Acta Diabetol (2007) 44:215–218
DOI 10.1007/s00592-007-0007-6

ORIGINAL ARTICLE

Effect of TNF-α inhibition on urinary albumin excretion in experimental diabetic rats

Yuji Moriwaki · Taku Inokuchi · Asako Yamamoto · Tsuneyoshi Ka · Zenta Tsutsumi · Sumio Takahashi · Tetsuya Yamamoto

Received: 31 January 2007 / Accepted: 7 May 2007 / Published online: 1 September 2007

Abstract The objective is to assess the effect of TNF-α inhibition on urinary albumin excretion in experimental diabetic rats. Male Wistar rats, 8-week-old, were categorized into four groups, which were the control (n = 9), diabetes (n = 9), infliximab-treated diabetes (n = 10), and FR167653-treated diabetes (n = 9) groups. Diabetes was induced by intraperitoneal injection of STZ (40 mg/kg). Thereafter, infliximab was injected intraperitoneally once a month (5.5 mg/kg) and FR167653 was administered orally by mixing with the rat chow (0.08%). The effects of infliximab and FR167653 on urinary albumin excretion were observed for 12 weeks. Body weight, blood sugar, 24-h urinary TNF-α, and 24-h urinary albumin/creatinine ratio (Ualb/Ucr) levels were determined at 1, 4, 8, and 12 weeks after the STZ-injection. Treatment of rats with STZ caused a significant loss of body weight, as well as polyuria and hyperglycemia within 1 week, while the urinary excretions of albumin and TNF-α were increased. Neither infliximab nor FR167653 affected body weight or blood sugar levels, whereas both decreased urinary albumin excretion, together with a modest decrease in the urinary excretion of TNF-α. These results suggest a role of TNF-α in the pathogenesis of diabetic nephropathy and show that TNF-α inhibition is a potential therapeutic strategy.

Keywords Diabetic nephropathy · Urinary albumin · TNF-α · FR167653 · Infliximab

Y. Moriwaki (✉) · T. Inokuchi · A. Yamamoto · T. Ka · Z. Tsutsumi · S. Takahashi · T. Yamamoto
Division of Endocrinology and Metabolism,
Department of Internal Medicine,
Hyogo College of Medicine,
Mukogawa-cho 1-1, Nishinomiya,
663-8501 Hyogo, Japan
e-mail: moriwaki@hyo-med.ac.jp

Introduction

Previous studies have reported that serum levels of pro-inflammatory cytokines, such as IL-1, IL-6, TNF-α, and IL-18, were increased in human and experimental animal models of diabetic nephropathy [1–4]. Further, Navarro et al. found positive associations of serum concentration and urinary excretion of TNF-α with urinary protein excretion in diabetes mellitus, as well as intrarenal TNF-α mRNA expression [5, 6]. These results suggest that TNF-α is involved in the development of diabetic nephropathy.

Prevention or treatment of diabetic nephropathy, a major cause of morbidity and mortality in diabetic patients, is extremely important. However, effective treatment modalities have not been established until recently. Infliximab, a chimeric type TNF-α monoclonal antibody has been utilized for the treatment of rheumatoid arthritis and Crohn's disease [7, 8], while FR167653 (1-[7-(4-fluorophenyl)-1,2,3,4-tetrahydro-8-(4-pyridyl)pyrazolo[5,1-c][1,2,4]triazin-2-yl]-2-phenylethanedione sulfate monohydrate), a potent inhibitor of p38 mitogen-activated protein kinase (MAPK), has been shown to inhibit TNF-α and IL-1 production in inflammatory cells [9, 10]. Therefore, we attempted to determine whether TNF-α inhibition by separate treatments of infliximab and FR167653 is effective for diabetic nephropathy, using a streptozotocin (STZ)-induced rat diabetic nephropathy model.

Materials and methods

Reagents

Streptozotocin was purchased from Sigma Company (St Louis, MO, USA). Infliximab was kindly provided by

Tanabe Phamaceutical Co. Ltd (Osaka, Japan). FR 167653 was a generous gift from Fujisawa Yakuhin Kogyo Co. Ltd (Osaka, Japan).

Animals

Male Wistar rats, 8-week-old, were purchased from Japan SLC Company (Shizuoka, Japan). Rats were kept under controlled conditions with a 12-h light:dark cycle at 25°C. All rats were fed CE-2 (CLEA Japan, Tokyo, Japan) with free access to water throughout the study. After a week of adaptation to the new environment, rats were categorized into four groups, which were the control ($n = 9$), diabetes ($n = 9$), infliximab-treated diabetes ($n = 10$), and FR167653-treated diabetes ($n = 9$) groups. All procedures were performed in accordance with the standards described in Hyogo College of Medicine Guidelines for the Care and Use of Laboratory Animals.

