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Comparative evaluation of torasemide and furosemide on rats with streptozotocin-induced diabetic nephropathy

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ABSTRACT

Nephropathy is one of the complications of diabetes mellitus in human and experimental animals. There are various pathological renal remodeling processes leading to diabetic nephropathy because of the chronic hyperglycemia during diabetes mellitus. Various reports suggest the involvement of oxidative stress, inflammation and fibrosis during this progression. As antihypertensive drugs are reported to provide renoprotection in various animal models and clinical studies, we have carried out this study to identify the effect of torasemide, a loop diuretic, against streptozotocin-induced diabetic nephropathy and compare with furosemide. Here we have performed the measurement of blood and urine parameters and renal protein expression levels for measuring the disease severity in streptozotocin-induced diabetic rats treated torasemide or furosemide and compared with the vehicle treated rats. Furosemide treatment significantly increased the urinary protein excretion when compared with the normal rats. Torasemide treatment has reduced the expression of mineralocorticoid receptor and oxidative stress marker p67phox levels with improved mRNA levels of heme oxygenase-1 in the kidneys. In addition, torasemide treated diabetic rats showed significantly reduced expression of renal fibrosis related proteins when compared with the vehicle treated diabetic rats. Although furosemide treatment has produced improvement, its effects are comparably less than that of torasemide. Thus with the present results, we can suggest that torasemide treatment can improve the diabetic kidney disease in this rat model and which is superior to furosemide against renal fibrotic remodeling.

Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders. Diabetics have a markedly greater incidence of cardiovascular disease and renal failure than the non-diabetic population. In recent years, a large body of evidence suggested oxidative stress as a mechanism underlying insulin resistance and diabetic complications. There are several mechanisms involved in the complications of diabetes mellitus and in each mechanism, oxidative stress might play a crucial role in the pathogenesis of cardiac abnormalities, as hyperglycemia may induce generation of oxygen free radicals. Various therapeutic strategies for the prevention or delay in the development of the above-mentioned complications include effective glycemic control, prevention of hyperlipidemia, oxidative stress and adverse tissue remodeling (Patel and Goyal, 2011).

Diabetic nephropathy (DN), also known as Kimmelstiel–Wilson syndrome, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis and accounts for approximately 50% of end-stage renal failure cases (Abe, 2011). DN is characterized by initial oxidative stress, inflammatory response, thickening of basement membranes, expansion of mesangial matrix and interstitial fibrosis, podocytes and renal cell death, increased albuminuria, and renal dysfunction (Cui et al., 2012). It occurs in 20–40% of patients with diabetes and is the single leading cause of end-stage renal disease. Persistent albuminuria has been shown to be the earliest stage of DN in type 1 diabetes. It is also a well-established marker of increased cardiovascular disease risk (American Diabetes Association, 2003).

Torasemide belongs to the pyridine-sulfonylurea class of loop diuretics. Its primary site of activity is the thick ascending limb of the

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loop of Henle, where it blocks active reabsorption of sodium and chloride, resulting in diuresis, natriuresis, and other effects. Torasemide has high bioavailability, a relatively long half-life, and a prolonged duration of activity (Fowler and Murray, 1995). Adverse effects due to torasemide are usually mild and transient in nature. No evidence of ototoxicity has been demonstrated in humans, and torasemide does not appear to affect blood glucose levels, serum uric acid concentrations, or serum potassium levels at dosages below 5 mg/day (Dunn et al., 1995).

Because of the importance of antihypertensive drugs against the treatment of diabetic complications, we have planned in this study to identify the effectiveness of torasemide on streptozotocin (STZ)-induced DN in rats and to compare with another loop diuretic furosemide. Both torasemide and furosemide are prescribed as diuretic agents in hypertension and other cardiovascular diseases. But their effectiveness in diabetic kidney disease is not identified completely. A comparison of the effects of these two common diuretics will assist in selecting the suitable drug for treating DN.

Materials and methods

Reagents and chemicals

All the reagents and chemicals used were of analytical grade and purchased from either Sigma or Wako (Tokyo, Japan), until mentioned otherwise. Torasemide was provided by Taisho Toyama Pharmaceutical Co., Ltd. (Toshima-Ku, Tokyo, Japan). Furosemide was purchased from Wako (Osaka, Japan).

Ethics statement

Animal protocols were carried out with approval from the Review Board for animal studies at our institute. All procedures were performed under minimize suffering.

