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Neu-P11 — a novel melatonin receptor agonist, could improve the features of type-2 diabetes mellitus in rats

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Abstract

Introduction: Melatonin (Mel) and its receptors are promising for glycaemic control in patients with type 2 diabetes mellitus (T2DM) and its complications, but there is significant heterogeneity among studies. This study aims to investigate the effects of Mel receptor agonist Neu-P11 on glucose metabolism, immunity, and islet function in T2DM rats.

Material and methods: In this study, SD rats were treated with a high-fat diet and streptozotocin (STZ) to establish a T2DM model. The glucose oxidase method was used to measure blood glucose levels. Glucose and insulin tolerance tests were used to assess glucose metabolism. Haematoxylin-eosin staining was used to observe pancreatic tissue injury. The apoptosis of islet β cells was analysed by TUNEL and insulin staining. Reactive oxygen species (ROS) levels and immune cell expression were analysed by flow cytometry. IF was used to analyse the activation of microglia. The immunoglobulins: IgA, IgG, IgM, tumour necrosis factor α (TNF-α), interleukins IL-10 and IL-1β, interferon γ (IFN-γ), C-peptide, and insulin levels were determined by ELISA. The expression of CD11b, CD86, cleaved caspase 3, p21, and P16 proteins were analysed by western blot.

Results: The results showed that the blood glucose level increased, insulin resistance occurred, spleen coefficient and ROS levels increased, humoral immunity in peripheral blood decreased, and inflammation increased in the model group compared to the control group. After Mel and Neu-P11 treatment, the blood glucose level decreased significantly, insulin sensitivity improved, spleen coefficient and ROS levels decreased, humoral immunity in peripheral blood was enhanced, and inflammation improved in T2DM rats. Brain functional analysis of T2DM rats showed that microglia cells were activated, TNF-α and IL-β levels were increased, and IL-10 levels were decreased. Mel and Neu-P11 treatment reversed these indexes. Functional analysis of islets in T2DM rats showed that islet structure inflammation was impaired, islet β cells were apoptotic, p21 and p16 protein expressions were increased, and blood C-peptide and insulin were decreased. Mel and Neu-P11 treatment restored the function of pancreatic β cells and improved the damage of pancreatic tissue.

Conclusion: Melatonin and its receptor Neu-P11 can reduce the blood glucose level, enhance humoral and cellular immunity, inhibit microglia activation and inflammation, and repair islet β cell function, and this improve the characterization of T2DM-related diseases.
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Key words: *melatonin; Neu-P11; type 2 diabetes mellitus; immunity*

Introduction

Diabetes mellitus (DM) is a multifactorial chronic health condition triggered by a variety of genetic and/or environmental factors [1, 2]. DM is characterized by an increase in blood sugar levels due to insufficient concentration and/or activity of insulin, which is a pancreatic hormone involved in controlling blood sugar [3]. The global report of the World Health Organization (WHO) on diabetes shows that since 1980 the number of adults with diabetes has reached 463 million people and is expected to increase to 700 million by 2045, among whom about 90–95% of diabetic patients suffer from Type 2 diabetes mellitus (T2DM) [4, 5]. T2DM is characterized by insulin resistance and abnormal β cell function [6]. There are still some problems in the current treatment of T2DM, such as hypoglycaemia, peripheral hyperinsulinaemia, weight gain, and postprandial hyperglycaemia, all of which are associated with inappropriate insulin supplementation, suggesting an important role of endogenous and physiological insulin secretion in the management of T2DM [7]. Therefore, finding endocrine targets for the rational regulation of insulin resistance and β cell dysfunction are the first choice for the treatment of T2DM.

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Melatonin (Mel) is a circulating hormone released mainly by the pineal gland [8]. Some studies have found that Mel is closely related to the immune system, and it is now considered to be one of the members of the neuroendocrine immune system, participating in innate immunity, cellular immunity, and humoral immunity of the body [9, 10]. A large number of in vivo and in vitro studies have found that Mel can reduce hyperglycaemia, insulin resistance, oxidative stress, and inflammation by regulating various intracellular signal transduction pathways or by participating in other pathophysiological targets of T2DM, so as to play an anti-diabetic role [11, 12]. Neu-P11 is a novel Mel agonist for the treatment of psychophysiological insomnia, belonging to a highly medically treatable G-protein coupled receptor superfamily [13, 14]. Intraperitoneal injection of Neu-P11 (10 mg/kg) and Mel (4 mg/kg) daily for 8 weeks showed that Neu-P11, like Mel, could effectively reduce weight gain and improve insulin sensitivity and metabolic status in obese rats [15]. Melatonin (20 mg/kg) and Neu-P11 (20, 10, and 5 mg/kg) normalize the over-activated hypothalamic-pituitary-adrenal axis and modulate metabolic profile and insulin sensitivity in high-fat diet and STZ-induced T2DM rats, which may impair insulin resistance and glucose homeostasis [16]. Therefore, it is urgent to clarify the precise role of melatonin and its receptors on glucose homeostasis in T2DM disease because melatonin is widely used in T2DM and its complications, either as a prescription drug or as a supplement without a medical prescription.

