MORPHO-CYTOPATHOLOGICAL ANALYSIS OF THE MIDGUT OF *Bombyx mori* L. INFECTED WITH BmNPV AND SUBMITTED TO THE ANTIVIRAL DRUG Bm5

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Abstract

The creation of silkworms for the production of cocoons for the industry is called sericulture. The biggest problems that threaten sericulture in Brazil and the world are diseases caused by various pathogens. Viral diseases are the most serious and represent a serious problem for the world's sericulture sector. *Bombyx mori* nuclear polyhedrosis virus (BmNPV) is a highly infective pathogen that causes serious disease in the species. Caterpillars become infected after ingesting viral occlusion bodies (OBs) present in contaminated mulberry leaves. After the death of the infected caterpillar, its integument easily disintegrates, releasing large amounts of OBs into the environment, which serves as an inoculum to infect other caterpillars. This process is facilitated by the synergistic interaction between the viral gene products *V-cath* (a viral cathepsin) and *chiA* (a viral chitinase). As the virus is polyorganotrophic, there are several target tissues, with the midgut being the primordial one, as it is related to the digestion and absorption of nutrients. In this work, the effects of the drug Bm5 (built through modeling and molecular docking to inhibit the viral cathepsin of BmNPV) on the cytopathological morphology of the midgut of *B. mori* caterpillars infected by BmNPV were analyzed. Morphological analyzes were performed following the techniques of histology and electron microscopy. The results revealed that more earnest alterations occurred in the tissue of caterpillars infected with BmNPV and not treated with the drug Bm5, indicating that it acted by minimizing the effects of the viral action in the midgut, thus reducing the viral proliferation of the contaminated host.

Introduction

Bombyx mori (Linnaeus, 1758) popularly known as silkworm is an insect belonging to the order Lepidoptera. Lepidoptera are holometabolic insects, that is, they have complete metamorphosis, and the caterpillar stage lasts about 27 days and is characterized by four seedlings and five instars. This insect is massively bred for commercial purposes, as the silk thread that serves as the raw material for making the cocoon where pupation will occur is widely used by the textile industry [1, 2]. Furthermore, it is widely studied and used as a model system in lepidopterans due to the rich repertoire of genetic information on mutations that affect their development, morphology and behavior [3].

The biggest problems that threaten sericulture in Brazil and in the world are diseases caused by various pathogens. Diseases of viral origin are the most serious and represent a serious problem for the world's sericulture sector. When *B. mori* is infected with *Bombyx mori* nuclear polyhedrosis virus (BmNPV), significant losses occur in sericulture, as there are no drugs available against this virus. Thus, the structure of viral cathepsin (BmNPV-Cath) was modeled in the presence of an inhibitor (MYP) and used as a target in virtual scanning simulations, aiming to identify potential molecules that could be used in the treatment of the infection. The virtual scan selected four better-ranked molecules compared to MYP, the molecule with the highest affinity to the enzyme, called Bm5, was used in in vivo infection models of *B. mori* caterpillars with BmNPV and the results showed that treatment with a dose of Bm5 dissolved in Pluronic F-127 (0.02%) was able to reduce the mortality of caterpillars by 22.6%, in addition [4].

Materials and methods

Light microscopy

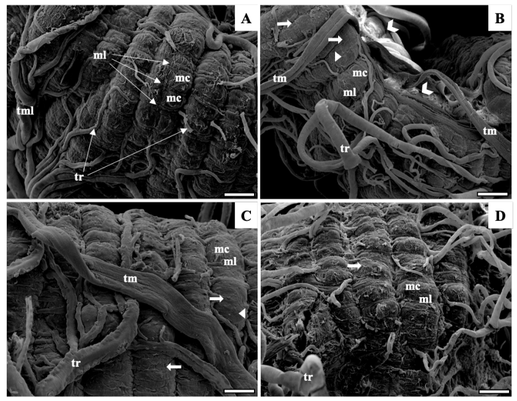
For histological analysis, samples were collected from the midgut and the caterpillars were cold sacrificed and dissected on the 7th day post-infection (dpi). The samples were fixed in Bouin aqueous solution (picric acid, formaldehyde, and acetic acid) for 24 hours at 4ºC, and routine histological processing was followed: dehydration in a growing series of alcohols, diaphanization in xylol, and embedding in paraffin [5]. Then, 6 µm sections were obtained in a Leica RM 2250 microtome, and the slides were stained with hematoxylin and eosin (HE) [6], as well as with modified Azan for observation of viral occlusion bodies [7]. The slides were analyzed under a light microscope (Olympus CBA) and the regions of interest were photographed using a digital camera.

