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Insulin Binding of Human and Porcine Monocomponent Insulin to Monocytes in Type 1 (Insulin-Dependent) Diabetic Patients and Control Subjects

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Summary. In the present study insulin binding properties of human semi-synthetic and porcine insulin were compared in Type 1 (insulin-dependent) diabetic patients treated from the onset of their disease either with human semi-synthetic insulin ($n=12$) or porcine insulin ($n=12$) and control subjects ($n=12$). In all three groups, insulin binding to circulating monocytes revealed no difference between human semi-synthetic and porcine insulin (specific insulin binding at tracer concentration: $5.7 \pm 0.5\%$ versus $5.4 \pm 0.5\%$ in control subjects, $5.5 \pm 0.4\%$ versus $5.4 \pm 0.4\%$ in Type 1 diabetic patients on porcine insulin, $5.3 \pm 0.4\%$ versus $5.6 \pm 0.4\%$ in Type 1 diabetic patients on human insulin). Treatment with human or porcine insulin did not have any significant influence on receptor binding properties of the two diabetic groups investigated. Absolute receptor number and affinity of both diabetic groups were within the range of healthy control subjects irrespective of treatment with human or porcine insulin.

Key words: Human insulin, insulin receptor binding, monocytes, porcine insulin, Type 1 diabetes.

It has been suggested that human insulin may offer advantages in insulin therapy of diabetes mellitus. Theoretical advantages of human insulin compared with porcine insulin may exist in terms of immunogenicity and/or biological potency [1, 2]. Furthermore, availability of biosynthetic human insulin may alleviate a possible shortage of animal pancreatic material. Biological differences in insulin preparations in different tissues could be caused by a change in binding to the cell surface receptor. Mutation of a single amino-acid in the insulin region thought to be involved in the binding of the receptor can cause marked impairment in binding to target tissues and subsequently in biological activity [3]. Bachmann et al. [4] reported that at lower insulin concentrations, binding of biosynthetic human insulin and purified human pancreatic insulin was significantly higher than that of porcine insulin. Other comparative binding studies of human and porcine insulin in a variety of human and animal tissues [5] revealed identical binding behaviour regarding affinity, association and dissociation kinetics and negative cooperativity.

In the present study, binding of semi-synthetic human insulin and porcine insulin to mononuclear leucocytes was compared among two groups of Type 1 (insulin-dependent) diabetic patients treated from the onset of their disease either with porcine or semi-synthetic human insulin and a group of control subjects.

Patients and Methods

Patients

Comparative insulin binding studies were performed in three groups of patients. Group 1 consisted of twelve healthy male adult volunteers (mean age: 26 years). All showed normal body weight and the hepatic and renal functions were within normal range. None took any drugs known to affect carbohydrate or insulin metabolism.

Insulin binding was compared in twelve Type 1 diabetic patients, who were treated from the onset of their disease with porcine insulin

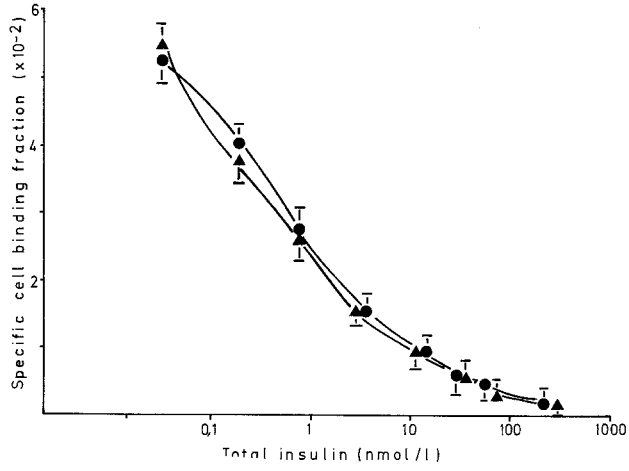

Fig. 1. Binding of human (●—●) and porcine (▲—▲) insulin in control subjects (mean ± SEM)

Table 1. Binding characteristics of human and porcine insulin to monocytes in control subjects, Type 1 diabetic patients treated with porcine insulin and Type 1 diabetic patients treated with human insulin

