Treatment With Insulin-Like Growth Factor I Alters Capillary Permeability in Skin and Retina

Mehboob A. Hussain, Kilian Studer, Elmar P. Messmer, and E. Rudolf Froesch

Treatment with insulin-like growth factor I (IGF-I) is accompanied by mild generalized and reversible edema. These changes may be due to increased capillary permeability. Therefore, we studied the effects of subcutaneous IGF-I treatment in healthy subjects on capillary permeability of the skin and the retina. Eight healthy subjects were treated with saline or recombinant human IGF-I (rhIGF-I) (10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ s.c.) in a randomized crossover fashion. Transcapillary diffusion of sodium fluorescein (NaF) was quantitated by video densitometry in the skin in all subjects on the 4th treatment day. In seven subjects, plasma penetration of NaF at the blood-retinal barrier was investigated using vitreous fluorometry (VF) on days 3 and 5. Fluorescent light intensities of the skin and plasma penetration determined by VF were significantly higher during IGF-I treatment as compared with those during the control situation. In conclusion, IGF-I treatment at the above dose is accompanied by increased transcapillary diffusion of NaF in skin and in retinal vessels. ***Diabetes*** **44:1209–1212, 1995**

Insulin-like growth factor I (IGF-I) has profound metabolic effects that offer a potential therapeutic option for treating growth hormone resistance, diabetes, catabolic states, and osteoporosis (1). Treatment with IGF-I has repeatedly been associated with mild and reversible generalized edema (2–4). IGF-I has also been shown to exert profound changes on endothelial function in vitro (5,6). We hypothesized that the clinically described edema seen during IGF-I treatment might be due to increased capillary permeability arising from effects of IGF-I on endothelial cells. Therefore, we treated healthy subjects with recombinant human IGF-I (rhIGF-I) and investigated microvascular skin blood flow and capillary permeability of sodium fluorescein (NaF) in skin and retinal vessels. The results suggest that IGF-I treatment is accompanied by increased capillary permeability. However, an effect of IGF-I on increased microvascular blood flow may also contribute to our findings.

From the Division of Endocrinology and Metabolism (M.A.H., E.R.F.), Department of Internal Medicine, and the Department of Ophthalmology (K.S., E.P.M.), University Hospital of Zürich, Zürich, Switzerland.

Address correspondence and reprint requests to Dr. Mehboob A. Hussain, Medical Clinic, Department of Internal Medicine, University Hospital of Zürich, Rämistr. 100, 8091 Zürich, Switzerland.

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AU, autonomous unit; BP, binding protein; FCI, fluorescence light intensity; IGF-I, insulin-like growth factor I; PR, penetration ratio; PU, perfusion unit; VF, vitreous fluorometry.

RESEARCH DESIGN AND METHODS

Subjects. The present studies were performed in conjunction with a study aimed at investigating metabolic effects of IGF-I (4). Eight healthy volunteers (five males, three females; age 28 ± 2 years, range 25–30) were studied. None had any evidence of somatic or mental illness as assessed by history, clinical and routine laboratory examination, and chest X ray. Glycosylated hemoglobin (HbA_{1c}) was within the normal range (0.04–0.64 mol/mol) in all subjects. Female participants were taking monophasic oral contraceptives. Written informed consent was obtained from each volunteer. The study protocol was approved by the ethical committee of the University Hospital of Zürich. The study consisted of two periods of 5 days each, during which the subjects received a continuous subcutaneous infusion of 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ recombinant human IGF-I (rhIGF-I; Ciba-Geigy AG, Basel, Switzerland) or 0.9% saline via a portable minipump (MRS-1 Disetronic AG, Burgdorf, Switzerland) in a crossover randomized fashion. An interval of 2.5 days was allowed between the two treatment periods. All studies were performed in a state of at least a 10-h fast.

