SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Apidra. For information on changes after approval please refer to module 8.

1. Introduction

This is a complete application for a marketing authorisation for the medicinal product Apidra submitted in accordance with Article 8.3 (i) of Directive 2001/83/EEC.

Apidra contains the new active substance insulin glulisine, an analogue of human insulin. Insulin glulisine is produced by recombinant DNA technology in *Escherichia coli (E. coli)* and differs from human insulin by two amino acid substitutions on the B chain of the protein. These give insulin glulisine a faster onset of action as they prevent the formation of inactive hexamers when injected. Insulins have a pronounced tendency to form hexamers that need to disintegrate to dimers and monomers to be pharmacologically active. The amino acid substitutions in insulin glulisine destabilise the hexamers and therefore enable a faster onset of action than that achieved with human insulin.

Apidra is indicated for the treatment of adult patients with diabetes mellitus.

Diabetes Mellitus is a chronic disorder characterised by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 1 diabetes mellitus is characterised by little or no insulin secretory capacity, and patients with type 1 diabetes mellitus require insulin for survival. In type 2 diabetes mellitus the combined effects of impaired insulin secretion and insulin resistance result in elevated blood glucose levels. In at least one-third of patients with type 2 diabetes mellitus the disease progresses to an absolute requirement for insulin therapy. In types 1 and 2 diabetes mellitus, hyperglycemia is a risk factor for microvascular complications exemplified by retinopathy, nephropathy, and neuropathy. Studies have shown that improving the control of blood glucose levels in patients with type 1 or type 2 diabetes mellitus reduces the risk of microvascular complications. Combinations of insulin preparations that differ both in their time of onset and duration of action are used to optimally control blood glucose concentrations in patients with diabetes mellitus. The most commonly used insulin regimens include a long-acting insulin to provide basal insulin requirements and control fasting and preprandial blood glucose values in combination with a short-acting insulin to control prandial blood glucose excursions.

Insulin glulisine displays a time-concentration and time-action profile with a more rapid onset, earlier peak effect in lowering blood glucose levels, and a shorter duration of action than the short-acting insulin preparation of regular human insulin. The time-concentration and time-action profiles of insulin glulisine define it as a member of the rapid-acting insulin subfamily of short-acting insulin preparations.

2. Chemical, pharmaceutical and biological aspects

Introduction

Apidra is a clear colourless solution for injection containing 100 units/ml of the active substance insulin glulisine. Apidra 100 units/ml is available in the following presentations:

- 10 ml injection vials
- 3 ml cartridges (for e.g. OptiPen Pro pen device)
- pre-filled pen, OptiSet (containing one 3 ml cartridge)

Vials and cartridges can also be used to refill external insulin infusion pumps.

The medicinal product contains as excipients metacresol, trometamol, sodium chloride, polysorbate 20, and water for injections. Hydrochloric acid (concentrated) and/or sodium hydroxide may be used to adjust the pH.

The two containers selected for this product are: (1) 10-ml injection vials made of colourless glass (Ph.Eur. Type 1), with aluminium cap and tear-off lid, and inserted rubber gasket; (2) 3-ml cartridges made of colourless glass (Ph.Eur. Type 1) with rubber stopper and aluminium cap, and inserted rubber gasket. Both, injection vials and cartridges are overfilled to ensure an adequate extractable volume.

Active Substance

General Information
Recommended INN: Insulin glulisine
Chemical name: 3^{B}Lys-29^{B}Glu -human insulin
Manufacturer's code: HMR 1964

Description of the active substance
Insulin glulisine is an analogue of human insulin and is manufactured from a fusion protein produced by *Escherichia coli* using recombinant DNA technology. The rapid acting time-action profile of insulin glulisine is achieved by exchange of two amino acids in the B –chain of human insulin. Asn is replaced by Lys at position 3^{B} and Lys is replaced by Glu at position 29^{B}. The molecular mass of insulin glulisine is 5923 Dalton.

The active substance is a powder of fine white to nearly clear white color. It is hygroscopic and shows a reversible uptake of atmospheric water. The solubility of the active substance is pH dependent. Insulin glulisine is practically insoluble in aqueous media around the isoelectric point of 5.1 and, as well, practically insoluble in methanol and ethanol.

- **Manufacture**

Development genetics
The genetic development has been extensively described and the rationale for the construction of the vector/host system has been given. The preparation of the production strain (*E. coli)* has been adequately described. In addition, the production strain has been described in sufficient detail.

Cell bank system
Description
For the production of insulin glulisine a two-tiered cell bank system has been established. The master cell bank (MCB) was prepared from the production strain. An aliquot of this MCB was used to prepare the first working cell bank (WCB) under the same culture conditions as for the MCB. All operations were performed under controlled conditions.

Testing
The identity, the microbiological purity, the viability of cells as well as the plasmid retention were determined for all cell banks. The growth characteristic and the ability to express the fusion protein were verified on the basis of lab-scale fermentation runs. Also, the identity of the plasmid and the maintenance of the correct coding sequence of the fusion protein and the relevant elements of the promoter and operator regions were verified by means of restriction enzyme and nucleic acid sequence analyses. Each subsequent working cell bank will be prepared and stored in the same manner as the current WCB.

