Original Article

Autophagy protects peripheral blood mononuclear cells from high glucose-induced inflammation and apoptosis

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ABSTRACT

Previous works have linked high concentrations of glucose to cellular toxicity through autophagy modulation. However, the ways in which high glucose (HG) regulates inflammation and apoptosis in peripheral blood mononuclear cells (PBMCs) have not been well characterized. In the present study, the role of autophagy in inflammatory responses and apoptotic death of PBMCs exposed to HG was investigated. 33mM glucose (HG) increased the level of LC3-II at 12h, 24h, and 48h. NH4Cl, a lysosome inhibitor that can block autophagic flux, further promoted LC3-II accumulation in HG-treated cells at 12h, 24h, and 48h. The protein level of p62 significantly decreased from 12h to 48h in HG-treated cells, suggesting an induction of autophagic flux in HG-treated PBMCs. Inhibiting autophagy with chloroquine (CQ) significantly augmented HG-induced PBMCs apoptotic death, as demonstrated by increased cleaved PARP and Cyt C levels and an increased percentage of apoptotic (YO-PRO-1 positive and PI negative) cells. Furthermore, CQ pretreatment exacerbated HG-induced TNF-α, IL-6, and IL-1β mRNA expression in PBMCs. In conclusion, this data revealed that the autophagy system could be activated in HG-treated PBMCs. The results also indicated that the induction of autophagy might play an adaptive and protective role in HG-induced inflammation and apoptotic death of PBMCs.

Keywords: High glucose, PBMC, autophagy, inflammation, apoptosis, LC3-II

Abbreviations: CQ: Chloroquine, FFA: Free Fatty Acids, TNF-α: Tumor Necrosis Factor alpha, IL-6: Interleukin 6, PBMC: peripheral blood mononuclear cell, TLR: Toll-like receptor, LC3: light chain 3, T2D: type 2 diabetes

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Introduction

Low-grade inflammation plays an important role in the pathogenesis of type 2 diabetes (T2D) (1). It is defined as an elevation of circulating pro-inflammatory cytokines and an increase in macrophage infiltration into adipose, liver, skeletal muscle, and pancreas tissues (2). Reports indicate that low-grade inflammation can induce insulin resistance and β cell dysfunction in patients with T2D (1). In addition to adipose tissue, which is considered the main site of pro-inflammatory cytokine production in the body, peripheral blood mononuclear cells (PBMCs) can

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be another source of inflammatory cytokine production in patients with T2D (3). The level of secreted TNF-α, IL-1β, and IL-6 were found to be elevated in PBMCs of T2D patients (4). Nutrient overload, particularly increased circulating free fatty acids and glucose levels, has been suggested as the main cause of enhanced pro-inflammatory signaling in PBMCs of diabetic patients. High glucose exposure to human monocytes and PBMCs results in increased production of pro-inflammatory cytokines in a toll-like receptor-4 (TLR-4)-dependent manner (5, 6).

Macroautophagy (here referred to as "autophagy") is an evolutionarily conserved dynamic pathway that primarily functions in a degradative manner (7). Autophagy plays a housekeeping role in removing aged and misfolded proteins and organelles such as mitochondria and endoplasmic reticulum (8). In autophagy, targeted proteins and organelles are delivered into double-membrane autophagosomes for lysosomal degradation (9). Implications of autophagy have been discovered in numerous cellular processes such as quality control of both proteins and organelles, cellular differentiation, development, survival, and metabolism (10). A basal level of autophagy occurs constitutively, but this process can be further induced in response to various types of stress including starvation, hypoxia, and hormonal stimuli. Through this basic mechanism, autophagy has a critical role in cellular homeostasis; however, either insufficient or excessive autophagy can seriously compromise cell physiology (10). In this regard, the dysregulation of autophagy has been associated with a wide range of diseases including cardiovascular diseases, cancer, and diabetes (11). Importantly, it was suggested that an autophagy defect in liver, skeletal muscle, and pancreatic β cells can contribute to diabetes development (12).

Autophagy has been reported to regulate immune signaling pathways (13). It has been suggested to represent an ancient form of innate immune response to infection. Autophagy improves host defense systems through several mechanisms, including the direct elimination of invading pathogens, control of adaptive immunity, induction of innate immune memory, and modulation of inflammation (14). In this regard, the engagement of various families of pattern-recognition receptors (PRRs) has been reported to induce autophagy in mouse and human macrophages (15). In addition to PRRs, several pro-inflammatory cytokines, including TNF-α (tumor necrosis factor alpha) and IL-1β (interleukin 1β), induce autophagy (16). On the other hand, pharmacological or genetic inhibitions of autophagy resulted in higher IL-1β production upon pathogen-associated molecular patterns (PAMPs) stimulation in macrophages (17). Therefore, any dysregulation of autophagy may play an essential role in the pathogenesis of inflammatory diseases such as atherosclerosis, cancers, diabetes, and Crohn's disease.

