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The Relationship Between the IGF-I System and Its Binding Proteins and Microvascular Reactivity in Type 1 Diabetes Mellitus

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Summary

The system of IGF-I and its binding proteins may be involved in the pathogenesis of vascular damage in Type 1 diabetes. The aim of this study was to analyze the relationship between this system and the microvascular reactivity in Type 1 diabetes as measured by laser-Doppler flowmetry. Twenty-two Type 1 diabetic patients (13 women and 9 men) with microangiopathy and fifteen healthy subjects (8 women and 7 men) were examined clinically, underwent laser-Doppler flowmetry and intima-media thickness measurements. Fasting serum levels of IGF-I, free IGF-I, IGFBPs and lipids were examined. The microvascular reactivity was impaired in Type 1 diabetic patients. Maximal perfusion during post-occlusive reactive hyperemia ($PORH_{max}$) and during thermal hyperemia (TH_{max}) was significantly decreased in Type 1 diabetes ($p<0.01$). Percentage perfusion increase in both tests (PORH and TH) was lower in Type 1 diabetes mellitus ($p<0.01$) and the reaction after heating was slower in diabetic patients ($TH_{max/t}$) ($p<0.01$). We did not find any significant dependence of microvascular reactivity on the parameters of IGF-I or its binding proteins. We conclude that the microvascular reactivity is impaired in Type 1 diabetes mellitus, but this impairment is not clearly dependent on the activity of the IGF-I system. It is probably only a complementary pathogenic factor.

Key words
IGF-I • IGFBP-1 • Microangiopathy • IDDM • Laser-Doppler flowmetry

Introduction

Insulin-like growth factor-I (IGF-I) is a single chain polypeptide that shares 48 % of its structural homology with proinsulin. It has many specific activities including the stimulation and regulation of cell growth, a mitogenic effect, and the modulation of various cell types functions (Baserga and Rubin 1993). In addition, it can also affect glucose, lipid and protein metabolism (Froesch *et al.* 1963). IGF-I circulating in the plasma is bound to binding proteins that determine its bioavailability and modulate its biological action. IGFBP-1 and IGFBP-3 are considered to be the most important of these insulin-like growth factor binding proteins (IGFBPs). Both of them have the major effect on the bioavailability of IGF-I. IGFBP-1 serum levels are tightly and inversely related to serum insulin levels in the portal vein (Murphy *et al.* 1991. Changes in serum IGF-I and IGFBP-1 concentrations in Type 1 diabetes are quite consistent and are characterized by a decrease of IGF-I and an increase of

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IGFBP-1 concentrations in the serum (Simpson *et al.* 1998). The IGF-I/IGFBPs system is thought to play an important role in the pathogenesis of vascular damage under various conditions, including diabetes mellitus. IGF-I was thought to increase and IGFBP-1 was suggested to decrease the risk of the development of macroangiopathy as well as that of cardiovascular morbidity and mortality (Janssen *et al.* 1997). However, recently published papers have also suggested a possible protective effect of IGF-I on the vessel wall in diabetic patients (Janssen *et al.* 1998, Janssen and Lamberts 2000, Feldmann *et al.* 2000).

Diabetic patients are at an increased risk of cardiovascular disease. However, some patients do not develop cardiovascular sequelae whereas others have devastating complications (Barrett-Connor and Wingard 1983). The early identification of patients at increased risk of vascular complications is very important for both the prevention and proper treatment.

The aim of the present study was to find a relationship between serum levels of IGF-I and its binding proteins, and the function of microcirculation in subjects with Type 1 diabetes in comparison with healthy controls. We used the intima-media thickness (IMT) as an early marker of macroangiopathy and the laser-Doppler flowmetry for detecting of the impairment of microcirculation (Rendell *et al.* 1989, Lehman *et al.* 1997).

Methods

Patients

Twenty-two patients (thirteen women and eight men, mean age 47±4 years) with Type 1 diabetes were included in the study. Microangiopathy was developed in all of them (retinopathy). Fifteen healthy subjects (eight women and seven men, mean age 50±4 years) served as the control group. The characteristics of diabetic patients and the controls are shown in Table 1. Smokers were excluded from the study. We also excluded patients with hypertension and moderate or severe hyperlipoproteinemia. The study was conducted with the approval of the Ethical Committee of the First Faculty of Medicine of Charles University and all participants gave their written consent.