Stability
The cell bank system is considered stable under defined storage conditions. The storage stability of the MCB and the WCB was determined by investigating relevant cell bank parameters, including microbiological, physiological, and molecular biological characteristics. After 5 years of storage, no changes have been observed in the investigated parameters. The product formation and growth of the production strain was adequately proven to be stable. No microbial contamination was detected. No decrease in product formation, viable cell count or plasmid retention was observed. The results of restriction enzyme analyses confirmed that the restriction patterns of the plasmid DNA remained unchanged. No insertions or deletions of the plasmid DNA were detected in the analyses. The complete sequence of the fusion protein and the sequence of the relevant elements of the promoter and operator region were confirmed for the master and the first working cell bank. The stability of the cell bank system will be controlled ongoing.

Genetic stability
The genetic stability of the production strain was proven during production and storage on the master and first working cell bank where the genetic stability of the expression system was adequately demonstrated by investigating production cells up to and beyond the generation number used in regular production. Cells from several regular fermentations and from two extended fermentations were examined for the genetic stability. The rate of plasmid retention was found to be 100 % during the complete cultivation time. The end of production cells were investigated by polyacrylamide gel electrophoresis, restriction enzyme, and nucleic acid sequence analysis demonstrating the genetic stability during production.

Fermentation and purification
The manufacturing process of insulin glulisine consist of 15 steps which can be divided into three major parts: cell culture and harvest, basic downstream processing and final purification. In summary, the insulin glulisine fusion protein is expressed by *E. coli* and stored in inclusion bodies within *E. coli* cells. The fusion protein is folded and then enzymatically converted during downstreaming processing. The product is thoroughly purified by chromatography.

Cell culture and harvest
The cell culture process (from WCB to harvest) has been adequately described and a flow chart submitted. In-process controls (IPC) assure appropriate cell growth and the absence of microbial contamination. In addition, the correct overall performance of the fermentation is verified by monitoring the production yield for which a lower limit has been set. Specifications for raw materials and a description of cell culture media have been provided. No human or animal derived materials are used in the cell culture process.

Basic downstream processing and final purification
The recovery and purification processes have been adequately described and flow charts have been submitted. Appropriate IPC have been justified and limits set to ensure consistency. In addition, upper and lower limits for the critical steps have been provided. All relevant buffers and reagents used during the purification process are described and specifications are presented.

Control of critical steps and intermediates
The manufacturing process of the active substance is monitored by appropriate in-process controls.

The basic downstream processing and final purification of the active substance are performed at two different plants. The transport of the intermediate to the final purification plant is carried out under controlled temperature conditions to maintain the quality of the product. Sufficient downstream in-process controls are in place to monitor the product in subsequent manufacturing.

Process validation

Adequate validation data derived from 4 consecutive production scale batches have been presented. All specifications were consistently met. The submitted data confirm that the applied procedures are adequately designed and controlled to yield a consistent quality of insulin glulisine. Studies to validate holding times of intermediates were provided and the presented data justify the proposed holding times. A sufficient cleaning validation study was performed for the active substance manufacturing process.

Viral validation studies were carried out on the production processes of the enzymes and on the insulin glulisine manufacturing process obtaining satisfactory results (see Adventitious Agents safety Evaluation).

Characterisation

The structure of insulin glulisine was satisfactorily characterised by state of the art methods using insulin glulisine primary reference standard: mass spectrometry, NMR spectrometry, infrared absorption spectrometry, ultraviolet absorption spectrometry, X ray diffractometry, amino acid sequence (Edman sequencing), UV circular dichroism spectrometry.

Impurities

A thorough discussion on process- and product-related impurities, e.g. host cell and vector DNA, host cell protein, enzymes, aggregates (high molecular weight proteins (HMWP)) and 21^{A}-Desamido-HMR1964) has been presented. The documentation submitted has sufficiently demonstrated the removal of the impurities. Further on, the impurities are either controlled by in process testing or by specification testing of the active substance. Alternatively adequate justification is provided. The tests used have been adequately described and validated. The results from the removal of the precursors and truncated forms during the purification steps of the process evaluation campaign 2002 have been submitted, which show that these impurities are also efficiently removed.

Specification of the active substance

Appropriate specifications have been set and justified for the active substance. Additional test parameters have been included in the active substance specification. The testing of these parameters may be discontinued, if supported by commercial scale data and after approval by a variation. The methods for release testing of the active substance are adequately described and validated according to ICH guidelines. Batch results presented show a high degree of batch-to-batch consistency.

Primary and secondary reference standards have been established in accordance with ICH Q6B requirements. Test results of both standards fully comply with the current active substance specifications. The stability of both reference standards is monitored ongoing.

Batch analysis

Batch results from 29 batches of insulin glulisine active substance used for toxicological and clinical trials as well as for primary stability studies were submitted including all batches manufactured at pilot and production scale. The results demonstrate a high degree of batch-to-batch consistency in the active substance manufacturing process.

Stability

The stability of the active substance is being investigated in an ongoing extensive study. The submitted results sufficiently show, that insulin glulisine is stable under long-term storage conditions (-20°C) and predict a 24-month storage period at –20°C.

