Early Pharmacokinetic and Pharmacodynamic Effects of Mixing Lispro With Glargine Insulin

Results of glucose clamp studies in youth with type 1 diabetes

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OBJECTIVE — Clinicians who treat children with type 1 diabetes often try to minimize the number of daily injections to reduce treatment burden and improve compliance. Despite the manufacturer's cautions against mixing glargine with rapid-acting insulin analogs, clinical studies have failed to demonstrate deleterious effects of mixing on glucose excursions or A1C levels. However, no formal glucose clamp studies have been performed to determine whether mixing with glargine has an adverse effect on the early pharmacodynamic action of rapid-acting insulin in humans.

RESEARCH DESIGN AND METHODS — To examine this question, euglycemic glucose clamps were performed twice, in random order, in 11 youth with type 1 diabetes (age 15.1 ± 3 years, A1C 7.6 ± 0.6%) with 0.2 units/kg lispro and 0.4 units/kg glargine, given either as separate or as a single mixed injection.

RESULTS — Mixing the two insulins shifted the time action curve to the right, with significantly lower glucose infusion rate (GIR) values after the mixed injections between 60 and 190 min and significantly higher values between 270 and 300 min, lowered the GIR_{max} (separate 7.1 ± 1 vs. mix 3.9 ± 1, $P = 0.03$), and markedly delayed the time to reach GIR_{max} (separate 116 ± 8 min vs. mix 209 ± 15 min, $P = 0.004$). The GIR area under the curve was significantly lower after the mixed injections. Mixing had similar effects on plasma insulin pharmacokinetics.

CONCLUSIONS — These data demonstrate that mixing lispro with glargine markedly flattens the early pharmacodynamic peak of lispro and causes a shift to the right in the GIR curve changes that might lead to difficulties in controlling meal-related glucose excursions.

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I nsulin glargine revolutionized the diabetes therapy by being the first soluble long-acting insulin analog without a pronounced peak and with a more prolonged time-action curve than NPH insulin (1–4). Basal-bolus therapy with glargine and rapid-acting insulin analogs provided patients with greater accuracy and precision in insulin dosing than was possible with split-mix regimens using intermediate-acting insulin suspensions (5), albeit at the expense of greater numbers of required daily insulin injections.

Poor compliance with the requirements of frequent injections is a major contributing cause of failure to achieve target plasma glucose and A1C levels in adolescents with type 1 diabetes using multiple daily injection regimes (6). Consequently, some pediatric diabetes providers have opted to decrease the number of daily injections in youth using multiple daily injection therapy by mixing glargine with rapid-acting insulin analogs, despite company (7) and U.S. Food and Drug Administration warnings against doing so.

Recommendations against mixing were based on preclinical glucose clamp studies in beagle dogs indicating that the maximal glucose-lowering effect of regular insulin was blunted and delayed by mixing with glargine (8). On the other hand, three clinical studies that examined mixing of glargine with rapid-acting analogs in youth with type 1 diabetes did not demonstrate a change in 24 mean glucose concentrations or A1C levels (9–11). However, glucose clamp studies have not been carried out to determine whether mixing of rapid-acting insulin analogs with glargine just before injection alters the early time-action profile of this combination in patients with type 1 diabetes. The present study was conducted to examine this question in adolescents with type 1 diabetes, since this is the population of patients with type 1 diabetes in whom mixing is most often considered.

RESEARCH DESIGN AND METHODS — Eleven subjects with type 1 diabetes (six male and five female) who attended the Yale Children's Type 1 Diabetes Clinic were studied. Eligibility criteria included a clinical diagnosis of type 1 diabetes for at least 1 year's duration, age ranging from 11 to 21 years, continuous subcutaneous insulin infusion therapy for at least 3 months, A1C <9.0%, BMI <95% for age and sex, and the ability to comprehend written and spoken English. Subjects were excluded for any other medical disease aside from type 1 diabetes or treated hypothyroidism; use of medications that might affect glycemic control; pregnancy or breast-feeding; not consistently using barrier methods or abstinence as contraception; or any other condition that in the judgment of the investigators would interfere with the subject's or parent's ability to provide informed consent or the investigator's ability to perform the study. The Yale University Human Investigation Committee approved the study.

