

Effect And Mechanism of Endoplasmic Reticulum Stress Induced By Tunicamycin In Trabecular Meshwork Cells

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Research Article

Keywords: Endoplasmic reticulum stress, trabecular meshwork , glaucoma

Posted Date: July 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-733716/v1>

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Abstract

Objective: To explore the role of endoplasmic reticulum stress in apoptosis of trabecular meshwork cells

Methods: Human trabecular meshwork cells (HTMCs) were cultured in vitro. When the cells grew to logarithmic phase, they were digested and resuspended, and then cultured in 96-well plate. When the cells are close to fusion, the culture is continued for 24h in a serum-free medium. The HTMCs were treated with different concentrations of tunicamycin (0mg/L, 1.25mg/L, 2.5mg/L, 5mg/L, 10mg/L, and 20mg/L) for different durations (0h, 6h, 12h, 24h, 36h, and 48h). The cytoactive was detected by CCK-8, and the optimal intervention concentration of tunicamycin was determined. Then we use the 5mg/L tunicamycin to interfere with HTMCs for different durations (0h, 6h, 12h, and 24h). The apoptotic rate was detected by flow cytometry, and the expression levels of GRP78, CHOP, Bcl-2 and Caspase-3 were detected by RT-PCR and Western blot.

Results: The number of RGCs in IRI group was significantly lower than that in NC group ($P < 0.05$), demonstrated by HE staining. Western blotting results indicated that the protein expression of LC3 and Beclin-1 in the IRI group were significantly elevated compared with those in the NC group ($P < 0.05$). However, with 3-MA treatment, the number of RGCs in 3-MA treated-IRI group was elevated and protein levels of LC3, Beclin-1 were downregulated, compared with those in the IRI group ($P < 0.05$). Further immunohistochemistry staining and Western blot showed that 3-MA treated-IRI group presented downregulated Caspase-3 and upregulated Bcl-2 protein expression with comparison of IRI group ($P < 0.05$).

CCK-8 results showed that the inhibition rate of HTMCs increased with the increase of tunicamycin concentration and duration of intervention. When the concentration of tunicamycin was 1.25mg / L and 2.5mg / L, there was no significant difference in cell inhibition rate compared with the control group ($P > 0.05$). When the concentration of tunicamycin was 5mg / L and the duration of action was 12h or more, the cells showed significant inhibition, of which the cell inhibition rates at 12h, 24h, 36h and 48h were $(21.11 \pm 1.23)\%$ and $(31.34 \pm 0.86, \text{ respectively}) \%$, $(44.37 \pm 1.01)\%$, $(51.47 \pm 0.36)\%$, compared with the 0h group, the differences were statistically significant ($P < 0.05$).

The results of flow cytometry suggest that with the increase of the time of action of tunicamycin, the early apoptosis rate and the late apoptosis rate of the cells increase significantly. Compared with the control group, the experimental group with different intervention durations had significantly higher early cell apoptosis rate and late cell apoptosis rate, and the difference was statistically significant ($P < 0.05$).

RT-PCR test results indicated that the expression levels of GRP-78, CHOP, and Caspase-3 mRNA in the experimental groups (6h, 12h, and 24h) with different intervention durations were all increased compared with the control group, and the difference was statistically significant ($P < 0.05$); BCL-2 mRNA expression in the experimental groups (6h, 12h, 24h) with different intervention durations was reduced compared with the control group, and the difference was statistically significant ($P < 0.05$).

Western blot test results showed that compared with the control group, GRP-78 and CHOP protein expressions of the experimental groups (6h, 12h, 24h) with different intervention durations increased, the difference was statistically significant ($P < 0.05$). Compared with the control group, the expression level of Caspase-3 protein increased in the 12h group and the difference was statistically significant ($P < 0.05$). The increase in the expression level of the remaining experimental group was not obvious, and the difference was not statistically significant ($P > 0.05$); 24h Compared with the control group, the expression level of BCL-2 protein was significantly reduced, the difference was statistically significant ($P < 0.05$), the expression level of BCL-2 protein in the remaining experimental group was not significantly reduced, and the difference was not statistically significant ($P > 0.05$).

Conclusion: Tunicamycin can increase the expression of endoplasmic reticulum stress proteins in trabecular meshwork cells and induce apoptosis. Our study demonstrated that endoplasmic reticulum stress played an important role in the apoptosis of trabecular meshwork cells.

Introduction

The glaucoma is an eye disease characterized by progressive loss of retinal ganglion cells and axons^[1]. The clinical symptoms of visual field loss caused by elevated intraocular pressure make glaucoma become the irreversible blinding eye disease with the largest number of patients in the world, Besides, it is predicted that the global glaucoma patients will reach 111.8 million in 2040^[2]. Primary open-angle glaucoma is a common type of glaucoma, with hidden onset and extremely slow progression. Therefore, it's difficult to diagnose in disease early. When patients seek the treatment, the disease has often progressed to the advanced stage, at which time the disease has caused irreversible damage to the patients. Thus, research on the pathogenic mechanism, early accurate diagnosis and effective treatment of POAG is of great significance.

