The Role of Mogroside IIE in Macrophage Diabetic Inflammation Models: A Postprint

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Abstract

To investigate the role of mogroside IIE in a diabetic inflammation model induced by the combination of lipopolysaccharide (LPS) and palmitic acid (PA) in mouse macrophages, the present study employed 1 ng · L-1 LPS and 100 mol · L-1 PA to jointly treat mouse macrophage RAW 264.7 cells to establish a diabetic inflammation model, and utilized qRT-PCR to detect the expression level changes of the inflammatory cytokine TNF-α mRNA in cells at six different time points (0, 1, 3, 6, 12, and 24 h). The results demonstrated that: (1) the combined treatment of mouse macrophage RAW 264.7 cells with 1 ng · L-1 LPS and 100 mol · L-1 PA produced the optimal inflammatory synergistic effect at 24 h; (2) subsequently, following 12 h of combined induction with 1 ng · L-1 LPS and 100 mol · L-1 PA, the cells were treated with 20 mol · L-1 mogroside IIE for 12 h, and qRT-PCR was applied to detect the expression changes of the cellular inflammatory cytokine TNF-α mRNA. It was found that 12 h treatment with 20 mol · L-1 mogroside IIE significantly reduced the TNF-α mRNA expression level induced by the LPS and PA combination, suggesting that mogroside IIE can effectively attenuate the inflammatory level of macrophages. In conclusion, mogroside IIE may exert a therapeutic role in diabetes treatment, providing a reference for the development of novel therapeutic strategies for diabetes.

Full Text

Preamble

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Abstract:
To investigate the effect of mogroside IIE on macrophages in a diabetes inflammatory model induced by lipopolysaccharide (LPS) and palmitic acid (PA), this study used combined treatment with 1 ng· L^{-1} LPS and 100 mol·L^{-1} PA on mouse macrophage RAW 264.7 cells to construct a diabetic inflammation model, and employed qRT-PCR to detect changes in inflammatory factor TNF-α mRNA expression levels at six different time points (0, 1, 3, 6, 12, and 24 h). The results showed: (1) Combined treatment of mouse macrophage RAW 264.7 cells with 1 ng· L^{-1} LPS and 100 mol·L^{-1} PA produced the best inflammatory synergistic effect at 24 h; (2) Following 12 h of combined induction with 1 ng· L^{-1} LPS and 100 mol·L^{-1} PA, treatment with 20 mol·L^{-1} mogroside IIE for 12 h significantly reduced the TNF-α mRNA expression level induced by LPS and PA, demonstrating that mogroside IIE can effectively decrease the inflammatory level in macrophages. In summary, mogroside IIE may play a certain role in diabetes treatment, providing a reference for proposing new therapeutic strategies for diabetes.

Keywords: diabetes inflammation model, mogroside IIE, lipopolysaccharide, palmitic acid, mouse macrophages

Introduction

Diabetes mellitus is a chronic metabolic disease with increasing incidence in recent years. According to statistics, its health impact ranks only behind cancer and cardiovascular diseases, making it the third major disease threatening human health and imposing a tremendous economic burden worldwide (Lassenius et al., 2011). Research has found that diabetic patients often exhibit lipid metabolism disorders, primarily characterized by elevated plasma free fatty acid (SFA) concentrations, particularly increased palmitic acid (PA) content (Ruiz-nez et al., 2016). SFAs and other factors can cause low-level elevation of lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls (Das et al., 2010; Hellmann et al., 2013). As circulating LPS and SFAs increase in the body, they may produce synergistic effects that promote a strong inflammatory response far exceeding that induced by LPS or SFAs alone. In recent years, the role of inflammatory responses in diabetes development has received widespread attention, with increasing numbers of inflammatory mediators and

immune markers, such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and plasminogen activator, being closely associated with diabetes and its complications, and considered predictive factors for obesity and diabetes. Therefore, attenuating the strong pro-inflammatory response generated by LPS and SFA synergy is crucial for diabetes treatment.

Siraitia grosvenorii, commonly known as monk fruit, is a traditional edible and medicinal plant endemic to Guangxi, China, belonging to the Cucurbitaceae family. Analysis has revealed that monk fruit contains abundant nutrients including proteins, vitamins, trace elements, and polysaccharides, along with various bioactive components such as cucurbitane triterpenoid saponins and flavonoids, with cucurbitane triterpenoids being the primary functional constituents. Modern pharmacological research has confirmed that monk fruit not only exhibits antitussive, expectorant, antioxidant, and anti-inflammatory effects, but also significantly ameliorates abnormal glucose and lipid metabolism. Studies have found that administering mogrosides to diabetic mice can prevent and reduce diabetic complications (Qi et al., 2008; Song et al., 2007; Suzuki et al., 2007). Mogroside IIE is a bitter triterpenoid saponin and a precursor of mogroside V, constituting the main component of immature monk fruit. However, the role of mogroside IIE in diabetes treatment remains unknown to date.

