Indian Journal of Experimental Biology
Vol. 60, November 2022, pp. 875-879
DOI: 10.56042/ijeb.v60i11.60264

Note

Royal jelly significantly alters inflammasome pathways in patients with chronic hepatitis B

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Received 14 February 2022; revised 17 October 2022

Royal jelly (RJ) plays immunomodulatory role in humans. Further, role played by inflammasomes against hepatitis B virus (HBV) and involvement in its complications are well known. Here, we evaluated the effects of RJ on the relative expression of apoptosis associated with speck-like protein (ASC), node like receptor (NLR) family pyrin domain containing 1 (NLRP1), NLRP3, S100 calcium binding protein A4 (S100A4), and S100A9, as the immune system-related molecules in patients with chronic hepatitis B infection. RJ was administrated for 1 month (@1 g/day), to the patients with chronic hepatitis B infection. The relative expressions of ASC, NLRP1, NLRP3, S100A4 and S100A9 were evaluated using Real-Time PCR. The results showed that RJ increased the expression of ASC, but decreased the expression of NLRP1 in the patients with chronic hepatitis B infection. Relative expressions of NLRP3, S100A4, and S100A9 were not altered following treatment with RJ. There were no significant differences between men and women regarding the relative expression of the molecules. The results suggest that RJ can modulate immune responses via downregulation of NLRP1. The roles played by ASC in other pathways suggest that the upregulation of ASC could be associated with its immunomodulatory potential.

Keywords: *Apis mellifera*, Bee products, Gene expression.

Inflammasomes play key roles in the induction of immunity against viral infections, such as hepatitis B, and their related complications, including liver fibrosis[1]. Among the inflammasomes, node like receptor (NLR) family pyrin domain containing 1 (NLRP1) and NLRP3 are the important intracellular sensors for the viral infections[1,2]. It has been demonstrated that the inflammasomes participate in neither defense against hepatitis B virus (HBV) nor induction of chronic inflammation, a main cause of liver cirrhosis and hepatocellular carcinoma (HCC)[3]. The most known members of inflammasomes, including NLRP1 and NLRP3, use apoptosis associated with speck-like protein (ASC) adaptor protein to activate their main target, caspase-1, as the protease for several proteins, including interleukin-1 beta (IL-1β) and IL-18[3]. Previous investigations have shown the critical roles played by NLRP1 and NLRP3, and their adaptor protein, ASC against HBV and also its related liver complications[4]. Additionally, it has been demonstrated that internal damage associated molecular patterns (DAMPs) are the main ligands for innate immunity receptors, including inflammasomes[5]. S100 calcium binding protein A4 (S100A4) and S100A9 are the main members of DAMPs[6]. It has been reported that the S100 molecule expressions and functions had significant positive correlations with activation of NLRP1 and NLRP3[7]. Additionally, the roles played by S100A4 and S100A9 in the pathogenesis of chronic hepatitis B infections have been documented previously[8,9]. Therefore, the factors that alter expression of the molecules may be considered as a therapeutic strategy against chronic hepatitis B infection.

Royal jelly (RJ), as a natural product from worker bees (*Apis mellifera*)[10], is widely used for dietary supplements[11]. RJ consists of several components, including water, protein, monosaccharides, fatty acids, 10-hydroxy-2-decenoic acid (10-HDA), antibacterial/ antibiotic components and some vitamins[12]. The component has potential antimicrobial, antioxidant and immunomodulatory roles[13-16]. RJ also promotes healthy aging and longevity[12], suppresses cancers[17], epidermal stress[18] and skeletal muscle atrophy[19]. Thus, it has been hypothesized that RJ may be useful to regulate immune responses in patients with chronic hepatitis B. Therefore, in this study, we evaluated the effect of royal jelly (RJ) treatment on the relative expressions of ASC, NLRP1, NLRP3, S100A4 and S100A9 in the CHB patients.

Material and Methods

Subjects

In this study, mRNA levels of ASC, NLRP1, NLRP3, S100A4, and S100A9 were analyzed in 30

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(13 men and 17 women) CHB patients, who were under RJ treatment. The patients were not under anti-HBV therapy and had normal liver enzyme serum levels and low HBV-DNA copy numbers. "Guide of Prevention and Treatment in Viral Hepatitis" was used to select the CHB diagnosis[20]. To eliminate the interference factors, the patients with other microbial co-infection, receiving antiviral and immunosuppressive components, liver disorders, aged 18 to 55 years, pregnant or breastfeeding, and mental disorders were excluded from the project. The project protocol was approved by Kerman University of Medical Sciences Ethical Committee (IR.IAU. Kerman. REC.1400.010) and Iranian Registry of Clinical Trials (IRCT202106-20051640N1).

