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Nephroprotective role of dipyridamole in diabetic nephropathy: Effect on inflammation and apoptosis

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ABSTRACT

Aims: Inflammation plays significant roles in developing diabetic nephropathy (DN). Adenosine, natural purine nucleoside, acts as potent endogenous anti-inflammatory agent. Extracellular adenosine usually disappears quickly due to rapid uptake into adjacent cells. In this regard; we investigated putative reno-protective effects of dipyridamole, nucleoside transport inhibitor, by exploring its anti-inflammatory mechanisms *in-vivo* and *in-vitro*.

Main methods: Daily 6 mg/kg/day dipyridamole was given to six-weeks streptozotocin-induced diabetic rats over two-week period in presence/absence of 10 mg/kg/day CGS15943, potent non selective adenosine receptors antagonist. Histological changes were assessed in kidney sections. Gene and protein expression of interleukin (IL)-1β, IL-10, IL-18, tumor necrosis factor (TNF)-α and intercellular adhesion molecule (ICAM)-1 was measured. Activation of apoptotic pathway was demonstrated by measuring the activity of caspase-3/8/9 and activation of c-Jun NH_2-terminal kinases (JNK)-mitogen-activated-protein kinase (MAPK). In addition, all markers were measured in human mesangial cells cultured in high glucose.

Key findings: Diabetes induced marked changes in the glomerular and tubular structure including focal glomerulosclerosis with marked shrinkage of some glomerular tufts. Diabetes resulted in enhanced production of IL-1β, IL-18, TNF-α and ICAM-1 associated with reduced IL-10 protein level, leading to activation of caspases-3/8/9 and pJNK/JNK *in-vivo* and *in-vitro*. Dipyridamole treatment restored diabetes-induced reduction in adenosine levels and resulted in mild glomerular effects and vacuolation of tubular epithelium. Dipyridamole reduced the adhesion molecule, ICAM-1, and restored the normal balance between pro- and anti-inflammatory cytokines *in-vivo* and *in-vitro*.

Significance: Dipyridamole prevented the progression of DN by elevating endogenous levels of protecting adenosine, leading to reduction in inflammation and intrinsic apoptosis.

1. Introduction

Supported by the recent reports, the global prevalence of diabetes will increase to 7.7% of the world population by 2030 [1]. Persisting high blood glucose level without appropriate treatment will develop life-threatening secondary disorders. Diabetic nephropathy (DN) is one of the most dangerous complications and secondary disorders of diabetes worldwide. DN represents a huge health and economic encumbrance [2]. Therefore, it is essential to develop novel strategies to prevent the incidence and progression of DN.

The pathogenesis of DN is multifactorial and complex. Inflammation performs major roles in developing DN. The major inflammatory mediators in DN include transcription factors, cytokines, chemokines and adhesion molecules. These inflammatory pathways activate and recruit macrophages and fibroblasts, which in turn initiate renal injury [3]. Therefore, targeting inflammation could be a potential treatment strategy for DN.

Adenosine is a natural purine nucleoside, which is formed from the breakdown of adenosine triphosphate (ATP) in response to stress to act as potent physiologic and pharmacologic regulator [4]. Adenosine is potent anti-inflammatory agent through coupling with specific G protein-coupled receptors on inflammatory and immune cells [5]. However, the beneficial effect of endogenous adenosine is short lived because extracellular adenosine usually disappears quickly due to rapid uptake into adjacent cells [6]. Adenosine uptake inhibitors block adenosine-active transporters on the cell membrane leading to elevated extracellular adenosine concentration [7]. Thus, inhibiting nucleoside transporters is a potential way to retard the disappearance of adenosine.

Ample evidence has accumulated that adenosine uptake inhibitors may have therapeutic applications in many diseases [8]. They enhance the protective effects of endogenous adenosine as well as avoiding the

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undesirable side effects associated with systemic administration of adenosine receptor agonists [9]. Indeed, adenosine uptake inhibitors have been reported to exhibit protective effects in different disease models including epilepsy, ischemic cardiac and cerebral injury, seizures, thrombosis, arrhythmia, insomnia, pain and inflammatory diseases [10]. Therefore, the present study was undertaken to investigate the putative reno-protective effects of dipyridamole, a nucleoside transport inhibitor in DN. Intriguingly, dipyridamole has been recently found to blunt DN in diabetic rats [11]. Hence, we aimed to explore the anti-inflammatory mechanisms involved the reno-protective effects of dipyridamole using STZ-induced experimental diabetic rat model and high glucose treated mesangial cells.

2. Methods

2.1. Animals and their treatment outlines

Experiments were performed according to a protocol approved by the ethical committee in Faculty of Pharmacy, University of Mansoura. Adult male Sprague Dawely rats weighing 180–230 g were maintained under standard conditions and allowed free access to food and water. Rats weight was recorded weekly (Fig. 1b). Rats were assigned randomly into:

Control group (n = 12). Rats received 0.5% carboxy methyl cellulose (CMC) in citrate buffer (0.01 mol/l pH 4.5) orally and served as vehicle control group.

Treated control group (n = 12). Rats received 6 mg/kg/day dipyridamole orally in 0.5% CMC for 2 weeks.