Glucose is the main source of energy in the form of ATP. In recent years, the importance of autophagy regulation by glucose has been highlighted. High glucose (HG), depending on the cell type, can activate or inhibit the autophagy pathway (18). High glucose, known as an inducer of pro-inflammatory responses, has also been implicated in the modulation of autophagy (19). In the present study, it was questioned whether autophagy is involved in high glucose-induced inflammatory responses in PBMCs of healthy subjects. Previous works in pancreatic β-cells and endothelial cells have linked high concentrations of glucose with cellular toxicity by autophagy modulation (20, 21). However, the ways in which this nutrient regulates apoptosis in PBMCs have not been well characterized. In this study, the aim was to investigate the role of autophagy in inflammatory responses and apoptotic death of PBMCs treated with HG. The stimulus was explored to mimic in vitro the metabolic alteration observed in T2D. It was found that HG induces autophagic flux in PBMCs. Inhibition of autophagy by treatment with chloroquine further promoted inflammatory responses and apoptotic death, indicating a protective role for autophagy in regulating inflammation and apoptosis in PBMCs exposed to HG.

Materials and methods

Materials

Fetal bovine serum (FBS) was purchased from Life Technologies (Gaithersburg, USA). Tissue culture flasks and disposable plasticware were purchased from Greiner Bio-One (Frickenhausen, Germany). Phenyl methyl sulfonyl fluoride (PMSF) and protease inhibitor cocktail were from Roche (Mannheim, Germany). Polyvinylidenedifluoride (PVDF) membrane was from Millipore (Schwalbach, Germany). ECL reagents were from Amersham Pharmacia Corp. (Piscataway, NJ, USA). Propidium Iodide and YO-PRO-1 were from Thermo Fisher Scientific (Waltham, MA, USA). All other reagents and chemicals were from Sigma Aldrich (Taufkirchen, Germany).

Subjects

This study was conducted on PBMC cells of 5 healthy male adults. Inclusion criteria were fasting blood glucose less than 100 mg/dl, age between 20 and 30, no current or history of acute or chronic inflammatory diseases, lipid disorders, malignant diseases, diagnosed or suspected endocrine disorders, no treatment with anti-inflammatory drugs such as aspirin, antioxidant supplements and vitamins, no history of smoking and alcohol consumption, and no family history of diabetes. The Ethics Review Board of Tehran University of Medical Sciences approved the study. Written informed consent was obtained from all participants.

Blood sampling

450 ml of venous blood (equivalent to one unit of blood) was taken from healthy volunteers at Tehran Blood Transfusion Organization. PBMCs were isolated from the buffy coat using Ficoll–Hypaque density-gradient centrifugation. The PBMCs were washed twice with PBS and resuspended in RPMI 1640.

Treatment with glucose

The cells were cultivated in DMEM medium, supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 µg/ml streptomycin. Cells were maintained at 37 °C (in an atmosphere of 5% CO2). PBMCs were treated with 5.5mM and 33mM as normal glucose (NG) and high glucose (HG), respectively, for 12h, 24h, and 48h. To eliminate the effect of osmolality on the measurements, 27.5 mM mannitol was added to the culture medium of 5.5mM glucose. For quantitative real-time polymerase chain reaction (qRT-PCR) experiments, 3×106 cells, for western blot detections 7×106, and for flow cytometry 1×106 cells were used. To evaluate the autophagy flux, 25 nM ammonium chloride (NH4cl) was added to the media 2h before harvesting the cells.

Real-time PCR

Total RNAs were extracted from treated PBMCs using GeneAll RibospinTM total RNA purification kit (GeneAll Biotechnology, South Korea). A total of 1 µg RNA was reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Gene expression analysis was evaluated using qRT-PCR. qRT-PCR was performed in triplicates using SYBR Green RealQ Plus 2x Master Mix Green (Ampliqon) on Corbett Rotor Gene 6000 Light Cycler (Qiagen, Hilden, Germany). The data were normalized against β-actin transcript level. The amplification protocol for 40 cycles was as follows: 15 min at 95 ºC for initial activation, 30s at 95 ºC for denaturation, and 60s at 60 ºC for annealing/extension. The delta-delta CT method was used to calculate relative expression. The sequences of the primers used in this study are shown in the supplementary file.

Western blot analysis

Cell lysate was prepared by homogenization in modified RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% Triton X-100, and 0.2% sodium deoxycholate, 0.2% SDS, 1 mM Na-EDTA, and 1 mM PMSF) supplemented with protease inhibitor cocktail. After determining protein concentrations, equal amounts of protein were subjected to SDS–PAGE, followed by transfer onto PVDF membrane. Blocking was carried out through 1h incubation at room temperature with 5% non-fat dry milk in TBS with 0.5% Tween-20. Blots were incubated overnight with antibodies against LC3-II, p62, cleaved PARP (all from Cell Signaling Technology Beverly, MA, USA), and β-actin (Abcam, Cambridge, MA, USA) at 4 °C. The bands after incubating with second HRP-conjugated antibodies were visualized using an enhanced chemiluminescent substrate (ECL).

