An inhibitory effect of camostat mesilate on urinary albumin excretion in streptozotocin diabetic rats

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It has been well known that kidney increases in size in diabetes mellitus of recent onset and in experimental diabetic animals[1~3]. In both human beings and animals the high glomerular filtration rate of diabetic is invariably accompanied by increased volume of the kidney[1,3~5]. Such renal hypertrophy and hyperfunction in early diabetes have been suggested as initiating or accelerating late diabetic nephropathy[6,7], although the exact mechanism is unknown. There has been a report that even in the NIDDM risk of progression into persistent proteinuria was higher in patients with enlarged kidneys than in those without them[8]. These suggest that an inhibition of initial renal hypertrophy prevents the subsequent occurrence of diabetic nephropathy. It has been known that protein restriction significantly reduced albuminuria in diabetic rats[9,10]. It has also been reported that, in some patients with diabetic nephropathy, camostat mesilate, one of protease inhibitors, is available for reducing the urinary protein excretion[11]. It was investigated in the present study that the effect of an administration of the protease inhibitor in experimental diabetic rats on the initial renal hypertrophy and the subsequent albuminuria. Besides the effect of the protease inhibitor was compared with that of the angiotensin converting enzyme inhibitor, which has been demonstrated to prevent from progression of diabetic nephropathy[12~16].

Materials and methods

Reagents

Enalapril was obtained from Pfeizer Co., Tokyo. Camostat mesilate was obtained from Ono Pharma. Co., Osaka. Streptozotocin was obtained from Sigma Chemical Co., St Louis, MO. Enalapril was dissolved in saline and brought to a concentration of 5 mg/ml.

Animals

Male Wistar albino rats weighing approximately 150 g were used in the present study. Diabetes was induced by the administration of intraperitoneal streptozotocin (50 mg/kg). Rats were divided into four groups; 1) non-diabetic control rats, 2) diabetic rats without treatment (diabetic rats), 3) diabetic rats treated with daily subcutaneous injection of enalapril (once daily 10 mg/kg at 4:00 pm, ACEI-rats), and 4) diabetic rats received rat chow containing 0.1% camostat mesilate (PI-rats). One day after the streptozotocin treatment, enalapril or camostat mesilate was administered.

Methods

Systolic blood pressure was measured with a photoelectric tail cuff device (Natume Co., Tokyo).

Table 1 Effect of camostat mesilate and enalapril on blood glucose, body weight, blood pressure, kidney weight, plasma renin activity, and urinary albumin excretion

		BG(mg/dl)	BW(g)	KW(mg)	KW/BW	PRA(ng/ml/hr)	BP(mmHg)	UAE(mg/day)
control	(3d)	120±15	195±12	708±78	3.6±0.4	1.5±0.4		
(n=8)	(7d)	120±16	208±10	836±63	4.0±0.3	2.1±0.5	115±4	
	(4wk)	119±15	275±15	963±47	3.5±0.1		120±4	0.50±0.05
	(8wk)	130±16	348±10	1009±73	2.9±0.1	1.9±0.3	130±3	0.81±0.11
	(12wk)	112±15	363±15	1089±67	3.0±0.1		130±3	0.74±0.08
DM	(3d)	491±96**	174±8**	835±53	4.8±0.3*	1.4±0.5		
(n=8)	(7d)	433±118**	163±5**	838±27	5.2±0.2*	2.0±1.1	125±2	
	(4wk)	545±96**	157±10**	832±78	5.3±0.2*		141±4*	2.01±0.22*
	(8wk)	547±118**	173±6**	969±73	5.6±0.4*	2.1±0.6	150±7*	2.61±0.34*
	(12wk)	522±96**	200±10**	1220±102*	6.1±0.3*		150±5*	3.75±0.42*
PI	(3d)	432±25**	169±14**	698±104	4.1±0.4#	2.4±1.3		
(n=8)	(7d)	562±71**	151±11**	702±65	4.6±0.2*#	3.0±0.8	129±3	
	(4wk)	531±25**	152±14**	730±83	4.8±0.2*		136±5*	1.40±0.19*#
	(8wk)	546±71**	163±14**	782±89	4.8±0.2*	2.5±0.7	146±4*	1.83±0.14*#
	(12wk)	528±25**	186±14**	930±121#	5.0±0.1*#§		150±8*	2.27±0.34*##
ACEI	(3d)	563±74**	174±15**	859±48	4.9±0.3*	4.8±2.3*		
(n=8)	(7d)	489±96**	153±12**	813±62	5.0±0.4*	6.1±1.4*	123±4	
	(4wk)	513±74**	162±19**	826±95	5.1±0.3*		121±4##¶¶	1.32±0.23*#
	(8wk)	557±96**	169±12**	980±113	5.8±0.4*	5.8±1.0*	130±4#¶	1.62±0.11*#
	(12wk)	520±74**	188±19**	1109±134	5.9±0.4*		132±2##¶	1.93±0.42*##

