



Full length article

Vinpocetine mitigates proteinuria and podocytes injury in a rat model of diabetic nephropathy

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ABSTRACT

Podocyte injury and glomerular basement membrane thickening have been considered as essential pathophysiological events in diabetic nephropathy. The aim of this study was to investigate the possible beneficial effects of vinpocetine on diabetes-associated renal damage. Male Wistar rats were made diabetic by injection of streptozotocin (STZ). Diabetic rats were treated with vinpocetine in a dose of 20 mg/kg/day for 6 weeks. Treatment with vinpocetine resulted in a marked decrease in the levels of blood glucose, glycosylated haemoglobin, creatinine, blood urea nitrogen, urinary albumin and albumin/creatinine ratio along with an elevation in creatinine clearance rate. The renal contents of advanced glycation end-products, interleukin-10, tissue growth factor-β, nuclear factor (NF)-κB and Ras-related C3 botulinum toxin substrate 1 (Rac 1) were decreased. Renal nephrin and podocin contents were increased and their mRNA expressions were replenished in vinpocetine-treated rats. Moreover, administration of vinpocetine showed improvements in oxidative status as well as renal glomerular and tubular structures. The current investigation revealed that vinpocetine ameliorated the STZ-induced renal damage. This beneficial effect could be attributed to its antioxidant and antihyperglycemic effects parallel to its ability to inhibit NF-κB which eventually modulated cytokines production as well as nephrin and podocin proteins expression.

1. Introduction

Diabetic nephropathy (DN) is a major complication of diabetes mellitus and the most common cause of the end stage renal disease (Takayanagi et al., 2011). DN is characterized by mesangial cells proliferation, mesangial matrix accumulation, renal hypertrophy and later glomerulosclerosis (Wu et al., 2012). Proteinuria is an important manifestation of glomerular injury as well as an independent mediator of progressive kidney damage in diabetic patients (Gorritz and Martinez-Castelao, 2012; Remuzzi et al., 2004). The glomerular filtration barrier of the kidney glomerulus that prevents plasma protein for leaking into primary urine comprises of endothelial cells, the glomerular basement membrane and visceral epithelial cells (podocytes) (Haraldsson et al., 2008; Pavenstadt et al., 2003). All three layers contribute substantially to the glomerular filtration barrier and damage to any layer might result in the development of proteinuria (Brinkkoetter et al., 2013).

Although the pathogenesis of DN is still far from being fully elucidated, hyperglycemia is a well distinguished contributing factor of chronic complications in diabetic mellitus. It does not only generate excessive free radicals but also could attenuate antioxidative

machineries (Giacco and Brownlee, 2010). Hence, oxidative stress has been considered to be a general pathogenic factor of diabetic complications including nephropathy. Therefore, a molecule possessing both hypoglycemic and antioxidant properties might be considered as a protective agent against DN.

Vinpocetine, a derivative of the alkaloid vincamine (Lorincz et al., 1976), has been found to be safe and effective in many disorders that could be seen as diabetic complications or associated with aging. These disorders include cerebrovascular disorders and cognitive impairment, such as stroke, senile dementia, and memory disturbances as well as atherosclerosis, neointimal hyperplasia and visual impairment secondary to arteriosclerosis (Bagoly et al., 2007; Patyar et al., 2011; Wang et al., 2014). These beneficial uses of vinpocetine are related to its selective phosphodiesterase (PDE)-1 inhibition (Dunkern and Hatzelmann, 2007), voltage-dependent sodium channels blockade (Sitges et al., 2005; Zhou et al., 2003), as well as its antioxidant (Herrera-Mundo and Sitges, 2013; Mendoza et al., 2007) and anti-inflammatory properties (Jeon et al., 2010; Medina, 2010). Furthermore, a recent study has revealed that vinpocetine also has an anti-hyperglycemic effect (El Sayed et al., 2014).

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Although the great work on vinpocetine in literatures, its effect on kidney diseases was no longer elucidated. Since a diabetic patient could have one or more of the aforementioned diseases together with DN, it would be of interest to study the effect of vinpocetine on renal disease. Therefore, the goal of the present study was to investigate whether vinpocetine could possess a beneficial effect against streptozotocin (STZ)-induced DN in rats.

2. Material and methods

2.1. Animals

Male Wister rats weighing 250–300 g were purchased from the animal facility of Faculty of Pharmacy, Cairo University. Rats were housed under the appropriate conditions of controlled humidity, temperature and constant light cycle and allowed free access to a standard rodent chow diet and water. The investigation complies with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication no. 85-23, revised 2011) and was approved by the Ethical Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (Permit Number: PT 1513).

2.2. Chemicals

STZ was obtained from Sigma Aldrich Chemicals (St. Louis, MO, USA). Vinpocetine powder was supplied as a gift from Global Napi pharmaceuticals (Egypt). All other chemicals were of analytical grade.

