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Study on Esmolol Treating Septic Cardiomyopathy by Regulating NLRP3 Inflammasome

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KEYWORDS Cardiomyopathy. Esmolol. NLRP3. Inflammasome. Septic. Treg Cells

ABSTRACT To expound the mechanism of esmolol in the treatment of septic cardiomyopathy, flow cytometry was used to observe changes in the levels of Treg cells. Ultrasound detection was performed to extract cardiac function in each group of sepsis rats. ELISA was applied to detect myocardial injury markers and inflammatory factors. Pathological detection of myocardial damage was performed, and the heart tissue protein was extracted. Western Blot was employed to detect the expression levels of NLRP3, ASC, casepase-1 and other antibodies in myocardial tissue. Esmolol can significantly improve the cardiac function of rats with septic cardiomyopathy. The experimental results suggested that its therapeutic effect is through regulating the balances between the level of Treg cells and the level of anti-inflammatory factors, as well as the expression level of NLRP3 inflammasome-related protein and its pro-inflammatory factors.

INTRODUCTION

Sepsis is currently defined as a severe, life-threatening host inflammatory response. Sepsis overreaction is usually accompanied by organ failure or even death. It is a common complication of severe trauma, burns, heatstroke, shock, and after major surgery. It also leads to severe illness and is one of the main causes of death of critically ill patients (Kim et al. 2019). Septic cardiomyopathy is an organ failure induced by sepsis. According to statistics, about forty to sixty percent of patients with sepsis have cardiac insufficiency (Kotecha et al. 2018; Yin J et al. 2021), and septic cardiomyopathy increases the mortality of sepsis without cardiac dysfunction from twenty to seventy percent (Frencken et al. 2018; Lu Nian Fang et al. 2020). Septic cardiomyopathy can affect the left and right ventricles, manifested as left ventricular systolic/diastolic dysfunction, abnormal right ventricular function, and abnormal wall motion (Shen and Wong 2016). However, due to the complexity of the cardiovascular system, the inability of a clear assessment method, and the changes in the state of the heart before and after infection, make it difficult to explain the causality. Currently there is no clear and unified definition of septic cardiomyopathy. For a long time, researchers have had a deeper understanding and prevention strategies for renal dysfunction and respiratory dysfunction caused by sepsis, but they have not paid enough attention to septic cardiomyopathy. Therefore, the study of septic cardiomyopathy has extremely important theoretical and clinical significance.

Esmolol is clinical drug, which can control the effects of atrial fibrillation, ventricular rate during atrial flutter, hypertension or tachycardia during operation. But there is no research on Esmolol in the treatment of septic cardiomyopathy, and this research has a certain degree of innovation.

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METHODOLOGY

Preparation and Grouping of Septic Cardiomyopathy Rat Models by Cecal Ligation and Perforation

SD rats were adapted to the environment for 1 week, fasted for 12 hours before operation, and anesthetised with chloral hydrate (0.5 ml/100 g). Then, the root of the cecum was ligated circularly with number 4 silk thread. After the ligation, the intestinal passage should be kept normal. Two places were punctured with an 18-gauge needle on the serosal surface of the intestinal wall of the cecum opposite to the mesentery. Erythromycin was applied to the wound to prevent infection. After the operation, 5 ml/100 g of normal saline was injected subcutaneously into the abdominal wall for fluid resuscitation. The grouping is as follows.

A: The Sham Group

The abdominal cavity was opened, and the cecum was pulled out and turned gently, and then returned to the abdominal cavity, which was sutured.

B: The Sepsis Rat Model 18-hour Group (CLP-18h)

The abdominal cavity was opened. The cecal ligation and puncture method was applied. The abdominal cavity was sutured and observed at 18 hours.

C: The Sepsis Rat Model 72-hour Group (CLP-72h)

The abdominal cavity was opened. The cecal ligation and puncture method was applied. The abdominal cavity was sutured and observed at 72 hours.

D: The Esmolol 18-hour Group (TGC-18h)

The abdominal cavity was opened, the cecum was pulled out and turned gently, and then returned to the abdominal cavity, which was sutured. After the operation, Esmolol of 0.5 mg/kg is given once a day. The observation continued at 18 hours.

