Abstract— Mast cells have been reported to be infiltrated in the kidney during renal diseases. The present study investigated the possible role of resident renal mast cells in the development of diabetes-induced nephropathy. The streptozotocin (STZ) (55 mg/kg, i.p., once) was administered in rats to induce experimental diabetes. The development of diabetic nephropathy was assessed using morphological, biochemical and histopathological assessments. Sodium cromolyn (24 mg/kg/day i.p.) or lisinopril (1 mg/kg, p.o., a standard agent) treatments were started in diabetic rats after 1 week of STZ administration and continued for 7 weeks. The single administration of STZ produced diabetes, which increased the renal oxidative stress and the density of resident renal mast cells and consequently produced nephropathy in 8 weeks. The treatment with sodium cromolyn, a mast cell stabilizer, significantly prevented the development of diabetes-induced nephropathy by reducing the density of resident renal mast cells and renal oxidative stress and consequently decreasing serum creatinine, blood urea nitrogen, proteinuria and glomerular and tubular injury. Treatment with lisinopril prevented the development of diabetes-induced nephropathy without affecting the density of resident renal mast cells. It may be concluded that diabetes-induced nephropathy is also associated with an increase in resident renal mast cell density and consequently decreasing serum creatinine, blood urea nitrogen, proteinuria and glomerular and tubular injury. Treatment with lisinopril prevented the development of diabetes-induced nephropathy possibly by preventing the degranulation of resident renal mast cells, reducing the renal mast cell density and protecting the diabetic kidney from oxidative insult.

Keywords— Diabetic nephropathy, Mast cells, Oxidative stress, Sodium cromolyn.

I. INTRODUCTION

Diabetic nephropathy is a leading cause of deaths in industrialised nations. Diabetic nephropathy is associated with persistent elevated albuminuria, declined glomerular filtration rate, and elevated arterial blood pressure and fluid retention [1], [2]. Numerous substances such as transforming growth factor-β (TGF-β), angiotensin-II (Ang-II), renin, endothelin-1 (ET-1) and advanced glycation end (AGE) products have been noted to be elevated during diabetic nephropathy [3]. Further, the induction and progression of diabetic nephropathy involves overexpression of various signalling molecules such as nuclear factor κB (NF-κB), tumor necrosis factor-α (TNF-α), poly (ADP-ribose) polymerase (PARP), Rho-Kinase, protein kinase C-β (PKC-β) and NADPH oxidase [4]. Limited therapeutic options are available for treating patients with diabetic nephropathy due to complexity in understanding the major signalling culprits involved in the disease pathogenesis. Mast cells are pluriopotent bone marrow derived cells that play a pivotal role in inflammation and tissue fibrosis [5], [6]. Mast cells precursors are progenitors of hematopoietic system that leave the bone marrow and migrate to injured tissue where they mature as resident mast cells [7], [8]. Mast cells were noted to be most abundant in the kidney of diabetic patients [9]. Mast cells have been reported to be infiltrated in the kidney during renal diseases [6], and mast cells contribute to renal deterioration by inducing chronic renal injury and glomerulonephritis [5]. Further studies suggest that mast cell density is increased significantly in the renal interstitium of diabetic kidney, which is correlated with relative interstitial volume, serum creatinine and urea in patients with diabetic nephropathy [10]. The degranulation of mast cells releases various pathological substances like TGF-β, chymase, tryptase, renin and various inflammatory cytokines [11], [12], which may play a detrimental role in the pathogenesis of diabetic nephropathy. Recently, we have suggested that resident renal mast cells may play a role in the pathogenesis and progression of diabetic nephropathy [13]. Sodium cromolyn is a well-known mast cell stabilizing agent, which prevents mast cell degranulation by averting transmembrane influx of calcium ions [14]. Moreover, sodium cromolyn reduces the increase in number of resident mast cells [15], [16]. Therefore, the present study has been undertaken to investigate the effect of sodium cromolyn, a mast cell stabilizer, in experimental diabetic nephropathy.

II. MATERIALS AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young wistar rats weighing about 220–270 g were employed in the present study. Rats were fed on standard chow diet and water ad libitum. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night.

A. Assessment of diabetes

The experimental diabetes mellitus was induced in rats by single injection of streptozotocin (STZ) (55 mg/kg i.p.), dissolved in freshly prepared ice cold citrate buffer (pH 4.5). The blood sugar level was monitored once daily for first week after administration of STZ. Then, at the end of the experimental protocol (8 weeks after
administration of STZ), the blood samples were collected and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. The serum glucose concentration was estimated by glucose oxidase peroxidase (GOD-POD) method [17] using the commercially available kit (Crest Biosystems, Goa, India).

