13 December 2012
EMEA/CHMP/BMWP/32775/2005_Rev. 1
Committee for Medicinal products for Human (CHMP)

Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues

Draft 1

Draft agreed by Biosimilar Medicinal Products Working Party (BMWP)	March 2005
Adopted by CHMP for release for consultation	May 2005
End of consultation (deadline for comments)	October 2005
Draft agreed by BMWP	January 2006
Adopted by CHMP	22 February 2006
Draft revision agreed by BMWP	November 2012
Adopted by CHMP for release for consultation	13 December 2012
Start of public consultation	14 December 2012
End of consultation (deadline for comments)	30 June 2013

This guideline replaces 'Guidance on similar medicinal products containing recombinant human soluble insulin' (EMEA/CHMP/BMWP/32775/2005).

Comments should be provided using this template. The completed comments form should be sent to BMWP.secretariat@ema.europa.eu

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

KEYWORDS	***recombinant human insulin, insulin analogues, similar biological medicinal products, biosimilar, comparability, non-clinical studies, clinical studies, insulin clamp, hyperinsulinaemic euglycaemic clamp, hyperinsulinaemic isoglycaemic clamp***

Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues

Table of contents

Executive summary

This guideline lays down the non-clinical and clinical requirements for recombinant insulin containing products, including human insulin and insulin analogues, claiming to be similar to another one already marketed.

The non-clinical section addresses the pharmaco-toxicological assessment. The clinical section addresses the requirements for pharmacokinetic, pharmacodynamic and safety studies as well as the risk management plan.

1. Introduction

The Marketing Authorisation (MA) application dossier of a new recombinant insulin claimed to be similar to a reference medicinal product already authorised shall provide the demonstration of comparability of the product applied for to this reference medicinal product.

Human insulin is a non-glycosylated, disulphide-bonded heterodimer of 51 aminoacids. Insulin analogues differ from human insulin by the substitution of aminoacids or other chemical changes such as addition of a fatty acid chain within the molecule. Insulin preparations differ mainly by their kinetic/pharmacodynamic profiles. They are usually classified as rapid-, short-, intermediate-, and long-acting preparations, and are used alone or as free mixtures or premixed preparations of rapid/short-acting insulin and intermediate/long-acting insulin in various proportions.

There is extensive experience with the production of insulin for therapeutic use from animal sources, in the form of semisynthetic insulin, and through different recombinant techniques. Physico-chemical and biological methods are available to characterise the primary, secondary and tertiary structures of the recombinant insulin molecule, as well as its receptor affinity and biological activity *in vitro* and *in vivo*. Current quality guidelines on comparability provide information on the characterisation and analysis of similar biological medicinal product and its comparator. For recombinant insulins, attention should be given to product related substances/impurities and process related impurities, and in particular to desamido forms and other forms that may derive from the expression vector or arise from the conversion steps removing the C-peptide and regenerating the three-dimensional structure.

Currently available insulins are administered subcutaneously or intravenously. The effects of insulin are mediated predominantly via stimulation of the insulin receptor but insulin is also a weak natural ligand of the insulin-like growth factor-1 (IGF-1) receptor. The same receptors are known to be involved in the mechanism of action relevant for the currently approved therapeutic indications of insulins.

Antibodies to insulin occur frequently, mainly as cross-reacting antibodies. These have been rarely described to have major consequences for efficacy or safety. The potential for development of product/impurity-specific antibodies needs to be evaluated. Possible patient-related risk factors of immune response are unknown.

2. Scope

The guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005) lays down the general requirements for demonstration of the similar nature of two biological products in terms of safety and efficacy.

This product-class specific guideline presents the current view of the CHMP on the non-clinical and clinical requirements for demonstration of comparability of two recombinant insulin-containing medicinal products. This guideline should be read in conjunction with the requirements laid down in the EU Pharmaceutical legislation and with relevant CHMP guidelines (see section 3 Legal Basis and relevant guidelines).

3. Legal basis and relevant guidelines

- Directive 2001/83/EC, as amended, in particular in Directive 2001/83/EC Art 10(4) and Part II of the Annex I of Directive 2001/83/EC, as amended.
- Guideline on similar biological medicinal products (CHMP/437/04)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005).
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: Quality issues (EMEA/CHMP/BWP/49348/2005) and EMA/CHMP/BWP/247713/2012)
- ICH guideline S 6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998)
- Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins (EMEA/CHMP/ 89249/2004)
- Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98)
- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006)
- Guideline on good pharmacovigilance practices (EMA/500020/2012)
- Guideline on good pharmacovigilance practices, Module V – Risk management systems (EMA/838713/2011)

4. Non-clinical studies

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in the response to the similar biological medicinal product and the reference medicinal product and should not just assess the response *per se*. The approach taken will need to be fully justified in the non-clinical overview.