Medicinal Product

Pharmaceutical Development

Product development
The development of the formulation and the choice of excipients has been fully described and justified. The excipients used in Apidra are well known, of pharmacopoeial grade and approved for parenteral use. Compatibility of insulin glulisine and the excipients has been satisfactorily addressed. The final, optimised formulation contains as excipients metacresol (antimicrobial preservative), trometamol (buffering agent), sodium chloride (tonicity agent), polysorbate 20 (stabilising agent) and water for injection (solvent). During the final formulation step, sodium hydroxide (alkalizing agent) and concentrated hydrochloric acid (acidifying agent) may be used for adjustment of pH.

Clinical trial formulations vs. intended market formulation
Four different formulations were used during development for the first pre-clinical and phase I clinical studies. Later phase I and all phase III studies were performed with batches, which qualitative and quantitative composition is identical to the medicinal product intended for the market.
Manufacturing process development
The manufacturing process remained largely unchanged throughout the process development. Only minor changes were introduced to simplify the process and to allow scaled up batch sizes. Stability studies show that these minor changes have no impact on the overall quality of the medicinal product.

Container closure system
Insulin glulisine solution for injection is supplied in two multiple-dose packages: 10 mL vials and 3 mL cartridges. Adequate specifications and routine tests have been established for these components and satisfactory batch analysis data have been submitted.

Manufacture of the Product
The manufacturing process is based on conventional dissolving, mixing, pH adjusting, filtration, filling and packaging techniques. These steps have been described in sufficient detail and a flow chart has been presented. Some Steps are performed under aseptic conditions. Sufficient data are provided to demonstrate that the manufacturing process is capable of removing endotoxins and controlling bioburden levels. Critical steps have been identified and are controlled. Recent certificates of analyses have been provided for all excipients besides water, for which the specification including frequency of testing was submitted. Appropriate in process controls have been set. All parts of the equipment and the primary packaging components that come in contact with the product are sterilised using a validated procedure.

Medicinal Product Specification
All methods for release testing of the medicinal product have been adequately described and are validated according to ICH guidelines. Appropriate medicinal product specifications have been set and justified. The acceptance criterion for total related impurities will be reviewed when 24 months stability data will be available. Any future changes to the limits for total related impurities and high molecular weight proteins in the medicinal product specification should be approved through a variation.

The activity of insulin glulisine will be labelled in units. The applicant has provided bioassay data which were obtained with the rabbit blood sugar method from the USP. Insulin human G USP was used as reference standard in the bioassay, and was used to calibrate the primary reference standard for insulin glulisine. In compliance with Ph.Eur. and ICH recommendations the activity of insulin glulisine will be labelled in units. The Ph.Eur. recommends the use of international units for human insulins but not for insulin analogues. ICH Q6B states that in case there is no international reference standard for a biological molecule, the potency of the molecule should be calculated against a characterised in-house reference material and the results should be reported as in-house units.

Process Validation
For process validation three consecutive batches of the bulk solution were manufactured at production scale and filled into 10 ml vials and 3 ml cartridges. The results from the validation study showed that the manufacturing process is capable of consistently producing Apidra 100 units/ml batches in production scale of the required quality.

Batch analysis data
Batch analysis data have been provided for laboratory, pilot and production scale batches of medicinal product manufactured between February 1998 and December 2002. Results have also been submitted for three process validation batches. All batches complied with the requirements of the medicinal product specification.

Stability of the Medicinal Product
The proposed shelf life for the 10 ml injection vials and the 3 ml cartridges is 24 months at 5°C. The applicant is carrying out an extensive, ongoing stability study over 24 months. Stability is monitored by a variety of test methods and results support the proposed shelf-life.

Based on results from the in-use stability study and in compliance with CPMP/QWP/159/96 corr., an in-use shelf life of 28 days at 25°C is approved for both, cartridges and vials.

According to the outcome of the photostability study, which revealed the photosensitivity of the medicinal product, Apidra should be stored protected from light.

Facilities and Equipment
Flow charts of the manufacturing process have been provided together with appropriate details of the equipment and facilities used for manufacture.

Adventitious Agents Safety Evaluation
No excipients of human or animal origin are used in the product manufacture and therefore there is no risk of contamination with viral or TSE agents by these ingredients. No animal ingredients are used for preparation of the cell banks or in the fermentation process. During downstream processing, enzymes are used, which are derived from porcine pancreas. All pigs used are suitable for human consumption and each batch of product must contain a certificate of animal origin and species in addition to the certificate of analysis. Viral validation studies were carried out to test the ability of the production process of the enzymes to remove and inactivate viruses. The submitted viral clearance study data demonstrate that the enzyme manufacturing processes are capable of inactivating/removing viruses. The choice of viruses, production steps investigated and the validity of the scale down process have been satisfactorily addressed. Based on these results, the possibility of viral contamination with the use of these enzymes in the production process of insulin glulisine is considered negligible.

Furthermore, a study on the viral clearance of the production process of insulin glulisine has been conducted. The study is adequately described and justified. Results demonstrate the ability of the insulin glulisine production process to significantly reduce possible viral contamination. Assurance has been provided that viruses potentially retained during chromatography will be eliminated by the sanitisation procedure. Data confirm the robustness of the production process. Parameters influencing the effectiveness of a production step on the inactivation and/or removal of viruses are controlled by appropriate process parameter ranges and acceptance limits. Based on the data provided Apidra is considered virologically safe.

