Biomedicine & Pharmacotherapy 101 (2018) 821–832

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Phloretin either alone or in combination with duloxetine alleviates the STZ-induced diabetic neuropathy in rats

Mohamed Balaha[a,*], Samah Kandeel[b], Ahmed Kabel[a]

[a] Pharmacology Department, Faculty of Medicine, Tanta University, Postal No. 31527, El-Gish Street, Tanta, Egypt
[b] Histology Department, Faculty of Medicine, Tanta University, Postal No. 31527, El-Gish Street, Tanta, Egypt

ARTICLE INFO

Keywords:
Phloretin
Duloxetine
STZ
Diabetic neuropathy
Oxidative stress
Inflammatory cytokine

ABSTRACT

Diabetic neuropathy (DN) is one of most disabling disorder complicating diabetes mellites (DM), which affects more than 50% of the all diabetic patients during the disease course. Duloxetine (DX) is one of the first-line medication that approved by FDA for management of DN, nevertheless, it is too costly and has many adverse effects. Recently, phloretin (PH) exhibited powerful euglycemic, antihyperlipidemic, antioxidant, and anti-inflammatory activities. Therefore, we investigated the *in vivo* possible antineuropathic activity of phloretin, besides, its modulating effects on duloxetine potency, in a rat model of DN. Twelve-week-old male Wistar rats received a single intraperitoneal injection of 55 mg/kg STZ to induce DM. Either DX (30 or 15 mg/kg dissolved in distilled water), PH (50 0r 25 mg/kg dissolved in 0.5% DMSO) or a combination of 15 mg/kg DX and 25 mg/kg PH, used daily orally for 4 weeks to treat DN, starting from the end of the 4th week of DM development, when DN confirmed. Our finding showed that both DX and PH dose-dependently improved behavioral parameters (with the superiority of DX), sciatic nerve tissue antioxidant state, and suppressed tissue inflammatory cytokine, besides, they abrogated the tissue histopathological changes (with the superiority of PH). Moreover, DX augmented the DM metabolic disturbance and hepatic dysfunction, however, PH effectively amended these disorders. Furthermore, the low-dose combination of both, had the merits of both medications, with the alleviation of their disadvantages. Therefore, phloretin could be a promising agent in the management of DN either alone or in combination with duloxetine.

1. Introduction

Diabetes mellitus (DM) is one of the worldwide major health problems, with many medical and financial burdens. One of its most disabling disorder is neuropathy, affecting more than 50% of the all diabetic patients during disease course [1]. Diabetic neuropathy (DN) could define as a neuropathic disorder that affects all types of peripheral nerves, including sensory, motor and autonomic nerves, thus affecting nearly all body organs and systems. Moreover, DN usually affects the long somatosensory nerves of hand and feet leading to either loss of sensation or severe pain [2]. DN pains clinically presented with a lot of symptoms and signs such as burning, numbness, tingling, stabbing sensation, shooting pain, allodynia and hyperalgesia, with the failure of the endogenous analgesic pathways for controlling the neuropathic pain [3]. It accounts for about 22% of diabetic patients, and resulted in poor quality of life, anxiety, depression and sleep disturbance [4,5]. Additionally, foot ulcers and Charcot neuroarthropathy are consequences of diabetic hypoesthesia that ends ultimately into amputation [6].

The persistent hyperglycemic state of diabetic patients is associated with, glycosylation and oxidative damage to important fats, proteins and nucleic acids resulting into functional and structural damage of nerve fibers, with the development of DN [2,7]. Moreover, DN development and progression may be immunologically mediated by activation of the inflammatory cascades through augmentation of glucose hexosamine pathway influx, stimulation of receptor for advanced glycation end products (AGEs), activation of nuclear factor kappa B (NFκB) and enhancement of inflammatory cytokine releases, such as TNF-α and IL-6. [8–11]. Furthermore, the diabetic dyslipidemia is involved in DN development as the increased free fatty acids directly damaging Schwann cells, meanwhile the increased oxidation of cholesterol to oxysterols inducing apoptosis, as well the raised level of LDL, which reformed in DM by either oxidation or glycation, induce apoptosis, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation [12,13]. Additionally, the loss of the neurotrophic function of insulin, through either a deficiency in type-1 DM or resistance and a relative deficiency in type-2 DM, enhance the development of diabetic neuropathy via neuronal mitochondrial dysfunction, apoptosis, and