E: The Esmolol 72-hour Group (TGC-72h)

The abdominal cavity was opened, the cecum was pulled out and turned gently, and then returned to the abdominal cavity, which was sutured. After the operation, Esmolol of 0.5 mg/kg is given once a day. The observation continued at 72 hours.

Two time points respectively at 18 hours and 72 hours after modelling were selected to observe the changes in cardiac function, and serum was collected to detect myocardial injury markers.

Detection of Rat Heart Function

At the corresponding time points, the rats in each group were selected and placed in an anaesthesia machine. After anaesthesia with isoflurane, the changes in the cardiac function of the rats were detected by ultrasound.

Detection of Rat Heart Injury Index

At the corresponding time point, each group of rats was selected. Blood was taken from the abdominal aorta, and the serum was retained after centrifugation. Myocardial injury markers were detected.

Detection of Inflammatory Factors in Rats

At the corresponding time points, each group of rats was selected. Blood was taken from the abdominal aorta, and the serum was retained after centrifugation. The serum enzyme-linked immunosorbent assay was used to determine the expression of various inflammatory factors in the serum.

Flow Cytometry to Detect Treg Cells

2 ml of abdominal aortic blood was collected with EDTA anticoagulation vacuum blood collection tube and mixed gently. Flow cytometer was used for detection according to the instructions.

Western Blot Detecting NLRP3 Pathway in Myocardial Tissues

Each group of myocardial tissues were obtained. NLRP3 pathway related proteins were detected according to the instructions.

Statistical Analysis

The experimental data is analysed using SPSS 23.0. If the measurement data conforms to the normal distribution, $x \pm s$ was used for statistical description. Multiple groups of data are tested by one-way analysis of variance. The multiple groups of measurement data conforming to the normal distribution and homogeneity of variance were tested using Dunett test, while the non-parametric Mann-Whitney U test was used to compare the two groups of measurement data that did not conform to the normal distribution and the homogeneity of variance. A p-value of less than 0.05 is considered to be statistically different.

RESULTS

The Effect of Esmolol on Heart Function

Ultrasound results showed that in the Sham group, the CLP-18h group, the CLP-72h group, the Esmolol-18h group, and the Esmolol-72h group, after surgical modelling, both the diastolic function indexes E/A and E/E and the systolic func-

tional indicators EF, FS, CO, VIDd, LVIDs, SV, EDV, and ESV conform to the normal distribution, suggesting that Esmolol can improve cardiac function, as shown in Figure 1.

