Absorption Kinetics and Action Profiles After Sequential Subcutaneous Administration of Human Soluble and Lente Insulin Through One Needle

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The effects of sequential administration through one needle of human soluble and human lente insulin on plasma insulin levels and action profiles, assessed with glucose clamping, were studied in six healthy volunteers. Insulin kinetics after administration of human soluble insulin (0.22 IU/kg) alone were compared with those after sequential administration of *1*) human soluble insulin followed by human lente insulin and *2*) human lente insulin followed by human soluble insulin. Total insulin dose in both sequences was 0.55 IU/kg, 40% of which was short-acting insulin.

Plasma insulin levels were not significantly different at any time point between 0 and 240 min after soluble insulin compared with either combination. Although insulin levels were slightly but significantly lower at 30 and 105 min after lente followed by soluble insulin compared with soluble followed by lente insulin, these differences probably reflect chance occurrences. Glucose requirements were not significantly different after either of the three administrations.

We therefore conclude that the unwanted retarding effect after mixing of human soluble insulin with human lente insulin in the syringe on the onset of action of the soluble insulin can be prevented by sequential subcutaneous injection of these insulins with two syringes through one needle. *Diabetes Care* 10:466–69, 1987

Since the late 1970s the aim of the treatment of patients with insulin-dependent diabetes mellitus (IDDM) has been to maintain a blood glucose value as close to normal as possible for as long as possible. In intensified conventional insulin treatment patients are often regulated with 2 injections/day with mixtures of soluble and intermediate-acting insulin. The efficacy of this treatment largely depends on the uncompromised action of the soluble insulin (1).

In studies with mixtures of purified porcine soluble and lente insulin, no appreciable loss of the soluble insulin bioavailability could be observed when the mixture was administered immediately after mixing (2–4). This was different, however, when mixing human soluble and lente insulin; a delayed onset of action of the soluble insulin was observed, with both U40 (4–6) and U100 insulins (7–9), even when the mixture was administered immediately after mixing.

This delay is probably caused by a binding of soluble insulin to the excess zinc present in lente (zinc) insulin. In U40 lente insulin the total zinc concentration is 0.08 mg/ml, with a free-zinc concentration of ~0.04 mg/ml; U100 lente insulin has a total zinc concentration of 0.13 mg/ml and an identical free-zinc concentration as U40 insulin. Thus U100 lente insulin contains proportionally less excess zinc (10). This is still enough to exert the reported delay in onset of action of soluble insulin when mixing U100 insulins, although probably to a lesser extent than with U40 insulins (8).

Some specific characteristic of human insulin must also play a role, however, because this immediate reaction does not occur in porcine insulin. It has been suggested that the slightly more hydrophilic nature of human insulins may accelerate the interaction (1,11). To avoid this specific interaction between human soluble and lente insulin, many patients are advised to administer these insulins either separately at two different injection sites, or sequentially, with separate syringes and injection through one needle that is left in situ while changing syringes.

We studied the effects of sequential subcutaneous administration of human soluble insulin and lente insulin on plasma

