Plasma Insulin and Glucose Levels in Maturity Onset Diabetics Treated with Chlorpropamide

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Summary. 19 diabetic patients taking various doses of chlorpropamide were studied throughout a normal day. Plasma chlorpropamide levels were related to the daily dose of the drug and were relatively constant throughout the day. Blood glucose concentration was highest and the plasma insulin concentration lowest in patients taking the largest dose of chlorpropamide. Patients on a small daily dose of chlorpropamide were well controlled and had higher than normal plasma insulin levels. — 12 patients were restudied 10 days after stopping chlorpropamide. Although diabetic control deteriorated in all patients, plasma insulin concentration did not change significantly. This suggests that the long-term hypoglycaemic action of chlorpropamide is not dependent on an effect on the concentration of insulin in peripheral venous blood.

Key words: Diabetes mellitus, maturity onset, chlorpropamide, plasma insulin, plasma chlorpropamide.

The sulphonylurea drugs have been shown to increase the secretion of insulin by the pancreas, both in vivo and in vitro and they are without effect in patients who have no endogenous secretion of insulin [1]. It has, therefore, been generally accepted that their hypoglycaemic action in patients with maturity-onset diabetes is due to stimulation of insulin secretion from the pancreatic B cells. A reliable method for measuring insulin was not available when sulphonylureas were first introduced and none of the early studies contained information about the plasma insulin concentration in relation to plasma concentrations of the drug and of glucose [2].

More recently, the plasma immuno-reactive in-

sulin (IRI) has been measured in response to sulphonylureas in various test situations in vivo [3, 4, 5, 6] and in vitro [7, 8]. These studies do not support the view that the long-term hypoglycaemic action of the sulphonylureas is due to a B-cell cytotoxic action, but suggest that it is extra-pancreatic. There is however, little information about the plasma concentrations of IRI in the course of a normal day in patients on long-term treatment with these drugs.

In this study, the plasma concentrations of chlorpropamide, glucose and IRI have been measured at intervals throughout a 24 hour period in diabetic patients taking chlorpropamide and 10 days after stopping the drug.

Patients and Plan of Study

19 diabetic patients being treated with various doses of chlorpropamide (given as a single daily dose before breakfast) were studied. Relevant clinical data relating to these patients are shown in Table 1. None were obese and all were taking a carbohy-

Table 1. Clinical characteristics

<i>Number of patients:</i>			
Male			10
Female			9
Taking 500 mg	Chlorpropamide daily		5 (Group A)
Taking 375–250 mg	Chlorpropamide daily		5 (Group B)
Taking 200 mg	Chlorpropamide daily		4 (Group C)
Taking 125–100 mg	Chlorpropamide daily		5 (Group D)
<i>Mean age:</i>			
69 years (range 54–86)			
<i>Mean duration of treatment with chlorpropamide:</i>			
3 years (range 2 weeks–8 years)			
<i>Mean weight as % of standard weight:</i>			
91 (range 79–103)			

Table 2. Mean plasma chlorpropamide levels in 4 groups of patients (± 1 SEM) $\mu\text{g/ml}$

Group	Fasting	Mid morning	Before lunch	Mid afternoon	Before supper	After supper	Bedtime	Fasting
A	161.3 ± 11.0	190.7 ± 21.3	194.9 ± 15.6	185.8 ± 16.8	183.1 ± 15.4	198.6 ± 7.0	192.0 ± 8.8	172.5 ± 13.1
B	115.2 ± 10.0	148.2 ± 16.2	157.4 ± 10.7	150.1 ± 6.7	144.6 ± 8.0	150.7 ± 4.0	135.9 ± 9.5	115.8 ± 8.3
C	56.3 ± 9.7	64.8 ± 4.1	62.4 ± 8.8	68.0 ± 10.2	65.5 ± 11.3	61.3 ± 7.1	55.5 ± 6.5	48.4 ± 4.9
D	50.3 ± 14.4	62.5 ± 13.2	64.5 ± 12.7	60.9 ± 12.4	61.3 ± 13.2	57.8 ± 11.7	62.7 ± 13.8	51.5 ± 11.6

Table 3. Mean (± 1 SEM) blood glucose and plasma insulin levels in patients in group A while on chlorpropamide