The data are expressed as the mean ± SD.
BG : blood glucose, BW : body weight, KW : kidney weight, PRA : plasma renin activity,
BP : systolic blood pressure, UAE : urinary albumin excretion, DM : diabetic rats,
PI : diabetic rats treated with camostat mesilate, ACEI : diabetic rats treated with enalapril.
* $p<0.05$ and ** $p<0.01$ vs corresponding control, # $p<0.05$ and ## $p<0.01$ vs corresponding DM,
§ $p<0.05$ vs corresponding ACEI, and ¶ $p<0.05$ and ¶¶ $p<0.01$ vs corresponding PI.

This device requires minimal warming of the rat (usually≤15 min) prior to blood pressure determination and a brief period of restraint in a plastic cage. For each animal, the systolic blood perssure was recorded for any given time represented the mean of four to six pressure recordings. Twenty-four-hour urine collections were obtained by rearing rats in standard metabolic cages, which permitted free access to rat chow and water. On day 1, 2, 3, 5, 7 and in week 4, 8, and 12, one rat out of every group was weighed, then resected its right kidney to wet-weigh and if necessary to extract insulin-like growth factor I (IGF-I) by the method of D'Ercole et al[17,18].

Measurements

Blood glucose was measured by glucose oxidase method. Plasma insulin, renin activity, and IGF-I[18] were measured by respective radioimmunoassay. Urinary albumin was measured by the double antibody method using rat albumin (Chemicon, CA), rabbit anti-rat albumin, and donkey anti-rabbit globulin (Organon Teknika Corp., West Chester, PA). The intra- and interassay coefficients of variation in albumin assay were 5 and 9%.

Statistical analyses

Both tests of variance analysis and of two-tailed Student's non-paired t were used.

Results

Changes in blood glucose

The data were summarized in Table 1. Blood glucose level in diabetic rats was significantly higher than that in controls, and that was not changed by the administration of enalapril or camostat mesilate.

Changes in body weight

The increase in body weight was significantly moderated in diabetic rats. Enalapril or camostat mesilate did not affect the moderation of body weight in diabetic rats.

Changes in kidney weight

Kidney weight in diabetic rats was slightly lower than in controls on day 7, in week 4 and 8. However, in week 12, kidney weight in diabetic rats was significantly greater than that in controls, and that in PI-rats was significantly lower than that in diabetic rats.

Although the ratio of kidney weight to body weight in diabetic rats was significantly higher than that in control rats, the ratio in PI-rats was significantly lower than that in diabetic rats on day 3 and 7, and in week 12. In week 12 the ratio of kidney weight to body weight in PI-rats was significantly lower than that in diabetic rats and ACEI-rats.

Changes in kidney tissue IGF-I

Kidney tissue IGF-I content in ACEI- and PI-rats was not significantly different from that in diabetic rats, although the data were not shown.

Changes in blood pressure and plasma renin activity

Systolic blood pressure was significantly increased after induction of diabetes. Enalapril treatment significantly inhibited the increase in blood pressure. Thus blood pressure in ACEI-rats was significantly lower than that in diabetic rats and PI-rats in week 4, 8, and 12.

Enalapril treatment significantly increased plasma renin activity (PRA), and PRA in ACEI-rats was significantly higher than in other groups of rats.

Changes in urinary albumin excretion

After induction of diabetes, urinary albumin excretion (UAE) was gradually and significantly increased during the course of study. However, the increase in UAE was moderated by the administration of enalapril or camostat mesilate, and that in ACEI- or PI-rats was significantly lower than that in diabetic rats in week 4, 8, and 12.

