Quercetin ameliorates tunicamycin-induced endoplasmic reticulum stress in endothelial cells

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Abstract

Objective: Endothelial dysfunction highlights that it is a potential contributor in the pathogenesis of vascular complications arising from endoplasmic reticulum stress (ER stress) and has been emerging as a main causative factor in vascular failure. Here, we hypothesize that the natural flavonoid, quercetin plays an effective role in reducing ER stress in human umbilical vein endothelial cells.

Materials and methods: Human umbilical vein endothelial cells were pre-treated with different concentrations of quercetin (0–100 μ M) before inducing ER stress using tunicamycin (TUN) (0.75 μ g/ml); cytotoxicity was assessed by MTT assay. Expression levels of ER stress responsive genes, antioxidant enzymes and apoptotic markers were assessed by qRT-PCR, while roles of caspase-3 and PARP cleavage were measured by western blot analysis.

Results: Quercetin pre-treatment at 25 and 50 μ M had a cytoprotective effect on cells against TUN-induced toxicity. Quercetin administration modulated expression level of ER stress genes coding for glucose-regulated protein 78 (GRP78) and C/EBP-homologous protein (CHOP), and antioxidant enzymes such as superoxide dismutase and catalase, along with free radical generation assessed by malondialdehyde assay. Induction of apoptosis was prevented with reduction in expression level of Bax, and concomitant increase in Bcl-2 levels, thus proving its potential against ER stress.

Conclusion: The current study indicates that quercetin modulated stress responsive genes GRP78 and CHOP, helping endothelial cells prevent TUN-induced ER stress.

Introduction

Endothelial dysfunction is a multifaceted disorder which results in massive alteration in endothelial cell phenotype, resulting in systemic disturbance in vascular homeostasis. This, in turn, contributes to development and clinical expression of pathological inflammatory processes and vascular diseases (1). Consequences of endothelial dysfunction arise as a secondary disorder in diabetes, atherosclerosis, hypertension and hypercholesterolemia (2), due to dysregulation of endothelium-derived NO, vasoconstriction, thrombosis and activation of transcription factors, such as NF κ B, protein kinase cascades and adhesion molecules as well as overexpression of growth factors (3). In addition to oxidative stress, several other factors contribute to endothelial dysfunction, including endoplasmic reticulum stress (ER stress), triggered under hyperglycaemic, hypoxic and shear stress conditions (4).

Induced ER stress elicits unfolded protein response (UPR), which functions adaptively and initiates the outcome by accumulation of misfolded proteins, leading to induction of a molecular chaperone, glucose-regulated protein 78 (GRP78). Under ER stress, GRP78 is released from ER transmembrane signal transducers, leading to sequential activation of ER-localized transmembrane proteins, including inositol-requiring enzyme 1 α (IRE1 α) and PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (5). These activated proteins in turn instigate UPR through launch of inflammatory and stress signalling pathways and finally lead to cell damage (6). Perturbations in ER function uphold induction of apoptosis by transcriptional activation of C/EBP-homologous protein (CHOP) (7).

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Several studies have documented that it negatively regulates cell population growth and endorses ER stress-induced apoptosis (8,9). Targeted disruption of CHOP has been shown to delay ER stress response, thereby promoting cytoprotection against oxidative injury (10). CHOP-deficient proximal tubular cells of diabetic mice have been reported to be resistant to ER stress-induced cell death (11). Similarly, CHOP over expressing cells have shown increased susceptibility to apoptosis mediated by ER stress (12,13). Identification of stress-associated ER protein expression gains importance in potential therapeutic targeting for ER stress-provoked disease. Furthermore, experimental ER stress responses have been induced using pharmacological agents such as thapsigargin, A23187 and brefeldin A, with upregulated levels of GRP78 and CHOP (14). One typical agent, tunicamycin (TUN), induces ER stress by interfering with N-linked protein glycosylation in ER (15).

Study of protection of endothelial cells by natural polyphenols is needed and has been demonstrated in both *in vitro* and *in vivo* models (16). Many plant polyphenolic compounds have beneficial effects against various diseases and disorders; their ameliorating properties on vascular homeostasis, antiplatelet and anti-inflammatory activities, have been validated (17,18). Resveratrol, a polyphenol found in red wine, protects against oxidized LDL-induced cytotoxicity in endothelial cells (19). Schroeder *et al.* documented beneficial effects of cocoa flavanol (–)-epicatechin, in murine aortic endothelial cells (20). Chronic intake of red-wine polyphenols has been reported to prevent aging-induced endothelial dysfunction in rats (16).