Diabetic group (n = 12). 36 rats were rendered diabetic by single intra-peritoneal (ip) injection of 50 mg/kg streptozotocin (STZ, Sigma Aldrich Chemicals Co., St. Louis, MO, USA) after fasting for 12 h. After 48 h, tail vein blood glucose level was measured using glucometer (OneTouch® System, LifeScan, USA). Rats with blood glucose level greater than 250 mg/dl were considered diabetic and kept for 6 and 8 weeks for further studies. Twelve diabetic rats were kept without any treatment to serve as diabetic group. Twenty four rats were treated with dipyridamole and CGS15934.

Dipyridamole treated diabetic group (n = 12). After 6 weeks of induction of diabetes, rats received 6 mg/kg/day dipyridamole (Tocris, UK) orally in 0.5% CMC for 2 weeks.

Dipyridamole + CGS15934 treated diabetic group (n = 12). After 6 weeks of induction of diabetes, rats received both 6 mg/kg/day dipyridamole (Tocris, UK) orally and 10 mg/kg/day CGS15934 (Tocris, UK), a potent non selective adenosine receptors antagonist, by ip injection in 0.5% CMC for 2 weeks.

The doses and time course of experiments used were in the range of those used in other studies [12,13]. In addition, the dose was determined after appropriate preliminary experiments.

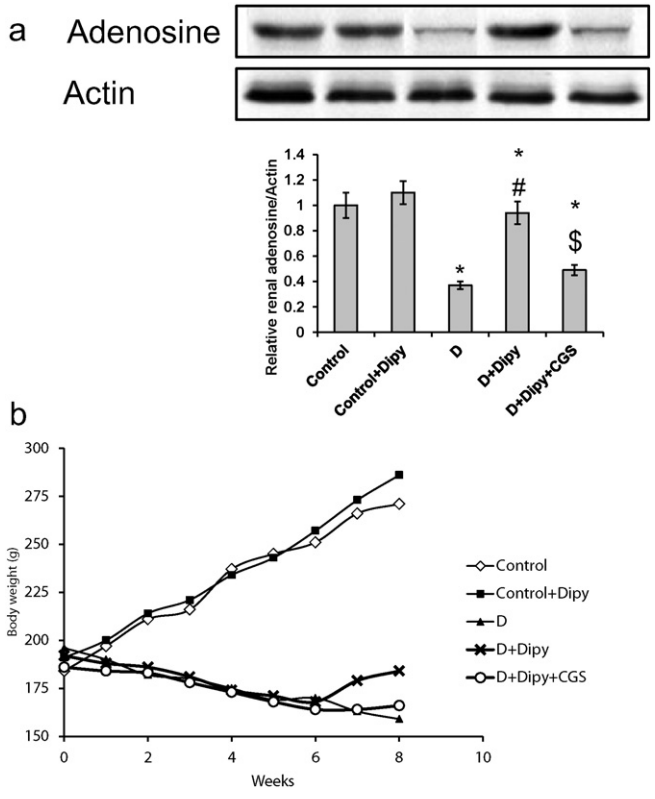

Fig. 1. Effect of dipyridamole on renal adenosine/actin ratio (a) and rats body weight (b). * Significant difference as compared with the control groups at $p < 0.05$. # Significant difference as compared with 8 weeks diabetic group at $p < 0.05$. $ Significant difference as compared with diabetic group treated with dipyridamole at $p < 0.05$. D, diabetic.

2.2. Collection of samples

At the end of treatment, rats in each group were individually housed in metabolic cage (Nalgene; Nalge Company, Rochester, NY, USA) for 24 h with free access to water and food. Total urine volume was measured. Then animals were sacrificed by decapitation. Rats' blood was collected and centrifuged at 3000 rpm for 5 min, then the formed sera were separated and stored at −80 °C. Rat kidneys were removed and weighed. The right kidney was used for histological analysis and the left one was immediately immersed in liquid nitrogen and stored at −80 °C. Renal tissue homogenate was prepared as previously described [14]. Briefly, one hundred milligrams of renal tissues and 4 volumes of 120 mM potassium chloride, 30 mM potassium phosphate, pH 7.4 buffer were sonicated for 1 min and then centrifuged at 600 g at 4 °C for 10 min. The supernatant was referred as homogenate, stored at −80 °C till used.

2.3. Histological analysis

As mentioned previously by our group [14], four rats were used from each group. The kidney was cut longitudinally; one half was fixed in 10% buffered formalin and embedded in paraffin. 5-μm thickness sections were cut and stained with Mayer's hematoxylin and eosin (H&E). Stained kidney sections were photographed using digital camera-aided computer system (Nikon digital camera, Japan).

2.4. Assessment of renal biochemical markers

It was assessed by commercially available kit for serum and urinary creatinine (Diamond Diagnostics, Egypt), serum urea nitrogen (Stanbio Company, TX, US) and urinary albumin excretion (ABC Diagnostics, Egypt).

2.5. Oxidative stress measurement

Oxidative stress was evaluated using the following parameters.

Thiobarbituric acid reactive species (*TBARs*). Renal homogenate level of TBARs, mainly malondialdehyde (MDA), was determined according to the reported method of Satoh [15] using a kit from Biodiagnostic Company.

Superoxide dismutase (*SOD*) *activity* was determined using phenazinemethosulfate (PMS) method [16].

2.6. Assessment of renal inflammatory cytokines and adhesion molecule

2.6.1. Enzyme-linked immunosorbent assay (ELISA)

The levels of ICAM-1, TNF-α, IL-1β and IL-10 were measured by ELISA assay using a commercially available ELISA kits (eBioscience Inc., San Diego, CA, USA) in accordance with the manufacturer's instructions.