Blood samples were collected before and one month after RJ treatment in anticoagulant pre-treated tubes. RJ (Pars Asal Company, Shiraz, Iran) was administrated 1 g/day, based on our previous investigation[21].

Evaluation of liver enzymes

The liver enzymes were evaluated using the commercial kits from MAN Company, Tehran, Iran, and based on the manufacturer's guidelines.

Extraction of mRNA

Total mRNA extraction was performed on the blood samples using a commercial kit (Karmania Pars Gene Company, Kerman, Iran). Briefly, the blood samples were lyzed by the lysis buffer and then the precipitation solution was added to the lyzate. After that, the component was added to the high absorbance column and after centrifuge, the column was washed using washing buffer. Finally, elution buffer was added to the column and total mRNA was separated from the column by centrifuge.

Complementary DNA (cDNA) synthesis

To convert the extracted mRNA to cDNA, a commercial kit from Karmania Pars Gene Company, Kerman, Iran, was used. Accordingly, 15 μL master mix was mixed with extracted mRNA (1 μg) and adjusted to 20 μL by RNase/DNase free water. The component was incubated at 40ºC for 3600 S and then 300 S at 70ºC.

Relative expression of ASC, NLRP1, NLRP3, S100A4 and S100A9

Relative expression of ASC (CN# KPG-ASCRT), NLRP1 (CN# KPG-NLR1RT), NLRP3 (CN# KPG-NLR3RT), S100A4 (CN# KPG-S100A4RT), and S100A9 (CN# KPG-S100A9RT) was evaluated using the commercial kits from Karmania Pars Gene Company, Kerman, Iran.

Data analysis and statistical methods

Dependent paired 't' test under SPSS software (version 18) was used for data analysis, mRNA levels of ASC, NLRP1, NLRP3, S100A4 and S100A9 before and after therapy with RJ. *P* value was considered significant at <0.05.

Results

The results demonstrated that all the patients were positive for HBV-DNA and all of them were under 20/000 copy per mL. Liver enzymes were in normal ranges in all of the participants, before and after RJ treatment.

The treatment with RJ led to significant up and downregulation of ASC (*P* value= 0.024) and NLRP1 (*P* value= 0.050), respectively, in the CHB patients. Accordingly, relative expressions of ASC were increased after RJ treatment to 2.20-fold. Relative expressions of NLRP1 were decreased 7.83-fold after RJ treatment. The results demonstrated that relative expressions of NLRP3 (*P* value= 0.349), S100A4 (*P* value= 0.246) and S100A9 (*P* value= 0.280) before RJ treatment were not changed after RJ treatment. Fig. 1 shows the relative expression of ASC, NLRP1, NLRP3, S100A4 and S100A9 before and after RJ treatment.

The results demonstrated that there were no significant differences between men and women regarding mRNA levels of ASC, NLRP1, NLRP3,

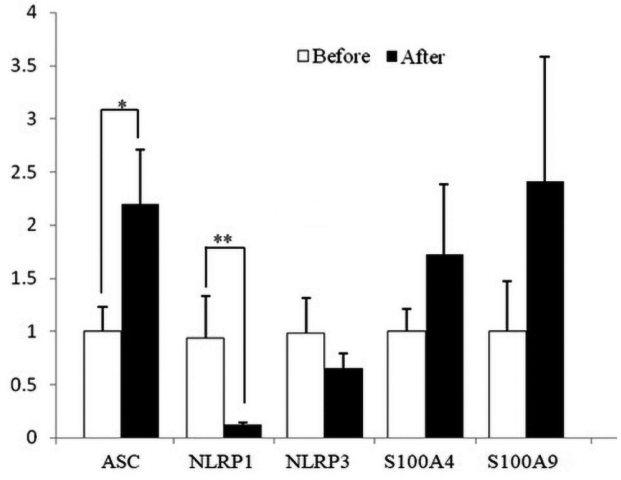

Fig 1 — Relative expression of apoptosis associated with speck-like protein (ASC), node like receptor (NLR) family pyrin domain containing 1 (NLRP1), NLRP3, S100 calcium binding protein A4 (S100A4), and S100A9 before and after treatment with royal jelly in the patients with chronic hepatitis B. [The figure shows that royal jelly therapy led to up regulation of ASC and down-regulation of NLRP1. * P value= 0.05, **P value= 0.025]