* Corresponding author.
E-mail address: Mohamed.Balaha@Med.Tanta.Edu.Eg (M. Balaha).

https://doi.org/10.1016/j.biopha.2018.02.135
Received 23 January 2018; Received in revised form 23 February 2018; Accepted 26 February 2018

oxidative damage [14]. These previous changes involved in the pathogenesis of microvascular dysfunction, with subsequent further stimulation of the macrophage recruitment, thus additional enhancement of inflammatory reactions, thereby, making a vicious circle that disturbed the nerve function and ends eventually in neuronal death that manifested clinically as DN [15].

Although, many therapeutic strategies have been developed for the management of painful DN, including, painkiller, vasodilator, anti-epileptic, anti-depressant, opioid, capsaicin and antioxidant agents, the course of disease development is still progressive and uncontrollable [16,17]. Also, all these drugs are aimed to reduce the pain, but not improve the hyperglycemic state, though the controlling of hyperglycemia proved to improve neuropathic pain in 78% and 10% in type 1 and type 2 diabetes mellitus patients respectively [6]. In addition, only 50% of diabetic patients feel 30% reduction of the neuropathic pain with these medications [18].

Duloxetine (DX) is one of the antidepressant drugs, which approved as a first-line therapy by the FDA, EMA, and Health-Canada, for the management of diabetic neuropathy [19]. However, it is too costly, and have many adverse effects including sedation, dizziness, drowsiness, somnolence, hypertensive crisis, myocardial infarction, cardiac arrhythmias, glaucoma, weight gain, decreased libido, severe hyponatremia, serotonin syndrome and neuroleptic malignant syndrome, in addition to hyperglycemia, increased glycosylated hemoglobin A1c (HbA1c), aspartate aminotransferase level (AST), total cholesterol and low-density lipoprotein (LDL). However, these associated problems are reduced with dose reduction, but at the same time, its therapeutic efficacy diminished [6,20,21]. Therefore, we are in great need to search for new agents that once combined with DX could reduce its dose, and hence its cost and adverse effects, meanwhile maintain its efficacy in controlling the neuropathic pain and could modulate the progressive course of the disease.

Phloretin (PH) is a naturally occurring dihydrochalcones flavonoid present in apple, especially in its peel, pear, strawberry, and vegetables. It has been proven to be a very effective inhibitor of sodium glucose co-transporter (SGLT) type 1 and 2, and intestinal glucose transporter (GLUT) -1 and 2. Thus, it inhibits glucose intestinal absorption and enhances urinary glucose excretion. Besides, it increases myotubes' glucose uptake by GLUT-4 and the Phosphatidylinositol-3 kinases/Protein kinase B (PI3K/Akt) pathway, meanwhile it suppresses AGEs production and improves insulin resistance [22–25]. Additionally, it exhibits strong antioxidant, anti-inflammatory, and antihyperlipidemic activities, along with maintaining normal mitochondrial function and endothelial integrity [22,23,26,27]. So, it's very effective in controlling the blood glucose level in DM and prevents diabetic complication such as nephropathy, retinopathy, and microvascular dysfunction. However, it's *in vivo* activity against diabetic neuropathy has never been evaluated. Therefore, in the present study, we assessed its activity against diabetic neuropathy *in vivo*, as well as its modulating effects on DX potency in the treatment of diabetic neuropathy.

2. Materials and methods

2.1. Agents and chemicals

Streptozocin (STZ), Phloretin, dimethyl sulphoxide (DMSO), duloxetine, Ellman's reagent, sodium dodecyl sulfate, pyridine, osmium tetroxide and epoxy resin obtained from Sigma, St. Louis, MO, USA. Moreover, formalin buffered saline, xylol, hematoxylin and eosin stain, phosphate buffered glutaraldehyde, sodium hydroxyl (NaOH), ethyl alcohol, Tris-HCl buffer, and citrate buffer purchased from El Gomhuria Co., Tanta, Egypt. Furthermore, pentobarbital sodium acquired from Abbott Lab., Chicago, IL, USA, thiobarbituric acid from Riedel-de Haën, AG., Germany, *n*-butanol and 1,1,3,3 tetramethoxypropane from VWR International Ltd., Ballycoolin, Dublin, Ireland. However, sulfuric acid gained from Misr Chem. Indust., Alexandria, Egypt, acetic acid from El-Nasr Pharmaceutical Co., Sohag, Egypt, 5% glucose solution from Egypt Otsuka Co., 10th of Ramadan, Egypt, and toluidine blue from Fluka Chemie, GmbH., Buchs, Switzerland.

