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Antibacterial properties of lucifensin in *Lucilia sericata* maggots after septic injury

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated that the antimicrobial peptide lucifensin was not found in the digestive tract of the larvae of the fly *L. sericata* as was believed and that lucifesin was isolated to the haemolymph and found higher antibacterial activity of such haemolymph in comparison to non-stimulated larvae.
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ABSTRACT

Objective: To investigate the antibacterial properties of lucifensin in maggots of *Lucilia sericata* after septic injury.
Methods: In our preliminary study we have shown that injuring the maggots with a needle soaked in lipopolysaccharide solution induced within 24 h lucifensin expression in the fat body and in the grease coupler of the salivary glands. It is assumed that lucifensin is secreted solely from this tissue into the haemolymph (similar to other insect defensins) and not into secreted/excreted products. We used high-performance liquid chromatography fractionation and radial diffusion assay to investigate the antibacterial properties of haemolymph extracted from larvae after septic injury.
Results: After septic injury, production of lucifensin in the haemolymph is increased. This led to higher antibacterial activity of such haemolymph in comparison to non-stimulated larvae.
Coclusions: These results suggest that beside the previously demonstrated role of lucifensin in the debridement therapy, lucifensin is simultaneously important as a part of the systematic immune response.

KEYWORDS
Lucilia sericata, Wound bacteria, Defensin, Lucifensin, Immune-challenge

1. Introduction

Lucifensin is one of the well-characterised antibacterial substance from maggots of *Lucilia sericata* (*L. sericata*) involved in maggot therapy[1]. It is assumed that it plays a role in the inhibition of some wound pathogens since it has been found in excretion/secretion of maggots. Lucifensin was originally isolated from larval guts and was subsequently detected in salivary glands, the fat body and haemolymph[1]. Using *in situ* hybridisation, expression of lucifensin has been confirmed in the salivary glands of all larval stages. Expression has been also occasionally

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detected in a few cells of the fat body and in the grease coupler of salivary glands. Surprisingly, no expression of lucifensin has been detected in the gut although lucifensin was originally purified from this tissue. This could mean that, after secretion from salivary glands into the environment, lucifensin is ingested by maggots along with food and passes through the digestive tract[2].

The antibacterial activity often results in a constitutive expression of antibacterial factors (produced at a constant level) or in an inductive expression of antibacterial factors upon bacterial stimulation[3]. It has previously been described that the larval immune system might be activated to induce production of antibacterial substances to survive in an infectious environment[4,5]. Synthesis of antimicrobial peptides in the fat body (a functional equivalent of the mammalian liver) and their rapid release into the haemolymph is important and the best characterised aspect of the insect immune response. Using *in situ* hybridisation, it has been shown that an infectious environment could increase the expression of lucifensin in the fat body of *L. sericata* larvae[2]. Lucifensin should be secreted solely from this tissue into the haemolymph (similar to other insect defensins) and not into excretion/secretion products. Injuring sterile maggots with a sterile needle increased fourfold the antibacterial activity of haemolymph within 24 h. When infected needle was used the antibacterial activity of haemolymph increased sixteenfold[6].

The aim of this study was to investigate the antibacterial properties of haemolymph extracted from the larvae after septic injury.

2. Materials and methods

2.1. Rearing of L. sericata larvae

Colonies of the green bottle fly (*L. sericata*) were maintained at the Institute of Zoology, Slovak Academy of Sciences under constant conditions. Imagos were exposed to 12 h light/dark photocycles at (25±1) °C and a relative humidity of 40-50%. Larvae were fed on ground beef liver mixed with bran.

2.2. Preparation of whole body larval extracts

The whole body extract from 4-day old larvae in the middle of third instars (n=300) was prepared as previously described with some modifications[1]. Briefly, larvae collected from beef liver, were washed and homogenised in grinding mortar using a methanolic extraction buffer (methanol/water/acetic acid: 90/9/1). The larval extract was vortexed and centrifugated at 10 700 r/min for 30 min at 4 °C to remove particular material. The supernatant was collected and lyophilized, and the obtained pellet was dissolved in 1 mL of ultrapure water.

2.3. Purification and identification of larval antibacterial lucifensin

Whole body larval extract was used for isolation of antibacterial peptide-lucifensin. The purification was performed as previously described with some modifications[1]. Briefly, extract was loaded onto HiTrap CM Sepharose HP column (GE Healthcare, UK) and eluted fractions with antibacterial activity were pooled and concentrated. This material was submitted to fractionation under reverse phase-high performance liquid chromatography (RP-HPLC) with a C18 column (250 mm× 4.6 mm; 5 μm) (Grace, IL USA) at a flow rate 0.3 mL/min by using a gradient from 0 to 90% (v/v) acetonitrile [containing 0.1% (v/v) trifluoroacetic acid], during 70 min, after initial 5 min at 0% acetonitrile. After lyophilisation, the fractions were dissolved in 100 μL of ultrapure water and tested for antibacterial activity.

Mass spectra of antibacterial fraction were acquired in positive ion mode using electrospray ionization on a Apex-Qe Ultra Fourier transform mass spectrometry instrument equipped with a 9.4 T superconducting magnet (Bruker Daltonics, Billerica, MA, USA).

2.4. Immune-challenge of L. sericata maggots

Second instar larvae of *L. sericata* were punctuated dorsolaterally with a needle that was contaminated with an lipopolysaccharide (LPS) solution (10 mg/mL, crude preparation of *Escherichia coli* endotoxin 0111: B4, Cat. No.: L2630, Sigma, Taufkirchen, Germany) and subsequently, 24 h post immune-challenge animals were used for collection of haemolymph.

2.5. Collection of haemolymph

Approximately 50 pieces of feeding larvae or larvae after 24 h post immune-challenge were removed from liver, thoroughly washed, then placed into a 50 mL Erlenmeyer flask and cut by scissors into multiple pieces and kept 1 h at 4 °C. The released liquid was decanted and centrifuged at 11 000 r/min for 5 min at 4 °C to remove all the debris before further processing.

2.6. RP-HPLC of haemolymph extracts

Haemolymph extracts were fractionated by using the same