Research Article

Immunomodulatory effect of melatonin supplementation in experimental diabetes

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Abstract

Aim: To investigate the effect of melatonin on the immunomodulatory response in experimental type 1 and 2 diabetes mellitus.

Methods: Experiments were performed on male rats (180–200 g), purchased from the Experimental Animal Holding,. Animals were maintained in standard diet conditions. Two pathological states were simulated on male rats: experimental type 1 and type 2 diabetes. Melatonin was introduced from 14 to 23 days of experiment intraperitoneally. Levels of immunoglobulin classes A, M and G (Ig A, M, G), circulating immune complexes (CIC), interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF-a) were measured.

Results: We demonstrated that melatonin in case of immune hyperactivity, can, provide a suppressive effect and is able to enhance immune reactivity under conditions of its limitation, indicating the immunostimulating activity. Furthermore, we found that administration of melatonin decreased inflammatory responses by mediating the levels of immunomodulatory factors, including TNF-α, IL-1β and IL-6.

Conclusion: Melatonin is a positive regulator of immune system, may be a potential therapeutic agent, it has no reported side effects.

Keywords

experimental diabetes, melatonin, immune system

Introduction

Diabetes mellitus (DM) as medical and social problem does not lose its relevance due to the lability of its course, the complexity of compensation, the early increased risk of complications that leads to the disability of patients (American Diabetes Association 2018.

The autoimmune processes play an important role in the development of the disease and its complications, however, regarding the role of the cellular and humoral parts of the immune system in the etiology and pathogenesis of diabetes, there are rather contradictory facts (Siraj et al. 2018).

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease with progressive destruction of the insulin-producing beta cells in the islets of Langerhans. The result of this process is the loss of tolerance to insulin. T1DM is basically a T-cell-mediated autoimmune response, the development of this disease results from complex interactions between the adaptive and inherent immune systems (Burrack et al. 2017).

Further research established that components of the immune system are also modified in type 2 diabetes mellitus (T2DM) with the most apparent changes occurring in adipose tissue, the liver, pancreatic islets, and these changes include altered levels of specific chemokines and cytokines, increased apoptosis and tissue fibrosis, therefore, all these changes suggest that inflammation participates in the pathogenesis of this disease (Donath and Shoelson 2011).

Melatonin (MT) (N-acetyl-5-metoxytryptamine) – epiphyseal hormone, which is synthesized by the pineal

gland from tryptophan, and an intermediate of its synthesis is serotonin (Munoz et al. 2009).

The effects of MT are realized through specific membrane (MT1 (MTNR1A) and MT2 (MTNR1B)) and nuclear (RZRα, RORα, RORα2 and RZRβ) receptors, which are present in many internal organs, including the liver (Pozo et al. 2004).

MT is one of the most powerful endogenic antioxidant. This hormone is able to bind free radicals and stimulate the enzyme activity of antioxidant system such as superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase. That is why the original and primary function of MT is as a free radical scavenger and antioxidant (Tan et al. 2015).

It is known, that melatonin is involved in the regulation of the immune system because of its essential role in the prevention of exacerbated immune responses (Srinivasan et al. 2005; Carrillo-Vico et al. 2013). MT reacts with peroxynitrite and nitric oxide to reduced toxicity to cells. This regulation has relevance to gene expression (Aydogan et al. 2006). Low melatonin level is associated with immune dysfunction.

Therefore the aim of investigation is to study the effect of melatonin on the parameters of immunological reactivity such as the cytokine profile and humoral immunity in animals with experimental type 1 and 2 diabetes mellitus.

Material and methods

Forty-two male Sprague-Dawley rats 8–10-week-old, 180–220 g weight were purchased from the Experimental Animal Holding. They were placed in standard diet conditions and given *ad libitum* access to water. All the animals received care in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985). Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal committee (Minutes No 40 dated 15 March 2017). There were no violations of ethical guidelines during carrying out this research.

