Are presently available insulin analogues clinically beneficial?

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Summary A number of insulin analogues have been developed by genetic engineering in order to improve the possibilities of substituting prandial and basal insulin requirements in diabetic patients by subcutaneous injection. For some short acting insulin analogues, in particular for [Lys(B28),Pro(B29)]-human insulin, preclinical and clinical trials have been performed. Despite the favourable pharmacokinetic and pharmacodynamic characteristics of these short-acting insulin analogues resulting in an attenuation of prandial hyperglycaemia following subcutaneous injection in diabetic patents, up to now, actual clinical benefits have not become apparent when they were used in clinical trials. [Diabetologia (1997) 40: S 91–S 97]

Keywords Insulin analogues, intensified insulin therapy, HbA$_{1c}$, hypoglycaemia, quality of life, insulin-dependent diabetes mellitus.

Beginning in the early 1980s, various strategies for intensified insulin therapy (IIT) were introduced in order to render subcutaneous insulin substitution somewhat more physiological; in principle, an attempt was made to separate the substitution of basal insulin requirements (usually provided by two injections of medium-long acting insulin in the morning and at bed-time; or by continuous subcutaneous infusion of regular insulin (CSII)) from prandial insulin needs (substituted preprandially as regular insulin injections). In many centres and in some countries, such forms of IIT have become the routine form of treatment for insulin-dependent diabetes mellitus (IDDM) [1]. As physicians and their patients gained experience with this type of insulin substitution, the drawbacks of available insulin preparations became more and more apparent. It was realised that the onset of action of regular insulin injected subcutaneously was too slow and its duration of action too long to mimic the physiological insulin secretion pattern during a carbohydrate containing meal [2]. On the other hand, available long-acting insulin preparations were obviously not suitable for providing a stable, continuous baseline insulin level; rather they led to peak serum insulin levels at around 3–4 h after subcutaneous injection and, furthermore, they appeared to be particularly variable with regard to their bioavailability, both inter- and intra-individually. Thus, clinical diabetologists were asking for regular insulin preparations with a more rapid onset of action and shorter duration of action and long-acting insulin preparations with more flat time-action profiles in order to improve insulin substitution therapies in the 1990 s [3].

As clear-cut as such requests might have been, the details of the absorption process of insulin from its subcutaneous injection depot into the circulation are only poorly understood. In fact, uncertainties exist as to the quantitative role of insulin degradation at the injection site [4], the contribution of the lymph-system and the molecular biology of the absorption

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Abbreviations: IIT, Intensified insulin therapy; CSII, continuous subcutaneous insulin infusion; IA, insulin analogue; NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus.

processes per se [5]. One aspect which might relate closely to the dynamics of the pharmacokinetics of subcutaneously injected insulin preparations, as described by Brange [6], was the fact that in commercially available preparations insulin molecules existed in aggregated forms of hexamers that needed to dissociate into dimers and monomers with dilution before the molecules could pass into the capillaries.

Current attempts to increase the speed of absorption of regular insulin preparations from the subcutaneous injection depot have used genetic engineering techniques to form insulin analogues which associate not at all or only loosely to hexamers. This was achieved by altering the amino acid sequence or composition of the insulin molecule at specific positions in order to decrease the adhesion tendencies leading to self-aggregation of the insulin molecules [6–9]. To this end, a considerable number of insulin analogues (IAs) have been manufactured by genetic engineering. Four of these analogues, [B9Asp,B27Glu]-human insulin (IA X2), [B10Asp]-human insulin (IA X10), [B28Asp]-human insulin (IA X14) and [Lys(B28),-Pro(B29)]-human insulin (insulin lispro (Humalog)) have undergone pre-clinical and clinical studies [8–10]; subsequently, further development of the IA X10 was discontinued after (at high doses) carcinogenicity was described in a rat model [11].