Discussion

In the present study, both enalapril and camostat mesilate significantly reduced UAE in streptozotocin diabetic rats, but they may act by different mechanisms each other. Enalapril significantly reduced systemic arterial pressure but did not affect renal weight. Because inhibitory effect of ACEI

on UAE has been demonstrated to be linked to a reduction in glomerular capillary pressure in diabetes mellitus[19~21], the present results may be responsible for the inhibitory effect of enalapril on glomerular capillary pressure. On the other hand, camostat mesilate significantly reduced renal hypertrophy as measured by kidney weight, suggesting that the beneficial effect of camostat mesilate on glomerular injury may correlate with a suppression of initial renal hypertrophy in streptozotocin diabetic rats.

It has been reported that in experimental diabetic rats reduction of the blood glucose level with insulin treatment was capable of checking the renal hypertrophy[22], and it has been known that oral administration of Trasylol, one of protease inhibitors, caused the decrease in blood glucose in alloxan diabetic rats[23,24]. However, the blood glucose level was not significantly different between diabetic rats and PI-rats in the present study.

The mechanisms by which camostat mesilate suppresses renal hypertrophy and reduces subsequent albuminuria are yet unknown, and not directly addressed by his study. It has been reported that increased IGF-I content in the kidney is responsible for the initial renal hypertrophy in streptozotocin diabetic rats[25,26]. In the present study, renal IGF-I content measured on day 1, 2, 3, 5, and 7 was significantly reduced in diabetic rats compared with control rats as we have previously reported[18], and renal IGF-I content was not changed by the administration of camostat mesilate.

Zats et al.[9] have reported that dietary protein restriction significantly reduced glomerular filtration rate in diabetic rats, and that kidney weight was higher in diabetic rats maintained on diets of higher protein content than that in diabetic rats maintained on diets of lower protein content and that in diabetic rats predisposed to marked and progressive albuminuria by the treatment of protein rich diet. Thus low-grade albuminuria and minimal glomerular injury were observed in diabetic rats maintained on diets of lower protein content. Camostat mesilate may inhibit the intestinal protease activity and subsequently inhibit the digestion and absorption of protein.

An inhibitory effect of enalapril on urinary albumin excretion may result from reduction in systemic arterial pressure (probably glomerular pressure), while camostat mesilate attenuates renal hypertrophy, suggesting the hypothesis that hypertension and renal hypertrophy are equally important in the genesis of progressive diabetic renal injury, and that each is amenable to pharmacologic manipulation. Although further studies should be needed to elucidate the mechanisms by which camostat mesilate inhibits renal hypertrophy and subsequent UAE in diabetic rats, combination therapy with ACEI might be more effective against diabetic renal desease.

Summary

It has been suggested that renal hypertrophy and hyperfunction in early diabetes can initiate or accelerate late diabetic nephropathy. In the present study, it was investigated the effect of an administration in streptozotocin-induced (50 mg/kg) diabetic rats of camostat mesilate, one of protease inhibitors, or enalapril, one of angiotensin converting enzyme inhibitors, on the initial renal hypertrophy subsequently albuminuria. The rats were divided into 4 groups; 1) non-diabetic control rats, 2) diabetic rats without treatment (diabetic rats), 3) diabetic rats treated with daily subcutaneous injection of enalapril (10 mg/kg, ACEI-rats), and 4) diabetic rats received rat chow containing 0.1% camostat mesilate (PI-rats). Camostat mesilate or enalapril was administered for 12 weeks.

The ratio of kidney weight to body weight in diabetic rats was significantly greater than that in

control rats. However, the ratio in PI-rats was significantly lower than that in diabetic rats on day 3, 7 and in week 12.

The blood pressure was significantly increased after induction of diabetes. However, enalapril treatment significantly inhibited the increase in blood pressure.

Urinary albumin excretion (UAE) was increased after induction of diabetes. However, UAE in ACEI- and PI-rats was significantly lower than that in diabetic rats in week 4, 8, and 12.

These results suggest that both hypertension and renal hypertrophy are equally important in the genesis of progressive diabetic renal injury, and that each is amenable to pharmacologic manipulation. Although the precise mechanisms by which camostat mesilate inhibits renal hypertrophy and subsequent UAE in diabetic rats are unclear, combination therapy with one of angiotensin converting enzyme inhibitors might be more effective against diabetic renal disease.

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