Laboratory methods

Blood samples for biochemical analysis were collected after an overnight fast between 07:00 and 08:00 h. The insulin-like growth factor-I (IGF-I) and free IGF-I serum levels were determined using commercial RIA kits (CIS, France). Insulin-like growth factor binding protein-1 (IGFBP-1), IGFBP-2, IGFBP-3 and IGFBP-6 serum levels were measured using commercial RIA kits (DLS, USA). Fasting plasma glucose was evaluated using a standard glucose oxidase method on Super GL ambulance analyzer (Dr. Müller Gerätebau, Freital, Germany). The plasma levels of glycated hemoglobin (HbA_{1C}) were determined by IMx GHb Assay System on Abbott Analyzer (Abbott, USA). Serum levels of total cholesterol and triglycerides were measured by a routine method on an automatic analyzer (Hitachi, Japan).

Table 1. The characteristics of group of Type 1 diabetic patients in comparison with the control group.

	Controls	**Type 1 diabetic patients**
Age (years)	49.9±4.4.0	47.3±4.3
BMI (kg/m^2)	23.5±2.3	24.7±2.2
Duration of diabetes (years)	Not Applicable	27.7±8.4
Fasting glycemia (mmol/l)	4.9±0.4	8.7±2.7**
Microalbuminuria (g/mol creatinine)	0.6±0.2	1.3±0.9*
HbA_{1C} (%)	4.7±0.3	8.1±1.0**
Retinopathy	None	All
Systolic Blood Pressure (mm Hg)	121±7	127±15
Diastolic Blood Pressure (mm Hg)	77±7	79±6

*** p<0.01, * p<0.05.*

Ultrasound examinations

The intima-media thickness (IMT) of the common carotid artery was determined using the ultrasound apparatus EcoCee (Toshiba, Japan) with a linear transducer (frequency 10 MHz). The measurement of the IMT was performed by one experienced person and values are expressed as the mean of ten values from both the right and left common carotid artery.

Laser-Doppler flowmetry

Laser-Doppler measurement were performed on the forearm in persons after at least a 20-min rest at room temperature (22 ^{o}C) in the sitting position by using Periflux 4001 apparatus (Perimed, Sweden). The basal flow was recorded at skin temperature of 32 ^{o}C maintained by a special thermostatic probe and the heating unit (Peritemp 4005, Perimed, Sweden). The

brachial artery was then occluded by inflating the sphygmomanometer tourniquet at pressure 20 mm Hg above the systolic blood pressure for three minutes. Post-occlusive reactive hyperemia (PORH) was recorded after sudden decompression of the tourniquet. Thermal hyperemia (TH) was measured after a stabilization period with the probe temperature set to 44 ^{o}C and maintained for the next 5 min.

The following variables of microvascular reactivity were evaluated: maximal flow during PORH ($PORH_{max}$) expressed in perfusion units (PU), the velocity of perfusion increase during PORH ($PORH_{max/t}$) and similarly the maximal flow during tissue heating (TH_{max}) and the velocity of perfusion during heating ($TH_{max/t}$) were recorded. The ratio of basal perfusion and the maximal perfusion measured during PORH and TH was calculated and expressed in percentages.

Statistics

Statistical analysis of the differences between both groups was performed using Student's t-test for unpaired data. Data are expressed as the mean ± S.D. Interdependence between variables inside separate groups was evaluated using the Spearman rank correlation test.

Results

Comparison of biochemical parameters between both groups

Some components of IGF-I/IGFBPs system were significantly different when comparing healthy controls to Type 1 diabetic patients. The results are shown in Table 2. Significantly lower serum concen-trations of IGF-I ($p<0.05$) and of free-IGF-I ($p<0.01$) were found in diabetic patients when compared to healthy controls. On the contrary, IGFBP-1 serum concentrations were significantly higher in patients with Type 1 diabetes than in control subjects ($p<0.001$). The IGFBP-3 serum levels were significantly lower in patients with Type 1 diabetes ($p<0.01$). No significant differences of IGFBP-2 and IGFBP-6 serum levels were observed between the two groups. There were also no significant differences in serum cholesterol and triglyceride levels. These results are shown in Table 2.

