Original Article
Renoprotective effect of lidocaine on streptozotocin-induced diabetic nephropathy

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Abstract: We assessed the renoprotective effect of lidocaine on a rat model of streptozotocin-induced diabetic nephropathy (DN). Diabetic nephropathy was induced with a single injection of streptozotocin (STZ, 60 mg/kg, i.p.). Lidocaine (5 mg/kg; 10 mg/kg; 20 mg/kg, p.o. 8 weeks) was administered to diabetic rats after 1 weeks of STZ treatment. Biochemical tests creatinine (Cr) and blood urea nitrogen (BUN) were performed to evaluate the renal functions. Oxidative stress was determined by estimating renal reactive oxygen species (ROS), malondialdehyde (MDA), total antioxidant capacity (T-AOC) and superoxide dismutase (SOD) levels. Inflammation was assessed by determining renal intercellular cell adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-18. Histological changes were also assessed in kidney sections. The results revealed that the STZ-treated deleterious alterations in kidney functions, enhanced oxidative stress, and caused inflammatory reaction. Administration of lidocaine significantly decreased biochemical parameters of inflammation and oxidative stress, and improved oxygenation and renal functions in a dose-dependent manner. These findings indicate that lidocaine exerts renoprotective effect on streptozotocin-induced diabetic nephropathy, and the renoprotective effect of lidocaine may be attributed to its antioxidative and anti-inflammation potential.

Keywords: Renprotective, lidocaine, diabetic nephropathy, oxidative stress, inflammation

Introduction

Diabetes is a metabolic disorder due to pancreatic dysfunction in insulin secretion and response, which has been identified as the third serious chronic disease to human health [1]. Long-term hyperglycemia leading to various diabetic chronic complications [2-4]. Diabetic nephropathy (DN) is the most common diabetic complications, which might initially develop into nephrotic syndrome, eventually leading to kidney failure and death [5]. It greatly increases the risk of premature death by end stage renal disease and is associated with increased cardiovascular mortality. Therefore, seeking for effectively therapeutic agent becomes urgently.

A number of key factors have been implicated in the pathogenesis of DN [6-9]. Among them, oxidative stress may act as trigger, modulator, and linker within the complex network of pathologic events [10, 11]. Furthermore, recent studies have clearly shown that kidney inflammation is crucial in promoting the development and progression of DN [12, 13]. Inflammation may be a key factor which is activated by the metabolic, biochemical, and haemodynamic derangements known to exist in the diabetic kidney. Therefore, compounds with anti-inflammatory or antioxidant features may be beneficial for DN.

Lidocaine is a widely used anesthetic in a variety of topically applied preparations, mostly as medicinal product [14]. A recent study showed that lidocaine exhibits antioxidant potential and anti-inflammatory properties [15, 16]. Thus, the aim of the present study was to examine the effect of lidocaine on streptozotocin (STZ)-induced DN and determine the potential underlying mechanisms in STZ-induced rats.

Materials and methods

Animals

Adult male Sprague-Dawley rats (N = 50) weighing 250-280 g were obtained from the Experi-

mental Animal Center of Suzhou Aiermaite technology Co. Ltd. (SPF grade, Certificate No. SCXK20140007). All of the rats were maintained in a 12 h light/dark cycle environment at $22 \pm 2°C$, with a relative humidity of $50 \pm 10\%$ and free access to water and food.

Ethics statements

All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures in this study were approved by the Institutional Animal Care Committee of Liaocheng People's Hospital.

Animal model and sample collection

The rats were randomly divided into five groups (n = 10): Group 1 (control), Group 2 (DN), Group 3 (DN + lidocaine 5 mg/kg), Group 4 (DN + lidocaine 10 mg/kg), and Group 5 (DN + lidocaine 20 mg/kg). In the DN and DN + lidocaine groups, diabetes was induced by a single intraperitoneal injection of 60 mg/kg freshly prepared streptozotocin (STZ, Sigma-Aldrich Co., Taufkirchen, Germany) dissolved in citrate buffer solution (0.1 M, pH 4.5). The animals in the control groups received the same volume of vehicle. 72 hours after injection, blood glucose level was quantified. Those with fasting blood glucose higher than 16.7 mM were defined as having diabetes. One week later, rats in the drug group received lidocaine via intraperitoneal injection (5 mg/kg; 10 mg/kg; 20 mg/kg, once daily for 8 weeks, purchased from Sigma-Aldrich Trading Co., Ltd, Shanghai, China), while control and DN group received equal volume of saline.

