Exogenous kallikrein protects against diabetic nephropathy

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The kallikrein-kinin system has been shown to be involved in the development of diabetic nephropathy, but specific mechanisms are not fully understood. Here, we determined the renal-protective role of exogenous pancreatic kallikrein in diabetic mice and studied potential mechanisms in db/db type 2 diabetic and streptozotocin-induced type 1 diabetic mice. After the onset of diabetes, mice were treated with either pancreatic kallikrein (db/db + kallikrein, streptozotocin + kallikrein) or saline (db/db + saline, streptozotocin + saline) for 16 weeks, while another group of streptozotocin-induced diabetic mice received the same treatment after onset of albuminuria (streptozotocin' + kallikrein, streptozotocin' + saline). Db/m littermates or wild type mice were used as non-diabetic controls. Pancreatic kallikrein had no effects on body weight, blood glucose and blood pressure, but significantly reduced albuminuria among all three groups. Pathological analysis showed that exogenous kallikrein decreased the thickness of the glomerular basement membrane, protected against the effacement of foot process, the loss of endothelial fenestrae, and prevented the loss of podocytes in diabetic mice. Renal fibrosis, inflammation and oxidative stress were reduced in kallikrein-treated mice compared to diabetic controls. The expression of kininogen1, tissue kallikrein, kinin B1 and B2 receptors were all increased in the kallikrein-treated compared to saline-treated mice. Thus, exogenous pancreatic kallikrein both prevented and ameliorated diabetic nephropathy, which may be mediated by activating the kallikrein-kinin system.

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Diabetic nephropathy (DN), a major cause of end-stage renal disease, has become a worldwide public health issue.[1,2] Several clinical studies provided convincing evidence that angiotensin-converting enzyme inhibitors, which inhibit angiotensin II formation and bradykinin degradation, had blood pressure–independent beneficial effects on DN in both types 1 and 2 diabetes.[3–6] This highlighted that the kallikrein-kinin system (KKS) was also involved in DN. In an Akita diabetic mouse model lacking both types I and II bradykinin receptors (B1R and B2R, respectively), Kakoki *et al.*[7] found that these mice displayed detrimental phenotypes, including urinary albumin excretion, glomerulosclerosis, and glomerular basement membrane thickening, In addition, overexpressing tissue kallikrein in mouse models ameliorated hypertension, insulin resistance, and DN.[8–10] These studies suggested that KKS might be a useful target for the treatment of DN.

Tissue kallikrein is a serine protease that converts kininogen to the peptide hormone bradykinin and kallidin (in humans) or kallidin-like peptide (in rodents).[11] Binding of bioactive bradykinin to B1R and/or B2R stimulates the production of nitric oxide (NO) and prostaglandins, which exerts protective effects on DN.[12] Kwak *et al.*[13] demonstrated that by implanting an osmotic bradykinin minipump, urinary albumin excretion in streptozotocin (STZ)-induced diabetic rats was reduced, and podocyte apoptosis was also ameliorated. Overexpressing human tissue kallikrein has been shown to prevent the development of DN in rats.[8–10] However, the mechanism through which kallikrein delayed the onset of DN was unclear.

To illuminate whether exogenous supplement of pancreatic kallikrein (PKK) could protect DN and to explore the mechanism, we used db/db mice, an obese type 2 diabetic mouse, and STZ-induced type 1 diabetic mouse, as our models. We showed that PKK treatment not only significantly prevented the development of DN when given at the onset of diabetes, but also ameliorated DN when given after the onset of albuminuria. PKK improved renal pathologic alterations, which were accompanied by decreased renal fibrosis, inflammation, and oxidative stress product accumulation in both types of diabetic mice. Moreover, PKK significantly increased the expression of kininogen 1, tissue kallikrein, B1R, and B2R in diabetic mice. Our results suggest that the renal protective effects of PKK are mediated, in part, through the activation of KKS.

RESULTS

Animal characteristics

Data on body weight (BW), kidney weight (KW), blood glucose (BG), blood pressure (BP), and lipid profiles for db/db and db/m mice are summarized in Table 1. BG, BW, and serum triglyceride levels of db/db+saline (db/db+NS) and db/db+PKK groups were significantly higher than in db/m mice ($P < 0.01$), whereas the KW/BW ratio was lower than db/m mice ($P < 0.01$). However, there was no difference between db/db+NS and db/db+PKK groups. Other indexes including systolic and diastolic BP, total cholesterol, high- and low-density lipoprotein, showed no statistical differences among all three groups. The characteristics of STZ-induced diabetic mice was presented in Supplementary Table S1. Noteworthy, compared with NDC group, the KW/BW ratio was increased in STZ+NS group (P<0.05), which was reversed in STZ+PKK group.