Two pathological states were simulated on male rats: experimental type 1 and type 2 diabetes. Modeling of type 1 diabetes was performed by one intraperitoneally injection of streptozotocin (STZ) («Sigma») at dose 50 mg / kg, type 2 diabetes in rats was performed by subcutaneous administration of dexamethasone solution («KRKA», Slovenia) at a dose of 0.125 mg / kg during 13 days (Barbera et al. 2001). Melatonin was introduced in dose (10 mg / kg) from 14 to 23 days of experiment intraperitoneally. Rats were randomly divided into 5 groups: control group (CON), model group 1 (DM1), model group 2 (DM2), model group 3 (DM1 + MT), and model group 4 (DM2 + MT); control group received an appropriate volume of isotonic solution, DM1 – experimental type 1 diabetes, DM2 – experimental type 2 diabetes, DM1 + MT – experimental type 1 diabetes plus melatonin and DM2 + MT – experimental type 2 diabetes plus melatonin.

The animals were starved for 12 h before experiments. Blood was collected from heart after sodium thiopental anesthesia during the day after the last injection; plasma was separated. Levels of immunoglobulin classes A, M and G (Ig A, Ig M, Ig G), circulating immune complexes (CIC), interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF-a) were measured by using ELISA method (Uscn, Life Science Inc), a standard set of reagents adapted for rats commercially available diagnostic kits according to the instruction.

Statistical analysis

Normality testing was conducted with the Kolmogorov-Smirnov test. Significant differences in values between independent quantitative values were determined in the normal distribution according to Student's t-test, in other cases – using Mann-Whitney U-test. All statistical calculations were performed with Statistica v. 10.0 and Excel for Windows-2010. Differences were considered significant if the probability of Type I error was less than 0.05. The data are presented as means (M) ± standard deviation (SD) unless indicated otherwise.

Results

The formation of experimental type 1 and type 2 diabetes was confirmed by increasing the levels of blood glucose in 4.7 times and 1.7 times, glycated hemoglobin levels – 1.5 and 1.4 times respectively in comparison with those of control group.

According to the results presented in Table 1, DM1 group has been complicated by an increase in the levels of the immunoglobulins A, M, G and circulating immune complexes, the level of Ig A increased by 43.3%, and the level of Ig M by 55.7% also, Ig G levels increased by 32% in comparison with CON group of animals. Thus, this data demonstrated that chronic hyperglycemia is the basis for the development of inflammatory response , that was probably the impetus for the enhanced production of immunoglobulins.

It is also important to note that there is a reciprocated relationship, with the increase in the level of immunoglobulins in blood serum of animals with DM1 group and the content of circulating immune complexes, which was also significantly higher by 28.5% in comparison with CON group of animals. This result demonstrated that to maintain immune homeostasis, both antigens and autoantigens are neutralized by binding to immunoglobulins to form CICs and subsequent excretion.

Overall, our data suggests that an impairment in the immune system of the body is due to the development of autoimmune reactions to the damage of β-cells of Langerhans pancreatic islets, which leads to suppression of insulin synthesis in case of DM1.

Dexamethasone diabetes unlike STZ-diabetes, was proceeded by the suppressed protective mechanisms with reduced the overall immunoreactivity of the body. According

Table 1. Changes in the immunological state of blood in rats with diabetes mellitus type 1 and after the administration of melatonin (M ± m, n = 7).

Indicator	Group		
	CON	DM1	DM1 + MT
Ig A, g/l	1.26 ± 0.04	1.80 ± 0.09 p<0.001	1.53 ± 0.07 p_1 < 0.05
Ig M, g/l	2.42 ± 0.09	3.77 ± 0.22 p < 0.001	2.55 ± 0.08 p_1 < 0.001
Ig G, g/l	5.84 ± 0.28	7.71 ± 0.12 p < 0.001	6.27 ± 0.11 p_1 < 0.001
CIC, conventional units	129.34 ± 2.09	166.23 ± 2.66 p < 0.001	146.55 ± 1.87 p_1 < 0.001

p – the probability of differences regarding the control group of animals; p_1 – the probability of differences in the group of animals with diabetes. CON – control group; DM1 – diabetes mellitus type 1; DM1 + MT – diabetes mellitus type 1 plus melatonin; Ig A – immunoglobulin A; Ig M – immunoglobulin M; Ig G – immunoglobulin G; CIC – circulating immune complex; g/l – gram per liter.