Blood samples were drawn at each occasion for measurements of plasma glucose and serum levels of total IGF-I, IGF binding protein (IGFBP)-1 and IGFBP-3, insulin, and creatinine. Plasma glucose was measured immediately after blood sampling using an automated glucose-oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA). Serum and plasma samples were stored at −20°C for later determinations. Total IGF (4) and IGFBP-1 and -3 (7) levels were measured by radioimmunoassay as previously described. Insulin was measured by a two-site immunometric assay (enzyme-linked immunosorbent assay, Dako, Copenhagen, Denmark; coefficient of variation 2.5%, lower detection limit 7.5 pmol/l). For determinations of creatinine clearance rates, 24-h urine was collected on the last days of the control and IGF-I treatment periods. Serum and urine creatinine and urine microalbumin levels were measured by a routine automated method (Hitachi 747). Creatinine clearance was calculated from serum and urine creatinine levels as in the Geigy Scientific Charts (8).

Studies of skin vessels. Measurements were performed on the right distal extremity, where marks were made in order to assure measurement in identical areas during control and treatment periods. All investigations were performed between 1:00 and 3:00 P.M. to control for possible diurnal variation of capillary permeability (9). Cutaneous capillary permeability was assessed by measuring the transcapillary diffusion of NaF by fluorescence video microscopy as previously described (9,10). This technique simultaneously assesses permeability of an average of 80 skin capillaries in a defined area of the skin (large window technique [9]). In brief, the arm was fixed in a vacuum cushion to minimize movement artifacts. Skin temperature was kept between 29 and 31°C with cold/hot packs. A fluorescence microscope (SM-Lux, Leica AG, Glattbrugg, Switzerland) with epi-illumination and planar fluotar objective (2.5 × 0.08) was adapted to the defined skin surface. A low-light level television camera (K 30, Siemens), a video monitor (Picture Monitor Model PM-171T, Ikegami Tsushinki, Japan), a tape recorder (S-VHS, Panasonic, AG-7500, Japan), a video densitometer (Colorado Video, Denver, CO), and a chart recorder (Gould 2600S, Cleveland, OH) were attached to the microscope. After an intravenous bolus injection of 20% NaF (0.3 ml/blood vol), appearance of the fluorescent dye was observed in the predefined skin area. The capillary filling and transcapillary diffusion were recorded and stored on videotape for later evaluation. Fluorescence light intensity (FLI) was measured over an area of 1.4 × 1.4 mm and quantitated at 5, 10, 20, 30, 60, 120, 180, and 300 s after first appearance of the dye in arbitrary units and in percentage of the maximum individual value obtained during 5 min of registration (9). The appearance time, defined as the time interval from

bolus injection of NaF into the cubital vein of the contralateral arm to first appearance of the dye, was measured.

Microvascular superficial skin blood flow was assessed with laser Doppler flux at rest and during postocclusive reactive hyperemia. A laser Doppler probe (2 mW, 632.8 nm, PeriFlux, PF 3, Standard Probe 108, Perimed, Stockholm, Sweden) emitting a light beam of 632.8 nm was attached at the dorsal wrist adjacent to the area selected for fluorescence microscopy. Arterial occlusion at the upper arm was accomplished by inflating a pneumatic cuff to 250 mmHg for 3 min. After calibration, resting values were taken during at least 5 min before deflating the cuff for measurement of peak flux. Flux was recorded continuously on a chart recorder (Gould 2600S, Cleveland, OH). Laser Doppler flux at rest and during hyperemia was expressed in perfusion units (1 PU = 10 mV). Time to peak flux was the interval between cuff deflation and peak flux. This method has been proven to be reliable as compared with the ^{133}Xe washout method (11,12).

