Rapid communication

Action profiles of fast onset insulin analogues

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Summary. Recombinant DNA technology allows the production of insulin analogues with faster absorption rates from subcutaneous tissue as compared to conventional human regular insulin. We report the time-action profiles of 12 U subcutaneously injected insulin analogues (B9Asp + B27Glu or B10Asp) as evaluated against human regular insulin by means of the euglycaemic clamp technique (blood glucose 5.0 mmol/l) in healthy men. After injection of 12 U of either insulin preparation identical values were found for maximal insulin action (maximal glucose infusion rate, time to peak action), total amount of glucose infused as well as area under the curve of glucose infusion rate. Half-maximal glucose in-

fusion rate was reached significantly earlier after injection of modified insulins (mean $\pm$ SD 38 ± 7 and 43 ± 5 min) as compared to regular insulin (56 ± 14 min, $p < 0.01$). Forty-five min after injection of both insulin analogues glucose infusion rate had increased by 7.4 ± 1.8 or 6.1 ± 1.8 mg $\cdot$ kg⁻¹ $\cdot$ min⁻¹, reflecting 83 ± 27 or $67 \pm 15\%$ of maximal regular insulin action. In conclusion, the two tested insulin analogues showed similar action profiles, but a significantly faster onset of action as compared to regular insulin.

Key words: Insulin action profiles, timing of injection, insulin pharmacodynamics.

Absorption of regular insulin from subcutaneous tissue is too slow to mimic the normal rapid increments of insulin in blood in response to nutrient intake [1]. In neutral solutions of short-acting insulin preparations hexameric self-association of the insulin molecules occurs. An inverse correlation between insulin self-association and the rate of absorption from the subcutaneous tissue has been reported [2]. Recombinant DNA technology [3] allows synthesis of insulin analogues (IA) in which one or two amino acids in the peptide's sequence have been substituted, in order to reduce intermolecular binding properties responsible for self-association. These IA remain mono- or dimeric in concentrations of 40–100 U/ml and two to three times faster absorption rates after s.c. injection were demonstrated as compared to regular insulin [3, 4].

In order to establish time action profiles after s.c. injection of such insulin analogues as compared to regular human insulin, the euglycaemic glucose clamp technique with a low dose basal i.v. insulin infusion in order to suppress endogenous insulin release was employed in this study [5].

Subjects and methods

Subjects

Studies were carried out in eight healthy male volunteers 26 ± 3 years of age (mean $\pm$ SD), body mass index 22.8 ± 1.5 kg/m². They were given a detailed description of the study and written con-

sent was obtained; the study was carried out according to the principles of the Declaration of Helsinki. Subjects were asked to abstain from alcohol, coffee, and physical exercise for the last 16 h prior to the studies.

Study protocol

In randomized order the IA (X2 $\Rightarrow$ B9Ser $\rightarrow$ Asp + B27Thr $\rightarrow$ Glu or X10 $\Rightarrow$ B10His $\rightarrow$ Asp) or regular human insulin (Actrapid HM, U100) (all provided by Novo Industri, Bagsvaerd, Denmark) were used. The concentration of the IA was 0.6 μ mol/l, corresponding to 100 U/ml for human insulin. The production and purification procedures of the IA are identical to those used in the production of human insulin by genetic engineering [3].

After connection to a Biostat (Life Science Instruments, Elkhart, Ind., USA) baseline glucose infusion rates (GIR) were monitored for 2 h at a glucose clamp level of 5.0 mmol/l before 12 U (0.12–0.17 U/kg body weight) of insulin was injected s.c. into the abdominal wall by an insulin pen (Novo Pen II, Novo Industri, Bagsvaerd, Denmark). Glucose infusion rates were monitored throughout the subsequent 6 h.

Plasma glucose was monitored by the glucose oxidase method (Glucose Analyzer, Beckman Instruments, Fullerton, Calif., USA). Insulin and C-peptide were measured with commercially available radioimmunoassay kits (Phadebas Co., Uppsala, Sweden; RIAGnost HC-Peptid, Behring-Werke, Marburg, FRG). Details of this methodology to determine action profiles of s.c. injected insulin have been reported previously [5].