	Fasting	Mid morning	Before lunch	Mid afternoon	Before supper	After supper	Bedtime	Fasting
Blood glucose (mg/100 ml)	161.2 ± 30.8	237.0 ± 30.8	223.0 ± 39.6	246.2 ± 43.4	226.8 ± 47.9	230.6 ± 44.9	222.0 ± 45.6	173.0 ± 30.0
Plasma insulin ($\mu\text{U/ml}$)	11.3 ± 0.8	15.1 ± 2.2	15.8 ± 2.7	18.5 ± 2.8	17.0 ± 2.3	15.5 ± 0.84	21.6 ± 2.1	4.8 ± 1.0

drate-restricted, weight-maintaining diet; they had no evidence of renal disease, and chlorpropamide was the only hypoglycaemic agent they were taking. Most patients studied had been treated with chlorpropamide for several years. In all cases treatment with chlorpropamide was started after an initial period of treatment with diet alone had failed to achieve adequate control. Patients were selected for study at random from those considered adequately controlled at regular clinic visits.

For the purpose of the study, they were admitted to a metabolic unit for 36 hours. An attempt was made to simulate a normal day in terms of both diet and activity. Venous blood was taken on 8 occasions in a 24 hour period and analysed for glucose, IRI and chlorpropamide. The sampling times were: resting and fasting; 1½ hours after breakfast; before lunch; 1½ hours after lunch; before supper; 1½ hours after supper; bedtime; and resting and fasting the following morning.

12 patients were studied twice: 4 each from groups B, C, and D. On the second occasion chlorpropamide was stopped 10 days before study: otherwise the procedure was identical. It was not considered ethical to ask patients in group A to discontinue treatment with chlorpropamide.

Laboratory Methods

Glucose (mg/100 ml) was measured in whole blood by an automated glucose-oxidase technique based

on the method described by Hill and Kessler [9]. The coefficient of variation was 0.78%.

Immunoreactive insulin ($\mu\text{U/ml}$) was measured in plasma by a double-antibody technique [10]. The coefficient of variation between assays was 12.6%.

Chlorpropamide ($\mu\text{g/ml}$) was measured in plasma by an adaptation of the method of Prescott and Redman [11]. A PYE 64 model Gas Chromatograph was used with flame ionisation detectors. 0.9 M glass columns were packed with 3.8% UCW 98 on Supelcoport (Chromatography Services Company), conditioned at 290° C for 24 hours. The column oven heat was 190° C, the injection heat 240° C and the detectors were at 350° C. The nitrogen flow rate was 40 ml/min; hydrogen flow 40 ml/min and airflow 900 ml/min.

Tolbutamide was used as an internal standard and, like chlorpropamide, was extracted in toluene and then methylated. The extract was redissolved in chloroform and 1.0 μl injected. The retention time for chlorpropamide was 1.4 minutes and for tolbutamide 1.9 minutes. Peak height ratios were used for calculation. The coefficient of variation between assays was 11.0%.

Results

For the purpose of analysis, patients have been grouped on the basis of their daily dose of chlorpropamide.

Table 2 shows the mean plasma chlorpropamide level at 8 sampling times for the 4 groups of patients in the first study. In general, the plasma levels rose as the daily dose increased, although there was no significant difference between the mean plasma chlorpropamide concentration of patients in groups C and D. Within each dosage group there was very little variation throughout the 24 hours and these levels are comparable with those previously reported.

Table 3 shows the blood glucose and plasma insulin levels in patients in group A when taking chlorpropamide. Corresponding values for groups B, C, and D are shown in Figures 1 and 2. Patients in groups D and C were well-controlled: in both groups the mean fasting blood glucose concentration was 103 ± 13.0 mg/100 ml and the peak values were 153 ± 11.3 mg/100 ml and 150 ± 23.4 mg/100 ml, respectively. Patients in group B were less well-controlled, with a mean fasting blood glucose of 119 ± 14.8 mg/100 ml and a mean peak value of 188 ± 18.9 mg/100 ml. Patients in group A were very poorly controlled throughout the 24 hours. In general, plasma IRI was inversely related to both blood glucose and plasma chlorpropamide concentration, being highest in the well controlled patients of group D, lowest in the poorly controlled patients in group A, and intermediate in patients in groups B and C. The correlation coefficient (r) between plasma chlorpropamide and plasma insulin was -0.3610 and between blood glucose and plasma insulin -0.1067 .

Figure 1 shows that diabetic control deteriorated markedly in all patients in groups B, C and D when chlorpropamide was stopped for 10 days. The greatest rise in blood glucose concentration occurred in patients previously taking the highest daily dose of chlorpropamide (group B).