Quercetin, a well-known antioxidant found abundantly in fruits and vegetables, has been reported to have a wide range of biological functions including anticarcinogenic, anti-inflammatory, anti-ulcer and anti-viral as well as to inhibit platelet aggregation and capillary permeability (21). In spite of its antioxidant potential, high doses of quercetin administration have elicited pro-oxidant effects in rats (22), due to auto-oxidation, which yields hydroxyl radicals within the cell, thereby inducing cytotoxicity. Previous studies have revealed protective effects of quercetin *in vitro* against apoptosis mediated by high glucose, H₂O₂ and oxidized LDL, in endothelial cells (23–25) by reversing cellular and nuclear damage induced by modulating anti-apoptotic potential of the cell. Moreover, evidence upholds that quercetin modulates ER stress provoked by calcium dynamic dysregulation in intestinal epithelial cells (14) and was found to modulate the ER stress. Hence, in view of the significance of ER stress in endothelial dysfunction, our current study has aimed to ascertain the protective nature of quercetin against TUN-induced ER stress by modulating

proapoptotic UPR and CHOP in human umbilical vein endothelial cells (HUVECs).

Materials and methods

Cell culture

Human umbilical vein endothelial cells were grown in EGM-2 (Clonetics; Lonza Ltd, Basel, Switzerland) containing vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor-1, epidermal growth factor, hydrocortisone, heparin, gentamicin sulphate, amphotericin B, 1% ascorbic acid and 2% foetal bovine serum (FBS), at 37 °C in a humidified atmosphere with 5% CO₂.

Cell viability assay

Cell viability was assessed using the MTT assay. HUVECs (1×10^4 cells/well) were cultured in 96-well plates at 37 °C for 24 h then pre-treated with different concentrations of quercetin (0–100 µM) for 24 h, followed by TUN (0.75 µg/ml) for 24 h. Spent medium was removed and 10 µl MTT solution (5 mg/ml) was added to 100 µl of respective growth medium without phenol red, and plates were incubated at 37 °C for 4 h in a humidified 5% CO₂ atmosphere. Then, formazan crystals formed by mitochondrial reduction of MTT were solubilized in dimethyl sulphoxide (100 µl/well) and absorbance was read at 540 nm using a microplate reader (BioRad, Hercules, CA, USA). Percent inhibition of cytotoxicity was calculated as a fraction of control (without quercetin) and expressed as percentage of cell viability (26).

Determination of malondialdehyde (MDA)

Human umbilical vein endothelial cells (1×10^6 cells/well) were seeded in 6-well culture plates and allowed to proliferate for 24 h, then were treated with quercetin (25 and 50 µM) for 24 h at 37 °C, followed by TUN (0.75 µg/ml) for 24 h. At the end of the experimental period, cells were centrifuged at 1000 *g* for 5 min (27) and pellets were homogenized according to the manufacturer's recommendations; they were then assayed for MDA using an MDA assay kit (Oxis Research, Portland, OR, USA).

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

A variety of polymerase chain reaction (PCR) primers was designed based on the NCBI human mRNA and genome DNA sequence database. DNA fragments of PCR products were designed to amplify within 200 bp

length. Forward and reverse primers were primarily designed from different exons to reduce background noise from genomic DNA contamination. Primer sequences are listed in Table 1. Cells were treated with 25 and 50 μM of quercetin for 24 h followed by TUN (0.75 $\mu\text{g}/\text{ml}$) for further 24 h, and qRT-PCR was performed following the manufacturer's protocol. Briefly, each reaction contained 2 μl of cDNA (0.1 μg of RNA equivalent), 0.8 μl primer (containing 100 pM forward and reverse primers), 2.2 μl of H_2O , and 5 μl of Sofast EvaGreen supermix. qRT-PCR was performed (BioRad) in a three-step program (95 $^{\circ}\text{C}$ for 15 s, 60 $^{\circ}\text{C}$ for 30 s and 72 $^{\circ}\text{C}$ for 45 s for 50 cycles). qRT-PCR data were analysed by CFX manager software (BioRad, Hercules, CA, USA). Gene expression fold change was obtained by dividing treated group signal by that of base expression level signal of corresponding genes in untreated cells. Results were normalized using qRT-PCR signal from β -actin of respective samples.