One of the anatomical characteristic changes of POAG is the degeneration of trabecular meshwork^[3]. Approximately eighty-five percent of the aqueous humor from anterior chamber is discharged through the trabecular meshwork tissue^[4]. The trabecular meshwork tissue consists of trabecular bundles formed by collagen fibers and trabecular meshwork cells that cover the single-layer mesh-like structure. Trabecular meshwork cells are involved in the synthesis, secretion, and metabolism of the extracellular matrix of trabecular meshwork tissue^[5, 6]. Meanwhile, it contained mitochondria^[7], secondary lysosomes^[8] and other organelles, which could eliminate cell debris, biological macromolecules and pigment particles attached to the trabecular meshwork tissue through phagocytosis^[9, 10]. The myofilament proteins contained in the trabecular meshwork cells can help the cells contract the mesh for keeping the patency of the aqueous humor and the stability of the intraocular pressure^[11]. Therefore, the change in the number and activity of trabecular meshwork cells can affect the patency of aqueous humor outflow, interfere with changes in intraocular pressure.^[12]

It is well established that trabecular meshwork can be surgically removed to save vision in patients with advanced open-angle glaucoma^[13]. However, the mechanism of apoptosis in trabecular meshwork in patients with open-angle glaucoma has not yet been elucidated. Recent studies have reported that endoplasmic reticulum(ER) stress may play an important role in the apoptosis of human trabecular meshwork cells [HTM] ^[14, 15]. However, the relationship between ER stress signaling and Apoptosis in primary HTM cells is unknown. We proposed for the first time that tunicamycin-induced ER stress-mediated apoptosis in HTM cells is associated with activation of the CHOP/Bcl-2 signaling pathway. Thus, in this experimental study, we aimed at exploring the relationship between CHOP /BCL-2 and apoptosis via establishing the model of ER stress-mediated apoptosis in HTM cells.

Materials And Methods

Primary Cell Culture and expansion

Human trabecular meshwork cells (HTMCs) were bought from Beina Chuanglian Biotechnology Research Institute. HTMCs were cultured in Dulbecco's Modified Eagle Medium: F-12 (DMEM/F12) supplemented with 1% penicillin/streptomycin (100U/mL), and 20% fetal bovine serum (Invitrogen-Gibco) at 5% CO₂ and 37°C. The culture medium is changed every 2 days, when the cells reach 80% confluence, cells were washed twice with PBS. The cells were digested by 0.25% pancreatin for 5min. Then add bovine serum-containing medium to terminate digestion and replat through 1:2 dilutions.

Detection of Cell toxicity

HTMCs were seeded into 96-well plates (1.0×10^4 cells/well). 24 hours later, cells were treated with tunicamycin [dissolved in dimethyl sulfoxide (DMSO)] at final concentrations of 1.25, 2.5, 5, 10 and 20mg/L as experimental group, while the cells without any treatment served as control group. Five wells were used for each dose. Subsequent to 6, 12, 24, 36 and 48h, 10 μ L of cell counting kit 8 (CCK8, TAKARA, Tokyo, Japan) for 2h. Ultimately, absorbance was measured at 490 nm by using a spectrophotometer. The inhibition rate was determined to figure out the suppressive effect of tunicamycin on HTMCs. The relative inhibition rate was calculated as a percentage, as follows: $(1 - A_{\text{experiment}} / A_{\text{control}}) \times 100\%$. Three independent experiments were performed.

Apoptosis assay

Cells were treated with tunicamycin at the concentrations of 5mg/L for 6, 12 and 24h, respectively. Then, we used an apoptosis detection kit (BD Co., Ltd., Nanjing, China) for an apoptosis assay. Cells were washed with PBS and resuspended gently with 1 \times Binding Buffer at a concentration of 1×10^6 cells/ml. Subsequently, pipette 100 μ l solution (1×10^5 cells) into a 5 ml culture tube, and then add 5 μ l FITC annexin V and 5 μ l PI to the cells suspension. Add 400 μ l 1 \times Binding Buffer to each test tube and incubate them in the dark at room temperature (25 ° C) for 15 minutes. The apoptosis level of cells in each group was measured using FACSCalibur flow cytometer.

Quantitative RT-PCR

Cells were treated as aforementioned in the previous paragraph (apoptosis assay). The western blotting experiment is the same. Cells were subsequently lysed in TRI Reagent (Total RNA Isolation Reagent, Biosharp, CN), and stored at -80°C. RNA was isolated following the manufacturer's instructions, including treatment with chloroform, precipitation and recovery with isopropanol, washing with ethanol, and finally adding DEPC water to dissolve RNA. Calculate the concentration of the isolated RNA sample at 260nm and 280nm by an ultraviolet spectrophotometer, and measure the OD260/OD280 ratio to judge the purity of the sample. If the ratio is between 1.8 and 2.0, the purity is acceptable. RNA was reverse transcribed into cDNA using Reverse Transcriptase kit (TAKARA, Japan). Quantitative PCR (qPCR) was performed using SYBR Green RT-PCR Reagents (TAKARA, Japan). The sequences of primers used in this study are presented in Table 1. Calculate the relative mRNA abundance by using the $2^{(-\Delta\Delta Ct)}$ method. Gene expression was normalized to GAPDH. The sequences of the primers were as follows,

GAPDH: 5'-AAATGGTGAAGGTCGGTGTGAAC-3'(forward);

5'-CAACAATCTCCACTTTGCCACTG-3'(reverse)

CHOP: 5'-AGGCACTGAGGGTATCATGTT-3'(forward);

5'-CTGTTTCCGTTTCCTGGTTC-3'(reverse)

Grp78: 5'-CATCACGCCGTCCTATGTCG-3'(forward);

5'-CGTCAAAGACCGTGTTCTCG-3' (reverse)

Bcl-2: 5'-ATTGTGGCCTTCTTTGAGTTCG-3'(forward);

5'-CATCCCAGCCTCCGTTATCC-3' (reverse)

Caspase-3: 5'-CATGGAAGCGAATCAATGGACT-3'(forward);

5'-CTGTACCAGACCGAGATGTCA-3' (reverse).

