Influence of aprotinin on insulin absorption and subcutaneous blood flow in Type 1 (insulin-dependent) diabetes

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Summary. Ten normal-weight Type 1 (insulin-dependent) diabetic patients (12 h postprandial) with normal insulin requirement were given ^{125}I -labelled soluble insulin (10 U) in the thigh together with aprotinin (10000 KIU) or its diluent on two consecutive mornings. Disappearance of ^{125}I -radioactivity was followed continuously for 3 h by external detection and plasma free insulin measured by radioimmunoassay. Subcutaneous blood flow following aprotinin or diluent was studied concomitantly in the contralateral thigh by external monitoring of locally injected ^{133}Xe . Plasma free insulin increased significantly faster ($p < 0.05$) and the insulin area under the

curve was significantly ($p < 0.05$) greater during the first hour after injection of insulin with aprotinin. Subcutaneous blood flow (rate constants for ^{133}Xe) was significantly higher with aprotinin ($p < 0.05$), the highest flow occurring early after injection. In conclusion, subcutaneously injected soluble insulin is more rapidly absorbed by addition of aprotinin to the insulin solution in Type 1 diabetes. Blood flow increase at the injection site may explain part of this effect.

Key words: Protease inhibitor, ^{133}Xe -clearance.

The protease inhibitor aprotinin has been shown to enhance insulin absorption from a subcutaneous injection site in healthy subjects when given as an additive to the insulin solution [1]. Inhibition of insulin degradation at the site of injection has been proposed as one possible mechanism behind this enhancing effect of aprotinin, but alteration in the local circulation is another suggested possibility [1]. In Type 2 (non-insulin-dependent) diabetic patients, on the other hand, no enhancing effect of the protease inhibitor on insulin absorption has been found [2]. The absorption kinetics of insulin following addition of aprotinin in Type 1 (insulin-dependent) diabetes have not yet been elucidated. The aim of the present study was therefore to investigate whether addition of aprotinin to the insulin solution enhances insulin absorption in Type 1 diabetes. We also wanted to study whether such an enhancing effect of aprotinin, if present, is coupled with an increased blood flow at the injection site.

Subjects and methods

Subjects

Ten Type 1 diabetic patients gave informed consent to participate in the study, which was approved by the Ethics Committee of Huddinge Hospital. Table 1 gives detailed clinical data on each patient. All had a normal serum creatinine value, but two patients (nos. 1 and 6) had proteinuria.

The last subcutaneous injection of insulin (soluble) was given 24 h before the study. The patients were admitted to the hospital 18 h before the study and received insulin intravenously. The infusion rate was adjusted to achieve a plasma glucose level of approximately 12 mmol/l by the following morning.

The subjects came to the laboratory at 0800 hours on two consecutive days, each after an overnight fast. To block thyroid uptake of ^{125}I iodide, potassium iodide solution (100 mg) was given orally 2 h before the study on the first experimental day, and 30 mg on each of the 4 consecutive days.

A cannula was inserted percutaneously into an antecubital vein and blood was collected for determination of basal plasma glucose and insulin. Light weight scintillation detectors (12.5 × 12.5 mm) (Leab, Mölnlycke, Sweden) were attached to the skin of each thigh, near the places to be injected, after which 0.25 ml (10 U; 20 kBq) ^{125}I -labelled soluble insulin (^{125}I -Actrapid, Novo Industri A/S, Copenhagen, Denmark) was injected subcutaneously on one side together with 0.5 ml aprotinin (10000 KIU, Novo Industrie GmbH Pharmaceutika, Mainz, FRG) or its diluent (thiomersal 20 µg/ml in saline, Pharmaceutical Department, Huddinge Hospital) in randomized order. The osmolality of the two additives was 294 and 296 mosmol for aprotinin and diluent solution, respectively. In the contralateral thigh the same additive as in the insulin solution was injected together with 0.25 ml (40 kBq) ^{133}Xe in saline (Byk Malinkrodt, Petten, Holland) to study the effect of aprotinin and diluent on the subcutaneous blood flow. The injections were performed over 2 min periods using an automatic injection device (Department of Biomedical Engineering, Huddinge Hospital). The needle (outer diameter 0.4 mm) was inserted at an angle of 45° with the skin to a depth of 5 mm beneath the skin surface. Registration of radioactivity started immediately after the first injection and was continued for 3 h with the patient supine. During the measurements the room temperature was $24 \pm 1^\circ\text{C}$. The two scintillation detectors were coupled to a four channel spectrometer (ND 600, Nuclear Data, Schaumburg, Illinois, USA) set to measure the photopeaks of ^{133}Xe and ^{125}I . Counts were accumulated during consecutive 60 s intervals and displayed on the screen of the spectrometer. Venous blood was sampled at 15-min intervals during the first hour and at 1.5, 2 and 3 h following injection and analyzed for plasma glucose and radioimmunoreactive free insulin.

Analytical procedures

Plasma glucose was analyzed by the glucose oxidase method on a glucose analyzer (Beckman Instruments, Fullerton, California, USA). Plasma free insulin was determined by radioimmunoassay [3] after immediate pretreatment of the plasma with polyethylene glycol [4]; all samples obtained from one and the same patient were analyzed in the same assay. The intra-assay method error was 3%.

