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Effectiveness of insulin suppositories in diabetic patients

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Abstract—Experimental insulin suppositories regulated postprandial hyperglycaemia in diabetic patients. The insulin suppositories seemed also to avoid hyperinsulinaemia. The formulation with more rapid dissolution of insulin, which contained a solid dispersed form of insulin, effectively reduced the insulin dose required.

Insulin treatment in diabetes leaves much room for improvement in the manner of its delivery to its site of action. A study, in which insulin was delivered via the hepatic portal vein to depancreatized dogs after regular feeding, demonstrated less hyperinsulinaemia than that observed in a peripheral infusion study (Goriya et al 1980; Ritschel & Ritschel 1984). This indicates the importance of insulin delivery via the portal vein in normalizing both blood glucose and insulin levels in the postprandial state. An oral or rectal insulin administration could have an advantage by achieving portal insulin delivery in a convenient way, but insulin is not normally absorbed from intestine. Recently, sodium salicylate and some of its derivatives, when administered as a solution or gel formulation, were found to enhance insulin absorption from the rectum and small intestine in dogs and rats (Nishihata et al 1981a, b, 1985, 1987).

Practically, the rectal route should be better than the oral route, because of insulin's rapid degradation enzymatically in the small intestinal lumen. However, it is difficult to achieve rapid dissolution of crystalline insulin from a suppository formulation within the rectal compartment, so that facilitated absorption may occur (Nishihata et al 1983). By overcoming this difficulty, an insulin suppository can effectively reduce the insulin dose required.

We have recently developed two types of insulin suppositories which facilitated dissolution of insulin (Nishihata et al 1986a, 1987). In the present report, we have examined effect of these insulin suppositories in diabetic patients as well as healthy humans.

Materials and methods

Suppositories were prepared according to the method reported previously (Nishihata et al 1986a, 1987). Briefly, Supp-1 was prepared by mixing 0·7 g suppository base (triglyceride, m.p. 33–35°C, containing 10% w/w natural soy lecithin), 0·3 g sodium salicylate powder and 50 μL of insulin solution (50, 75, 100, or 150 units). Supp-2 was prepared by mixing suppository base (described above), and 0·3 g sodium salicylate powder containing insulin (30 units, 45 units, or 60 units) in solid dispersed form. Human insulin (27·3 units mg^{-1}, NOVO) was used. As a third formulation, Supp-0 was prepared by mixing 0·7 g suppository base (triglyceride alone), 0·3 g sodium salicylate and 150 units insulin powder.

The dissolution of insulin from 1 g suppositories in 3 mL saline (0·9% NaCl) for 1 h at 37°C in the method described previously (Nishihata et al 1987) was 12·6 ± 4·2% from Supp-0 (n = 6), 56·4 ± 8·9% from Supp-1 (n = 6, $P < 0·01$ v Supp-0, Students t-test) and 92·6 ± 4·1% from Supp-2 (n = 6, $P < 0·01$ both Supp-0 and Supp-1). Thus, Supp-1 and Supp-2 displayed significantly improved insulin dissolution rates compared with Supp-0. Therefore, Supp-0 was not examined in human studies.

For the clinical investigation the experimental conditions and insulin dose are described in captions to the figures. The healthy subjects were allowed only a drink of water or Japanese tea after dinner (20.00 h) on the day before the experimental date, and the administration of the suppository was at 09.00 h. (i.e. under fasting conditions). Five healthy subjects received Supp-1 and four healthy subjects Supp-2. Only two subjects had both Supp-1 and Supp-2 at one week's interval. Thus, a direct comparison of Supp-1 and Supp-2 may be difficult because of the problems of producing the same condition in subjects between both groups.

The diabetic patients were allowed only a drink of water after dinner (17.00 h) on the day before the experiment, a suppository being administered at 08.00 next day (i.e., a 16 h fast). In the tolerance test, patients ate a regular Japanese meal (270 g of boiled rice) 15 min after administration of a suppository. The results of the tolerance test without insulin when patients were diagnosed as diabetic are described below.

