Proinsulin and A-Component Antibodies in Diabetics after Long-Term Monocomponent Insulin Treatment

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Summary. In newly diagnosed diabetics treated with Monotard® (porcine monocomponent (MC) Lente insulin) for five years, no antibodies against porcine or bovine proinsulin were observed, but 2 of 13 subjects developed a-component antibodies. In newly diagnosed diabetics, treatment for the same period with conventional Lente insulin induced both proinsulin and a-component antibodies (in 5 and 8 of 10 cases, respectively). In 31 patients transferred from conventional Lente to Monotard, proinsulin and a-component antibody levels were significantly lower than in 22 patients maintained on conventional Lente after the 5-year follow-up period. No significant differences were noted between bovine and porcine proinsulin antibodies. Insulin antibody production was similar to that of proinsulin antibody.

Key words: Proinsulin antibody, anti a-component antibody, insulin antibody, monocomponent insulin, Monotard (MC-Lente).

The immunogenicity of insulin preparations cannot be completely assessed by determination of insulin binding IgG alone, as patients treated with conventional (recrystallized) insulin develop specific antibodies against bovine and porcine proinsulin [1], and a-component [2], as well as antibodies against insulin [3]. This is due to the presence of non-insulin-like immunoreactive determinants in the contaminating protein substances e.g. proinsulin. Thus immunisation of rabbits with a- and b-component is known to produce specific antibodies against these components, in addition to insulin antibodies [3]. Residual amounts of impurities may account for the occurrence of specific proinsulin and insulin antibodies

during treatment with Single Peak (SP) (chromatographed) insulin [4] and, to a lesser and generally transient extent, with the monocomponent (MC) (twice chromatographed) insulin preparations used hitherto [5, 6, 7, 8, 9].

The purpose of this paper was to examine the specific antibody production against porcine/bovine proinsulin and porcine a-component in diabetics after a five-year period of treatment with Monotard® (porcine MC Lente) in comparison with diabetics treated for the same period with bovine/porcine conventional Lente.

Materials and Methods

Patients

From 1970 through 1975, 76 insulin-dependent subjects with juvenile diabetes were studied, subdivided into the following groups:

Group 1. 13 newly diagnosed diabetics, 5 men and 8 women, aged 7–42 years, started during 1970 on treatment with 16–48 IU Monotard daily.

Group 2. 10 newly diagnosed diabetics, 5 men and 5 women, aged 9–43 years, started during 1970 on treatment with 16–48 IU conventional Lente daily.

Group 3. 31 long-term diabetics, 12 men and 19 women, aged 7–47 years, previously treated with conventional insulin for 5–11 years and transferred during 1970 to Monotard (24–52 IU daily). Patients were selected on the basis of clinical conditions such as high insulin requirement (14 cases), unstable diabetes (3 cases), insulin allergy (3 cases), insulin lipodystrophy (4 cases), retinal and/or renal microangiopathy (7 cases).

After initial hospitalisation the patients' clinical control was checked at two-monthly intervals and IgG insulin binding was determined according to Christiansen's radioimmuno-electrophoretic technique [14]. At the end of 1975 sera from the patients were assayed for IgG against porcine and bovine proinsulin (PIB) and for IgG against porcine a-component (AB), as well as for IgG against bovine insulin (IB) (detection limit: 0.10 mU/ml; ref. 7 and 8).

Prior to the determinations of proinsulin and a-component binding to IgG, all insulin binding IgG was removed by adding a suspension of MC-bovine insulin covalently bound to Sepharose and checking the supernatant for absence of insulin antibodies [13]. The determination of porcine and bovine proinsulin as well as a-component binding to IgG was performed using the radioimmuno-electrophoretic separation method described by Christiansen [14] in agarose gel containing H-chain specific anti-human IgG from rabbits.

In 10 normal sera the mean non-specific binding was 0.65 ng/ml (1 SD = 0.28 ng/ml). The results were corrected for this non-specific binding and only values > 1 ng/ml (~ 3 SD) were considered positive.

The concentration of I-125 a-component used was 1250 ng/ml and in 23 normal sera the mean nonspecific binding was 32 ng/ml (1 SD = 7 ng/ml). The results were corrected for the nonspecific binding in normal sera and only values > 25 ng/ml (~ 3 SD) were considered positive.

This method does not allow measurement of a-component antibody concentration but only indicates the presence of a-component antibodies relative to a detection limit [13]. Further data on reproducibility and precision of proinsulin and a-component assays are reported in (2) and (13).

The significance of the differences between the values of proinsulin binding (PIB) and a-component binding (AB), as well as for insulin binding (IB), in group 1 and 2, respectively, group 3 and 4, as well as between porcine and bovine proinsulin binding in groups 2, 3, 4 was analysed by Wilcoxon's rank sum test.

Proinsulin Antibodies

In group 2, treated for five years with porcine/bovine conventional Lente, the mean porcine PIB value was 4.1 ng/ml, range 0.1–12.0 ng/ml; the mean bovine PIB value was 7.5 ng/ml, range 0.2–49.7 ng/ml.