Apoptosis quantified by flow cytometry

To assess apoptosis, 1×106 cells were plated onto culture plates and treated with HG for 12h. After treatment, the cells were collected and rinsed twice with cold PBS and then resuspended in 0.5 mg/ml propidium iodide and 10µM YO-PRO-1. The suspension was incubated for 30 min in the dark at room temperature. YO-PRO-1 or PI-positive cells were determined using a flow cytometer (Becton Dickinson FACs caliber, Canada). The fluorescence of YO-PRO-1 and PI was measured in the FL1 channel (488 nm) and FL3 channel (488 nm), respectively. The results were analyzed using FlowJo software (Treestar, USA).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 22 (IBM, USA). Comparisons among all groups were performed with an unpaired student's t-test or one-way analysis of variance (ANOVA) test. If statistical significance was found, the Tukey post hoc test was performed. Values of $P < 0.05$ were considered statistically significant. Results are expressed as mean ± SEM of five independent experiments.

Table 1: Sequences of primers used in this study

Name	Forward primer	Reverse Primer
TNF-α	AGGACCAGCTAAGAGGGAGA	CCCGGATCATGCTTTCAGTG
IL-6	TTCGGTCCAGTTGCCTTCTC	GAGGTGAGTGGCTGTCTGTG
β-Actin	GCAAGCAGGAGTATGACGAG	CAAATAAAGCCATGCCAATC
IL-1β	CCTGTCCTGCGTGTTGAAAGA	GGGAACTGGGCAGACTCAAA
Beclin	CCAGGATGGTGTCTCTCGCA	CTGCGTCTGGGCATAACGCA
LAMP2	GCACAGTGAGCACAAATGAGT	CAGTGGTGTGTATGGTGGGT

Results

The Effect of Glucose on the Expression of Inflammatory Cytokines

The mRNA expression of TNF-α, IL-6, and IL-1β in PBMCs exposed to 5.5mM (NG) and 33mM (HG) glucose for 12h, 24h, and 48h was first studied. As shown in Fig. 1, 33mM glucose (HG) caused a time-dependent induction of TNF-α and IL-6 expression, whereas the increase in IL-1β mRNA expression peaked at 24h. HG treatment for 12h increased the expression of IL-6, IL-1β, and TNF-α by 44%, 42%, and 34%, respectively. HG treatment for 24h caused an induction of the expression of IL-6, IL-1β, and TNF-α by 64%, 83%, and 62%, respectively. The increases in the expression of IL-6, IL-1β, and TNF-α genes after 48h of HG treatment were 86%, 65%, and 93%, respectively.

High glucose induces autophagic flux in PBMCs

To examine whether autophagy was affected by HG treatment in PBMCs, several markers of autophagy were investigated. The mRNA expression of Beclin-1 and LAMP-2 (Fig. 2A-B) was significantly upregulated from 12h to 48h. In a further experiment, the conversion of LC3-I to LC3-II, a hallmark of autophagy activation, was assessed. Western blot analysis showed that the levels of LC3-II were time-dependently increased in HG-treated cells compared to the cells treated with NG (Fig. 3C). These findings suggest an increase in the number of autophagosomes in HG-treated cells. Accumulated LC3-II could be attributed to increased autophagosome formation or decreased lysosomal fusion and degradation. To verify whether autophagosome accumulation by HG resulted from impaired clearance due to defective fusion with lysosomes or from true autophagic flux, LC3-II accumulation was detected in the presence or absence of ammonium chloride (NH4Cl), a lysosome inhibitor that can block autophagic flux. Compared with cells treated only with HG, the cells treated with HG and NH4Cl for 12, 24, and 48h had more accumulation of LC3-II (Fig. 3D). The difference in the amount of LC3-II without blocking lysosomal degradation from the value with lysosomal degradation indicates that HG stimulates autophagic flux in PBMCs. This result, however, does not rule out the possibility of a subsequent impairment of lysosomal degradation. To rule out impairment of lysosomal degradation, p62, an indication of autophagic degradation in HG-treated cells, was detected. p62 is a