At the initial enrollment visit, the risks and benefits of the study were ex-

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plained; informed consent from the parents and informed assent from the subjects were obtained; history and physical examinations were performed and A1C was measured.

Procedures

Subjects were admitted to the Yale–New Haven Hospital Research Unit on the evening before the euglycemic clamp to monitor blood glucose levels. An intravenous catheter was placed to measure blood glucose levels hourly overnight and insulin dose was adjusted via insulin pump to achieve glucose levels between 80 and 120 mg/dl on the morning of the euglycemic clamp.

A second intravenous catheter was placed on the contralateral arm for infusion of exogenous glucose the following morning, and subjects were randomized to receive 0.2 units/kg lispro and 0.4 units/kg glargine in a mixed or nonmixed fashion. Subjects who received both insulins mixed in a syringe before the initial clamp were given separate injections before the second euglycemic clamp performed within 4 weeks of the first clamp and vice versa. Insulin lispro and glargine were mixed in the same syringe (Becton Dickinson insulin syringe with an ultrafine needle, 8 mm, 31 gauge; Becton Dickinson, Franklin Lakes, NJ) at room temperature immediately before the injection into the deep subcutaneous tissue of the left arm through a two-finger pinch of skin at a 45–90° angle. Subjects were given insulin glargine from the left arm and insulin lispro from the right arm on the day that they were randomized to receive insulins separately. The infusion of insulin via the insulin pump was suspended just before the administration of lispro and glargine.

Plasma glucose levels were measured every 5 min, and a 20% dextrose infusion was adjusted to clamp plasma glucose concentrations between 80 and 90 mg/dl during 5 h of the study, as previously described (12). Blood for measurement of plasma insulin levels was collected every 10 min for the first 90 min, every 15 min for the next 90 min, and every 30 min for the last 120 min.

Biochemical methods

A1C was measured by the DCA Vantage Analyzer (Siemens Medical Equipment, Malvern, PA), plasma glucose by the YSI Glucose Analyzer, and plasma insulin by the Mercodia (Mercodia, Uppsala, Sweden) iso-insulin enzyme-linked immu-
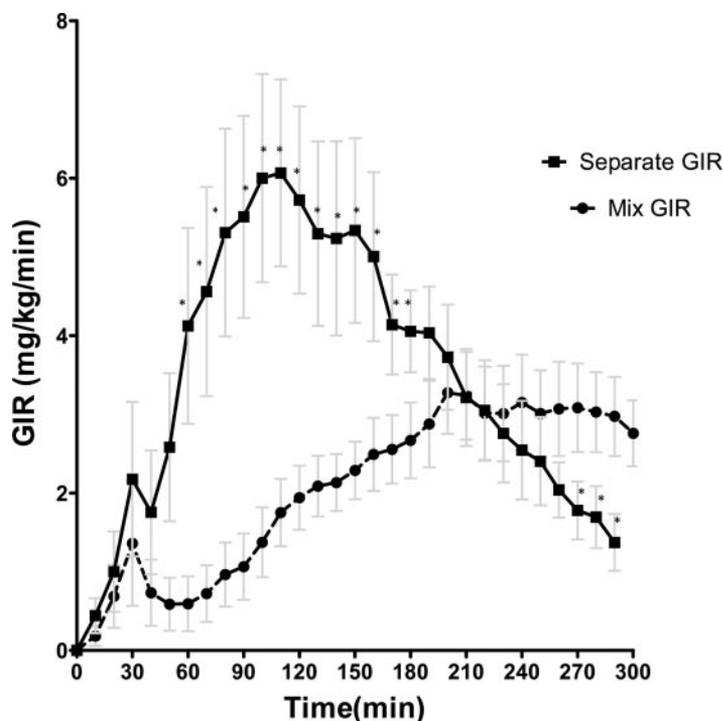

Figure 1—*Pharmacodynamic profiles. Insulin action, as expressed as GIR, required to maintain euglycemia after a standard bolus of 0.2 units/kg insulin lispro and 0.4 units/kg of insulin glargine mixed or separate during the 5 h of study are shown. Data are means ± SEM. *Time points where differences between the two studies were statistically significant ($P < 0.05$–0.001).*

nosorbent assay (ELISA) test. This assay has a reported cross-reactivity of 89% with insulin lispro and 44% with insulin glargine (13).