Comparison of vascular examination between both groups

The microvascular reactivity was impaired in patients with Type 1 diabetes mellitus. Maximal perfusion in PORH ($PORH_{max}$) and in TH (TH_{max}) were significantly decreased in Type 1 diabetes ($p<0.01$).

Table 2. The comparison of biochemical variables between the control group and the group of Type 1 diabetic patients.

	Controls	**Type 1 diabetic patients**
Fasting glycemia (mmol/l)	4.9±0.4	8.7±2.7**
HbA_{1C} (%)	4.7±0.3	8.1±1.0**
Microalbuminuria (g/mol creatinine)	0.6±0.2	1.3±0.9*
Cholesterol (mmol/l)	5.8±1.1	5.4±0.8
Triglycerides (mmol/l)	1.1±0.5	1.3±0.5
IGF-I (μg/l)	215.6±57.5	148.6±68.8*
Free IGF-I (μg/l)	1.1±0.5	0.63±0.3**
IGFBP-1 (μg/l)	31.7±12.1	85.7±34.8**
IGFBP-2 (mg/l)	0.58±0.33	0.82±0.42
IGFBP-3 (mg/l)	4.0±0.5	3.3±0.6**
IGFBP-6 (μg/l)	281.4±103.1	252.8±147.2

*IGF-I – insulin-like growth factor-I; IGFBP (1 to 6) – insulin-like growth factor binding proteins (1 to 6); * p<0.05, ** p<0.01, *** p<0.00*

Similarly, percentage perfusion increase in both tests (PORH and TH) was lower in type 1 diabetes mellitus ($p<0.01$) and the reaction after heating was slower in this group of patients ($TH_{max/t}$) ($p<0.01$). The comparisons are given in detail in Table 3.

Relationship of biochemical and laser Doppler variables

The interdependence between variables is given in the Tables 4 and 5. Table 4 shows the analysis within the control group. We only found a weak negative correlation between serum free-IGF-I levels and $PORH_{max}$ ($p<0.05$) and $TH_{max/t}$ ($p<0.05$) and between serum IGFBP-3 serum levels and TH ($p<0.05$). No other significant relationship was found between the IGF-I and its binding proteins and the microvascular reactivity in the control group. Table 5 shows the analysis within the group of patients with Type 1 diabetes mellitus. It is clearly shown that we did not find any significant relationship between IGF-I and its binding proteins and microvascular reactivity.

Table 3. The comparison of intima-media thickness IMT and the parameters of microvascular reactivity measured by laser-Doppler in Type 1 diabetic patients and controls.

	Controls	Type 1 diabetic patients
IMT (mm)	0.59±0.09	0.61±0.16
PORH (%)	610.3±291.1	342.1±109.1**
$PORH_{max}$ (PU)	56.4±21.4	34.2±10.7**
$PORH_{max/t}$	4.63±2.41	3,67±4.02
TH (%)	1766.2±846.8	1009.0±353.11**
TH_{max} (PU)	131.15±56.6	84.71±28.8**
$TH_{max/t}$	1.89±0.70	1.17±0.54**

*PORH – percentage perfusion increase in the test with postocclusive reactive hyperemia; $PORH_{max}$ – maximal perfusion in the test with postocclusive reactive hyperaemia, $PORH_{ma/t}$ – velocity of perfusion increase in the test with postocclusive reactive hyperemia; TH – percentage perfusion increase in the test with thermally induced hyperaemia; TH_{max} – maximal perfusion in the test with thermally induced hyperemia; $TH_{max/t}$ – velocity of perfusion increase in the test with thermally induced hyperemia. *p<0.05, **p<0.01, ** p<0.001, NS non-significant.*

Discussion

In the present study, we evaluated the relationship between the system of IGF-I and its binding proteins and the microcirculation in Type 1 diabetic patients. While comparing the differences between the control group and Type 1 diabetic patients, typical features of Type 1 diabetes were observed. This means that a significant reduction was found in both IGF-I and free-IGF-I serum levels. The significant increase in IGFBP-1 serum levels that are inversely regulated by insulin concentrations in portal blood is typical for Type 1 diabetes and this was also confirmed in our group of patients (Snyder and Clemmons 1990). The decrease of IGFBP-3 concentration in Type 1 diabetes could be explained by the functional resistance to the growth hormone as the main regulator of IGFBP-3 expression and production. All changes described above are typical for Type 1 diabetes and have been described previously (Cusi and DeFronzo 1995).