Data analysis

Subcutaneous blood flow was followed by local clearance of ^{133}Xe [5]. For the calculation of blood flow in absolute units, the partition coef-

Table 1. Characteristics and clinical data on the diabetic patients studied

Patient no.	Sex	Age (years)	Duration of diabetes (years)	Body mass index (kg/m ²)	Plasma C-peptide (nmol/l)	Insulin dose		Eye examination
						U/day	U/kg ⁻¹ ·d ⁻¹	
1	M	45	24	19.5	<0.05	28	0.46	Proliferative retinopathy
2	M	38	11	24.2	<0.05	52	0.67	Background
3	M	23	3	22.6	<0.05	68	1.01	Normal
4	M	40	7	23.9	0.08	20	0.29	Normal
5	F	24	22	22.1	<0.05	56	0.89	Background
6	M	53	26	23.5	<0.05	62	0.97	Proliferative retinopathy
7	F	54	30	21.7	<0.05	32	0.60	Background
8	F	24	20	23.6	<0.05	32	0.56	Proliferative retinopathy
9	M	41	3	23.4	0.30	24	0.32	Normal
10	M	35	6	22.7	<0.05	44	0.61	Normal
Mean		38 ± 4	15 ± 3	22.7 ± 0.4		42 ± 5	0.64 ± 0.08	
± SEM

Fig. 1. Residual ¹²⁵I-radioactivity after subcutaneous injection of ¹²⁵I-labelled soluble porcine insulin (10 U) with aprotinin (●—●) or diluent (○—○). First order disappearance rate constants of ¹²⁵I during consecutive 15-min periods in the two groups. During the first 30 min the rate constants in the aprotinin group tended to be higher than those in the diluent group ($p=0.07$)

ficient for ¹³³Xe in the tissue is needed. Addition of aprotinin or diluent to the ¹³³Xe solution will unpredictably alter this coefficient. Therefore, only rate constants are given in this study as an index of the subcutaneous blood flow and conclusions have been drawn, not from absolute rate constants, but from within and between group comparisons. An Apple II microcomputer (Apple Computer Inc., Cupertino, California, USA) was used to calculate the fractional disappearance rates of ¹³³Xe and ¹²⁵I using linear regression analysis of the natural logarithms of the counts accumulated over consecutive 15-min intervals. The insulin area-under-curve was calculated by trapezoidal integration of the incremental values.

Statistical analysis

Significant differences between groups were determined by using the paired t-test. All data are presented as mean ± SEM.

Results

Absorption kinetics of ¹²⁵I-radioactivity

During the initial 15 min following injection the absorption rate of ¹²⁵I in the aprotinin group was higher than later after injection, when the rates stabilized (Fig. 1). In the diluent group, the rate constants tended to be lower than those in the aprotinin group during the first 30 min after injection ($p=0.07$), but the reverse pattern was seen later after injection. The time until 50% of the radioactivity was eliminated was, however, similar in the aprotinin and diluent groups, 143 ± 13 and 138 ± 16 min respectively.

Plasma immunoreactive free insulin and C-peptide

Plasma free insulin was significantly higher in the aprotinin compared to the diluent group during the first 45 min following injection after which the two curves were superimposable (Fig. 2, $p<0.05$). There was a significant difference between the areas under the curves above basal level in the aprotinin and diluent groups from time of injection up to 60 min following injection (Fig. 2; $p<0.05$). In the two patients with residual insulin production the plasma C-peptide levels were similar on the 2 study days and did not change after insulin injection.

Subcutaneous blood flow

Disappearance of ¹³³Xe from the depots of aprotinin and diluent is shown in Figure 3. The first order rate constants in the aprotinin group were significantly higher than those in the diluent group during the entire period of registration ($p<0.05$). This difference between the two groups was most pronounced during the first hour following injection.

There was a low insignificant correlation between the disappearance rate constants of ¹²⁵I and ¹³³Xe as well as between the plasma insulin areas and the rate constants of ¹³³Xe.

Fig. 2. Plasma immunoreactive free insulin after subcutaneous thigh injection of ^{125}I -labelled soluble porcine insulin (10 U) with aprotinin (●—●) or diluent (○—○). The values in the aprotinin group were significantly higher than those in the diluent group during 45 min following injection ($p < 0.05$). Area-under-curve above basal plasma immunoreactive free insulin during the time periods indicated in the figure in the aprotinin (hatched bars) and diluent (white bars) groups. During all four time periods there was a statistically larger area in the aprotinin group ($p < 0.05$).

Fig. 3. First order disappearance rate constants of ^{133}Xe during consecutive 15-min periods following subcutaneous injection with aprotinin (●—●) or diluent (○—○) in the contralateral thigh. The rate constants in the aprotinin group were significantly higher than those in the diluent group during the entire period of registration ($p < 0.05$).

Plasma glucose

Mean basal plasma glucose measured 15.0 ± 0.9 mmol/l in the aprotinin group and 13.5 ± 1.3 mmol/l in the diluent group (NS). Following insulin injection the falls in plasma glucose were similar.

