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Lysates of Locusta migratoria brain exhibit potent broad-spectrum antibacterial activity

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Sir,

In recent years, the burden of infectious diseases has exacerbated with the emergence of antimicrobial resistance and lessening efficacy of the available antimicrobial compounds. Vancomycin-resistant enterococci and methicillin-resistant Staphylococcus aureus (MRSA) have recently emerged as major threats to public health. In fact, the drug of choice to treat MRSA is vancomycin, but it has emerged recently that vancomycin-intermediate S. aureus is exhibiting some levels of resistance against vancomycin. In addition, there are reports from the USA of MRSA showing high-level resistance to glycopeptides due to acquisition of the vanA gene complex. Thus, new antimicrobial agents are required to meet the challenge posed by the emergence of multidrug-resistant microorganisms. The search for new antibiotic compounds originating from natural resources is an important research area. Insects are the largest (80% of all fauna) and most widespread group within the Animal Kingdom, and synthesize a variety of antibacterial compounds, including the defensins and cecropins, as part of their innate immune response to infection. Up to 50% of the reported antimicrobial substances are identified in invertebrates (predominately in insects). We investigated the possible presence of antimicrobial activity in the various tissues of a locust, Locusta migratoria.

Briefly, locust brains were isolated by making a sagittal cut through the base of the left antenna as described previously. Brains were pooled in batches of 30, resuspended in 500 µL PBS and subjected to four cycles of freeze–thaw. The brains were disrupted using a Cole-Parmer cup-horn sonicator and tested for antimicrobial activity. In addition, fat body and dorsal longitudinal flight muscle tissue (equivalent to that of 30 pooled brains) were removed and lysates prepared. A 100 µL aliquot of brain lysate (equivalent to six brains) or lysate from muscle or fat body was incubated with ~10⁶ bacterial cells [Escherichia coli K1 (a cerebrospinal fluid isolate from a meningitis patient; 018:K1:H7), Staphylococcus epidermidis, S. aureus and MRSA]. All bacteria tested here are clinical isolates that were obtained from Birkbeck culture collection (available upon request). Tubes were incubated for 2 h at 37°C, and bacterial counts were determined by plating on nutrient agar plates. For controls, bacteria were incubated in PBS without the brain lysates. The percentage bactericidal effect was determined as the percentage of bacteria surviving relative to the control as follows: 100 – (cfu in brain lysates/cfu in PBS × 100). The results revealed that brain lysates killed more than 99% E. coli K1, while muscle and fat body lysates had no effect (Table 1), suggesting that the active compound(s) are specific to the brain tissue. The brain lysates had broad-spectrum activity as demonstrated by potent bactericidal effects against E. coli K1, MRSA, S. aureus and S. epidermidis (Table 1).

To determine the potency of the compound(s) in the locust brain lysates, serial dilutions of the brain lysates were made and tested against E. coli K1. Aliquots of brain lysate ranging from 5, 10, 50 and 100 µL were bactericidal, i.e. 99.82%, 99.98%, 99.99% and 99.997%, respectively, whereas volumes of brain lysate <1 µL did not show bactericidal activity. When the brain lysates are heated at 100°C for 10 min, the potency remains the same. Perhaps, there is more than one compound present in the brain lysates, and heating activates one or more of these compounds, or heating increases the efficiency of extraction, thus increasing the antimicrobial potency of the lysates above that of non-heated brain lysates. When brain lysates were treated with 1% SDS and boiled as above, the bactericidal activity disappeared (Table 1), suggesting that the active components of brain lysates are proteinaceous in nature.

To determine the effect of the locust brain lysates on human brain microvascular endothelial cells (HBMECs), cytotoxicity assays were performed using a cytotoxicity detection kit (Roche Applied Science) as described previously. HBMECs grown in 24-well plates were incubated with different volumes of brain lysate from 25 to 200 µL for 24 h at 37°C in a 5% CO₂ incubator. Next, the supernatant of each well was collected and percentage cell death determined. It was demonstrated that the locust brain lysates had no cytotoxic effect on HBMECs (data not shown), suggesting that the putative target(s) for the active component(s) may be absent in eukaryotes. In support, our preliminary studies suggest that the brain lysates have no
amoebicidal effects against *Acanthamoeba castellanii* and *Balamuthia mandrillaris* (N. A. K., K. O. and G. J. G., unpublished data).

Future work will include identification of the nature of the compound(s), such as the chemical structure and properties, as well as its mode of action, and whether this is a known or novel compound.

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**Transparency declarations**

None to declare.

**References**


**Antifungal activity against Candida albicans of nikkomycin Z in combination with caspofungin, voriconazole or amphotericin B**

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Sir,

Until the last decade, antifungal therapy was based mostly on drugs acting on the fungal membrane, such as amphotericin B and azoles, and the rationale for the use of combination therapy remained questionable.1 Thus, the only drug combination of two antifungals with two modes of activity used clinically, primarily in cryptococcosis, was amphotericin B and 5-fluorocytosine.2 The introduction of echinocandins, which act on the fungal cell wall by inhibiting glucan synthesis, opened the approach to explore different drug combinations, such as echinocandins and polyenes, or echinocandins and azoles,3,4 for various mycoses.

Nikkomycin Z inhibits chitin synthesis, by acting as a competitive analogue of chitin synthase substrate UDP-N-acetylglucosamine.5 Since chitin is found in most fungal cell walls,