B. Assessment of renal oxidative stress

The development of oxidative stress in the kidney was assessed by estimating renal thiobarbituric acid reactive substances (TBARS) and reduced form glutathione (GSH). The renal TBARS, an index of lipid peroxidation, was estimated according to the method described earlier [18]. The GSH level in the kidney was estimated by the method as described earlier [19]. The renal protein content was estimated by Lowry’s method [20].

C. Assessment of diabetes-induced nephropathy

The diabetes mellitus-induced nephropathy was assessed biochemically by estimating serum creatinine, blood urea nitrogen and proteinuria. The serum creatinine concentration was estimated by alkaline picrate method [21], blood urea was estimated by Berthelot method [22], proteinuria was assessed by pyrogallol red method [23] by using the commercially available kit (Crescent biosystems, Goa, India). The early diabetic changes in glomeruli were assessed histologically as previously described [24].

D. Toluidine blue staining for mast cells

The renal sections were stained using toluidine blue to assess the density of mast cells as described previously [6], and the mast cell density was quantified by counting number of toluidine blue positive mast cells per field at 100 fold magnification. At least 15 fields were included from each slide for counting and the mean mast cell density was calculated [6], [16].

E. Experimental protocol

Six groups were employed in the present study and each group comprised of 10 animals. Group I (Normal control), rats were maintained on standard food and water and no treatment was given. Group II (Diabetic Control), rats were administered streptozotocin (STZ) (55 mg/kg, i.p, once) dissolved in citrate buffer (pH 4.5). Group III (Sodium Cromoglycate per se), rats were administered sodium cromoglycate (24 mg/kg, i.p.) for 7 weeks. Group IV (Lisinopril per se), rats were administered Lisinopril (1 mg/kg, p.o.) for 7 weeks. Group V (Sodium Cromoglycate Treated Diabetic Group), the diabetic rats after 1 week of STZ administration were treated with sodium cromoglycate (24 mg/kg, i.p.) for 7 weeks. Group VI (Lisinopril Treated Diabetic Group), the diabetic rats after 1 week of STZ administration were treated with lisinopril (1 mg/kg, p.o.) for 7 weeks.

F. Statistical analysis

All values were expressed as mean ± S.E.M. The data obtained from various groups were statistically analyzed using one way ANOVA followed by Tukey’s multiple comparison test. The p value of less than 0.05 was considered to be statistically significant.

G. Drugs and chemicals

Streptozotocin was purchased from Sigma-Aldrich Ltd, St. Louis, USA. Sodium cromoglycate was obtained from Pharmascience INC., Montreal, Canada. Lisinopril was obtained from Dr. Reddy laboratories Pvt. Ltd., Hyderabad, India. 1,1,3,3-tetra methoxypropane was purchased from V. K. Chemicals and Instruments, Ambala, India. All other chemicals and reagents used in the study were of analytical grade and freshly prepared.

III. RESULTS

Administration of STZ (55 mg/kg, i.p., once) produced hyperglycemia after 72 hours (serum glucose >180 mg/dL). After 7 days of STZ administration, the rats showed blood glucose level of greater than 260 mg/dL were selected and were named as diabetic rats. Sodium cromoglycate (24 mg/kg i.p.,) and lisinopril (1 mg/kg p.o.,) were administered to diabetic rats after 7 days of single injection of STZ and their treatments were continued for 7 weeks. All the parameters were assessed at the end of 7 weeks in normal and diabetic rats with or without drug treatments. Administration of sodium cromoglycate (24 mg/kg i.p., 7 weeks) or lisinopril (1 mg/kg p.o., 7 weeks) to normal rats did not produce any significant per se effects on various parameters assessed in the present study. The kidney weight (KW) to body weight (BW) ratio (mg/g) was noted to be increased markedly in diabetic rats (5.94 ± 0.41) as compared to normal rats (4.07 ± 0.34). However, treatment with either sodium cromoglycate (24 mg/kg i.p., 7 weeks) (4.91 ± 0.27) or lisinopril (1 mg/kg p.o., 7 weeks) (4.73 ± 0.31) significantly reduced diabetes-induced increase in KW/BW (mg/g). Less than 10% of mortality rate was observed in diabetic rats with or without drug treatments.

