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The Clinical Significance of Highly Purified Pig-Insulin Preparations

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Summary. Side effects of insulin treatment (red spots at the injection site, lipoatrophy and insulin antibody formation) are primary related to the purity and the species of the insulin preparations used in treatment. To evaluate the clinical significance of these factors daily insulin requirements were registered in 1685 protamine insulin treated cases; red spots and lipoatrophy recorded in 500 NPH insulin treated patients and insulin antibodies determined in 173 patients. 51 of the patients were treated for more than 12 months with highly purified porcine insulin (proinsulin contamination <0.5 mmol/mol). 96 patients were treated for 1–4 years with porcine NPH insulin (proinsulin contamination 12–13 mmol/mol), and most of the remaining patients have been treated with mixed porcine-bovine insulin preparations in earlier

periods. It was shown that high purification of porcine NPH insulin is of importance in reducing insulin dose, lipoatrophy and insulin binding capacity of serum. Thus insulin antibodies could be demonstrated in only 3 of 51 patients after 1 ½ year of treatment with highly purified porcine NPH insulin. None of these patients developed red spots at the injection site and only one patient developed a small shallow depression of the subcutaneous tissue on one thigh. The daily insulin requirement was significantly lower when compared with the insulin requirement of a porcine NPH insulin treated control group.

Key words: Porcine insulin, insulin antibodies, lipoatrophy, skin reactions, insulin requirement.

Since insulin treatment started in 1922 several side effects have been described repeatedly. These are insulin allergy [23], lipodystrophy [25] and insulin antibody formation with or without the development of insulin resistance [15].

It is well known that these side effects primarily are related to the purity and the species specificity of the insulin preparations. Thus Jorpes [18] was able to demonstrate a significant reduction of local allergic reactions after recrystallization of insulin preparations and several authors were able to demonstrate reduced formation of insulin antibodies in patients treated with pure porcine insulin preparations compared to patients treated with insulin preparations containing beef insulin [3, 8, 12, 21, 30].

Since diabetic patients at the Steno Memorial Hospital have been treated with porcine insulin preparations of increasing purity for several years, we might be able to give some informations regarding the effect of treatment with pure porcine insulin preparation on insulin dose, skin reactions and insulin antibody formation.

Material and Methods

From 500, 506, and 615 consecutive records concerning insulin treated patients, covering the period from 1942–52, 1962–67 and 1971–73 respectively, the daily insulin requirements of the last day of hospitalization were registered. Only the records of patients which fulfilled the following condition were used:

age over 18 years
non-obese
normal blood urea and creatinine
non-pregnant
insulin treatment for more than one year.

The principle of dietetic treatment during these periods were identical, but during the period 1942–52 the fat content of the diet was higher than during the two following periods, whereas the opposite was the case with the carbohydrate content. All patients were out of bed and walked around. None of the patients received oral antidiabetic drugs. During the period 1942–52 the patients were treated with a mixture of porcine (85%) and beef (15%) protamine insulin Leo® (amorphous [16]), during the period 1962–67 the patients were treated with a mixture of porcine (85%) and bovine (15%) NPH Leo® (crystalline [20]), and during the period 1971–73 the patients were treated with porcine NPH Leo® insulin not contaminated with bovine insulin, since all Leo insulin preparations commercially available since 1968 were prepared from porcine insulin crystals, manufactured at a part of the factory separated physically from the part preparing bovine insulin. Furthermore daily insulin requirement and body weight was recorded in 1973 in 64 well controlled consecutive adult non-obese patients from the outpatient clinic. All patients had been treated with porcine insulin for 12 to 15 months. 31 patients were from the beginning of the treatment treated with commercial available porcine NPH Leo® insulin (proinsulin contamination 12–13 mmol/mol insulin). 33 patients were from the beginning of the treatment treated with a highly purified porcine NPH insulin, supplied

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to the patients by Nordisk Insulin Laboratorium. This insulin preparation was prepared from highly purified porcine insulin crystals. The purification of these crystals was achieved by multiple chromatographic procedures resulting in a reduction of the proinsulin content to less than 0.5 mmol/mol insulin. To eliminate variations in body weight in this rather small sample, insulin requirement in these population was expressed in units/kg bodyweight, also.

For registrations of skin reactions 500 consecutive patients from the out patient clinic were examined on both legs and other injection sites in august–september 1973. All patients were treated with porcine NPH insulin. Red spots more than 5 mm in diameter and lipoatrophic areas were registered. Even the slightest depression of subcutaneous tissue at the injection sites was recorded as lipoatrophy. The examination was made by 5 different doctors, 1–4 hrs after the last insulin injection was given.

