Inhibition of *Plasmodium falciparum* Cysteine Proteases by Sugarcane Cystatins

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Abstract

Introduction: Malaria is caused by *Plasmodium* parasites and affects millions of people. Plasmodium proteases are involved in invasion, erythrocytes egress and degradation of host proteins. Falcipains are cysteine peptidases located in the parasite’s food vacuole. Canecystatins are natural cysteine protease inhibitors present in sugarcane, which can inhibit cathepsins B, L, V and S and kill pathological microorganisms. The aim of this study is to assay canecystatins as inhibitors of *P. falciparum* cysteine proteases evaluating its potential use as new antimalarial drug, since proteases are a target to kill parasites. Material and Methods: The inhibitory action of canecystatins upon the *P. falciparum* cysteine proteases was determined using the fluorescent substrate Z-FR-MCA. Western blotting, fluorescent microscopic and mass spectrometry elucidated the inhibition in parasites cells. Results: The recombinant canecystatins CPI-1, CPI-2, CPI-3, CPI-4 and CPI-4 TAT are able to inhibit the proteolytic activity of *P. falciparum* parasites and recombinant falcipain-2 and falcipain-3 with IC₅₀ in nanomolar range. The binding of the canecystatins to the falcipains was evaluated by mass spectrometry confirming the binding of these proteins. Western blotting analysis showed that CPI-1, CPI-2, and CPI-3 were slightly hydrolyzed by parasite, but not CPI-4 and CPI-4 TAT. Using FITC-labeled CPIs, it was observed that canecystatins can reach the parasite, suggesting that they can exert the inhibitory function inside the parasites. Only infected erythrocytes were able to uptake the labeled inhibitors. Conclusion: The recombinant canecystatins here presented are promising inhibitors to arrest parasite’s development. Studies are in progress to better define the inhibition of plasmodia proteases by canecystatins *in vivo*.

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