A. Effect of mast cell stabilizer and lisinopril on serum glucose

The marked increase in serum glucose level was noted in diabetic rats as compared to normal rats. Treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) did not alter the serum glucose concentration in diabetic rats. However, treatment with lisinopril (1 mg/kg, p.o., 7 weeks) slightly reduced the glucose level in diabetic rats, but results were not statistically significant (Table 1).

B. Effect of mast cell stabilizer and lisinopril on renal oxidative stress

Diabetic rats after 7 weeks (8 weeks after STZ administration) showed marked increase in renal TBARS as compared to normal rats. In addition, the renal concentration of GSH was noted to be decreased in diabetic rats as compared to normal rats. Treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) or lisinopril (1 mg/kg p.o., 7 weeks) markedly attenuated diabetes-induced increase in renal TBARS and consequent decrease in renal GSH (Table 1).

C. Effect of mast cell stabilizer and lisinopril on serum creatinine and blood urea nitrogen

The serum creatinine and blood urea nitrogen levels were noted to be increased markedly in diabetic rats as compared to normal rats (Figs. 1 and 2). Treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) partially but significantly reduced the diabetes-induced increases in serum creatinine and blood urea nitrogen. On the other hand, treatment with lisinopril (1 mg/kg p.o., 7 weeks) markedly reduced the elevated levels of serum creatinine and blood urea nitrogen in diabetic rats (Figs. 1 and 2).

D. Effect of mast cell stabilizer and lisinopril on proteinuria

The marked elevation in proteinuria was noted in diabetic rats as compared to normal rats. Treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) partially but significantly reduced the incidence of proteinuria in diabetic rats. However, treatment with lisinopril (1 mg/kg p.o., 7 weeks) markedly reduced the diabetes-induced elevation in proteinuria (Fig. 3).
E. Effect of mast cell stabilizer and lisinopril on renal histological study

The diabetic rats after 7 weeks developed pathological changes in the glomeruli such as glomerular capillary size reduction, mesangial expansion and tubular injury as compared to normal rats. Pharmacological treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) prevented the diabetes-induced renal pathological changes by improving the glomerular capillary size, and reducing the mesangial expansion and tubular injury in diabetic rats. Moreover, treatment with lisinopril (1 mg/kg p.o., 7 weeks) markedly protected the diabetic kidney from renal pathological changes (Fig. 4).

F. Effect of mast cell stabilizer and lisinopril on renal density of mast cells

The renal mast cell density was noted to be markedly increased in diabetic rats as compared to normal rats. Pharmacological treatment with sodium cromoglycate (24 mg/kg i.p., 7 weeks) significantly reduced the diabetes-induced increase in number of mast cells. However, treatment with lisinopril (1 mg/kg p.o., 7 weeks) did not affect the elevated renal mast cell density in diabetic rats (Fig. 5).

IV. DISCUSSION

Diabetic nephropathy is an insidious disorder associated with renal dysfunction followed by renal failure. The treatment option for managing diabetic nephropathy is limited due to incomplete understanding of the major signalling culprits involved in the pathogenesis of this disease. The present study explored the novel role of mast cell stabilizer in preventing the development of diabetic nephropathy. Creatinine, a non-protein waste product of creatinine phosphate metabolism in the skeletal muscle, is freely filtered by the kidney and the serum creatinine level is elevated as a result of diminished glomerular filtration rate [25]. Thus, the ability of the kidney to filter creatinine is reduced during renal dysfunction [26]. The urea formed in the liver is excreted by the kidney and the nitrogen obtained during protein metabolism combines with urea to form urea nitrogen, whose elevation in the blood is considered to be an indication of renal dysfunction [27]. The degree of proteinuria correlates with the progression of glomerulosclerosis and tubulointerstitial fibrosis, and the renal hemodynamic changes have been suggested to be an important inducer of proteinuria [28]. The elevated levels of serum creatinine and blood urea nitrogen and the incidence of proteinuria have been documented to be an index of renal dysfunction in experimental diabetic nephropathy [29]. In addition, the chronic diabetic kidney is associated with structural changes such as glomerulosclerosis, glomerular capillary size reduction, mesangial expansion and tubular injury [24], [30]. In the present study, the levels of serum creatinine and blood urea nitrogen were noted to be increased in diabetic rats in 7 weeks. Moreover, the diabetic rats exhibited the incidence of proteinuria and marked renal structural changes such as glomerular capillary size reduction, mesangial expansion and tubular injury. These renal structural and functional abnormalities in diabetic rats observed in the present study indicate the development of nephropathy. The pharmacological treatment with sodium cromoglycate halted the development of nephropathy in diabetic rats by preventing the mast cell degranulation and markedly reducing the number of resident renal mast cells. Sodium cromoglycate is a well-known mast cell stabilizing agent, which prevents mast cell degranulation by inhibiting transmembrane influx of calcium ions [14]. Moreover, sodium cromoglycate reduces the increase in number of resident mast cells [15], [16]. Thus, it may be suggested that the resident renal mast cells play a detrimental role in the development of diabetes-induced nephropathy.

