

C-Peptide Does Not Affect Ocular Blood Flow in Patients With Type 1 Diabetes

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OBJECTIVE — The aim of the present study was to investigate the effect of intravenous C-peptide infusion on ocular blood flow in patients with type 1 diabetes under euglycemic conditions.

RESEARCH DESIGN AND METHODS — The study was performed in a randomized, placebo-controlled, double-masked, two-way, crossover design in 10 type 1 diabetic patients. C-peptide was intravenously administered at two different dosages (dosage 1: 25 pmol · kg⁻¹ · min⁻¹ bolus followed by 5 pmol · kg⁻¹ · min⁻¹ continuous infusion; dosage 2: six times higher than dosage 1), each for 60 min. Physiologic saline solution was used as a control for C-peptide on a different study day. On both study days, euglycemic clamps were performed. To assess retinal blood flow, laser Doppler velocimetry (blood flow velocities) and retinal vessel analyzer (vessels diameters) measurements were performed. Laser interferometric measurements of fundus pulsation were used to assess pulsatile choroidal blood flow. Blood velocities in the ophthalmic artery were measured using color Doppler imaging.

RESULTS — Eight patients (two female and six male) completed the study according to the protocol and without adverse events. One patient developed an anaphylactic reaction to C-peptide, which resolved without sequelae. The following results originate from the remaining eight subjects. Systemic hemodynamic parameters remained stable during both study days. Infusion of C-peptide did not affect any ocular hemodynamic parameter.

CONCLUSIONS — The data of the present study indicate that exogenous C-peptide exerts no effect on ocular hemodynamic parameters in type 1 diabetic patients under euglycemic conditions. The maximum detectable change in these parameters was <25%.

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In the proinsulin molecule, the α - and β -chains of active insulin are linked by a third polypeptide chain called the C-peptide (1). After C-peptide is cleaved off from proinsulin, it is stored in secretory granules in the pancreatic β -cells. In response to β -cell stimulation, C-peptide is secreted with insulin in equimolar amounts into the bloodstream.

It has generally been thought that C-

peptide is biologically inactive. However, recent studies demonstrate that C-peptide exerts vascular effects (2,3) and that the lack of endogenous C-peptide may be associated with the development of diabetic microvascular complications (4,5). The influence of C-peptide on ocular hemodynamics in humans has not been examined up to now. Ido et al. (6) found, however, that subcutaneously in-

jected biosynthetic C-peptide in streptozotocin-induced diabetic rats markedly damped diabetes-induced increases in blood flow in the anterior uvea, retina, and sciatic nerve and also prevented increased albumin permeation in these tissues and in the aorta. The aim of the present study was to investigate the effect of systemic infusion of C-peptide in patients with type 1 diabetes on ocular blood flow under euglycemic conditions.

RESEARCH DESIGN AND METHODS

The present study was performed in adherence to the Declaration of Helsinki and the Good Clinical Practice guidelines. After approval of the study protocol by the ethics committee of the Vienna University School of Medicine, the nature of the study was explained and all patients gave written consent to participate. All patients passed a prestudy screening during the 4 weeks before the first study day, which included medical history and physical examination; 12-lead electrocardiogram; complete blood count; activated partial thromboplastin time; thrombin time; clinical chemistry (sodium, potassium, creatinine, alanine aminotransferase, γ -glutamyltransferase, total bilirubin, and total protein); hepatitis A, B, C, and HIV serology; urine analysis; and an ophthalmic examination. Patients were excluded if any abnormality was found as part of the pretreatment screening, unless the investigators considered an abnormality to be clinically irrelevant. After the screening, 10 patients with type 1 diabetes were enrolled and randomized to the present study.