The primers were designed using NCBI.

Western Blotting

The prepared cells were added with RIPA cell lysis buffer together with protease inhibitor and phosphatase inhibitor and transferred to EP tubes which were placed on ice for 30 minutes, followed by cell lysis using a centrifuge and centrifugation at 13000 rpm for 5 minutes. The supernatant prepared for western blot analysis was then transferred to another sterile EP tube. The membranes were blocked with 10% fat-free milk and incubated with the primary antibody selected from ab108615, ab179823, ab32124, and ab184787 (Abcam, UK) overnight at 4 C. Goat anti-rabbit IgG (1:5000, ab6721, Abcam, UK) was used for the secondary antibody.

Results

3.1 The effect of tunicamycin on the activity of HTMCs

CCK8 assay showed that the inhibitory effect of HTMCs treated with 1.25mg/L and 2.5mg/L tunicamycin was not obvious compared with control group 0mg/L ($P > 0.05$). However, the inhibitory effect appeared a significantly increase when the treatment concentration was higher than 5mg/L. Moreover, under the 5mg/L of tunicamycin treatment, the HTMCs' proliferation was remarkably inhibited when treated duration last for 12 hours or more. The result showed that the inhibitory effect on HTMCs treated with tunicamycin of 12, 24, 36 and 48 hours ($21.11 \pm 1.23\%$, $31.34 \pm 0.86\%$, $44.37 \pm 1.01\%$, $51.47 \pm 0.36\%$, respectively) were remarkably enhanced when compared to the control group 0 hours ($P < 0.05$; Fig. 1)

3.2 The effect of tunicamycin on apoptosis of HTMCs

According to the results of the above CCK8 assay, HTMCs were previously treated with 5mg/L tunicamycin for 0, 6, 12 and 24 hours and marked with Annexin V -FITC/ PI dye. The effect of tunicamycin on HTMCs apoptosis was analysed by flow cytometry analysis. Annexin V -FITC(+) and PI(-) cells were classified as early apoptosis, while Annexin V -FITC(+) and PI(+) cells were classified as late apoptosis. The results showed that with the increase of treatment duration, the early apoptosis rate and late apoptosis rate increased gradually (Fig. 2 and Table 1).

Early apoptosis results: There was a statistical difference in the early apoptosis rate of each HTMCs groups ($F = 1024.457$, $P < 0.001$). The early apoptosis rates of group 6, 12, 24 hours ($7.43 \pm 0.78\%$, $8.59 \pm 0.89\%$, $34.22 \pm 0.87\%$, respectively) were higher than negative control ($4.68 \pm 0.82\%$, $P < 0.05$). The early apoptosis rate of 24h group was significantly higher than that of group 6 hours and group 12 hours ($P < 0.05$), while there was no statistical difference in the early apoptosis rate between the other groups pairwise comparison ($P > 0.05$).

Late apoptosis results: There was a statistical difference in the early apoptosis rate of each HTMCs groups ($F = 81.475$, $P < 0.001$). The late apoptosis rates of group 6, 12, 24 hours ($5.03 \pm 0.90\%$, $6.57 \pm 0.93\%$, $10.53 \pm 1.00\%$, respectively) were higher than negative control ($0.52 \pm 0.09\%$, $P < 0.05$). The late apoptosis rate of 24h group was significantly higher than that of group 6 hours and group 12 hours ($P < 0.05$), while there was no statistical difference in the late apoptosis rate between the other groups pairwise comparison ($P > 0.05$).

Table 1 The apoptosis rate of HTMCs treated with tunicamycin at different time points ($\bar{X} \pm S$)

treatment duration hours	0	6	12	24	F	P
Early apoptosis rate%	4.68±0.82	7.43±0.78*	8.59±0.89*	34.22±0.87*	1024.457	0.000
Late apoptosis rate%	0.52±0.09	5.03±0.90#	6.57±0.93#	10.53±1.00#	81.475	0.000

Note :* represents the early apoptosis rate of each experimental group and the control group (0h), the difference was statistically significant (P < 0.05).

represents the late apoptosis rate of each experimental group and the control group (0h), the difference was statistically significant (P < 0.05).

3.3 Effect of tunicamycin on ERS-related mRNA expression in HTMCs

HTMCs were previously treated with tunicamycin (0, 6, 12,24hours) and then extracted total RNA. The relative mRNA expression levels of HTMCs GRP-78,CHOP,Bcl-2,Caspase-3 were detected by RT-PCR.Cells treated for 0 hours was the negative control group, and the rest was the experimental group.