Western blotting

Expression of caspase-3 was measured by western blot analysis as previously described (28), with modifications. HUVECs were treated with 25 and 50 μM quercetin for 24 h followed by TUN 0.75 $\mu\text{g}/\text{ml}$ for a further 24 h. Then, cells were harvested, washed once in ice-cold phosphate-buffered saline, gently lysed in ice-cold lysis buffer (250 mM sucrose, 1 mM EDTA, 0.05% digitonin, 25 mM Tris, pH 6.8, 1 mM dithiothreitol, 1 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ pepstatin, 1 $\mu\text{g}/\text{ml}$ aprotinin, 1 mM benzamide and 0.1 mM phenylmethylsulphonyl fluoride) for 30 min, and centrifuged at 12 000 g at 4 $^{\circ}\text{C}$. Protein concentration was measured using BioRad Bradford protein assay reagent (29), and subjected to SDS-PAGE. Proteins were transferred to polyvinylidene fluoride membranes and incubated successively in 5% bovine serum albumin in Tris-buffered saline – Tween 20 buffer (TTBS) (25 mmol/L Tris, pH 7.5, 150 mmol/L NaCl and 0.1% Tween 20) for 1 h, then incubated

overnight at 4 $^{\circ}\text{C}$ with Caspase-3 antibody (Cell Signaling Technology, Danvers, MA, USA) or PARP antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by reaction with horseradish peroxidase-labelled secondary antibody (Santa Cruz Biotechnology) for 1 h. After each incubation, membranes were washed extensively in TTBS and the immunoreactive band was detected using ECL-detecting reagents.

Statistical analysis

Results are presented as mean $\pm$ SD of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test; $P < 0.05$ was taken to indicate significant difference between groups.

Results

Effect of quercetin on TUN-induced cytotoxicity in HUVECs

To evaluate cytoprotective effect of quercetin against TUN-induced apoptosis, we performed the MTT assay. TUN at 0.75 μg for 24 h exposure reduced cell viability by up to 60%. However, quercetin pre-treatment (5–100 μM) for 24 h before TUN (0.75 μg) showed quercetin concentration-dependent increase in viability in the order of 73%, and 79% at 25 and 50 μM concentrations respectively (Fig. 1). This illustrates the protective effect of quercetin against TUN-induced cell death. As the effect was predominant at 25 and 50 μM , we restricted concentrations up to 50 μM .

Effect of quercetin on MDA level

To determine the role of free-radicals on induction of ER stress, we measured levels of MDA, an end product of lipid peroxidation was used as an indirect index of oxidative injury. As shown in Fig. 2, TUN-induced

Table 1. q-PCR primer sequences for validated targets

Gene name	Short name	Forward primer 5'–3'	Reverse primer 5'–3'
Glucose regulated protein 78	GRP78	TCTGCTTGATGTGTCTCTCTT	GTCGTTCACCTTCGTAGACCT
CCAAT-enhancer-binding protein homologous protein	CHOP	GGAGAAGGAGCAGGAGAATGA	AGACAGACAGGAGGTGATGC
Superoxide dismutase1	SOD1	GAAGGTGTGGGGAAGCATTA	ACATTGCCCAAGTCTCCAAC
Catalase	CAT	TCATGACATTTAATCAGGCA	GTTGTCAGGATAGGCAAAAAG
B-cell lymphoma 2	BCI-2	GCTGAGGCAGAAAGGTTATG	GCCCCCTTGAAAAAGTTCAT
Bcl-2 associated X protein	BAX	AGGGTTTCATCCAGGATCGAGCAG	ATCTTCTCCAGATGGTGAGCGAG
Actin- Beta	ACT	GGCGGACTATGACTTAGTTG	AAACAACAATGTGCAATCAA

Figure 1. Cytoprotective effect of quercetin on human umbilical vein endothelial cells against tunicamycin (TUN)-induced toxicity. Data are presented as mean $\pm$ SD of three independent experiments. Significance compared to control, * $P < 0.05$, and compared to control + TUN group, # $P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test.