Since we were unable to discriminate between lipoatrophic areas of long and short duration, the patients were divided in 4 groups:

- A. 341 patients who were treated with mixed porcine-bovine insulin before pure porcine insulin treatment started between 1968 and 69.
- B. 96 patients never treated with other insulin preparations than porcine insulin and treated for more than 1 year.
- C. 12 patients treated for less than 1 year with either porcine insulin or highly purified porcine insulin.
- D. 51 patients never treated with insulin preparations other than highly purified porcine insulin and treated for more than 1 year.

Determination of insulin antibodies were done on sera from three different insulin treated populations.

1. 98 patients treated for more than 6 months with mixed porcine (85%) beef (15%) NPH-Leo® insulin preparations. 97 of the patients have been treated for more than 12 months. The sera were sampled and analyzed during the period 1960–64.

2. 24 patients treated for more than 6 months with porcine NPH-Leo® insulin (porcine proinsulin contamination 12–13 mmol/mol insulin). 22 of the patients have been treated between 12 and 24 months. The sera were sampled during the period 1968–70. The patients had never been treated with other insulin preparations before.

3. 51 patients treated for 12–24 months with a highly purified porcine NPH-Insulin (porcine proinsulin contamination less than 0.5 mmol/mol insulin). The sera were sampled during the period 1971–73. The patients had never been treated with other insulin preparations before.

Insulin antibodies in sera from patients of group A were determined by immunoelectrophoresis and autoradiography as reported earlier [11]. No quantitation of the insulin binding capacity was possible by

this method, which determined the binding of labelled porcine insulin to immunoglobulins. Immunoglobulins from sera of non-insulin treated patients and from non-insulin treated non-diabetics do not bind labelled material [11].

Insulin antibodies in sera from patients of group B and C were determined by the method of Ortved Andersen [1]. In this method the insulin binding capacity of non-iodinated insulin is determined. The method is a double antibody technique based on the competition between guinea-pig anti-pig insulin antibodies, and human insulin antibodies to bind labelled porcine insulin. The determination is not influenced by free or antibody bound plasma insulin in the concentration usually found. The intra-assay coefficient of variation was 5–12%, the inter-assay coefficient of variation 8–28%, depending on high or low insulin binding capacity. The range of porcine insulin binding capacity in sera from non-insulin treated newly diagnosed diabetics was 0–32 μ units/ml, from well controlled diabetics treated for 14 days with insulin was 0–27.0 μ units/ml [1]. Porcine proinsulin was determined radioimmunologically by a double antibody technique [17] with purified porcine proinsulin as standard and tracer. The intra-assay coefficient of variation was 10.4%, the inter-assay coefficient of variation 20.2%. Comparison of two means were done by Students T-test.

Results

Fig. 1 gives the distribution curves of the daily insulin requirement of diabetic patients treated with insulin in 3 different periods. The figure demonstrates a shift of the distribution curve to the left from

Fig. 1. Distribution of daily insulin requirement of adult diabetic patients treated in different periods with different protamine insulin preparations. 1942–52: amorphous porcine (85%) bovine (15%) protamine insulin. 1962–67: crystalline porcine (85%) bovine (15%) NPH insulin. 1971–73: crystalline porcine NPH insulin

1942–52 to 1971–73. Whereas during the period 1942–52 (amorphous porcine-bovine protamine insulin) 18.2% of the patients were treated with less than 30 units/day and 9.0% with more than 60 units/day, during the period 1971–73 (crystalline porcine NPH insulin) only 4.8% received more than 60 units/day and 32.0% less than 30 units/day. Only 1 patient (in 1949) received more than 100 units/day. Insulin requirements in out-patients treated with highly purified porcine insulin were significantly lower than in-patients treated with commercially available porcine NPH insulin, whether calculated in international units or in international units/kg bodyweight (Table 1). Insulin resistance of immunoge-

ment. Red spots were never observed neither by the patient nor by the doctor.

Whereas 56 (16.4%) of the patients in group A and 5 (5.2%) of group B demonstrated lipoatrophic areas, only 1 out of 51 patients in group D had this lesion. In this patient we found a small shallow depression of the subcutaneous tissue on one thigh. 113 of the 500 patients were 20 years of age or below. Only 2 of these patients had lipoatrophic areas (1.8%). It was remarkable that nearly half of the patients with lipoatrophic areas (30 patients) had been treated with insulin for 25 years or more and therefore started insulin treatment with amorphous porcine-bovine protamine insulin.

