATERIALS AND METHODS

Bee venom: Venom was collected by the method of Benton, Morse, and Stewart (6). The crystalline venom was pooled and separated into components on a Sephadex G50 column (2).

Melittin: To establish the purity of the melittin fraction the ultra-violet absorption spectrum of melittin was obtained prior to and after the experiments. A comparison of the two spectra showed the melittin had not been degraded by air oxidation.

Organisms: Most of the organisms tested were isolated from various animal sources. The three strains of Staphylococcus aureus were obtained from the collection of Drs. V. Hurst and V. Sutter of the University of California Medical Center. The 9 unidentified organisms were isolated from the gastro-intestinal tract of a strain of mice routinely used in the laboratory. A total of thirty organisms were tested - fifteen Gram positive organisms and fifteen Gram negatives.

The organisms to be tested were inoculated from stock cultures into 10 ml of Brain Heart Infusion (BHI) broth, and incubated for 18 hours at 37°C. (An exception to this was Pseudomonas fluorescens which was incubated at 25°C).

Test Method: The method used in testing the antibacterial activity of bee venom and melittin was similar to that used for standard antibiotic testing. Fresh BHI agar plates, dried in an incubator at 37°C for one-half hour, were flooded with a suspension of the organism. The

excess fluid was removed with a Pasteur disposable pipette, and the plates allowed to air dry. Three plates per organism were used in each experiment.

Sensitivity discs were made from #7 filter paper. These discs measured approximately 7 mm in diameter, and held 10 µl of fluid. Sterile discs were immersed in the bee venom or melittin test solution and placed on the dry plates with sterile forceps. The plates were then incubated overnight. The diameter of the zone of inhibition was measured and the mean values computed.

In order to evaluate the antibacterial effect of whole bee venom and of the melittin fraction, their inhibitory effect at a concentration of 30 mg/ml was compared to that of a standard antibiotic, Penicillin. Ten-fold dilutions in sterile water were made of buffered Potassium Penicillin G - 200,000 units (Squibb). These dilutions were tested in the same manner as the bee venom and melittin solutions.

RESULTS

Each of the thirty organisms used in these experiments was tested several times for its sensitivity to whole bee venom and to the melittin fraction. The results of these tests are given in Tables I and II. The Gram positive organisms (Table I) proved to be more sensitive to the test substances than the Gram negatives (Table II) - 86.6% compared to 46.6%.

TABLE I

COMPARISON OF EFFECT OF WHOLE BEE VENOM (30 mg/ml) AND

OF THE MELITTIN FRACTION (30 mg/ml) ON GRAM POSITIVE ORGANISMS

ORGANISM	ZONE OF INHIBITION (Diam., mm)	
	Bee Venom	Melittin
Strep. fecalis	8.5	9.5
Strep. liquefaciens	8.0	7.8
Staph. aureus - Strain 3A	9.0	9.3
" " " 53	8.1	8.3
" " " 80	8.0	8.3
Corynebacterium sp.	10.5	12.0
gram pos. cocci #1	8.8	9.3
2	8.5	8.5
3	8.8	9.0
4	8.8	9.8
5	8.3	9.3
gram pos. rods #1	8.8	9.8
2	11.8	11.8
3	0	0
4	0	0

TABLE II

COMPARISON OF EFFECT OF WHOLE BEE VENOM (30 mg/ml)

AND OF THE MELITTIN FRACTION (30 mg/ml) ON GRAM NEGATIVE ORGANISMS

ORGANISM	ZONE OF INHIBITION (Diam., mm)	
	Bee Venom	Melittin
Aerobacter aerogenes	8.0	7.5
Aerobacter cloacae	8.0	8.0
Bethesda - Ballerup	7.6	8.0
Citrobacter freundii	0	0
Citrobacter freundii (aberrant)	0	0
E. coli	0	0
Mima polymorpha	10.5	12.0
Proteus mirabilis	0	0
Proteus morganii	0	0
Pseudomonas aeruginosa	7.8	9.0
Pseudomonas fluorescens	0	0
Pseudomonas maltophilia	8.0	7.9
Salmonella derby	0	0
Salmonella newport	7.8	8.5
Serratia marcescens	0	0

In most cases a slightly higher inhibitory effect was achieved with the melittin fraction. However, this difference is not large enough to be considered significant, and it can therefore be concluded that the antibacterial activity of the melittin fraction is of the same magnitude as that of whole bee venom.

The zones of inhibition created by bee venom or melittin are the same size for most sensitive organisms with the average being 8.5 mm. A few cultures showed a greater sensitivity to the two substances — Corynebacterium sp., Gram positive rod #2 and Mima polymorpha.

A comparision of the activity of melittin and penicillin against three strains of Staph aureus is shown in Table III. All three strains were sensitive to melittin to the same degree while the magnitude of response to penicillin varied greatly with the strain and the concentration. The experimental technique previously mentioned was varied slightly for one experiment. The test cultures were incubated for 5 hours in a water bath rather than 18 hours in an incubator prior to use. It was thought that with a lighter lawn the zones of inhibition would be larger and more easily measured. However, there was no significant difference between the zones created on the 5 hour culture and those on the 18-hour culture.

