# Effects of Cicaprost and Fosinopril on the Progression of Rat Diabetic Nephropathy

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**We have studied the effects of chronic therapy with cicaprost (a $PGI_2$ analog), fosinopril (a converting enzyme inhibitor), and the combination of both drugs on the progression of experimental diabetic nephropathy. Uninephrectomized streptozotocin-induced diabetic rats were maintained for 8 months with plasma glucose between 13.7 and 22.0 mmol/L to hasten renal damage. Systemic and renal parameters were measured periodically, and at sacrifice structural and morphometrical renal studies were performed to evaluate diabetic injury.**

**Control rats exhibited characteristic features of this model, such as high blood pressure and plasma creatinine and urinary albumin excretion, together with prominent alterations in the kidney (renal and glomerular hypertrophies, mesangial matrix expansion, and tubular alterations). The three therapies attenuated equivalently the progression of diabetic renal injury, as estimated by lower urinary albumin excretion, renal and glomerular hypertrophies, and a better renal architectural preservation. No synergistic action was appreciated with the combined therapy. However, renal preservation achieved with cicaprost was not linked to reductions in systemic blood pressure, whereas in the groups treated with fosinopril the hypotensive effect of this drug could have contributed to the positive outcome of the therapy. Therefore, nephroprotection exerted by this $PGI_2$ analog in this model seems more related to the derangement of renal local mechanisms than to systemic blood pressure control. Finally, the possibility that an impaired prostacyclin synthesis or bioavailability is involved in the pathogenesis of the diabetic nephropathy in this model underlies our results.**
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KEY WORDS: **Cicaprost, fosinopril, diabetic nephropathy, streptozotocin, rat.**

During the last years diabetes mellitus and nephrosclerosis have become the main causes of end-stage renal failure (ESRF), with growing prevalence both in the US[1] and in Europe.[2] Because of the high sociosanitary repercussion of ESRF, the National High Blood Pressure Council encouraged the development of new pharmacological interventions directed at retarding renal damage,[3] particularly in diabetes mellitus.

Diabetes mellitus causes both micro- and macroangiopathy,[4] which constitute the major causes of the increased morbidity and mortality in diabetics. The presence of arterial hypertension has been shown as a recognized concomitant risk factor for the progression of diabetic damage. Although diverse pharmacological antihypertensive approaches have been shown to slow the progression of renal disease,[5,6] variable effectiveness in providing renal protection has been

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reported in the literature.[7] Higher efficacy of angiotensin converting enzyme inhibitors (CEI) in both experimental and human diabetic nephropathies has been clearly demonstrated,[8-10] even in the case of a similar level of blood pressure reduction.[9,11] Positive actions of CEI have been mainly attributed to their blockade of angiotensin II (ang II) generation, with consequent decreases in both systemic and intraglomerular blood presures, although the possibility that other mechanisms could be involved in their protective effect has also been suggested.[7] Indeed, CEI are able to improve insulin resistance,[12] reduce sympathetic overactivity,[13] and potentiate bradykinin and arachidonic acid-prostaglandin systems,[14] through which these drugs might exert additional positive effects.

Recently, the existence of abnormalities involving the endothelial function in diabetic patients has been reported.[15] These alterations include an impaired endothelium-dependent vasodilation that could be related to an imbalance between endothelial vasoconstrictor and vasodilator substances. In this regard, the existence of a decreased vascular $PGI_2$ level has been reported in experimental[16] and human[17] diabetes. In addition, we have previously reported some renal protective effects of an oral stable prostacyclin analog (cicaprost) in a model of renal mass ablation, and high sodium and protein intake.[18] On the basis of this finding, and on the hypothesis that a derangement in $PGI_2$ production could be partially responsible for diabetic injury, we decided to compare the effects of chronic cicaprost and fosinopril (a CEI) treatments in a model of uninephrectomized streptozotocin-induced diabetic rats. Furthermore, we have studied the combination of both monotherapies in an attempt to disclose synergistic positive effects that could improve the outcome of the individual therapies.

