European Journal of Pharmacology 819 (2018) 80–88

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

Quercetin improves endothelial function in diabetic rats through inhibition of endoplasmic reticulum stress-mediated oxidative stress

Natarajan Suganya[a], Sireesh Dornadula[b], Suvro Chatterjee[c], Ramkumar Kunka Mohanram[b,*]

[a] Department of Biotechnology, SRM University, Kattankulathur 603203, Tamil Nadu, India
[b] SRM Research Institute, SRM University, Kattankulathur 603203, Tamil Nadu, India
[c] Vascular Biology Lab, AU-KBC Research Centre, Anna University, Chromepet, Chennai 600044, Tamil Nadu, India

ARTICLE INFO

Keywords:
ER stress
Endothelial dysfunction
Nitric oxide
cGMP
Quercetin
Streptozotocin

ABSTRACT

Endoplasmic reticulum (ER) stress attributes a crucial role in diabetes-induced endothelial dysfunction. The present study investigated the effects of quercetin, a potent antioxidant on the attenuation of ER stress-modulated endothelial dysfunction in streptozotocin (STZ)-induced diabetic rats. Oral administration of quercetin for six weeks to diabetic rats dose-dependently reduced the blood glucose levels and improved insulin secretion. Histopathological examination of pancreatic tissues in diabetic rats showed pathological changes such as shrunken islets, reduction in islet area and distorted β-cells, which were found to be restored by quercetin treatment. In addition, quercetin reduced the pancreatic ER stress-induced endothelial dysfunction as assessed by immunohistochemical analysis of C/ERB homologous protein (CHOP) and endothelin-1 (ET-1). Moreover, quercetin administration progressively increased the expression of vascular endothelial growth factor (VEGF) and its receptor, VEGFR2 in diabetes rats. Quercetin-mediated decrease in the nitric oxide (NO·) and cyclic 3′,5′-guanosine monophosphate (cGMP) levels were also observed in the diabetic rats. Quercetin treatment reduced the lipid peroxidation in the diabetic rats, meanwhile increased the total antioxidant capacity in the pancreas from diabetic rats. Altogether, these results demonstrated the vasoprotective effect of quercetin against STZ-induced ER stress in the pancreas of diabetic rats.

1. Introduction

Diabetes characterized by hyperglycemia arbitrates to the progressive destruction of insulin-producing pancreatic β-cells. Moreover, hyperglycemia-induced overproduction of reactive oxygen species mediates the oxidative stress condition which underlies the onset, progression, and pathological consequences of diabetes. It mediates the induction of various pathways including the activation of protein kinase C (PKC) isoforms, aldose reductase pathway and increased the formation of advanced glycation end products (AGEs), which in turn provoke pancreatic β-cell dysfunction (Giacco and Brownlee, 2010). It is notable that the pancreas is susceptible to oxidative stress because of low expression of antioxidant enzymes in the islets as compared to other tissues (Lei and Vatamaniuk, 2011). Moreover, the tissue antioxidant status is hampered during diabetes, with increased oxidative damage of membranes leading to tissue injury (Genet et al., 2002). In addition, oxidative stress upholds the nitric oxide (NO·) production thereby increasing the peroxynitrite levels in the tissues, which relies on its ability to trigger lipid peroxidation (Pitocco et al., 2013). Evidence highlights that in addition to oxidative stress, nitrosative stress also mediates the β-cell dysfunction (Watson and Loweth, 2009).

Recent reports suggested that endoplasmic reticulum (ER) stress plays an important role in the pathogenesis of diabetes and its complications (Kozuka et al., 2015; Takatani et al., 2016). Hyperglycemia perturbs ER homeostasis leading to the chronic activation of unfolded protein responses (UPR) resulting in cell death by triggering the expression of the transcription factor C/EBP homologous protein (CHOP), c-Jun N-terminal kinase (JNK) and Caspase-12 (Xu et al., 2005, Pirot et al., 2008). The demand for insulin biosynthesis under hyperglycemia predisposes β-cells to provoke ER stress responses (Kim et al., 2012). Additionally, silencing of CHOP attenuates streptozotocin (STZ)-induced β-cells apoptosis as demonstrated in both *in vitro* (RINm5F cells) and *in vivo* ($CHOP^{-/-}$ knockout mice) models (Oyadomari et al., 2002; Zhang et al., 2010). Further few reports provided a comprehensive view on the induction of ER stress which leads to islet deterioration, initiation of UPR response and disruption of calcium homeostasis (Ahn et al., 2015; Wang et al., 2015; Zhu et al., 2014).