At the end of the experimental period, rats were sacrificed and collected for 24-hour blood and renal tissue samples.

Biochemical analysis

Blood glucose level, along with creatinine (Cr) and blood urea nitrogen (BUN) was quantified by automatic biochemical analyzer. The 24-hour total urea protein was determined by Biuret method.

The renal tissues were homogenized in physiological saline solution and the 10% homogenate was centrifuged at 4000 r/min for 15 min at 4°C. The supernatant was collected and quantitatively assayed for the activities of ROS, T-AOC and SOD, and the levels intercellular cell adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-18. The detection of these substances used enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer instructions (Nanjing Jiancheng Co.).

Histological evaluation

At the end of experiment, the kidneys were removed from the rats and fixed in 10% formaldehyde, embedded in paraffin, and cut into 5-μm thick sections. Then, the sections were stained with hematoxylin and eosin (HE), and examined under a light microscope.

Statistical analysis

The data were expressed as mean ± SD. SPSS 13.0 software package was used to perform all statistical analysis. Differences between groups were analyzed by one-way analyses of variance (ANOVA) followed by Dunnett's test. The significant level was defined as 0.05.

Results

Effect of lidocaine on renal functions

The body weight, kidney index, blood glucose level, Cr, BUN and 24-hour urine protein of the rats were shown in **Table 1**. Controlled rats had significantly elevated body weight than the other groups (P < 0.05). DN group had increased blood glucose, kidney index, Cr, BUN and 24-hour urea protein levels compared to control group (P < 0.05, respectively). Conversely, diabetic rats treated with lidocaine showed an attenuated renal function injury (Cr, BUN and 24-hour urea protein) in comparison with the STZ-induced DN group rats (**Table 1**).

Effect of lidocaine on renal oxidative stress parameters

STZ-induced oxidative stress in kidney was examined by measuring the level of ROS, T-AOC, SOD and MDA. As shown in **Figure 1**, the results showed a significant decline in the levels of T-AOC and SOD, along with the noticeable rise of the levels of ROS and MDA. Those dates were observed in DN group compared with control group (P < 0.05, respectively). While treatment with lidocaine increased the renal levels of T-AOC and SOD, simultaneously decreased the renal levels of ROS and MDA.

Table 1. General condition and biochemical indexes

Index	Control	DN	DN + lidocaine 5 mg/kg	DN + lidocaine 10 mg/kg	DN + lidocaine 20 mg/kg
Body weight (g)	457.6 ± 19.9	318 ± 12*	334 ± 8.4#	325 ± 8#	336 ± 11.4#
Kidney index (mg/g)	2.64 ± 0.09	6.64 ± 0.2*	5.82 ± 0.11#	5.39 ± 0.12#	4.68 ± 0.12#
Blood glucose (mM)	6.23 ± 0.46	26.63 ± 0.21*	21.67 ± 0.23#	18.42 ± 0.09#	16.5 ± 0.22#
Cr (mmol/L)	68.86 ± 0.66	156.46 ± 2.23*	127.99 ± 1.16#	107.27 ± 1.41#	96.93 ± 0.9#
BUN (mmol/L)	6.68 ± 0.2	15.88 ± 0.27*	13.73 ± 0.09#	12 ± 0.43#	8.48 ± 0.15#
24-hour urine protein (mg)	8.22 ± 0.99	65.57 ± 1.87*	41.92 ± 1.07#	37.18 ± 0.61#	32.03 ± 1.46#

