Original Article

C-peptide and captopril are equally effective in lowering glomerular hyperfiltration in diabetic rats

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Abstract

Background. C-peptide has been shown to reduce glomerular hyperfiltration, glomerular hypertrophy and urinary albumin excretion in type 1 diabetes, but its effect has not been compared with that of an angiotensin-converting enzyme inhibitor (ACEI) in the early stage of renal involvement in diabetes.

Methods. Glomerular filtration rate (GFR) was measured in terms of inulin clearance and renal blood flow, using ultrasound technique, in four groups of streptozotocin-induced diabetic rats before and after a 60 min infusion of C-peptide (D-Cp), captopril (D-ACEI), C-peptide and captopril (D-Cp–ACEI) or placebo (D-placebo). In addition, a non-diabetic control group was studied before and after captopril infusion (C-ACEI).

Results. GFR was 37–51% higher in the diabetic groups than in the control animals. GFR decreased after treatment in the D-Cp, D-ACEI and D-Cp–ACEI groups, but did not change in the D-placebo group. Blood flow increased by 26–32% in the three groups receiving captopril and by 5% in the diabetic groups treated with C-peptide alone or placebo. The increase in blood flow in the three ACEI-treated groups was significantly greater than in the D-placebo group. Filtration fraction fell significantly in all groups, but only in the combined D-Cp–ACEI group did it fall significantly more than in the D-placebo group.

Conclusions. C-peptide and captopril lower diabetes-induced glomerular hyperfiltration to a similar extent, but the influence of captopril on blood flow is greater than that of C-peptide, suggesting different mechanisms of action. No statistically significant additive effects of C-peptide and captopril were shown in this acute infusion study.

Keywords: captopril; C-peptide; diabetic nephropathy; glomerular hyperfiltration; renal blood flow

Introduction

In recent years, several studies in C-peptide-deficient type 1 diabetes patients and diabetic rats have provided evidence of physiological effects of C-peptide, suggesting that C-peptide deficiency in type 1 diabetes might contribute to the development of late diabetic complications. Thus, C-peptide replacement has been shown to partially prevent diabetic polyneuropathy in rats [1,2] and humans [3,4]. Early signs of diabetic nephropathy are also affected by C-peptide. Young type-1 diabetic patients with glomerular hyperfiltration [5] and microalbuminuria showed less marked glomerular hyperfiltration and diminished urinary albumin excretion after 3 months replacement of C-peptide [6,7]. C-peptide has also been administered acutely for 1 h and during a 2 week infusion study in rats with streptozotocin-induced diabetes; hyperfiltration and albuminuria or proteinuria were prevented in the C-peptide treated group [8,9]. Furthermore, the renal and glomerular hypertrophy that accompanies diabetes was partially prevented by a 2 week infusion of C-peptide [8].

Specific binding of C-peptide to cell membranes has been demonstrated for several cell types, notably renal tubular cells [10]. There is evidence that indicates that the binding site is a G-protein coupled receptor [10,11]. Binding of C-peptide is accompanied by a prompt rise in the intracellular Ca2+ concentration, which, in turn, results in stimulation of endothelial nitric oxide synthase (eNOS) and activation of a
MAP-kinase-dependent signalling pathway, leading to activation of Na\(^+\)K\(^+\)ATPase [11]. Stimulation of eNOS and Na\(^+\)K\(^+\)ATPase may contribute to C-peptide's renal effects, but the precise mechanism by which C-peptide improves renal function, minimizing hyperfiltration and albumin excretion, and limits glomerular hypertrophy is not fully understood.

This study was undertaken to compare the haemodynamic and renal effects of C-peptide with those of an angiotensin-converting enzyme inhibitor (ACEI), a well-established drug in the treatment of type 1 diabetic patients with renal involvement [12–14]. We addressed the question of whether there were additive effects of the two drugs in these respects.

Subjects and methods

Forty-two 8-week-old male Sprague-Dawley rats (Møllegaard, Copenhagen, Denmark) with an initial weight of \(\sim 200\) g were studied in five groups. Four diabetic groups were treated with either isotonic NaCl (D-placebo group, \(n = 7\)), rat C-peptide II (D-Cp, \(n = 13\); Genosys Biotechnologies, UK), captopril (D-ACEI, \(n = 9\); Sigma-Aldrich Sweden AB, Stockholm, Sweden) or C-peptide and captopril (D-Cp–ACEI, \(n = 8\)). We also studied a healthy control group treated with captopril (C-ACEI, \(n = 5\)). Diabetes was induced by an intravenous injection of streptozotocin (Sigma-Aldrich Sweden AB) at a dose of \(55\) mg/kg body weight. The experiments were performed at 2 weeks after induction of diabetes. During these 2 weeks, no treatment was given. All animals had free access to tap water and standardized chow (R36; Lactamin, Vadstena, Sweden). The study protocol was reviewed and approved by the institutional animal ethics committee.