In addition, some studies have shown that the main factor affecting the quality of life of diabetic patients is not diabetes itself, but a variety of acute and chronic complications of diabetes, especially diabetes associated with infection and cardiovascular disease, which has become one of the important death factors of diabetic patients [17]. Several studies have proved that there are changes in humoral and cellular immune function in diabetic patients, and the low immune function will lead to the susceptibility of the body to various infections [18–21]. Previous studies have shown that melatonin has a certain anti-infection ability, but its half-life in the body is short [22, 23]. Neu-P11 has superior pharmacokinetics and a longer half-life than Mel, but its effect on DM complicated with infection and immunodeficiency is unknown. This study aimed to investigate the effects of melatonin and its receptor agonist Neu-P11 on the phenotype of T2DM and its associated diseases, to provide a theoretical basis for T2DM treatment and the development and application of Mel-related drugs.

Material and methods

Experimental animals and groups

In this experiment, 120 male Sprague Dawley (SD) rats with a body weight of 180–220 g were selected and fed adaptively for one week under the conditions of temperature 20–24°C and relative humidity 40–50% with a 12/12h light/dark cycle. A T2DM rat model was established by combined treatment of high fat diet (HFD) and low-dose STZ (30 mg/kg). After 4 weeks of HFD, the rats were injected with STZ (30 mg/kg). One week after the injection, blood was collected from the tail vein to check that the blood glucose level was ≥ 250 mg/dL, indicating that the modelling was successful. The study was approved by the Medical Ethics Committee of the Hunan University of Medicine (2017062201).

Twelve male SD rats comprised the normal group (control). A total of 60 male T2DM rats were randomly divided into T2DM + inject normal saline (model), T2DM + inject Mel (MEL, 10 mg/kg), T2DM + inject low dose Neu-P11 (Neu-L, 5 mg/kg), T2DM + inject middle dose Neu-P11 (Neu-M, 10 mg/kg), and T2DM + inject high dose Neu-P11 (Neu-H, 20 mg/kg) groups, 12/group. All rats were treated once a day for 21 days. Blood samples were collected from the tail tip at 0, 7, 14, and 21 days for 6 hours after each feeding, and glucose oxidase method was used to measure blood glucose concentration. In the experiment, the weight of the test rats in each group was measured. Rats were euthanized by intraperitoneal injection of 150 mg/kg pentobarbital. The spleen tissue was weighed and recorded, and the spleen coefficient was calculated.

Glucose and insulin tolerance tests

For the glucose tolerance test (GTT), all rats were fed after the injection of Neu-P11 at 14 days and injected with 50% glucose (2.5 g/kg) by intraperitoneal injection 6 hours later. Blood samples were collected from the tail tip at 0, 30, 60, and 120 minutes to measure blood glucose levels. For the insulin tolerance test (ITT), insulin was injected intraperitoneally (0.5 U/kg) at 6 hours after feeding on day 21, and blood was collected from the tail tip at 0, 30, 60, and 120 minutes to measure blood glucose levels.

The isolation of primary spleen cells

Spleen tissues of rats in different treatment groups were collected after the experiment. The spleen tissues of mice were cut and ground with RPMI-1640 medium, 200-mesh sterile cell screen, sterile ophthalmic scissors, and a sterile glass syringe piston. 2 mL RPMI-1640 culture solution was used to wash the screen, so that all the scattered mononuclear cells could enter the culture solution. The mixture was centrifuged at 400 g for 10 min. The supernatant was then discarded , and 5 times the volume of sterile erythrocyte lysate was added to the cell precipitate, mixed, and left for 8 min. Then centrifugation was performed at 400 g for 5 min, followed by washing with 10 mL sterile PBS and resuspension to obtain the splenic tissue mononuclear cell suspension.

Haematoxylin-eosin (HE) staining

The pancreases of rats were taken and fixed in 4% paraformaldehyde for 24 h, followed by gradient dehydration with 20% and 30% sucrose solutions. The pancreatic tissue of rats was sliced, dehydrated, and embedded in paraffin. Coronal sections were successively made using a paraffin slicer and connected to the treated glass slides. The sections were roasted at 60°C for 12 h. The sections were placed in xylene for 10 min × 2 times. The sections were placed in 100%, 100%, 95%, 85%, and 75% ethanol successively for 5 min at each stage, and then soaked in distilled water for 5 min. The haematoxylin (Wellbiology, China) was stained for 1 min, washed with distilled water, and blue again with PBS. The sections were stained with eosin (Wellbiology, China) for 0.5 min and then washed with distilled water. The sections were dehydrated with alcohol gradient (95–100%), 5 min for each grade. The sections

were placed in xylene for 10 min × 2 times, sealed with neutral glue (Sigma) and observed under a microscope (BA210T, Motic).

TUNEL apoptosis assay

Spleen tissues of rats in each group were collected for paraffin sections, which were dewaxed to water. The slices were immersed in EDTA buffer (pH 9.0), heated in microwave oven for 23 min, cooled to room temperature, and washed with 0.01 M PBS (pH 7.2–7.6) for thermal antigen repair. The tissue sections were placed in sodium borohydride solution for 30 min and Sudan black dye solution for 5 min at room temperature. The tissue sections were sealed with 5% BSA for 60 min. Then 100 μL 1 × Equilibration Buffer was dropped for each tissue section to completely cover the sample area and incubate it at room temperature for 10–30 min. 50 μL TDT incubation buffer was added to the sample to be detected, and it was incubated at 37°C for 60 min in the dark. The tissue sections were rinsed with PBS, followed by dripping with suitably diluted Insulin (15848-1-AP, 1:100, Proteintech, USA) at 4°C overnight. The PBS was used to rinse tissue sections for 5 min × 3 times. The tissue sections were dropped with 50–100 μL anti-rabbit-IgG-labelled fluorescent antibody, incubated at 37°C for 90 min, and washed with PBS for 5 min × 3 times. The tissue sections were dyed with DAPI working solution at 37°C for 10 min and washed with PBS for 5 min × 3 times. The tissue sections were sealed with buffered glycerine and observed under a fluorescence microscope.