2.3. Experimental design

Diabetes was induced by a single intraperitoneal injection of STZ in a dose of 40 mg/kg, freshly prepared in 0.1 M citrate buffer (pH 4.5) (Aloud, 2016; Ramesh and Pugalendi, 2006; Srinivasan et al., 2014). The STZ-treated rats were allowed to drink 5% glucose solution during the first 24 h of diabetes induction to overcome STZ-induced hypoglycemia. Blood glucose concentrations of peripheral blood from the tail vein were measured using an analyzer (Roche Diagnostic Accu-Check test strips, Germany) after 48 h of STZ injection. Animals with blood glucose level > 250 mg/dl were considered as diabetic. Rats were randomly divided into four groups of 12 animals each as follows: diabetic untreated group (STZ control), diabetic vinpocetine treated group (STZ + vinpocetine), Normal vinpocetine treated group (Normal + vinpocetine) and a fourth group was injected with the corresponding volume of citrate buffer and served as normal control.

Vinpocetine was administered daily in a dose of 20 mg/kg, p.o. (El Sayed et al., 2014; Zaki and Abdelsalam, 2013) starting from the third day after STZ injection and continued for 6 weeks.

Immediately after the last dose of treatment, animals were housed in the metabolic cages for 24 h with free access to tap water without food to collect urine samples. Animals were weighed and blood samples were collected from each rat. Animals were killed by cervical dislocation under thiopental sodium (5 mg/kg) anesthesia (Onk et al., 2016) and the kidneys were excised, washed with saline and weighed. One kidney from each rat was fixed in 10% formalin and preserved for histopathological examination. The other kidney was homogenized in ice-cold normal saline to obtain a 20% homogenate that was divided into several aliquots and stored at -80°C for the later assessment of the chosen biochemical parameters.

2.4. Urine sampling and analysis

The volume of the collected urine samples was measured. After centrifugation, the urine supernatant was used to determine the levels of albumin and creatinine using specific kits obtained from Biodiagnostic (Dokki, Giza, Egypt). Albumin/creatinine ratio (ACR) was calculated according to the following equation:

$$ACR(\text{mg/g}) = \frac{\text{urineal bumin}(\text{mg/dl})}{\text{urine creatinine}(\text{mg/dl}) \times 1000}$$

2.5. Blood sampling and analysis

Blood samples were withdrawn in heparinized tubes from the retro-orbital sinus of all rats. Serum was separated for immediate measurement of fasting blood glucose (FBG) and the remaining serum was divided into small aliquots that were stored at -80°C to be used for estimation of blood urea nitrogen (BUN) and creatinine using specific kits obtained from Biodiagnostic (Dokki, Giza, Egypt).

Creatinine clearance (Ccr) was calculated on the basis of urinary creatinine, serum creatinine, urine volume and body weight using the following equation (Pan et al., 2014):

$$Ccr(\text{ml/min/kg}) = \frac{\text{urine creatinine}(\text{mg/dl}) \times \text{urine volume}(\text{ml})}{\text{serum creatinine}(\text{mg/dl}) \times 1440(\text{min})} \times \frac{1000}{\text{bodyweight}(\text{g})}$$

Fresh blood samples were used to determine the percentage of glycosylated haemoglobin (HA1c) using commercially available kit (Spectrum Diagnostics, Egypt).

In addition, Interleukin (IL)-10 was measured in serum using the specific enzyme linked immunosorbent assay (ELISA) kit (Elabsience Biotechnology Co, Ltd., Wuhan, Hubei, China).

2.6. Estimation of renal hypertrophy

Kidney hypertrophy index (KI) was estimated by comparing the wet weight of the kidney to the body weight as follow:
 $KI = \text{kidney weight}(\text{g})/\text{body weight}(\text{g})$

2.7. Histopathological examination

Kidney samples fixed in 10% formalin were prepared in paraffin blocks. Transverse sections of 4–6 μm were obtained, stained with hematoxylin and eosin (H & E) and Periodic Acid Schiff (PAS). The sections were examined under a light microscope and graded individually by a pathologist blinded to the treatment regimen. In H & E staining, each section was assigned a damage score between 0 and 3 for each of six parameters, namely, vacuolar degeneration of tubular epithelium, vacuolation of endothelial lining glomerular tuft, thickening of glomerular basement membrane, hypertrophy of glomerular tuft and glomerulitis, periglomerular inflammatory cells infiltration, as well as focal tubular necrosis associated with inflammatory cells infiltration. The scores for the six parameters measured for each rat were summed to obtain the “total histology score”, being maximally 18 (three being the maximum for the six parameters examined).

Sections stained with PAS stain were observed for glycogen deposits in renal tubular epithelium and basement membrane thickening. The degree of basement membrane thickening was graded and assigned a score between 0 and 3.

2.8. Biochemical measurements in renal tissues

2.8.1. Measurement of secreted cytokines

Interleukin (IL)-10 was measured in kidney homogenate using the specific ELISA kit (Elabsience Biotechnology Co, Ltd., Wuhan, Hubei, China). Kidney homogenate was used also for estimation of transforming growth factor (TGF)- β using the corresponding ELISA kit (Kamiya biomedical company, Seattle, WA 98168, USA).

2.8.2. Lipid peroxidation measurement

Kidney lipid peroxidation products were estimated by determination of the level of thiobarbituric acid reactive substances (TBARS)

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