Study of the blood-retinal barrier: vitreous fluorometry (VF). All subjects had slit lamp, fundoscopic, and fluorescein angiograms before the study. One of the eight subjects was excluded from VF measurements because of abnormal fundoscopic findings before the study. VF measurements were performed on days 3 and 5 of IGF-I and saline treatment according to previously described methods (13) on a Fluorotron Master (Coherent Radiation, Palo Alto, CA). This method has been successfully used for the characterization of early changes in diabetic retinopathy (14). Briefly, a beam of exiting blue light was focused by a lens system that picks up the fluorescent light emitted from the fluorescein in the eye and directs it to a photomultiplier. The light source is a halogen tungsten incandescent bulb. Barrier filters consist of double interference filters allowing for negligible spectrum overlap. The photomultiplier is operated in a single photon-counting mode. The excitation beam is delivered through one part of the lenses, and the fluorescence is gathered through another part. The intersection of these two parts creates a volume of measurement at the focal plane. The motion of the second lens causes this volume to be translated along the axis of the eye bulb, thus sampling at different positions in the vitreous solution of the eye. A reading is obtained for each discrete step, starting at a position appearing to be posterior of the retina and ending at a position anterior to the cornea. Each measurement is calibrated by an automatic reading of an internal fluorescent glass. Scans were taken before administration of 20% NaF (14 mg/kg i.v. bolus) and 5, 15, and 60 min later. Plasma fluorescence was measured simultaneously in samples drawn from a cubital vein. The measured scan was corrected by subtracting the preinjection scan. The mean posterior vitreous fluorescein concentration that averaged 2–4 mm in front of the chorioretinal peak was then divided by the integral of the plasma fluorescence to obtain the posterior vitreous penetration ratio (PR). Decay of fluorescein in plasma can be expressed as a logarithmic function [$\log(\text{plasma}) = a + b \log(t)$] where a and b are constants that are determined by two or more measurements and t is the time of measurement after intravenous injection of the dye. The integral of the plasma concentration over time was regarded as the amount of fluorescein to which the blood-retinal barrier was exposed until the measurement scan was performed.

Statistical analysis. All data are given as means ± SE. Comparisons were performed using Wilcoxon's rank-sum test (two-tailed) for paired differences and analysis of variance for repeat measurements. $P < 0.05$ was considered statistically significant.

RESULTS

Metabolic parameters. Fasting plasma glucose levels were unchanged during IGF-I treatment. Total IGF-I levels were within normal values during the control period (20.9 ± 0.8), rose to 101.3 ± 3.1 nmol/l after 24 h of IGF-I treatment, and remained at that level thereafter ($P < 0.01$). IGFBP-1 levels rose from 3.9 ± 0.8 during control to 18.4 ± 2.5 μg/l during treatment ($P < 0.01$), whereas IGFBP-3 levels remained unaltered (2,963 ± 207 during control and 3,354 ± 342 μg/l during IGF-I). Fasting insulin levels, 45.0 ± 3.6 during the control period, decreased significantly by the second day of IGF-I treatment to 29.4 ± 3.6 pmol/l ($P < 0.03$) and remained suppressed. Creatinine clearance was 122.6 ± 2.1 during control and 145.9 ± 3.7 ml/min ($P < 0.02$) on the last day of the protocol. No microalbuminuria was detectable during the control and treatment phases.