Pharmacodynamic studies

In vitro studies

In order to assess any differences in properties between the similar biological medicinal product and the reference medicinal product, comparative studies such as *in vitro* bioassays for affinity, insulin- and IGF-1-receptor binding assays, as well as tests for intrinsic activity should be performed. Partly, such data may already be available from bioassays that were used to measure potency in the evaluation of physico-chemical characteristics. It is important that assays used for comparability testing are demonstrated to have appropriate sensitivity to detect minute differences and that experiments are based on a sufficient number of dilutions per curve to characterise the whole concentration-response relationship.

In vivo studies

Comparative study(ies) of pharmacodynamic effects would not be anticipated to be sensitive enough to detect differences not identified by *in vitro* assays, and are normally not required as part of the comparability exercise.

Toxicological studies

Generally, separate repeated dose toxicity studies are not required. In specific cases, e.g. when novel or less well studied excipients are introduced, the need for additional toxicology studies should be considered

Studies regarding safety pharmacology and reproduction toxicology are not required for non-clinical testing of a biosimilar containing insulin or insulin analogues. Studies on local tolerance are not required unless excipients are introduced for which there is no or little experience with the intended route of administration. If other *in vivo* studies are performed, local tolerance may be evaluated as part of these studies.

5. Clinical studies

Pharmacology studies

Demonstration of similar pharmacokinetic and pharmacodynamic profiles is considered the mainstay of proof of similar efficacy of the biosimilar and the reference insulin. For this purpose, cross-over, preferably double-blind insulin clamp studies using single subcutaneous doses of the test and reference agents and performed at an interval of a few days to a few weeks are considered suitable. The time-concentration and time-action profiles may be studied separately or, preferably, simultaneously (in the same clamp study). Separate pharmacology studies for intravenous use, if applicable, are not required.

Study population

The study population should be homogenous and insulin-sensitive to best detect potential product-related differences and may consist of normal-weight healthy volunteers or patients with type 1 diabetes.

Besides their better availability, healthy volunteers have the advantage of relatively consistent fasting blood glucose levels but the disadvantage of presence of endogenous insulin which cannot be distinguished from exogenously administered insulin by the available assays, except for some insulin analogues. Methods for suppressing endogenous insulin or adjusting measured insulin serum concentrations for estimated endogenous insulin should be considered (see below).

Patients with type 1 diabetes recruited into clamp studies should have their serum C-peptide concentration measured to ensure absence of relevant remaining endogenous insulin secretion. It is important to establish stable and comparable baseline blood glucose and insulin levels for some time (e.g. one hour) prior to the study intervention in order to achieve comparable baseline conditions in all experiments, which is usually more difficult in patients with type 1 diabetes compared to healthy subjects.

Insulin sensitivity in women may vary during the menstrual cycle and it is unclear whether this may affect study results. Thus, inclusion of only men in the studies would be justified.

Insulin clamp studies

There is general agreement that the euglycaemic or isoglycaemic hyperinsulinaemic clamp technique is the best available method for the measurement of insulin action. In these clamp experiments, the plasma insulin concentration is raised (e.g. by subcutaneous injection of insulin) and the blood-glucose level maintained (“clamped”) at a pre-defined level by means of a variable infusion of glucose. Measurements of plasma insulin concentrations and glucose infusion rate (GIR) allow an estimation of the time-concentration and time-action profile and, if investigated in the same clamp study, of the dose-response relationship of an insulin preparation. For the purpose of comparing the pharmacokinetic and pharmacodynamic profiles of a biosimilar and its reference insulin, these clamp experiments will need to be conducted by experienced investigators under highly standardised conditions.

Different clamp methods and feedback algorithms for maintaining blood glucose levels exist. Clamp studies can be performed manually or using an automated procedure, e.g. the Biostator. With a Biostator the blood glucose concentration is measured continuously (every minute), and the glucose infusion rates are calculated in a computerised manner by means of a negative feedback algorithm. The major disadvantage of the Biostator appears to be its age (successor models are under development) and difficulties to maintain the system. Manual clamps, on the other hand, are associated with higher blood loss when blood glucose measurements are performed with standard laboratory methods (typical measurement intervals 5 to 10 min) and have a considerable demand for manpower. Manual clamps are also more prone to bias by the examiner compared to automatic clamps. A double-blind design is therefore strongly recommended or, if this is not possible, other means to effectively reduce potential investigator-related bias. Both techniques require substantial experience. However, both methods have been reported to provide similar and reproducible results as long as there are no rapid changes in glucose requirements, which may not be recognised in time depending on the length of intervals between the blood glucose measurements during the manual clamp.