Pen Devices
Insulin glulisine solution for injection filled into 3 mL cartridges can either be used in a reusable pen, e.g. OptiPen Pro, which is a medical device or be assembled in a disposable pen OptiSet. OptiPen Pro is a class IIb medical device, approved in the EU since 1998 under CE mark 0123. It is already commercialised for other insulins marketed by Aventis Pharma Deutschland GmbH. A copy of the EC certificate issued by the German Notified Body TÜV Product Service GmbH has been provided as well as an EC Declaration of conformity from Aventis Pharma.

A dose accuracy study has been carried out with Apidra in OptiPen Pro and OptiSet. The results obtained with both devices showed compliance with the requirements of ISO 11608-1 under the test conditions uesd. Based on these data, both Optiset and OptiPen Pro may be used with insulin glulisine solution for injection filled in cartridges.

Preservative efficacy in compliance with Ph.Eur. criteria A requirements was proven for cartridges stored at 25 °C for 6 months and 37 °C for one month. The content of M-cresol did not show a significant change during the in-use period, regardless of the storage conditions. No microbial contamination was detected. At the end of the in-use period the total viable aerobic count remained unchanged at < 1 cfu/ml.

The cartridges for use in the Optiset devices should be stored at 2 - 8 °C before use, and below 25 °C during use.

Pump Devices
Insulin glulisine solution for injection can be infused subcutaneously by external insulin pumps. Compatibility studies were performed to support the use of Apidra in infusion pumps and their corresponding infusion sets, when transferred into the cartridges and reservoirs recommended by the pump manufacturers. The results of the compatibility studies confirm the chemical and physical integrity of Apidra under the ordinary use in these pumps and infusion systems.

- Insulin Pumps and Infusion Sets Used in Compatibility Studies

Pump (manufacturer and type)	**Delivery System (Reservoir/Cartridge)**	**Infusion Set (manufacturer and type)**
Disetronic D-Tron	3ml cartridge (colourless glass)	Disetronic Rapid D CR12
		Disetronic Ultraflex soft
		Disetronic Tender
Disetronic H-Tron plus V100	3.15ml Cartridge (polypropylene)	Disetronic Tender
Medtronic Minimed 508	3ml Reservoir (Polypropylene)	Medtronic Minimed Sof-Set Ultimate QR
		Medtronic Minimed Quick Set QR

Discussion on chemical, pharmaceutical and biological aspects
In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The information provided in the application demonstrated consistent batch-to-batch production of Apidra achieving a well-defined quality for the active substance and the medicinal product. The fermentation, basic down-stream processing and purification of the active substance, insulin glulisine, are adequately controlled and validated. Appropriate active substance specifications have been set. The active substance has been well characterised using state-of the-art methods with regard to its physicochemical characteristics. The manufacturing process of the medicinal product has been described and validated in sufficient detail. The quality of the medicinal product is controlled by adequate test methods and specifications. No excipients of human or animal origin are used in the product manufacture and therefore there is no risk of contamination with viral or TSE agents by these ingredients. No animal ingredients are used for the cell culture process, the basic downstream processing and purification with the exception of porcine enzymes. Sufficient virus validation data were provided for the manufacturing processes of porcine enzymes and, as well, for the manufacturing process of insulin glulisine to consider that Apidra is virologically safe. On the basis of the data provided and the agreed follow-up measures, the quality of the product is satisfactory for the grant of a Marketing Authorisation.

3. Non-clinical aspects

Introduction

The non-clinical development program was primarily designed to compare the pharmacological and toxicological properties of insulin glulisine to soluble human insulin. In some studies, the rapid-acting insulin analogues insulin lispro and/or insulin aspart and/or AspB10 were used as comparators as well. The applicants point out that the program was performed in compliance with the ICH guideline on "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals", the CHMP "Points to Consider Document on the Non-Clinical Assessment of the Carcinogenic Potential of Insulin Analogues", the CHMP "Points to Consider on the Need for Assessment of Reproductive Toxicity of Human Insulin Analogues" and other applicable guidelines.
The Applicant states that all non-clinical safety studies were GLP-compliant, except for the safety pharmacology study in conscious telemeter dogs. This study, which was completed before the effective date of the CHMP "Note for Guidance on Safety Pharmacology Studies for Human Pharmaceuticals", was not audited but meets all other principles of current GLP regulations. Scientific advice was previously requested to the CHMP in 2000, regarding non-clinical aspects.

Pharmacology

Insulin glulisine (3^{B}Lys-29^{B}Glu-human insulin) is a rapid-acting analogue of human insulin in which the asparagine moiety at position B3 is replaced by lysine and the lysine moiety at position B29 by glutamic acid. Thus, insulin glulisine is a close structural relative of human insulin. 1 U of insulin glulisine equals 34.9 μg, and 1 ng equals 28.6 μU.

- Primary pharmacodynamics

-In vitro

Primary pharmacodynamics included *in vitro* tests for insulin receptor binding, effects on glucose transport and lipogenesis.
Insulin glulisine and human insulin had comparable insulin receptor association kinetics and maximal binding in transformed rat embryo fibroblasts over-expressing the insulin receptor. Insulin glulisine had a slightly lower affinity for the insulin receptor than human insulin in human insulin receptor preparations.
Lipogenic activity and glucose transport in isolated rat adipocytes was slightly lower than human insulin, but reached the same maximum obtainable effect as human insulin at higher concentrations. In contrast to human insulin, which exerted a prominent and equal activation of IRS-1 and IRS-2 in rat and human cardiomyoblasts and adult rat cardiomyoctyes, insulin glulisine produced a marginal activation of IRS-1 in all three cell systems, and showed a predominant activation of IRS-2 in adult rat cardiomyocytes expressing a high level of insulin receptors as well as in cardiomyoblasts with a high density of IGF-1 receptors. Stimulation of glucose transport was equal for both insulin glulisine and human insulin in this cellular assay system independent of the IRS-1 or IRS-2 pathway.