The data expressing the equivalency of the number of units of penicillin to 1 mg/ml of bee venom is given in Table IV. Only those organisms sensitive to both bee venom and penicillin are considered in this table. It is not surprising to find more Gram positive than

TABLE III

COMPARISON OF ANTIBACTERIAL ACTIVITY OF MELITTIN AND PENICILLIN AGAINST THREE STRAINS OF STAPH. AUREUS

ORGANISM	ZONE OF INHIBITION (mm)						
Staph aureus	Melittin (mg/ml)	Penicillin (Units/ml)					
	30	50,000	5000	500	50	5	0.5
Strain 3A	9.3	35	32	27	22	13	0
" 53	8.3	18	13	0	0	0	0
" 80	8.3	0	0	0	0	0	0

TABLE IV

COMPARISON OF INHIBITORY ACTION OF PENICILLIN AND OF BEE VENOM (and melittin)

ORGANISM	UNITS/ml OF PENICILLIN EQUIVALENT TO 1 mg/ml of WHOLE BEE VENOM (or melittin)
Gram Negative	
Aerobacter cloacae	1,700
Bethesda - Pallerup	93
Mima polymorpha	930
Salmonella newport	93
Gram Positive	
Staph. aureus - Strain 3A	0.093
" " " 53	93.0
Strep. fecalis	1.70
Strep. liquefaciens	17.0
Gram positive cocci #1	0.093
2	0.093
3	0.093
4	0.093
5	0.093
Gram positive rods #1	0.093
2	0.16

Gram negative organisms listed since penicillin is known to be very effective against Gram positive organisms, and this results in the small equivalence ratios of the Gram positives. The large equivalence ratios exhibited by the Gram negative bacteria reflect the relative impotency of penicillin towards these microorganisms. Among the Gram positives, the antibacterial effect of a 1:10 dilution of whole liquid bee venom was equal to that of penicillin at a concentration of 0.093 to 17.0 units/ml. The same dilution of bee venom when tested against Gram negative organisms compared to a higher range of penicillin values-93 to 1,700 units/ml.

DISCUSSION

These experimental data confirm the previous work that bee venom can inhibit bacterial growth. In addition, we have found that a specific chromatographic fraction of the venom, identified as melittin, is the component responsible for this antibacterial activity. More Gram positive organisms are sensitive to melittin than Gram negatives. Presumably, this antibiotic action of melittin is associated with its polypeptide structure.

A penicillin-resistant strain of Staph aureus, strain 80, was found to be sensitive to the action of melittin. It is possible that other drug-resistant microorganisms may exhibit a similar property. This sensitivity of strain 80 to melittin suggests an extension of the present study--in vivo testing of the antibacterial activity of melittin using animals infected with Staph. aureus strain 80.

The relative sensitivities of the bacteria were qualitatively estimated by measuring the zones of inhibition. Ortel and Markwardt (4) quantitatively determined the zones of inhibition. They found that Gram positive organisms were sensitive at lower concentrations of bee venom than Gram negatives.

The question as to the mechanism of action of bee venom, i.e. whether bacteriocidal or bacteriostatic, has not been fully explored. Schmidt-Lang (3) found it to be bacteriocidal but Benton, et al (5) considered it to be primarily bacteriostatic. However, Ortel and Markwardt (4) concluded that the venom had both effects.

Attempts have been made previously to identify the component(s) of venom responsible for its antibacterial activity. Ortel and Markwardt separated bee venom electrophoretically into two fractions. They found the inhibitory action confined to one fraction - a polypeptide (4), the characteristics of which were suggestive of melittin, as described by Beard (7).

In order to interpret the present results in terms of the antibacterial activity of a single bee sting an attempt was made to estimate the venom content of a single sting. Other investigators (8,9,10) have estimated numerically the venom content of one bee sting, and have arrived at varying results. Venom content appears to be influenced by 2 factors--the time of the year venom is being collected and the age of the bee. Hahn and Ostermayer (8) extracted the venom from the stings of summer bees and winter bees. They calculated the venom content of summer bees as 465 µg/bee sting, and that of winter bees as 250 µg/bee sting. This work was repeated by Hahn and Fernholz (9), and they calculated the venom content of the sting of a winter bee as 256 µg. Other investigators, using unspecified bees, found the venom concentration to be 339 ug/bee sting (10). The average value of the venom content of a bee sting based on the above data, is 320 $\pm$ 129 µg. However, in all these reported cases the entire bee gland was subjected to a crude extraction process so that

the resulting substance did not represent pure venom. Therefore, we conclude that the venom content of one bee sting is below the estimated 320 μg.

For our experimental purposes, one bee sting was defined as 100 μg of solid venom in a solution volume of 0.3 μl. From the experimental results it can be calculated that a single bee sting has the antibiotic potency of 0.93 to 170 units/ml of penicillin for a variety of Gram positive bacteria and a range of 930 to 17,000 units/ml of penicillin when measured against a selected group of Gram negative organisms.

In view of the present results, it would be of considerable interest to determine whether the antibacterial property of melittin, particularly that against penicillin resistant Staphylococci, is associated with the whole polypeptide macromolecule, or whether smaller molecular fragments would also exhibit this antibacterial activity. We plan to pursue further work along these lines, subjecting mellitin to partial enzymatic hydrolysis, followed by column chromatographic separations and isolation of the resultant peptides and their biological assay. The potential clinical usefulness of such chemically-characterized products is self-evident.

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