Figure 2. Effect of quercetin on levels of malondialdehyde in human umbilical vein endothelial cells. Data are presented as mean $\pm$ SD of three independent experiments. Significance compared to control, * $P < 0.05$, and compared to control + TUN group, # $P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test.

free-radical generation was markedly suppressed in a concentration-dependent manner by pre-treatment with quercetin (25 and 50 μ M) for 24 h.

Effect of quercetin on ER stress marker

To confirm the protective effect of quercetin against TUN-induced ER stress in HUVECs, we assessed expression of ER stress markers GRP78 and CHOP by qRT-PCR. TUN upregulated expression of GRP78 and CHOP, confirming induction of ER stress. Pre-treatment with quercetin (25 and 50 μ M) reduced expression of GRP78 and CHOP in TUN-induced cells (Fig. 3a,b) and at 50 μ M this was at its maximum effect compared to 25 μ M. Quercetin alone had no modulation in expression level of CHOP even after 24-h exposure. On the other hand, treatment with different concentrations of quercetin alone (25 and 50 μ M) induced GRP78 expression.

Effect of quercetin on antioxidant gene expression

To study effects of quercetin on antioxidant genes, such as those coding for superoxide dismutase 1 (SOD1) and catalase (CAT), we assessed mRNA expression levels by qRT-PCR. Upon TUN treatment, expression of SOD1 and CAT were found to be downregulated, and quercetin pre-treatment (25 and 50 μ M) effectively modulated its expression levels up to near normal (Fig. 4a, b). In untreated control cells, quercetin treatment increased expression of SOD1 and CAT, proving its antioxidant property.

Anti-apoptotic effect of quercetin on TUN-treated HUVEC cells

Bcl-2 and Bax are major regulatory proteins associated with apoptosis. As depicted in Fig. 5a and 5b, Bcl-2, an apoptotic suppressor, was markedly lower after treatment with TUN for 24 h. In contrast, Bax, a pro-apoptotic protein, expression was significantly higher after treatment with TUN. However, TUN-induced Bcl-2 reduction and Bax increase were reversed in a concentration-dependent manner by pre-treatment with quercetin for 24 h. As

Figure 3. Effect of quercetin on (a) GRP78 and (b) CHOP expression levels in human umbilical vein endothelial cells. Significance compared to control, * $P < 0.05$, and compared to control + TUN group, # $P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test.

Figure 4. Effect of quercetin on (a) SOD1 and (b) CAT expression levels in human umbilical vein endothelial cells. Significance compared to control, $*P < 0.05$, and compared to control + TUN group, $\#P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test.

Figure 5. Effect of quercetin on (a) Bcl-2 and (b) Bax expression levels (c) Bax/Bcl-2 ratio in human umbilical vein endothelial cells. Significance compared to control, $*P < 0.05$, and compared to control + TUN group, $\#P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test.

shown in Fig. 5c, ratio of Bax and Bcl-2 was reduced by pre-treating with quercetin in TUN-induced HUVECs.

Western blot analysis of caspase expression and PARP cleavage

To gain insight into mechanisms of antiapoptotic effects of quercetin in TUN-induced cells, we examined levels of caspase-3 and PARP cleavage, using western blot analysis. PARP plays an important role in DNA repair but can also lead to cell death by depleting the cell NAD pool. To investigate whether the mechanism of action by quercetin may depend on modulation of PARP activity, we carried out western blot analysis to detect cleaved products of PARP. We found that exposure of cells to TUN resulted in PARP cleavage and activation of caspase-3. Further quercetin pre-treatment in TUN-induced cells resulted in significant reduction in caspase-3 levels and indicated reduction in cleavage of PARP (Fig. 6a,b).

Discussion

Loss of balance between endothelial cell apoptosis and regeneration results in discontinuity of endothelial layers, which favours initiation and progression of micro- and macrovascular complications (30). Increased production of oxidative stress in diseased states, such as hyperglycaemia, relates to endothelial dysfunction and has been reported in both *in vitro* and *in vivo* models (31,32). In addition, metabolically active endothelial cells inclined by various pathological conditions resulted in ER stress. (33). Disruption of ER function has been linked to development of several disorders including Alzheimer's disease, Huntington's disease and Parkinson's disease, type-1 diabetes mellitus and hepatic steatosis (34–38). In the case of diabetes mellitus, endothelial cells fail to overcome the hyperglycaemic state induced by ER stress. Kassan *et al.* (39) reported that inhibition of ER stress