On the other hand, a variety of alterations of the insulin molecule by genetic engineering are being used to retard and stabilise absorption kinetics for long-acting insulin preparations. Thus, apart from increasing the self-aggregation tendencies by changing certain amino acids, the introduction/addition of basic amino acid residues at several locations of the A and B chain was performed in order to elevate the isoelectric point. Thus, the injection of a clear acid solution into the neutral pH subcutaneous tissue will result in a crystallisation of insulin molecules retarding the absorption of the IA into the circulation. Of the many potential long-acting IA, only NovoSol Basal [12] and Gly(A21),Arg(B31), Arg(B32)-human insulin (HOE901) have been used in clinical investigation, so far. Due to unacceptably low bioavailability of NovoSol Basal further studies with this compound have been discontinued. Presently, HOE 901 is being pursued in preclinical [13] and clinical studies; and a number of other preparations (such as acylated insulins which reversibly bind to albumins for protracted action [14]) will probably be subjected to clinical trials in the near future. Even with regard to HOE 901, the available information is still too fragmentary to draw any conclusions as to its clinical benefits.

The following is therefore restricted to describing the available evidence of the benefit of short acting IA. Of those, only X14 and insulin lispro are being pursued at present in ongoing clinical trials or are on the market. It is the purpose of this paper to review their clinical benefit. Reports available from published manuscripts and abstracts up to the end of 1996 have been screened to ascertain the clinical benefit provided by these new analogues.

Pharmacokinetic/pharmacodynamic evaluations of IA

In a number of studies using the euglycaemic glucose clamp technique it was clearly demonstrated that the IA X10, IA X14 and insulin lispro do have a favourable time-action profile when compared with regular human insulin preparations [15–17]. All of these IA are characterised by substantially shorter intervals between injection and onset of action and peak action, by somewhat higher biological effects at peak time, and a shorter duration of action [18]. These favourable pharmacodynamic characteristics of IA were compatible with data on the pharmacokinetics of IA, i.e. circulating insulin/IA levels, as reported in some of these investigations [17].

Evaluation of IA in clinical physiology

These studies have clearly documented that the changes introduced into the insulin molecule by genetic engineering have resulted for these IA in the intended alterations of the time-action profile when compared with regular human insulin. This would be expected to result in an improvement of glycaemic excursions during and after a carbohydrate rich meal in IDDM patients.

Pharmacokinetic and pharmacodynamic studies on the IA insulin lispro have confirmed these favourable properties in IDDM patients; furthermore, no changes in counterregulation, symptoms and deterioration of cognitive function after IA-induced hypoglycaemia were observed in comparison to regular human insulin [19, 20]. Consistent with the shorter duration of action, post-hypoglycaemic hyperglycaemia was more pronounced after insulin lispro [19] and the exercise-induced hypoglycaemia was more marked if exercise was performed early (40 min) and attenuated if performed 180 min after the injection of the IA [21]. In accordance with the different time-action profile of the IA insulin lispro prandial hyperglycaemia was attenuated when compared with regular insulin, even when regular insulin was given 30 min before and the IA only 5 min before breakfast [21].

Circulating fasting concentrations of glucagon and adrenaline were elevated in IDDM patients treated with insulin lispro when compared to regular insulin. This phenomenon which could not be explained by differences in fasting glycaemia or insulin levels [21] is potentially disturbing and needs to be re-examined in further studies.

In order to study the potential of IA to cover better the prandial insulin requirements in IDDM patients, we have carried out the following experiment in our clinical physiology laboratory in 10 patients with well-controlled IDDM on IIT or CSII [22]. On two occasions, the patients consumed a mixed meal which was rich in fast-absorbable carbohydrates, i.e. a pizza, 330 ml of (classic) Coca-Cola and a serving of *tiramisu,* altogether 140 g of carbohydrate, within 20 min. In a randomised, double blind fashion, the patients injected either regular human insulin or insulin lispro in identical individual dosages of approximately 15 IU immediately before consuming the meal. In accordance with the study hypothesis, the postprandial hyperglycaemic excursion was significantly less pronounced after the insulin lispro injection when compared to regular insulin (decreases of the area under the blood glucose-time$_{0-240\,min}$-curve to 78%, $p < 0.01$; of prandial peak hyperglycaemia, 9.9 ± 1.4 vs 11.9 ± 2.8 mmol/l, $p < 0.05$; and of mean time interval to return to baseline glycaemia, 108 vs 218 min, $p < 0.05$) with respective alterations in the radio-immunoassayable serum insulin/IA profiles [22]. Clearly, with IA insulin lispro prandial glycaemia can be substantially reduced after a mixed meal rich in rapidly absorbable carbohydrates. Based on this experimental study, insulin lispro has the potential to allow for a further reduction in dietary restrictions for diabetic patients on IIT.