Assays of glucose and insulin were carried out enzymatically, using an assay kit (Wako Pure Chemicals Co., Osaka, Japan), and a radioimmunoassay method, using an assay kit (Daiichi Radioisotope Co., Tokyo, Japan) (Nakagawa et al 1971), respectively.

Results and discussion

The administration of Supp-1 containing 100 units of insulin to five healthy male human subjects, caused a significant increase of plasma insulin concentration, along with a significant decrease in the plasma glucose concentration (Fig. 1). High plasma

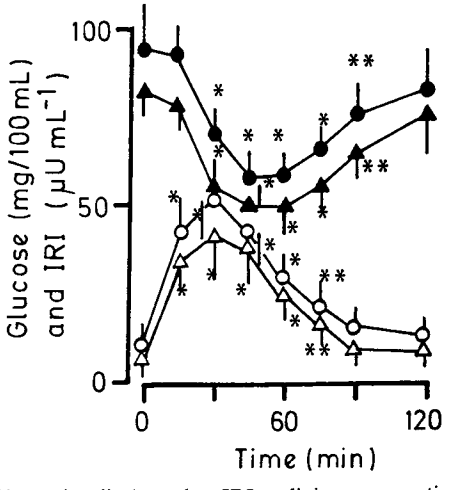

FIG. 1. Plasma insulin (○ and △, IRI; radioimmunoreactive insulin) and glucose (● and ▲) concentrations in healthy men (54 to 76 kg and 24 to 36 years) after administration of Supp-1 containing 100 units of insulin (five subjects, ○ and ●) or Supp-2 containing 45 units of insulin (four subjects, △ and ▲). Each value represents the mean ± s.d. *, $P < 0·05$ versus the value before administration (Student's t-test); **, $P < 0·1$ versus the value before administration.

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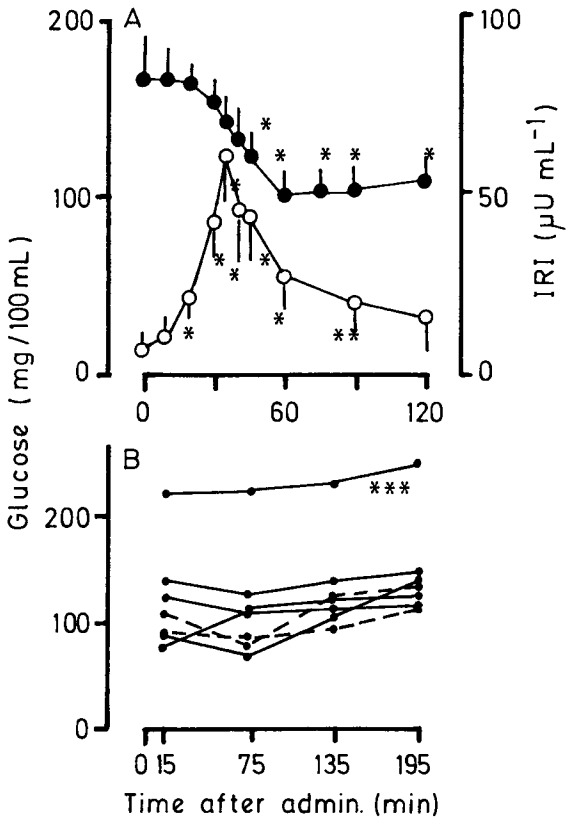