## METHODS

**Animals** Forty age-matched male Wistar rats, with body weight (BW) ranging 300 to 350 g, were subjected to left unilateral nephrectomy under pentobarbital anesthesia. Three weeks later, every animal was made diabetic by a single intraperitoneal injection of 70 mg/kg body weight of streptozotocin (Sigma Chemical Co., St. Louis, MO). After 48 h, successful diabetes mellitus induction was confirmed by tail blood glucose measurement, using a reflectometer device (Lifescan, Milpitas, CA). The rats were fed a standard pellet laboratory chow with 24% of protein and allowed free access to water. Each one received a daily injection of ultralente insulin, adjusted to attain blood glucose levels of 13.7 to 22.0 mmol/L (250 to 400 mg/dL). The rats were randomly divided into four groups (n = 10) according to the treatment. The control group (DIAB) was given no additional treatment but insulin. The cicaprost group (CICA) was treated with a single oral dose of 15 μg/kg BW/day of cicaprost (Schering AG, Berlin, Germany). This dose was previously determined in order to avoid any short- or long-term hypotensive effect in the animals. The fosinopril group (FOSI) was treated with a single oral dose of 0.25 mg/kg BW/day of fosinopril (Bristol-Myers Squibb, Princeton, NJ). The fourth group (F + C) was treated with the combination of fosinopril plus cicaprost, using the same doses as in monotherapy. Treatment other than insulin was administered by gastric tube.

**Analysis** Periodically, blood glucose (BG), body weight, systolic blood pressure (SBP) by tail-cuff method (Panlab, Barcelona, Spain), and plasma creatinine (PC) by a reflectometer device (Reflotron Creatinine, Boehringer, Mannheim, Germany) were measured. Every 8 weeks, 24-h urine was collected, by housing the animals in metabolic cages with free access to water and food, to measure sodium, potassium, and albumin excretion by a radial immunodiffusion commercial kit specific for rat albumin (The Binding Site, Birmingham, England).[19] At week 32, all the animals were killed and blood samples were collected for creatinine and glycosylated hemoglobin ($HbA_1$) determination. The remaining kidney from each rat was removed, weighed, and processed for light microscopy.

**Light Microscopy** Samples from every remnant kidney were fixed by immersion in Carnoy's solution and embedded in paraffin. Sections from every case were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), and Masson trichrome. Percentage of focal or diffuse glomerulosclerotic lesions was determined by scoring at least 75 superficial and midcortical glomeruli in two coronal sections. Glomerular volume estimation and cellular counts were performed by using an image analyzer (Microm, Barcelona, Spain), and values were obtained by measuring the same glomeruli used to evaluate histological damage. Maximum and minimum glomerular diameters between the internal edges of Bowman's capsule were measured and the mean value of both was considered to be the diameter of each glomerulus. From this value, individual volumes were calculated for each glomerulus from the formula $4/3\pi r^3$. Glomerular cellularity was considered either normal or high, and tubular lesions were evaluated semiquantitatively by the degree of tubular atrophy as: no (−), slight (+), moderate (++), and intense (+++).

**Statistical Analysis** A Wilcoxon's signed rank test for paired comparisons was used to analyze differences within the groups, whereas differences between them were tested using analysis of variance for multiple groups. $P < .05$ was considered significant. In the text, data are presented as mean ± SD.

## RESULTS

Clinical and biochemical parameters (Table 1). Blood glucose increased drastically 48 h after diabetes melli-

tus induction, and it was maintained within the range of 13.7 to 22.0 mmol/L during the follow-up without significant differences among the four groups. This fact was confirmed by similar high $HbA_{1c}$ levels shown by every group at death. No significant difference was observed in BW during the study either within or among groups. After diabetes mellitus induction, SBP significantly increased ($P < .05$) in DIAB and CICA groups, whereas FOSI and F + C groups remained around basal values during the 8 months of follow-up. Plasma creatinine levels were below the detection limit of the analyzer (0.5 mg/dL) in the four groups before week 16, whereas after that time, PC started to differentiate between groups and at week 32 it was significantly lower ($P < .05$) for all the treatments compared to the DIAB group.

