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The Effect of Serum Ca^{2+} Compensation on the Efficacy of Chlorpropamide in Alloxan-diabetic Rabbits

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Abstract—A decrease in serum Ca^{2+} concentration was observed in alloxan-diabetic rabbits, with recovery of serum Ca^{2+} levels achieved following insulin therapy. This suggested that the diabetic condition directly relates to the decrease in serum Ca^{2+} levels. The efficacy of chlorpropamide in alloxan-diabetic rabbits after intravenous injection (150 μmol kg^{-1}) was less than that in normal rabbits as measured by the serum insulin levels, and the efficacy did not change when the dose was increased. However, compensation of serum Ca^{2+} levels in alloxan-diabetic rabbits caused an increase in the efficacy of chlorpropamide observed as an increase in serum insulin and a decrease in serum glucose levels.

The pharmacokinetics of oral anti-diabetic drugs such as sulphonylureas in alloxan-diabetic animals has been widely investigated (Nishihata et al 1978a,b,c,d, 1979). It has been reported that the different pharmacokinetics of chlorpropamide in alloxan-diabetic rabbits compared with normal rabbits was partly due to differences in the blood chemistry in the diabetic condition (Nishihata et al 1978b, 1979). Alloxan has been used to develop diabetes in animals by causing damage to the pancreas, but complete damage of the pancreas can be avoided by adjusting the dose of alloxan (Nishihata et al 1978b). Thus, alloxan-diabetic rabbits with partial damage to the pancreas still show sensitivity to sulphonylureas, such as chlorpropamide. Using alloxan-diabetic rabbits which were sensitive to chlorpropamide, this study on the efficacy of chlorpropamide in experimental diabetes was carried out. Because the abnormal behaviour of chlorpropamide in alloxan-diabetic rabbits may be due to changes in blood chemistry (Nishihata et al 1979), this investigation may give information for the development of effective medication.

It has been reported that Ca^{2+} plays an important role in the secretion of insulin from pancreatic β-cells (Hellman 1975, 1976; Devis et al 1975; Ashcroft 1980; Grapengiesser et al 1988); a rise in the cytoplasmic concentration of Ca^{2+} is a key event in the initiation of insulin secretion. Further, the increase in acetone bodies observed in alloxan-diabetic rabbits and the changes in drug distribution to the red blood cell as a result of this was reversed by the addition of Ca^{2+} (Nishihata et al 1978c, 1979). Thus, the Ca^{2+} status of the alloxan-diabetic rabbits may have important implications for the efficacy of chlorpropamide.

In the present study, the serum Ca^{2+} profile of alloxan-diabetic rabbits was investigated, and the efficacy of chlorpropamide in relation to serum Ca^{2+} status was determined.

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Materials and Methods

Materials

Chlorpropamide was obtained from Sigma Chemicals (MO, USA). Insulin zinc suspension (Japanese Pharmacopoeia XI) was used for insulin treatment. Other reagents used were of analytical grade.

Preparation of alloxan-diabetic rabbits

Alloxan-diabetic rabbits were prepared as reported in the previous paper (Nishihata et al 1978b). Twenty percent aqueous solution of alloxan, freshly prepared with saline (0·9% NaCl) was intravenously injected into the ear vein of male albino rabbits, 2·0–3·0 kg, at a dose of 0·5 mL kg^{-1}. The concentration of blood glucose was measured once a day for several days. Rabbits which were sustained with 150 mg% glucose under fasting conditions given at 0900 h from the seventh to the tenth day after alloxan administration were used for the following experiments. During the experiments, food was given at 1000 h.

Insulin treatment of alloxan-diabetic rabbits

Administration of insulin to alloxan-diabetic rabbits was as follows: insulin zinc suspension was injected into the femoralis muscle for 3 weeks at a total dose of 0·4 units kg^{-1} day^{-1}. Insulin at a dose of 0·2 units kg^{-1} was given at 1000 and 2200 h and glucose, Ca^{2+} and insulin levels in serum were monitored at 0900 h.

Determination of glucose, Ca^{2+} and insulin in serum

Blood samples were collected from an ear vein and were centrifuged at 4°C, 3000 rev min^{-1} for 10 min to obtain serum. The serum glucose concentration was determined with an assay kit (Glucose B-Test Wako, Wako Pure Chemicals, Osaka, Japan) based on the enzyme method. Serum Ca^{2+} concentration was determined by an assay kit (Calcium C-Test Wako, Wako Pure Chemicals). Serum insulin concentration was determined using a radioimmuno assay kit with a Sephadex-bound antibody (Phadebas Insulin Test, Pharmacia Co. Ltd, Tokyo, Japan).