FIG. 2. (A) Plasma insulin (○, IRI) and glucose (●) concentrations in five male diabetic patients (51 to 64 kg and 42 to 64 years old), who were fasted for 16 h before administration, after administration of Supp-1 containing 100 units of insulin. Each value represents the mean ± s.d. *, $P<0.05$ versus the value before administration; **, $P<0.1$ versus the value before administration. (B) The effect of rectal administration of Supp-1 containing 150 units of insulin (dashed line), or Supp-2 containing 60 units of insulin (solid line), on plasma glucose concentration of seven diabetic patients (six male and one female) after a regular Japanese meal (270 g of boiled rice) for the tolerance test. The suppository was administered 15 min before the meal (patients ate at the 15 min point in the Figure). Plasma glucose concentrations under fasted condition, which were measured two days before the administration of suppositories, were between 140 to 180 mg/100 mL for six patients, and 310 mg/100 mL for one patient marked***.

insulin levels were observed from 15 to 75 min after the administration. Supp-1 containing 75 units of insulin was also effective (data not shown). However, Supp-1 containing 50 units of insulin caused only a slight decrease in plasma glucose and a slight increase in plasma insulin concentration (maximum of 26.4 ± 4.2 μunits mL^{-1} at 30 min against the value of 16.7 ± 5.2 μunits mL^{-1} before the administration; slightly elevated concentrations were also observed at 15 and 45 min).

The administration of Supp-1 containing 100 units of insulin to five, fasted diabetic patients, produced an increase in plasma insulin concentration along with a significant decrease in plasma glucose (Fig. 2A).

The administration of Supp-1, containing 150 units of insulin, to two diabetic patients eating a regular meal for the tolerance test was found to inhibit postprandial hyperglycaemia (Fig. 2B) (for two patients given Supp-1, plasma glucose concentrations for the tolerance test without insulin, at the time they were diagnosed as diabetic, were between 200 and 240 mg/100 mL). It is therefore considered that Supp-1 is an effective dosage form for the treatment of diabetes, especially with regard to inhibiting postprandial hyperglycaemia.

An attempt to reduce the insulin dose in the suppository was made by the administration of Supp-2, since the dissolution of insulin from Supp-2 was more complete than that from Supp-1. Supp-2, containing 45 units of insulin, gave high plasma insulin concentrations from 15 to 75 min, along with a significant decrease in plasma glucose in healthy subjects, similar to the effect after administration of Supp-1 containing 100 units of insulin (Fig. 1). Supp-2, containing 30 units of insulin also caused a significant decrease in plasma glucose in healthy subjects (minimum levels of 71.3 ± 4.9 mg/100 mL at 45 min, $n=5$, $P<0.01$ versus 92.3 ± 8.1 mg/100 mL before the administration), along with an increase in plasma insulin concentrations (maximum 32.7 ± 6.9 μunits mL^{-1} at 15 min, $n=3$, $P<0.01$ against the value of 12.3 ± 5.1 μunits mL^{-1} before the administration). Thus, in terms of the insulin dose, Supp-2 appears to be a more effective suppository formulation.

Supp-2 was also formulated with 60 units insulin, and was found to inhibit postprandial hyperglycaemia in five diabetic patients (Fig. 2 B) (for the five patients examined with Supp-2, plasma glucose concentrations for the tolerance test without insulin administration at the time they were diagnosed as diabetic, were 180 to 25 mg/100 mL in four patients and >300 mg/100 mL in the other patient).

The insulin suppositories, Supp-1, and Supp-2, could control postprandial hyperglycaemia in diabetics, and peripheral insulin profiles were similar to those in healthy subjects after eating. The observations suggest that the insulin suppository can control postprandial hyperglycaemia in diabetic patients in a more natural manner compared with conventional insulin therapy.

There may also be some incidental advantage in the use of sodium salicylate as an absorption-promoting agent for insulin. It has been reported that salicylate is an effective agent to treat a vascular disease which often occurs in the diabetic condition, because it inhibits glycosylation of collagen (Yue et al 1984) and because it suppresses cyclooxygenase activity, acting as an antiplatelet agent (Yue et al 1985). Salicylate also inhibits gluconeogenesis from L-lactate and L-alanine (Woods et al 1974; Nishihata et al 1986b).