Urinary albumin excretion increased significantly ($P < .0005$ *v* week 0) during the study in the four groups, but lower levels ($P < .05$) were observed for all treated groups at any week when compared to DIAB group (Figure 1).

**Macroscopical Findings (Table 2)** At death, the remaining kidney from animals in any of the treatment groups appeared to be less hypertrophic, as shown by their significantly lower weight, and KW/BW indices than the ones from DIAB animals ($P < .05$).

**Histological Findings (Table 2 and Figures 2 and 3)** Within the DIAB group changes in the glomerular tufts were most prominent. Most of them showed variable, though predominantly intense, degrees of diffuse mesangial matrix expansion that merged into frank diffuse glomerulosclerosis in 17% of the glomeruli. Focal adhesions to the glomerular capsule were also occasionally observed associated with focal sclerotic lesions. Both glomerular cellularity and glomerular volume were significantly increased in the DIAB group as compared to the three treatment groups. However, rats in the CICA group showed no indication of established glomerulosclerosis, with preserved glomeruli and scarce (7% of sclerotic glomeruli). Morphological findings in the kidney of rats included in the F + C group (with 5% of sclerotic glomeruli) were indistinguishable from those described in the CICA group. The changes observed in the FOSI group were somewhat intermediate between those observed in the DIAB group and the F + C group (with 4% of sclerotic glomeruli). In spite of a predominance of preserved glomeruli, the presence of mesangial expansion was more frequent than in both the CICA and F + C groups. Glomerular volume and cellularity were significantly lower in all of the treated groups as compared to the DIAB group (Table 2). A moderate degree of tubular changes was observed in the DIAB group. These consisted of tubular atrophy and metaplasia. No or only slight tubular alterations were observed in the remaining treatment groups, with no significant variation among them.

## DISCUSSION

In the present study we have tested the effectiveness of three different therapies on the progression of experimental diabetic renal disease in rats. In this model, chronic cicaprost treatment, in the absence of changes in BP, exerted nephroprotective actions, as estimated by lower albumin excretion rate, renal and glomerular hypertrophies, and better architectural preservation when compared with diabetic untreated animals. The level of renal protection in cicaprost was

TABLE 1. CLINICAL AND BIOCHEMICAL PARAMETERS

| Group | Week | BW (g) | SBP (mm Hg) | BG (mmol/L) | $HbA_{1c}$ (%) | PC ($\mu$mol/L) |
|---|---|---|---|---|---|---|
| DIAB | 0 | 448 ± 63 | 112 ± 3 | 4.1 ± 0.8 | — | <44.2 |
| | 16 | 454 ± 39 | 132 ± 5* | 16.3 ± 4.2† | — | <44.2 |
| | 32 | 464 ± 44 | 140 ± 6* | 19.0 ± 1.5† | 8.4 ± 0.4 | 51.6 ± 2.35 |
| CICA | 0 | 429 ± 73 | 110 ± 4 | 4.0 ± 0.7 | — | <44.2 |
| | 16 | 416 ± 31 | 130 ± 4* | 15.2 ± 3.8† | — | <44.2 |
| | 32 | 468 ± 23 | 138 ± 7* | 19.0 ± 4.2† | 8.7 ± 0.5 | 44.6 ± 0.78** |
| FOSI | 0 | 416 ± 17 | 114 ± 4 | 4.2 ± 0.6 | — | <44.6 |
| | 16 | 420 ± 35 | 117 ± 4‡ | 14.3 ± 3.5† | — | <44.6 |
| | 32 | 471 ± 24 | 119 ± 5‡ | 18.7 ± 3.2† | 8.9 ± 0.5 | 46.1 ± 3.9** |
| F+C | 0 | 445 ± 47 | 112 ± 4 | 4.3 ± 0.8 | — | <44.6 |
| | 16 | 426 ± 33 | 116 ± 3‡ | 15.7 ± 3.9† | — | <44.6 |
| | 32 | 456 ± 32 | 117 ± 6‡ | 19.8 ± 4.0† | 8.6 ± 0.4 | 47.7 ± 3.1** |