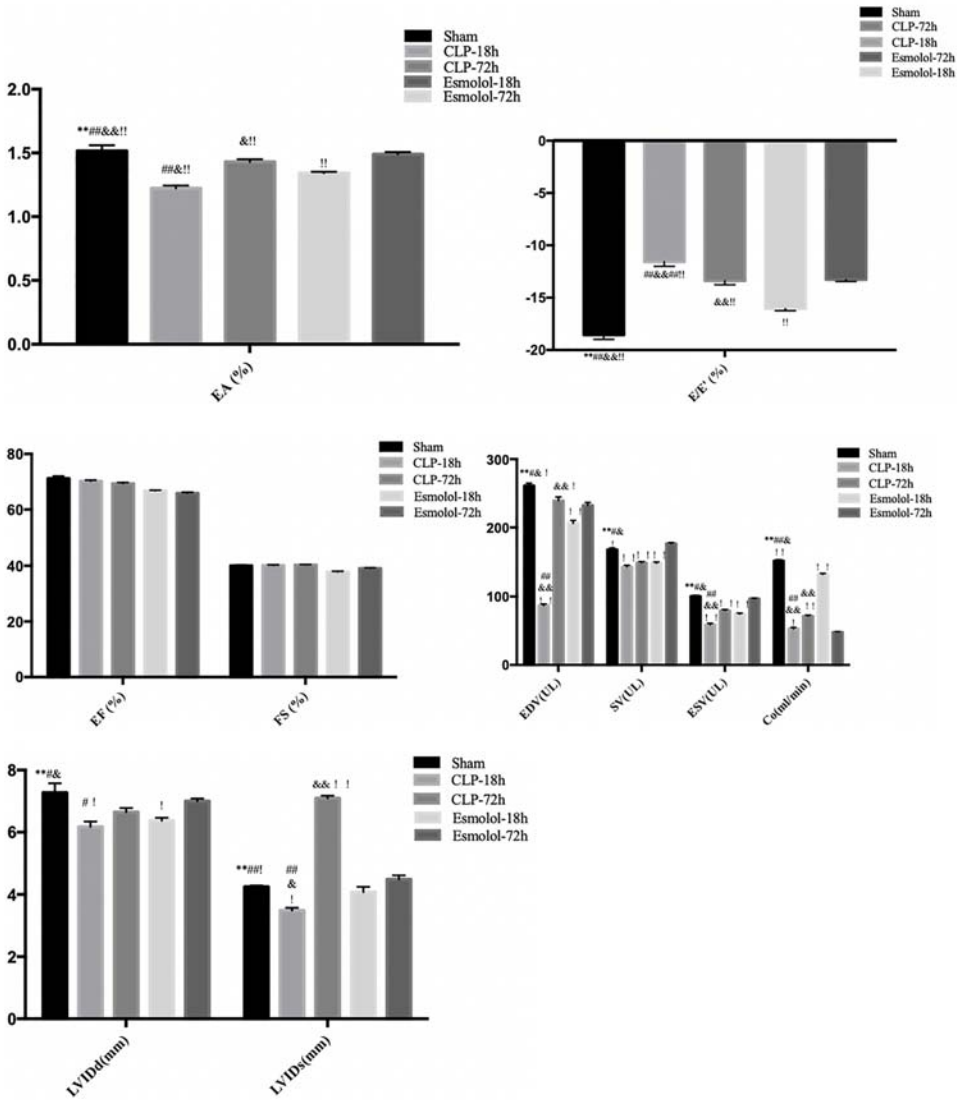

Fig. 1. The effect of Esmolol on cardiac function
** P < 0.01 vs. CLP-18h
P < 0.01 vs. CLP-72h
# P < 0.05 vs. CLP-72h
&& P < 0.01 vs. Esmolol-18h
&P < 0.05 vs. Esmolol-18h
!!P < 0.01 vs. Esmolol-72h
!P < 0.05 vs. Esmolol-72h

The Effect of Esmolol on Serum Myocardial Injury Markers in Rats with Septic Cardiomyopathy

The results showed that after the surgical modelling, the myocardial injury markers CK-MB, LDH, and cTnT in the serum of sepsis rats in the Sham group, the CLP-18h group, the CLP-72h group, the Esmolol-18h group, and the Esmolol-72h group all conformed to the normal distribution. Esmolol can reduce the expressions of CK-MB, LDH and cTnT, as shown in Figure 2.

The Effect of Esmolol on Serum Inflammatory Factors in a Septic Cardiomyopathy Rat Model

The ELISA results showed that the expression levels of IL-6, IL-lf3, IL-10, IL-18, and TGF-P1 in the serum of septic rats in each group decreased significantly after surgical modelling, as shown in Figure 3.

Treg Cell Level Analysis

In each group of sepsis rat models, after flow cytometry, it was found that the levels of Treg cells in the Sham group, the CLP-18h group, the CLP-72h group, the Esmolol-18h group, and the Esmolol-72h group all showed a normal distribution, as shown in Figure 4.

Changes in the Expression Level of NLRP3 Pathway Protein in Myocardial Tissue of Rats with Septic Cardiomyopathy

Compared with the Sham group, the expression levels of NLRP-3, ASC, and Casepase-1 proteins in the myocardial tissue of septic rats in the CLP-18h and CLP-72h groups were significantly different. Compared with the CLP-18h and CLP-72h groups, the expression of each protein in the