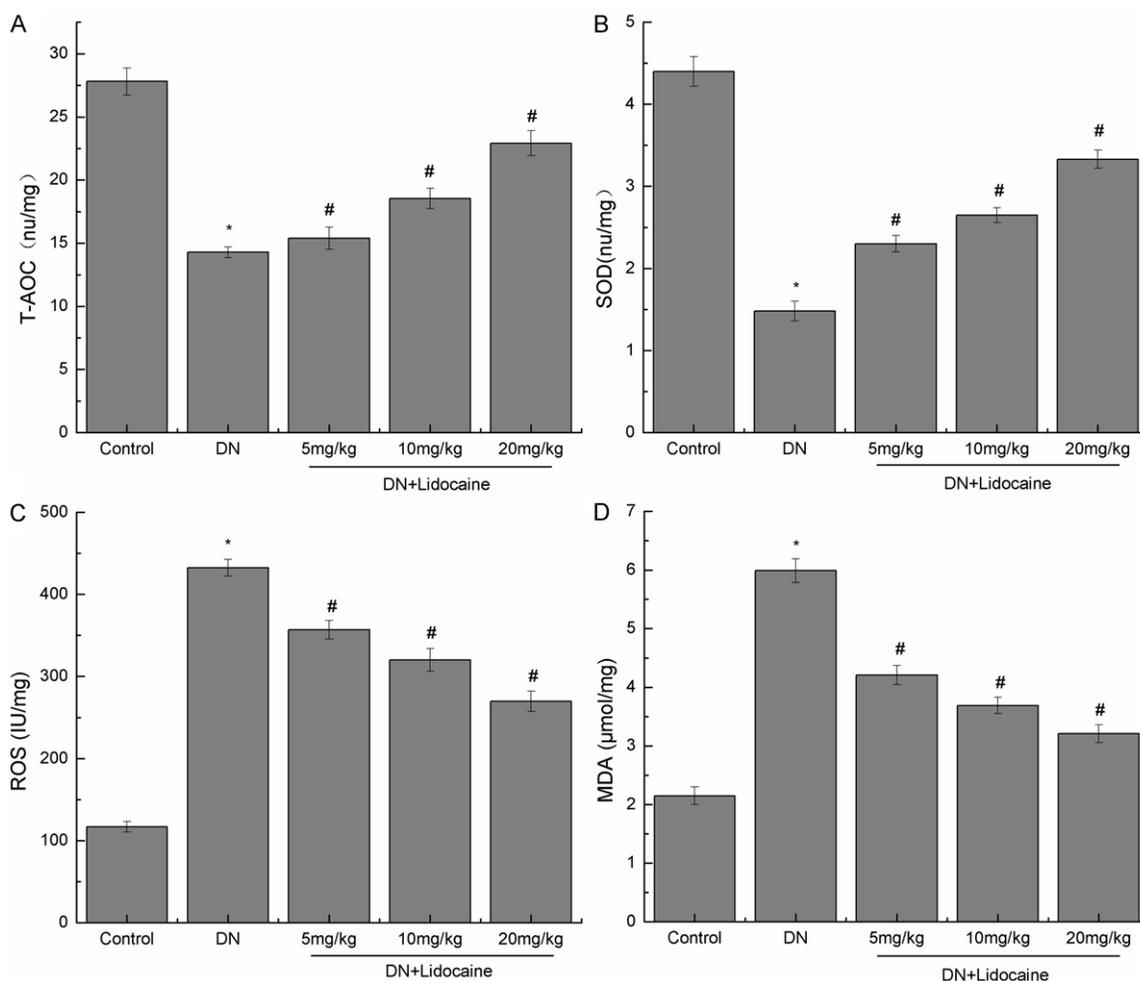Values are mean ± SD. *$P < 0.05$ vs. Control group, #$P < 0.05$ vs. DN group.

Figure 1. Effects of lidocaine on renal oxidative stress parameters. Lidocaine notably increases the T-AOC (A) and SOD (B) content suppressed by STZ and reduces the ROS (C) and MDA (D) content elicited by STZ. Data were shown as mean ± SD. *$P < 0.05$ vs. control group, #$P < 0.05$ vs. DN group.