Clinical evaluations of IA

The next step in describing a potential clinical benefit was to look at daily profiles of circulating glucose (and insulin) levels under IIT using IAs compared to human regular insulin to substitute prandial insulin requirements. So far as an attenuation of postprandial hyperglycaemia was strongly expected based on the unanimous results from pharmacokinetic and pharmacodynamic studies earlier findings using the IA X14 were disappointingly inconclusive [23, 24]; and using the IA X10, Nielsen et al. [25] reported – despite clearly elevated prandial insulinaemia – and attenuation of prandial hyperglycaemia only after breakfast without any significant improvement of glycaemia after lunch or after dinner. A somewhat similar phenomenon was described by Jacobs [20] comparing insulin lispro (given immediately before main meals) and human insulin Actrapid (given 30 min before main meals) in studies with 12 hospitalised IDDM patients on IIT: whereas using insulin lispro the serum insulin profile was substantially closer to the physiological pattern with mean maximal peaks at meal times 50–70% higher than following human regular insulin, the differences in glycaemic profiles were surprisingly less pronounced. In fact, only following lunch were prandial blood glucose levels significantly lower on insulin lispro, whereas after breakfast and dinner levels were similar [20].

Trials to document clinical benefit

Even though expectations were somewhat sobered by the reports on daily glycaemic profiles, evidence such as the results of the experimental studies [10, 22] was expected to translate into a clinical benefit for patients on insulin therapy in daily life. However, a definite *clinical benefit* can only be documented if the use of IA in the framework of optimised insulin treatment strategies (IIT for IDDM patients; and possibly conventional insulin therapy for non-insulin-dependent diabetic patients (NIDDM) results in an improvement of the overall outcome. The outcome of IIT can be quantified based upon three endpoint parameters: [1] mean values of HbA$_{1c}$, [2] incidence of severe hypoglycaemia (with the incidence of diabetic ketoacidosis being negligible, possibly except for patients on CSII); and [3] quality of life measures (mainly related to the degree of flexibility of lifestyle and nutrition). In order to prove a clinical benefit of IA their use must be associated with an improvement of any one or more of these parameters. Based upon a prospective 6-year follow up of IDDM patients treated with IIT as a routine therapeutic procedure we have proposed the following standards achievable under non-experimental routine conditions in our German health care system (using human regular and NPH insulin preparations): mean HbA$_{1c}$ over the entire observation period 7.6%, mean incidence of severe hypoglycaemia (defined as hypoglycaemia followed by treatment with glucose i.v. or glucagon injections) 0.17 cases per patient per year [26, 27]. Patients were instructed to inject regular insulin immediately before meals, i.e. not to keep injection-meal time intervals of more than 10–15 min, and they were not given any meal plan. In fact, after 6 years, 85% of the patients habitually consumed sugar or sugar containing nutrients and 53% skipped even main meals [26, 27]. Under central European health care systems, in order to prove a clinical benefit of insulin therapy with IA, such outcome measures would have to be improved.

Obviously, data potentially related to a clinical benefit of IA are only available for shorter periods of time. Nevertheless, taken together they can serve to answer the question of whether IA have the potential to improve the outcome of IIT in IDDM patients or more conventional insulin treatment strategies in elderly NIDDM patients.

In one randomised, double-blind study the question of a clinical benefit of IAs in IDDM patients on IIT was addressed without injection-meal time intervals (of more than 5 min) in either 2-month experimental period: the results were absolutely unequivocal: there

was no difference between regular human insulin Actrapid and the IA X10 with regard to any one of the outcome parameters documented [25]. Likewise, Jacobs found in his non-blinded study comparing regular human insulin Actrapid (injected 15–30 min before main meals) and insulin lispro (injected immediately before main meals) for periods of 4 weeks each in 12 IDDM patients on IIT, no difference with regard to the incidence of hypoglycaemia, and, quite unexpectedly, an improvement of HbA_{1c} levels associated with regular human insulin [20].