Table 2. Changes in the immunological state of blood in rats with diabetes mellitus type 2 and after the administration of melatonin (M ± m, n = 7).

Indicator	Group		
	CON	DM2	DM2 + MT
Ig A, g/l	2.28 ± 0.10	1.71 ± 0.06 p < 0.001	1.90 ± 0.04 p_1 < 0.01
Ig M, g/l	2.95 ± 0.06	2.57 ± 0.08 p < 0.01	2.69 ± 0.07 p_1 < 0.05
Ig G, g/l	4.30 ± 0.08	3.56 ± 0.08 p < 0.001	3.97 ± 0.06 p_1 < 0.01
CIC, conventional units	147.06 ± 5.99	114.16 ± 3.55 p < 0.01	123.83 ± 1.54 p_1 < 0.01

p – the probability of differences regarding the control group of animals; p1 – the probability of differences in the group of animals with diabetes. CON – control group; DM2 – diabetes mellitus type 2; DM2 + MT – diabetes mellitus type 2 plus melatonin; Ig A – immunoglobulin A; Ig M – immunoglobulin M; Ig G – immunoglobulin G; CIC – circulating immune complex; g/l – gram per liter.

to the results of research presented in Table 2, levels of Ig A decreased in 1.3 times, Ig M – 1.15 times, Ig G – 1.21 times and CIC – 1.18 times in animals with modeled dexamethasone diabetes. It demonstrates the inhibition of protective mechanisms and reduction of the total immunoreactivity.

Daily injections of melatonin to animals with experimental DM1 decreased the concentration of immunoglobulins in the serum, such as IgA by 14.8%, Ig M – 32.3%, Ig G by 18.7%, and the level of the CIC by 11.8%. The effect of MT on the immune system is presented in Fig. 1.

Our study has shown that after 10 days of melatonin supplementation, increased the concentration of immunoglobulins in the blood serum, namely Ig A by 16.7%, Ig M by 8.7%, Ig G by 7.7%, and the level of CIC by 8.6% in the experimental DM2 group animals. Melatonin administration might beneficial to the activation of the humoral immune response.
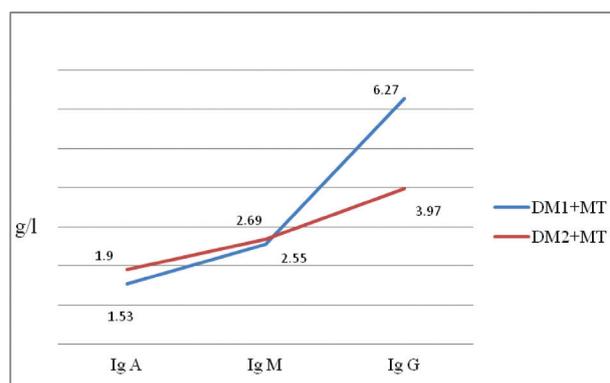
Figure 1. Immunoglobulins A, M, G data diversity at different diabetes models with melatonin introduction. DM1 + MT – diabetes mellitus type 1 plus melatonin; DM2 + MT – diabetes mellitus type 2 plus melatonin; Ig A – immunoglobulin A; Ig M – immunoglobulin M; Ig G – immunoglobulin G; g/l – gram per liter.

Table 3. Changes in the cytokine profile of blood in rats with diabetes mellitus type 1 and after the administration of melatonin (M ± m, n = 7).