Moreover, evidence emphasized the molecular interaction between the islets and the intraislet endothelial cells, in which their mutual signaling aids in the induction of pancreatic development,

* Corresponding author.
E-mail address: ramkumar.km@res.srmuniv.ac.in (R.K. Mohanram).

https://doi.org/10.1016/j.ejphar.2017.11.034
Received 30 April 2017; Received in revised form 8 November 2017; Accepted 20 November 2017
Available online 21 November 2017

differentiation and maintenance of islet function, mainly in insulin gene expression and glucose-stimulated insulin secretion in β-cells (Cao and Wang, 2014; Peiris et al., 2014). The endothelium regulates the blood flow, angiogenesis and also acts as an immunological barrier between the blood and the pancreatic tissues (Cano et al., 2007). It has also been reported that vascular endothelial growth factor (VEGF), an important angiogenic factor has been abundantly expressed in the β-cells and plays a vital role in islet vascularization and its function (Brissova et al., 2006). Together with insulin, the secretion of VEGF from the pancreatic β-cells promotes endothelial cell proliferation and contribute to islet vascularization. Evidence highlighted that inhibition of VEGF signaling promotes rapid regression of islet vasculature meanwhile VEGF over expression improves the islet mass during islet transplantation (Reinert et al., 2013).

The pathophysiology of the islets is largely influenced by the availability of endocrine signals, particularly the imbalance between the vasodilator and vasoconstrictor substances. Together with augmented oxidative stress, this mediates endothelial dysfunction, which is an initial event in diabetes-mediated complications (Ogita and Liao, 2004). Bojunga et al. reported that increased oxidative stress together with increased concentration of vasodilator, NO· triggers its biologically active form, cyclic 3′,5′- guanosine monophosphate (cGMP) arbitrating to endothelial dysfunction in STZ-induced diabetes (Bojunga et al., 2004). Additionally, elevated NO· has been reported to promote apoptosis intervening to endothelial damage (Levonen et al., 2001) and also demonstrated to induce β-cell dysfunction in experimental diabetes model with STZ (Rydgren and Sandler, 2002), which exerted its effect by triggering ER stress (Oyadomari et al., 2001). Moreover, ER stress and UPR induction links the upregulation of inflammatory and stress signaling networks intervened through the effect of triggered reactive oxygen species and NO· (Cullinan and Diehl, 2006; Gotoh and Mori, 2006). The association between ER stress and endothelin-1 (ET-1), a potent vasoconstrictor have been reported in human microvascular endothelial cells (Kalani, 2008; Lenna et al., 2013) and also found to be increased in diabetic patients (Schneider et al., 2002; Seligman et al., 2000). ET-1 has also been evidenced as an effective stimulus of NO· production (Stricklett et al., 2006) and associated with pancreatic damage in STZ-induced animals (González et al., 2000). With respect to this, ER stress elicits oxidative and nitrosative stress-related mechanisms thereby play a foremost role in mediating diabetic vascular complications.

Considering the significance of ER stress, attenuation of UPR pathway inclined to be a novel therapeutic strategy to attenuate vascular complications in diabetes (Galán et al., 2014). Evidence emphasizes the effect of antioxidant molecules against ER stress and UPR induction (Malhotra et al., 2008). Additionally, few polyphenolic compounds such as curcumin, proanthocyanidin and astragaloside IV have been reported to ameliorate ER stress in STZ-induced diabetes (Afrin et al., 2015; Ding et al., 2013; Wang et al., 2015). Our earlier study with quercetin, a flavonoid compound showed the protection against ER stress and re-established ER homeostasis by reducing the apoptosis and increasing antioxidant status, in endothelial cells (Suganya et al., 2014). In this present study, we made an attempt to evaluate the effect of quercetin against STZ-induced ER stress in the pancreas, *in vivo* with emphasis on its role in reversing endothelial dysfunction.