**Determination of blood pressure, GFR and RBF**

After 14 days of diabetes without any treatment, the rats were anaesthetized by an intraperitoneal injection of Inactin\(^®\) (sodium 5-carbox-butyryl-5-ethyl-2-thioibarbiturate; Sigma-Aldrich Sweden AB) \((120\) mg/kg body weight) and then placed on a servo-controlled heating pad to maintain the body temperature at \(\sim 37.5^\circ\)C. A cannula was inserted into the trachea to facilitate spontaneous breathing. The right femoral artery was cannulated for blood sampling and continuous measurement of the arterial blood pressure. The latter was measured by a transducer (GOULD Statham P23XL; Millar Instruments, Houston, TX) connected to a printer (BBC Goertz Metrawatt SE 460; Brown Boveri, Sweden) and is presented as mean arterial blood pressure \([1/3 \times (systolic \text{ pressure} – \text{diastolic \text{ pressure}})] + \text{diastolic \text{ pressure}}\). The right femoral vein was cannulated for infusions. The bladder was catheterized via a suprapubic incision. Thereafter, the left kidney and renal artery were exposed via a flank incision and dissected free of adherent fat and connective tissue before the kidney was carefully placed in a cup and covered with oil. Subsequently, the ureter on the same side was ligated as distally as possible and catheterized, which deviated the urine from the left kidney and enabled glomerular filtration rate (GFR) measurements from each individual kidney. The renal artery was exposed and an ultrasound recorder probe (Transonic\(^®\) T 206; Transonic Syst. Inc., Ithaca, NY) was positioned to measure renal blood flow (RBF).

Immediately after surgery, arterial blood was drawn to determine the blood glucose level. Thereafter, an infusion of isotonic saline containing \([^{3}\text{H}]\)-inulin was started. The infusion rate was \(5\) ml \(\text{hr}^{-1} \cdot \text{kg}^{-1} \) body weight following a bolus dose of \(1\) ml. When steady state had been reached, after \(\sim 45\) min, urine samples were collected at 15 min intervals for analyses of volume, osmolality, albumin excretion rate, sodium and potassium concentrations and \([^{3}\text{H}]\)-inulin. Plasma samples \((\sim 60\) \(\mu\)l) for analyses of \([^{3}\text{H}]\)-inulin were obtained at the midpoint of each 15 min urine collection period, making calculations of GFR possible. After two 15 min periods when the basal GFR was measured, infusion of either saline as placebo, C-peptide \((50\) pmol kg\(^{-1}\) min\(^{-1}\)) in a combination of C-peptide and captopril was started and maintained for the remaining 60 min of the experiment.

GFR was measured individually for each kidney, a prerequisite for calculations of the filtration fraction changes on the left side where the blood flow was measured. However, the GFR and urine variables for each individual kidney are not presented in the text or figures, since total values for both kidneys are more relevant in that aspect.

**Analyses**

Levels of \([^{3}\text{H}]\)-inulin in the plasma and the urine were determined by liquid scintillation counting (PW 4700; Philips, the Netherlands). The sample \((1\) ml urine or \(10\) ml plasma) was mixed in \(1\) ml water and then \(3\) ml scintillation fluid (Pico-Flour 40TM, CiAB; Chemical Instruments AB, Lidingö, Sweden) was added. A radioimmunoassay technique (Linco Research Inc., USA) was used to measure the plasma concentrations of rat C-peptide. Urine volumes were measured gravimetrically. Urinary sodium and potassium concentrations were determined by flame photometry (IL 543; Instrumentation Lab., Milan, Italy) and urine osmolality by a freezing-point depression method (Model 3 MO; Advanced Instruments, MA). Blood glucose concentrations were analysed using a MediSense Pen\(^®\) and Precision Plus Electrodes\(^®\) (Abbot Scandinavia AB, MediSense Produkter, Solna, Sweden).

**Statistical methods and presentation of data**

Analysis of variance followed by Tukey’s post-hoc test was used to detect changes within and between groups before and after treatment. Data in the text, figures and tables are presented as means ± SEM.