Figure 6. Effect of quercetin on caspase-3 and PARP cleavage. (a) Western blot analysis of caspase-3 and PARP cleavage. (b) Corresponding histogram of caspase-3 protein expression in western blot analysis. Significance compared to TUN group, $*P < 0.05$, and compared to control group, $^{\#}P < 0.05$ determined by one-way ANOVA followed by Duncan's multiple range test. (Lane 1: Control; Lane 2: Quercetin – 50 μM; Lane 3: TUN – 0.75 μg; Lane 4: TUN + Quercetin – 25 μM; Lane 5: TUN + Quercetin – 50 μM).

improves vascular endothelial function in type 1 diabetic mice.

The study described here contemplated possible involvement of ER stress in endothelial dysfunction that arbitrates vascular complications and demonstrated by TUN-induced ER stress in model systems. TUN is a nucleotide sugar analogue and a naturally occurring antibiotic produced mainly by *Streptomyces* species. TUN has been reported to inhibit first steps of N-linked protein glycosylation catalysed by DPAGT1 (UDP-GlcNAc: dolichol phosphate *N*-acetylglucosamine-1-phosphotransferase) by targeting the major facilitator domain containing the 2A (MFSD2A) transporter (40). This causes extensive protein misfolding and activation of UPR, thereby inducing apoptosis (41). TUN has also been reported to increase intracellular calcium levels in bovine aortic endothelial cells (42).

Here, we observed concentration-dependent cytoprotective effects of quercetin against TUN-induced toxicity in HUVEC cells. Furthermore, the effect of quercetin on activation of ER stress markers was studied, to attain a precise aspect to ameliorate the ER stress response. Under ER stress conditions, GRP78 rescues cells from effects of accumulated proteins, but in acute unresolved ER stress, this may lead to pathology by inducing cell death. GRP78 has been reported to be increased in endothelial cells in diabetic conditions (43) and also with an ER stress inducer, thapsigargin (33). We found elevated expression of GRP78 by TUN treatment, which is supported by previous reports, for example, of Natsume *et al.* (14). This revealed that upregulation of GRP78 in intestinal epithelial cells by

ER stressors, including TUN, results in induction of genes, such as *PERK*, *ATF6* and *IRE1α*, which coordinately regulate ER stress responses. Pre-treatment with quercetin in TUN reduced up-regulated GRP78 mRNA in HUVECs, thus re-establishing ER homeostasis.

C/EBP-homologous protein, a mediator of ER stress-induced apoptosis was considered to be minimal and was undetectable under normal conditions (44). Following GRP78 induction, ER stress signals lead to activation of CHOP and has been reported to sensitize cells to apoptosis (45). Elevated expression of CHOP has been observed in both *in vitro* and *in vivo* models such as in cystic fibrosis bronchial epithelial cells (46) and in retinitis pigmentosa (47). In the present study, TUN treatment was found to elevate expression of CHOP mRNA comparably as reported for other ER stress inducers such as A23187, thapsigargin and brefeldin A (14). Downregulation of CHOP and cells lacking CHOP, was significantly protected from lethal ER stress (48). One such report demonstrated that inhibition of ER stress CHOP-signalling pathway protects macrophages from ox-LDL-induced apoptosis (49). We found the reduction in *CHOP* gene expression arbitrated by quercetin, which might be a reason for anti-apoptotic effects in HUVECs. Effect of quercetin to restrain expression of GRP78 and CHOP was concurrent with suppression of ER stress in epithelial cells (14). As quercetin prevents expression of GRP78 and CHOP induction, mediated by calcium dynamics, it was also found to inhibit the PI3K pathway, thereby ameliorating ER stress. The effect of quercetin in HUVECs was comparable with that of other polyphenols, such as kaempferol (50), vaticanol B (51)

and methoxyflavones (52); these compounds have been reported to regulate expression of ER stress proteins GRP78 and CHOP and also to improve the ER homeostasis and restore ER membrane integrity.