*Abbreviations: BW, body weight; SBP, systolic blood pressure, BG, blood glucose; $HbA_{1c}$, glycosylated hemoglobin; PC, plasma creatinine; DIAB, control group; CICA, cicaprost group; FOSI, fosinopril group; F+C, fosinopril + cicaprost group.*

* $P < .05$ v *0 week.*

† $P < 0.001$ v *0 week.*

** $P < 0.05$ v *DIAB 32 week.*

‡ $P < 0.05$ v *DIAB & CICA.*

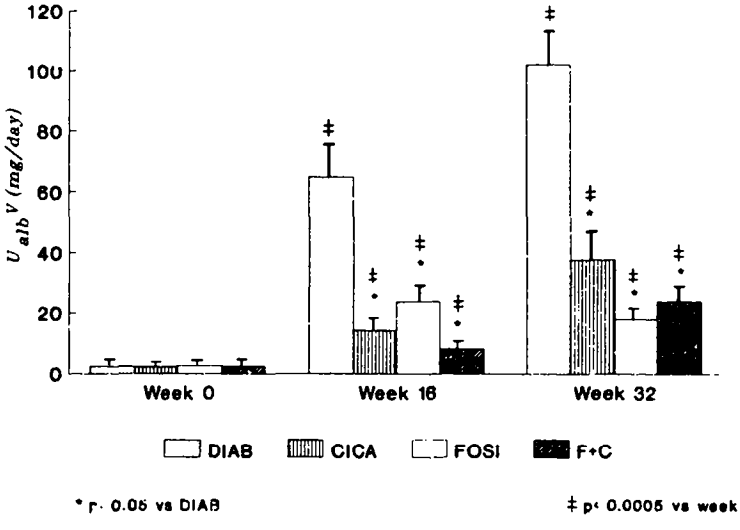


**FIGURE 1.** *Evolution of 24-h urinary albumin excretion ($U_{alb}V$).*

similar to those achieved with fosinopril or fosinopril + cicaprost treatments, although in these last two groups the reduction observed in blood pressure might have contributed to the beneficial outcome. Finally, no synergistic action with the combination of both drugs was observed.

Diabetic condition involves a wide range of alterations (systemic, renal, and vascular changes), which, all together, contribute to the development of the clinical complications associated with this pathology. In this regard, systemic hypertension is a recognized concomitant risk factor for the progression of diabetic nephropathy and the development of microalbuminuria, which is considered as an early marker of the diabetic renal damage.[20-22] However, reduction of blood pressure and proteinuria seem not to be a condition sufficient to prevent renal injury, as not all antihypertensive therapies have shown equivalent efficacy.[6,23] Indeed, some calcium antagonists[24] and selective $\alpha$-1 and $\beta$-blockers[25,26] have shown lower nephroprotective effect when compared to CEI, which are so far considered the elective therapy in diabetic nephropathy both in the presence[27] or even in the absence[28] of systemic hypertension. The beneficial effect of the CEI can be attributed to selective blockade of ang II, and this possibility has been recently supported with early positive results using specific nonpeptide ang II receptor blockers.[29,30] Our results with fosinopril are in agreement with previous data[6] regarding blood pressure control, renal histological preservation, and evolution in urinary albumin excretion.