Statistical analysis

Statistical comparisons were performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA) and SAS version 9.2 (SAS Institute Cary, NC). Data are expressed as means ± SEM. Exogenous glucose infusion rate (GIR) analyzed every 10 min was adjusted for changes in the glucose space, as previously described (14). The pharmacodynamic parameters that were calculated for each clamp study included area under the curve of the glucose infusion rate ($AUC_{GIR\ 0-300}$), maximum glucose infusion rate (GIR_{max}), and time to maximum glucose infusion rate (T_{GIRmax}). Pharmacokinetic parameters included AUC_{ins}, peak concentration of insulin ($C_{ins-max}$), and time to $C_{ins-max}$ ($T_{ins-max}$). Plasma glucose, pharmacodynamic, and pharmacokinetic parameters were compared using paired t tests. A mixed-model repeated-measures analysis was used to analyze differences in GIR and plasma insulin levels between the two studies across time points. Because there was a significant

group × time effect in both GIR ($P < 0.001$) and plasma insulin ($P < 0.001$) responses, paired t tests were used to localize the effects.

RESULTS — A total of 11 subjects with type 1 diabetes, age 15.1 ± 2.9 years and A1C of 7.6 ± 0.6%, were enrolled and completed both clamp studies. Plasma glucose levels were similar during the mixed and separate injection studies at baseline (121 ± 9 vs. 126 ± 12 mg/dl) and during the 5 h of the clamp (95 ± 2 vs. 99 ± 2 mg/dl, respectively). As shown in Fig. 1, compared with separate injections, mixing the two insulins significantly shifted the time action curve to the right, with significantly lower GIR values after the mixed injections between 60 and 190 min and significantly higher values between 270 and 300 min. As shown in Table 1, mixing significantly reduced the GIR_{max} ($P = 0.03$), delayed the T_{GIRmax} ($P < 0.0001$), and decreased overall $AUC_{GIR\ 0-300}$ ($P = 0.03$). As shown in Fig. 2 and Table 1, mixing lispro with glargine had pharmacokinetic effects that were similar to the changes in pharmacodynamics; namely, plasma insulin levels were lower between 10 and 170 min,

Table 1—*Pharmacodynamic and pharmacokinetic summary measures after subcutaneous injection of insulin glargine and lispro in separate or mixed injections*

	Separate injection	Mix injection	P
Pharmacodynamics			
GIR_{max} $(mg \cdot kg^{-1} \cdot min^{-1})$	7.1 ± 1	3.9 ± 1	0.04
T_{GIRmax} (min)	116 ± 8	209 ± 15	<0.001
$AUC_{GIR\ 0-300}$ (min)	$1,050 \pm 202$	613 ± 109	0.03
$AUC_{GIR\ 0-90}$ (min)	250 ± 32	64 ± 72	0.04
$AUC_{GIR\ 210-300}$ (min)	201 ± 36	273 ± 48	0.1
Pharmacokinetics			
$C_{ins-max}$	149 ± 38	53 ± 9	0.04
$T_{ins-max}$ (min)	55 ± 6	106 ± 19	0.04
$AUC_{insulin\ 0-300}$ (min)	$27,134 \pm 6,088$	$16,354 \pm 4,101$	0.01
$AUC_{insulin\ 0-90}$ (min)	$10,195 \pm 1,965$	$3,934 \pm 857$	0.0007
$AUC_{insulin\ 210-300}$ (min)	$4,346 \pm 1,107$	$4,775 \pm 1,398$	0.5

Data are means $\pm$ SE. *P* values refer to the significance of differences between separate and mixed injections.

overall AUC_{ins} and $C_{ins-max}$ were reduced, and $T_{ins-max}$ was delayed.

CONCLUSIONS — Our data demonstrate that the pharmacokinetic and pharmacodynamic (PKPD) profiles of lispro insulin are markedly altered when lispro is mixed with glargine insulin. Mixing caused a marked delay in the peak insulin action compared with when lispro and glargine were given as separate injections in this study and in comparison to the timing of peak lispro action previously reported in continuous subcutaneous insulin infusion–treated adolescents after a similar 0.2 units/kg bolus of lispro insulin alone (12). Moreover, mixing significantly diminished the peak lispro effect (GIR_{max}), as well as the overall $AUC_{GIR\ 0-300}$, in comparison to corresponding values when the two insulins were given as separate injections. Compared with separate injections, mixing the two insulins resulted in increased GIR values during the last hour of the clamp, which was limited to 5 h because of the difficulty of extending the duration of clamp procedure further in this age-group.