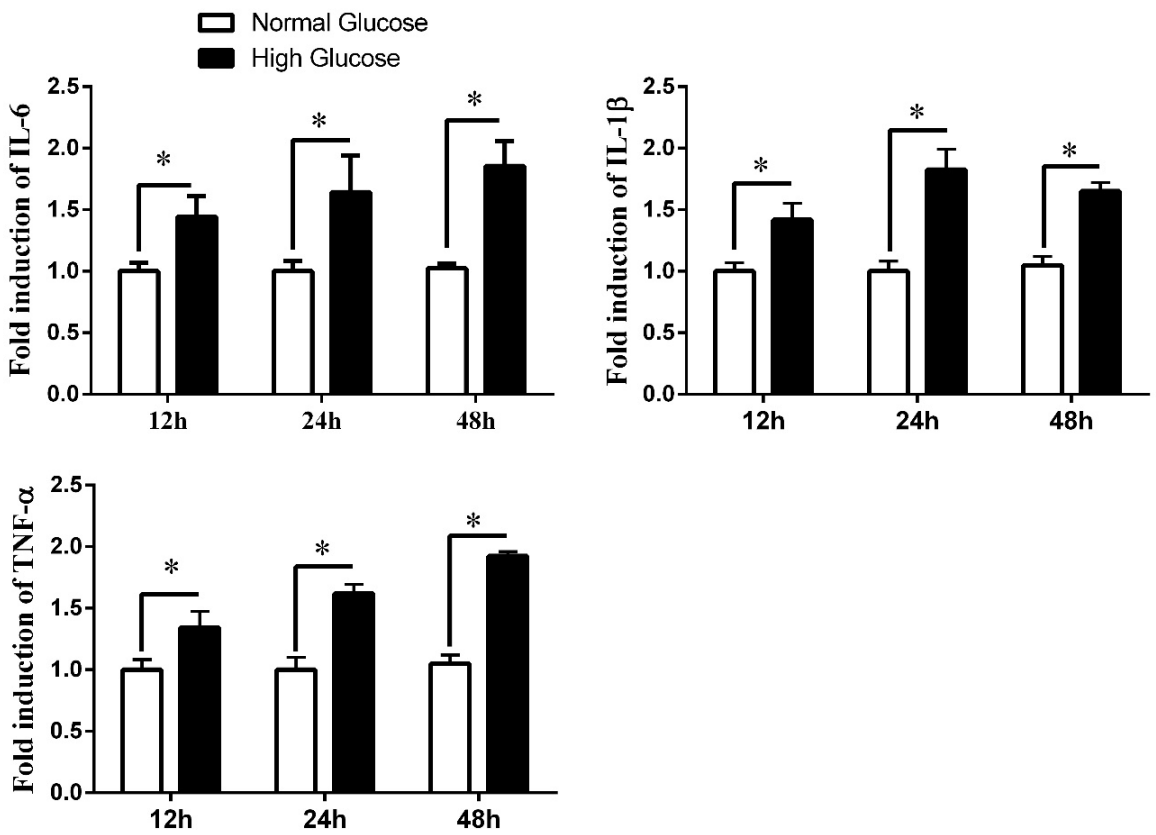

Figure 1: Effect of high glucose (HG) on the expression of IL-6, IL-1β and TNF-α in PBMCs of the healthy subjects. PBMCs were treated with 5.5mM (NG) and 33mM (HG) glucose for time points (12h, 24h, and 48h). The expression level of IL-6, IL-1β and TNF-α was examined by quantitative real-time PCR. The results are expressed as mean ± SEM of five independent experiments. *$p < 0.05$ vs. the control group. NG: normal glucose; HG: high glucose