Table 1 — Relative expression of ASC, NLRP1, NLRP3, S100A4, and S100A9 in the men in comparison to women

Target gene	ASC B	ASC A	NLRP1 B	NLRP1 A	NLRP3 B	NLRP3 A	S100A4 B	S100A4 A	S100A9 B	S100A9 A
Men	0.89±0.38	1.39±0.50	0.86±0.57	0.12±0.03	0.51±0.36	0.31±0.11	1.70±0.97	3.88±2.32	1.56±0.75	10.28±8.98
Women	1.09±0.30	9.78±6.90	1.02±0.57	0.12±0.03	1.32±1.07	0.39±0.27	0.38±0.12	2.10±1.19	0.49±0.18	12.93±10.98
P value	0.687	0.273	0.850	0.987	0.506	0.801	0.226	0.479	0.209	0.866

[The table compares the relative expression of apoptosis associated with speck-like protein (ASC), node like receptor (NLR) family pyrin domain containing 1 (NLRP1), NLRP3, S100 calcium binding protein A4 (S100A4), and S100A9 in the men in comparison to women at both before (B) and after (A) RJ treatment. The t test revealed that relative expression of ASC, NLRP1, NLRP3, S100A4, and S100A9 were not different when compared men to women either before or after RJ treatment]

S100A4 and S100A9 either before or after RJ treatment. Table 1 illustrates the details of the relative expression of the molecules in the men and women.

Discussion

The results demonstrate that receiving RJ for a period of one month was associated with increased expression of ASC and decreased expression of NLRP1. Previous investigations proved the critical roles played by NLRP1 in the pathogenesis of chronic hepatitis B[4]. Our results show that RJ can modulate expression of NLRP1 and it may be hypothesized that the natural component may confer immunoregulatory effects in the patients with chronic hepatitis B. It has been reported that ASC not only makes a link between the inflammasomes and caspase-1, but also it plays other roles in immune system, including regulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and activation of mitogen-activated protein kinase (MAPK), as well as regulation of the dedicator of cytokinesis 2 (Dock2) mRNA stability[22]. MAPK, and Dock2 pathways play significant roles during immune responses and make immune response more effective against microbes, including viruses. However, it has been reported that RJ can suppress phosphorylation of NF-κB and other related signaling pathways, such as p38 and c-Jun NH2-terminal protein kinase (JNK)[23-26]. Hence, it may be hypothesized that although mRNA levels of ASC were increased following RJ treatment, it may not result in activation of the pathway. However, based on the immunomodulatory effects of RJ, increased expression of ASC may show the immunoregulatory effects of the molecules, except its known pro-inflammatory roles. Thus, it seems that RJ via down-regulation of NLRP1 can modulate the pathogenesis of the molecule during chronic form of hepatitis B and it may be associated with increased functions of other pathways in ASC dependent ones.

Based on our knowledge, this is the first study on RJ treatment in the chronic hepatitis B and on the expression of ASC, NLRP1, NLRP3, S100A4, and S100A9 molecules. Our results revealed that RJ was unable to change expression of NLRP3 as the most known inflammasome, and also S100A4 and S100A9, as DAMPs. However, the increased sample size may be associated with alteration in the expression of these molecules. Further investigation with higher sample size and increased RJ doses may clarify the mechanism associated with alteration in expression of the molecules. In parallel with our results, investigations revealed that RJ can relieve the necrotic hepatocytes via downregulation of tumor necrosis factor (TNF) -α, mixed lineage kinase domain-like protein (MLKL) and intracellular reactive species, the most important agents of hepatic necrosis, and suppresses HBV entry and replication in the hepatocytes[27,28].

The results also demonstrated that relative expression of ASC, NLRP1, NLRP3, S100A4, and S100A9 were not different in the men and women. Our previous investigations also proved the fact that gender did not affect expression of immune related molecules in the patients with chronic hepatitis B[29-31]. Therefore, it appears that gender cannot be considered as an important factor to alter expression of the immune system-related molecules in the Iranian chronic hepatitis B patients.

Conclusion

The above study has demonstrated that the Royal Jelly (RJ) from the worker bees of *Apis mellifera* could modulate immune responses in patients suffering from chronic hepatitis B through downregulation of NLRP1. Due to the roles played by ASC in other pathways, upregulation of the ASC might be associated with immunomodulatory roles. It has also been shown that the gender is not a potential factor for relative expression of ASC, NLRP1, NLRP3, S100A4 and S100A9.

Conflict of Interest

Author declares no competing interests.

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