The study was performed in a randomized, placebo-controlled, double-masked, two-way, cross-over design. Two trial days were scheduled for each subject. The washout period between the study days was at least 7 days. All patients were asked to refrain from alcohol and caffeine for at least 12 h before trial day. On the trial days, subjects arrived after sleeping for 7–8 h, having a light breakfast, and having their usual morning insulin dosing. Intravenous cannulas (Venflon) were inserted into antecubital veins of each arm for simultaneously monitoring plasma concentrations of glucose and taking

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Abbreviations: CDI, color Doppler imaging; LDV, laser Doppler velocimetry.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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blood samples (1. Venflon) and for drug infusion (2. Venflon). After topical instillation of one drop of a mydriaticum (Acephal) into the eye under study and after a resting period of at least 20 min in a sitting position, a euglycemic insulin clamp was started. Baseline parameters of ocular blood flow were obtained when the blood glucose level reached 5.56 mmol/l with a tolerance of $\pm 20\%$ but not < 30 min after the start of the euglycemic clamp. Thereafter, C-peptide or placebo administration was started. The hemodynamic measurements were performed every 30 min throughout the C-peptide/placebo infusion period. Blood pressure was measured in 10-min intervals. Pulse rate and a real-time electrocardiogram were continuously monitored. Subjects were monitored throughout the infusion period and until all parameters returned to baseline.

Study medication and measurements

All intravenous infusions were prepared by an experienced nurse using appropriate aseptic technique.

Euglycemic insulin clamp. Euglycemic clamps were performed according to DeFronzo et al. (7). Insulin (Huminsulin Normal; Lilly, Fegersheim, France) was infused at a rate of $0.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Glucose (glucose 10%, Leopold Infusionsflaschen; Leopold Pharma, Linz, Austria) was infused at a rate necessary to maintain the blood glucose level at 5.56 mmol/l with a tolerance of $\pm 20\%$. Blood samples for glucose concentration were drawn every 5 min from the contralateral arm.

C-peptide infusion. C-peptide infusions were prepared using fully synthetic produced C-peptide powder (Clinalpha, L  ufelingen, Switzerland) and physiologic saline solution as the vehicle according to the manufacturer's information. We administered C-peptide in two dosages, each during 60 min: 1) loading dose: $25 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 3 min as a bolus given intravenously, maintenance dosage: $5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and 2) loading dose: $150 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 3 min as a bolus given intravenously, maintenance dosage: $30 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Physiologic saline solution was given as placebo control for C-peptide on the placebo day.

Laboratory analyses. Insulin and C-peptide plasma concentrations were determined by standard procedures (Department of Medical and Chemical Laboratory Diagnostics, Medical Univer-

sity of Vienna, Vienna, Austria). Glucose plasma concentrations during the study were measured immediately using a Beckman glucose analyzer. Arterialized venous blood samples were chosen for the convenience of the subjects because it has previously been shown that capillary glucose is almost similar to arterialized venous glucose (8).

Systemic hemodynamics. Brachial artery blood pressure (systolic and diastolic blood pressure) was monitored on the upper arm by an automated oscillometric device. Pulse rate was automatically recorded from a finger-pulse oxymeter device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Laser Doppler velocimetry. The principle of erythrocyte velocity measurement by laser Doppler velocimetry (LDV) is based on the optical Doppler effect. Laser light, which is scattered by moving erythrocytes, is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte velocity (V_{max}) (9). From V_{max} , mean blood velocity in retinal vessels (V_{mean}) may be calculated as $V_{\text{mean}} = V_{\text{max}}/2$. In the present study, we used a fundus camera-based system with a single-mode laser diode at a center wavelength of 670 nm (Oculix Sarl, Arb  z, Switzerland). LDV provides a reliable and reproducible technique for retinal blood velocity measurement (9). In the present study, V_{mean} was determined for a major retinal vein draining into the optic disc. All measurement locations were within one to two disc diameters from the center of the optic disc.

Retinal vessel analyzer. The vessels diameters (D) at the same measurement locations were determined from retinal images recorded with a commercially available system that comprises a fundus camera (IMEDOS, Jena, Germany), a video camera, a video monitor, and a personal computer with analyzing software for the accurate determination of retinal arterial and venous diameters (10). Every second, a maximum of 25 readings of vessel diameter can be obtained. For this purpose, the fundus is imaged by the video camera. The consecutive fundus images are digitized using a frame grabber. Our previous data (11) indicates excellent reproducibility of measurements with the retinal vessel analyzer. With this system, changes between 3 and 5% in retinal venous diameters can be detected (11).