GRP-78 results:The GRP-78 relative mRNA expression levels of groups 0 ,6,12,24 hours were respectively 1.00 ± 0.00 , 54.83 ± 2.96 , 37.47 ± 1.34 , 33.21 ± 1.87 .There was a statistical difference in the GRP-78 relative mRNA expression levels of each HTMCs groups (F = 432.895, P < 0.001).The GRP-78 relative mRNA expression levels of group 6,12,24hours increased compared with group 0 hours(P<0.05),in which the expression of group 6 hours reached the peak.The difference in the relative expression of GRP78 mRNA between each experimental group was statistically significant. (P < 0.05).(Fig. 3A and Table 2)

CHOP results:The CHOP relative mRNA expression levels of groups 0 ,6,12,24 hours were respectively 1.00 ± 0.00 , 15.94 ± 1.95 , 13.94 ± 0.81 , 23.80 ± 3.14 .There was a statistical difference in the CHOP relative mRNA expression levels of each HTMCs groups (F = 73.898, P < 0.001).The CHOP relative mRNA expression levels of group 6,12,24hours increased compared with group 0 hours(P<0.05),in which the expression of group 24 hours reached the peak.The difference in the relative expression of CHOP mRNA between each experimental group was statistically significant. (P < 0.05).(Fig. 3B and Table 2)

Bcl-2 results:The Bcl-2 relative mRNA expression levels of groups 0 ,6,12,24 hours were respectively 1.00 ± 0.00 , 0.52 ± 0.11 , 0.40 ± 0.07 , 0.37 ± 0.09 .There was a statistical difference in the Bcl-2 relative mRNA expression levels of each HTMCs groups (F = 67.967, P < 0.001).The Bcl-2 relative mRNA expression levels of group 6,12,24hours decreased compared with group 0 hours(P<0.05),in which the expression downregulation of group 24 hours was most significant.The difference in the relative expression of Bcl-2 mRNA between each experimental group was statistically significant. (P < 0.05).(Fig. 3c and Table 2)

Caspase-3 results:The Caspase-3 relative mRNA expression levels of groups 0 ,6,12,24 hours were respectively 1.00 ± 0.00 , 1.65 ± 0.29 , 1.62 ± 0.11 , 2.66 ± 0.40 .There was a statistical difference in the

Caspase-3 relative mRNA expression levels of each HTMCs groups ($F = 22.406, P < 0.001$).The Caspase-3 relative mRNA expression levels of group 6,12,24hours increased compared with group 0 hours($P < 0.05$),in which the expression of group 24 hours increased compared with group 6,12 hours($P < 0.05$),while there was no statistical difference in Caspase-3 relative mRNA expression between the other groups pairwise comparison ($P > 0.05$).(Fig. 3D and Table 2)

Table 2 Expression of ERS-related mRNA in HTMCs treated with tunicamycin at different time points($\bar{X} \pm S$)

Treatment duration(hours)	GRP-78	CHOP	Bcl-2	Caspase-3
0	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
6	54.83±2.96*	14.94±1.95*	0.52±0.11*	1.65±0.29*
12	37.47±1.34*	13.94±0.81*	0.40±0.07*	1.62±0.11*
24	33.21±1.87*	23.80±3.14*	0.37±0.09*	2.66±0.40*
<i>F</i>	432.895	73.989	67.967	22.406
<i>P</i>	0.000	0.000	0.000	0.000

Note :Compared with the control group (0h),* $P \leq 0.05$

3.4 Effect of tunicamycin on ERS-related protein expression in HTMCs

HTMCs were previously treated with tunicamycin (0, 6, 12,24hours) and then extracted total protein. The protein expression levels of HTMCs GRP-78,CHOP,Bcl-2,Caspase-3 were detected by Western Blot.Cells treated for 0 hours was the negative control group, and the rest was the experimental group.

GRP-78 results:The GRP-78 protein expression levels of groups 0 ,6,12,24 hours were respectively $0.34 \pm 0.04, 0.44 \pm 0.03, 0.44 \pm 0.04, 0.46 \pm 0.05$.There was a statistical difference in the GRP-78 protein expression levels of each HTMCs groups ($F = 4.973, P < 0.05$).The GRP-78 protein expression levels of group 6,12,24hours increased compared with group 0 hours($P < 0.05$),while there was no statistical difference in GRP-78 protein expression between the other groups pairwise comparison ($P > 0.05$).(Fig. 4A and Table 3)

CHOP results:The CHOP protein expression levels of groups 0 ,6,12,24 hours were respectively $0.23 \pm 0.03, 0.53 \pm 0.02, 0.68 \pm 0.03, 0.59 \pm 0.03$.There was a statistical difference in the CHOP protein expression levels of each HTMCs groups ($F = 45.000, P < 0.001$).The CHOP relative mRNA expression levels of group 6,12,24hours increased compared with group 0 hours($P < 0.05$).The difference in the relative expression of CHOP protein between each experimental group was statistically significant. ($P < 0.05$).(Fig. 4B and Table 3)

Bcl-2 results: The Bcl-2 protein expression levels of groups 0, 6, 12, 24 hours were respectively 0.17 ± 0.03 , 0.14 ± 0.02 , 0.13 ± 0.02 , 0.09 ± 0.02 . There was a statistical difference in the Bcl-2 protein expression levels of each HTMCs groups ($F = 6.158$, $P < 0.05$). The Bcl-2 protein expression levels of group 24 hours decreased compared with group 0 hours ($P < 0.05$), while there was no statistical difference between the other groups pairwise comparison ($P > 0.05$). (Fig. 4C and Table 3)

