Insulin Aspart in a 30/70 Premixed Formulation

Pharmacodynamic properties of a rapid-acting insulin analog in stable mixture

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OBJECTIVE — To study the pharmacodynamic properties of a 30/70 premixed formulation of the rapid-acting insulin analog insulin aspart (B28Asp) and its protamine-retarded preparation (30/70 IA) in comparison with a respective mixture of soluble human insulin and NPH insulin (30/70 HI).

RESEARCH DESIGN AND METHODS — In this single-center double-blind euglycemic glucose-clamp study, 24 healthy male volunteers (age, 26 ± 2 years; BMI, 23.7 ± 1.7 kg/m^2) received single subcutaneous injections of 0.3 U/kg body wt of either 30/70 IA or 30/70 HI on 2 study days in randomized order. Glucose infusion rates (GIRs) were determined over a 24-h period after administration.

RESULTS — The injection of 30/70 IA resulted in an earlier onset and more pronounced peak of action (t_{max}, 127 ± 24 min; GIR_{max}, 9.7 ± 2.3 mg · kg^{-1} · min^{-1}) than 30/70 HI (t_{max}, 185 ± 52 min; GIR_{max}, 7.4 ± 1.7 mg · kg^{-1} · min^{-1}) ($P < 0.001$). The metabolic activity of 30/70 IA (measured as the sum of the glucose infused) within the first 4 h after injection was 37% greater than that of 30/70 HI ($P < 0.0001$), while the total metabolic potencies over 24 h of both preparations were comparable.

CONCLUSIONS — The 30/70 premixed formulation of insulin aspart shows a significantly greater metabolic effect in the first 4 h after subcutaneous injection than the 30/70 mixture of human insulin. Insulin aspart retains its pharmacodynamic properties in a premixed 30/70 formulation.

Premixed formulations of regular and NPH insulins are the most often prescribed insulin preparations, at least among type 2 diabetic patients. Because of the slow onset of the regular insulin, it is difficult to adequately cover the prandial insulin requirements in patients with such insulin mixtures. Subcutaneous injection of rapid-acting insulin analogs (IAs) provides better prandial insulin substitution, resulting in the reduction of postprandial hyperglycemia (1,2). Insulin aspart (B28Asp) is a novel rapid-acting insulin analog in which the amino acid proline at position 28 of the B-chain is replaced by aspartic acid. This change results in a faster absorption of the insulin molecules due to a lower self-association tendency to hexamers (1).

The time-action profiles of premixed formulations with this rapid-acting IA have not been studied so far. When insulin aspart and NPH insulin remain in prolonged contact within a mixture, an exchange between the soluble analog and protamine-bound human insulin takes place. To avoid such an exchange, the protamine-retarded portion of the premixed formulation has to be formulated with the IA. The novel protamine-retarded formulation of insulin aspart allows for the formulation of a stable 30/70 mixture (30/70 IA, consisting of 30% insulin aspart and 70% of its protamine-retarded formulation).

In a euglycemic glucose-clamp study, we investigated the pharmacodynamic properties of 30/70 IA in comparison with the respective mixture of human insulin (30/70 HI) after subcutaneous injection in healthy subjects to determine whether the rapid onset of action of insulin aspart is retained when combined with its protamine-retarded formulation.

RESEARCH DESIGN AND METHODS

— This study was a single-center randomized double-blind crossover trial. The protocol was approved by the local ethical committee, and the study was carried out according to the Declaration of Helsinki. Twenty-four healthy male volunteers (age, 26 ± 2 years; BMI, 23.7 ± 1.7 kg/m^2) received in random order a single injection of either 30/70 IA or 30/70 HI (Actraphane, Novo Nordisk, Bagsvaerd, Denmark) on 1 of 2 study days. All subjects were nonsmokers for at least 3 months and free of concomitant illness and medication and of a family history of diabetes. Subjects were connected to a Biostator (Life Science Instruments, Elkhart, IN), and a euglycemic glucose clamp was established (intravenous insulin infusion of 0.15 mU · kg^{-1} · min^{-1}) (3). After a baseline period of 2 h, the subjects received a subcutaneous injection of 0.3 U/kg body wt of either insulin preparation into a paraumbilical skinfold by means of a syringe (Low-Dose Micro-Fine IV, Becton-Dickinson, Heidelberg, Germany). Glucose infusion rates (GIRs) necessary to keep blood glucose levels constant at 5.0 mmol/l were monitored during the subsequent 24 h. Blood samples were drawn at 30-min intervals for the estimation of serum insulin concentrations, measured by a commercial radioimmunoassay kit (similar affinity of the antibody to human insulin and insulin

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Abbreviations: AUC, area under the curve; CV, coefficient of variation; GIR, glucose infusion rate; HI, human insulin; IA, insulin analog.

aspart). Plasma glucose concentrations were estimated by use of the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, CA). Serum C-peptide levels were measured by a standard commercial enzyme-linked immunosorbent assay kit. After subcutaneous injection of either insulin formulation, mean C-peptide levels remained <0.3 nmol/l.