2.2. Animals

Twelve-week-old male Wistar rats (Tanta Faculty of Medicine's Animal House, Egypt) weighing 150–200 g were used. The animals were housed in plastic cages at room temperature with 12 h light/dark cycle, fed a standard laboratory diet and gave water *ad libitum*. All procedures were carried out between 9:00 A.M. and 5:00 P.M. All animals were accommodated for one week prior to the experimentation. All experiments were carried out following the guideline for the care and use of experimental animals in Tanta Faculty of Medicine with an approval of the Research Ethics Committee of the Faculty.

2.3. Induction of diabetic neuropathy

DN induced according to Kandhare et al. In brief, DM-induced by a single intraperitoneal (i.p.) injection of 55 mg/kg STZ (dissolved in cold citrate buffer, pH 4.4, 0.1 M) in overnight fasted rats. Meanwhile, control rats received citrate buffer instead. Then, rats drink 5% glucose solution rather than water for 24 h to reduce STZ-induced hypoglycemic death. Forty-eight hrs later, blood collected by a heparinized capillary tube through the retro-orbital puncture, and the blood glucose level estimated using an automated glucometer (Ridgtest™ GM300, Bionime Co., Taichung, Taiwan). Rats with fasting blood glucose level more than 250 mg/dl considered diabetic, and used for further experimentation. Then, DN confirmed by behavioral parameters at the end of the 4th week of DM development [28].

2.4. Experimental design

Eighty rats used in the present study and divided into 8 groups. Before any experimental handling, 10 rats randomly selected and assigned as group 1 (CON), which was the normal, non-diabetic rats that received cold citrate buffer (pH 4.4, 0.1 M) in a corresponding amount to STZ group. The remaining 70 rats were randomly divided into 7 groups, after confirmation of DN development. Group 2 (STZ) was STZ-induced DN group. Group 3 (VEH) was DN group that received distilled water and 0.5% DMSO daily orally in a corresponding amount to the DX and PH treatment. Group 4 (DXH) was DN group that treated orally with 30 mg/kg/d DX (dissolved in distilled water) [29]. Group 5 (DXL) was DN group that treated orally with 15 mg/kg/d DX (dissolved in distilled water). Group 6 (PHH) was DN group that treated orally with 50 mg/kg/d PH (dissolved in 0.5% DMSO) [30]. Group 7 (PHL) was DN group that treated orally with 25 mg/kg/d PH (dissolved in 0.5% DMSO). Group 8 (DXL&PHL) was DN group that treated orally with 15 mg/kg/d DX (dissolved in distilled water), and 25 mg/kg/d PH (dissolved in 0.5% DMSO). All treatments freshly prepared daily, and continued for 4 weeks, starting from the end of the 4th week, when DN confirmed. Moreover, all treatment introduced daily between 9:00 and 11:00 A.M., after two hrs of animal fasting.

2.5. Sample collection and tissue preparation

Twenty-four hrs after the last treatment, behavioral parameters were evaluated, then animals immediately anesthetized with i.p. injection of 50 mg/kg pentobarbital sodium, and blood collected through the retro-orbital puncture, centrifuged at 5000 rpm for 10 minutes (min), sera harvested and stored at −80 °C for further biochemical analysis. For measurement of HbA1c, 0.5 ml of blood collected in an EDTA coated tube. Soon afterward, animals sacrificed by cervical dislocation, dissected, and both sciatic nerves reaped. The right sciatic nerve weighed with PS-750.R1, RADWAG Wagi Electronics, Poland, then homogenized in 2 ml of 0.1 mM Tris-HCl buffer (pH = 7). After