The effect of melatonin on interleukins 22 and 13 in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass: A Clinical Trial

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Research note

Keywords: Interleukin 13, Interleukin 22, Coronary Artery Bypass Graft (CABG)

Posted Date: September 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-57283/v1

Abstract

Objective: Coronary artery bypass graft surgery with cardiopulmonary bypass (CABG) is one treatment for patients with coronary artery disease. After CABG, a series of inflammatory processes occur which affect other organs of the body and even cause severe organ damage and subsequently a worse prognosis. The purpose of this study was to evaluate the effect of pre-surgically administered melatonin on interleukins 22 and 13 in patients undergoing CABG.

Results: In this study, 22 patients were evaluated, 10 patients (45.5%) in the Melatonin group, and 12 patients (54.5%) in the control group. The mean level of IL-22 at all time-points in T2, T3, and T4 was significantly lower in the Melatonin group ($P < 0.05$). There was also a significant increase in IL-22 in both groups at T4 compared to T1. There was no significant difference between the two groups in IL-13 at any time, but the Melatonin group at T2 had a significant decrease in IL-13 compared to T1. This study showed that preoperative melatonin administration could prevent the increase of IL-22 but not IL-13.

Introduction

After Coronary artery bypass graft surgery (CABG), a series of inflammatory processes occur and these stressors affect other organs of the body and even cause severe organ damage and subsequently a worse prognosis (1).

Interleukins are play an important role in the immune system and inflammation. The role of interleukin-13 is to stimulate the growth and differentiation of B lymphocytes, inhibition of T1 helper lymphocyte (TH1) production, and inflammatory macrophage cytokine production. Interleukin-22 also plays an important role in the production of acute-phase proteins (2). Studies have shown that these interleukins increase after coronary artery bypass graft surgery and play a key role in systemic inflammation, immune response, and post-operative tissue damage (3).

There have been many studies about the beneficial effects of melatonin on the cardiovascular system. Melatonin has a protective effect against ischemia by reducing sympathetic tone and rhythmic changes in heart rate, blood pressure, and cardiac output. Melatonin also increases collagen in myocardial ischemia-induced scarring through its direct effect on fibroblasts (4, 5). Melatonin may play a role in decrease ventricular hypertrophy and preventing heart failure (6). Melatonin supplementation ameliorated the amount of myocardial ischemic-reperfusion injury (7).

Melatonin acts as an antioxidant and anti-inflammatory in the body and increases defense immune system mechanisms (8). Melatonin can be used as an anti-inflammatory drug to boost the immune system (9) since inflammatory processes and oxidative stress are produced after cardiopulmonary bypass (CBP), and because the melatonin is safe (10), it is a choice antioxidant to reduce these complications (11).

It is shown that IL-22 was induced in cardiac surgery with CPB and it may interactively contribute to systemic inflammatory responses after CPB (12). In a study, it is determined that IL-13 may be involved in the development of coronary artery disease via different mechanisms under different conditions (13).

Thus, if we can reduce the inflammatory processes caused interleukins 13 and 22 by melatonin, we can decrease the complications after cardiopulmonary bypass and coronary artery bypass graft surgery. Since there are practically a few methods to control the complications after coronary artery bypass graft surgery and little research have been done in this regard, it seems necessary to conduct this study. This study aims to evaluate the effect of pre-surgically administered melatonin on interleukins 22 and 13 in patients undergoing CABG.

Materials And Methods

This clinical trial (RCT: 20141009019470N83) study was performed in Namazi Hospital of Shiraz university of medical science, shiraz, Iran. The study population was 20 patients undergoing elective coronary artery bypass graft surgery.

The inclusion criteria were age between 40 and 75 years and having consent to enter the study. The exclusion criteria were included kidney failure, pulmonary disease, cerebrovascular disease, and history of stroke, using the immunosuppressant drug, mental illness, smoking more than 5 packages per year, emergency surgery, pregnancy or lactation, anticoagulation, concomitant cardiac surgery, non-sinus rhythm in electrocardiogram (ECG), and ejection fraction less than 45%. Patients were randomly and using a random number table divided into two groups after signing the written consent.

Two weeks before surgery, patients were admitted to the hospital and the necessary explanations were given by the resident. Then 5 cc of blood (T1) was taken to measure serum levels of IL-13 and IL-22 and sent to the laboratory and stored at -70 ° C. The experimental group was asked to take 10 mg of melatonin 1 hour before bedtime and placebo (5% dextrose dissolved in water) was given to the control group (7, 14). After 2 weeks of taking melatonin and placebo, the second sample was taken on the surgery day before the induction of anesthesia (T2). Also, the third sample (T3) 6 hours after removal of the pump by the cardiac surgeon (T3) and the fourth sample 2 days after surgery (T4). All samples were stored in the laboratory refrigerator immediately after blood sampling and in the same condition and analyzed simultaneously.

IL-13 and IL-22 levels were measured using the Human Th cytokine panel (13 plex) BioLegend USA kit, using a Bead Based assay.