Immunofluorescence (IF)

Brain tissues of rats in different treatment groups were collected to prepare paraffin sections, which were dewaxed to water and heated to repair antigens. Tissue sections were placed in sodium borohydride solution for 30 min and Sudan black dye solution for 5 min at room temperature. The treated tissue sections were sealed at 5% BSA at 37°C for 60 min. Tissue sections were dropped with appropriately diluted primary antibodies. The primary antibodies for incubation included anti-CD11b (66519-1-Ig, 1:100, Proteintech, USA), anti-CD86 (13395-1-AP, 1:100, Proteintech, USA), and anti-IBA1 (ab5076, 1:100, abcam, USA). Tissue sections were then incubated with anti-Mouse IgG (SA00013-7, Proteintech, USA), anti-Rabbit IgG (SA00013-8, Proteintech, USA), and anti-Goat IgG (SA00003-3, Proteintech, USA) at 37°C for 90 min and rinsed with PBS (Wellbiology, China). Sections were sealed with buffered glycerine (Wellbiology, China) and observed under a fluorescence microscope.

Reactive oxygen species (ROS)

An ROS detection kit (S0033S, Beyotime Biotechnology) was used to detect the ROS level in the spleen. 1 × 10^6 cells were taken into a 1.5 mL centrifuge tube, centrifuged at 400 g for 5 min, then the supernatant was discarded and the cells were washed once with PBS (SH30256.01, Hyclone). The diluted DCFH-DA was added to the cell precipitation to resuspend the cells, and the cells were incubated in the dark at 37°C for 20 min, and the cells were mixed every 5 min. Cell precipitates were collected by centrifugation at 400 g, and the cells were washed twice in serum-free medium. The green fluorescence of ROS-FITC (Ex = 488 nm, Em = 530 nm) was detected by flow cytometer (A00-1-1102, Beckman) through an FITC channel (FL1).

Enzyme-linked immunosorbent assay (ELISA)

Whole blood samples were placed for 2 h at room temperature, centrifuged at 2–8°C in 1000 g for 15 min, and the supernatant was taken for immediate detection. According to the manufacturer's instructions, the IgA (CSB-E07987r, CUSABIO, China), IgG (CSB-E07981r, CUSABIO, China), IgM (CSB-E07978r, CUSABIO, China), TNF-α (CSB-E11987r, CUSABIO, China), IFN-γ (CSB-E04579r, CUSABIO, China), IL-1β (CSB-E08055r, CUSABIO, China), IL-10 (CSB-E04595r, CUSABIO, China), C-Peptide (CSB-E05067r, CUSABIO, China), and Insulin (KE20008, Proteintech, USA) were used for detection. The optical density (OD value) was measured at 450 nm using an enzyme plate analyser.

Western blot

After the experiment, the brain tissues and pancreas tissues of rats were collected, and the total proteins were extracted by radio immunoprecipitation analysis (RIPA) and lysis buffer solution. The protein concentration was determined by bicinchoninic acid (BCA) method. A total of 200 μg protein samples were separated by 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The isolated proteins were transferred to a polyvinylidene fluoride membrane that had been activated by methanol and blocked by 5% skim milk and dried at room temperature for at least 1 h. The primary antibodies were then incubated overnight at 4°C. The primary antibodies for incubation included anti-CD11b (66519-1-Ig, 1:2000, Proteintech, USA), anti-CD86 (13395-1-AP, 1:1000, Proteintech, USA), anti-cleaved-caspase 3 (19677-1-AP, 1:1000, Proteintech, USA), anti-p21 (10355-1-AP, 1:1000, Proteintech, USA), anti-P16 (10883-1-AP, 1:1000, Bioss, China), and anti-β-actin (60008-1-Ig, 1:5000, Proteintech, USA). They were then incubated with anti-mouse IgG (SA00001-1, 1:5000, Proteintech, USA) and anti-rabbit IgG (SA00001-2, 1:6000, Proteintech, USA) at 37 °C for 90 min. Chemiluminescence (Millipore, USA) was visualized and analysed using imaging software (GE Healthcare, Life Sciences, USA).

Flow cytometry

The expression levels of T cells (CD4+, CD8+, CD3+, CD4+/ /CD8+), B cells (CD86 and CD80), and NK cells (CD16) in immune cells were analysed by flow cytometry. The cells were divided into 1.5 mL EP tubes at 1 × 10^6/100 μL, with the addition of 0.5 μg CD3-APC antibody (17-0032-82, eBiosciences), 0.125 μg CD19-APC antibody (17-0193-82, eBiosciences), and 0.125 μg CD161-APC antibody (17-5941-82, eBiosciences) for CD3, CD19, and CD161, respectively. The cells were incubated at room temperature in the dark for 30 min, then 1 mL PBS was added to wash the cells once, and the supernatant was discarded after centrifugation at 350 g for 5 min. 150 μL PBS was added to resuspend the precipitate, and a flow cytometer (A00-1-1102, Beckman) was used for testing.