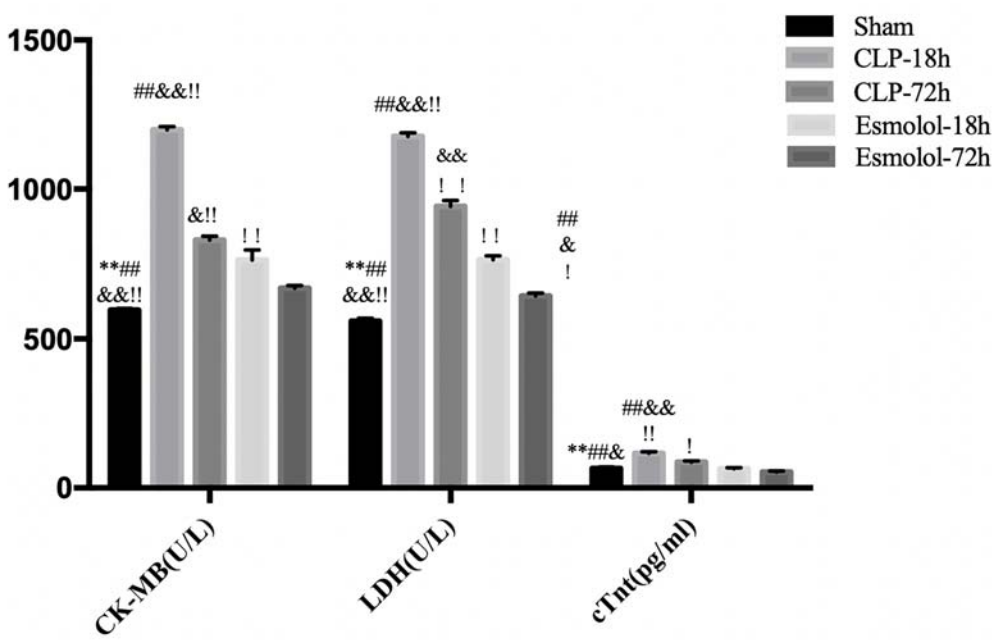

Fig. 2. The effect of Esmolol on serum myocardial injury markers in rats with septic cardiomyopathy
$^{**}P < 0.01$ vs. CLP-18h
$^{\#\#}P < 0.01$ vs. CLP-72h
$^{\&\&}P < 0.01$ vs. Esmolol-18h
$^{\&} P < 0.05$ vs. Esmolol-18h
$^{!!} P < 0.01$ vs. Esmolol-72h

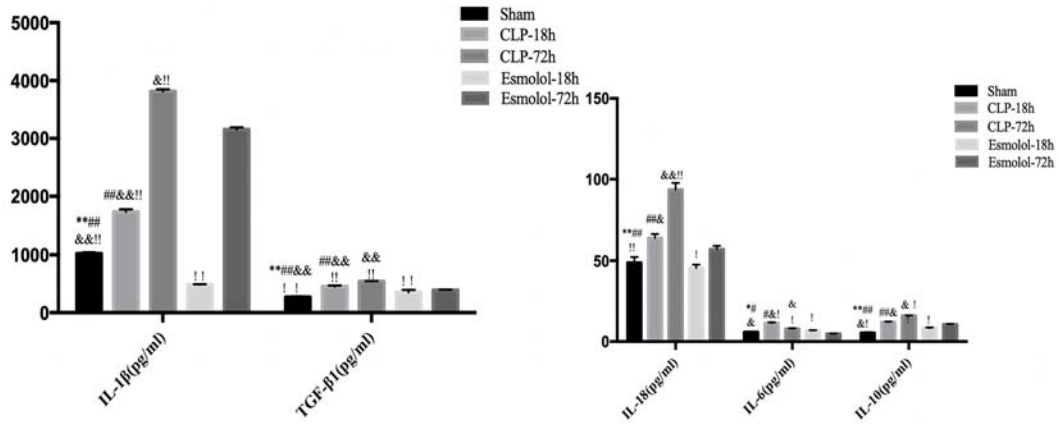

Fig. 3. The effect of Esmolol on inflammatory factors in the serum of septic cardiomyopathy model rats

** P < 0.01 vs. CLP-18h
* P < 0.05 vs. CLP-18h
P < 0.01 vs. CLP-72h
# P < 0.05 vs. CLP-72h
&& P < 0.01 vs. Esmolol-18h
& P < 0.05 vs. Esmolol-18h
!! P < 0.01 vs. Esmolol-72h
! P < 0.05 vs Esmolol-72h