Results are given as mean ± SD throughout the text and as mean ± SE in the figure. An exponential function was fitted to each of the individual GIR profiles (4), allowing for the calculation of the following pharmacodynamic summary measures: maximal GIR (GIR_{max}), time to GIR_{max} (t_{max}), time to early and late half-maximal GIR values (early $t_{50\%}$ and late $t_{50\%}$), and the area under the GIR curves (AUCs) for different time intervals. A two-sided paired t test was used for statistical comparison of the summary measures. The pharmacokinetic summary measures were evaluated by fitting a polynomial function to the individual serum insulin concentration profiles with subsequent graphic estimation of the following parameters: maximal serum insulin concentration (C_{max}) and time to early half-maximal (early $t_{50\%}$), maximal (t_{max}), and late half-maximal (late $t_{50\%}$) C_{max}. AUCs were calculated for different time periods under the individual serum insulin profiles by means of the trapezoidal rule.

RESULTS — Maximal metabolic activity after subcutaneous injection of 30/70 IA was higher and was reached earlier than with 30/70 HI (Fig. 1*A*, Table 1). The onset of action (early $t_{50\%}$) was more rapid as well. This resulted in greater glucose consumption (AUCs) within the first 4 h. Beyond 8 h, AUCs were lower with 30/70 IA than with 30/70 HI (AUC, 480–1,440 min, 1.42 ± 0.86 vs. 2.11 ± 0.93 $g \cdot kg^{-1} \cdot 960\ min^{-1}$; $P < 0.02$). However, the overall metabolic effect of both insulin preparations did not differ.

Serum insulin concentrations peaked at higher values and were reached earlier after injection of 30/70 IA than after 30/70 HI (Fig. 1*B*, Table 1). The AUCs under the serum-insulin concentration profiles were different in the first 10 h after injection, but were comparable over 24 h.

Mean blood glucose concentrations during the clamps with 30/70 IA (4.9 ± 0.3 mmol/l [coefficient of variation (CV) = 4.7%]) were comparable to those with 30/70 HI (5.0 ± 0.2 mmol/l [CV = 4.9%]). No blood glucose values <4.0 mmol/l were observed.