Methods

Diabetes was induced by intraperitoneal injection of streptozotocin (40 mg/kg) dissolved in physiological saline. Control rats were injected with an equal volume of vehicle. Infliximab was injected intraperitoneally once a month (5.5 mg/kg), which is the dose recommended to inhibit TNF-α in human Crohn's disease. FR167653 was administered orally by mixing with the rat chow (0.08%). The dose of FR167653 has been validated by a previous study [11]. The effects of infliximab and FR167653 on urinary albumin excretion were observed for 12 weeks. All tests were performed between 0800 and 1100 hours.

Measurements

Rats were housed once monthly in metabolic cages for 24-h urine collections to measure urinary excretions of albumin, creatinine, and TNF-α. Body weight, 24-h urinary TNF-α excretion, and 24-h urinary albumin/creatinine ratio (Ualb/Ucr) levels were determined at 1, 4, 8, and 12 weeks after the STZ-injection. Blood samples were also drawn at that time, and fed blood glucose levels (0900 to 1100 hours) and HbA1c were measured. Urinary excretions of TNF-α were measured with Rat TNF-α Immunoassay Kit (Biosource International, CA, USA), while urinary albumin excretion was measured with nephelometry using goat anti-rat serum albumin. When albumin level was less than 0.39 mg/dL, this value was used for statistical analysis. Blood glucose level was measured by glucose oxidase method using Glutest Neo (Sanwa Kagaku Kenkyusho Co. Ltd). Stable HbA1c was measured using Micromat II Hemoglobin A1c Test (Bio-Rad Laboratories, CA, USA). Urinary creatinine level was measured by the enzymatic method.

Statistical analyses

Log transformation was used when necessary (TNF-α) to obtain a normal distribution of data. All data are presented as the mean ± SE and observed differences among groups were compared by one-way ANOVA with Fisher's correction, with significance defined as $P < 0.05$.

Results

Infliximab and FR167653 do not change metabolic parameters during diabetes

As indicated in Table 1, treatment of rats with STZ caused a significant loss of body weight, as well as polyuria and hyperglycemia within 1 week. Hemoglobin A1c was already increased within 1 week. An increase in body weight was suppressed similarly in diabetic rats and infliximab-treated diabetic or FR167653-treated diabetic rats, though body weight of FR167653-treated diabetic group was lower than other diabetic groups. Accordingly, infliximab and FR167653 had no effect on body weight during diabetes. Blood glucose levels and HbA1c concentrations were significantly increased in diabetic rats compared to control rats throughout the study. Overall, neither infliximab nor FR167653 improve blood sugar levels and HbA1c concentrations.

Infliximab and FR167653 decrease urinary TNF-α excretion

As indicated in Table 1, urinary TNF-α excretion was significantly increased in diabetic rats on weeks 1, 4, 8, and 12, compared to control rats ($P < 0.05$, respectively). Administration of infliximab significantly decreased urinary TNF-α excretion on weeks, 1, 4, and 12, compared to diabetic rats ($P < 0.05$, respectively). Similarly, administration of FR167653 significantly decreased urinary TNF-α excretion on week 1, compared to diabetic rats ($P < 0.05$). However, urinary TNF-α excretions in both infliximab-treated diabetic rats and FR167653-treated diabetic rats did not reach the levels in control rats, except in the values of weeks 1 and 12 in infliximab-treated diabetic rats ($P < 0.05$, respectively) and weeks 8 and 12 in FR167653-treated diabetic rats ($P < 0.05$, respectively).

Table 1 Effects of TNF-α inhibition on urinary albumin excretion

	Group	1 week	4 weeks	8 weeks	12 weeks
Body weight (g)	Control (n = 8)	248 ± 14	303 ± 21	338 ± 25[a]	379 ± 25[a]
	STZ (n = 9)	211 ± 13[a]	215 ± 26[a]	212 ± 27[a]	208 ± 30[a]
	STZ + infliximab (n = 6)	225 ± 17	234 ± 27[a]	179 ± 6[a]	171 ± 8[a]
	STZ + FR167653 (n = 7)	184 ± 3[a,b]	171 ± 4[a]	163 ± 6	152 ± 9[a]
Blood sugar (mg/dL)	Control (n = 8)	79 ± 3	81 ± 4	77 ± 5	83 ± 5
	STZ (n = 9)	413 ± 27[a]	492 ± 42[a]	424 ± 21[a]	414 ± 40[a]
	STZ + infliximab (n = 6)	409 ± 22[a]	401 ± 68[a]	368 ± 56[a]	325 ± 11[a]
	STZ + FR167653 (n = 7)	446 ± 31[a]	365 ± 13[a,c]	405 ± 16[a]	404 ± 41[a]
Ualb/Ucr (μg/mg)	Control (n = 8)	63.7 ± 17.2	62.9 ± 9.9	81.7 ± 19.6	175.1 ± 49.4
	STZ (n = 9)	126.6 ± 37.9	259.6 ± 107.2[a]	247.5 ± 63.3[a]	521.7 ± 199.5[a]
	STZ + infliximab (n = 6)	80.2 ± 15.5	132.7 ± 46.9[c]	151.4 ± 34.0	188.7 ± 37.0[c]
	STZ + FR167653 (n = 7)	60.8 ± 6.3[c]	67.3 ± 10.9[c]	89.0 ± 19.6[c]	149.3 ± 35.4[c]
Log TNF-α (pg/day)	Control (n = 8)	1.9 ± 0.1	1.7 ± 0.2	1.9 ± 0.3	1.8 ± 0.3
	STZ (n = 9)	3.7 ± 0.0[a]	3.2 ± 0.1[a]	3.3 ± 0.1[a]	3.3 ± 0.1[a]
	STZ + infliximab (n = 6)	2.5 ± 0.5[c]	2.4 ± 0.3[a,c]	2.7 ± 0.3[a]	2.3 ± 0.4[c]
	STZ + FR167653 (n = 7)	2.7 ± 0.3[a,c]	2.6 ± 0.3[a]	2.6 ± 0.3	2.2 ± 0.4