Studies have shown that macrophages play a key role in host defense mechanisms and can be activated upon exposure to pro-inflammatory cytokines and bacterial LPS (Xie et al., 1993; Zhang & Ghosh, 2000). Activated macrophages produce large quantities of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, as well as NO and PGE2. These pro-inflammatory factors play critical roles in inflammatory diseases (Vane et al., 1994; Marks-konczalik et al., 1998). Since increased palmitic acid (PA), an abundant fatty acid in plasma, can cause dyslipidemia in type II diabetes, PA represents an important fatty acid involved in diabetes pathogenesis. Meanwhile, clinical studies have reported elevated lipopolysaccharide (LPS) in the blood circulation of obese patients, which can trigger inflammatory responses and activate tissue inflammatory factors through the TLR4 signaling pathway. Therefore, diabetic patients with comorbid obesity may concurrently exhibit elevated PA and LPS levels. Consequently, this study employed combined treatment with LPS and PA on mouse macrophage RAW 264.7 cells to establish a diabetes inflammation model. This model objectively simulates the inflammatory response caused by PA and LPS dysregulation in diabetic patients, while enabling simple, economical, rapid, and objective observation of experimental results, thus possessing certain innovative value. Building upon this diabetes model, we subsequently investigated the effect of mogroside IIE on intracellular inflammatory factor TNF-α mRNA expression, aiming to explore the potential therapeutic possibility of mogroside IIE for diabetes and provide a reference basis for proposing new diabetes treatment strategies.

Materials and Methods

1.1 Materials

Palmitic acid (PA) and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich. Mogroside IIE was obtained from Chengdu Must Bio-Technology Co., Ltd. (http://www.cdmust.com/) and dissolved in methanol to prepare a 20 $mol \cdot L^{-1}$ solution.

1.2 Preparation of PA Stock Solution

A quantity of PA was added to 70% ethanol containing 0.1 $mol \cdot L^{-1}$ NaOH, dissolved in a 70°C water bath, mixed thoroughly, and cooled to room temperature for subsequent use.

1.3 Cell Culture

Mouse macrophage RAW 264.7 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai). Cells were seeded in DMEM complete medium containing 10% fetal bovine serum (Gibco) and cultured at 37°C in a humidified incubator with 5% CO_2.

qRT-PCR Detection of TNF-α mRNA Expression Levels in Treated Cells

RAW 264.7 cells in good condition were collected 24 h after drug treatment. Total RNA was extracted using the Trizol method, and cDNA was synthesized using a reverse transcription kit. Using cDNA as template, SYBR Green Gene Expression Assay kits were employed to detect TNF-α and reference gene GAPDH mRNA expression in each group of cells after transfection. The experiment employed 40 cycles with the following amplification program: predenaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 10 s, and extension at 72°C for 30 s. After amplification, CT values for each group were recorded, and relative TNF-α mRNA expression levels in RAW 264.7 cells were calculated using the $2^{-}\Delta\Delta CT$ method. TNF-α primer sequences were F: 5' -CTT CAG GGA TAT GTG ATG GAC TC-3' and R: 5' -GGA GAC CTC TGG GGA GAT GT-3' ; GAPDH primer sequences were F: 5' -GGTGCTGAGTATGTCGTG-3' and R: 5' -TTCAGCTCTGGGATGACC-3' .

Statistical Analysis

Experimental data are expressed as mean ± standard deviation ($\bar{x} \pm s$). Data analysis was performed using SPSS 21.0 software. Differences among multiple groups were analyzed by one-way ANOVA, while differences between two groups were analyzed by SNK-q test. $P < 0.05$ was considered statistically significant.

Results

2.1 Changes in TNF-α mRNA Expression Levels in RAW 264.7 Macrophages Induced by LPS and PA

The experimental portion of this study consisted of four groups: untreated control (NC), PA alone induction group, LPS alone induction group, and combined PA and LPS induction group. After the aforementioned treatments, cells were collected at six different time points (0, 1, 3, 6, 12, and 24 h), and qRT-PCR was used to detect changes in inflammatory factor TNF-α mRNA expression levels. As shown in [Figure 1: see original paper], TNF-α mRNA expression levels induced by 1 ng $\cdot$ L^{-1} LPS were significantly higher than those in the untreated control (NC) group at 1, 3, 6, and 12 h time points, with statistically significant differences ($P < 0.05$). TNF-α mRNA expression levels induced by 100 mol $\cdot$ L^{-1} PA showed no significant differences from the untreated control (NC) group at 1, 3, 6, 12, and 24 h time points ($P > 0.05$). Intracellular TNF-α mRNA expression levels after combined LPS and PA induction were significantly higher than those in the LPS or PA alone groups at 1, 6, and 24 h time points, with statistically significant differences ($P < 0.05$), and the difference was most pronounced at the 24 h time point. This indicates that combined treatment of RAW 264.7 cells with 1 ng $\cdot$ L^{-1} LPS and 100 mol $\cdot$ L^{-1} PA produced the optimal synergistic effect at 24 h. Therefore, we selected 24 h as the optimal induction time for combined LPS and PA treatment.