Laser interferometric measurement of fundus pulsation. With this technique, pulse-synchronous distance changes between cornea and retina are assessed. For this purpose, the eye is illuminated by the beam of a single-mode laser diode ($\lambda = 783 \text{ nm}$) along the optical axis (12). The light is reflected at both the front surface of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be evaluated. This distance change is observed because the volume of blood entering the eye through the arteries exceeds the volume of blood leaving the eye during systole. Hence, a reduction in corneo-retinal distance is observed during systole, whereas the distance during diastole slightly increases. The fundus pulsation amplitude, which is the maximum distance change between cornea and retina during the cardiac cycle, has been shown to be an estimate for pulsatile ocular blood flow (13). In the present study, the measurements were performed in the fovea to assess pulsatile choroidal blood flow.

Color Doppler imaging of the ophthalmic artery. The color Doppler imaging (CDI) examinations were performed using a Vivid 7 Color Doppler Ultrasound device (GE Vingmed Ultrasound). The probe was placed on the closed upper eyelid following the application of contact jelly (methylcellulose 2%). To minimize the exertion of pressure on the globe, the examiner supported his hand on patient's forehead. The ophthalmic artery was measured anteriorly, at the point where it crosses the optic nerve, $\sim 25 \text{ mm}$ posterior to the globe. Peak systolic flow velocity and end diastolic flow velocity were determined (14). From these parameters, mean flow velocity (mean flow velocity = integral of the Doppler curve/duration of the cardiac cycle) was calculated.

Data analysis

Blood flow through a retinal vein (Q) was calculated from LDV and retinal vessel analyzer measurements as $Q = V_{\text{mean}} \times \pi \times D^2/4$, where V_{mean} is the mean blood velocity and D is the diameter. Mean flow velocity in the ophthalmic artery was calculated as the mean of two consecutive measurements. All data are presented as absolute values (means \pm SE). Repeated-measures ANOVA was used to assess statistical significance of the intervention-induced effects. A P value < 0.05 was considered significant. Statistical analysis

Table 1—Patient demographics

	Age (years)	Sex	BMI (kg/m ²)	Diabetes duration (years)	HbA _{1c} (%)	Diabetic retinopathy in the index eye	Concomitant diseases and their therapy	Smoking (cigarettes per day)
Patient 1	38	Male	25	15	7.1	Microaneurysms	Hypertension (enalapril)	No
Patient 2	36	Male	27	24	6.5	Microaneurysms	None	No
Patient 3	33	Male	23	20	6.9	Microaneurysms	Hypertension (lisinopril/ hydrochlorothiazid)	No
Patient 4	25	Male	25	10	6.1	None	None	<5
Patient 5	33	Male	24	19	8.2	None	Hypertension (enalapril)	No
Patient 6	34	Male	30	5	7.9	None	Hypertension (lisinopril, amlodipin, and terazosin)	No
Patient 7	23	Female	26	14	6.4	None	Hypertension (enalapril)	No
Patient 8	26	Female	18	22	5.7	None	None	No

was carried out using CSS Statistica for Windows (Statsoft, Tusla, CA).

RESULTS

Patient disposition, demographics, and disease characteristics

Eight patients, including two women and six men, completed the study according to the protocol and without adverse events. One patient was excluded on his 1st study day because of insufficient target fixation during ocular hemodynamic measurements. Another patient (female, 21 years old) developed an anaphylactic reaction to C-peptide, which resolved without sequelae. A few minutes after the infusion was started, the patient began to experience hot flush, itchiness, urticaria, mild respiratory distress, and lip swelling progressing to severe face swelling. All study medications were immediately discontinued, and the therapy for anaphylaxis was initiated. After 3.5 h, the patient was asymptomatic and could return home. Due to this serious adverse reaction, the present study was terminated

prematurely. Table 1 refers to the baseline demographic characteristics of the remaining eight subjects.