Mast cells have been shown to be infiltrated in the kidney and degranulated during renal diseases [6]. The increase in number of renal mast cells is associated with an induction of fibrosis and accumulation of extracellular matrix protein in the kidney of patients with diabetic nephropathy [9]. Further, mast cells involve in renal deterioration by inducing tubulointerstitial injury in patients with glomerulonephritis [5]. Moreover, the mast cell density was shown to be increased in the renal interstitium of diabetic kidney and the increase in mast cell density is well-correlated with the relative interstitial volume, serum creatinine and urea in patients with diabetic nephropathy [10]. TGF-β has been reported to stimulate fibroinectin synthesis in mesangial cells by activating extracellular regulating kinase (ERK) pathway [31]. Further, TGF-β involves in the incidence of albuminuria by reducing the renal uptake of albumin and decreasing the renal expression of albumin binding receptor megalin in diabetic rats [32]. Recently, the detrimental role of TGF-β in diabetic nephropathy was demonstrated in a study in which TGF-β enhanced the expression of interleukin-18 in human renal proximal tubular epithelial cells through an activation of MAPK pathway [33]. It is worthwhile to note that degranulation of mast cells releases TGF-β [34]. Chymase is an enzyme which is involved in the conversion of angiotensin-I to angiotensin-II, activation of TGF-β and alteration of the lipid metabolism [35]. Chymase has been shown to be upregulated in diabetic nephropathy and its upregulation is associated with glomerulosclerosis and tubulointerstitial fibrosis in patients with diabetic nephropathy [36]. Mast cells are the chief source of chymase, which is released during degranulation of mast cells [37]. Mast cell released tryptase has been demonstrated to play a role in the development of renal interstitial fibrosis by increasing the production of extracellular matrix protein [38]. Renin upregulates TGF-β1 gene expression in mesangial cells of the rat kidney by activating ERK1/2 pathway [39]. It is noteworthy that minor amount of renin is released during mast cell degranulation [12]. The overexpression of TNF-α is implicated in the development of renal lesions in patients with diabetic nephropathy [40]. In addition, the elevated level of TNF-α was shown to induce renal hypertrophy in rats with diabetic nephropathy [41]. It is interesting to note that mast cell degranulation releases TNF-α [42]. Taken together, it may be suggested that the resident renal mast cells degranulation-mediated release of various mediators such as TGF-β, chymase, tryptase, renin and TNF-α may play a pathogenic role in the development of diabetes-induced nephropathy. Thus, it is strongly suggested that the diabetes-induced significant increase in number of resident renal mast cells and their subsequent degranulation may account for the induction and progression of nephropathy in diabetic rats. This contention is strongly supported by the results obtained in the present study that treatment with sodium cromoglycate markedly reduced the diabetes-induced increase in mast cell density and their degranulation in the kidney and thus halted the progression of diabetic nephropathy. The diabetic rats developed renal hypertrophy as assessed in terms of increase in KW/BW ratio, which was markedly prevented by treatment with sodium cromoglycate, suggesting the additional beneficial effect of sodium cromoglycate in halting the development of diabetic nephropathy.