Table 1. *Daily insulin requirement of two comparable groups of patients treated either with non-highly purified porcine NPH insulin or highly purified porcine NPH insulin*

Insulin	Number of patients	Duration of treatment (months)	Daily insulin requirement (mean $\pm$ SEM)	
			i. u.	i. u./kg bodyweight
Porcine-NPH-Leo®	31	13.4	32.9 $\pm$ 2.4 ^a	0.53 $\pm$ 0.04 ^b
Highly purified porcine-NPH	33	13.7	24.6 $\pm$ 2.0 ^a	0.38 $\pm$ 0.03 ^b

^a $p < 0.05$

^b $p < 0.01$

Table 2. *Cutaneous side effects of insulin treatment in 4 groups of patients. A. Patients treated with mixed porcine-bovine insulin preparations before conversion to porcine NPH insulin. B. Patients treated with porcine NPH insulin. C. Patients treated with porcine NPH insulin for less than 1 year. D. Patients treated with highly purified porcine NPH insulin*

Treatment	Number of patients	Duration of treatment (years $\pm$ SEM)	Cases with red spots	Cases with lipoatrophy	Duration of treatment in cases with lipoatrophy (years $\pm$ SEM)
Porcine NPH-Leo®					
group A	341	16.1 $\pm$ 0.5	5	56 (16.4%)	21.1 $\pm$ 1.5
B	96	2.5 $\pm$ 0.1	1	5 (5.2%)	2.3 $\pm$ 0.5
C	12	0.5 $\pm$ 0.06	1	0 (—)	—
Highly purified porcine NPH					
group D	51	1.5 $\pm$ 0.05	0	1 (2%)	2.0

nic origin has not been seen at the Steno Memorial Hospital since 1950 (more than 2000 insulin treated patients a year).

The results of the examination of the injection sites are summarized on Table 2. Only 7 out of 500 patients showed red spots of more than 5 mm in diameter. In 2 cases the duration of insulin treatment had been one year or less, in the other cases insulin was given for more than 5 years. None of the patients were aware of the spots nor disturbed by them, and none of the spots were warm or tender. In 5 cases the spots disappeared spontaneously but in 2 cases not until the injection technique was corrected. The 51 patients of group D who were treated with highly purified porcine NPH insulin were examined on several occasions from the beginning of insulin treat-

ment. Table 3 shows the number of patients in 3 different groups demonstrating insulin-antibodies. Since 98% of patients had demonstrable insulin antibodies against porcine insulin in group 1 (porcine-bovine NPH insulin), only 6% had demonstrable antibodies in group 3 (highly purified porcine NPH insulin). In group 2 (porcine NPH insulin) the figure was intermediate. The insulin binding capacity after treatment of the patients in group 3 with highly purified porcine NPH insulin was not significantly different from the insulin binding capacity of sera before the beginning of treatment. Table 4 gives the results of antibody determination of group 3 in more detail. It is seen that some patients do have a significantly raised insulin binding capacity after 6–9 months of treatment but thereafter returned to a normal value. The

insulin binding capacity of the 3 patients who were found to have insulin antibodies after 1 year of treatment are given in brackets in Table 4.

Discussion

The study has shown, that daily insulin requirement was lower in patients treated with crystalline NPH-insulin compared to patients treated with amorphous protamine insulin. Furthermore daily insulin requirement in patients treated with highly purified porcine NPH-insulin was lower than in patients treated with porcine NPH-insulin of the

when the injection technique was corrected. Local allergic reaction after injections of insulin appear usually during the first weeks of treatment. The absence of red spots months or years after the onset of treatment does therefore not exclude a transient skin allergy during the first few months of insulin treatment in group A and B, since only group C and D were observed during the early phase of insulin treatment. Renold *et al.* found a frequency of lipoatrophy in insulin treated diabetics of 24.2%; 44.4% in patients below the age of 20, and 14.9% in patients above the age of 20 [25]. In our material only 1.8% of the patients ≤ 20 years of age had lipoatrophic areas,

Table 3. Frequency of insulin antibodies in patients treated with different insulin preparations. Insulin binding capacity was measured by the method of Orved Andersen [1]. Upper control limit 27 μ units/ml. The logarithm of the values of group 2 are normally distributed. The standard deviation of the insulin binding capacity of group 3 is not given, since the values were neither normally nor logarithmically distributed

Group	Number of patients	Treatment	Insulin antibodies detectable		Insulin binding capacity (μ -units/ml)	
			Number of patients	%	mean (m)	range of $m \pm s$
1	98	Porcine/bovine NPH-insulin Leo®	96	98%	—	—
2	24	Porcine NPH-insulin Leo®	18	75%	89	27—289
3	51	High purified porcine NPH insulin	3	6%	18	—

Table 4. Frequency of insulin antibodies and insulin binding capacity in 51 patients before and after treatment with highly purified porcine NPH insulin. Upper control limit 27 μ units/ml. The figures in brackets (3rd line, last column) represent the insulin binding capacity of the 3 patients who had detectable insulin antibodies

Duration of treatment (months)	0	2—4	5—7	8—10	11—27 (mean: 17.6)
Number of patients investigated	39	39	43	38	51
Mean insulin binding capacity (μ units/ml)	5.1	11.2	15.8	17.9	(61) 17.6 (445) (60)
Number of patients with insulin binding capacity > 27 μ U/ml	0	1	5	5	3

usual degree of purity. Thus the degree of purification of the insulin preparations seems to be of significance for the daily insulin requirement. It is well known that insulin preparations of high purity are less immunogenic than insulin preparations of low purity [9, 26, 30]. Furthermore the positive correlation between insulin antibody titer and insulin requirement is well known [2].