Statistical analysis

The statistical analysis of the data in this study was performed using Graphpad Prism 8.0 statistical software. Statistical significance between two groups within experiments was determined by unpaired two-tailed Student's t-tests, and among more than two groups using analysis of variance (ANOVA). All the data are presented as means ± standard deviation (SD). P value < 0.05 was considered to be statistically significant. All the experiments were repeated at least three times with similar results.

Results

Neu-P11 affects the fasting blood glycose in T2DM rats

Blood samples were collected from tail tip to measure blood glucose concentration 6 h after feeding on 0, 7, 14, and 21 days, respectively. The results showed that the blood glucose level in the model group was significantly higher than that in the control group (Fig. 1). After Mel and Neu-P11 treatment, the blood glucose level was decreased compared with that in the model group (Fig. 1). Compared with the model group, the blood glucose level of the Mel, Neu-M, and Neu-H groups decreased significantly at 21 d (Fig. 1). These results indicate that

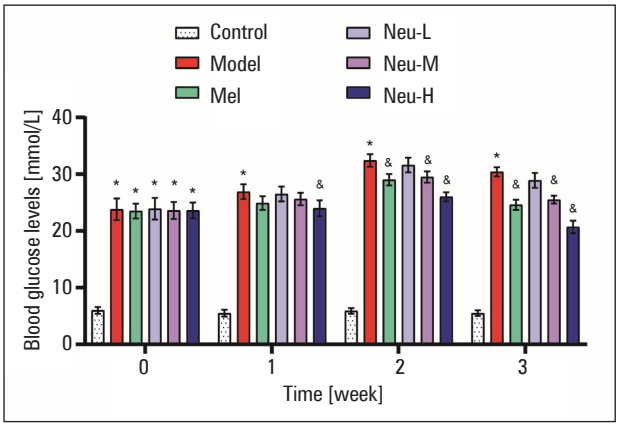

Figure 1. *Neu-P11 regulated blood glucose levels in type 2 diabetes (T2DM) rats. *p < 0.05 vs. control group; &p < 0.05 vs. model group*

high-dose Neu-P11 could significantly reduce the blood glucose level in T2DM patients.

Study of glucose metabolism in T2DM rats under Neu-P11 treatment

To further determine the effect of Neu-P11 on blood glucose in T2DM patients, we continued to conduct GTT and ITT. GTT showed that blood glucose levels in the model group were significantly higher than those in the control group, while after Mel and Neu-P11 treatment, blood glucose levels were significantly decreased relative to the model group (Fig. 2A). ITT showed that, compared with the control group, the model group showed increased blood glucose concentration and insulin resistance, while after Mel and Neu-P11 treatment, insulin sensitivity was improved and blood glucose concentration was reduced compared with the model group, and the effect of a high dose of Neu-P11 was significantly stronger than that of Mel (Fig. 2B). These results further demonstrate that Neu-P11 could be used as a potential drug for the treatment of T2DM.

Neu-P11 improved the immune function in T2DM rats

After the experiment, the spleen coefficients of rats in different treatment groups were analysed, and it was shown that, compared with the control group, the spleen coefficients of the model group increased, while the spleen coefficients of the Neu-P11 group decreased (Fig. 3A). Flow cytometry was used to detect the ROS level in the spleen, and the results showed that the ROS level of the model group was significantly increased, while the ROS level of the Neu-P11 group was decreased (Fig. 3B). In addition, the expression of T cell, B cell, and NK cell markers were analysed by flow cytometry, and it was found that the expression of CD3+ T cells, NK cells, and CD19 B cells were decreased in T2DM, while the expression of immune cells was promoted by high-dose Neu-P11 treatment, indicating that the overall level of cellular immunity was increased (Fig. 3C). At the same time, ELISA was used to analyse blood immunoglobulin levels, which showed decreased IgG and IgM expressions and increased IgA expression in T2DM rats (Fig. 3D–F). However, Neu-P11 treatment decreased the IgA level and increased the IgG level, but IgM changes were not obvious, which proved that the humoral immune function of the rats was enhanced (Fig. 3D–F). Compared with the control group, the expression of pro-inflammatory cytokine TNF-α was increased, while the secretion of interferon IFN-γ was decreased in the model group (Fig. 3G–H). Neu-P11 treatment promoted the expression of IFN-γ but decreased the TNF-α level (Fig. 3G–H). These results indicate that Neu-P11 could restore the humoral and cellular immune functions of T2DM rats.