Caspase-3 results: The Caspase-3 protein expression levels of groups 0, 6, 12, 24 hours were respectively 0.76 ± 0.02 , 0.80 ± 0.01 , 0.96 ± 0.02 , 0.80 ± 0.05 . There was a statistical difference in the Caspase-3 protein expression levels of each HTMCs groups ($F = 7.548$, $P < 0.05$). The Caspase-3 protein expression levels of group 12 hours increased compared with group 0 hours ($P < 0.05$), while there was no statistical difference when group 6, 24 hours were compared with group 0 hours ($P > 0.05$). The Caspase-3 protein expression levels of group 12 hours increased compared with group 6 hours ($P < 0.05$), and protein of group 24 hours decreased compared with group 12 hours ($P < 0.05$). There was no statistical difference between the other groups pairwise comparison ($P > 0.05$). (Fig. 4D and Table 3)

Table 3 Expression of ERS-related protein in HTMCs treated with tunicamycin at different time points ($\bar{X} \pm S$)

Treatment duration (h)	GRP-78	CHOP	BCL-2	Caspase-3
0	0.34 ± 0.04	0.23 ± 0.03	0.17 ± 0.03	0.76 ± 0.02
6	$0.44 \pm 0.03^*$	$0.53 \pm 0.02^*$	0.14 ± 0.02	0.80 ± 0.01
12	$0.44 \pm 0.04^*$	$0.68 \pm 0.03^*$	0.13 ± 0.02	$0.96 \pm 0.02^*$
24	$0.46 \pm 0.05^*$	$0.59 \pm 0.03^*$	$0.09 \pm 0.02^*$	0.80 ± 0.05
<i>F</i>	4.973	45.000	6.158	7.548
<i>P</i>	≤ 0.05	0.000	≤ 0.05	≤ 0.05

Note: Compared with the control group (0h), * $P \leq 0.05$

Discussion

Although it has been reported that endoplasmic reticulum stress plays an important role in the pathogenesis of glaucoma^[16], the role of ER stress in trabecular meshwork cells has not been reported yet. In present research, we investigated the effect of endoplasmic reticulum stress in trabecular meshwork cells. Tunicamycin is often used in establishing the model of endoplasmic reticulum stress^[17, 18]. Here, we found that the apoptosis of trabecular meshwork cells may go through affecting the interaction between CHOP and Bcl-2 protein which induced by tunicamycin.

Protein homeostasis plays an important role in the normal function of cells. In eukaryotic cells, endoplasmic reticulum is involved in the synthesis, folding and maturation of proteins^[19, 20]. When in

some physiological or pathological conditions, such as nutritional deficiency, infection, chemical damage, DNA mutation and so on^[21], can trigger the abnormal function of endoplasmic reticulum (ER), which leads to the protein misfolding and accumulating^[22], which is called endoplasmic reticulum stress (ERS). Abnormal proteins accumulate in the endoplasmic reticulum and activate a series of protective intracellular signaling pathways, which are called the unfolded protein response (UPR). UPR includes reducing protein translation, activating chaperone transcription, inducing endoplasmic reticulum degradation of misfolded proteins and inducing apoptosis^[23, 24]. Whether UPR promotes cell survival or apoptosis depends on the duration and severity of ERS^[25, 26]. Under transient and mild ERS conditions, UPR promotes cell survival, whereas persistent and severe ERS promotes cell death by activating downstream apoptosis signaling molecules, including IRE1 / ASK1 / JNK pathway³³, Caspase-12 kinase pathway³⁴ and C/EBP-homologous protein or growth arrest and DNA damage-inducible gene153 (CHOP/GADD153) pathway^[25, 27, 28].

The 78-kDa glucose-regulated protein (GRP78), also known as binding-immunoglobulin protein (BiP)^[29], is localized in the ER. It can bind to unfolded or misfolded proteins in ER, and is considered to be a marker of the ER-stress cascade^[30]. Chop is a transcription factor involved in ERS and has been confirmed as a proapoptotic protein^[31]. Chop encoded by DDIT3 gene, induces the expression of proapoptotic genes such as DR5, TRB3, Bim and puma, and inhibit the expression of BCL2^[32], thus triggering cell apoptosis during ER stress.^[33]

There are more and more evidences ^[34, 35]suggested that the endoplasmic reticulum stress may play an important role in the pathogenesis of glaucoma. Ganglion cells undergo apoptosis in glaucoma leading to progressive loss of vision - the loss of RGCs is the principal endpoint in experimental glaucoma^[36]. Peter et al^[37] demonstrated that high level of ER-stress related-proteins can be detected in the human trabecular meshwork tissue. In the experiment conducted by Zode et al^[38], found that the reduction of ocular-specific ER stress can rescue glaucoma in murine model of glucocorticoid-induced. Besides, the deletion of CHOP in ER-stress pathway can reduce ER stress and prevented dexamethasone-induced ocular hypertension.

Recently, accumulating evidence suggests that endoplasmic reticulum stress may play an important role in the survival and apoptosis of cell. however, the effect of endoplasmic reticulum stress for cell in vivo is controversial, some studies show that endoplasmic reticulum stress may play a protective role^[39, 40]. But, continuous endoplasmic reticulum stress can cause cells apoptosis^[41, 42]. In the current experiment, we found that Endoplasmic reticulum stress pathway activated by the tunicamycin induction might activate the apoptotic pathway and lead to the apoptosis of HTMCs successively. which is consistent with previous studies^[43].

