

Research Article

Polymethoxyflavones in Citrus Regulate Lipopolysaccharide-Induced Oscillating Decay of Circadian Rhythm Genes by Inhibiting Nlrp3 Expression

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The aim of this study is to compare the regulatory abilities of citrus flavonoids on the oscillating expression of circadian genes. Seven varieties of citrus fruits and twenty-five citrus flavonoids were selected and evaluated. Per2 luciferase bioluminescence report system and serum shock were used to induce circadian gene expression in mouse microglia BV-2 cells. In vivo experiments were carried out using C57BL/6 mice to evaluate the regulation of flavonoids on the oscillatory expression of liver biorhythm genes. Lipopolysaccharide was used to interfere the gene oscillating expression. QRT-PCR was performed to detect the expression of circadian rhythm-related genes, including Clock, Bmal1, Per1, Per2, Per3, Cry1, Cry2, Rev-erb, Rev-erb, Ror, Dbp, and Npas2. The results show that the polymethoxy flavones (PMFs) exerted stronger circadian gene regulatory capability, while the flavonoids containing glycosides showed no biological activity. Also, all tested flavonoids decreased LPS-induced nitric oxide release, but only polymethoxy flavones inhibited circadian rhythm disorder. PMFs inhibited Nlrp3 in ammasome-related genes and proteins, including Nlrp3, IL-1, ASC, and Caspase1, while other flavonoids only affected IL-1 and Caspase1 expression. This mechanism was preliminarily verified using the Nlrp3 inhibitor INF39.

1. Introduction

The circadian rhythm, also called the circadian clock, is the intrinsic rhythm of the life activity of organisms determined by the temporal structure, as well as specific genes and proteins [1]. Homeostasis of the circadian rhythm is vital to the organism. The functional patterns of the circadian genes, such as expression and phase, affect basal metabolism [2], organ function [3], and system homeostasis [4]. The biological clock is in a dynamic equilibrium state, and it can be affected by many factors, such as photoperiod [5], inflammatory factors [6], and nutrient intake [7]. An excessive inflammatory response is a common cause of circadian rhythm disturbance [8]. Studying the effects of environmental factors on the circadian rhythm, especially the interaction of ubiquitous inflammatory factors and natural products with the circadian clock, would contribute to revealing the mechanism

of circadian clock regulation and guide the consumption of natural products.

Natural products are primary or secondary metabolites of animals, plants, or microorganisms. Flavonoids are an important class of natural products, in which the main structure consists of a C6-C3-C6 ring at its core and substituents at different positions. The substituents of flavonoids mainly include hydroxy groups, methoxy groups, and glycosides [9]. Citrus is one of the daily intake sources of flavonoids. Interestingly, the distribution of flavonoids in citrus is tissue-specific and variety-specific. Flavonoids with high methoxy content (usually equal to or above 4 methoxy moieties), also known as polymethoxy flavones (PMFs), are present in the ravedo of citrus fruits [9], which may be related to their lipophilicity. Flavonoids with low methoxy content, such as hesperidin and narirutin, are detectable in the whole citrus fruit [9]. For example, PMFs exhibit stronger cancer inhibition activities than flavanones [10, 11], while the