The influence of Neu-P11 on microglia in T2DM rats

Based on the model of T2DM rats, the influence of Neu-P11 on the brain function of T2DM rats was in-

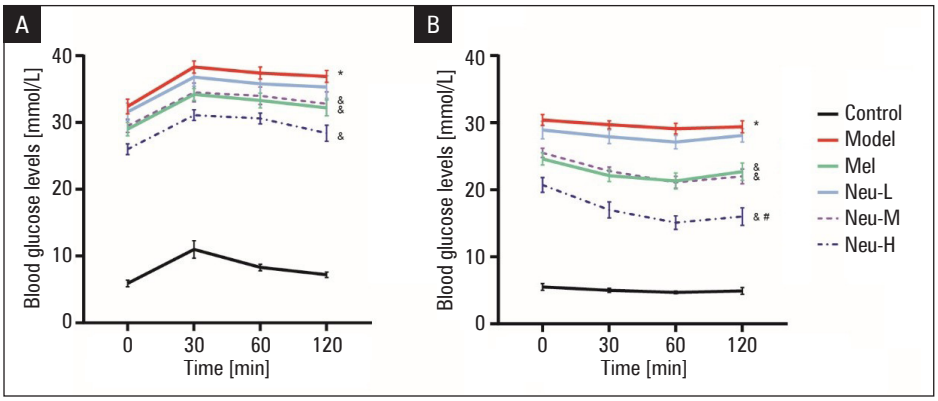

Figure 2. *Effect of Neu-p11 on glucose and insulin sensitivity in type 2 diabetes (T2DM) rats.* **A.** *Intraperitoneal glucose tolerance test.* **B.** *Insulin tolerance test. *p < 0.05 vs. control group; &p < 0.05 vs. model group; #p < 0.05 vs. Mel group*

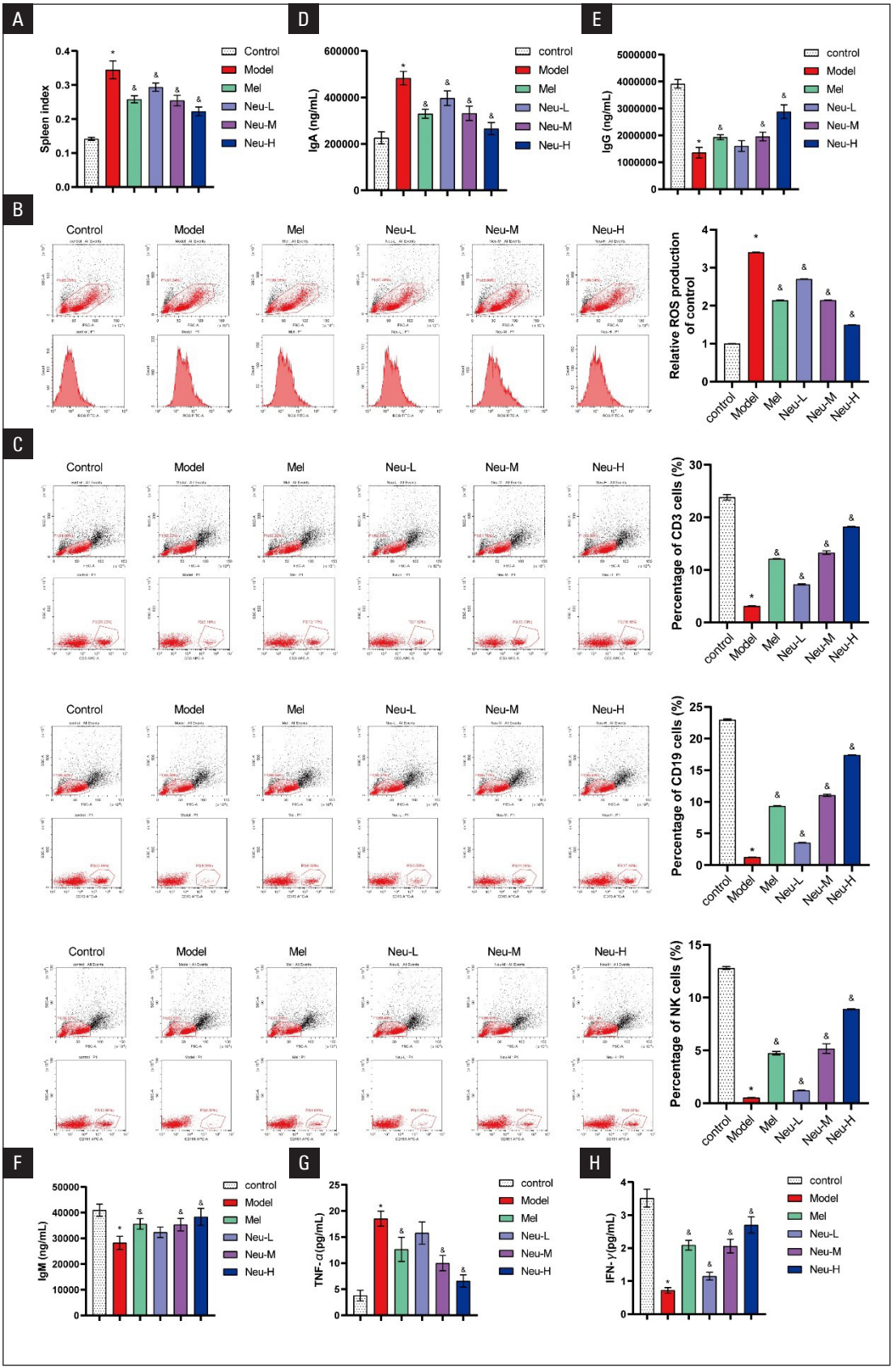

Figure 3. *Neu-p11 improved the immune function in type 2 diabetes (T2DM) rats.* **A.** *Spleen coefficient statistics of rats in different treatment groups.* **B.** *Flow cytometry was used to detected ROS levels.* **C.** *Flow cytometry was used to analyse the levels of T cells, B cells, and NK cells.* **D–H.** *The levels of IgA, IgG, IgM, TNF-α, and IFN-γ were detected by ELISA.* $^{*}p < 0.05$ *vs. control group;* $^{\&}p < 0.05$ *vs. model group*