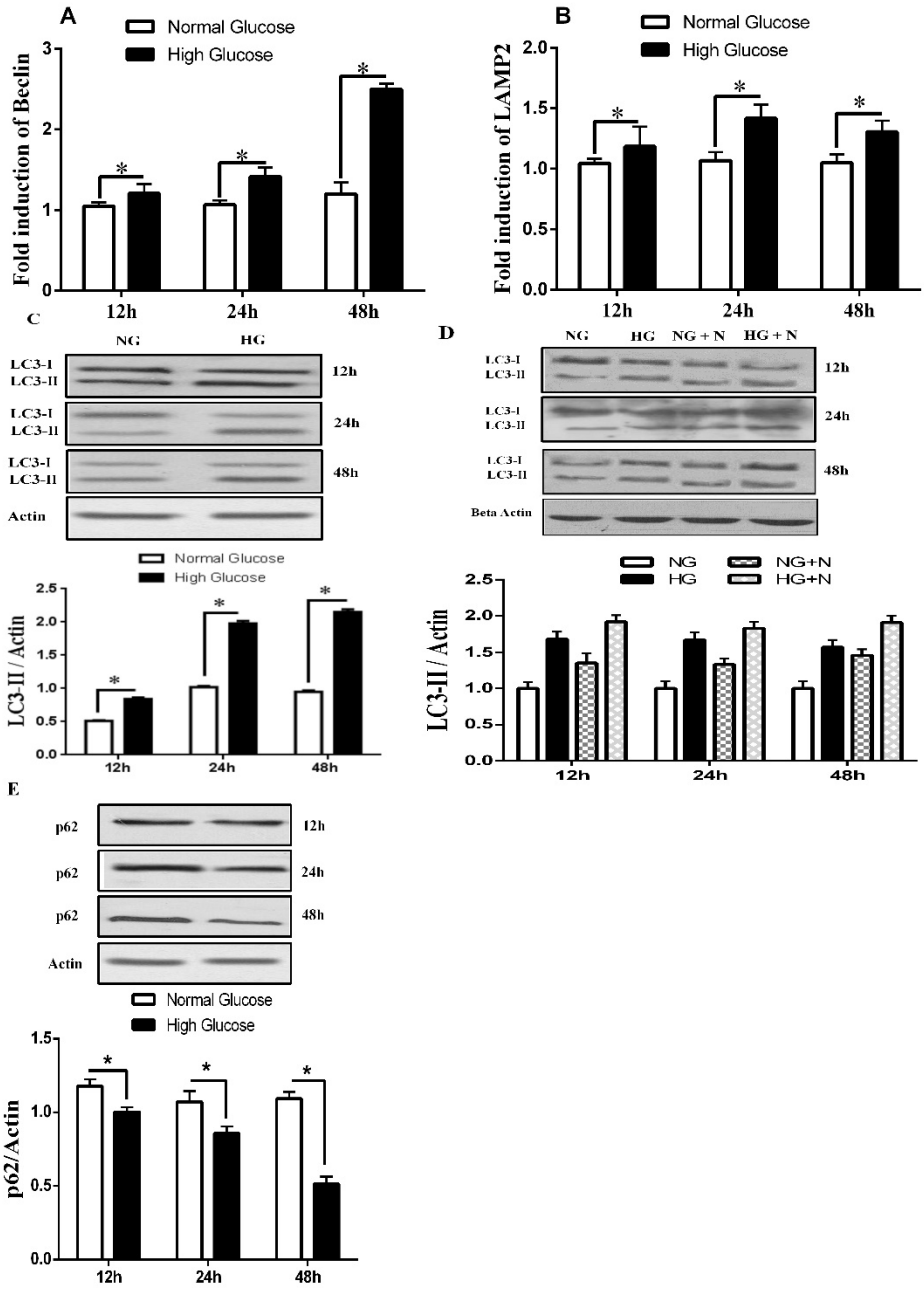

Figure 2: Effect of high glucose (HG) on autophagic flux in PBMCs. A-B: Effects of HG on the expression of Beclin-1 and LAMP2 in PBMCs of the healthy subjects. PBMCs were treated with 5.5mM (NG) and 33mM (HG) glucose for time points (12h, 24h, and 48h). The expression level of Beclin-1, LAMP2 was examined by quantitative real-time PCR. C: Western blot analysis of LC3-II after treatment of PBMCs with HG for 12h, 24h and 48h. D: Western blot analysis of LC3-II after treatment of PBMCs with HG for 12h, 24h and 48h and then incubation with NH4cl in the last 2 hours before harvesting. E: p62 protein levels after treatment of PBMCs with HG for 12h, 24h and 48h. The results are expressed as mean ± SEM of five independent experiments. *p < 0.05 vs. the control group. NG: normal glucose; HG: high glucose

key substrate of autophagy whose primary function is to be involved in turnover of ubiquitinated proteins (22). Treatment with HG significantly decreased p62 protein level at 12h, 24h, and 48 h (Fig. 3E), suggesting that HG increased autophagic flux in PBMCs. Taken together, the data suggest that exposure of PBMCs to HG leads to increased autophagic flux in PBMCs.

Inhibition of autophagy exacerbates HG-induced inflammation in PBMCs

To investigate the relationship between HG-induced inflammation and autophagy, chloroquine (CQ), an inhibitor of autophagic flux, was used. The cells were first pretreated with 50μM of CQ. As shown in Fig.