In conclusion, our experiment show that under tunicamycin, ER stress can induce CHOP expression, whereas CHOP can lead cells to death by decreasing BCL-2 level and upregulation of CHOP may bring trabecular meshwork cells with harsh survival. These findings highlight the importance of apoptosis

regulated by ER stress in trabecular meshwork cells, disclose new mechanisms of POAG, and suggest CHOP as a potential anti-POAG target.

Declarations

Compliance with Ethical Standards:

Disclosure of potential conflicts of interest: The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals: there is no human participants or animals involved in this study

Informed consent: Not applicable.

Funding: This work was funded by Quanzhou Program of High-level Talents Innovation Entrepreneurship(2018C055R) and the Provincial medical innovation project in Fujian province (2020CXA043)

Compliance with Ethical Standards:

Conflict of Interest: The authors declare that they have no conflict of interest.

Author contribution:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xiaorui Wang and Yuyu Wu. The first draft of the manuscript was written by Xiaorui Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

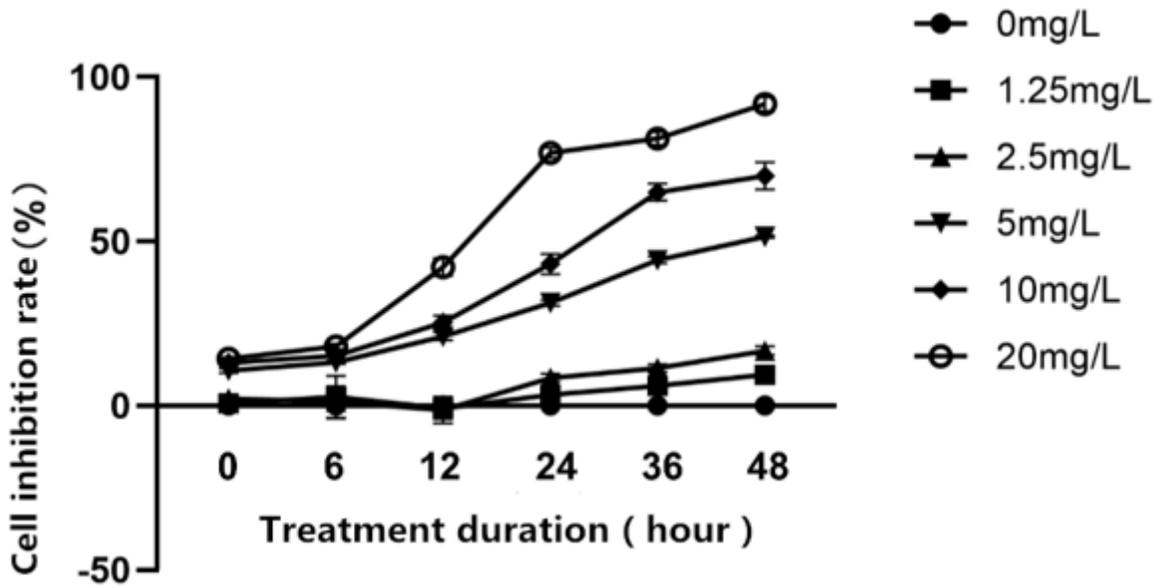


Figure 1

Cell inhibition rates of HTMCs at different time under different concentrations of capsulomycin