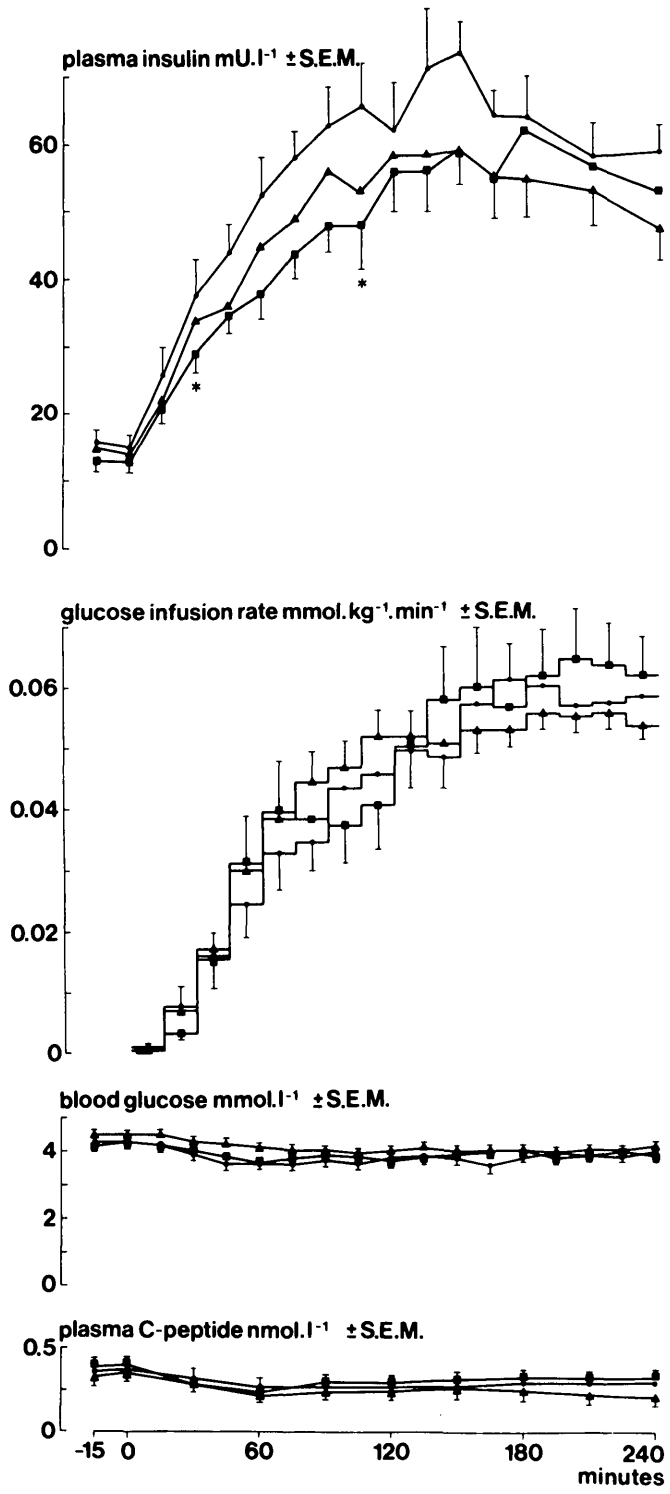

FIG. 1. Plasma insulin levels, glucose infusion rates, blood glucose values, and C-peptide levels after injection of 0.22 IU/kg human soluble insulin (▲), 0.22 IU/kg human soluble insulin followed by 0.33 IU/kg human lente insulin (●), and 0.33 IU/kg human lente insulin followed by 0.22 IU/kg human soluble insulin (■). *P < .05 for soluble then lente insulin vs. lente then soluble insulin.

insulin levels and glucose infusion rates and compared the results with those after administration of soluble insulin alone, with the same dosages as in an earlier study (5). To avoid counterregulatory responses to hypoglycemia and assess insulin action, we used the manual euglycemic clamp technique, in which the glucose infusion rate needed to maintain fasting blood glucose levels after the insulin injection is a measure of the hypoglycemic effect of insulin (12,13).

MATERIALS AND METHODS

Subjects. We studied six healthy male volunteers aged 20–28 yr (mean 23.2 yr) with a body mass index of 19.4–23.0 kg/m^2 (mean 21.8 kg/m^2). Informed consent was obtained from all subjects. Our study was approved by the local ethical committee.

Insulins. Two semisynthetic human insulins were used, Actrapid (40 IU/ml) as soluble insulin and Monotard (40 IU/ml) as lente insulin (Novo, Bagsvaerd, Denmark). Zinc content of the lente insulin was 0.08 mg/ml.

Protocols. The following protocols were used. *1*) Administration of soluble insulin alone (0.22 IU/kg body wt) with a perpendicular injection with a 13-mm needle. 2) Administration of soluble insulin (0.22 IU/kg body wt), immediately followed by lente insulin (0.33 IU/kg body wt), with a 45° angle injection with a 20-mm needle left in situ while changing the separate insulin syringes. 3) Injection of the same amounts of insulin and with the same technique as in protocol 2, but with lente insulin immediately followed by soluble insulin.

The tests of each subject were separated by at least 1 wk, and the order of the protocols was randomized.