	Control subjects ($n=12$)		Porcine insulin-treated patients ($n=12$)		Human insulin-treated patients ($n=12$)	
	R_o	K_E ($M^{-1}/10^8$)	R_o	K_E ($M^{-1}/10^8$)	R_o	K_E ($M^{-1}/10^8$)
Human insulin	13100±2300	4.50±0.31	12900±2400	4.43±0.48	13900±2200	4.42±0.35
Porcine insulin	12700±2100	4.41±0.19	13300±2600	4.31±0.40	14100±2300	4.02±0.29

Results expressed as mean ± SEM. * R_o = number of binding sites per cell, K_E = the limiting high affinity state was determined using the model of negative cooperativity

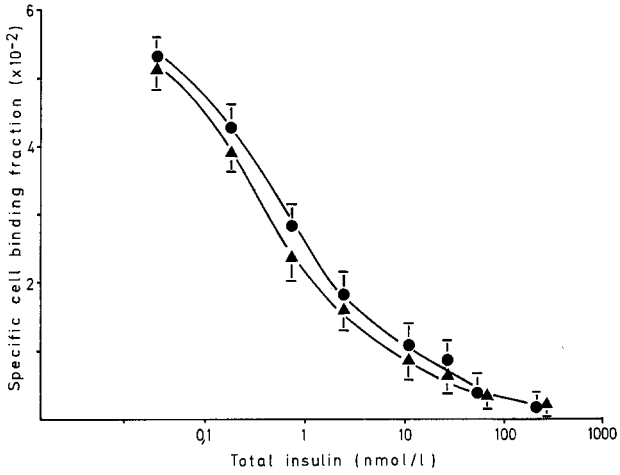

Fig. 2. Binding of human (●—●) and porcine (▲—▲) insulin in Type 1 diabetic patients treated with porcine insulin (mean ± SEM)

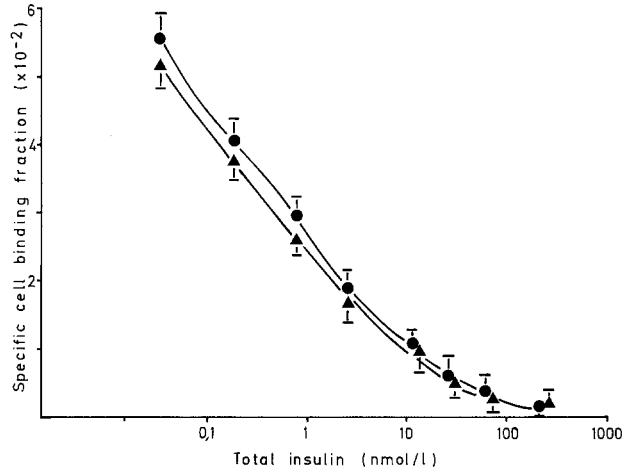

Fig. 3. Binding of human (●—●) and porcine (▲—▲) insulin in Type 1 diabetic patients treated with human insulin (mean ± SEM)

(group 2). All were within ideal body weight and their ages ranged from 17 to 36 years (mean 30±2 years). The duration of diabetes was from 2 to 11 years.

Group 3 consisted of 12 Type 1 diabetic patients. Their age ranged from 16 to 32 years (mean age 23±1 years). This patient group was treated with human insulin from the onset of their disease. The mean duration of diabetes in this group varied between 2–9 months. Body weight was within normal range.

At the time of investigation all the diabetic patients were in fairly good metabolic control (estimated by measurement of glycosylated haemoglobin: 8.9±0.9% in group 2 and 8.5±0.7% in group 3) and none presented with ketonuria. Both groups of diabetic patients fulfilled the classical criteria of insulin-dependent diabetes mellitus [6]. Endogenous insulin secretion, estimated by measurement of basal C-peptide levels, was not detectable in any group 2 patients. In the diabetic patients treated with human insulin, basal C-peptide levels were below 0.2 pmol/ml. The mean daily insulin requirement of the diabetic patients was 0.81±0.04 U/kg body weight in group 2 and 0.69±0.05 U/kg body weight in group 3.