-In vivo

Primary pharmacodynamics included *in vivo* tests for evaluating the effects on blood.

Insulin glulisine administered to rats s.c. was as effective as insulin lispro and showed a slightly greater hypoglycaemic activity than human insulin. In euglycaemic clamp studies in dogs insulin glulisine demonstrated the pharmacodynamic properties of rapid-acting insulin. The time-action profile of insulin glulisine displayed a faster onset and shorter duration of action than regular human insulin and was not statistically different from the profile of insulin lispro.

Based on hypoglycaemic activity of various mixtures of human regular insulin or rapid-acting insulin analogues with human NPH insulin, it appears that insulin glulisine is suitable to be mixed with human NPH insulin immediately before injection

- Secondary pharmacodynamics

Secondary pharmacodynamics investigated the effects on IGF-1 and mitogenic activity.
Insulin and IGF-I have a high degree of sequence homology and share a spectrum of metabolic and mitogenic activities. At very high concentrations, insulin can bind to the IGF-I receptor and mediate the effects of IGF-1. Such concentrations are not reached in human therapy.
The IGF-1 receptor affinity of insulin glulisine was significantly lower than that of human insulin in human osteosarcoma cells and slightly reduced in rat cardiomyoblasts. Insulin glulisine induced a higher IGF-1 receptor autophosphorylation than human insulin in rat cardiomyoblasts at a concentration of 500 nmol/L.
Stimulation of MAP kinases activation (related to mitogenic effects) was even lower for insulin glulisine relative to human insulin. In the final pathway, stimulation of Shc protein and DNA synthesis (thymidine incoporation) was equal for insulin glulisine and human insulin, and lower than Asp(B10) insulin. In rat fibroblasts over-expressing the human insulin receptor, insulin glulisine and human insulin did not differ with respect to stimulation of thymidine incorporation at concentrations of 0.01 nmol/L and 0.1 nmol/L. By contrast Asp (B10) insulin enhanced mitogenesis. In a human breast cancer cell line (MCF10) over a concentration range from 0.01 to 100 nmol/L insulin glulisine showed lower mitogenic activity than human insulin at concentrations above 1 nmol/L.

- Safety pharmacology

The safety pharmacology tests investigated the effects on the CVS and respiratory system. The clamp studies in conscious telemetered dogs did not reveal any clinically relevant effect on vital functions.
Subcutaneous injection of up to 1.0 U/kg insulin glulisine caused a decrease in systolic blood pressure and an increase in heart rate and breathing rate. These inotropic and chronotropic effects are known to be caused by insulin-induced hypoglycaemia.
The observed increase in QTc is well known from insulin-induced hypoglycaemia in man.

- Pharmacodynamic drug interactions

When insulin glulisine was mixed with human NPH insulin immediately before s.c. administration in healthy dogs there were no adverse effects. The mixture had a later onset and MTA but no loss of hypoglycaemic effect.
Insulin glulisine is expected to behave like other insulins. The class labelling applied to section 4.5 of the proposed SPC on interactions with other medicinal products is appropriate.

- Summary of salient findings

Primary pharmacodynamics comprised *in vitro* tests for insulin receptor binding, effects on glucose transport and lipogenesis. *In vivo* studies evaluated effects on blood glucose. *In vitro*, insulin glulisine behaved similarly to human insulin with respect to insulin receptor binding, insulin receptor autophosphorylation and single-cell carrier-mediated glucose transport and lipogenesis, although generally with a slight shift to the right of the dose-response curve.
In vivo, insulin glulisine caused a slightly more pronounced hypoglycaemia than human insulin in rats and had an earlier onset of action in dogs.
Secondary pharmacodynamics investigated the effects on IGF-1 and mitogenic activity. The results did not raise concern at the expected concentrations in human therapy
The safety pharmacology tests investigated the effects on the CVS and respiratory system, and the results did not raise any major concern. Because of close similarity between insulin glulisine and

human insulin, other tests for adverse pharmacodynamic effects on other vital organs were not considerd necessary.
It can be concluded that the general pharmacological profile of Insuline glulisine is favourable.

Pharmacokinetics

Pharmacokinetic studies were conducted in rats and dogs using unlabelled insulin glulisine or drug substance labelled with I^{125} in the tyrosine residue at position A14. The in-vitro stability of insulin glulisine was determined in human and rat plasma.
Hepatic metabolism and biodegradation pathways were not investigated on the grounds that insulin glulisine differs little from human insulin and such differences do not involve the known cleavage sites.
The studies conducted were single dose only. Information on the pharmacokinetics of insulin glulisine following repeated administration was obtained from toxicokinetic investigations conducted during toxicology studies.