Table 4. The interdependence between the serum levels of IGF-I and its binding proteins and the microvascular reactivity in the group of control subjects.

	PORH (%)	**$PORH_{max}$ (PU)**	**$PORH_{max/t}$**	**TH (%)**	**TH_{max} (PU)**	**$TH_{max/t}$**
IGF-I	r=0.39	r=0.35	r=0.28	r=0.17	r=0.13	r=-0.33
(μg/l)	p=0.19	p=0.24	p=0.35	p=0.58	p=0.68	p=0.28
f-IGF-I	r=-0.32	r=-0.56	r=-0.43	r=-0.35	r=-0.51	r=-0.59
(μg/l)	p=0.29	p=0.05	p=0.14	p=0.25	p=0.08	p=0.03
IGFBP-1	r=-0.14	r=-0.12	r=-0.07	r=-0.03	r=-0.19	r=0.34
(μg/l)	p=0.65	p=0.70	p=0.82	p=0.92	p=0.54	p=0.26
IGFBP-2	r=0.11	r=0.30	r=0.17	r=0.21	r=0.36	r=0.37
(mg/l)	p=0.73	p=0.33	p=0.58	p=0.48	p=0.22	p=0.21
IGFBP-3	r=-0.52	r=-0.25	r=0.01	r=-0.57	r=-0.36	r=0.24
(mg/l)	p=0.07	p=0.41	p=0.99	p=0.04	p=0.23	p=0.43
IGFBP-6	r=0.03	r=0.09	r=0.30	r=-0.22	r=-0.11	r=-0.25
(μg/l)	p=0.93	p=0.78	p=0.32	p=0.47	p=0.72	p=0.42

IGF-I – insulin-like growth factor-I; f-IGF-I – free insulin-like growth factor-I; IGFBP (1 to 6) – insulin-like growth factor binding proteins (1 to 6); PORH – percentage perfusion increase in the test with postocclusive reactive hyperemia; $PORH_{max}$ – maximal perfusion in the test with postocclusive reactive hyperaemia, $PORH_{max/t}$ – velocity of perfusion increase in the test with postocclusive reactive hyperemia; TH – percentage perfusion increase in the test with thermally induced hypeaemia; TH_{max} – maximal perfusion in the test with thermally induced hyperemia; $TH_{max/t}$ – velocity of perfusion increase in the test with thermally induced hyperemia.

The importance of IGF-I and its binding proteins in the development of vascular damage in various situations is generally accepted. However, the detailed mechanism of their action under different pathological conditions is not completely understood and the conclusions of studies are contradictory. Many papers in the past confirmed the fact that IGF-I is the factor enhancing the risk as well as the progression of angiopathy under different circumstances, especially in diabetic patients (Janssen *et al.* 1997, Merimee 1997). In spite of this, it has also been suggested that IGF-I may exert a possible protective effect and has been reviewed recently by Janssen and Lamberts (2000). The differences between various studies depend on a variety of factors such as the type of angiopathy (macroangiopathy vs. microangiopathy), methodology used (morphological studies vs. functional studies, *in vivo* vs. *in vitro* studies), the evaluated parameters (IGF-I vs. free IGF-I) etc. Moreover, it is also important to take into account the group of patients which has been selected for the study, because particular parameters of the IGF-I/IGFBP system differ in various situations (e.g. Type 1 diabetes vs. Type 2 diabetes vs. acromegaly). There are also other methodological problems to be considered, namely differences between total IGF-I and free IGF-I serum levels and also differences between concentrations in systemic circulation and those at the tissue levels which are also regulated by paracrine and autocrine mechanisms (Rajkumar *et al*. 1996).

Table 5. The interdependence between the serum levels of IGF-I and its binding proteins and the microvascular reactivity in the group of patients with Type 1 diabetes mellitus.