**Results**

**Basal data**

Table 1 shows the basal data in the study groups after 2 weeks of diabetes (except for the non-diabetic C-ACEI group). The mean glucose levels (Table 1) in the four diabetic groups ranged from 20.6 to 22.6 mmol/l. The blood glucose level in the D-Cp group was 22.6 ± 0.6 mmol/l, which was slightly higher than in
the D-placebo group ($P<0.05$). Body weight did not differ significantly between the groups, but the diabetic animals tended to have the lowest weight in absolute terms.

**C-peptide levels**

Plasma C-peptide levels at the end of the experiment in the D-placebo and D-ACEI groups were 0.46±0.12 and 0.19±0.03 nmol/l, respectively. The C-ACEI group had a normal C-peptide level of 1.25±0.14 nmol/l. The D-Cp and D-Cp–ACEI groups had C-peptide levels after treatment of 46±13 nmol/l (range: 0.3–120 nmol/l) and 50±20 nmol/l (range: 10–160 nmol/l), respectively.

**Urine variables**

Urine flow (Table 2) was significantly higher in the diabetic groups than in the non-diabetic group, as expected. In all groups, urine flow decreased significantly after treatment. Urinary osmolality, sodium excretion and potassium excretion are presented in Table 2.

**Blood pressure**

Mean arterial blood pressure (MAP; Figure 1) in the basal state did not differ between the study groups and it fell significantly in all study groups after treatment. In the D-placebo group MAP decreased by 7±2%, in the D-Cp group by 9±2% and in the C-ACEI, D-ACEI and D-Cp–ACEI groups by 15±4%, 13±1% and 14±3%, respectively. The relative fall in blood pressure tended to be greater in the ACEI-treated groups, but not significantly so.

**Glomerular filtration rate**

All the diabetic groups presented statistically significantly increased GFR in the basal state compared with the healthy C-ACEI group (Figure 2). GFR in the C-ACEI group and in the D-placebo group did not change significantly after treatment. In all the other diabetic groups, GFR decreased significantly following treatment with either C-peptide or ACEI or a combination of the two. Thus, GFR in the D-Cp group decreased by 17±4% ($P<0.01$), in the D-ACEI group by 14±5% ($P<0.01$) and in the D-Cp–ACEI group by 18±7% ($P<0.01$). After treatment, GFR in these groups was no longer significantly higher than in the healthy C-ACEI group. There was no statistically significant relationship between the plasma concentration of C-peptide and the changes in GFR or blood flow, respectively, within each C-peptide treated group.

**Blood flow**

Blood flow increased in the C-ACEI group by 26±3% ($P<0.01$), in the D-ACEI group by 27±4% ($P<0.001$) and in the D-Cp–ACEI group by 32±6% ($P<0.001$). Blood flow in the D-Cp group and in the D-placebo group increased by 5±2% ($P<0.05$) and 5±1% ($P<0.01$), respectively. The rise in blood flow in the D-Cp group did not differ from the D-placebo group.

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**Table 1.** Basal data. Data from the final experiment (2 weeks after diabetes onset) in each study group. Blood glucose and body weight were measured before the study.

<table>
<thead>
<tr>
<th></th>
<th>C-ACEI ($n=5$)</th>
<th>D-placebo ($n=7$)</th>
<th>D-Cp ($n=13$)</th>
<th>D-ACEI ($n=9$)</th>
<th>D-Cp–ACEI ($n=8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>6.7±0.3</td>
<td>20.6±0.7</td>
<td>22.6±0.6</td>
<td>22.3±0.5</td>
<td>21.8±0.3</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>280±14</td>
<td>265±7</td>
<td>254±7</td>
<td>264±8</td>
<td>240±12</td>
</tr>
</tbody>
</table>

Data are presented as means±SEM.

**Table 2.** Urine variables. Data from the final experiment (2 weeks after diabetes induction) in each study group. Urine variables were measured before (basal) and after treatment.