Serum concentrations of insulin, including insulin glulisine and endogenous insulin, were determined by a RIA method. This method was validated with regard to human, rat and dog serum. The cross-reactivities with human insulin, rat insulin and insulin lispro were 80.2%, 106.9% and 75.7%, respectively. Anti-insulin glulisine antibodies were determined by a validated semi-quantitative ^{125}I-insulin glulisine tracer test.

- Absorption- Bioavailability

Insulin glulisine was rapidly and completely (rat) or well (dog) absorbed following subcutaneous administration to male animals, and has a short elimination half-life in all relevant species.

The absolute bioavailability, T_{max} and $T_{½}$ were 105%, 0.17 hours and 0.35 hours in the rat; 42%, 1.0 hours and 1.1 hours in the dog; and 71%, 1.0 hours and 0.70 hours in healthy human volunteers. Dose-proportionality and accumulation were not studied formally in animals; however, in the toxicokinetic studies discussed below, AUC and C_{max} generally increased with dose without gender differences or cumulative effects.

- Distribution

Following ^{125} I-insulin glulisine administration to rats, radioactivity was distributed throughout the body, with low radioactivity in the CNS. The highest levels of radioactivity were found at the injection site, followed by the thyroid gland. This study may be of uncertain value due to the rapid metabolism or degradation of the radiolabelled substance in the rat. Data from published literature indicated a similar distribution pattern in rats after administration of ^{125} I human insulin by the same route. However, there was a more rapid rate of disappearance from the injection sites in the case of insulin glulisine.

- Metabolism (in vitro/in vivo)

In vitro stability studies with insulin glulisine in human and rat plasma showed no degradation to des-(B1-B3) insulin glulisine, which would result from cleavage at the 3B-Lys modification. The Applicant assumed that the endogenous degradation of insulin glulisine is the same as for human insulin therefore no further metabolism studies were conducted.

- Excretion

The excretion of ^{125}I-insulin glulisine was investigated in rats. After a single subcutaneous administration, 87% and 8.9% of the radioactivity was excreted in the urine and faeces respectively. The excretion of TCA insoluble radioactivity suggested that almost all the radioactivity excreted in the urine was free ^{125}I or low molecular ^{125}I-labelled compounds.

- Summary of pharmacokinetic parameters

Rats and dogs were the main species used to investigate pharmacokinetic parameters of insulin glulisine. Drug substance labelled with I^{125} in the tyrosine residue at position A14 and also unlabelled drug were used.
Insuline glulisine showed a rapid absorption and a short half-life in all relevant species. It is distributed throughout the body, with no major differences in the distribution of human insulin, except for a faster clearance of the former from the injection site.
Endogenous degradation of insulin glulisine appears to be the same as for human insulin.
Insuline glulisine is mainly excreted in the urine, showing 87% of the radioactivity after single subcutaneous administration.

Toxicology

Insulin glulisine was evaluated for single-dose and repeated-dose toxicity; toxicity to reproduction; genotoxicity; carcinogenic potential; antigenicity and for effects on the environment.

- Single dose toxicity

Studies were conducted in mice, rats and dogs
Single dose toxicity was comparable for mice and rats after subcutaneous (s.c.) and intravenous (i.v) injection. Mortality and other abnormal findings were dose-related and consistent with the sequels of severe hypoglycaemia. Hypoglycaemia was verified by blood glucose measurement.

- Repeat dose toxicity (with toxicokinetics)

Repeated dose toxicity studies were conducted using the intended therapeutic route, that is the subcutaneous route. All studies used normoglycaemic animals dosed once or twice daily whereas insulin glulisine is administered three times daily to hypoglycaemic diabetic patients.

Repeat-dose toxicity studies were conducted in rats and dogs for up to 12 and 6 months, respectively. The 12-month rat study included satellite groups treated with regular human insulin as a reference drug. Toxicokinetics was included in the 1-month and the 6-month studies, and a 2-week study served to estimate exposure in the 12-month rat study.

Signs of chronic systemic toxicity were due to exaggerated pharmacodynamic effects and similar to those recorded in other insulin studies. The observed effects, and the deaths at high dose levels, in non-diabetic animals are not considered to be predictive of toxicity in diabetic patients. Four-week recovery periods were included in the 6-month rat and 1-month dog study. In the rat study, relative liver weight remained marginally decreased in high-dose males. All other findings pertaining to systemic toxicity were fully reversible in both species.
Safety margins based on systemic exposure (AUC or Cmax) were at least 7 fold (dog) or 18 fold (rat) based on a human dose of 0.3 U/kg.

Insulin antibody levels were determined during the course of both 6-month studies and at the end of the 12-month rat study. In rats, no antibodies to insulin glulisine were detected.
In dogs, there was a slight transient increase in antibodies to insulin glulisine in 1/8 mid-dose and in 2/10 high-dose animals, a marked and persistent increase in 1/7 mid-dose animals and a slight increase on day 176 but not on day 30 in 1/10 high-dose animals. These findings indicate that insulin glulisine is not immunogenic in rats, but elicits antibody formation in dogs. Given the sporadic occurrence of antibody formation in the dogs, it is unlikely to have confounded the toxicity studies in this species.

