

Physalin B inhibits *Rhodnius prolixus* hemocyte phagocytosis and microaggregation by activation of endogenous PAF-acetylhydrolase activities

Castro,D.P.<sup>1</sup>, Figueiredo,M.B.<sup>1</sup>, Genta,F.A.<sup>1,3</sup>, Ribeiro,I.M.<sup>2</sup>, Tomassini,T.C.B.<sup>2</sup>, Azambuja,P.<sup>1,3</sup>, Garcia,E.S.<sup>1,3</sup>

danicastro@ioc.fiocruz.br

<sup>1</sup>IOC-FIOCRUZ; <sup>2</sup>FarManguinhos-FIOCRUZ; <sup>3</sup>INEM, CNPq

The effects of physalin B (a natural secosteroidal chemical from *Physalis angulata*, Solanaceae) on hemocyte phagocytosis and microaggregation, in experiments using 5<sup>th</sup>-instar larvae of *Rhodnius prolixus*, were investigated. Hemocyte phagocytosis and microaggregation are induced by the platelet-activating factor (PAF) or arachidonic acid (AA) and regulated by phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and PAF acetyl hydrolase (PAF-AH) activities. Hemocyte phagocytic activity and formation of hemocyte microaggregates were strongly blocked by oral treatment of this insect with physalin B (1 µg/mL of blood meal). These inhibitions, induced by physalin B, were reversed by exogenous arachidonic acid (10 µg/insect) or PAF (1 µg/insect) applied by hemocelic injection.

We measured the activities of PLA<sub>2</sub> and PAF-AH in cell-free plasma and hemocytes of control and physalin fed *R. prolixus* nymphs. Total plasmatic PLA<sub>2</sub> activity showed a significant (P<0.001) increase (1.5x) with physalin treatment, mainly due to the increase (2x, P<0.001) in the iPLA<sub>2</sub> form, as the cPLA<sub>2</sub> did not change significantly (P>0.05). PAF-AH showed a dramatic increase in insects that were fed with physalin (6x, P<0.005).

Following the treatment with physalin B, the hemocyte PLA<sub>2</sub> activity was not affected. Neither the total PLA<sub>2</sub> nor any of PLA<sub>2</sub> isoforms (iPLA<sub>2</sub> or cPLA<sub>2</sub>) showed any significant differences between insects treated with physalin and the controls (P>0.05). In these hemocyte samples a very low PAF-AH activity was detected and this enzyme was more active (3x) in the physalin treated insects when compared to the controls (P<0.05).

These results show that physalin B inhibits hemocytic activity by depressing insect PAF analogous (iPAF) levels in hemolymph and confirm the role of PAF-AH in the cellular immune reactions in *R. prolixus*.

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