Glucose, insulin, and C-peptide plasma concentrations

As a result of the euglycemic clamp, all patients had glucose plasma concentrations within or slightly below the normal range during both study days (Table 2). C-peptide plasma concentrations were highly increased during infusions of C-peptide (Table 2).

Systemic hemodynamics

The values of systemic blood pressure and pulse rate during the study are shown in Table 3. Neither euglycemic clamp nor C-peptide affected these parameters.

Ocular blood flow

During the infusion of C-peptide, no significant changes were observed in any of the ocular hemodynamic parameters (Table 4).

CONCLUSIONS — Data of the present study indicate that exogenous C-peptide exerts no effect on systemic and ocular hemodynamic parameters in type 1 diabetic patients under euglycemic conditions. In contrast, previous investigations showed improvement of blood flow in different vascular compartments during infusion of C-peptide at comparable doses. Hansen et al. (3) demonstrated that a 60-min infusion of 6 pmol · kg⁻¹ · min⁻¹ C-peptide improved impaired myocardial function and perfusion in diabetic patients. After C-peptide infusion, myocardial velocities, as assessed using CDI, increased ~12% during both early diastole and systole. Forst et al. (15) showed that replacement of human C-peptide (8 pmol · kg⁻¹ · min⁻¹, 60-min infusion) in type 1 diabetic patients leads to redistribution in skin microvascular blood flow levels to those in healthy subjects. The capillary blood cell velocity, as measured with videophotometric capillaroscopy, increased ~9.5% during infusion of C-peptide (15). The measurements of subpapillary blood flow using

Table 2—Insulin, glucose, and C-peptide plasma concentrations on both study days

	Time of measurement	Insulin (pmol/l)		Glucose (mmol/l)		C-peptide (nmol/l)	
		Placebo day	C-peptide day	Placebo day	C-peptide day	Placebo day	C-peptide day
	Baseline	192 ± 42	240 ± 60	4.1 ± 0.3	4.4 ± 0.4	0.04 ± 0.02	0.04 ± 0.02
C-peptide (5 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	30 min	174 ± 42	180 ± 42	3.9 ± 0.2	4.3 ± 0.4	0.03 ± 0.02	1.14 ± 0.08*
	60 min	198 ± 48	162 ± 30	4.3 ± 0.3	4.6 ± 0.3	0.03 ± 0.02	1.12 ± 0.14*
C-peptide (30 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	30 min	186 ± 42	180 ± 48	4.2 ± 0.3	4.7 ± 0.4	0.03 ± 0.02	6.8 ± 0.2*
	60 min	174 ± 42	186 ± 54	4.0 ± 0.2	4.4 ± 0.3	0.03 ± 0.02	6.55 ± 0.6*

Data are means ± SE. n = 8. *Significant differences versus baseline and versus placebo.

Table 3—Systemic hemodynamic parameters on both study days

	Time of measurement	Mean arterial blood pressure (mmHg)		Pulse rate (bpm)	
		Placebo day	C-peptide day	Placebo day	C-peptide day
C-peptide (5 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	Baseline	82 ± 4	79 ± 3	78 ± 3	75 ± 2
	30 min	80 ± 3	82 ± 3	75 ± 4	76 ± 2
	60 min	83 ± 3	78 ± 2	73 ± 3	70 ± 3
C-peptide (30 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	30 min	85 ± 4	79 ± 4	71 ± 4	73 ± 3
	60 min	79 ± 2	78 ± 4	70 ± 3	71 ± 3

Data are means ± SE. n = 8.

laser Doppler fluxometry did not show any significant changes (15). Physiological concentrations of C-peptide increase resting blood flow, improve capillary diffusion capacity, and stimulate oxygen uptake in forearm skeletal muscle in type 1 diabetes (16,17). C-peptide infusion increased basal blood flow in the forearm by 35% (6 pmol · kg⁻¹ · min⁻¹, 60-min infusion), as measured with CDI (16), and by 25% (5-pmol · kg⁻¹ · min⁻¹ infusion), as assessed using venous occlusion plethysmography (17). The cellular mechanism underlying the vasoactive effects of C-peptide has not been fully established. Preliminary evidence suggests that C-peptide, via an increase in the intracellular Ca²⁺ concentration, stimulates endothelial nitric oxide synthase activity (18).