Statistical Analysis

SPSS software version 19 was used for data analysis. Descriptive statistics and inferential statistics were used for analysis. Kolmogorov Smirnov test was used to check for normality. Student t-test, U Mann-Whitney, chi-square, and repeated measure tests were used to compare the variables between the two groups. Amounts were significant at $P < 0.05$.

Results

In this study, 22 patients were evaluated, 10 patients (45.5%) in the melatonin group, and 12 patients (54.5%) in the control group. Appendix 1 shows the CONSORT diagram (Figure S1).

The mean age of patients was 60.22 ± 6.36 years. Table 1 shows the demographic features. The mean serum levels of IL-22 and IL-13 were similar between the two groups before intervention. Serum IL-22 levels in second (T2), third (T3), and forth (T4) samples were significantly different between the two groups (Table 2). The serum levels of IL-22 were significantly decreased in the melatonin group after 2 weeks (Table 3).

Table 1
Demographic information of patients

Parameters	Melatonin n = 10	Control n = 12	P-value
Age, y (mean ± SD)	61.20 ± 8.02	59.41 ± 4.79	0.52
Sex			0.37
Male	5 (50%)	9 (75%)	
Female	5 (50%)	3 (25%)	

Table 2
Mean IL-22 levels in both groups

IL-22 (ng/l)	Melatonin	Control	P-value
IL-22 (ng/l)			
IL-22 (T1)	381.57 ± 33.81	379.37 ± 15.88	0.84
IL-22 (T2)	364.31 ± 16.97	387.73 ± 22.98	0.01
IL-22 (T3)	408.92 ± 13.87	434.53 ± 17.04	0.001
IL-22 (T4)	402.12 ± 17.10	418.82 ± 19.04	0.04
P value*	0.03	0.001	
IL-13 (ng/l)			
IL-13 (T1)	14.25 ± 6.55	11.41 ± 7.25	0.35
IL-13 (T2)	10.84 ± 5.95	10.89 ± 6.50	0.98
IL-13 (T3)	15.03 ± 6.13	17.02 ± 11.28	0.64
IL-13 (T4)	12.96 ± 4.13	15.06 ± 7.49	0.43
P value*	0.12	0.05	
*Repeated measure test result			

Table 3
The mean difference between the different IL-22 and IL-13 measurement steps

		T2		T3		T4	
		Melatonin	Control	Melatonin	Control	Melatonin	Control
IL-22							
T1	Mean difference	-17.26	8.35	27.34	55.15	20.55	39.44
	P-value	0.78	0.40	0.05	< 0.001	0.04	< 0.001
T2	Mean difference			44.60	46.80	37.81	31.09
	P-value			< 0.001	< 0.001	< 0.001	0.001
T3	Mean difference					-6.79	-15.70
	P-value					0.37	0.07
IL-13							
T1	Mean difference	-3.41	-0.51	0.75	5.06	-1.29	3.65
	P-value	0.01	0.83	0.75	0.18	0.24	0.06
T2	Mean difference			4.54	5.76	2.12	4.17
	P-value			0.06	0.04	0.06	0.09
T3	Mean difference					-2.02	-1.14
	P-value					0.31	0.77

According to the results in Table 2, no significant difference was observed in serum levels of IL-13 between the two groups in all 4 steps.

Like IL-22 after 2 weeks of melatonin administration, serum IL-13 levels also decreased significantly. The trend of IL-13 changes is shown in Table 3.

Discussion

In the present study, the two groups at the beginning of the study were matched for serum levels of interleukins 13 and 22 to compare the two groups correctly. On the day of surgery and before induction, serum IL-22 levels were significantly decreased in the Melatonin group, whereas in the control group serum IL-22 levels were not significantly changed. Given that they had taken melatonin 2 weeks before surgery, the decrease in IL-22 was justified in this group. Also in the control group, no change in IL-22 was observed. There was also a significant difference between the two groups at this stage and the level of IL-22 in the Melatonin group was significantly lower.

In the third measurement step (T3), 6 hours after the pump was removed by the cardiac surgeon, both groups showed an increase in serum IL-22 levels, which was significant in relation to T2 in both groups and T1 only in the Melatonin group.

In the fourth measurement, serum levels of IL-22 did not change significantly in the two groups compared to the third stage, but compared to the T1 stage, both groups showed a significant increase in T4. It can be argued that the acute

phase of IL-22 occurs at T3 and reaches its maximum level after which its level has decreased but its decrease has not been significant. Inter-group differences in the T4 level of IL-22 were also significantly higher in the control group than in the Melatonin group. Given that patients in the Melatonin group at the T3 stage also had lower IL-22, this is justified because no significant change in T4 over T3 was observed in either group.

In the present study, serum levels of IL-13 at T1 were similar between the two groups. But unlike IL-22, this cytokine did not show a significant difference between the two groups at all stages of T2 to T4. In the intra-group analysis in this study, IL-13 at T2 compared to T1 in the Melatonin group showed a decrease in IL-13 level, which was significant. But no significant changes were observed in the control group. A non-significant increase in IL-13 serum level was observed in T3 compared to T2 in both groups, which is justified since it is after CABG. There was a decrease in IL-13 in both groups at T4 compared to T3, which was not significant in either group.