Discussion

Addition of the protease inhibitor aprotinin to the insulin solution induced a similar early enhancing effect on the absorption of insulin from its injection site in our group of Type 1 diabetic patients, as previously reported for healthy subjects [1]. Thus, we found a significantly earlier insulin entry into the circulation. The disappearance rate of the radiolabelled insulin from the injection site was also higher with aprotinin early after injection, although the difference was not statistically significant. The most likely explanation for this minor discrepancy between the two methods used to study insulin absorption is a statistical type 2 error. However, it cannot be entirely excluded that the discrepancy could

be due to the fact that the two methods are not fully comparable as a measure of insulin absorption, as has been discussed earlier [6].

The possibility that the early enhancement of insulin absorption by aprotinin could be due to effects on the circulation at the injection site was elucidated by injecting the flow indicator ^{133}Xe in the contralateral thigh together with aprotinin or diluent. There was in fact a significantly higher elimination rate of ^{133}Xe in the aprotinin group during the whole measurement. Since the elimination of an inert freely diffusible substance is influenced not only by the local blood flow but also by the physical solubility of the substance in tissue relative to blood, the so called partition coefficient [7, 8], a difference in this coefficient would theoretically cause a difference in the elimination rate between the groups. However, since the volume of the injectates was standardized and the osmolality of the additives was found to be similar in the present study, it is unlikely that the solubility for ^{133}Xe would differ significantly between the two experimental groups. Therefore, the faster elimination rate of ^{133}Xe with aprotinin is most probably due to a higher blood flow in this group.

By using a photoelectric measuring principle, hyperaemia in the tissues beneath the measuring probe following the injection of aprotinin has also recently been reported [9]. The photoelectric technique, however, does not allow discrimination between blood flow changes in the skin and the subcutaneous tissue. Therefore, the flow increase registered by Williams et al. [9] might well have been an effect of the injection trauma in the cutaneous tissues. The local ^{133}Xe clearance technique, on the other hand, reflects the circulation selectively within the depot area and, thus, shows that blood flow was indeed higher in this area when aprotinin was given with the insulin.

There was a greater difference in the elimination rates of ^{133}Xe between the two groups during the first hour after injection than later, indicating a more pronounced hyperaemia in the aprotinin group early after injection. This coincided in time with the enhanced insulin absorption in this group, which might imply a causal relationship between increased blood flow and

insulin absorption. However, no significant correlation was found between the rate constants of ^{125}I -radioactivity and ^{133}Xe nor between the plasma insulin areas and the rate constants of ^{133}Xe . Although some authors have found a correlation between subcutaneous blood flow and insulin absorption [10, 11] others [12] have found this to be true only under basal blood flow conditions, whereas during higher flow rates the influence of other factors became more important. The absence of such a correlation could have several explanations and does not exclude the possibility that an increase in blood flow may have contributed to the enhancement of insulin absorption by aprotinin.

It is obvious that the enhancing effect of aprotinin was not quantitatively important enough to influence plasma glucose significantly in our diabetic subjects. Berger et al. [1] found a significantly earlier onset of the hypoglycaemic action of insulin following aprotinin in their group of healthy volunteers. However, Type 1 diabetic patients are on the whole more insulin resistant than healthy subjects [13]. This may be especially true at the plasma glucose levels found in the present study at the beginning of the investigation. A recent study by Lunetta et al. [14] showed no effect on plasma glucose of aprotinin compared with diluent in Type 1 diabetes, which is in agreement with our results. However, that study did not report data on plasma insulin and, thus, an effect of aprotinin similar to that found in the present study may also have been present.

Deckert et al. found no effect of aprotinin on plasma insulin in Type 2 (non-insulin-dependent) diabetic patients given approximately 4 U soluble insulin [2]. The discrepancy between the results from the present study and that of Deckert et al. [2] might be due to the different insulin doses given. Lower doses are absorbed more rapidly [15] and it is possible that an enhancing effect of aprotinin might be more difficult to detect at a higher basal absorption rate. Conversely, it seems possible that patients requiring high doses of insulin would have a more pronounced effect on insulin absorption than patients using lower doses by the addition of an agent causing hyperaemia at the site of injection. High dose requiring patients also often receive more concentrated insulin solutions which per se are known to be absorbed more slowly [16]. In fact, it has been shown that Type 1 diabetic patients requiring very high doses of insulin by the subcutaneous route can reduce their insulin dose considerably by the addition of aprotinin to the insulin solution [17]. Inhibition of insulin degradation at the site of injection has been proposed as one possible mechanism behind the beneficial effect of aprotinin in these patients [17]. On the basis of the present results, however, it may be suggested that at least part of the effect of aprotinin in these patients requiring unusually high doses of insulin could be due to hyperaemia at the injection site.

In summary, the present study has shown an enhancing effect of the protease inhibitor aprotinin on the absorption of soluble porcine insulin from a subcutane-

ous injection site after injection in Type 1 diabetic subjects. Higher subcutaneous blood flow was also found following aprotinin and may explain part of this enhancing effect.

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