In the present study, levels of C-peptide measured under study medication reached values clearly above normal plasma concentrations seen in healthy humans and even higher than in other trials (15–17) in which a vasoactive response to intravenous C-peptide was seen. However, we observed no tendency toward changes in any of the hemodynamic parameters obtained. Accordingly,

it seems unlikely that either the selected dose was too low or the period of observation was too short. Based on the variability of the outcome parameters during the placebo day, we calculated the minimum changes we could have detected in our sample size with an α -error of 5% and a β -error of 20%. To obtain retinal blood flow, we measured vessel diameters using the retinal vessel analyzer and flow velocities using a laser Doppler velocimeter. A retinal vessel analyzer provides excellent reproducibility (11). In contrast, LDV measurements require good fixation and cooperation, leading to a generally lower reproducibility. Using the data from eight patients, we are not able to detect statistically significant changes in the calculated retinal blood flow <25%. Choroidal microcirculation was assessed by laser interferometry. This technique provides very good reproducibility; hence, the data from our study allow detecting changes as small as 5%. An important limitation is, however, that only the pulsatile blood flow component can be measured with this technique (13). The reproducibility of CDI measurements in the present study would have allowed detecting velocity

changes of ~15%. Accordingly, we assume that C-peptide-induced changes in ocular perfusion are small and unlikely to be of physiological or pathophysiological relevance.

We cannot completely rule out the possibility that C-peptide did not alter ocular blood flow in our patients because of structural morphological changes in diabetic blood vessels. We deem this, however, unlikely because a variety of previous studies (19–22) have shown that the ocular vasculature in patients with diabetes is still capable of reacting to vasoactive stimuli. In addition, we cannot entirely exclude that the antihypertensive medication itself reduced the reactivity to C-peptide. Again, this is unlikely because most patients included in the present trial were on ACE inhibitors, which normalize retinal blood flow in patients with diabetes (23). In addition, it has been shown that angiotensin 1 receptor blockers improve endothelium-dependent vascular reactivity in the retinal vasculature (24). In conclusion, our data do not indicate a major role of C-peptide in the regulation of ocular blood flow.

Table 4—Ocular blood flow parameters during the study

	Time of measurement	Fundus pulsation amplitude (pulsatile choroidal blood flow) (au)		Retinal blood flow (μ l/min)		Mean flow velocity in ophthalmic artery (cm/s)	
		Placebo day	C-peptide day	Placebo day	C-peptide day	Placebo day	C-peptide day
Baseline	4.3 ± 0.6	4.4 ± 0.6	11.4 ± 2.9	9.8 ± 2.1	13.9 ± 1.8	13.4 ± 1.0	
C-peptide (5 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	30 min	4.4 ± 0.6	4.4 ± 0.6	12.2 ± 2.7	9.8 ± 2.1	Not done	Not done
	60 min	4.4 ± 0.6	4.3 ± 0.6	10.9 ± 3.0	9.8 ± 2.3	13.4 ± 0.9	12.6 ± 0.7
C-peptide (30 pmol · kg ⁻¹ · min ⁻¹) or placebo infusion	30 min	4.5 ± 0.6	4.4 ± 0.6	10.1 ± 2.2	9.1 ± 1.7	Not done	Not done
	60 min	4.5 ± 0.6	4.5 ± 0.6	12.7 ± 2.8	8.9 ± 1.4	14.3 ± 1.1	12.2 ± 0.8

Data are means ± SE. n = 8.

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