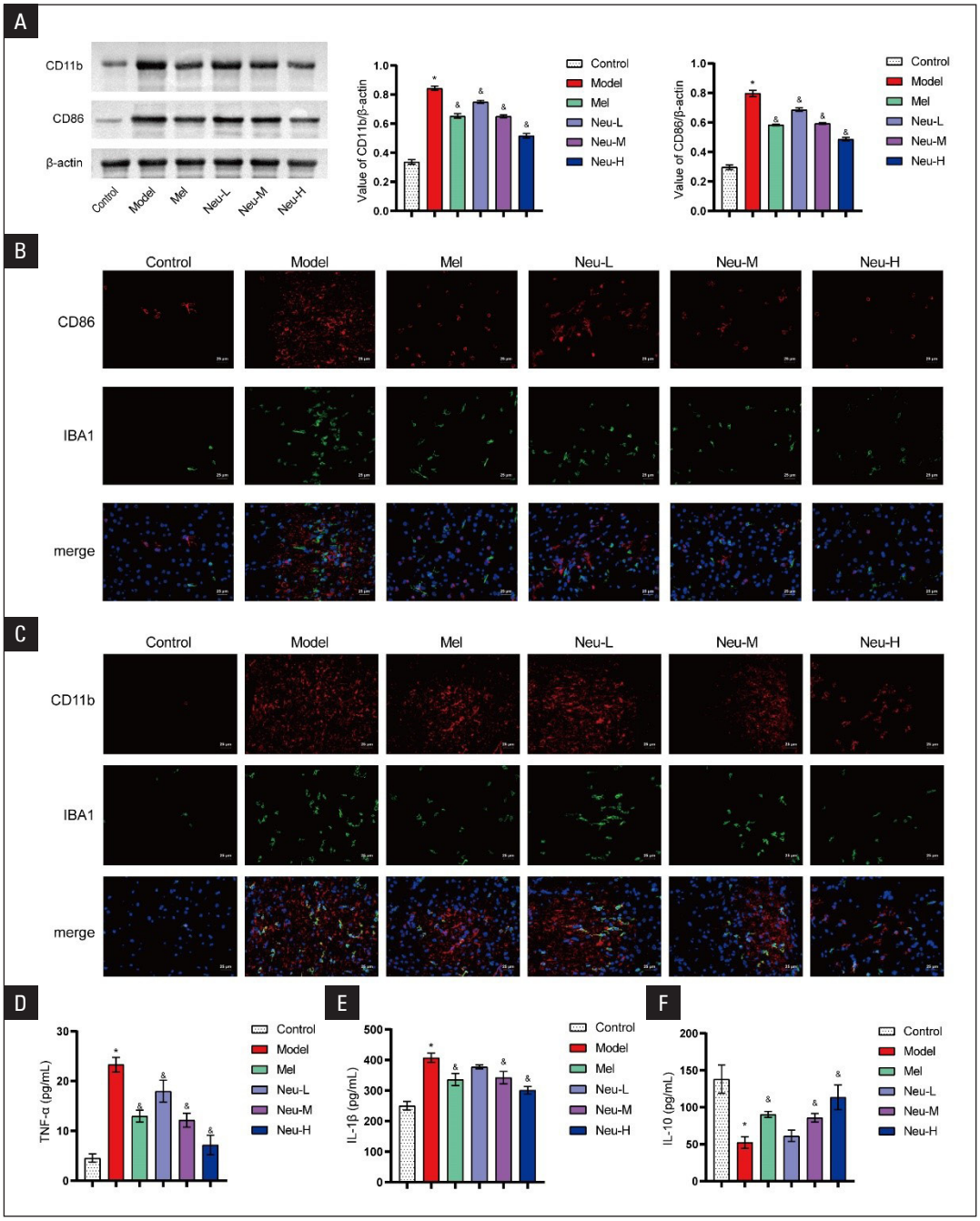

Figure 4. *The influence of Neu-p11 on the activation of microglia in type 2 diabetes (T2DM) rats.* **A.** *The expression of the activated microglia markers CD11b and CD86 was detected by western blot.* **B, C.** *The distribution of double-stained IBA1 and CD86, IBA1, and CD11b was observed by IF.* **D–F.** *The levels of TNF-α, IL-b, and IL-10 were analysed by ELISA. Scale bar = 25 μm; The magnification is 400×; *p < 0.05 vs. control group; &p < 0.05 vs. model group*

vestigated. The expression of the activated microglia markers CD11b and CD86 was detected by western blot, and it was found that microglia were significantly activated in the model group, while Neu-P11 had certain inhibitory effects on the activation of microglia cells in the brain (Fig. 4A). In addition, we also double-stained IBA1, CD11b, and CD86 by IF and found that co-localization of IBA1 and CD11b, as well as IBA1 and CD86, increased in the model group, while Neu-P11 could reduce its co-localization (Fig. 4B–C). ELISA assay showed that the inflammation level was significantly increased in the model group, which was reflected in the increased TNF-α and IL-β, while Neu-P11 treatment inhibited these inflammatory markers (Fig. 4D–E). The results also show that IL-10 expression was decreased in the model group, while Neu-P11 treatment could significantly increase the expression of IL-10, thus inhibiting inflammation (Fig. 4F). These