Administration of chlorpropamide to alloxan-diabetic rabbits
An aqueous solution of chlorpropamide (0·1 M) was prepared with saline, and was intravenously injected into the ear vein of diabetic rabbits at a dose of 150 μmol kg^{-1} (300 μmol kg^{-1}). After administering the drug, one mL blood samples were taken from the opposite ear vein at designated time intervals. Blood samples were immediately centrifuged for 10 min, 3000 rev min^{-1}, 4°C, and then supernatant serum layers were frozen pending analysis.

Calcium chloride treatment (calcium compensation)
Calcium chloride solution (50 mg mL^{-1} in saline) was subcutaneously administered to the dorsum (0·5 mL kg^{-1}) of alloxan-diabetic rabbits at 12 h intervals for 5 days.

Assay of chlorpropamide
Extraction of chlorpropamide from plasma samples with ethyl acetate was performed according to the method reported previously (Nishihata et al 1978c). Serum (0·5 mL) was mixed with 0·5 mL of 0·1 M acetate buffer (pH 3) containing 60 μg mL^{-1} tolbutamide as an internal standard for the HPLC assay. After centrifugation for 5 min at 3000 rev min^{-1}, 4 mL of the organic layer was collected and evaporated to dryness. The residue was dissolved in 0·2 mL mobile phase using the assay conditions for HPLC described previously (Nishihata et al 1978c).

Results and Discussion

Profiles of serum glucose, Ca^{2+}, and insulin concentration in alloxan-diabetic rabbits
A significant increase in serum glucose concentration and a significant decrease in serum insulin concentration under fasting conditions were observed 3 weeks after alloxan administration, as shown in Fig. 1A, B. A gradual decrease in serum Ca^{2+} concentration was also observed after the alloxan administration (Fig. 1C). On insulin treatment, serum glucose levels decreased rapidly to the normal range (Fig. 1A, B). However, the recovery of serum Ca^{2+} levels occurred slowly (Fig. 1C). When the insulin treatment was stopped, the increase in serum glucose levels and decrease in serum Ca^{2+} and insulin levels were observed again. Because serum insulin levels in hyperglycaemia in alloxan-diabetic rabbits was between 5 and 10 units mL^{-1}, this indicated that the pancreas of alloxan-diabetic rabbits only partly maintained the ability to secrete insulin (Fig. 1B).

Effect of calcium chloride administration on the efficacy of chlorpropamide administered intravenously to alloxan-diabetic rabbit
Alloxan-diabetic rabbits 16 weeks after alloxan administration were used for this study. Administration of chlorpropamide increased serum insulin levels (about 15 μ units mL^{-1} at maximum) and decreased serum glucose levels, but no recovery of serum Ca^{2+} levels was observed (Fig. 2). Furthermore, the recovery of serum insulin was insufficient, because insulin levels in normal rabbits was between 18 and 25 μ units mL^{-1} in this study. When the efficacy of chlorpropamide by intravenous injection in alloxan-diabetic rabbits, was compared with that in normal rabbits, in terms of the serum insulin levels, the greatest increase in serum insulin levels was observed in normal rabbits (normal rabbits