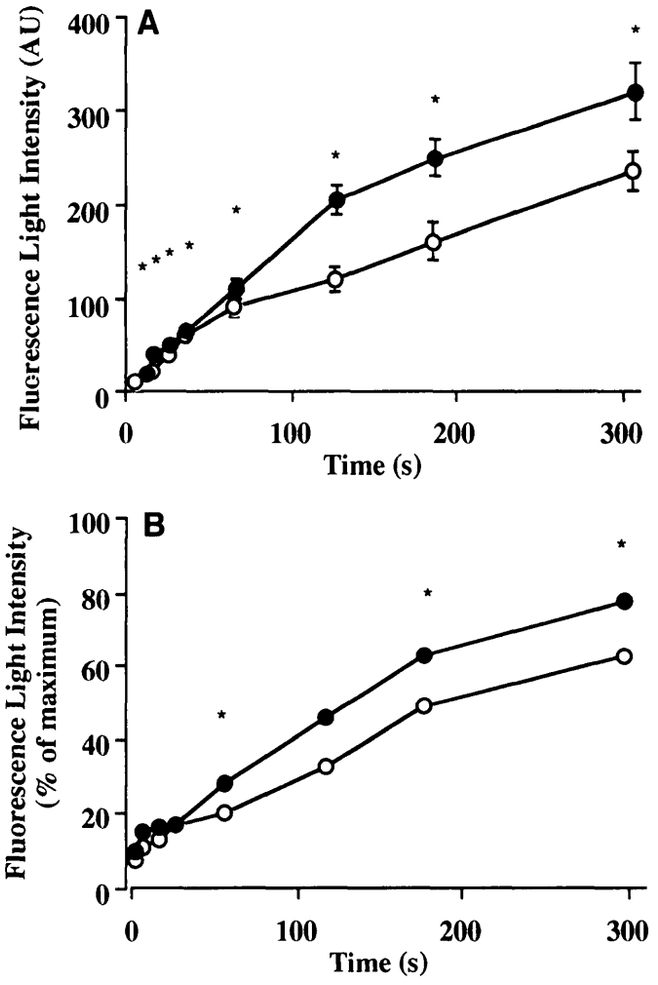

FIG. 1. Mean values of FLIs in AUs (*A*) and in percentage of the individual maximum (*B*) on day 5 of saline (○) and IGF-I (10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ s.c.; ●) in healthy subjects measured at different times after first appearance of intravenously injected sodium fluorescein. Data are means ± SE (Wilcoxon's rank-sum test; $n = 8$). *$P < 0.05$.

Transcapillary diffusion in skin. FLI, expressed in arbitrary units (AUs), is shown in Fig. 1. The FLI was already significantly higher ($P < 0.05$) 5 s after appearance of the dye during IGF-I treatment. For FLI given as percentage of the maximum intensity, the difference was significant at 60 s. During IGF-I, the FLI remained higher throughout the observation period, suggesting accumulation of dye in the subcutaneous interstitial tissue. Mean appearance time of NaF was 42.4 ± 1.0 s during saline and significantly ($P < 0.02$) shorter (32.0 ± 1.1 s) during IGF-I treatment.

Laser Doppler flux of skin microvasculature. Results are summarized in Table 1. Mean flux at rest during saline was 7.6 ± 0.7 PU and 10.7 ± 1.0 PU during IGF-I. Peak fluxes were 24.1 ± 1.7 PU (saline) and 32.2 ± 1.1 PU (IGF-I). Resting and peak flux tended to be higher during IGF-I treatment but a statistical significance was not reached. Mean time to peak flux was 15.2 ± 1.3 s (saline) and 13.9 ± 1.0 s (IGF-I, NS).

Vitreous fluorophotometry. Plasma PR was 20% and 23% higher on days 3 and 5, respectively, of IGF-I treatment as compared with control. The change in PR from day 3 to day 5 during IGF-I treatment was not significant. PR had returned to baseline 14 days after termination of IGF-I treatment (not shown). Subjects did not complain of impaired visual acuity at any time during the entire protocol.

TABLE 1
Laser Doppler fluxmetry at rest (LDF_{rest}) and peak (LDF_{peak}) of skin microcirculation in healthy subjects on day 5 of saline and rhIGF-I ($10\mu g \cdot kg^{-1} \cdot h^{-1}$ s.c.)

	Treatment		
	NaCl	IGF-I	*P*
LDF_{rest} (PU)	7.6 ± 0.7	10.7 ± 1.0	0.176
LDF_{peak} (PU)	24.1 ± 1.7	32.2 ± 1.1	1.0
Time to peak(s)	15.2 ± 1.3	13.9 ± 1.0	0.813

Data are means ± SE (Wilcoxon's rank-sum test; $n = 8$). For details, see METHODS.

None of the parameters of capillary permeability or microvascular blood flow correlated with levels of IGF-I or IGFBP-1 or -3 levels (not shown).

DISCUSSION

The present study demonstrates increased permeability of skin and retinal capillaries during a short-term (5 days) treatment with rhIGF-I. The FLI technique used to demonstrate this effect has successfully been applied for detection of increased capillary permeability in diabetic subjects (9–14).