Treatment with quercetin alone induced GRP78 expression; meanwhile, no modulation was observed in expression level of CHOP in HUVECs. One possible mechanism is that quercetin strengthened cell's defence by upregulating chaperone GRP78, which plays an important role in cell adaptation and survival. Our results are supported by previous reports explaining that quercetin induces GRP78 mRNA expression but does not alter expression of CHOP in a concentration and time-dependent manner (14). Quercetin had similar effects in HUVECs, which clarify that quercetin alone does not play a role in ER stress induction.

The mechanism of cell death triggered as the result of ER stress is distinct. Although proapoptotic CHOP targets activation of numerous genes, including those which code for Bcl-2, GADD34, endoplasmic reticulum oxidoreductin 1 and Tribbles-related protein 3 in apoptosis induction, *Bcl-2* is the main downstream target gene of CHOP (45). CHOP is known to represses the promoter of *Bcl-2*, thus downregulating anti-apoptotic events and rendering the cell more sensitive to pro-apoptotic signals (53). Studies have reported that overexpression of Bcl-2 can block CHOP-mediated apoptosis (13). Also, overexpression of CHOP is known to be associated with activation and mitochondrial translocation of Bax. In our study, TUN treatment increased expression of pro-apoptotic Bax and reduced Bcl-2 in HUVECs, and significant alteration was noticed with quercetin pre-

treatment, which correlated well with findings reported previously in macrophages (54).

Next, our study showed that the ER-CHOP pathway activated caspase-3, leading to caspase-dependent PARP cleavage and, consequently initiating apoptosis. PARP, which is considered to be the endpoint of a number of cell signalling pathways, is known to be involved in DNA repair, transcription, and DNA methylation. It also controls many physiological and pathological outcomes, and is a key component of immunity and inflammation (55). In a *Parp-1*^{-/-} knockout mouse model resistance to various modes of inflammation has been revealed. (56). Reduction in induction of caspase-3 and PARP cleavage in quercetin pre-treatment indicates their role in protecting cells from ER stress response.

Under normal physiological conditions, antioxidant systems balance reactive oxygen species (ROS) production and prevent oxidative damage encountered by cells. In particular, enzymatic antioxidants such as SOD, CAT and glutathione peroxidase play main roles in neutralizing ROS produced in pathological conditions. Cells responding to various diseased conditions and ER stressors accumulate ROS with impaired antioxidant responses. However, maintaining balance is essential for homeostasis of the cell. In particular, SOD catalyses breakdown of superoxide ($O_2^{\cdot-}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2), preventing formation of $OH^{\cdot}$, and thus, is an essential defence against toxicity induced by free radicals. ROS scavenging activity of SOD is more effective with actions of other enzymes, such as CAT. Our results indicate reduced levels of SOD1 and CAT after TUN administration and were

Figure 7. Hypothetical scheme representing the protective effect of quercetin against endoplasmic reticulum stress-induced endothelial dysfunction.

found to be normalized upon quercetin pre-treatment. Concurrently, quercetin has been acknowledged as being a scavenger of peroxynitrite and superoxide radicals in various model systems (57,58).

Increased oxidative stress was reflected with increased MDA level (59). MDA, a final product of lipid peroxidation, marker for oxidative stress and anti-oxidant status of cells, was found to be elevated in our study, after TUN administration. In contrast, quercetin tended to reduce TUN-induced MDA level. Quercetin had a comparable effect to that found in a previous report, in which reduced glutathione reduced MDA level in palmitate-mediated ER stress response, in hepatocytes (60). In addition, role of quercetin on carbonyl pathway has been documented *in vitro* metal-catalysed oxidation of myofibrillar proteins (61). Protein oxidation resulted in lower antioxidant functions with reduction in levels of glutathione peroxidase and ascorbic acid, as reported by Altomare *et al.* (62). Recent studies have demonstrated that quercetin scavenges peroxynitrite and prevents sarcoendoplasmic reticulum calcium ATP-ase carbonylation (63).

In conclusion, exposure of HUVECs to TUN lead to apoptosis through activation of ER stress *in vitro*. We also demonstrated concentration-dependent protective effects of quercetin on TUN-induced apoptosis. Thus, with an antioxidant property, protective effects of quercetin against ER stress is considered important, and it appears to be a potent regulatory element in TUN-evoked apoptotic signalling pathways (Fig. 7). Furthermore, PARP may at least in part, mediate protective effects of quercetin in HUVECs. Further pre-clinical studies will be required to explore potential efficacy of quercetin against development and progression of conditions associated with ER stress.

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