It has been shown that the positive effects of melatonin in ischemia-reperfusion damages are related to the activation of the Nrf2 pathway. Nrf2 is a transcription factor. It plays its antioxidant role by binding to DNA antioxidant response element (ARE) and the Nrf2-ARE pathway has an important protective effect in ischemia-reperfusion injury (15). Nrf2 suppresses IL-13 and IL-22 secretion (16, 17). IL-13 and IL-22, upregulate the STAT3 pathway and cause inflammation (18, 19). By the decrease of IL-13 and IL-22, STAT3 is downregulated and prevent inflammation.

These changes in serum levels of cytokines after cardiac surgery have also been shown in other studies. Brull's study showed that 6 hours after CABG, serum IL-6 levels peaked. In that study, IL-6 levels reached 45 times their basal level, 6 hours after CABG (20). In the present study, as in the Brull study, peak levels of both IL-22 and IL-13 were observed 6 h after CABG. In the study of Czerny et al., patients with CABG had the highest serum IL-6 and IL-10 levels at the fourth hour and eighth hours after CABG, respectively (21). Nathan et al. evaluated the serum levels of IL-10, IL-4, and IL-13 in patients who had cardiopulmonary bypass (CPB). In that study, IL-10 peaked at the sixth hour after CPB. However, IL-13 peaked after 24 hours of CPB completion (22). In the Sablotzki study, serum levels of IL-10 were highest in CPB patients at the skin closure stage after CPB completion. However, there was a significant decrease of 6 h after CPB (23). The findings of these two studies are not in line with ours. The main reason for this difference may be the difference in the study population and the type of surgery. A study by Kawamura et al. investigated the changes in IL-10, IL-8, and IL-6 cytokines after aortic declamping. The results of this study showed that the peak of all three interleukins occurs 3 hours after aortic declamping (24). Hsing et al. showed that levels of IL-22 and IL-19 peaked after 8 hours of CPB (12). In the Wan study, it has been reported that the elevation of IL-10 begins 2 hours after CABG (25). The pattern of IL-10 changes in this study is similar to the pattern of IL-22 changes in our control group. IL-22 is functionally similar to IL-10 in that the two cytokines are in the same group (12), so the trend of changes in these two cytokines in the present study and the Wan study is justified. In Struber et al.'s study, serum IL-8 and IL-6 levels peaked at the eighth hour after CABG. C1-INH, TNF-R1, and TNF-R2 cytokines were also measured in this study, all of which increased after CABG surgery and peaked within 2 to 8 hours after surgery (26). Surgery causes a range of metabolic, endocrine, and immune alterations (27). The inflammatory response in cardiac surgery is made by complex interactions with several pathways such as production or activation of complement, neutrophils, thrombin, cytokines, mast cells, and some other multiple inflammatory mediators (28). Mechanisms such as exposure of blood to nonphysiologic surfaces, anesthesia, trauma, body temperature alterations, and ischemia or reperfusion injury may be responsible for these pathological effects (29), which results in immunologic reactions and release proinflammatory cytokines, arachidonic acid metabolites, platelet-activating factors, endothelins, endothelial, and leukocyte adhesion molecules which induce the overproduction of reactive oxygen species (30, 31).

During the inflammatory process, the stimulation of inflammation-related genes can happen as a result of activation of the nuclear transcription factor-kappa B (NF-κB) (32). Many studies have revealed that melatonin modulates the NF-κB signaling pathway throughout inflammation (33–35). Reports recommend that melatonin performs its anti-inflammatory effects by modulating both pro- and anti-inflammatory cytokines in various pathophysiological situations (36, 37). It was displayed that the presence of melatonin’s receptors in a mast cell line by inhibiting the release of TNF-α modulates an

anti-inflammatory pathway (38). Other anti-inflammatory activities of melatonin are including prevention from the synthesis of prostaglandins, production of adhesion molecules (39, 40), and downregulation of cyclooxygenase 2 expressions in macrophages (41), and the decrease of the polymorphonuclear cell recruitment to the inflammation location (39, 42). Melatonin also counteracts inflammatory processes by scavenging free radicals, which contributes to inflammation (43–45).

Conclusion

Finally, this study found that preoperative melatonin administration could prevent the increase of IL-22 but not IL-13.

Limitation

The small number of patients is a limitation of this study.

Abbreviations

CABG: Coronary artery bypass graft surgery

LV: Left Ventricular

ECG: electrocardiogram

ASA: American Society of Anesthesiologists

Declarations

Acknowledgments

There is no acknowledgment for the present study.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study is approved by the ethics committee of Shiraz University of Medical Sciences with reference number IR.SUMS.MED.REC.1396.17. Written consent was obtained from participants. The study is performed according to the ethics principals of the Declaration of Helsinki.

Funding

There is no funding for the present study.

Competing Interest

There are no conflicts of interest for the present study.

Consent for publication

Not applicable.

Authors' Contributions

MJ designed and supervised the study, RJ and MJ did the field and laboratory activities. Also, RJ and SK wrote the draft of the manuscript and MJ finalized the Draft. SK analyzed the data. All authors read and approved the final manuscript.

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