<table>
<thead>
<tr>
<th></th>
<th>C-ACEI ($n=5$)</th>
<th>D-placebo ($n=7$)</th>
<th>D-Cp ($n=13$)</th>
<th>D-ACEI ($n=9$)</th>
<th>D-Cp–ACEI ($n=8$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal After treatment</td>
<td>Basal After treatment</td>
<td>Basal After treatment</td>
<td>Basal After treatment</td>
<td>Basal After treatment</td>
</tr>
<tr>
<td>Urine flow (µl/min)</td>
<td>16±2 9±2$^{a}$</td>
<td>46±8 29±5$^{b}$</td>
<td>59±6 33±3$^{c}$</td>
<td>57±5 40±3$^{b}$</td>
<td>68±6 43±3$^{c}$</td>
</tr>
<tr>
<td>U$_{Osm}$ (mOsm/kg)</td>
<td>852±60 1325±161$^{a}$</td>
<td>1056±45 1250±52$^{b}$</td>
<td>1014±45 1215±31$^{c}$</td>
<td>981±31 1172±29$^{c}$</td>
<td>875±32 1066±30$^{c}$</td>
</tr>
<tr>
<td>U$_{Na}$V</td>
<td>1.80±0.31 0.84±0.29$^{a}$</td>
<td>1.02±0.20 0.74±0.14</td>
<td>0.92±0.17$^{b}$</td>
<td>1.44±0.28 1.66±0.38</td>
<td></td>
</tr>
<tr>
<td>U$_{K}$V</td>
<td>1.82±0.31 1.80±0.42</td>
<td>1.14±0.18 1.53±0.24</td>
<td>1.33±0.10 1.50±0.13</td>
<td>1.17±0.14 1.43±0.16</td>
<td>1.46±0.28 1.81±0.27$^{b}$</td>
</tr>
</tbody>
</table>

Statistically significant changes from the basal state ($^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$). Data are presented as means±SEM.
In contrast, the blood flow changes in the three groups receiving captopril were significantly greater than those in the D-placebo group (Figure 3).

**Filtration fraction**

The filtration fraction decreased significantly in all diabetic groups as well as in the C-ACEI group (Figure 4). Thus, the filtration fraction in the D-placebo group decreased by 12 ± 5%, in the D-Cp group by 20 ± 4%, in the D-ACEI group by 31 ± 5% and in the D-Cp–ACEI group by 36 ± 7%. Only in the latter group was the fall significantly greater than in the D-placebo group (P < 0.05).

**Discussion**

C-peptide, earlier thought to be without physiological effects, recently has been proven to effectively lower glomerular hyperfiltration, diminish albuminuria and reduce glomerular hypertrophy in diabetic rats and humans [5–9]. It has been hypothesized that C-peptide could be an additional treatment to insulin in type 1 diabetes patients to retard or prevent the development of diabetic nephropathy. ACEI treatment is currently a well-established therapy in type 1 diabetes patients with renal involvement, particularly when hypertension and/or microalbuminuria occurs [12–14]. The aim of this study, therefore, was to compare the renal effects...
of C-peptide with those of ACEI administration or a combination of the two. The objective was also to compare the haemodynamic effects of the drugs in an attempt to discern if they act via similar or different mechanisms and to evaluate possible additive effects.

Glomerular hyperfiltration is considered to be an independent risk factor for the development of diabetic nephropathy [15–17]. In the present study we found that the diabetic rats, as expected, showed increased levels of GFR in the basal state, ranging from 37 to 51% in the different groups compared with the healthy (C-ACEI) group (Figure 2). Treatment with C-peptide (D-Cp), captopril (D-ACEI) or a combination of the two (D-Cp–ACEI) decreased the hyperfiltration significantly compared with placebo (D-placebo). Thus, GFR decreased by 17% in the D-Cp group, 14% in the D-ACEI group and 18% in the D-Cp–ACEI group. The magnitude of these changes did not differ statistically significantly between the three groups. The data show that C-peptide and captopril are equally effective in lowering GFR in diabetic rats with glomerular hyperfiltration, but an additive effect of the two agents could not be detected. A contributory factor for the decrease in GFR may be the lower MAP observed in all study groups during

**Fig. 3.** Relative renal blood flow (%) changes after drug administration in each study group: healthy captopril-treated rats (C-ACEI, n = 5), diabetic placebo-treated rats (D-placebo, n = 7), diabetic C-peptide-treated rats (D-Cp, n = 13), diabetic rats treated with captopril (D-ACEI, n = 9) and diabetic rats treated with the combination of C-peptide and captopril (D-Cp–ACEI, n = 8). Analysis of variance followed by the Tukey post-hoc test was used to statistically detect differences in blood flow change from the D-placebo group after treatment. Whiskers represent SEM. Statistically significant differences from the D-placebo group are shown: *P < 0.05, **P < 0.01, ***P < 0.001.

**Fig. 4.** Filtration fraction changes (%) after treatment in each study group: healthy captopril-treated rats (C-ACEI, n = 5), diabetic placebo-treated rats (D-placebo, n = 7), diabetic C-peptide-treated rats (D-Cp, n = 13), diabetic rats treated with captopril (D-ACEI, n = 9) and diabetic rats treated with the combination of C-peptide and captopril (D-Cp–ACEI, n = 8). Analysis of variance followed by the Tukey post-hoc test was used to statistically detect differences in filtration fraction change compared with the D-placebo group. Whiskers represent SEM. A statistically significant difference from the D-placebo group is shown: *P < 0.05.
the treatment period compared with the basal state (Figure 1). Thus, in the ACEI-treated diabetic rats MAP fell by 13–14% and GFR decreased by 14–18%. However, this can only partly explain the lowered GFR since MAP fell most in the C-ACEI group (15%), while the fall in GFR in this group was small (3%). The reason for the consistent decrease in blood pressure in all diabetic study groups is probably that their urine production during the anaesthesia exceeded the rate of saline infusion, resulting in a mild hypovolaemia.