Recent evidence has documented a pivotal physiological role for the endothelial cells, which, through complex endocrine, paracrine, autocrine, or intracrine mechanisms, and in association with other structural components of arterial wall, contribute to cardiovascular homeostasis.[31] Prostacyclin participates actively

**TABLE 2. MACRO AND MICROSCOPICAL RENAL PARAMETERS**

| | KW (g) | KW/BW (g/kg) | Glomerular Volume ($\mu m^3$) | Glomerular Cellularity | Tubular Lesion |
|---|---|---|---|---|---|
| DIAB | 4.85 ± 0.75 | 9.72 ± 1.1 | 3.1 ± 0.31 | High | + + |
| CICA | 3.77 ± 0.65* | 8.0 ± 1.3* | 2.77 ± 0.24* | Normal | + / − |
| FOSI | 3.73 ± 0.37* | 7.9 ± 0.8* | 2.48 ± 0.20* | Normal | + / − |
| F+C | 3.57 ± 0.71* | 7.8 ± 1.1* | 1.93 ± 0.21* | Normal | + / − |

*Abbreviations: KW, Kidney Weight; KW/BW, Kidney Weight/Body Weight. (−) No, (+) Slight, (+ +) Moderate; (+ + +) Intense.*

*Other abbreviations as in Table 1.*

* $P < .05$ v *DIAB*.

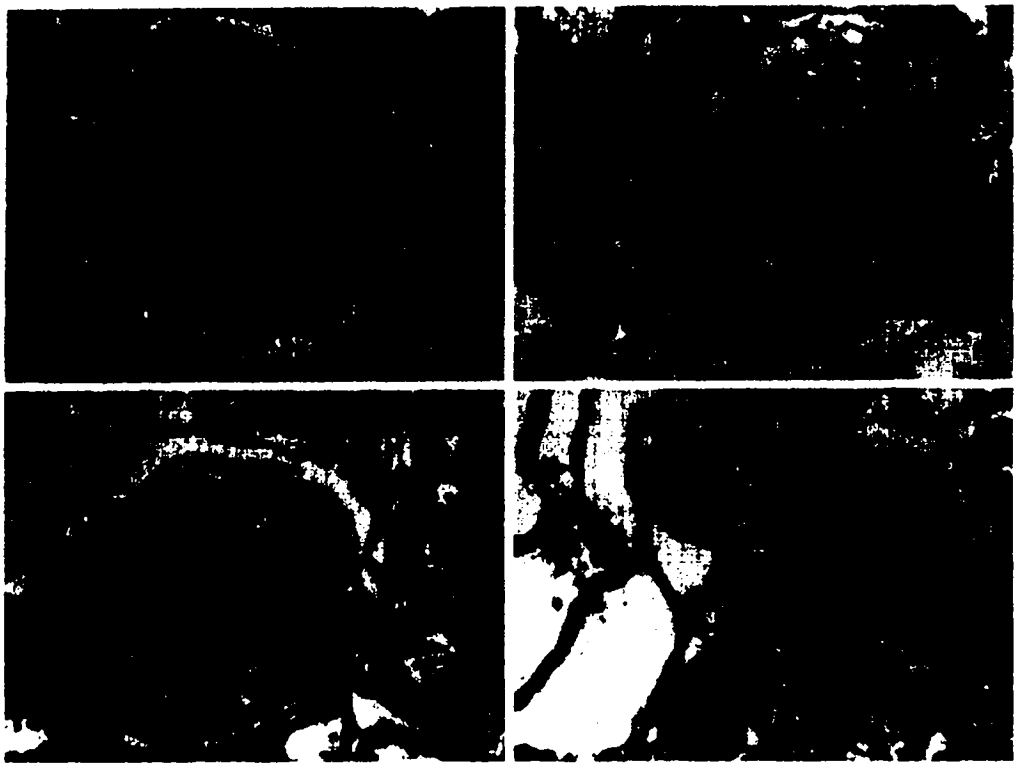

**FIGURE 2.** *The predominant glomerular lesion in each treatment group is depicted, showing various degrees of glomerular hypercellularity and mesangial matrix expansion with deposition of PAS-positive material. A: DIAB group; B: cicaprost group; C: fosinopril group; D: fosinopril + cicaprost group (PAS, ×400).*