Fig. 2. Pork proinsulin binding in 31 long-term insulin treated diabetics after 5 years of transfer to MC Lente (Monotard), compared with 21 long-term insulin treated diabetics maintained on conventional Lente for the same period

Fig. 3. Ox proinsulin binding in 31 long-term insulin treated diabetics after 5 years of transfer to MC Lente (Monotard), compared with 22 long-term insulin treated diabetics maintained on conventional Lente for the same period

The difference between group 2 and 1 is highly significant for porcine PIB ($p < 0.001$).

Figures 2 and 3 show that, in the majority of patients with long-standing insulin treatment, porcine and bovine PIB was below or just around the detection limit in group 3, five years after transfer to Monotard treatment. The mean porcine PIB value was 1.10 ng/ml, range 0–11.4 ng/ml; the mean bovine PIB value was 1.00 ng/ml, range 0–10.3 ng/ml. However, the distribution of the values was very skewed.

In group 4, continued on conventional Lente for the same period of observation, the mean porcine PIB value was 11.8 ng/ml, range 0–66.5 ng/ml; the mean bovine PIB value was 6.4 ng/ml, range 0–51.2 ng/ml.

The difference between group 4 and 3 is statistically significant for both porcine PIB ($p < 0.001$) and bovine PIB ($p < 0.002$).

There was no statistically significant difference between porcine PIB and bovine PIB in patients of group 2 and 4 treated with conventional Lente (bovine/porcine).

A-Component Antibodies

Figure 4 shows that 2 out of 13 patients of group 1, treated from the beginning with Monotard produced a-component antibodies (AB), definitely above the detection limit. Three further cases had values just below the limit considered as positive. The case presenting with the highest AB value (69 ng/ml) was a young woman with severe pollen allergy. The mean AB value was 10.9 ng/ml, range 0–69 ng/ml.

All patients of group 2, treated with conventional Lente, developed a-component antibodies, except for two cases with AB values of 0.0 ng/ml. The mean AB value was 66.9 ng/ml, range 0–148 ng/ml.

The difference in porcine a-component binding between group 2 and 1 was statistically significant ($p < 0.01$).

Figure 5 shows that in 19 out of 31 patients transferred to Monotard (group 3) the AB values were below the limit of significance. The mean value was 25.4 ng/ml, range 0–98 ng/ml.

In patients of group 4 maintained on conventional Lente the mean AB value was 95.2 ng/ml,

Fig. 4. Pork a-component binding in 13 newly diagnosed diabetics after 5 years of treatment with MC Lente (Monotard), compared with 10 newly diagnosed diabetics treated with conventional Lente for the same period. The dotted line represents the limit of significance

Fig. 5. Pork a-component binding in 31 long-term insulin treated diabetics after 5 years of transfer to MC Lente (Monotard), compared with 21 long-term insulin treated diabetics maintained on conventional Lente for the same period

range 0–185 ng/ml. In 6 out of 22 cases a-component antibody was not detectable.

The difference in porcine a-component binding between group 4 and 3 was significant ($p < 0.001$).

Insulin Antibodies

At the end of the five year observation period, the mean IB values in patients considered in the study were as follows:

Group 1: 0.10 mU/ml, range 0–0.33 mU/ml.

Group 2: 0.80 mU/ml, range 0.10–3.25 mU/ml

Group 3: 0.40 mU/ml, range 0.05–2.25 mU/ml

Group 4: 1.25 mU/ml, range 0.10–3.90 mU/ml

The differences between group 2 and 1, and between group 4 and 3 were significant ($p < 0.001$).

No correlations could be established between immunological results and clinical conditions, such as vascular complications.

Discussion

A five-year period of treatment with Monotard did not induce proinsulin binding antibodies in diabetics never previously treated with insulin in contrast to

patients treated exclusively with bovine/porcine Lente of conventional purity for the same length of time.

These results are similar to those published by Schlichtkrull et al. [3]; however, these patients were only followed for a treatment period of 8 months.

Transfer to treatment with Monotard apparently reduced IgG proinsulin antibody formation significantly after five years' treatment, in comparison to that seen in long-term diabetics treated with conventional insulin (Lente or Rapiard) for the same period.

Similarly, patients treated with Monotard did not produce significant titres of IgG insulin antibodies, in contrast to patients treated with conventional Lente; and patients changed from conventional to MC treatment showed significantly lower values of insulin binding antibody than patients maintained on conventional treatment.

Formation of a-component antibody was found in patients treated during the last five years with monocomponent insulin only. This finding was reported by Yue and Turtle [11], in contrast with other previous observations [12]. Since other explanations, such as errors in storage and administration of insulin can be

excluded, the reason may be that the first batches of Monotard did contain minor amounts of a-component which then induced the formation of a-component antibody. It appears that the purity of the MC-preparations used until 1974, as judged by their content of proinsulin like immunoreactivity (PLI) (300 ppm) was not sufficient to avoid AB in a few patients. Whether such an antibody production is possible with the improved quality of MC-insulin containing less than 1 ppm of PLI remains to be seen [15].

Even if these results differ from those of Yue and Turtle [11] in relation to proinsulin antibody production, they seem to indicate the role possibly played by large molecular weight contaminants in the immunogenicity of MC insulin preparations.

Non-reactivity of proinsulin antibodies with insulin makes it unlikely that these antibodies interfere with the insulin requirement [10]. In contrast, the antibodies against a-component have been shown to cross-react with human a-component [2], but the significance of this to the patient is unknown.

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