Ubiquitin/Proteasome System is Involved in Host Hormone Melatonin Action in the Human Malaria Parasite *Plasmodium falciparum*

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The ubiquitin/proteasome system (UPS) plays a fundamental role in eukaryotic cells including signal transduction, cell cycle and transcription regulation. We have investigated if melatonin could modulate ubiquitination in human malaria parasite, *Plasmodium falciparum*.To evaluate modulation of UPS genes by melatonin we performed real time PCR (RT-PCR) using 14 selected *Plasmodium* genes. Synchronized *P. falciparum* 3D7 strain were treated with melatonin 10µM and 100 nM at 24 hours post invasion and after 3-6 hours treatment transcriptional changes of UPS genes were analyzed by RT-PCR. Student *t* test analysis was used to assess $\log_2$ of relative expression ($2^{-??CT}$) changes. We have also performed western blot (WB) and immunoprecipitation assays (IP) to analyze *P. falciparum* protein ubiquitination profile changes of parasites treated with melatonin. *P. falciparum* culture at trophozoite stage was submitted to melatonin 10 µM after 24 hours of the invasion for 6 hours. The anti-Ubiquitin antibody (AbCam, UK) was used for IP experiments and WB. Immunocomplexes were subjected to mass spectrometric analysis at the TUFTS University Core Facility (Boston, MA). Our results show that nine UPS genes were up-regulated at 5hs of melatonin treatment. In addition, after 6 hours of *Plasmodium falciparum* infected red blood cells treatment with the hormone, we observed a differential protein ubiquitination profile as indicated by WB and IP assays. Mass spectrometry analysis reveals the identities of protein ubiquitination triggered by melatonin on *P. falciparum*. We concluded that UPS genes are involved in melatonin action through cell cycle of *P. falciparum* parasite.

Key words: *malaria, melatonin, Plasmodium falciparum, ubiquitin*
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