Indicator	Group		
	CON	DM1	DM1 + MT
IL-1β, pg / ml	5.07 ± 0.38	25.5 ± 0.80 p < 0.001	14.46 ± 0.79 p_1 < 0.001
IL-6, pg / ml	5.22 ± 0.52	23.98 ± 1.03 p < 0.001	18.56 ± 1.18 p_1 < 0.01
TNF-α, pg / ml	5.73 ± 0.25	29.76 ± 1.40 p < 0.001	24.97 ± 1.25 p_1 < 0.05

p – the probability of differences regarding the control group of animals; p1 – the probability of differences in the group of animals with diabetes. CON – control group; DM1 – diabetes mellitus type 1; DM1 + MT – diabetes mellitus type 1 plus melatonin; IL-1β – interleukin 1β; IL-6 – interleukin 6; TNF-α – tumor necrosis factor; pg / ml – picogram per mililiter.

As expected, content of proinflammatory mediators such as TNF-α, IL-1β, IL-6 were obviously increased in blood serum of animals with both experimental DM1 and DM2.

The data presented in Table 3 indicated that the content of proinflammatory TNF-α increased by 5.2-fold, the concentration of other studied cytokines, namely IL-1β and IL-6 in blood serum, were significantly higher in 5 times and 4.6 fold, respectively, in animals with experimental DM1 compared to the control group.

According to the results of the study, the content of TNF-α increased in 4.4 times in animals with experimental DM2 and also the concentration of other studied cytokines, such as IL-1β and IL-6 in blood serum, was significantly higher in 4.8 times and 4.2 times respectively, compared to the control group (Table 4).

Melatonin treatment significantly inhibited the production and expression of proinflammatory cytokines TNF-α, IL-1β and IL-6 in blood serum of animals with both experimental DM1 and DM2. Overall MT demonstrated the anti-inflammatory activity via the reduction of the proinflammatory cytokines.

Table 4. Changes in the cytokine profile of blood in rats with diabetes mellitus type 2 and after the administration of melatonin (M ± m, n = 7).

Indicator	Group		
	CON	DM2	DM2 + MT
IL-1β, pg / ml	4.64 ± 0.43	22.26 ± 0.84	15.39 ± 0.9
		$p < 0.001$	$p_1 < 0.001$
IL-6, pg / ml	4.94 ± 0.31	21.67 ± 1.63	17.17 ± 1.01
		$p < 0.001$	$p_1 < 0.05$
TNF-α, pg / ml	5.81 ± 0.31	24.16 ± 1.60	19.31 ± 1.33
		$p < 0.001$	$p_1 < 0.05$

p – the probability of differences regarding the control group of animals; p1 – the probability of differences in the group of animals with diabetes. CON – control group; DM2 – diabetes mellitus type 2; DM2 + MT – diabetes mellitus type 2 plus melatonin; IL-1β – interleukin 1β; IL-6 – interleukin 6; TNF-α – tumor necrosis factor; pg / ml – picogram per mililiter.

Discussion

DM characterized by immune dysfunction, manifested as chronic pro-inflammatory state, in response to metabolic and hemodynamic disorders, it triggers activation of endothelial, immune cells and releases a large variety of cytokines (IL-1β, IL-6, and TNF-α), which further exacerbate the inflammatory response (Verhulst et al. 2019).

Therefore, immune dysfunction itself can also be one of the causes of DM, such as DM type 1 is a form of diabetes that is caused by autoimmune destruction of pancreatic β cells (Zheng et al. 2017), DM type 2 is characterized by a progressive status of chronic, low-grade inflammation which is attributable to an autoimmune phenomenon (De Candia et al. 2019).

A recent study showed that melatonin has different immune functions in various physiological and pathological processes, displayed anti-inflammatory effects by modulating the balance between pro-inflammatory and anti-inflammatory cytokines (Hu and Li 2019). We found the protective role of melatonin which is associated with immunomodulation, the ability of melatonin to stimulate the synthesis of certain cytokines, and suppress the production of TNF-α, IL-1 β, IL-6.

MT as a potent antioxidant has shown appropriate immune regulatory properties, the beneficial effect is stimulation or suppression of some immune cell responses (Najafi et al. 2017).

Our studies demonstrated that an increase in the levels of the immunoglobulins is one of the most common features of DM type1, at the same time, the growth of IgA may indicate the development of autoimmune disease, Ig M – a defeat of connective tissue and Ig G – the immune response of the body to the inflammatory processes (Arazi and Neumann 2010).