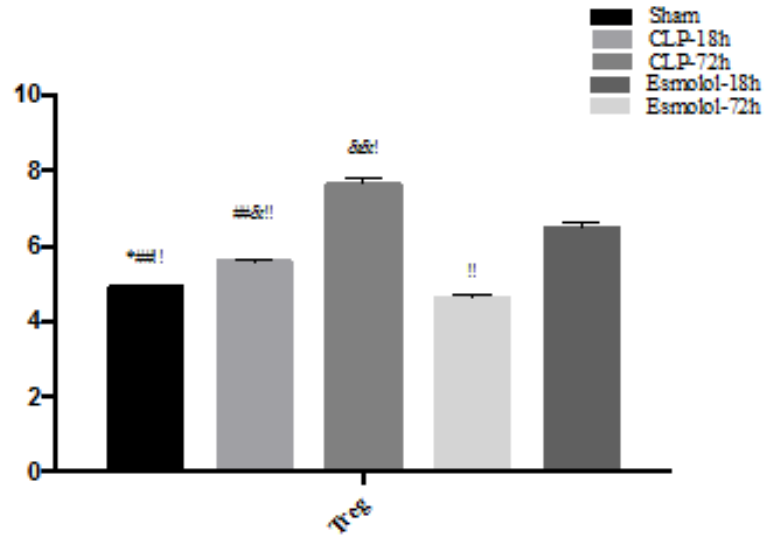

Fig. 4. The effect of Esmolol on the expression level of Treg cells

* P < 0.05 vs. CLP-18h
P < 0.01 vs. CLP-72h
&& P < 0.01 vs. Esmolol-18h
& P < 0.05 vs. Esmolol-18h
!! P < 0.01 vs. Esmolol-72h
! P < 0.05 vs. Esmolol-72h

Esmolol-18h and Esmolol-72h groups was also different, as shown in Figure 5.

DISCUSSION

Dilatant cardiomyopathy is an organ damage accompanied by sepsis, manifested as myocardial injury with elevated cardiac biomarkers, echocardiographic myocardial dysfunction, and hemodynamic instability. Septic cardiomyopathy is a common complication of sepsis, with a general incidence of forty to sixty percent and particularly poor prognosis. It can involve the left ventricle, the right ventricle, or both. Septic cardiomyopathy can cause death from sepsis. Mortality of sepsis without heart dysfunction has increased from twenty to seventy percent. Studies have found that septic cardiomyopathy can be manifested as systolic dysfunction, left ventricular diastolic dysfunction, right ventricular systolic dysfunction, global hypokinesia or local ventricular wall motion abnormalities. Studies have also found that most patients with septic shock have overall left ventricular hypokinesia, some patients have systolic left ventricular dysfunction, and some patients have diastolic dysfunction. In the early stage of sepsis, the left ventricular ejection fraction may be normal, but the stroke volume will decrease. As the disease progresses, the EF value can still maintain a normal level, and the left ventricular end diastolic volume will decrease (Zhou et al. 2018; Sciences NAO 2017; Sato et al. 2016; Claudia et al. 2017 Hollenberg and Singer 2021).

Regulatory T cells are a group of T cells that regulate the immune system. They mainly play a negative regulatory role in the immune system, and maintain immune homeostasis by inhibiting the function of T cells. There are two types of regulatory T cells. The first type is the Treg cells, which express CD4, CD25 and FOXP3 that enter the peripheral lymphatic tissues after the thymus matures. They are called natural Treg cells. They play a broad role in autoimmunity and tolerance that have been studied extensively. The other type is derived from the activation and differentiation of mature CD4+CD25 T cells in the periphery, called inducible regulatory T cells, which have been shown to inhibit T cell proliferation and experimental autoimmune diseases. Through antigen stimulation and secreting cytokines IL-10 and TGF-β, it exerts immunosuppressive effects (Patel et al. 2018; Cox and Weiner 2018). The host's unregulated response to infection in the latest definition of sepsis refers to the host's immune dysfunction. More and more data reveal the importance of immune dysfunction in patients with sepsis and