Altogether around 2000 insulin-treated diabetic patients have been subjected to the treatment with insulin lispro within the framework of clinical trials for up to 2 years' duration [28, 29]. Unfortunately, most of these studies – which should be relevant to the issue of clinical benefit of IAs – are available in abstract form only or else such results are being referred to in the context of non-original communications. Nevertheless, the following conclusions may provisionally be drawn while a more detailed analysis will have to await the full publication of all of these studies. A cross-over study including 1057 IDDM patients on IIT compared parameters of metabolic control when either human regular insulin (injected 30–45 min before main meals) or insulin lispro (0–15 min before main meals) was used for the substitution of prandial insulin needs for 3-month periods each. Mean fasting blood glucose concentrations were around 11.5 mmol/l and HbA_{1c} levels around 8.2 %; whereas prandial glycaemic excursions were attenuated, there was a slight reduction of (undefined) hypoglycaemic episodes (6.4 ± 7.6 vs 7.2 ± 8.1 per 30 days) during insulin lispro treatment. HbA_{1c} levels, however, remained unchanged [29]. Similar findings were reported for 722 NIDDM patients treated with IIT subjected to an identical study protocol: again during 3 months' insulin lispro therapy postprandial blood glucose levels were improved; but HbA_{1c} and fasting blood glucose levels remained unchanged at around 8.2 % and 10.5 mmol/l, respectively, whereas the frequency of hypoglycaemic episodes was comparable [30]. Another study compared the use of insulin lispro and human regular insulin in the context of conventional (twice daily free admixtures of NPH insulin and short acting insulin) insulin therapy in 49 NIDDM patients using an open-label 2-month cross-over design. In this study, the difference for the injection-meal interval was only 15 min (0 min during the insulin lispro vs 15 min during the control period). There were no differences with regard to glycaemic profiles, HbA_{1c}, or frequency of hypoglycaemic episodes [31]. In a randomised unblinded study with 39 IDDM patients treatment with insulin lispro did result in a decrease of 2 h postprandial glycaemia, but no improvement in HbA_{1c} [32].

From these preliminary data, Trautmann and Anderson [33] have concluded that extensive clinical trials with insulin lispro in more than 2000 IDDM and NIDDM patients have demonstrated decreased postprandial glucose excursions whereas no worsening of HbA_{1c} and no increase in rates of hypoglycaemic episodes were observed [33]. In another report describing the results with insulin lispro in Germany, the frequency of self-reported hypoglycaemic episodes was lower while using insulin lispro [34]. Analysis of the frequency of hypoglycaemic events of 1008 patients with IDDM showed a lower frequency with insulin lispro ($n = 16184$) than with regular insulin ($n = 22513$; $p < 0.001$); however, the frequencies were not different when more valid criteria to define hypoglycaemic events were used [35].

In a more recent double-blind cross-over study with 30 IDDM patients using CSII for their insulin therapy, use of insulin lispro resulted in a moderate but significant improvement of glycated haemoglobin from a basal value of 8.03 % to 7.65 % ($p = 0.0041$), while no decrease was seen with regular insulin (7.99 %) [36]. CSII allows a better substitution of the basal insulin requirements, probably under such circumstances the improvement in postprandial control by using a rapid acting insulin analogue may result in an improvement in glycated haemoglobin. Use of insulin lispro in patients with CSII resulted in better metabolic control; however, interruption of insulin infusion results in an earlier increase in glycaemia with insulin lispro [37].

The problem of an insufficient substitution of basal insulin requirements with the conventional long-acting insulin preparation was also demonstrated in another double-blind cross-over study [38]. Eighty-seven IDDM patients on an IIT from 10 different centres in Great Britain participated in this study. The use of insulin lispro for 3 months did not result in an improvement in HbA_{1c} in comparison to regular human insulin (7.44 vs 7.51 %). The patients injected once daily NPH-insulin in the evening. Due to the short duration of action of insulin lispro, insulin substitution during the daytime was insufficient, resulting in a significant increase of the preprandial glycaemia (lunch 7.6 vs 7.2 mmol/l ($p = 0.033$); dinner 9.1 vs 7.9 mmol/l ($p = 0.002$)).