[a] Compared to control

[b] Compared to STZ + infliximab

[c] Compared to STZ

Infliximab and FR167653 ameliorate urinary albumin excretion in diabetic rats

Urinary albumin excretions were increased in diabetic rats, as compared with control rats (1 weeks, NS; 4 weeks, $P < 0.05$; 8 weeks, $P < 0.05$; 12 weeks, $P < 0.05$). Treatment with infliximab and/or FR167653 decreased urinary albumin excretion of diabetic rats, as indicated by reductions in Ualb/Ucr (μg/mg) (Infliximab 1 week, NS; 4 weeks, $P < 0.05$; 8 weeks, NS; 12 weeks, $P < 0.05$; FR167653 1 week, $P < 0.05$; 4 weeks, $P < 0.05$; 8 weeks, $P < 0.05$; 12 weeks, $P < 0.05$), together with a modest decrease in the urinary excretion of TNF-α. Urinary albumin excretions of infliximab-treated diabetic and FR167653-treated diabetic rats were comparable to those of control rats (Table 1). Urinary excretions of TNF-α were not different throughout the experimental periods. Neither infliximab nor FR167653 affected urinary excretion of IL-6 (data not shown).

Discussion

Diabetic nephropathy is one of the serious complications of diabetics, affecting their prognosis and mortality. However, the exact underlying mechanism and effective treatment of diabetic nephropathy still remain to be elucidated.

Several reports have indicated that serum concentrations of TNF-α, IL-1, IL-6, and IL-18 were increased in type 2 diabetes patients [1–4]. Moreover, clinical investigations have implicated the roles of TNF-α and IL-6 in the development of diabetic nephropathy, suggesting that inhibition of those cytokines is promising remedy to ameliorate diabetic nephropathy.

Infliximab, a chimeric monoclonal antibody directed against TNF-α, has been used in the treatment of Crohn's disease [8] and rheumatoid arthritis [7]. In addition, the effectiveness of infliximab in the treatment of sciatica due to experimental disc herniation has been described [12]. The family of mitogen-activated protein kinases (extracellular signal-regulated kinase [ERK], c-Jun N-terminal kinase, and p38) may be implicated in the various pathological processes, and it has been reported that pretreatment with FR167653 suppressed various glomerulopathies with puromycin or adriamycin nephropathy [13]. However, to the best of our knowledge, there has been no detailed study of their effects on diabetic nephropathy.

In the present study, TNF-α inhibition with infliximab and FR167653 decreased urinary albumin excretion, as indicated by the reductions in Ualb/Ucr, as with the previous report of the beneficial effects of pentoxifyline, a non-specific inhibitor of TNF-α production [14] on urinary protein excretion in diabetic patients in the clinical studies [5, 15].

Although glomeruli, as well as proximal renal tubules are considered to be the sites of urinary TNF-α production during diabetic nephropathy [16, 17, 18], the precise mechanism of decreased urinary albumin excretion by infliximab and FR167653 remains to be elucidated. However, recently, beneficial effect of infliximab on insulin resistance and insulin sensitivity is reported in patients with RA [19]. Since albuminuria is considered a marker of insulin resistance syndrome, decrease in urinary albumin excretion by infliximab administration may in part reflect improvement of insulin resistance. Further studies are needed to clarify the mechanism of decreased albumin excretion in diabetic nephropathy by infliximab and FR167653.

In conclusion, these results suggest TNF-α is partially involved with the pathogenesis of diabetic nephropathy and show that TNF-α inhibition may be effective in preventing and/or treating diabetic nephropathy.

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