Malignant fibrous histiocytoma and benign or malignant mammary gland tumours were found in the rat studies. Spontaneous mammary gland tumours are common in aged Sprague-Dawley rats and occurred in 3-17% of the applicant's historical controls. The incidence of such tumours in the insulin-treated groups was similar to the historical range. The incidence of malignant fibrous histiocytomas was 10% in the controls and was not significantly higher in any of the treatment groups. The controls were injected with insulin-free vehicle and practically all rats had pronounced chronic inflammatory lesions at the injection site. Therefore, these tumours were attributed to the irritant effects of the vehicle, which was injected twice daily at the same site for 12 months.

The in vitro data on receptor binding and on mitogenicity and the in vitro proliferation studies on mammary glands indicated a lack of mitogenic potential of insulin glulisine.

- Genotoxicity *in vitro* and *in vivo*

Insulin glulisine was tested for gene mutations in bacteria and for chromosome aberrations in vitro and in vivo. As expected, there was no evidence of genotoxic potential in any of these tests.

- Carcinogenicity

Conventional carcinogenicity studies are not warranted as neither the tumour findings in the 12-month rat toxicity study nor any of the tests for insulin and IGF-1 receptor binding, genotoxicity, in-vitro mitogenic potency or in-vivo proliferation raise concerns about the carcinogenic potential of insulin glulisine
One-year study in rats was performed especially aimed at investigating the carcinogenic potential of insulin glulisine. The tumours detected were not considered to be treatment related.

- Reproductive and developmental studies

A complete reproduction and developmental toxicity programme was conducted in rats and rabbits. The results of these studies for insulin glulisine were similar to that of regular human insulin, and there were no indications that insulin glulisine had any adverse effects on reproduction and development over and above those caused by disruption of glucose homeostasis.

- Local tolerance

Local tolerance was investigated in a conventional test in rabbits in order to assess the tolerability of insulin glulisine after single subcutaneous and intravenous injection, the routes intended for therapeutic use and after intramuscular and paravenous injection to reflect the consequences of faulty injections. In a 12-month repeated dose study in rats, subcutaneous injections of insulin glulisine produced severe inflammatory lesions and malignant fibrous histiocytomas at the injection site.
In short, insulin glulisine showed to be well tolerated following i.v. and s.c. administration, whereas paravenous or i.m. injection resulted in moderate inflammatory and/or necrotic lesions.

- Other toxicity studies

Three different batches of insulin glulisine were used for toxicity studies. The contents of related impurities and high molecular weight proteins in these batches were higher than or equal to the contents in active substance produced by the commercial production process. Therefore, these impurities can be considered qualified.

With regard to the environmental risk assessment the results demonstrated no acute toxic effects of insulin glulisine in algae, daphnia and fish. A ready biodegradability in activated sludge was indicated. It can be concluded that insulin glulisine poses a negligible risk to the environment

- Summary of salient findings

Toxicology studies were performed in mice, rats, dogs and rabbits. High single doses of insulin glulisine appeared to cause death by hypoglycaemia.
Repeat-dose toxicity studies were conducted in rats and dogs. The effects observed appeared to be exagerated pharmacodynamic effects, and were fully reversible in both species. Moreover, they are not considered to be predictive of toxicity in diabetic patients. Acceptable margins of exposure were achieved in all repeat-dose toxicity studies.
Appropriate studies showed that the incidence of malignancies in any of the treatment groups compared to the controls was not significantly higher.
There was a complete package of tests for toxicity to reproduction. The toxicity of insulin glulisine was similar to that of human regular insulin.
Local tolerability of insulin glulisine in rabbits following s.c. administration was good.
The experimental data indicated that the therapeutic use of insulin glulisine does not pose an immediate concern to the environment.
In conclusion, insulin glulisine has undergone sufficient toxicity testing to conclude that its safety profile in experimental animals is very similar to that of regular human insulin, and the discussion has been appropriately reflected in section 5.3 of the SPC.

4. Clinical aspects

Introduction

Apidra contains the active ingredient insulin glulisine, a recombinant human insulin analogue– which differs from human insulin by the replacement of asparagine in position B3 by lysine, and replacement of lysine at position B29 by glutamic acid. It is produced by recombinant DNA technology in E-coli bacteria.
Apidra is claimed to have a more rapid onset, earlier peak effect and shorter duration of effect. The product is intended for "the treatment of adult patients with diabetes mellitus". The applicant proposes the product to be used in conjunction with a basal long acting insulin. They also propose the product to be used with oral hypoglycaemic agents in type II diabetics and it may be mixed in the same syringe as NPH insulin.

Apidra is proposed for administration by subcutaneous injection either intermittently or by a continuous infusion pump.

The Applicant claims that all clinical studies have been conducted in accordance with good clinical practice (GCP), as required by the International Conference on Harmonization (ICH) E6 Guideline for Good Clinical Practice, 1 May, 1996, in agreement with the Declaration of Helsinki.

The CHMP have previously adopted a Guideline concerning the clinical development of medicinal products for diabetes mellitus (CPMP/EWP/1080/00). The clinical aspects of this application have been discussed together with the requirements of this document.