Figure 2 shows that although the mean plasma IRI concentration is lower in all groups when chlorpropamide is stopped, the difference is not significant at any point. Consistent with this the coefficient of correlation (r) between glucose and insulin was 0.1389.

The glucose and insulin results were analysed for each individual patient on and off treatment using Students' paired t-test (Table 4). In 7 of 8 patients in groups B and C, there was a significant rise in blood glucose concentration when chlorpropamide was stopped. In only one patient, MB, was there an equally significant fall in insulin. In 5 patients there was no significant change in insulin, and in JB the insulin fell, but there was no corresponding rise in glucose.

In group D, 3 patients were well controlled with-

Fig. 1. Mean blood glucose concentration in three groups of patients with maturity-onset diabetes, 1. taking chlorpropamide, 2. 10 days after stopping chlorpropamide

out chlorpropamide and 2 have since been maintained without it. One patient, AB, showed a significant rise in blood glucose when chlorpropamide was stopped, but no corresponding fall in plasma insulin.

Discussion

The critical concentration of chlorpropamide in plasma which stimulates the release of insulin from the B cells of the pancreas in acute experiments has

Fig. 2. Mean plasma immuno-reactive insulin concentration in three groups of patients with maturity-onset diabetes, 1. taking chlorpropamide, 2. 10 days after stopping chlorpropamide

Table 4. Significance of change in glucose in insulin in individual patients

Dose group	Patient	Glucose	Insulin
B	NB	**	*
	MB	**	**
	JK	**	NS
	MM	*	NS
C	JB	NS	*
	JBu	**	NS
	GR	**	NS
	FT	*	NS
D	AB	**	NS
	JD	NS	*
	JF	NS	NS
	DM	NS	*

NS = Not significant

* = $0.01 < p < 0.05$

** = $p < 0.01$

reasonable to infer that chlorpropamide was exerting a hypoglycaemic effect by increasing the secretion of insulin. The fact that, when chlorpropamide was withdrawn and diabetic control deteriorated, the concentration of insulin in the peripheral venous blood did not change significantly in any of the groups of patients (representing different degrees of B cell dysfunction within the spectrum of insulin-independent diabetes), makes this unlikely.

Feldman and Lebovitz [14] have listed possible mechanisms which would explain why the long-term hypoglycaemic effect of chlorpropamide depends on an ability to secrete some endogenous insulin and yet is not associated with an increased concentration of insulin in the peripheral blood. They point out that while numerous effects involving many organs other than the pancreas have been attributed to sulphonylureas, the majority of these effects have been demonstrated in *in vitro* experiments using drug concentrations far in excess of those existing in the plasma of diabetic patients on long-term treatment with these drugs.

Reviewing the available evidence derived from experiments where the plasma concentration of sulphonylurea was comparable to that achieved in the clinical situation, one or more of three possible mechanisms seem likely to account for most of the long-term hypoglycaemic effect of sulphonylureas in maturity-onset diabetes. They may 1. increase the sensitivity of the liver to the action of endogenous insulin so that increased hepatic uptake of glucose occurs [15]; 2. have a direct effect on the liver causing reduced hepatic release of glucose [16]; 3. potentiate the action of insulin on the peripheral tissues so as to increase glucose uptake [14].

Other extra-pancreatic effects which have been observed to occur in the absence of insulin may also contribute to the long-term hypoglycaemic effect of the sulphonylureas *in vivo*. For example, it has been shown that sulphonylureas inhibit leucine aminotransferase in the liver [17], and lipase activity in both liver [18] and adipose tissue [19], resulting in decreased gluconeogenesis and lipolysis respectively.

Finally, it is possible that chlorpropamide could exert a hypoglycaemic effect by altering the insulin/glucagon ratio [20].

Further work is needed to clarify the exact mechanism by which chlorpropamide controls maturity-onset diabetes. In particular, immunoassay of C-peptide (the chain of aminoacids released when proinsulin is converted to insulin) in peripheral blood may give a more sensitive and accurate indication of B cell activity than the immunoassay of insulin, since unlike insulin, C-peptide is not bound significantly by the liver.

been shown to be between 20 and 30 $\mu\text{g/ml}$. [12, 13] Since the plasma concentration of chlorpropamide was above this level in all our patients and the degree of diabetic control achieved appeared to be positively related to the insulin concentration in peripheral blood, it would not be un-

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