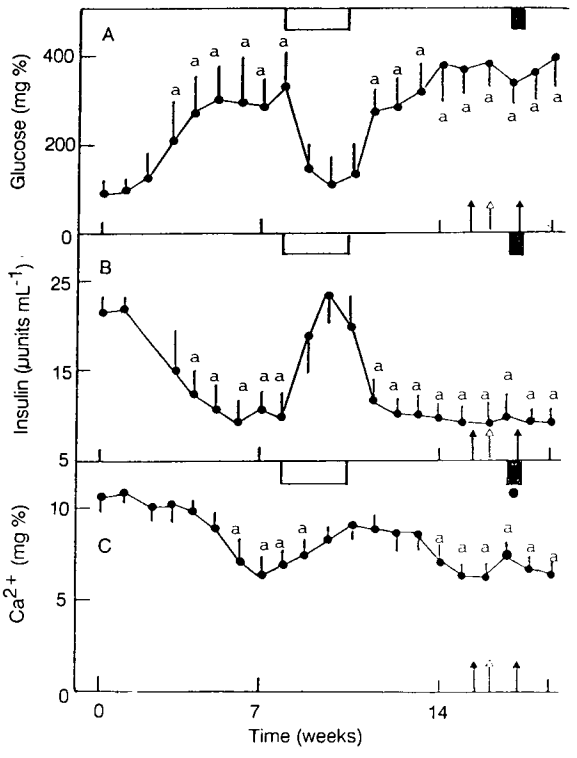

FIG. 1. Serum profiles of glucose (A), insulin (B) and Ca^{2+} (C) levels in rabbits after the administration of alloxan (see Methods section). Alloxan was administered at week 1. □ Indicates the period of insulin treatment (see Methods section); ■ indicates the period of Ca^{2+} compensation (see Methods section); and arrows (▲ and △) indicate the day when the chlorpropamide challenge study was performed at a dose of 150 μmol kg^{-1} and 300 μmol kg^{-1}, respectively. ● Indicates the serum Ca^{2+} levels three days after starting Ca^{2+} compensation. Each value represents the mean ± s.d. (n = 4) a: $P < 0.01$ vs the value before alloxan administration.

60 μ units mL^{-1} at maximum, Fig. 3; alloxan-diabetic rabbits 15 μ units mL^{-1} at maximum, Fig. 2). When the dose of chlorpropamide was increased to 300 μmol kg^{-1} in alloxan-diabetic rabbits (1 week after the first chlorpropamide challenge experiment), the profiles of serum glucose, Ca^{2+} and insulin were not different from those after chlorpropamide (150 μmol kg^{-1}) administration.

Administration of Ca^{2+} for 5 days in alloxan-diabetic rabbits was started 1 week after the investigation of efficacy of chlorpropamide alone. The efficacy study with chlorpropamide was repeated 4 days after starting the administration of calcium chloride (chlorpropamide was administered 2 h after administration of calcium chloride.

As seen in Fig. 2, the administration of chlorpropamide to alloxan-diabetic rabbits with Ca^{2+} supplementation showed greater decreases in serum glucose levels and greater increases in serum insulin levels compared with alloxan-diabetic rabbits without Ca^{2+} supplementation, indicating that the efficacy of chlorpropamide was greater in the alloxan-diabetic rabbits by compensating serum deficient Ca^{2+}.

Serum chlorpropamide profiles in alloxan-diabetic rabbits after intravenous administration
It has been reported that higher plasma chlorpropamide levels are maintained in alloxan-diabetic rabbits compared

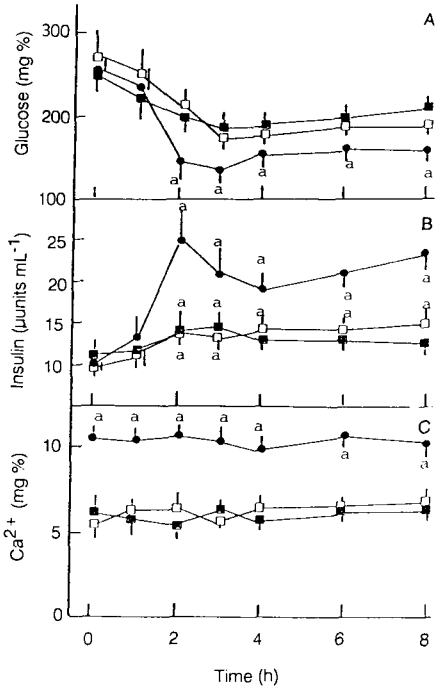

FIG. 2. Serum profile of glucose (A), insulin (B) and Ca^{2+} (C) levels in alloxan-diabetic rabbits (■ and □, at weeks 16 and 17 after the alloxan administration, respectively) and in alloxan-diabetic rabbits with compensation of serum Ca^{2+} (●, 18·6 weeks after alloxan administration), after administration of chlorpropamide at a dose of 150 μmol kg^{-1} (■ and ●), 300 μmol kg^{-1} (□). Each value represents the mean ± s.d. (n = 4) a: $P < 0.01$ vs the levels obtained in alloxan-diabetic rabbits without Ca^{2+} compensation.

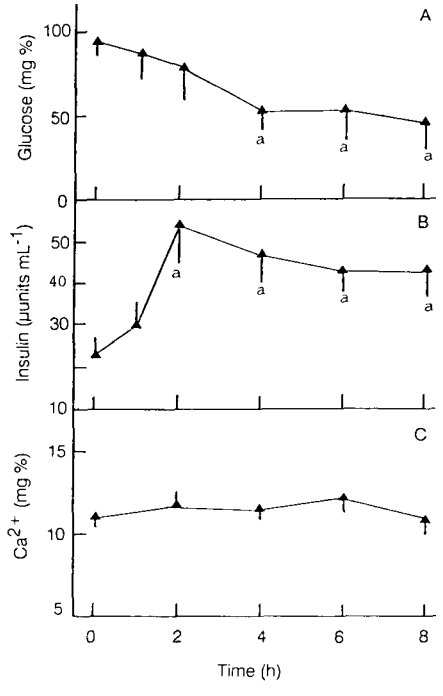

FIG. 3. Serum profile of glucose (A), insulin (B) and Ca^{2+} (C) levels in normal rabbits after administration of chlorpropamide at a dose of 150 μmol kg^{-1}. The study was performed before the administration of alloxan. Each value represents the mean ± s.d. (n = 4). a: $P < 0.01$ vs the value at zero time.