Previous studies in humans have shown that ACEI reduces renal vascular resistance and increases renal blood flow [18] due to a dilatation of predominantly the efferent arterioles. This results in reduced intraglomerular pressure, which, in turn, may lead to reduced GFR. In the case of C-peptide, the possible mechanism of action is less apparent. Recently, it has been shown, in an acute infusion study in rats with human C-peptide, that C-peptide does not significantly affect renal blood flow [19]. Following an acute infusion of C-peptide to diabetic patients, renal plasma flow increased slightly [5]. In the present study, renal blood flow increased by 26–32% in the ACEI-treated groups, whereas the increase in the group treated with C-peptide alone was only 5% and similar to that in the D-placebo group (Figure 3). Likewise, C-peptide administration did not result in a significantly greater fall in MAP than that seen in the placebo-treated group. Accordingly, the C-peptide effect on glomerular hyperfiltration is unlikely to be due to a change in renal haemodynamics. A possible theoretical objection might be that a dilatation of the efferent arteriole, matched by a constriction of the afferent arteriole, could reduce intraglomerular pressure with only a minor effect on total renal blood flow. It is, however, more likely that, as proposed earlier [19], C-peptide affects the other major determinant of the filtration fraction, namely the glomerular ultrafiltration coefficient, which is the product of the surface area for filtration and the permeability of the glomerular capillary wall. Thus, in this study, the GFR reduction could be due to either decreased capillary surface area or to decreased glomerular capillary wall permeability. To further explore these mechanisms, which are indeed important, micropuncture studies are necessary.

The filtration fraction decreased in all study groups compared with baseline before drug infusion started (Figure 4). The largest decrease (36%) was seen in the group treated with the combination of C-peptide and captopril (D-Cp–ACEI). It was only in this group that the filtration fraction fell significantly more than in the diabetic placebo-treated group (D-placebo). This interesting finding raises the question of whether further studies with long-term treatment could reveal other additive effects of C-peptide and captopril in incipient diabetic nephropathy. At this stage, we can only speculate about the exact mechanisms of action of C-peptide in the glomerulus; micropuncture studies are essential to evaluate this issue further.

The C-peptide levels during treatment were low, as expected, in the D-placebo and D-ACEI groups. However, the levels in the diabetic animals remained measurable, showing that β-cell function was not completely abolished. The C-peptide levels in the C-ACEI group were in the normal range. In the C-peptide treated groups, the observed C-peptide levels ranged from physiological to supraphysiological concentrations. The background to this variability is not apparent. The infusion pump was validated repeatedly and the residual volume in the syringe was checked after each experiment. Since the same volume of the stock solution was used for each experiment, the variability in C-peptide concentration between the animals cannot have arisen from a calculation error in the process of diluting the C-peptide for infusion. An alternative explanation could be layering of C-peptide in the stock solution. However, visual inspection of the stock solution showed no sign of this. In accordance with an earlier dose-response study of human C-peptide in rats [19], in which no relationship was found between the plasma C-peptide concentration in the supraphysiological range (5–225 nmol/l) and the effects on GFR or RBF, there was, in the present study, in a similar supraphysiological concentration range, no relationship between the plasma C-peptide concentration and the magnitude of the effects. This can be explained by the observation that maximal C-peptide binding to cell surfaces seems to be obtained at a physiological C-peptide concentration of ~1 nmol/l.

In conclusion, C-peptide and captopril are equally effective in lowering glomerular hyperfiltration in rats with experimental type 1 diabetes without insulin treatment. No statistically significant additive effects on GFR or blood flow of C-peptide and captopril could be demonstrated in this short-term study. Captopril acts, at least partly, via vascular effects, while C-peptide does not seem to affect renal haemodynamics. Thus, the mechanisms of action are probably different for the two agents and long-term studies are warranted to further investigate whether a combined treatment may be beneficial in type 1 diabetic patients.

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Conflict of interest statement. J Wahren and K Ekberg hold stock in and are employed by Creative Peptide Inc., Stockholm, a biotech company that promotes research and development of C-peptide.

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