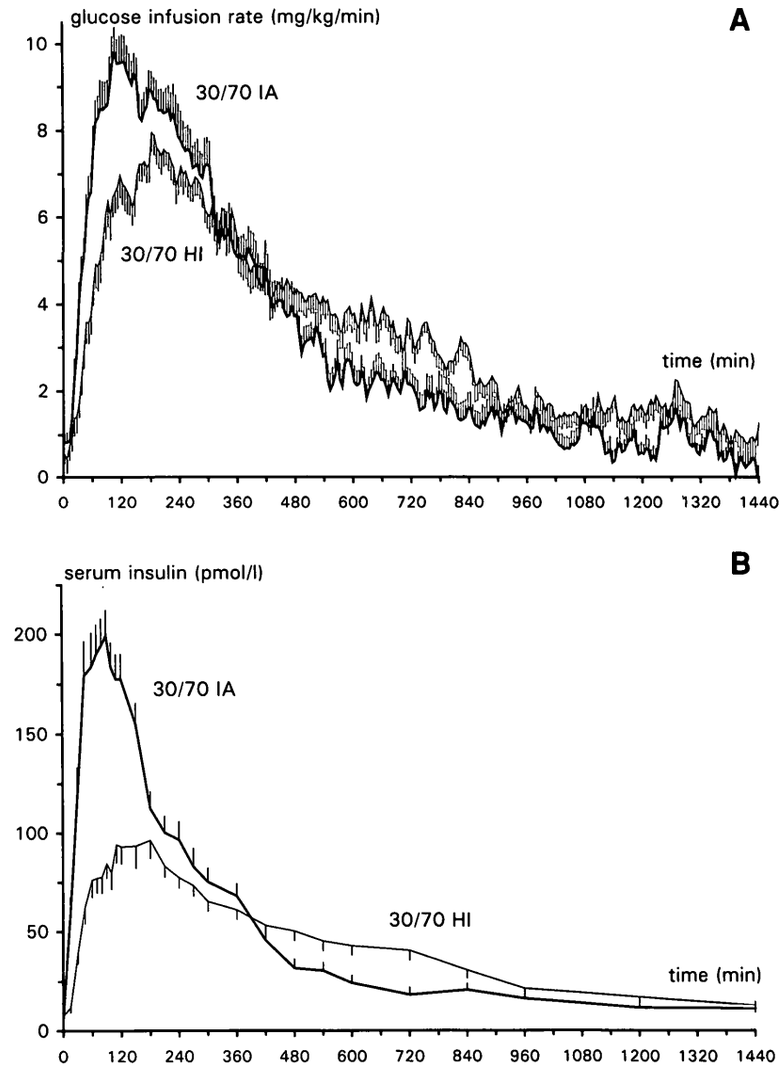

Figure 1—*Glucose infusion rates (A) and serum insulin concentrations (B) after the subcutaneous injection of 0.3 U/kg body wt (mean dose, 22.8 ± 2.6 U) of 30/70 stable mixtures, formulated with either the rapid-acting IA insulin aspart or with regular human insulin in 24 healthy male volunteers. Data are means ± SE.*

CONCLUSIONS — This study shows that the potentially beneficial pharmacodynamic properties of the rapid-acting IA insulin aspart (i.e., the faster onset and higher peak of action) are preserved in a premixed 30/70 mixture (3,5). Hence, the metabolic activity within the first 4 h after injection, most important for the control of postprandial glycemia, was considerably higher (37%) with 30/70 IA than with the 30/70 mixture formulated with human insulin. A comparable result has been described for a 25/75 mixture of insulin lispro and NPL-insulin, the protamine-retarded preparation of this rapid-acting analog (6). However, in this study, the two components were manually mixed immediately before injection.

Our study also shows that even the use of a rapid-acting IA in a premixed formulation does not result in a "biphasic" time-action profile, which is often thought to be characteristic for premixed insulins. The relatively rapid onset of action of the protamine-retarded insulin adds in such a manner to the glucose-lowering effect induced by the soluble part, that the result is a protracted decline of the activity, but not a second separate peak.

Beyond 8 h after injection of 30/70 IA, when the soluble part of insulin aspart is completely absorbed, the time-action pro-

Table 1—*Pharmacodynamic and pharmacokinetic summary measures of the time-action profiles of a 30/70 premixed formulation of insulin aspart (30/70 IA) or regular and NPH insulin (30/70 HI) registered in 24 healthy male volunteers during euglycemic glucose clamps*

	30/70 IA	30/70 HI	Difference	*P* value
Pharmacodynamic summary measures				
GIR_{max} (mg · kg^{-1}/min^{-1})	9.7 ± 2.3	7.4 ± 1.7	2.3 (1.3 to 3.2)	<0.0001
t_{max} (min)	127 ± 24	185 ± 52	−57 (−81 to −33)	<0.0001
Early $t_{50\%}$ (min)	41 ± 15	57 ± 22	−15 (−26 to −4)	<0.01
Late $t_{50\%}$ (min)	360 ± 147	574 ± 273	−220 (−321 to −119)	<0.0002
AUC_{0-90} (g · kg^{-1} · 90 min^{-1})	0.44 ± 0.15	0.26 ± 0.14	0.18 (0.09 to 0.26)	<0.001
AUC_{0-240} (g · kg^{-1} · 240 min^{-1})	1.77 ± 0.43	1.29 ± 0.34	0.48 (0.28 to 0.68)	<0.0001
AUC_{0-600} (g · kg^{-1} · 600 min^{-1})	3.45 ± 0.92	3.11 ± 0.68	0.33 (−0.11 to 0.77)	NS
$AUC_{0-1,440}$ (g · kg^{-1} · 1,440 min^{-1})	4.49 ± 0.15	4.74 ± 1.29	−0.26 (−1.11 to 0.59)	NS
Pharmacokinetic summary measures				
C_{max} (pmol/l)	183 ± 12	101 ± 8	82 (57 to 107)	<0.0001
t_{max} (min)	115 ± 3	177 ± 13	−63 (−89 to −37)	<0.0001
Early $t_{50\%}$ (min)	20 ± 2	40 ± 3	−20 (−26 to −14)	<0.0001
Late $t_{50\%}$ (min)	276 ± 8	534 ± 51	−258 (−364 to −153)	<0.0001
AUC_{0-90} (nmol · kg^{-1} · 90 min^{-1})	12.5 ± 5.3	7.8 ± 2.8	4.7 (3.2 to 6.2)	<0.0001
AUC_{0-240} (nmol · kg^{-1} · 240 min^{-1})	32.9 ± 11.2	21.1 ± 7.6	11.7 (7.7 to 15.7)	<0.0001
AUC_{0-600} (nmol · kg^{-1} · 600 min^{-1})	50.5 ± 17.9	41.3 ± 12.5	9.2 (2.0 to 16.5)	<0.02
$AUC_{0-1,440}$ (nmol · kg^{-1} · 1,440 min^{-1})	59.3 ± 24.7	61.9 ± 16.1	−2.6 (−13.3 to 8.2)	NS