Test conditions for a comparative clamp study need to be strictly standardised. Study subjects should undergo the clamp experiments after an overnight fast (usually 10 to 12 hours, only water allowed) and remain fasting throughout the tests to avoid a confounding effect on study results. In patients with diabetes, carry-over effects from the participants’ last pre-study insulin injection should be prevented and intravenous insulin infusion started at least 4-6 hours prior to study insulin administration to attain steady-state baseline glucose levels. Ideally, the clamp glucose target should be reached at least one hour before study insulin administration without any glucose infusion during this last hour. Standardisation of clamp technique and factors influencing insulin sensitivity such as time of day, physical activity and food intake/diet, avoidance of alcohol, caffeinated drinks, smoking or medication other than the study medication and absence of intercurrent illness/infection or mental stress are important. Standardisation of habits may be relevant up to several days preceding the day of examination. In the test facility, the subjects should be allowed to adapt to the experimental situation (e.g. for 2 hours prior to the test) to establish a comparable metabolic situation and should stay in bed throughout the experiment in a quiet and pleasant environment. This highlights that even small details are very important. There is, however, evidence that, despite such standardisation, the first of the two clamps may be associated with a somewhat decreased insulin sensitivity, possibly due to an unavoidable increase in the test-related stress level of study subjects with the first clamp.

When healthy volunteers are used for the clamp studies, their endogenous insulin production can be suppressed, although usually not entirely, by a priming dose of rapid- or short-acting insulin, followed by a basal rate (e.g., 0.10 to 0.15 mU/min/kg). Alternatively, somatostatin has been used for maximal suppression of endogenous insulin, glucagon and growth hormone during the test period but it should be noted that somatostatin reduces insulin clearance by about 20%, thus prolonging the duration of insulin action artificially. Setting the target glucose level below the patient's fasting glucose also helps suppress endogenous insulin production. Serum C-peptide should be measured in parallel to insulin concentrations to estimate the extent and consistency of suppression of endogenous insulin throughout the experiment. In the absence of insulin suppression, C-peptide correction methods have also been proposed but their value is unclear. Regardless which method is used, it should be justified and consistent throughout the clamp studies to ensure comparable test conditions.

The subcutaneously administered dose of the test and reference insulin should reflect commonly used therapeutic doses. For rapid-/short-acting insulins doses of 0.2 to 0.3 U/kg bodyweight and for intermediate-/long-acting insulins doses of 0.3 to 0.4 U/ kg bodyweight are frequently used. The mid-physiological range of hyperinsulinaemia (60-70 µU/ml), which represents the typical insulin concentration after a standard meal, has been shown to correspond to the steepest part of the dose-response curve of insulin and can thus be expected to be most sensitive to detect potential differences in the time-action profiles of two insulins. All injections should preferably be performed by the same experienced investigator in order to ensure a reproducible subcutaneous injection. The site of injection, known to potentially influence the rate of absorption of insulin, should also be the same to decrease variability.

In healthy subjects the blood glucose concentrations are usually clamped 5 mg/dL below the subjects fasting glucose or at 80-100 mg/dL (4.4-5.6 mmol/L). In patients with type 1 diabetes blood glucose concentrations may also be clamped in the euglycaemic range or at typical/target fasting blood glucose levels (isoglycaemic clamp), which may exceed the normal range for healthy subjects. Glucose levels below approximately 60 mg/dL should be avoided because they result in the stimulation of counterregulatory hormones (epinephrine, glucagon, cortisol, growth hormone) to increase blood glucose concentrations and lead to a rapid and pronounced worsening of insulin sensitivity, thus influencing the estimated time-action profile of the investigated insulin preparation.

The duration of the clamp studies needs to take into account the known duration of action of the investigated insulin preparation and its dose-dependency. The duration of action in glucose clamp studies may be defined as the time from insulin injection to GIR returning to baseline or, in patients with diabetes, of blood glucose values exceeding a predefined threshold, e.g. 150 mg/dL (8.3 mmol/l). Clamp durations of 8 to 10 hours for rapid- and short-acting insulins and of 24 hours and more for long-acting insulins have been reported for healthy volunteers or patients with type 1 diabetes when using therapeutic doses. A rationale for the selection of the clamp duration should be provided in any case.

Endpoints/statistical analyses

Pharmacokinetics

Comprehensive comparative data should be provided on the time-concentration profiles of the biosimilar and the reference insulin with AUC and C_{max} as the primary and T_{max}, early and late $T_{50\%}$, and $T_{1/2}$ as secondary pharmacokinetic endpoints. Alternatively to early and late $T_{50\%}$, other measures (e.g. $AUC_{0\text{-}Tmax}$) may be used, as appropriate. For the primary endpoints AUC and C_{max}, the 90% confidence interval of the ratio test/reference should lie within 80% to 125%, the conventional acceptance range for bioequivalence, unless otherwise justified. For the other parameters descriptive statistics would be appropriate.