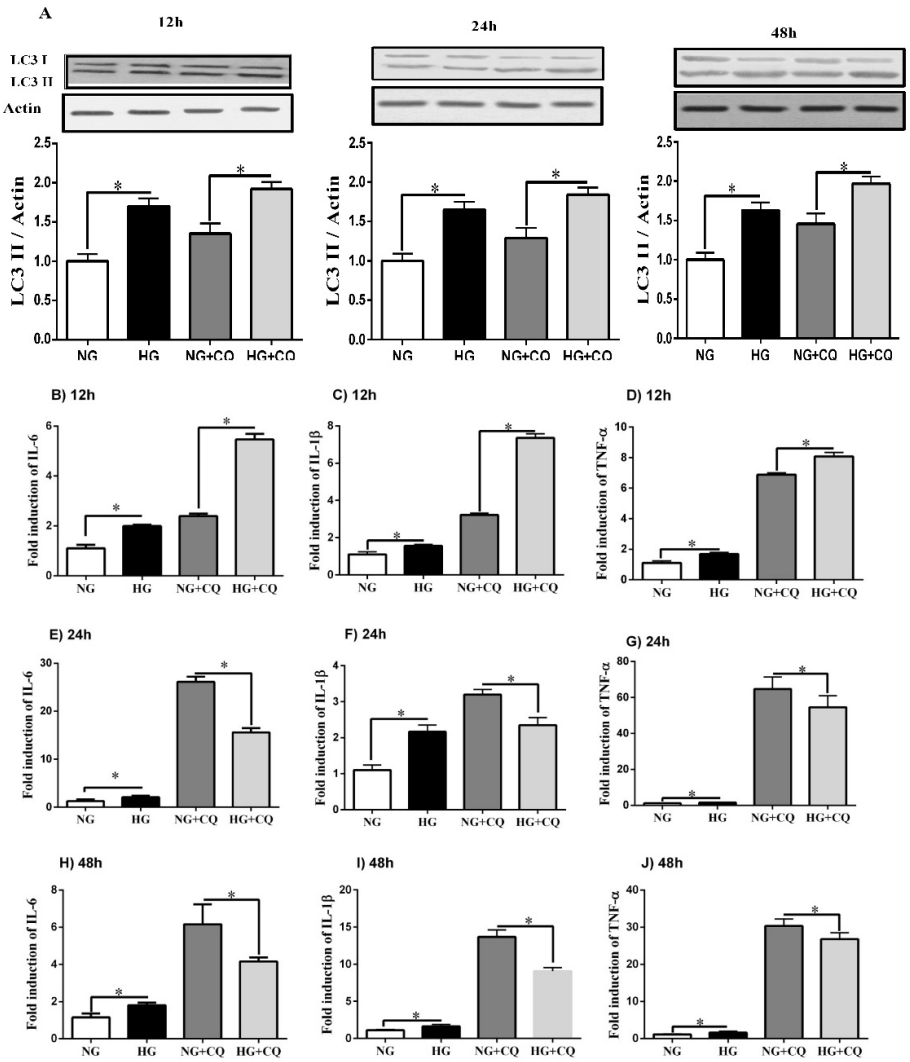

Figure 3. Effect of autophagy inhibition on pro-inflammatory cytokines expression in HG-exposed PBMCs. A: Western blot analysis of LC3-II after treatment of PBMCs with 50μM chloroquine (CQ) for 12h, 24h and 48h. B-D: Effect of 50μM CQ on the expression of TNF-α, IL-6 and IL-1β for 12h in PBMCs of the healthy subjects. E-G: Effect of 50μM CQ on the expression of TNF-α, IL-6 and IL-1β for 24h in PBMCs of the healthy subjects. H-J: Effect of 50μM CQ on the expression of TNF-α, IL-6 and IL-1β for 48h in PBMCs of the healthy subjects. The results are expressed as mean ± SEM of five independent experiments. *$p < 0.05$ vs. the control group. NG: normal glucose; HG: high glucose

4A-C, pretreatment with CQ further enhanced the accumulation of LC3-II in HG-treated cells for 12h, 24h, and 48h, suggesting inhibition of autophagic flux in CQ-treated cells. Treatment of PBMCs with 50μM CQ for 12h significantly increased the mRNA expression levels of TNF-α, IL-6, and IL-1β compared to the cells treated with HG alone, indicating the key role of autophagy in regulating HG-induced inflammation in PBMCs (Fig. 4D). However, after 24h and 48h of HG treatment, a significant reduction in the expression of TNF-α, IL-6, and IL-1β was observed in CQ-treated compared to HG-treated cells (Fig. 4E-F). Furthermore, the expression of pro-inflammatory markers was elevated by treatment with CQ alone. This implies that basal autophagy plays a key role in controlling inflammatory responses in PBMCs.

Augmentation of HG-induced cell death by blockage of autophagy

To investigate whether impaired autophagy exacerbated or prevented HG-induced cell death, the autophagic flux in HG-treated at the stage of lysosomal degradation was decreased and the effect of autophagy inhibition on HG-induced cell death was then investigated. PBMCs were first treated with CQ for 12h. As shown in Fig. 4A, the level of cleaved PARP and Cyt C was augmented by co-treatment of HG and CQ compared with HG and CQ alone. This finding was confirmed by staining the cells simultaneously with YO-PRO-1 and PI. YO-PRO-1 is a sensitive marker of early apoptotic events and PI is a marker of necrosis (23). The results of flow cytometry showed that treatment of