Animals and induction of diabetes

Male Sprague–Dawley rats weighing between 250 and 280 g were obtained from Charles River Japan Inc., Kanagawa and allowed to acclimatize to the local *vivarium*. The rats were housed on 12-h light–dark cycle and were allowed free access to standard laboratory diet and drinking water. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg). The rats were considered diabetic and used for the study only if they had hyperglycemia (≥300 mg/dL) at 72 h after STZ injection.

Experimental protocol

Rats were divided into four groups, namely normal (N, n = 6), diabetic (DN, n = 8), diabetic treated with torasemide (DN + Tor, n = 7) and diabetic treated with furosemide (DN + Fur, n = 7) groups. Five weeks after induction of diabetes, treatment was started with daily single oral dose of drug suspended in 0.5% methyl cellulose (vehicle) (Torasemide 3 mg and furosemide 30 mg/kg body weight/day) for 2 weeks. We have selected the doses based on our previous study using rat dilated cardiomyopathy animal model (Veeraveedu et al., 2008). Blood glucose and insulin levels were measured by tail vein bleeding.

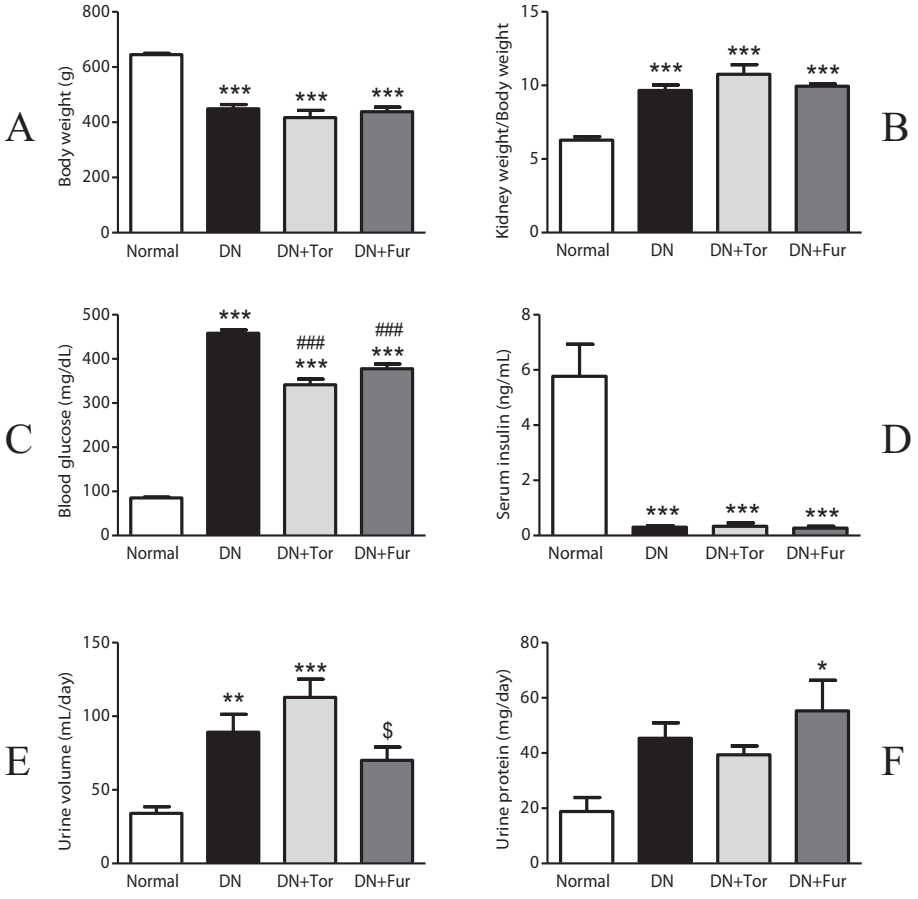

Fig. 1. Bar graphs depicting the values as on the final day of the experiment. A, Body weight comparison. B, Ratio of kidney weight to body weight analysis. C, Blood glucose level. D, Serum insulin levels. E, 24 h urine volume. F, Urine protein concentration. Normal, age matched control rats received vehicle (0.5% methyl cellulose); DN, diabetic rats received vehicle treatment; DN + Tor, diabetic rats received 3 mg/kg bw oral dose of torasemide; DN + Fur, diabetic rats received 30 mg/kg bw oral dose of furosemide; all the values are expressed as mean ± SEM, n = 6 to 8 rats per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Normal; $^{\#\#\#}p < 0.001$ vs DN; $^{\$}p < 0.05$ vs DN + Tor.