Study design. All subjects were asked to maintain their usual diet and have an overnight fast of at least 10 h before each study. On the morning of each study at time −30 min, an intravenous cannula (Venflon, Viggo, Helsingborg, Sweden) was inserted into an antecubital fossa vein of each arm. One cannula was used for intermittent sampling of blood for blood glucose, plasma insulin, and plasma C-peptide levels and was kept open with saline (0.9% solution, 0.15 M). The second cannula was used for the infusion of glucose (20% solution, 1.11 M) by a variable-rate volumetric infusion pump (Imed 928, Abingdon, Oxon, UK). At 0 min insulin was injected subcutaneously, either with a 1-ml Plastipak syringe with a 13-mm needle (Becton Dickinson, Dublin, Ireland) or separate syringes with a detachable 20-mm needle

TABLE 1
Glucose requirements expressed as areas under curve

Insulin	Time (h)		
	0–1	0–3	0–4
Soluble	0.76 ± 0.11	7.07 ± 0.59	10.71 ± 0.70
Soluble plus lente	0.67 ± 0.23	7.37 ± 0.89	10.20 ± 1.11
Lente plus soluble	0.61 ± 0.20	6.73 ± 1.45	10.82 ± 1.94

Values are in mmol/kg (means ± SE).

(Terumo, Tokyo). Throughout the experiments the subjects remained in a supine position and abstained from food, drink, and smoking.

After insulin injection, blood glucose values were maintained at the fasting blood glucose level −0.5 mM by a manual euglycemic clamp as described by Poncher et al. (12). Blood glucose values were measured every 5 min. Plasma insulin levels were measured every 15 min during the 1st 180 min and every 30 min thereafter. Plasma C-peptide levels were measured every 30 min. The glucose clamps were maintained for 4 h.

Methods. Blood glucose was measured by a glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin was assessed by radioimmunoassay with human insulin as standard (Sorin Biomedica, Saluggia, Italy; sensitivity 2.5 mU/L, intra-assay coefficient of variation 8.2%). Plasma C-peptide was measured by radioimmunoassay with ethanol precipitation with human C-peptide as standard (Novo; sensitivity 0.06 nM, intra-assay coefficient of variation 8.1%) (14).

Calculations. A quantitative estimate of insulin action was provided during the euglycemic clamps by the glucose requirement, expressed as mmol · kg^{-1} · min^{-1}. Results are expressed as means ± SE unless indicated otherwise. For the statistical evaluation, Student's *t* test for paired observations was used for comparisons between protocols.

RESULTS

Clamp values were well maintained; mean coefficient of variation of the blood glucose values was 6.2 ± 1.8% (mean ± SD) during the clamps (Fig. 1). Plasma C-peptide levels remained below fasting concentrations throughout the clamps, excluding the occurrence of stimulated endogenous insulin secretion. No differences were found in plasma insulin levels, comparing soluble insulin alone with either sequence of soluble and lente insulin. After injection of soluble followed by lente insulin, plasma insulin levels were significantly higher at 30 and 105 min compared with lente followed by soluble insulin (38 ± 5 vs. 29 ± 3 mU/L and 66 ± 6 vs. 48 ± 6 mU/L, respectively; $P < .05$).

Integrated plasma insulin levels at 0–2 and 0–4 h did not show significant differences between soluble followed by lente insulin and lente followed by soluble insulin (3985 ± 547 vs. 2890 ± 331 mU/L and 9768 ± 1046 vs. 8160 ± 920 mU/L, respectively). Glucose infusion rates did not differ at any time point. Glucose requirements expressed as area under curve after 1, 3, and 4 h were not different (Table 1).

DISCUSSION

This study demonstrates that the delay in the bioavailability of human soluble insulin, when mixed in the syringe with human lente insulin, can be prevented by injecting the insulins sequentially with two separate syringes and leaving the injection needle in situ. Because the differences in insulin levels when comparing both sequences of administration of human soluble and lente insulin are not reflected in either the integrated plasma insulin levels or the glucose requirements, the two significant differences at 30 and 105 min probably reflect chance occurrences.

Although results in previous studies suggest a quick interaction between the human soluble insulin and the excess zinc in the human lente insulin with both U40 and U100 insulins (4–9), this interaction does not seem to occur when administering both insulins sequentially and subcutaneously through the same needle with two separate syringes. Possible explanations for the absence of a delay after sequential administration are either a rapid dispersion of the insulin through the subcutaneous tissue, thus preventing an interaction, or a binding of the excess zinc in lente insulin by phosphate, which is abundantly present in the subcutaneous fluid, thus preventing precipitation with the added soluble insulin. In conclusion, patients treated with combination of human soluble and lente insulin should be advised to administer their insulins separately, or sequentially with two syringes and one needle, to prevent the interaction between human soluble and lente insulin.

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