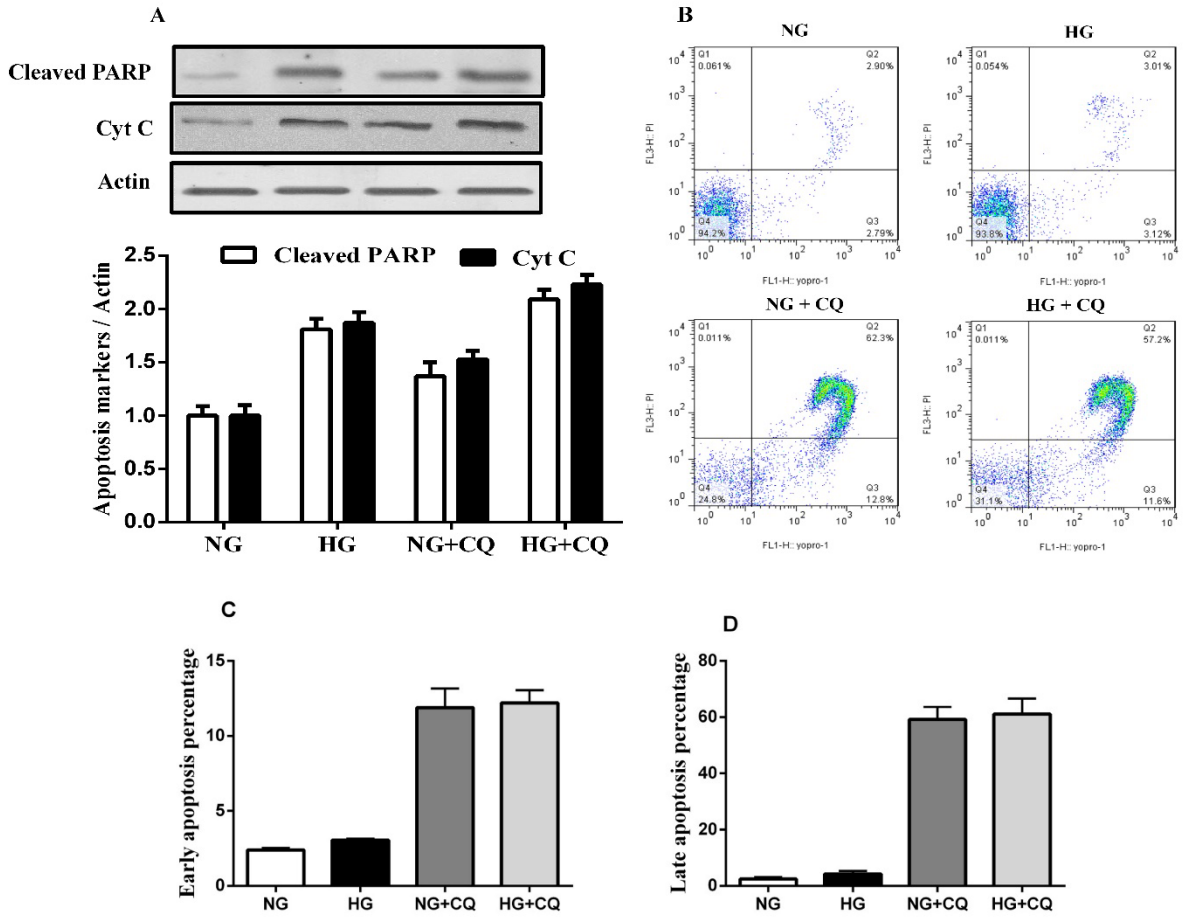

Figure 4. Effect of autophagy inhibition on apoptotic death of HG-exposed PBMCs. A: Western blot analysis of apoptosis-related protein, cleaved PARP and Cyt C, after exposing the cells to 50μM of CQ for 12 h. B: After treatment with HG and 50μM CQ for 12h, induction of apoptosis was measured by YO-PRO1/PI double-staining assay followed by flow cytometry analysis. C: The level of apoptotic cells (early apoptosis) according to the flow cytometry results. D: The level of apoptotic cells (late apoptosis) according to the flow cytometry results. The results are expressed as mean ± SEM of five independent experiments. *p < 0.05 vs. the control group. NG: normal glucose; HG: high glucose

the cells with CQ and HG alone for 12h significantly increased the percentage of apoptotic (YO-PRO-1 positive and PI negative) events compared to untreated cells (Fig. 4B-C). Moreover, co-treatment of PBMCs with CQ and HG for 12h significantly promoted the level of apoptotic cell death. However, a remarkable increase in the number of necrotic cells (YO-PRO-1 positive and PI positive) was found in simultaneous treatment with CQ and HG.

Discussion

It has become well known that chronic low-grade inflammation contributes to the development of T2D. Increased exposure to HG has been demonstrated as one of the key activators of both altered metabolic and immune signaling in diabetes (1). Furthermore, HG causes metabolic abnormalities and cell death in different cell types, including β cells, hepatocytes, adipocytes, PBMCs, and cardiomyocytes (24). The mechanisms by which HG induces toxicity are not completely known. Autophagy has been suggested to play a role in HG-induced apoptosis and inflammation (13). However, the function of autophagy in inflammatory responses and apoptotic death of PBMCs exposed to HG is not well understood. Importantly, it is not clear how HG, as observed in patients with T2D, modulates autophagy in PBMCs. The present study was designed to examine the importance of autophagy in regulating the inflammatory responses and apoptotic death of PBMCs treated with HG.