Concluding comments

Based upon impressive data on the pharmacokinetics and pharamcodynamics, the short acting IAs should allow substantial improvement in prandial insulin substitution in the context of IIT.

Despite these clear-cut advantages, so far relevant clinical benefits have not become apparent although clinical trials have been performed with insulin lispro in some 2000 insulin-treated diabetic patients worldwide. Whereas prandial blood glucose excursions have been unequivocally attenuated, there was no

improvement in HbA$_{1c}$ levels. A tendency for a reduction in the rate of mild hypoglycaemic episodes was observed in most studies, significant in some studies only, but there was no reduction of the rate of severe hypoglycaemic episodes.

Subsequently, it has been argued that insulin lispro was associated with an increased quality of life because in most clinical trials patients were asked to keep a 30–45 min injection-time interval when on human regular insulin whereas insulin lispro was to be injected immediately before meals – something which was seen as a considerable improvement for the patients' lifestyle. In fact, as there was no other significant benefit, the possibility of omitting the injection-meal interval has been proposed as a specific advantage of insulin lispro by some investigators. Based upon our own experience this does not appear to be justified: ever since introducing IIT as a routine treatment for IDDM in the early 1980s we have advised our patients against fixed injection-meal-intervals of more than 10–15 min [39]. As the biological effect of human regular insulin starts 10–15 min after its subcutaneous injection [2, 5] such advice seemed necessary to protect patients who were aiming at near-normalisation of metabolic control from preprandial hypoglycaemia. The relatively low incidence of severe hypoglycaemia in our patient cohorts [1, 26, 27] may – at least to some extent – be related to our practice of not recommending injection-meal intervals which may have been suitable before the introduction of IIT. On the other hand, the inconsistent tendency to more frequent hypoglycaemic episodes during the control periods of insulin lispro trials [30] may be related to the recommendation of fixed injection-meal intervals of around 40 min during treatment with human regular insulin. In daily life, most patients seem to ignore the dogma of fixed injection-meal intervals, anyway [40]. In our opinion, patients on IIT should not be advised to keep fixed injection-meal intervals of more than 10–15 min independent of which regular insulin preparation they use; fast acting IAs are not required to abandon such an obsolete dogma.

It is surprising that in previous clinical trials HbA$_{1c}$ levels and incidence rates of hypoglycaemia were not consistent with a clinical benefit of fast acting IAs despite the clear-cut reductions in prandial hyperglycaemic excursions associated with the use of these IAs. This discrepancy may be related to the inadequate substitution of basal insulin requirements in most of these studies: with only once-daily injections of medium or long-acting insulin, such as NPH insulin preparations, any potential benefit of short-acting IAs may have been obscured. With a more adequate substitution of basal insulin requirements by twice daily injections of insulin preparations with retarded action profiles, such as NPH insulin, or by CSII, the advantages of short-acting IAs may become more readily apparent. In addition, future clinical trials on short-acting IAs should be carried out in a double-blinded fashion with no fixed injection-meal intervals in either arm of the trial. With these two provisos, a clinical benefit of short-acting IA may become demonstrable. Available long-acting IAs for an improvement in the substitution of basal insulin requirements look promising with regard to pharmacokinetic/pharmacodynamic evaluations. However, results from clinical trials to document potential clinical benefits concerning endpoint objectives of diabetes therapy will hopefully be available soon.

Very much against general expectations and despite voluminous trials world-wide, it has not been possible to document clear-cut clinical benefits of short-acting IAs. While the demonstration of clinical benefit is still pending the potential of side effects needs to be pointed out rigorously. As to unwanted potential growth-promoting effects, mitogenicity [41] and immunogenicity [42], "no matter which experimental models are used, in the end only long-term clinical trials will be able to confirm whether a recombinantly produced analogue" is safe. This caveat applies in particular to concerns about a potentially harmful side-effect of IAs' increased affinity to the insulin-like growth factor-1 receptor with regard to the development and progression of microangiopathic complications.

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