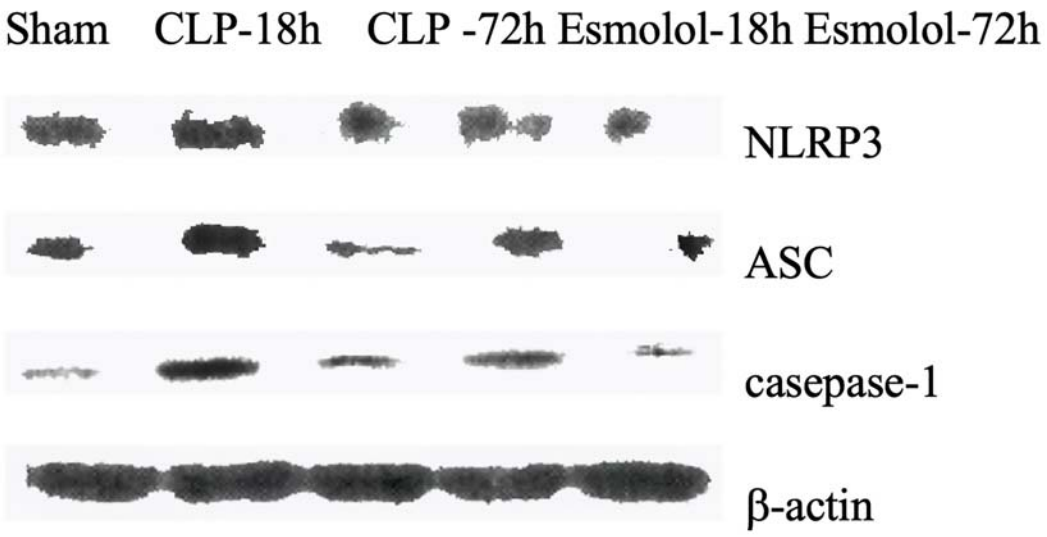

Fig. 5. The effect of Esmolol on the expression of NLRP3 pathway protein in myocardial tissue of rats with septic cardiomyopathy

septic shock. Although Treg cells account for only a small part of the total number of T lymphocytes in the immune system, these cells have powerful regulatory properties, making them important participants in immunosuppression during sepsis. It has been reported that the sequential monitoring of Treg cells in the peripheral blood of patients with sepsis may be helpful for the evaluation of the patient's condition and prognostic judgment. A deep understanding of Treg cells and their physiological functions will help develop new treatment methods that regulate the immune response in severe sepsis and septic shock (Beesley et al. 2018; Ehrman et al. 2018).

The activation of NLRP3 inflammasomes causes the release of pro-inflammatory factors IL-1β and IL-18, which is an important link in the activation phase of sepsis inflammation, as well as a precursor to serious consequences such as septic shock and organ failure. The level of Treg cells and the release of its anti-inflammatory factors IL-10 and TGF-β are the key links in the immunosuppressive phase of sepsis. The activation of NLRP3 inflammasomes and Treg cell-mediated immunosuppression maintain a stable and balanced state in the host body, which is a protection against sepsis. In the rat model of septic cardiomyopathy prepared by CLP, with the prolongation of the operation time, the surviving septic rats gradually showed a balance between the activation of NLRP3 inflammasomes and the immunosuppression mediated by Treg cells. Drug intervention undoubtedly promoted the rapid balance of the two in the host's body, thus playing a therapeutic effect (Haak and Ffiersinga 2017; Phimister and Tilg 2018 Sanfilippo et al. 2017; Li 2020; Barry 2020; Liu 2020).

The researchers' experiment found that after Esmolol injection, the rat heart function improved, and the level of myocardial injury markers decreased significantly. Esmolol intervention significantly reduced the level of peripheral blood Treg cells, and the expression levels of related proteins NLRP-3, ASC, and Casepase-1 decreased. The level of inflammatory factors in the serum decreased significantly.

CONCLUSION

Esmolol can significantly improve the heart function of rats with septic cardiomyopathy. The experiment showed that its therapeutic effect is through regulating balances between the level of Treg cells and the level of anti-inflammatory factors, as well as the expression levels of NLRP3 inflammasome-related protein and its pro-inflammatory factors.

RECOMMENDATIONS

By regulating the balances between the level of Treg cells and the level of anti-inflammatory factors, as well as NLRP3 inflammasome-related protein and the pro-inflammatory factors, Esmolol was expected to achieve the treatment of septic cardiomyopathy and provide new ideas and new targets.

CONTRIBUTIONS AUTHORS

Yongxia Li, Jiamei Jiang and Qiming Feng designed expeiriments, all authors performed testing, laboratory support and wrote the manuscript, Yongxia Li and Jiamei Jiang, Yongxia Li and Jiamei Jiang provided resources and analysis methods, and Yongxia Li and Jiamei Jiang contributed equally to this work.

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