Pharmacodynamics

The glucose-infusion rate (GIR) over time describes the time-action profile of an insulin preparation. GIR_{AUC} and GIR_{max} should be measured as primary and T_{GIRmax}, and early and late $T_{GIR50\%}$ as secondary pharmacodynamic endpoints. Alternatively to early and late $T_{GIR50\%}$, other measures (e.g. GIR-AUC_{0-Tmax}) may be used as appropriate. Calculation of 95% confidence intervals will be required for PD parameters. Equivalence margins should be pre-defined and justified.

It is not easy to control the blood glucose concentrations during the clamp study. Depending on the measurement intervals and feedback algorithm, and due to the inherent measurement delay between sampling and resetting the glucose infusion and the subsequent delay of change in blood glucose levels in response to GIR changes, blood glucose values usually do not correspond to the exact target value but vary around it. In response to that, variations ("noise") in GIR occur. The Applicant should provide an estimate of the quality of the performance of the clamp study, e.g. by calculating the coefficient of variation of the blood glucose concentrations. The mean intra-individual coefficient of variation of well executed euglycaemic hyperinsulinaemic clamps should usually not exceed 10% for glucose infusion rate. The noise of the GIR measurements can be reduced by fitting a mathematical model. The algorithm for GIR adjustment should be predefined and the appropriateness of the applied smoothing method demonstrated.

Specifics of long-acting insulin preparations

Long-acting insulin preparations are intended to produce a time-concentration profile which, as far as possible, approximates physiological basal insulin secretion. For long-acting insulins with a very flat pharmacokinetic profile, determination of C_{max} and T_{max} (for insulin and GIR) may be difficult to assess and may even become meaningless. For long-acting insulins with a slow decline in insulin action, together with the unavoidable variations of the GIR, it may be difficult to determine the duration of action, particularly in healthy subjects with interfering endogenous insulin. Therefore, patients with type 1 diabetes are more suitable to determine the time-action profile of long-acting insulins. Insulin sensitivity may increase over time in long-term clamp studies, which may affect GIR. However, when strict standardisation of the test conditions (as described above) is implemented, a similar increase in insulin sensitivity over time in the same individual would be expected in both treatment phases of the cross-over study and should thus not impair the comparison of the biosimilar with the reference insulin.

Despite these limitations and the increased intra-subject variability of long-acting compared to short-acting insulins, the hyperinsulinaemic euglycaemic clamp has been successfully used for the comparison of the pharmacokinetic and pharmacodynamic profiles of currently approved long-acting insulin preparations. It should be noted that clamp studies for long-acting insulins may need to be of substantial duration (e.g. for insulin glargine in the clinically relevant dosage range, the duration of action is close to 24 hours in patients with type 1 diabetes).

Taken together, hyperinsulinaemic euglcycaemic/isoglycaemic insulin clamps, with some limitations, may be appropriate to compare the time-concentration and time-action profiles of long-acting biosimilar and reference insulins but will usually require a relatively large sample size and a long duration for the purpose of demonstrating similarity

Clinical efficacy

There is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered sensitive enough for the purpose of showing biosimilarity of two insulins.

Clinical safety

Convincing demonstration of similar physicochemical characteristics, pharmacokinetic and pharmacodynamic profiles of the biosimilar and the reference insulin will already provide reasonable reassurance that adverse drug reactions which are related to exaggerated pharmacological effects (e.g. hypoglycaemia) can be expected at similar frequencies. Therefore, the main focus of the safety study is the evaluation of immunogenicity, although similarity in the adverse event profile, e.g. with regard to hypoglycaemia and local tolerability, of the biosimilar and the reference product should also be confirmed.

Immunogenicity studies should always include a reasonable number of patients with type 1 diabetes. If a mixed population is included, stratification for type of diabetes and pre-existing anti-insulin antibodies is necessary. The study duration should be at least 12 months, including a comparative phase of at least 6 months. The primary outcome measure should be the incidence and titres of antibodies to the test and reference medicinal products but there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity. The potential impact of antibodies, if detected, on glycaemic control, insulin requirements and safety, especially local and systemic hypersensitivity reactions, should be investigated, and the necessity for further characterisation, e.g. with regard to their neutralising potential, considered.

6. Pharmacovigilance plan

Within the authorisation procedure the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should take into account identified and potential risks associated with the use of the reference product and, if applicable, safety in indications licensed for the reference product that are claimed based on extrapolation. In addition, it should be discussed in detail how these safety concerns will be addressed in post-marketing follow-up.

7. Extrapolation of indication

Demonstration of similar pharmacokinetic and pharmacodynamic profiles of the biosimilar and the reference product and absence of safety issues such as excessive immunogenicity with subcutaneous use will allow extrapolation of efficacy and safety data to intravenous use, if applicable, and to other indications and patient populations licensed for the reference product.