Data are means ± SD, difference with 95% CIs.

file of 30/70 IA was lower in comparison with 30/70 HI. As the time-action profile of pure protamine-retarded insulin aspart has not yet been studied, it remains to be determined whether its metabolic profile is different from that of NPH insulin.

Whether the time-action profile of premixed insulin aspart provides a relevant improvement of meal-related metabolic control in diabetic patients, compared with premixed human insulin preparations, remains to be studied in clinical trials. So far, this has only been demonstrated for soluble rapid-acting insulin analogs (7,8). A previous attempt to enhance the initial metabolic effect of insulin mixtures, based on an increase of the proportion of regular insulin (from 30/70 to 50/50), resulted under glucose-clamp conditions in a 62% higher glucose consumption within the first 4 h after injection in healthy volunteers (9). However, comparing the postprandial hyperglycemic response to a standard breakfast, the injection of a preformulated 50/50 instead of a 30/70 mixture did not improve postprandial metabolic control in elderly type 2 diabetic patients (10). In agreement with this finding, the use of either a preformulated 30/70 mixture or extemporary mixtures of regular and NPH insulin at ratios from 20/80 to 40/60 did not result in differential metabolic control in an 8-week crossover study in type 1 diabetic patients (11).

In conclusion, the rapid-acting insulin analog insulin aspart retains its potentially beneficial pharmacodynamic properties when prepared in a stable 30/70 premixed formulation. The clinical relevance of these findings remains to be studied.

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References

1. Kang S, Creagh FM, Peters JR, Brange J, Vølund A, Owens DR: Comparison of subcutaneous soluble human insulin and insulin analogues (Asp^{B9},Glu^{B27}; Asp^{B10}; Asp^{B28}) on meal-related plasma glucose excursions in type I diabetic subjects. *Diabetes Care* 14:571–577, 1991
2. Heinemann L, Heise T, Wahl C, Trautmann ME, Ampudia J, Starke AAR, Berger M: Prandial glycaemia after a carbohydrate-rich meal in type I diabetic patients using the rapid acting insulin analogue [Lys(B28), Pro(B29)]. *Diabet Med* 13:625–629, 1996
3. Heinemann L, Heise T, Jorgensen LN, Starke AAR: Action profile of the rapid acting insulin analogue B28Asp. *Diabet Med* 10:535–539, 1993
4. Bender R, Heinemann L: Fitting non-linear regression models with correlated errors to individual pharmacodynamic data using SAS software. *J Pharmacokinet Biopharm* 23:87–100, 1995
5. Heinemann L, Kapitza C, Starke AAR, Heise T: Time-action profile of the insulin analogue B28Asp. *Diabet Med* 13:683–684, 1996
6. Radziuk J, Bradley B, Welsh L, DeFellipis MR, Roach P: Profiles of biological activity after subcutaneous administration of mixtures of Lys^{B28}-Pro^{B29} human insulin (Lispro) in soluble and neutral protamine formulations (Abstract). *Diabetes* 45 (Suppl. 2):218A, 1996
7. Anderson JH, Brunelle RL, Vignati L: Insulin lispro improves postprandial glucose control and reduces hypoglycemia rate in type 1 diabetes (Abstract). *Diabetologia* 38 (Suppl. 1):3, 1995
8. Round PM, Olsen KJ, Home PD, for the B28Asp UK Study Group: Improved blood glucose control with insulin analogue B28Asp (Abstract). *Diabetologia* 39 (Suppl. 1):88, 1996
9. Woodworth JR, Howey DC, Bowsher RR, Brunelle RL, Rowe HM, Compton J, Cerimele B: Comparative pharmacokinetics and glucodynamics of two human insulin mixtures. *Diabetes Care* 17:366–371, 1994
10. Brodows R, Chessor R: A comparison of premixed insulin preparations in elderly patients. *Diabetes Care* 18:855–857, 1995
11. Cucinotta D, Mannino D, Lasco A, Di Cesare E, Musolino C, Alessio R: Premixed insulin at ratio 3/7 and regular + isophane insulins at mixing ratios from 2/8 to 4/6 achieve the same metabolic control. *Diabete Metab* 17:49–54, 1991