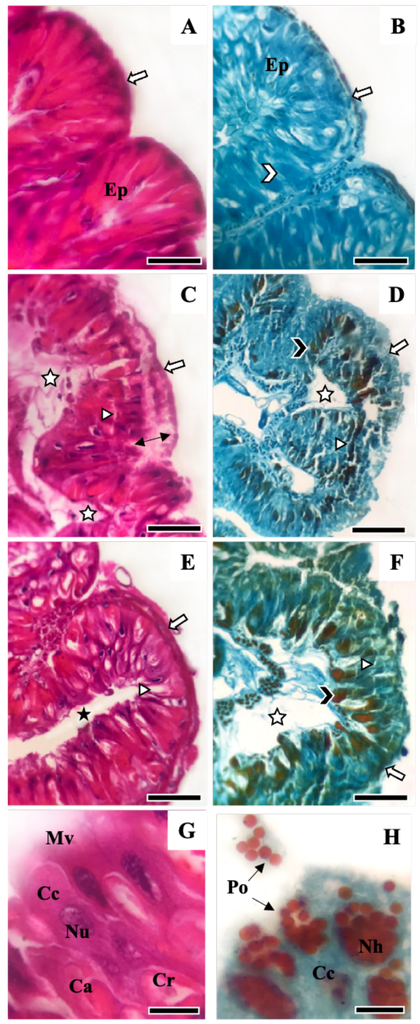
Scanning Electron Microscopy (SEM)

For SEM, the midgut was dissected and fixed in Bouin aqueous solution for 24 hours at 4ºC, and then washed in 0.1 M sodium phosphate buffer. Afterward, the samples were dehydrated in an increasing series of alcohols (50 %, 70%, 80%, 90%, 95%, and 100%), subjected to a critical point (Leica CPD 030), and metalized with gold powder in the Shimadzu IC-50 metallizer. Analyzes were performed using the QUANTA 250-FEI Scanning Electron Microscope from the Microscopy Center of the Research Support Centers Complex (CMI-COMCAP) at the State University of Maringá/Paraná/Brazil.

Results

The cytopathology of the infection caused by BmNPV in the intestinal epithelial cells of infected caterpillars that were not treated with the drug revealed a major internal disruption of the epithelium, with ruptured cells and basal lamina detachment, in addition to spacings between the epithelium (Fig. 1C and D), which coincides with the events caused in the intestinal musculature of these caterpillars, which were loose, with dilation of circular fibers and deformation of longitudinal fibers, causing a displacement of the basal lamina where the intestinal epithelium rests (Figs. 2B and C). Cytopathological events also showed hypertrophic columnar cell nuclei with the presence of polyhedra stained in red by the Azan technique (Fig. 1H).





In caterpillars infected and treated with the drug, the intestinal epithelium showed the same degenerations, however, in a regressed way compared to the epithelium of caterpillars without Bm5 (Fig. 1E and F), and the less loose musculature (Fig. 2D).

The control group caterpillars did not show structural changes in the musculature and continued with an intact epithelium, and in the cell nucleus, there was no presence of polyhedra, nucleocapsids, or any structures or components indicative of viral infection (Figs. 1A, B, G, and 2A).

Figure 2. Electron microscopy of the midgut musculature of *Bombyx mori* on the 7th day of the 5th instar. **A** control; **B** and **C** BmNPV; **D** BmNPV treated with the drug Bm5. Longitudinal musculature (ml), circular musculature (mc), tracheas (tr), Malpighian tubules (tm), loose longitudinal musculature (white arrow), and epithelial disruption (white chevron). Scale = 200 µm.

Figure 1: Photomicrographs of *Bombyx mori* midgut epithelial cells on day 7th of the 5th instar. **A**, **B**, and **G** control; **C**, **D**, and **H** BmNPV; **E** and **F** BmNPV treated with Bm5. Epithelium (Ep), musculature (white arrow), epithelial cells without infection (white chevron), epithelial cells with infection (black chevron), epithelial disruption (white star), epithelial spacing (black star), basal lamina detachment (arrow double), cell disruption (isosceles triangle), microvilli (Mv), columnar cells (Cc), goblet cells (Ca), regenerative cells (Cr), normal nucleus (Nu), hypertrophic nucleus (Nh), and mature polyhedra in the nucleus and extracellular environment (Po). Color: Hematoxylin and eosin (A, C, E, and G); Modified Azan for occlusion bodies (B, D, F, and H). Scale = 50 µm.

Conclusion

Based on the results obtained, it is clear that the morphology is less unstructured and corrupted in caterpillars infected with BmNPV and treated with a dose of the drug Bm5 compared to the morphology observed in caterpillars infected with BmNPV without the drug, which is indicative that the drug is acting by minimizing the effects of viral action on different tissues, reducing the disorder caused in cells and tissues during BmNPV infection.

Recommendations

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Acknowledgements

The authors thank to the company Fiação de Seda BRATAC, for supplying *Bombyx mori* caterpillars; the Microscopy Center of the Research Support Centers Complex (COMCAP) of UEM, and CAPES for the financial support.