The changes seen in our study may be attributable to different mechanisms. Specific receptors for IGF-I have been demonstrated on endothelial cells (15,16). In vitro studies have demonstrated specific receptor-mediated effects of IGF-I on endothelial cells (6,16). Thus, it may be likely that the effects of IGF-I seen in the present study may be due to direct effects on capillary endothelium. Another mechanism that may account for the changes seen in this study is the effect of IGF-I on enhanced microvascular blood flow resulting in an increased filtration rate of the applied dye. In the present study, a trend toward increased microvascular blood flow (although not significant) was demonstrable with the laser Doppler flux measurements of skin vessels (Table 1). A tendency toward enhanced microvascular blood flow is also supported by the significantly reduced appearance time of skin fluorescent dye after intravenous administration during IGF-I treatment. This parameter mainly reflects reduced arteriolar resistance. Increased forearm blood flow and reduced arterial resistance has been shown during acute

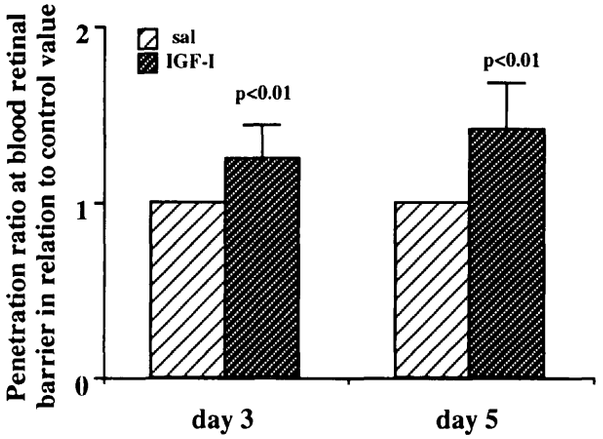

FIG. 2. Penetration ratio of sodium fluorescein at the blood-retinal barrier as assessed by fluorophotometry on days 3 and 5 of IGF-I (10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ s.c.) treatment. Values measured during IGF-I are given in relation to control values. Data are means ± SE (Wilcoxon's rank-sum test; $n = 7$).

(17,18) and short-term (19) IGF-I treatment in humans. Renal blood flow has also been shown to be elevated during IGF-I treatment (20,21). Lack of microalbuminuria in the present study indicates that renal glomeruli were unaffected by IGF-I treatment in the face of increased creatinine clearance rates.

Alterations in IGFBP levels may also have a potential role in the changes found in the present study. Indeed, IGFBP-1 and -3 have been associated with retinal microangiopathy (7). However, it remains uncertain by which mechanism IGFBPs may be responsible for the changes (22).

IGF-I has been suggested to play a role in diabetic micro- (7,23–25) and macroangiopathy (5). Elevated levels of IGF-I have been positively correlated to rapid progression of diabetic microangiopathy by some (23,25) but not other (26) researchers. Administration of IGF-I directly into rabbit eyes has previously been shown to stimulate angiogenesis (24). Moreover, IGF-I has recently been associated with the development of retinal neovascularization in nondiabetic as well as diabetic humans (7). Increased permeability of retinal (14) and skin (10) capillaries is considered to be an early sign of diabetic microvascular complications. Whether the changes in capillary permeability seen during the pharmacological administration of IGF-I, as in the present study, ultimately would lead to angiopathy as seen in diabetic subjects remains unknown.

Various side effects have been reported during IGF-I treatment in humans (i.e., syncopal attacks, pseudotumor cerebri, and Bell's palsy) (3,27,28). Some of these may be explained by increased capillary permeability and microvascular blood flow, which would preferentially occur only in certain tissues.

In summary, the present study demonstrates increased permeability in skin and retinal capillaries during a 5-day IGF-I treatment in normal subjects. These effects are probably due to both increased microcirculatory blood flow and increased capillary permeability and are possibly responsible for the mild subcutaneous edema seen during IGF-I treatment. Whether these changes would ultimately lead to changes similar to those of diabetic microangiopathy remains unknown.

Administration of IGF-I at lower doses than used in the present study may still be accompanied by the known beneficial metabolic effects (4) without the side effects of capillary permeability. Indeed, recent reports (29) and ongoing studies in our laboratory do support the contention that lower doses of IGF-I may be adequate for large-scale clinical applications.

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