Elevated concentrations of glucose have been demonstrated to modulate the autophagy pathway in adipocytes, hepatocytes, and pancreatic β cells (22, 25, 26). In this study, PBMCs were treated with HG to model the pathological stress of T2D. The autophagy pathway in PBMCs was first investigated by measuring the expression level of two important genes, Beclin-1 and LAMP2. Beclin-1 and LAMP2 are involved in initial nucleation/formation and maturation of the

autophagosome, respectively (25). The data showed that the expressions of these genes were time-dependently increased after HG treatment. Furthermore, the protein level of LC3-II as a surrogate indicator of autophagosome abundance and formation was measured. Compared with control-treated cells, PBMCs exposed to HG for 12h, 24h, and 48h had an increased protein level of LC3-II. Since LC3-II is continually turned over during autophagy, LC3-II level was evaluated in the presence of an autophagic inhibitor such as NH4cl. Treatment of the cells with NH4cl led to further accumulation of LC3-II protein in the cells exposed to HG. Eventually, protein levels of p62, a selective substrate for autophagy, were detected. In general, p62 protein accumulates when autophagy is impaired (27). Compared with control cells, PBMCs treated with HG from 12h to 48h had more accumulated p62 protein levels. Taken together, these results demonstrate that autophagic flux is induced in HG-treated PBMCs. Similar to these findings, it was reported that HG could induce the autophagy pathway in different cells such as pancreatic β cells and podocytes (28, 29).

Autophagy has been closely linked to the control of innate and adaptive immune responses, in part by regulating cytokine production (30). Recently, studies have clearly indicated that defects in autophagy contribute to the inflammatory response in T2D. For instance, autophagy inhibition by genetic ablation of the regulator Atg16L1 or ATG-7 enables lipopolysaccharides (LPS)-dependent inflammasome activation, suggesting that autophagy normally counters inflammasome activation by LPS (31, 32). Defective hypothalamic autophagy directs the central pathogenesis of obesity by induction of inflammation in mice fed a high-fat diet (33). Depletion of the autophagic proteins LC3-II and Beclin-1 also enhanced the activation of caspase-1 and secretion of IL-1β and IL-18 in macrophages (34). Induction of autophagy in diabetic and in C57BL/6 mice fed a high-fat diet revealed low-grade systemic inflammation (35). Excessive autophagic activation by palmitate resulted in an unexpected significant increase in IL-1β, IL-6, and TNF-α production in pancreatic β cells (32). In addition, inhibition of autophagy in human and mouse adipose tissue explants led to a significant increase in IL-1β and IL-8 mRNA expression and protein secretion (36). To investigate whether autophagy induction by HG was an important contributor to increased expression of pro-inflammatory genes, the expression levels of these genes were first detected in PBMCs exposed to HG. The expression level of IL-6, TNF-α, and IL-1β was induced in PBMCs after 12h, 24h, and 48h treatment with HG. In agreement with these results, it was reported that hyperglycemia increases the expression of inflammatory TNF-α and IL-6 cytokines in PBMCs (37). Pharmacological inhibition of autophagy by CQ for 12h further increased cytokine expression levels in HG-treated cells, indicating that autophagy has a protective role against HG-induced pro-inflammatory responses of PBMCs. Compared to 12h treatment, lower expression of inflammatory cytokines was observed in PBMCs co-treated with HG and CQ for 24 and 48h. These observations could be due to higher apoptotic death of PBMCs in longer time exposures. Consistent with what has been mentioned above, previous studies have supported the hypothesis that autophagy has a protective role in pro-inflammatory responses (31, 32). For instance, it was reported that autophagy inhibition by 3-Methyladenine (MA-3) or shRNA against Beclin1 or Atg5 causes an increase in TNF-α production in keratinocytes (38). Taken together, the findings that autophagy inhibition by CQ could augment pro-inflammatory responses in HG-exposed PBMCs suggest autophagic dysregulation as a new mechanism linking HG exposure to inflammation in PBMCs.

Accumulating evidence has suggested a paradoxical role of autophagy in controlling cell death and survival under various stimulus conditions (39). On one side, autophagy has been proposed to have a crucial role in the maintenance of normal cellular function and survival, and its dysregulation might contribute to the pathogenesis of diseases such as T2D (35, 40). On the other hand, recent studies indicate that autophagy itself may be a mechanism of caspase- and apoptosis-independent cell death (41). After observing that autophagy is induced in HG-treated PBMCs, the question of whether autophagy could play a role in HG-induced cell death was addressed. Several markers of apoptosis were evaluated and it was found that HG could increase apoptosis of PBMCs. This was demonstrated by increased cleaved PARP and Cyt C level and YO-PRO1/PI positive cells in flow cytometry. To demonstrate the role of autophagy in HG-mediated apoptotic cell death, the autophagy inhibitor CQ was utilized to disrupt lysosomal function and prevent completion of autophagy. Pretreatment with CQ further promoted apoptosis in HG-treated PBMCs, indicating a protective role for autophagy against HG-induced apoptosis in PBMCs.

In conclusion, this study revealed that the autophagy system could be activated in HG-treated PBMCs. The results also indicated that induction of autophagy might play an adaptive and protective role in HG-induced inflammation and apoptotic death of PBMCs. Therefore, the autophagy mechanism in PBMCs may be a novel mechanism that connects high glucose to low-grade inflammation in patients with T2D.

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