The safety of salicylate in the insulin suppository, is supported by reports that sodium salicylate did not change the rectal mucosa epithelium in rats as assessed by light- and electron-microscopic observations (Sithigorngul et al 1983). No pathological changes were detected in rats, to which 100 mg sodium salicylate kg^{-1} day^{-1} was administered rectally for 60 days (Nishihata et al 1982).

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Vasocontractile action of daunorubicin

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Abstract—Daunorubicin (35·5–142 μmol L^{-1}) induced in rat aortic strips a contraction with slow onset and a gradual development of tension on which neither phentolamine nor bromophenacyl bromide pretreatment had an effect. The contraction was not altered by removal of the endothelium but it was suppressed in calcium-free solution or by preincubation with nifedipine. These results suggest that daunorubicin directly stimulates vascular smooth muscle and induces a contractile response which is mainly dependent upon extracellular calcium.

Daunorubicin (daunomycin) is a potent antineoplastic anthracycline derivative, but its cytotoxicity to cardiac muscle limits its use in antineoplastic therapy (Tan et al 1967). It generally displays chronic depressive action on cardiac muscle, but in some species, it has an acute positive inotropic effect (Gibbs 1985), the precise mechanism of which, like its effect on the vascular system, remains unknown. We have investigated the acute effect of the drug on rat aortic strips and found it to have a vasocontractile action.

Materials and methods

Contraction study. Thoracic aortas were excised from male Wistar rats (400–450 g) and placed in Krebs-Ringer bicarbonate solution (mM: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 10). After removal of the connective tissue, helical strips (2 mm wide × 15 mm long) were prepared and suspended vertically in 10 mL organ chambers filled with the above solution (37°C, pH 7·4) through which 95% O_2–5% CO_2 was bubbled. After 1 h of equilibrium with a resting tension of 1 g, changes in isometric force were recorded. In some preparations, the endothelium was removed by gentle abrasion of the intimal surface with sandpaper. Denudation of the endothelium was confirmed functionally by the disappearance of the 10^{-5} M acetylcholine-induced relaxing response of the 10^{-7} M noradrenaline-precontracted vessel (Wakabayashi et al 1987). Contractile responses to daunorubicin were expressed in terms of the percentage of contraction by 60 mM KCl in each strip. The concentration of each drug was expressed as the final concentration in the organ bath.

Substances. Nifedipine (Sigma) was dissolved in ethanol to give a stock solution of 10^{-3} M. The final concentration of ethanol in 10^{-6} M nifedipine was 0·1%, which did not affect the contractile response to daunorubicin. All experiments with nifedipine were conducted in the dark. Daunorubicin (Daunomycin, Meiji Seika) and phentolamine (Ciba-Geigy) were dissolved in 0·9% saline. Bromophenacyl bromide (Wako) was dissolved in ethanol. The concentration of ethanol in 3×10^{-6} M bromophenacyl bromide was 0·3%, which did not affect the contraction by daunorubicin.

Statistical analysis. All the values represent mean ± s.e.m. The data were analysed by Student's t-test and $P < 0.05$ was defined as significant.

Results

Daunorubicin induced a contractile response of the rat aorta which showed a slow onset and a gradual increase in tension. About 1 h was required for this contraction to reach a plateau. Incubation of the aortic strip with 0·9% saline, a solvent of daunorubicin, for 60 min did not affect the basal vascular tone (Fig. 1A). The vasocontractile action of daunorubicin was initiated at a concentration of 35·5 μM and the maximal contraction was attained at 142 μM (Fig. 1B). Figure 1C shows the effects of several inhibitors and conditions on the daunorubicin-induced contractile response of the rat aorta. This response was suppressed by pretreatment of the aorta with nifedipine and also in calcium-free solution. Pretreatment with either phentolamine or bromophenacyl bromide did not affect the contractile

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