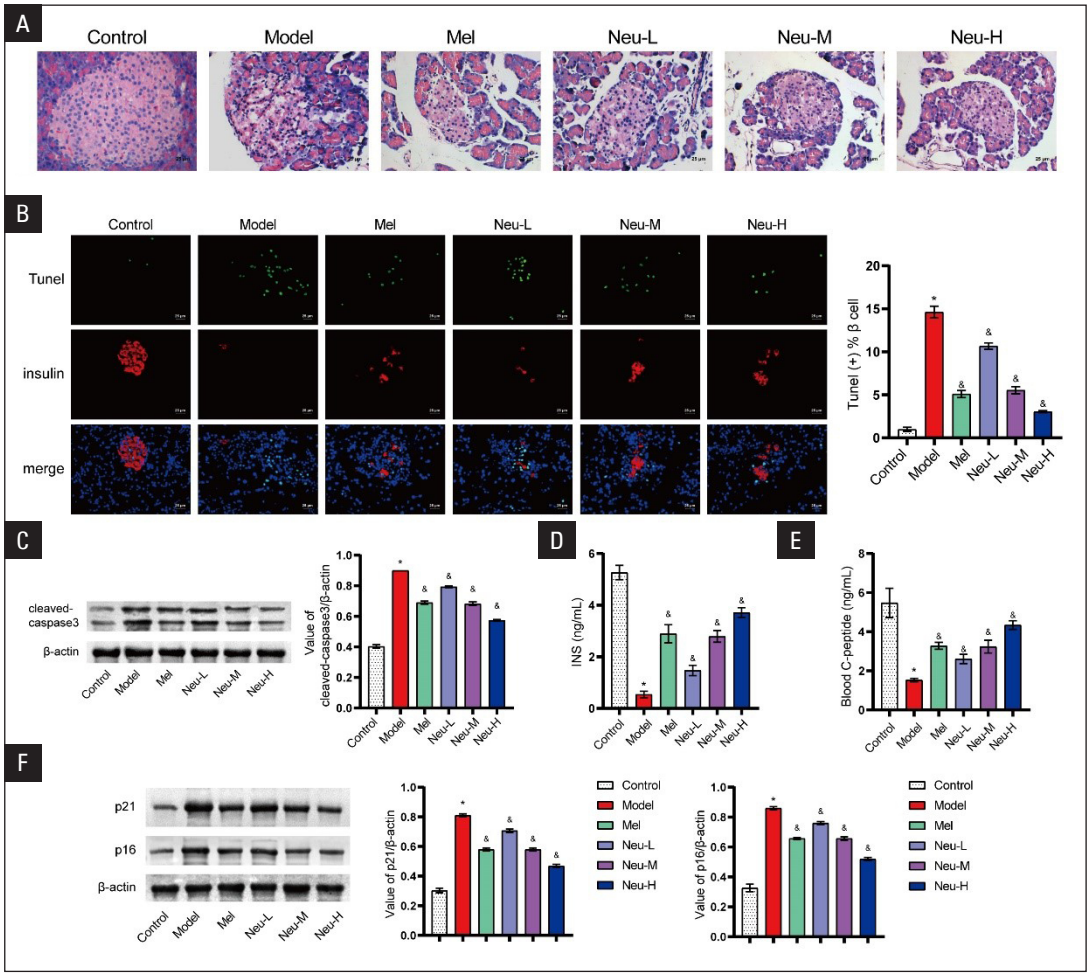

Figure 5. *The influence of Neu-p11 on pancreas in type 2 diabetes (T2DM) rats.* **A.** *HE staining was used to observe the pathological changes of pancreatic tissue in rats.* **B.** *The apoptosis of islet* b *cells was observed by TUNEL staining.* **C.** *Western blot was used to analyse cleaved-caspase-3 expression.* **D, E.** *Blood insulin (INS) and serum C-peptide levels were measured by ELISA.* **F.** *The expression of* $p21^{WAF}$ *and* $p16^{LNK4a}$ *in islet* b *cells was detected by western blot. Scale bar = 25 μm; The magnification is 400×; *p < 0.05 vs. control group; &p < 0.05 vs. model group*

results suggest that Neu-P11 could regulate microglia activation and inflammation and improve T2DM related brain dysfunction.