2.2 Morphological Changes in RAW 264.7 Macrophages After 24 h Induction with LPS and PA

As shown in [Figure 2: see original paper], mouse macrophage RAW 264.7 cells exhibited morphological changes after 24 h of individual induction with LPS or PA, as well as combined induction with LPS and PA. The results revealed that the LPS+PA combined induction group had the fewest cell numbers with some dead cells present, while the LPS alone induction group had the highest cell numbers, further demonstrating that combined treatment of mouse macrophages with 1 ng $\cdot$ L^{-1} LPS and 100 mol $\cdot$ L^{-1} PA for 24 h produced the best inflammatory synergistic effect. Therefore, 24 h was selected as the optimal induction time for combined LPS and PA treatment.

Figure caption:
A. Negative control; B. Palmitic acid; C. Lipopolysaccharide; D. Palmitic acid + Lipopolysaccharide. All scale bars = 200 m.
Fig. 2 Morphological changes in mouse macrophages induced by LPS and PA for 24 h

2.3 Mogroside IIE Effectively Counteracts the Inflammatory Synergistic Effect Induced by LPS and PA

qRT-PCR was used to detect TNF-α mRNA expression levels of inflammatory factors at four different time points (0, 6, 12, and 24 h) [Figure 3: see original

paper]. The results showed that 12 h treatment with 20 $\mu mol \cdot L^{-1}$ mogroside IIE significantly reduced the TNF-α mRNA expression level generated by combined LPS and PA induction, with statistically significant differences ($P < 0.05$), while showing no significant effect on TNF-α mRNA expression levels induced by LPS or PA alone ($P > 0.05$). This demonstrates that mogroside IIE can inhibit the cellular inflammatory response induced by combined LPS (1 $ng \cdot L^{-1}$) and PA (100 $\mu mol \cdot L^{-1}$), thereby effectively reducing the inflammatory level in macrophages.

Figure caption:
Fig. 3 Changes of TNF-α mRNA expression in mouse macrophages induced by PA and LPS alone or in combination with mogroside IIE for 12 h in each group

Discussion and Conclusion

The regulation of diabetes-related inflammation is crucial for diabetes treatment. Guariguata et al. (2014) demonstrated that diabetes is a complex metabolic disease characterized by hyperglycemia that severely impacts patients' quality of life. Wellen et al. (2005) showed that this metabolic disorder is closely associated with abnormal production of inflammation and activation of inflammatory signaling pathways. The concentrations of LPS and PA selected in this study to simulate the diabetes inflammation model in mouse macrophages are consistent with those reported by Xiao et al. (2020), and our experimental results also demonstrate that the combination of these two agents at these concentrations can induce an inflammatory synergistic effect. Moreover, inflammatory responses in diabetic patients can cause damage to human cells, indicating that the selected concentrations are feasible.

Our research group previously published findings by Dr. Xiao Juan in 2020 showing that 20 $\mu mol \cdot L^{-1}$ mogroside could ameliorate pancreatitis in both cell models and mice by downregulating the IL-9/IL-9 receptor pathway (Xiao et al., 2020). Due to the numerous experimental groups involved in the current study, we only examined TNF-α mRNA expression changes induced by combined LPS and PA at different time points when treating the mouse macrophage inflammation model with mogroside IIE. The present study found that 12 h treatment produced the optimal effect, with 20 $\mu mol \cdot L^{-1}$ mogroside IIE significantly reducing the synergistic inflammatory response induced by combined PA and LPS in cells, while showing no significant effect on inflammation induced by PA or LPS alone. However, the specific underlying mechanism remains unclear and warrants further in-depth investigation.

Mogroside IIE is extracted from the edible monk fruit, and the sugars in monk fruit produce weak glycemic effects in humans. Based on this characteristic, mogroside IIE could become an important direction for adjuvant therapy in diabetic patients. Therefore, the findings of this project will provide important guidance for developing effective anti-diabetic inflammatory drugs and improving the quality of life for diabetic patients. Additionally, mogroside IIE is the

most abundant glycoside in immature monk fruit, and a certain proportion of immature monk fruit is harvested each season. The results of this study will not only help improve the utilization value of immature monk fruit but also benefit the enhancement of the economic value of mature monk fruit.

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Note: Figure translations are in progress. See original paper for figures.