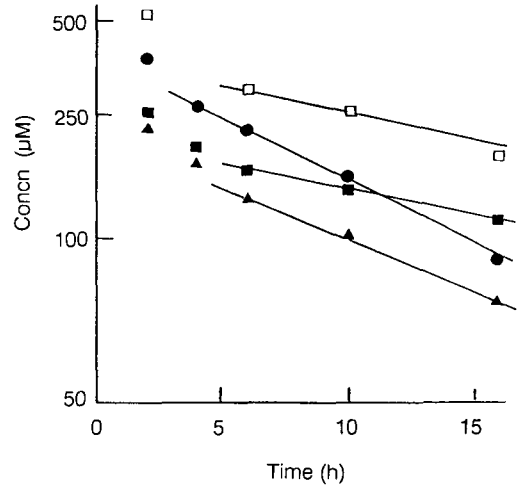

FIG. 4. Serum chlorpropamide profile in normal rabbits (▲; see Fig. 3) and alloxan-diabetic rabbits (●; with compensation of serum Ca^{2+} levels and ■, □; without compensation of serum Ca^{2+} levels, see Fig. 2) after administration at a dose of 150 μmol kg^{-1} (▲ ● ■) or 300 μmol kg^{-1} (□). Each value represents the mean ± s.d. (n = 4).

with normal rabbits, because of decreased renal clearance of chlorpropamide (Nishihata et al 1979). This decrease in renal clearance of chlorpropamide may occur by a decrease in the tubular secretion of chlorpropamide and an increase in the tubular reabsorption of chlorpropamide (Nishihata et al 1978d, 1979).

When chlorpropamide was intravenously administered to the alloxan-diabetic rabbits with compensation for deficient serum Ca^{2+}, higher serum chlorpropamide concentrations were observed at an early stage (up to 5 h), followed by rapid elimination from plasma (Fig. 4). Pharmacokinetic parameters of serum chlorpropamide are summarized in Table 1. The elimination rate constant (k_{el}) returned to the value in normal rabbits when alloxan diabetic rabbits were treated with Ca^{2+}. However, the distribution volume (Vd) of the central compartment of alloxan-diabetic rabbits decreased significantly by treatment with Ca^{2+}.

Table 1. Pharmacokinetic parameters of chlorpropamide in alloxan-diabetic rabbits after intravenous administration of chlorpropamide (150–300 μmol kg^{-1}).

Experiment	Weight (kg)	Concn of serum[a] Ca^{2+} (mg%)	k_{el}[b] (h^{-1})	Vd[c] (L)
1 Normal (1 week before alloxan administered): 150 μmol kg^{-1}	2·4 ± 0·5	10·96 ± 0·7	0·076 ± 0·018	1·24 ± 0·31
2 Alloxan-diabetic rabbits (16 weeks after alloxan administered): 150 μmol kg^{-1}	2·2 ± 0·4	6·21 ± 1·1	0·046 ± 0·0092	1·11 ± 0·20
3 Alloxan-diabetic rabbits (1 week after experiment 2): 300 μmol kg^{-1}	2·3 ± 0·3	5·94 ± 0·8	0·044 ± 0·0061	1·19 ± 0·14
4 Alloxan-diabetic rabbits with administered Ca^{2+} (Ca^{2+} compensation was started 2 weeks after experiment 3)	2·2 ± 0·4	10·11 ± 1·6	0·095 ± 0·021	0·85 ± 0·14

[a] Concn of serum Ca^{2+} was determined 10 min before chlorpropamide administration.

[b] k_{el}: apparent serum elimination constant of chlorpropamide calculated in the β-phase.

[c] Vd: distribution volume of central compartment of chlorpropamide. Mean ± s.d. (n = 4) values are given.

In summary, we consider that the increase in chlorpropamide's efficacy to increase insulin secretion in alloxan-diabetic rabbits is related directly to the compensation of serum Ca^{2+} concentration rather than the increase in serum chlorpropamide concentration.

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