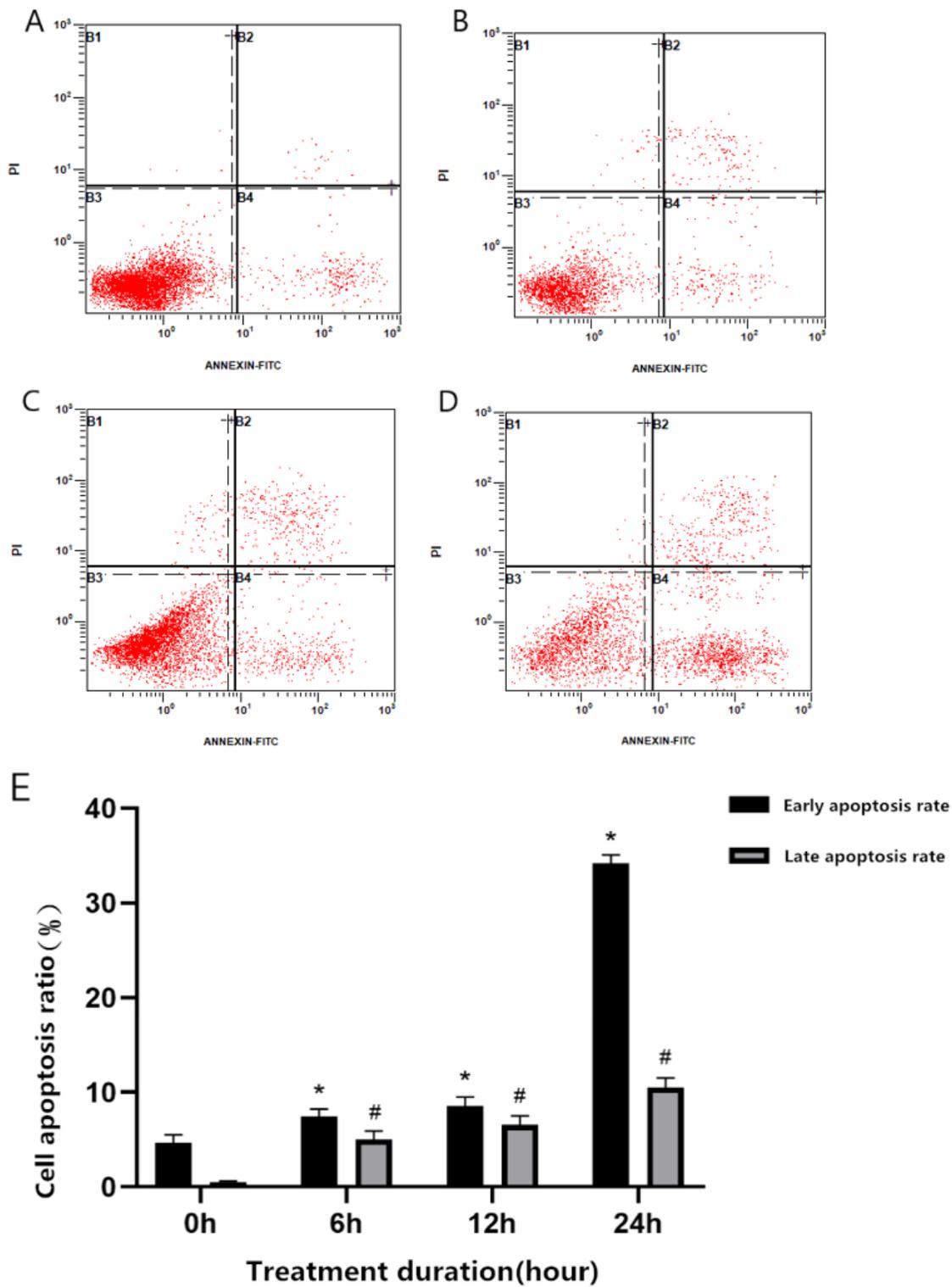


Figure 2

Apoptosis rate of HTMCs treated with tunicamycin at different time points Note :A-D : The treatment duration of tunicamycin were 0h, 6h, 12h, 24h. E : The apoptosis rate of HTMCs at different time points treated with itacamycin.