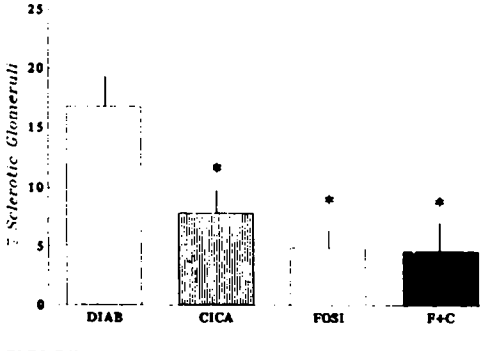


**FIGURE 3.** *Mean percentage values of sclerotic glomeruli in diabetic rats (DIAB), and diabetic rats treated with cicaprost (CICA), fosinopril (FOSI) and fosinopril plus cicaprost (F + C). * P < .05 v DIAB.*

in the glomerular hemodynamics,[32] firstly, by attenuating the contractile response of the mesangial cells to ang II[33,34] and, secondly, through its vasodilator effects on pre- and postglomerular vasculature.[35] In this regard, a decreased level of $PGI_2$ has been described in both experimental and human diabetes.[16,17,36] In our model, cicaprost induced a level of renal protection similar to that achieved with fosinopril or fosinopril + cicaprost. Nevertheless, this positive outcome was afforded in spite of no reduction of SBP levels. Prostacyclin and some of its analogues have been reported to decrease vascular resistances in both afferent and efferent arterioles.[35] Therefore, we can hypothesize that cicaprost, as a $PGI_2$ analog, may have improved renal function by a dilatory effect on the glomerular vasculature, thus reducing intraglomerular pressure and albumin excretion rate, even in the absence of changes in systemic blood pressure. Additional mechanisms can also contribute to the positive effects of this drug. A modified eicosanoid syn-

thetic pattern has been reported in diabetic models[37]: after an initial proportional increase in the production of vasodilatory prostanoids, which has been correlated with the early hyperfiltration period of the disease, there is a preferential enhancement of the production of vasoconstrictor elements, mainly thromboxane.[37] The implication of this prostanoid in the pathogenesis of albuminuria and glomerular morphologic changes, by stimulation of mesangial matrix protein production, has been demonstrated in diabetes mellitus.[38,39] Cicaprost, chronically administered, could counteract the negative effects of the diabetic eicosanoid unbalance by favoring dilatation.

The existence of a comparable degree of renal preservation with (fosinopril/ fosinopril + cicaprost) or without (cicaprost) blood pressure control reinforces the possibility that not only systemic, but also local mechanisms are playing a major role in the pathogenesis of diabetic glomerulopathy. Previous results from our group using a different experimental model of mild renal failure (surgical renal mass reduction and high salt and protein intake) have also shown nephroprotective properties of cicaprost therapy without reductions in BP.[18]

Although both independent therapies yielded positive results, no synergistic action was observed when the drugs were used in combination. Final outcome of the fosinopril + cicaprost group was mostly comparable to those obtained with any of the individual therapies. This fact may suggest the possibility that, through different mechanisms, both drugs might exert similar positive effects (reduction of intraglomerular pressure, antiproliferative action, etc).

In summary, we have demonstrated that the use of cicaprost, an orally stable $PGI_2$ analog, in a model of experimental diabetes mellitus exerted a degree of renal preservation comparable to that afforded by fosinopril, as estimated by several biochemical and histological parameters. This nephroprotection was not related to the reduction of systemic blood pressure in the case of cicaprost, whereas the beneficial effect of fosinopril came associated to BP control. These data suggest the existence of impaired $PGI_2$ production or bioavailability as one of the underlying defects responsible for some of the pathological features linked to this pathology. Finally, no additive effect was achieved with the combination of both drugs. Our data seems to support the hypothesis that local hemodynamic rather than systemic or metabolic factors are implicated to a major degree in the genesis of the diabetic nephropathy.

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