Effects of lidocaine on renal inflammatory cytokines

In this study, we observed the effects of lidocaine on the levels of ICAM-1, TNF-α, IL-6 and IL-18 in kidney of rats. In comparison to the control group, rats with diabetic demonstrated increased ICAM-1, TNF-α, IL-6 and IL-18 levels (P < 0.05, respectively). However, treatment with lidocaine markedly decreased levels of these cytokines in the kidney at all doses (**Figure 2**).
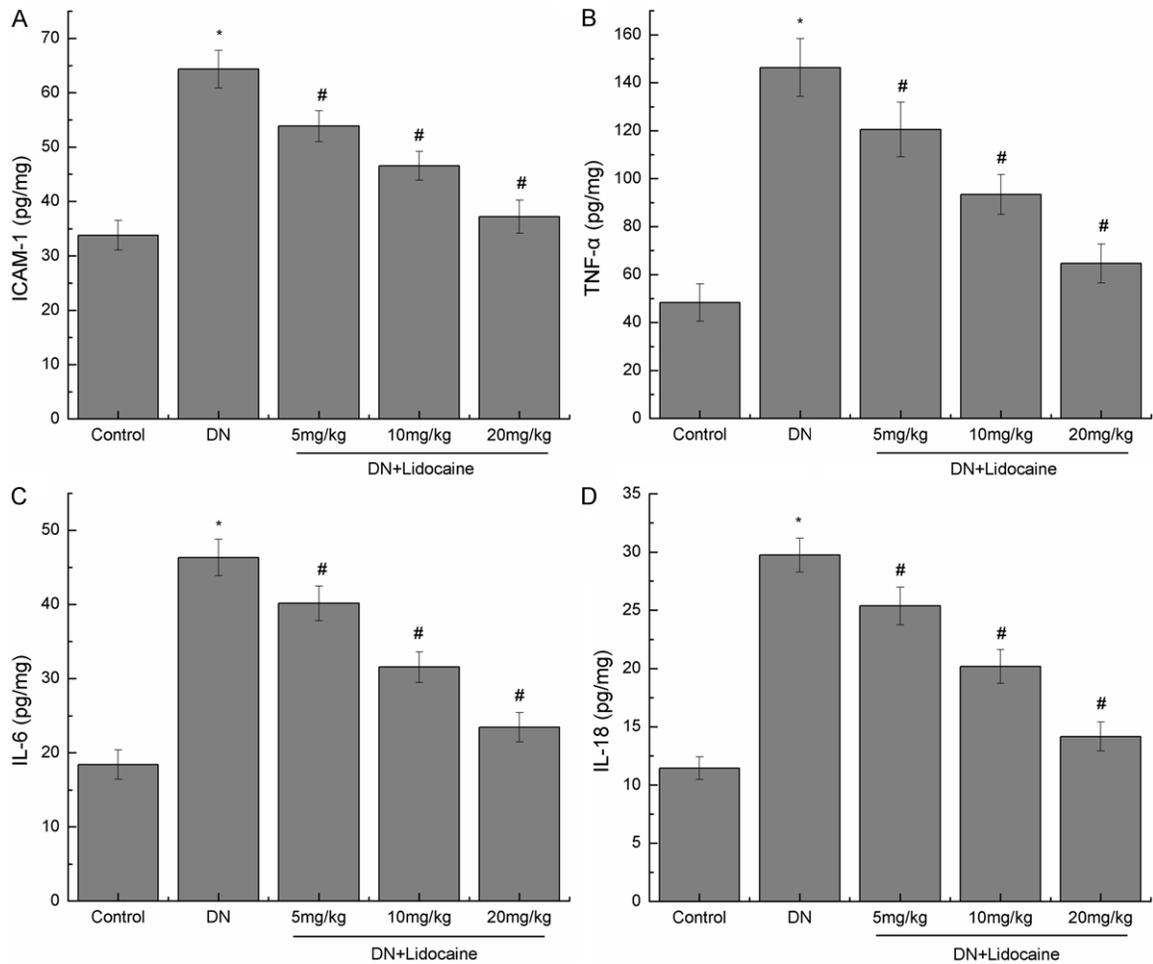
Figure 2. Effects of lidocaine on renal inflammatory cytokines. Lidocaine markedly decreased the levels of ICAM-1 (A), TNF-α (B), IL-6 (C) and IL-18 (D) in the rats elicited by STZ. Data were shown as mean ± SD. *P < 0.05 vs. control group, #P < 0.05 vs. DN group.

Histopathological evaluation

Figure 3 showed representative images of H&E stained kidney of all groups. The normal glomerulus surrounded by the Bowman's capsule, proximal, and distal convoluted tubules without any inflammatory changes. No histopathological alterations were observed in control kidney (Figure 3A). Compared with the control group, in kidney of the DN group, many histopathological changes such as degenerated glomeruli, the inflammatory cells in the glomeruli, and thickening of the basement membrane were observed (Figure 3B). However, in DN + lidocaine group, although the changes mentioned above also could be observed, nevertheless, STZ-induced these pathological damages of kidneys were markedly attenuated by lidocaine treatment (Figure 3C-E).

Discussion

DN is a major diabetic complication that leads to the severe end-stage renal diseases. There is still no effective preventative or therapeutic approaches until now. In this study, we generated a diabetic rat model via STZ induction and intraperitoneal injection of lidocaine to observe the renoprotective effect of lidocaine on DN, further to explore its possible mechanism. In model rats, we found significantly elevated BUN, Cr and 24-hour urea protein, all of which suggested the occurrence of renal damage. After the treatment of lidocaine, all those indexes were significantly depressed. These results demonstrate that lidocaine has renal protection function.