PKK decreased the urinary albumin/creatinine ratio in both types 1 and 2 diabetic mice

Albuminuria is considered as an important indicator of renal damage. Compared with db/m mice, db/db+NS mice showed a significantly increased urinary albumin to creatinine (Alb/Cre) ratio (Figure 1a), which was reduced by PKK. In STZ-induced diabetic mice, PKK showed the same effect (Figure 1b).

PKK protected the kidney structure in DN

Previous studies demonstrated that the increase in glomerular basement membrane (GBM) thickness was one of the most important pathological changes in DN.[14,15] To verify whether PKK could protect the glomerulus of diabetic mice, we examined the ultramicrostructure with transmission electron microscopy (TEM). Our results showed that GBM thickness was significantly increased in the db/db+NS group compared with db/m mice, whereas PKK protected against this pathologic change (Figure 2a, red arrow). This was confirmed by semiquantitative analysis (Figure 2b). In addition, PKK also protected the endothelial fenestrae from fusion or loss (Figure 2c and d, yellow arrow), which partially explained the protective role of PKK against albuminuria. Similar results were observed in STZ-induced diabetic mice (Supplementary Figure S1).

Hematoxylin and eosin, periodic acid–Schiff (PAS), and Gomori trichrome reagent (Masson) staining were performed to examine the pathologic changes. Hematoxylin and eosin staining showed the inordinate arrangement of renal capillary in db/db+NS mice compared with db/m mice, whereas exogenous PKK prevented this pathologic disorder (Figure 2e). The percentages of PAS- or Masson-positive material, indicative of mesangial expansion and collagen deposition, respectively, and the glomerular sclerosis index were significantly increased in the diabetic glomeruli compared with db/m controls (Figure 2f–i). However, both were significantly attenuated in diabetic mice treated with PKK. Similar results for PAS and Masson staining were also observed in STZ-induced diabetic mice (Supplementary Figure S2).

PKK protected against the dysfunction and loss of podocytes in DN

To verify whether PKK protected podocytes from injury and dysfunction, we examined the ultrastructure of the foot process with TEM and Wilms tumor (WT-1), a marker of a podocyte, with immunohistochemistry (IHC), respectively. Figure 3a shows that PKK protected against the effacement of the foot process in diabetic mice (Figure 3a and c;

Table 1 | Basic characteristics of mice

	db/m ($n = 8$)	db/db+NS ($n = 8$)	db/db+PKK ($n = 8$)	F value	P value
BW (g)	24.25 ± 3.06	42.64 ± 4.76[a]	42.64 ± 4.76[a]	38.82	<0.001
BG (mM)	5.43 ± 0.64	27.37 ± 4.63[a]	26.90 ± 3.04[a]	86.35	<0.0001
SBP (mm Hg)	115.75 ± 8.25	110.20 ± 8.8	115.2 ± 8.76	0.99	>0.05
DBP (mm Hg)	86.25 ± 7.31	84 ± 5.4	84.4 ± 4.92	0.62	>0.05
KW (mg)	307.8 ± 18.83	380.5 ± 13.52[b]	415.0 ± 15.44[b]	11.7	<0.001
KW/BW (mg/g)	12.52 ± 2.27	9.02 ± 0.99[a]	9.83 ± 0.93[b]	7.27	<0.01
TG (mM)	2.11 ± 0.3	4.06 ± 1.4[c]	3.68 ± 1.12[c]	6.88	<0.01
TCH (mM)	4.50 ± 0.63	5.31 ± 1.44	4.47 ± 0.75	1.82	>0.05
HDL (mM)	2.19 ± 0.25	2.68 ± 0.71	1.99 ± 0.31	4.94	>0.05
LDL (mM)	1.76 ± 0.62	1.53 ± 0.56	1.51 ± 0.57	0.45	>0.05

BG, blood glucose; BW, body weight; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; KW, kidney weight; KW/BW, kidney weight/body weight; NS, saline; PKK, pancreatic kallikrein; SBP, systolic blood pressure; TCH, total cholesterol; TG, triglyceride.
Data are mean ± SEM.
[a]$P < 0.001$ versus db/m group.
[b]$P < 0.01$.
[c]$P < 0.05$.