Methods

Cell binding studies: Insulin binding studies were carried out in the morning following an overnight fast. The last insulin dose before the studies was given in the previous evening. Blood (120 ml) was drawn into tubes containing EDTA (dipotassium-salt). Mononuclear leucocytes were isolated by gradient centrifugation [7]. Insulin binding studies to mononuclear leucocytes were performed according to the method of Beck-Nielsen et al. [8]. The cells were washed twice and incubated in Hepes buffer (100 mmol/l, pH 7.8 at 15 °C) at a concentration of approximately 5×10^7 ml for 100 min with ^{125}I-human and ^{125}I-porcine insulin at a concentration of 34 pmol/l (0.2 ng/ml). The specific activity of the tracer was approximately 223 μCi/μg and 225 μCi/μg, respectively. Native semi-synthetic or native porcine insulin was added in increasing amounts to the incubation medium for the competition studies. Cell bound and free insulin were separated by centrifugation after the incubation period. The specific cell binding fraction was defined as total cell binding fraction minus non-specific cell binding, i.e. radioactivity which remained bound in the presence of an excess of native insulin (10 μmol/l). This fraction averaged approximately 8% of the total binding. Monocytes were identified by alpha-naphthyl-acetyl-esterase staining [9] and specific cell binding fraction was adjusted to a standard concentration of monocytes of 1.0×10^7 ml using the formula described by Beck-Nielsen et al. [8]. We could not observe any statistically significant difference between the numbers of monocytes in the two groups of the diabetic and control subjects.

Analysis of binding data: The results of the binding studies are presented as specific cell bound fraction, plotted as a function of total insulin concentration (competition curve). Binding data were further analyzed by the average affinity model of De Meyts and Roth [10].

Haemoglobin A_1 was estimated by microcolumn chromatography [11] and immunoreactive C-peptide by radioimmunoassay [12].

Statistical methods: For comparison of human and porcine insulin bindings paired Student t-test was considered appropriate. Comparative binding studies between the two groups of diabetic and control subjects were performed by the unpaired t-test.

Results

The healthy control subjects showed no statistically significant difference between the binding of human and porcine insulin at any insulin concentrations tested (Fig. 1). Absolute receptor number and average affinity did not differ significantly between human and porcine insulin either (Table 1).

The comparative binding data of human and porcine insulin in Type 1 diabetic patients treated from the onset of their disease with porcine insulin are summarized in Figure 2. In analogy to healthy control subjects, we found no significantly difference in insulin binding of human and porcine insulin to monocytes. There was also no difference between human and porcine insulin binding with respect to absolute receptor number or average affinity (Table 1).

In Type 1 diabetic patients treated from the onset of their disease with human semi-synthetic insulin (Fig. 3), we found no significant difference between human and porcine insulin with respect to insulin binding behaviour. None of the patients tested revealed any significant difference in insulin binding between porcine and human insulin to circulating monocytes. Average affinity and absolute receptor number were also identical between human and porcine insulin binding (Table 1).

Comparing the insulin binding properties of the Type 1 diabetic patients treated either with porcine or human insulin, we found no significant difference with respect to absolute receptor number and average affinity (Table 1). Furthermore, neither Type 1 diabetic patients treated with porcine nor patients with human insulin showed a significantly different binding behaviour compared with healthy subjects.

Discussion

In the present study insulin receptor behaviour was studied for the first time in newly diagnosed Type 1 diabetic patients treated from the onset of their disease with semi-synthetic human insulin. Insulin receptor number and insulin receptor affinity of these patients did not differ from that of control subjects, which is in accordance with previous reports of insulin binding studies in Type 1 diabetic patients with good metabolic control [13, 14]. Furthermore, by comparing the binding of semi-synthetic human insulin and porcine insulin to isolated mononuclear leucocytes, identical binding properties in terms of receptor number and receptor affinity were found in both groups of diabetic and control subjects. These data are in contrast to recent findings by Bachman et al. [4], who reported significant differences of insulin binding at lower insulin concentrations. Two factors should be taken into consideration with respect to the observed findings of Bachmann et al. [4]. Erythrocytes were used as targets in this study. Recent comparative studies of insulin binding to monocytes and erythrocytes indicate that insulin receptor data determined in the erythrocyte model might not be an exact reflection of the insulin receptor status [15–17].

The observed different binding of porcine und human insulin in the study of Bachmann et al. [4] could be due to the great difference in the specific radioactivity of the labelled insulins used.

We conclude that the binding behaviour of semi-synthetic human insulin and porcine insulin is completely identical. Furthermore, well-controlled Type 1 diabetic patients treated from the onset of their disease with semi-synthetic human insulin did not differ from those treated with porcine insulin with regard to their insulin receptor status. There seems to be no risk in changing from porcine to human insulin treatment considering peripheral insulin binding.

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