The frequency of local allergic reactions at insulin injection sites is quite variable, in Joslin's book figures from 1.0 to 55.8% are reported [23]. In most of the patients who demonstrated red spots at the injection site, we felt that the spots were the consequence of a bad injection technique. The spots were neither warm nor tender and disappeared in a few days

whereas the frequency of lipoatrophy was 12.0% in patients above the age of 20. The explanation for the low frequency of lipoatrophy in our youngsters and the relatively high frequency in patients above 20 years of age is probably the higher purity of modern insulin preparations. In accordance with this, lipoatrophy in insulin treated patients could be reduced by treatment with highly purified insulin preparations [19]. It is unlikely that the duration of treatment itself should be the explanation of the varying frequency of lipoatrophy in the group A—D, since lipoatrophy in susceptible patients is described to develop during the first years of insulin treatment [29].

Insulin crystals are contaminated by different substances as shown by Deckert [11] and others [9, 27].

One of the most important impurities is proinsulin detected by Steiner [28]. High purification of insulin results in insulin preparation of low immunogenicity as shown by Deckert [11] and Schlichtkrull *et al.* [27]. Patients treated with long acting insulin preparations, prepared from highly purified porcine insulin, developed insulin antibodies significantly less than patients treated with non-highly purified insulin preparations [6, 10, 14, 19, 26, 30]. Thus Andreani and coworkers demonstrated insulin antibody formation in only 2 of 7 patients treated with MC-Lente insulin for one year or more [6].

Korp & Levett were unable to demonstrate insulin antibodies in 4 of 22 patients treated with monocomponent porcine insulin for one year. In another 8 patients the insulin binding capacity was very low [19]. Similar results were found by Wegmüller & Fankhauser [30], Fankhauser & Michl [14] and by Schlichtkrull *et al.* [26].

Only 3 of our 51 patients treated with highly purified porcine insulin had demonstrable insulin antibodies after 1–2 years of treatment. This low figure is not due to a low sensitivity of the method used for insulin antibody determination since the sensitivity of the method of Ortvad Andersen was found to be comparable with the method of Hein-Christiansen and Skom & Talmage [4].

The results seem to indicate, that long acting porcine insulin preparations can be considered of low immunogenicity in man if the content of proinsulin and other impurities in the insulin crystals is reduced by a considerable degree. Furthermore the clinical results indicate, that treatment of diabetics with highly purified porcine NPH-insulin results in an appreciable reduction of insulin dose and of side effects such as red spots and lipoatrophy.

The question as to whether insulin requirement will fall when insulin treatment is changed from conventional porcine insulin to highly purified porcine insulin could not be answered by our investigation. However, marked reductions in insulin binding capacity and insulin requirements were seen when patients treated with bovine insulin were changed to treatment with highly purified porcine insulin [6, 7]. It is not clear whether or not treatment with highly purified porcine insulin leads to benefits other than a reduction in insulin requirement, insulin allergy, insulin resistance and lipoatrophy. Ortvad Andersen has shown, that the quality of metabolic regulation in younger diabetics is significantly correlated to the insulin binding capacity of serum [2]. He also was able to demonstrate that patients not producing insulin antibodies had a remission period of longer duration than a comparable group of patients who did develop insulin antibodies [5]. Dixon *et al.* [13] however found labile metabolic state in patients with low insulin binding capacity and stable metabolic state in patients with high insulin binding capacity.

It is not known whether or not circulating soluble

insulin-antibody complexes are of significance for the development of late diabetic manifestations. It has however to be mentioned that as well labelled insulin [24] as immunoglobulins [22] have been demonstrated in vessel walls of insulin treated diabetics. Furthermore Wehner *et al.* [31] have found basement membrane alterations in the capillaries of glomeruli from mice treated with immunogenic insulin preparations, whereas no alterations could be found in mice treated with low immunogenic insulin preparations. Most of the insulin preparations in the world are however prepared from bovine insulin. It is hoped that bovine insulin in the near future can be purified to such an extent that not only skin reactions but also insulin antibody formation can be eliminated.

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