The increase in lipid peroxidation and decrease in GSH have been documented to be an index of oxidative stress [43]. In the present study, the lipid peroxidation assessed in terms of measuring TBARS has been noted to increased, which was accompanied with consequent reduction in GSH in diabetic rats with nephropathy. The reactive oxygen species (ROS) are known to activate mast cells
through intracellular Ca2+ mobilization [44], [45]. The ROS generated by mast cells are not exported out of the cells due to their strong antioxidant defence system [46]. However, TGF-β, Ang-II and TNF-α released from the degranulated mast cells may further augment the ROS production [47], [48] in the kidney, which may further deteriorate the structure and function of diabetic kidney. Thus, mast cell stabilization may reduce the renal oxidative stress in diabetic rats. This contention is supported by the results obtained in the present study that treatment with sodium cromoglycate partially decreased the renal TBARS levels and increased the GSH levels. The renoprotective effect of lisinopril, an inhibitor of angiotensin converting enzyme, has been well reported in basic and clinical studies [49], [50]. Therefore, lisinopril has been employed as a standard drug in the present study to compare the renoprotective potential of sodium cromoglycate. In the present study, treatment lisinopril prevented the development of diabetes-induced nephropathy without affecting the density of resident renal mast cells. On the basis of the above discussion, it may be concluded that diabetes-induced nephropathy is associated with an increase in resident renal mast cells density and consequent degranulation of mast cells with augmented renal oxidative insult. Sodium cromoglycate, being a mast cell stabilizer, may have halted the development of diabetes-induced nephropathy possibly by preventing the degranulation of resident renal mast cells, reducing the renal mast cell density and protecting the diabetic kidney from oxidative insult.

REFERENCES


TABLE I

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<tr>
<th>Assessments</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>SCG Per se</th>
<th>Lisino Per se</th>
<th>SCG Treat Diabetic Group</th>
<th>Lisino Treat Diabetic Group</th>
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<tr>
<td><strong>KW/BW (mg/g)</strong></td>
<td>0.34 ± 0.01</td>
<td>0.54 ± 0.01</td>
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<td>0.42 ± 0.01</td>
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<tr>
<td><strong>Serum Glucose (mg/dl)</strong></td>
<td>94.7 ± 14.3</td>
<td>344.9 ± 14.3</td>
<td>101.4 ± 14.3</td>
<td>97.27 ± 14.3</td>
<td>313.7 ± 14.3</td>
<td>310.7 ± 14.3</td>
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<tr>
<td><strong>Renal TBARS (nmol/mg protein)</strong></td>
<td>0.04 ± 0.01</td>
<td>0.64 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td><strong>Renal GSH (nmol/mg protein)</strong></td>
<td>21.97 ± 1.04</td>
<td>21.21 ± 1.04</td>
<td>24.22 ± 1.04</td>
<td>17.23 ± 1.04</td>
<td>17.05 ± 0.98</td>
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</table>

All values were represented as mean ± SEM. a = P < 0.05 versus normal control; b = P < 0.05 versus diabetic control. SCG indicates sodium cromoglycate; Lisino indicates lisinopril; KW/BW indicates kidney weight/body weight ratio (mg/g); TBARS indicates thiobarbituric acid reactive substances; GSH indicates reduced form of glutathione.

Fig. 1. Effect of mast cell stabilizer on serum creatinine (mg/dl). All values were represented as mean ± SEM. a = P < 0.05 versus normal control; b = P < 0.05 versus diabetic control; c = P < 0.05 versus sodium cromoglycate treated diabetic group.
Fig. 2. Effect of mast cell stabilizer on blood urea nitrogen (mg/dl). All values were represented as mean ± SEM. a = p < 0.05 versus normal control; b = p < 0.05 versus diabetic control; c = p < 0.05 versus sodium cromoglycate treated diabetic group.

Fig. 3. Effect of mast cell stabilizer on proteinuria (mg/24 hr). All values were represented as mean ± SEM. a = p < 0.05 versus normal control; b = p < 0.05 versus diabetic control; c = p < 0.05 versus sodium cromoglycate treated diabetic group.

Fig. 4. Effect of mast cell stabilizer on diabetes-induced renal pathological changes. SCC indicates sodium cromoglycate. The section of 3 µM in thickness were made and stained with hematoxylin and eosin to assess the pathological changes of glomeruli using the light microscopy (400 X). The diabetic rats developed renal pathological changes such as glomerular capillary size reduction, mesangial expansion and tubular injury as compared to normal rats. The treatment with sodium cromoglycate or lisinopril markedly prevented the diabetes-induced renal pathological changes by improving the glomerular capillary size, and reducing the mesangial expansion and tubular injury.

Fig. 5. Effect of mast cell stabilizer on elevated renal mast cell density in diabetic rats (Number of mast cells per mm2). All values were represented as mean ± SEM. a = p < 0.05 versus normal control; b = p < 0.05 versus diabetic control; c = p < 0.05 versus lisinopril treated diabetic group.