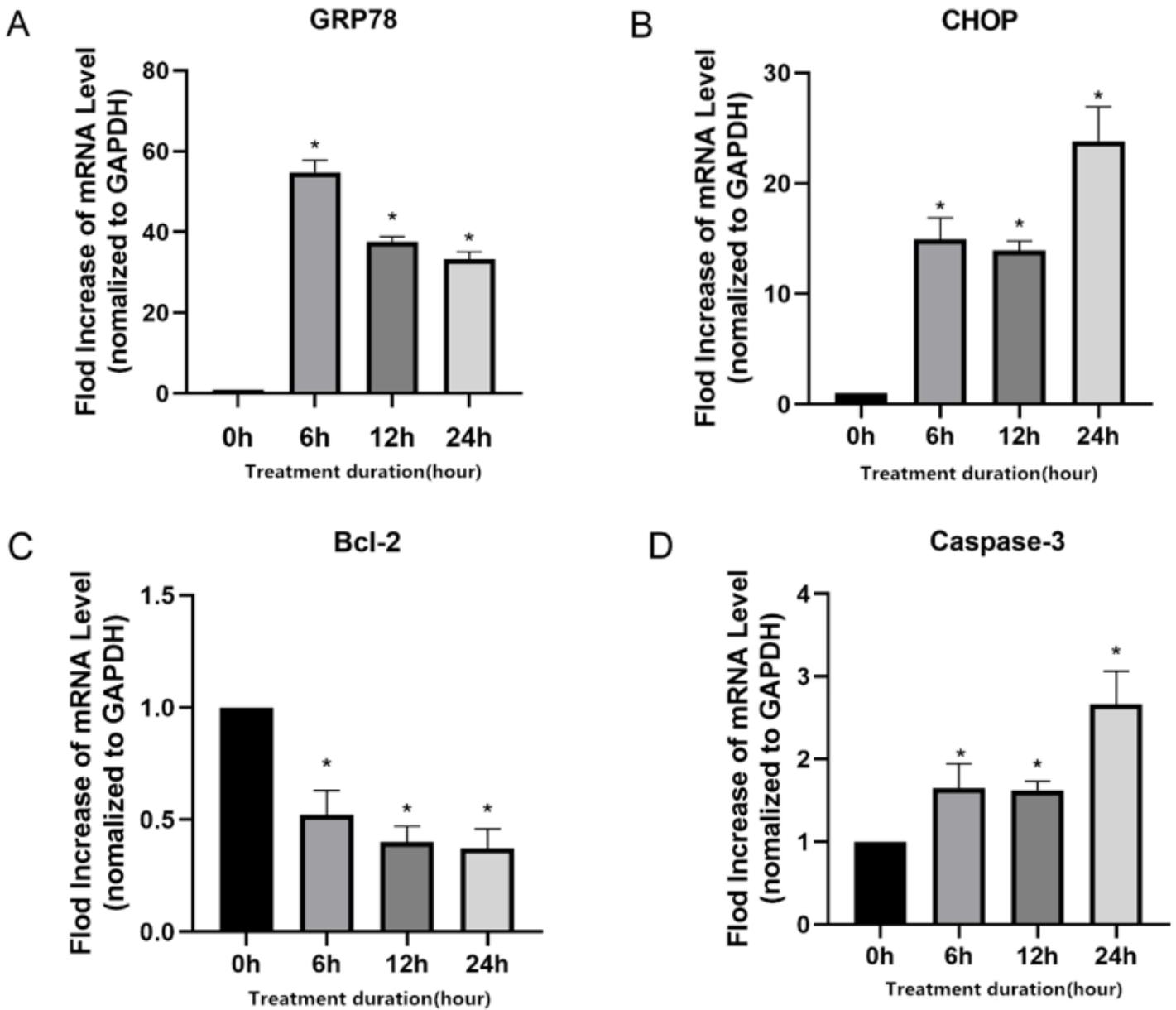


Figure 3

Expression of ERS-related mRNA in HTMCs treated with tunicamycin Note :A-D represents mRNA expression of GRP78, CHOP, BCL-2 and Caspase3 respectively, Compared with the control group (0h),*P < 0.05

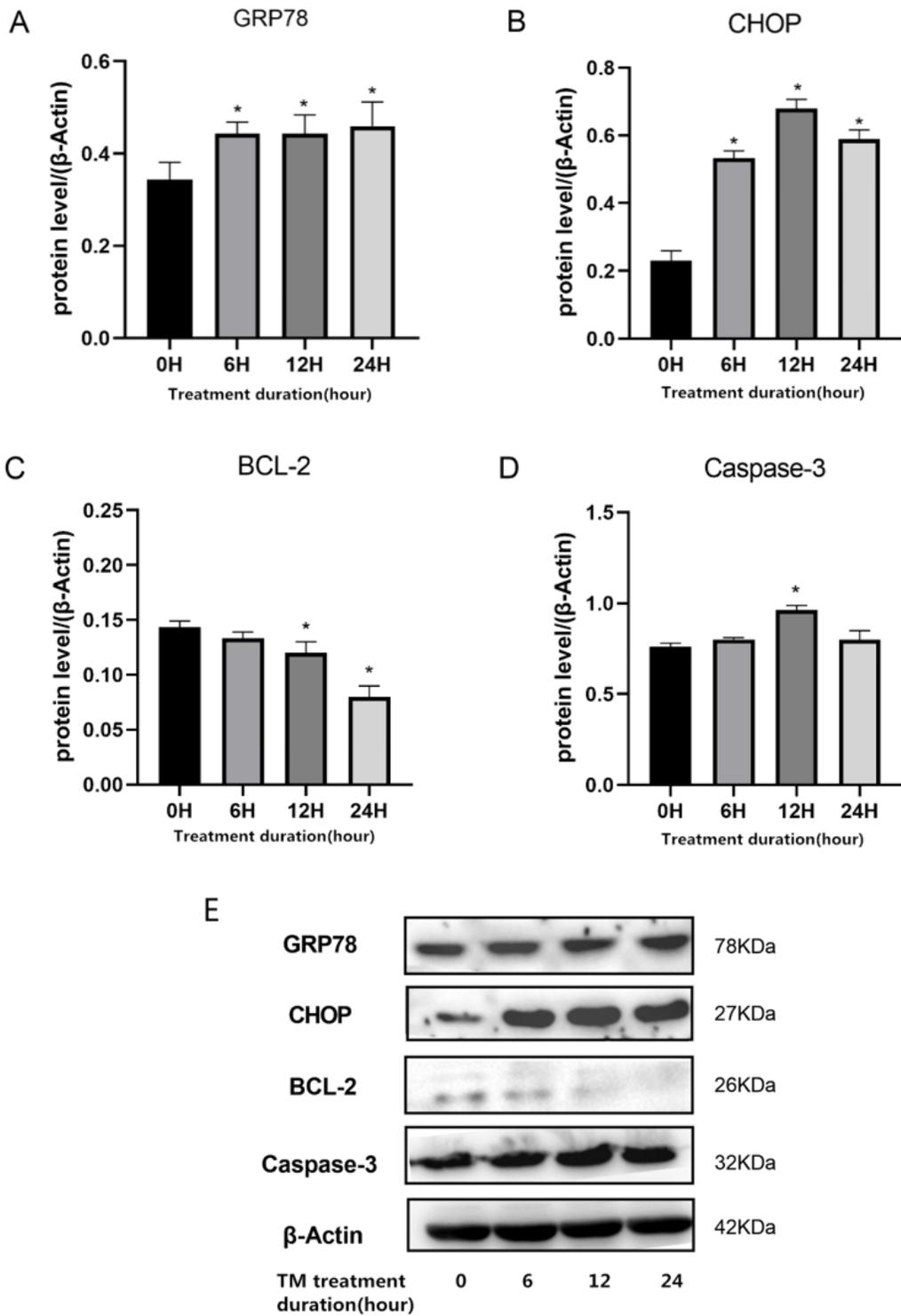


Figure 4

Expression of ERS-related protein in HTMCs treated with tunicamycin at different time points Note:A-D represents GRP78, CHOP, Bcl-2, Caspase-3 , respectively.The ratios of above protein to β -actin indicate the relative expression levels of GRP78 , CHOP , Bcl-2 and Caspase-3 proteins.Compared with the control group (0h),* $P < 0.05$ E represents GRP78, CHOP, Bcl-2, Caspase3 proteins imprinting strip.