Statistical analysis

Results are given as means $\pm$ SD. The glucose infusion rate was printed every min by the Biostat and mean values of 5 min periods were calculated. Differences between glucose infusion rates of dif-

Table 1. Mean $\pm$ SD baseline glucose infusion rates, insulin-, and C-peptide-concentrations, onset of insulin action, peak glucose infusion rates (GIR), fractional glucose infusion, and area under the curve (AUC) during euglycaemic clamps after s.c. injection of 12 U of regular insulin or insulin analogue. Differences between treatments and subjects were analysed employing two-factorial ANOVA for randomized blocks. Significance is given for 'treatment'-blocks (insulin analogues vs regular insulin) while ^a indicates significant ($p < 0.05$) 'subject'-block differences. 'Maximal GIR' indicates the mean of highest GIR recorded individually during the 6 h study period

Parameter	Unit	Regular insulin	X2 insulin analogue (B9Ser + B27Glu)	X10 insulin analogue (B10Asp)	
Baseline glucose requirements (60 min prior to injection)	$\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	0.9 $\pm$ 0.9	0.8 $\pm$ 0.9	1.1 $\pm$ 1.2	NS
Baseline insulin concentration (at time of injection)	$\mu\text{U/ml}$	11.9 $\pm$ 4.2	11.7 $\pm$ 2.4	13.0 $\pm$ 4.6	NS
Baseline C-peptide concentration (at time of injection)	nmol/l	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	NS
Time and GIR when GIR had increased by 2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ over baseline after insulin injection	min $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	40 $\pm$ 13 4.0 $\pm$ 1.7	28 $\pm$ 6 4.7 $\pm$ 1.8	27 $\pm$ 7 3.8 $\pm$ 1.6	$p < 0.01$ NS
Time to half-maximal GIR after insulin injection	min	56 $\pm$ 14	38 $\pm$ 7	43 $\pm$ 5	$p < 0.01$
Maximal GIR was reached after (corrected for baseline)	min $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	138 $\pm$ 40 9.8 $\pm$ 2.5	114 $\pm$ 44 11.6 $\pm$ 2.3	106 $\pm$ 31 11.4 $\pm$ 1.9	NS NS
Glucose infused for 60 min after insulin injection (without baseline)	g	11 $\pm$ 7	20 $\pm$ 6	17 $\pm$ 5	$p < 0.01^a$
Glucose infused for 90 min after insulin injection (without baseline)	g	26 $\pm$ 15	40 $\pm$ 9	33 $\pm$ 9	$p < 0.01^a$
Glucose infused for 120 min after insulin injection (without baseline)	g	43 $\pm$ 23	61 $\pm$ 14	58 $\pm$ 14	$p < 0.01^a$
Maximal slope at time	min	0.1 $\pm$ 0.1 56 $\pm$ 14	0.2 $\pm$ 0.1 37 $\pm$ 20	0.2 $\pm$ 0.1 35 $\pm$ 18	$p < 0.05$ $p < 0.05$
AUC after insulin injection but without baseline glucose requirements	$(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \cdot \text{min}$	1962 $\pm$ 585	2172 $\pm$ 441	2116 $\pm$ 252	NS
AUC 60 min after insulin injection (without baseline)	$(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \cdot \text{min}$	115 $\pm$ 70	219 $\pm$ 65	187 $\pm$ 57	$p < 0.01$
AUC 90 min after insulin injection (without baseline)	$(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \cdot \text{min}$	295 $\pm$ 141	472 $\pm$ 92	436 $\pm$ 103	$p < 0.01^a$
AUC 120 min after insulin injection (without baseline)	$(\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) \cdot \text{min}$	501 $\pm$ 214	733 $\pm$ 136	694 $\pm$ 148	$p < 0.01^a$

ferent insulin preparations were tested by two way analysis of variance adjusted for repeated measurements (Statistical Analysis System, SAS Institute, Raleigh, NC, USA). F-values were calculated from two-factorial ANOVA for randomized blocks. Tukey's test for multiple comparisons was used to test between the insulin preparations.