We also demonstrated a significant increase of circulating immune complexes that was observed in blood serum of animals with DM1 group that exhibit functional and structural abnormalities is due to the development of autoimmune reactions to the damage of β-cells of Langerhans pancreatic islets.

Despite the fact that melatonin is classically associated with the regulation of circadian rhythms following its night-time release by the pineal gland it influences on the regulation of the immune system through specific receptors (Pfeffer et al. 2017). Both membrane and nuclear melatonin receptors have been identified on leukocytes (Pandi-Perumal et al. 2008). However, MT can be released by different cell types, including natural killer cells, astrocytes, eosinophilic leukocytes, platelets, mast cells, epithelial cells, endothelial cells, and bone marrow cells (Anderson and Maes 2014; Paltsev et al. 2016). This indicates a potential paracrine and intracrine role of MT on immune regulation.

Importantly, we found that treatment with MT decreases the concentration of immunoglobulins and the level of the CIC suggesting that it can counteract activation of cell apoptosis and regulates the proliferative response.

The present study also examined the effect of melatonin on the suppression of defense mechanisms and the reduction of overall immunoreactivity in DM type 2. Melatonin has shown appropriate immune regulatory properties by increasing the concentration of immunoglobulins and the level of the CIC. MT, having antioxidant action, through nuclear receptors (ROR, RZR, etc.) can indirectly affect the immune system because these receptors are known for its free radical scavenging actions and might be indirectly controlling the immune function (Haldar and Ahmad 2010), it is also important to consider the metabolic properties of the protective effect of melatonin, these data were published in our previous study (Oleshchuk et al. 2019).

The systemic increase in the level of proinflammatory cytokines in diabetes mellitus and their role in the formation of metabolic changes indicate that this pathology is a chronic inflammatory process and pro-inflammatory cytokines play a significant role in the loss of beta-cells of insulin-producing function, they are also involved in the formation of insulin resistance of peripheral tissues (Greenberg and McDaniel 2002).

A recent study reports that the immunomodulatory effect of the MT may be mediated by specific receptors MT1, MT2 and MT3 on the function of lymphoid cells and cellular elements of the blood. (Singh et al. 2019). Another study (Srinivasan et al. 2008) showed that melatonin not only stimulates the production of natural killer cells, monocytes and leukocytes, but also alters the balance of relevant cytokines, induces the synthesis of interferon.

One of the immunomodulatory factors of melatonin which involved in immune responses is the anti-inflammatory effect through regulation of cytokine-related inflammation (Mauriz et al. 2013). The results of our study have demonstrated that the administration of melatonin decreased inflammatory response by mediating the levels of immunomodulatory factors, including TNF-α, IL-1β and IL-6 in blood serum of animals with both experimental DM1 and DM2.

Although MT acted as a prototype anti-inflammatory compound, reducing exacerbations, but we can see opposite effects in immunosuppressive type 2 diabetes, which causes compensatory response.. These findings open up a new field of diabetes research that may lead to

the use of melatonin as a treatment, the greatest advantage of it, is that it has no reported side effects and could be used for treating various diseases.

Importantly, the results of our research suggest that the use of melatonin for correction of the simulated pathological process leads to a significant improvement of all investigated parameters of immunological reactivity in comparison with similar in the group of animals with diabetes. Through direct intervention in the function of immunocompetent cells, or indirectly, melatonin is able to enhance immune reactivity under conditions of its limitation, indicating the immunostimulating activity, in case of immune hyperactivity, it can, provide a suppressive effect.

In conclusion, our studies have shown the immunoregulatory activity of melatonin in animals with experimental models of type 1 and type 2 diabetes. It is the result of melatonin effects through specific receptors by modulating the proliferative response of stimulated lymphocytes and also studies demonstrated an immunoenchancing activity via the nuclear receptor. To summarize, melatonin is a positive regulator of immune system and is a potential substance for the treatment of proinflammatory disorders according to it anti-inflammatory effect by inhibiting the immune response and reducing the production of proinflammatory cytokines.

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