Oxidative stress has been found to play an important role in the development and progres-
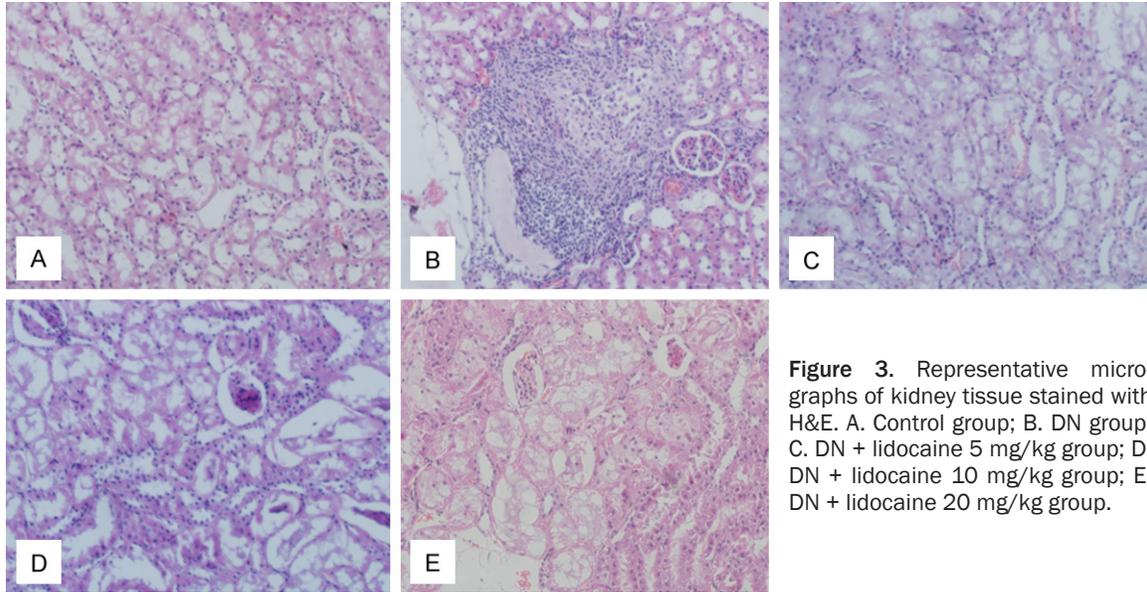
Figure 3. Representative micrographs of kidney tissue stained with H&E. A. Control group; B. DN group; C. DN + lidocaine 5 mg/kg group; D. DN + lidocaine 10 mg/kg group; E. DN + lidocaine 20 mg/kg group.

sion of DN, while high glucose is the main cause of the formation of ROS, which is involved in the production of oxidative damage [17, 18]. T-AOC and SOD are antioxidant enzymes that can resist oxidative damage [19]. Lipid peroxidation was assessed by measuring the MDA level [20]. In the experiment, STZ treatment induced a significant increase in the levels of ROS and MDA compared with that in the control group. While the levels of T-AOC and SOD were significantly reduced. Lidocaine treatment could significantly attenuate the ROS and MDA levels, simultaneously increased the renal levels of T-AOC and SOD in the kidney tissues of STZ-induced rats. These results indicated that the antioxidant capacity of lidocaine may play an important role in DN rats.

Inflammation in the STZ-induced diabetic rats was ascertained in the present study. Previous studies have indicated that inflammatory cytokines play an important role in the development and progression of DN [21]. ICAM-1 is a cell surface glycoprotein can be induced by hyperglycaemia, AGEs, oxidative stress, hyperlipidaemia and hyperinsulinaemia [22]. Resident renal cells are able to produce TNF-α, and TNF-α could induction cytotoxicity, apoptosis, and necrosis [23, 24]. Experimental studies have shown IL-6 over expression in diabetic kidneys, which correlate with kidney hypertrophy and albumin excretion [25]. IL-18 is a potent inflammatory cytokine that induces the production of other proinflammatory cytokines, upregulation of ICAM-1, and apoptosis of endo-

thelial cells [26, 27]. The levels of ICAM-1, TNF-α, IL-6 and IL-18 were increased in the renal tissues of diabetic mice. However, treatment with lidocaine markedly decreased levels of these cytokines. Those results suggested that lidocaine shows potential as a DN treatment via anti-inflammatory effects.

In conclusion, lidocaine treatment significantly ameliorates DN in STZ-induced diabetic rats by regulating oxidative stress parameters and inflammatory cytokines. These beneficial effects appear to be mediated by its antioxidative and anti-inflammation capacity. These results demonstrated that lidocaine could be a promising new therapeutic agent to prevent DN.

Disclosure of conflict of interest

None.

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