Results

Baseline glucose requirements, insulin- and C-peptide-concentrations were not significantly different prior to each insulin preparation under investigation (Table 1). Glucose concentration was held constant at 5.0 mmol/l with a coefficient of variation of less than 5% and C-peptide levels remained suppressed below 0.3 nmol/l throughout the three different study protocols.

The complete time action profiles of both IA and regular human insulin are given in Figure 1. The time required until GIR was 2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ above basal rate after injection of the three insulin preparations was significantly shorter ($p < 0.01$) after both IA as compared to regular insulin. Maximal and half-maximal GIR for regular insulin

were not different from those for both analogues, however, half-maximal GIR was reached significantly earlier after the injection of the two IA as compared to regular insulin ($p < 0.01$) (Table 1).

Similarly, the total amount of glucose infused 60, 90 and 120 min after insulin injection – corrected for baseline requirements – was significantly higher after both analogues as compared to regular insulin ($p < 0.01$). The area under the curve for 60, 90 and 120 min after injection of IA revealed significantly higher values at the 1% confidence level. The maximal slope of increasing GIR after injection revealed significant differences between analogues and regular insulin as a measure of rapid onset of insulin action due to rising insulin or analogue concentrations during the first 60 min ($p < 0.05$). Forty-five and 60 min after injection GIR had increased by 3.2 ± 1.9 and $5.1 \pm 2.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ after regular insulin – reflecting 33 ± 14 and $50 \pm 20\%$ of maximal action of $9.8 \pm 2.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. However, 45 and 60 min after injection of insulin analogue X2 GIR had increased by 7.4 ± 1.8 and $8.6 \pm 2.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, reflecting 83 ± 27 and $92 \pm 25\%$ of maximal regular insulin action. Similarly, 45 and 60 min after injection

Fig. 1. Mean glucose infusion rates before and after the s.c. injection (▼) of 12 units of regular insulin (shaded area = SEM), insulin analogue X2 (◇—◇) or X10 (—) in eight healthy subjects. The variation of glucose infusion rates was comparable for the three investigated insulin preparations and superimposable for both insulin analogues. Blood glucose concentration was clamped at 5.0 mmol/l

tion of insulin-analogue X10 GIR had increased by 6.1 ± 1.8 and $8.1 \pm 2.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, reflecting 67 ± 15 and $88 \pm 33\%$ of maximal regular insulin action.

Discussion

Reduction of self-association of insulin molecules in subcutaneous tissue significantly changed the pharmacodynamic properties of the insulin analogue preparations investigated in this study in comparison to regular human insulin. Half-maximal action was obtained within 40 min after s.c. injection of both analogues as compared to nearly 60 min following the administration of regular human insulin. Forty-five and 60 min after injection of either IA the GIR necessary to maintain euglycaemia was $3.0\text{--}4.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ higher than the GIR required to neutralize the action of regular insulin. The most obvious differences vs the bioavailability of regular human insulin occurred between 30 and 120 min following s.c. injection. Thus, the area under the curve as well as the amount of total glucose infused were significantly higher 60, 90, 120 min after injection for both analogues. However, the overall bioavailability of equimolar amounts of regular insulin and the two insulin analogues did not show significant differences, as evident from the total amount of glucose infused.

Plasma concentrations of insulin or respective insulin analogue are not reported, since their statistical evaluation would be doubtful from a pharmacodynamic point of view due to in vitro receptor affinities of 20% (X2) and 300% (X10) that of regular human insulin [6].

In conclusion, the two tested insulin analogues showed similar overall action profiles, but a significantly faster onset of action as compared to regular insulin. Thus, these insulin analogue preparations might be helpful to achieve a more rapid insulin action after its s.c. injection, as they

may meet physiological prandial insulin demands more closely than regular human insulin preparations.

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