2. Materials and methods

2.1. Animals and diet

Male albino Wistar rats (170–200 g BW), averaging 5–7 weeks old were used in this study. The animals were maintained under a constant 12 h light and dark cycle at 21 ± 3 °C; with relative humidity 30–70% in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad. The study was approved by the Institutional Animal Ethical Committee (72/IAEC/2013), SRM University. Throughout the experimental period, the animals were fed with a commercially available balanced pellet diet and water *ad libitum* (Hindustan Lever Ltd., Mumbai, India).

2.2. Experimental induction of diabetes

Diabetes was induced by intraperitoneal injection of STZ (50 mg/kg BW) for five consecutive days, with freshly prepared in 0.1 M sodium citrate buffer (Verspohl, 2002). After 7 days, rats with moderate diabetes (i.e., fasting blood glucose concentration range of 200–300 mg/dl) that exhibited glycosuria were selected for the experiments.

2.3. Experimental design

The rats were divided into six groups (n = 6): (1) control; (2) control rats treated with quercetin (50 mg/kg BW); (3) STZ-induced diabetic rats; (4) diabetic rats treated with quercetin (25 mg/kg BW); (5) diabetic rats treated with quercetin (50 mg/kg BW) (Machha et al., 2007) (6) diabetic rats treated with gliclazide (10 mg/kg BW) (Vallejo et al., 2000). Gliclazide, an oral antihyperglycemic agent reported to improve endothelial function in diabetic rats (Vallejo et al., 2000) has been used as a reference drug. Quercetin and gliclazide were dissolved in carboxymethyl cellulose (0.5%) (Seufi et al., 2009) and orally administered to the respective groups for six weeks. Fasting blood glucose of experimental animals was periodically recorded by Accu-Chek Glucometer (Roche Diagnostics, Indianapolis, USA). After the experimental period, animals were fasted overnight and killed by cervical decapitation, blood samples were collected by cardiac puncture and the pancreas was dissected out and stored for further analysis.

2.4. Analytical procedures

Serum insulin levels were determined with an ELISA kit using rat insulin as a standard (Mercodia, Uppsala, Sweden) and cGMP levels were measured using the cGMP ELISA kit (Abcam, Cambridge, MA, USA). The serum NO· levels were indirectly measured as serum nitrite using Greiss assay (Miranda et al., 2001). The pancreas was perfused with ice-cold 0.9% NaCl to completely remove blood, followed by homogenization using ice-cold 0.15 mol/l KCl buffer (pH 7.4) and were used for the estimation of malondialdehyde (MDA) (Buege and Aust, 1978) and lipid hydroperoxides (LPO) (Jiang et al., 1992). In addition, the rat pancreas was rapidly homogenised in cold phosphate buffer (pH 7.4) and used for the estimation of enzyme activities including superoxide dismutase (SOD) (Kakkar et al., 1984), catalase (CAT) (Aebi, 1984), glutathione peroxidase (GpX) (Rotruck et al., 1973) and reduced glutathione (GSH) (Davidson and Hird, 1964).

2.5. Histology and immunohistochemistry of pancreas

The pancreatic tissues obtained from all the experimental animals were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. The organ was processed in graded series of alcohol and embedded in paraffin wax. The spleenic region of the pancreas was sectioned (5 μm) using a microtome (Reichert-Jung, Buffalo, NY, USA), mounted on glass slides, stained with hematoxylin and eosin and images were captured using a microscope (Carl Zeiss, Thornwood, NY, USA).

Immunohistochemical analysis was performed using primary antibodies such as insulin (sc-9168; Santa Cruz Biotechnology Inc. CA, USA), CHOP (sc-575; Santa Cruz Biotechnology Inc. CA, USA) and endothelin-1 (ET-1) (ab117757, Abcam, USA). The 5 μm thick paraffin sections were deparaffinized in xylene and hydrated with ethanol. The hydrated sections were then treated with 3% H_2O_2 in methanol for 30 min to block any endogenous peroxidase and washed with 0.01 M phosphate buffer for 10 min. The sections were further processed by an