Melatonin inhibits Gram-negative pathogens by targeting citrate synthase

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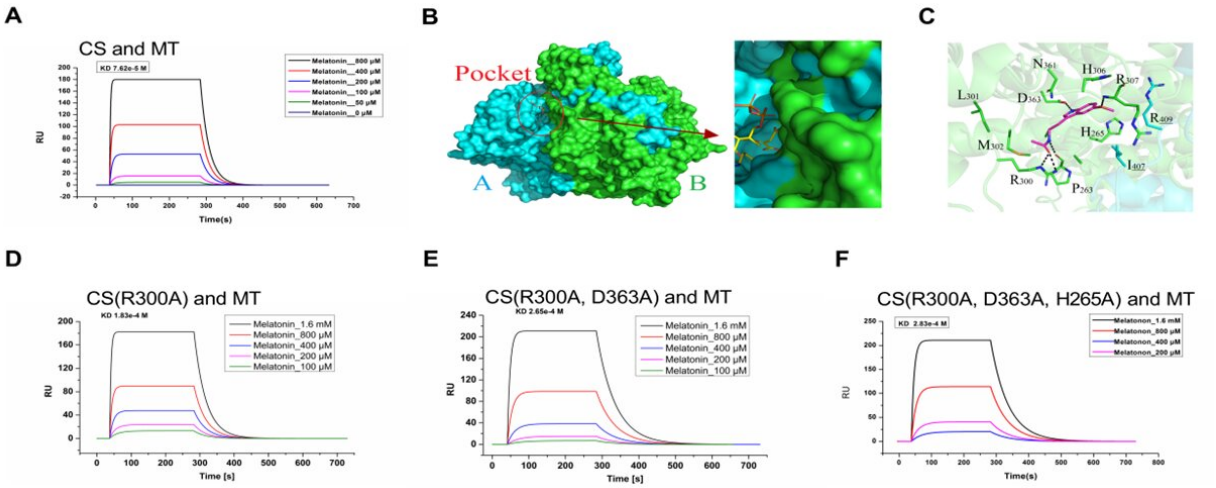

Melatonin targets citrate synthase in P. multocida. A: The concentration gradient binding curves of type II citrate synthase with melatonin. B: The binding pocket of predicted citrate synthase with melatonin and small molecule binding site region. C: Interactions between melatonin and the amino acid residues of the binding sites in type II citrate synthase. D-F: The concentration gradient binding curves of melatonin with type II CS (R300A) (D), type II CS (R300A, D363A) (E) and type II CS (R300A, D363A, H265A) (F). Credit: Science China Press

Infection caused by pathogenic bacteria (especially Gram-negative pathogens), including Escherichia coli, Klebsiella pneumonia, Pasteurella multocida and Pseudomonas aeruginosa, severely threatens public health worldwide. Historically, the discovery and application of

efficacious and safe antibiotics/natural products undoubtedly played an indispensable role in fighting against bacterial infections. However, the overuse and misuse of these antibiotics in medicine and as growth-promoting agents in livestock and poultry farming have irreversibly resulted in the emergence and prevalence of antibiotic resistance (for example, many of Gram-negative pathogens have multidrug resistance features). Alarmingly, plasmid-mediated horizontal transfer of antibiotic resistance genes between intra- and inter-species accelerates the rapid spread of resistance genetic elements. In addition, the residue of antibiotics in environment and animal products also leads to serious environmental pollution and poses a big threat for human health. Therefore, more eco-friendly, safe and effective compounds are urgently required for the control of bacterial infectious diseases.

"We first use the model of P. multocida (one of the important pathogens in mammals) infection to evaluate the efficacy of concomitant administration of melatonin before and after infection for the treatment of bacteremia in mice." said Dr. Fang He, co-first author for this work. The results of this research indicated that melatonin is a promising compound for developing a preventive and therapeutic regimen against P. multocida infection. "This regimen improved survival rate of mice compared with control group, and an approximate 2-log10 reduction of bacterial burden (CFUs/g) was observed at the dose of 120 mg/kg." said He.

"Given that the marked anti-infection effects of melatonin in vivo (especially reducing the bacterial loads in mouse lungs), we next investigated its antibacterial activity." said Professor Yuan Liu, co-first author for this work. The results demonstrated the potential antibacterial efficacy of melatonin against pathogenic bacteria, particularly for P. multocida. "To better elucidate the modes of action of melatonin, we first compared the differentially expressed genes (DEGs) of bacteria in the absence or presence of melatonin by transcriptome analysis." said

Liu. Consequently, a total of 259 DEGs was observed, including 138 up-regulated and 121 down-regulated genes. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis demonstrated that the DEGs were mainly involved membrane transport and bacterial metabolic processes, particularly carbohydrate metabolism. "Considering that the majority of DEGs were clustered in functions of bacterial membrane, we focus on bacterial cell membranes", said Liu. Permeability of bacterial cell membrane, SEM and TEM results showed that melatonin kills bacteria by damaging the bacterial membrane and inducing leakage of intracellular contents.

"Our earlier transcriptome analysis demonstrated that a large number of DEGs were clustered on pathways related to bacterial metabolism. To clarify which metabolic pathway of bacteria is specifically affected by melatonin to achieve its antibacterial activity, we performed non-targeted metabonomics of P. multocida treated with or without melatonin" said Professor Yuanyi Peng, the co-corresponding author. Melatonin treatment resulted in a large number of differential metabolites, and KEGG analysis showed that these metabolites were mainly concentrated in pyruvate metabolism, citrate cycle and tryptophan metabolism. "Interestingly, we found that pyruvic acid and acetyl-CoA in melatonin-treated P. multocida cells were markedly up-regulated, whereas citric acid was significantly down-regulated. This observation implied that the pathway from acetyl-CoA to citric acid might be blocked by melatonin, leading to the accumulation of pyruvic acid and acetyl-CoA. Thus, we focused on citrate synthase, a vital enzyme responsible for the biosynthesis of citric acid.", said Peng. Further experiments indicated that melatonin exhibits antibacterial activity by inhibiting the activity of citrate synthase to reduce the synthesis of citric acid.

"We evaluated whether melatonin has a similar inhibitory effect on different citrate synthases in various bacteria or eukaryotes." said

Professor Nengzhang Li, the co-corresponding author. Experimental results implied that melatonin inhibits the activity of citrate synthase in Gram-negative pathogens rather than Gram-positive pathogens or eukaryotes.

"To further explore the interaction between melatonin and citrate synthase, type II citrate synthase was expressed and purified through the E. coli expression system", said Professor Wenkai Ren, the leading corresponding author. LSPR assay showed that melatonin can directly inhibit type II citrate synthase, but has little effect on type I citrate synthase. Meanwhile, in silico docking and LSPR analysis revealed that the hydrogen bond between R300 in type II citrate synthase and melatonin plays a crucial role in their interaction.

The discovery of melatonin as an antimicrobial agent against Gram-negative pathogens provides a feasible rationale for identifying compounds for other uses to fight pathogenic bacteria. The present findings of melatonin's effects on type II citrate synthase may be regarded as a proof or principle concerning this enzyme as a future target for drugs to be developed, which may be structurally different from an indolic compound. However, more studies are needed to better understand the modes of action of melatonin in diverse organisms, and its preventive and therapeutic effect in vivo. Nevertheless, we believe that melatonin presents a new mechanistic class of antimicrobial agents to address life-threatening infections caused by pathogenic bacteria.

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