The influence of Neu-P11 on islet β cells in T2DM rats

T2DM disease is often accompanied by insulin resistance and impaired islet function, but the influence of Neu-P11 on islet β cells in T2DM rat models is unknown. HE staining showed the regular structure and uniform arrangement of a large number of intact islet β cells in the control group (Fig. 5A). In contrast, inflammation of the islet structure was impaired, including cytolysis and apoptosis, accompanied by a sharp decline in the number of islet β cells in the model group (Fig. 5A). TUNEL and insulin staining showed that islet β-cell apoptosis was significantly increased in the model group, while Neu-P11 treatment inhibited islet β cell apoptosis in T2DM rats (Fig. 5B). The expression of cleaved-caspase 3 protein was increased in the model group, while Neu-P11 treatment inhibited its expression (Fig. 5C). In order to detect the function of pancreatic islet β cells, blood C-peptide and insulin were detected by ELISA, and the results showed that the levels of C-peptide and INS in the model group were significantly lower than those in the control group (Fig. 5D-E). However, the levels of C-peptide and INS in Neu-P11 treatment were significantly increased compared with those in the model group, suggesting that the function of islet β cells was restored (Fig. 5D-E). Western blot also showed that the fluorescence of $p21^{WAF}$ and $p16^{LNK4a}$ in islet β cells was increased in the model group, while the expression of $p21^{WAF}$ and $p16^{LNK4a}$ was decreased after treatment with Neu-P11 (Fig. 5F). These results suggested that Neu-P11 could restore the function of islet β cells and improve the treatment of T2DM with islet injury.

Discussion

Type 2 DM is a global epidemic that is predicted to continue to increase [24]. Clinical studies have shown that patients with T2DM are more often associated with sleep dysfunction than the general population, which is mainly associated with cardiometabolic changes such as hypertension, increased sympathetic nervous system activity, and systemic insulin resistance [25]. Neu-P11, an agonist of Mel, is used to treat physiological insomnia and has similar functions to Mel [26–28]. It is known that Neu-P11 may improve insulin resistance by affecting the activity of insulin signalling pathway [26–28]. Our study confirmed that Mel and Neu-P11 treatment of T2DM rats reduced blood glucose levels, improved insulin sensitivity, decreased spleen coefficient and ROS levels, and enhanced humoral and cellular immunity in peripheral blood. Previous studies have reported that Mel boosts the function of the immune system by stimulating the production of certain immune factors [9]. These studies proved that Mel and its receptor Neu-P11 can effectively improve insulin resistance and glucose homeostasis in T2DM rats and can be used in the treatment of T2DM.

In addition, there is growing evidence that people with T2DM are prone to brain dysfunction and cognitive deficits [29]. Factors such as hyperglycaemia and insulin resistance are associated with impaired neural congruence, leading to cognitive decline [30]. Multiple ultrastructural images of the brain in female diabetic db/db model rats showed significant cellular remodelling of parietal cells and microglia, suggesting that T2DM may increase the vulnerability of brain neurovascular units, glial cells, and neurons [31]. In our study, we found that brain microglia were significantly activated in T2DM rats, TNF-α and IL-β levels were increased, and IL-10 levels were decreased. Mel and Neu-P11 treatment could effectively improve the activation state of microglia and inflammation levels. In addition, analysis of the IL-10 protein profile and expression in organs and tissues in male Wistar rats showed that mutations in the IL-10 gene sequence in the brain and stomach of T2DM rats increased IL-10 expression in ileum, brain, and liver [32]. These studies have proven that Mel and its receptor Neu-P11 can effectively improve T2DM-related brain dysfunction or provide a theoretical basis for the treatment of T2DM-related brain diseases.

Apoptosis plays a key role in the pathophysiology of T2DM [33]. Loss of functional beta cell mass is a major cause of T2DM [34]. Pancreatic β cells play an important role in glucose homeostasis by secreting insulin, and impaired insulin secretion can lead to chronic hyperglycaemia characterized by T2DM [35]. In this study, islet function analysis in T2DM rats revealed impaired islet inflammation, apoptosis of islet beta cells, increased p21 and p16 protein expression, and decreased blood C-peptide and INS. Pancreatic β-cell dysfunction, the failure to deliver insulin at a sufficient concentration to control blood sugar, is central to the aetiology of type 2 diabetes [36]. It has been reported that activation of melatonin signalling in human islets can restore glucose-stimulated insulin secretion in islets exposed to chronic hyperglycaemia and in T2DM islets [37]. In this study, it was confirmed that Mel and Neu-P11 treated with T2DM rats could inhibit the apoptosis of islet β cells, restore the function of islet β cells, and improve the pancreatic tissue damage. Mel and its Neu-P11 receptor may affect insulin secretion of pancreatic islets by activating G protein-coupled melatonin receptors 1 and 2 expressed in islet β cells [38]. All the studies proved that Mel and its Neu-P11 receptor improved pancreatic islet damage and repaired pancreatic β cell function.

Melatonin agonists have been shown to reliably regulate circadian rhythms for the treatment of rhythm disturbances, circadian sleep disorders, and depression with aetiological circadian dysfunction [39]. Neu-P11 also modulated metabolic characteristics and insulin sensitivity and reduced insulin resistance caused by sleep restriction [40]. Neu-P11 may ameliorate impaired glucose metabolism and prevent insulin resistance by normalizing the function of the hypothalamic-pituitary-adrenal (HPA) axis [41]. In conclusion, exogenous melatonin and Neu-P11 can affect diabetes mellitus and related metabolic disorders not only by regulating insulin secretion, but also by protecting islet β cell function.

Conclusions

Melatonin and its receptor agonist Neu-P11 can reduce blood glucose levels, enhance humoral and cellular immunity, inhibit microglia activation and inflammation, and repair islet injury and apoptosis β cell function, improving the characterization of T2DM related diseases.

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Conflicts of interest

To the best of our knowledge, no conflict of interest, financial or other, exists.

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