	PORH (%)	**$PORH_{max}$ (PU)**	**$PORH_{max/t}$**	**TH (%)**	**TH_{max} (PU)**	**$TH_{max/t}$**
IGF-I	r=-0.14	r=0.14	r=-0.01	r=-0.25	r=-0.02	r=0.15
(μg/l)	p=0.57	p=0.56	p=0.98	p=0.29	p=0.95	p=0.53
f-IGF-I	r=0.17	r=0.15	r=-0.11	r=0.09	r=0.09	r=-0.12
(μg/l)	p=0.47	p=0.54	p=0.65	p=0.69	p=0.70	p=0.63
IGFBP-1	r=0.04	r=-0.14	r=0.17	r=0.35	r=0.08	r=0.06
(μg/l)	p=0.85	p=0.57	p=0.48	p=0.13	p=0.75	p=0.81
IGFBP-2	r=-0.29	r=-0.07	r=0.21	r=-0.11	r=0.14	r=0.17
(mg/l)	p=0.22	p=0.79	p=0.39	p=0.65	p=0.56	p=0.47
IGFBP-3	r=-0.05	r=-0.05	r=-0.08	r=0.08	r=0.08	r=-0.13
(mg/l)	p=0.85	p=0.83	p=0.75	p=0.73	p=0.75	p=0.58
IGFBP-6	r=-0.11	r=0.01	r=-0.13	r=-0.24	r=-0.04	r=0.11
(μg/l)	p=0.64	p=0.98	p=0.58	p=0.31	p=0.88	p=0.65

IGF-I – insulin-like growth factor-I; f-IGF-I – free insulin-like growth factor-I; IGFBP (1 to 6) – insulin-like growth factor binding proteins (1 to 6); PORH – percentage perfusion increase in the test with postocclusive reactive hyperemia; $PORH_{max}$ – maximal perfusion in the test with postocclusive reactive hyperemia, $PORH_{max/t}$ velocity of perfusion increase in the test with postocclusive reactive hyperemia; TH – percentage perfusion increase in the test with thermally induced hyperemia; TH_{max} – maximal perfusion in the test with thermally induced hyperemia; $TH_{max/t}$ – velocityof perfusion increase in the test with thermally induced hyperemia.

The present study considers the functional changes in microcirculation in Type 1 diabetic patients with proven retinopathy. Our results confirm the results of some previous studies showing a significant impairment of microcirculation in Type 1 diabetes (Škrha *et al.* 2001). A particular aim of our study was to find out a relationship between serum levels of IGF-I and its binding proteins and the function of microcirculation. The latter was analyzed using laser-Doppler flowmetry that was successfully used in previous studies for early detection of microcirculatory impairment (Rendell *et al.* 1997). A number of studies have analyzed the role of the IGF-I system and its binding proteins in the pathogenesis of vascular damage in different pathological situations.

However, results of these studies are not unequivocal (Janssen *et al.* 1997, Feldman *et al.* 2000). Studies suggesting that IGF-I contributes to the development of angiopathy under different circumstances usually explain this effect by proliferative and mitogenic effects of IGF-I. The most important seems to be stimulation of smooth muscle cell proliferation in the vessel wall (Chen *et al*. 1998). This effect could possibly play an important role only after the endothelium had been damaged and a series of pathogenic processes in the vessel wall is in progress. IGFBP-1 could play a protective role in these processes by means of binding to IGF-I and thus decreasing its bioavailability (Gibson *et al.* 1996). Recently it has been suggested that IGF-I could exert a protective effect on the development of angiopathy possibly by stimulation of NO synthase by IGF-I (Janssen *et al.* 2000). In our opinion, this mechanism requires a well functioning endothelium which is not seriously damaged. Our complex analysis of the relationship of this system to the microcirculation as measured by laser-Doppler flowmetry failed to confirm any significant influence of this system on the function of microcirculation in either Type 1 diabetic or control subjects.

We conclude that IGF-I does not seem to be significant factor influencing the function of microcirculation in Type 1 diabetes mellitus. It probably only plays a complementary role in the pathogenesis of microangiopathy together with many other pathogenic factors. Further studies are necessary to elucidate the role of IGF-I and its binding proteins in the pathogenesis of angiopathy under different circumstances and pathological conditions. Different aspects of this complex problem also fall within the scope of our present studies.

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