Paradoxically, determination of the pharmacokinetic effects of mixing lispro with glargine is more challenging in many ways than determining the changes in pharmacodynamics. Many of the available insulin assays measure only a small fraction of the total circulating concentrations of analog insulins or have substantial cross-reactivity between analog insulins. We used the Mercodia iso-insulin assay in this study because it detects >80% of circulating lispro concentrations and because cross-reactivity with glargine would be expected to have only a small effect on the early rise in plasma insulin levels that was being investigated in this study. Based on the first-dose pharmacokinetics of glargine (4) and the 44% cross-reactivity of glargine in the iso-insulin assay, the maximum contribution of glargine to the measured plasma levels was likely to be <10 μU/ml at any time during the 5 h of the study.

Nevertheless, it is important to emphasize that this study does not delineate the PKPD of insulin glargine due to the low specificity of this assay for insulin glargine and the confined 5-h duration of our clamp procedure. Despite these limitations, the pharmacokinetic effects of mixing lispro with glargine paralleled the changes in pharmacodynamics. It is noteworthy that the effects of mixing lispro insulin with glargine in adolescents with type 1 diabetes in this study were similar to those observed with mixing of regular insulin and glargine in prior animal studies (3).

The findings of this study are not incompatible with those of previous clinical trials that failed to observe deterioration in A1C levels or daily glucose profiles in children and adolescents with type 1 diabetes when lispro was mixed with glargine. We only studied a fixed 1:2 ratio of lispro to glargine, whereas the fraction of lispro can be increased in clinical studies to compensate for the delayed and diminished peak action of lispro when it is mixed with glargine. Moreover, in practice, the potential adverse effect of mixing lispro and glargine at dinner may be offset by greater compliance with the other two to four injections of lispro insulin that are required in multiple daily injection regimens. Nevertheless, the alterations in the time action profiles that we have observed in this study raise serious concerns that there will be a greater risk of early postprandial hyperglycemia after mixed doses of lispro and glargine. As important, when this mixture is used at dinnertime, the delayed increase in the action of lispro ≥ 5 h after the dose, corresponding to 3–4 h after the bedtime of young children and possibly when parents are also asleep,
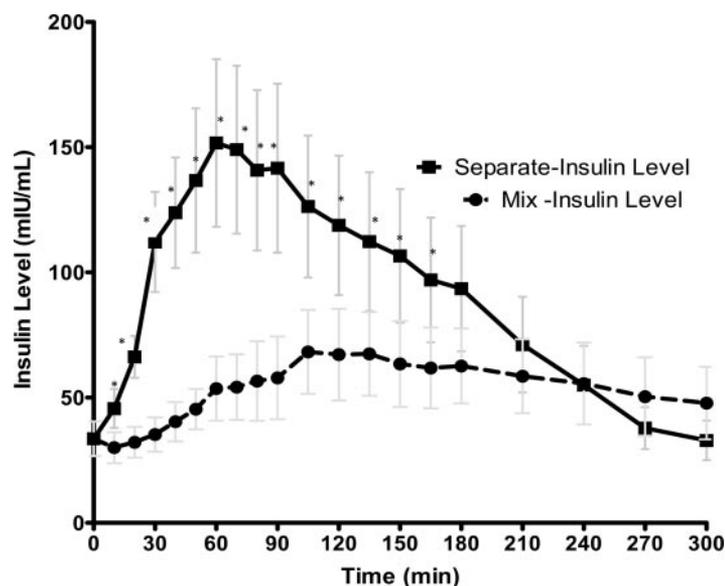

Figure 2—*Pharmacokinetic profiles. Insulin concentration, measured by ELISA with a reported cross-reactivity of 44% for insulin glargine, for separate and mixed injections depicted in means ± SEM for separate and mixed insulin glargine-lispro injections. *Time points where differences between the two studies were statistically significant (P < 0.05–0.001).*

may increase the risk of episodes of nocturnal hypoglycemia.

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