Scientific Advice to the CHMP was requested concerning clinical issues in 2000. The CHMP was of the view that phase II dose ranging studies were not warranted for this product, bearing in mind its pharmacodynamic similarity to insulin lispro (Humalog) – but the CHMP requested that its pharmacodynamics and pharmacokinetics should be adequately characterised in normal volunteers and in type I and type II diabetics – in addition to information on the ease of dose titration. The CHMP also expressed concern that the data then available did not provide reassurance that insulin glulisine was equipotent with other insulins and a steady state infusion study was therefore requested.
With respect to the adequacy of the phase III package, the CHMP agreed with the company that the 2 pivotal phase III studies proposed (in type I and type II diabetes respectively) together with their proposed 12 month extensions should be adequate to establish the efficacy of insulin glulisine. However, the CHMP expressed some concern regarding the size of the database in relation to safety and immunogenicity – in particular the concern that these data were sufficient in scope to adequately assess the incidence of cross-reacting antibodies for insulin glulisine compared to other similar products and for an assessment of the clinical impact of the development of these antibodies.
The CHMP agreed with the company that measurement of E-coli protein antibodies in the phase III trials would not be necessary, provided that the limit of these proteins did not exceed 10 ppm in any batch.

The adult clinical program for glulisine consisted of 14 clinical pharmacology studies and 4 international, completed, and controlled Phase III studies (3 active-controlled studies that evaluated the efficacy and safety of s.c. administered glulisine, plus 1 active-controlled safety study to support the use of glulisine administered by continuous s.c. insulin infusion). The Phase III efficacy studies were specifically designed to evaluate the safety and efficacy of glulisine in subjects with type 1 and type 2 diabetes, and also to evaluate the immediate post-meal dosing of glulisine. A total of 950 subjects with type 1 diabetes and 435 subjects with type 2 diabetes received glulisine in completed Phase III studies. At the time of the submission, there were 3 ongoing or completed/not reported studies in the Phase III adult program, including 2 long-term extension studies. Approximately 180 subjects had been exposed to 1 year of glulisine treatment at the time of the submission.
The clinical pharmacology program included non-diabetic subjects, obese non-diabetic subjects, subjects with type 1 or type 2 diabetes, and non-diabetic subjects with varying degrees of renal function. An additional completed clinical pharmacology study evaluated glulisine in paediatric subjects with type 1 diabetes.
The total number of subjects exposed to glulisine was more than 1500.

Pharmacokinetics

Fourteen clinical trials are presented supporting the clinical pharmacology of insulin glulisine. In these trials 248 adults received more than one dose. These individuals comprised 195 non-diabetics, and 53 diabetics (37 with type I diabetes and 16 with type II diabetes).

The studies included healthy male volunteers of Caucasian and Japanese origins; patients suffering from type 1 and type 2 diabetes; subjects with renal impairment, and pediatric patients with type 1 diabetes.
The pharmacokinetic properties of glulisine are presented for the following trials totalling 248 adult subjects and 20 pediatric subjects who received glulisine:

- Healthy subjects: Study 1001, 1002, 1003, 1004, 1009, 1010, 1011, 1012, 1013, 1016.
- Subjects with type 1 diabetes: Study 1005 and 1008.
- Subjects with type 2 diabetes: Study 1006.
- Special populations
- Children and adolescents with type 1 diabetes: Study 1017
- Subjects with renal impairment: Study 1011
- Japanese expatriates living in Europe: Study 1013
- Obese non-diabetic subjects: Study 1010

A human insulin radioimmunoassay was used to determine insulin glulisine concentrations in trials 1001, 1002 and 1003. For other trials, a specific RIA for glulisine was used. Human insulin and insulin lispro concentrations were determined using an human insulin radioimmunoassay. C-peptide concentrations were measured in all trials. The CHMP considered the analytic methods used reasonably suitable for their purposes and well validated
Pharmacokinetic parameters have been studied in normal volunteers, (study 1009) type I diabetics (study 1005) and type II diabetics (study 1006).
In these studies, a comparator with a similar time-action profile has been employed in line with the CHMP Guideline in this area. The data presented also satisfy the terms of the previous CHMP Scientific Advice where PK data were stated to be required in healthy volunteers, and in type I and type II diabetics.

- Absorption – Bioavailability

In study 1009, insulin glulisine was observed to be absorbed more rapidly than regular insulin (t_{max} 56 min vs. 99 min). The maximal concentration was 2 fold higher and the duration of action as assesssed by MRT shorter at 105 as opposed to 182–min. Total AUC was 35% higher but this difference was not significant. The parameters were similar to those obtained for insulin lispro.
Similar findings were apparent in type I diabetics (study 1005) and in type II diabetics (1006).

The CHMP was of the opinion that the absorption of glulisine has been adequately described upon single-dose s.c. administration in healthy volunteers and in the target population. The influence of various injection sites has been satisfactory documented

- Bioequivalence

The absolute bioavailability of insulin glulisine was assessed in study 1004. Compared with iv injection, the bioavailability based upon AUC was as follows for the differing anatomical areas studied:

Femoral area	68%
Deltoid area	71%
Abdominal area	73%

The clinical trial report from study recommends that the abdominal area is the preferred site for injection and indeed this area has been used in the phase III studies.
The SPC proposes however that all 3 sites may be used in practice. The CHMP is of the view that, bearing in mind the slight difference in bioavailability across the sites, this is acceptable. The SPC

and PIL include the above data to inform prescribers and recipients of the variability associated with injection in differing anatomical sites.

The possible influence of food was examined in Study 1008. Subjects with type 1 diabetes were administered glulisine at different times relative to a standard meal. The pharmacokinetic parameters of glulisine were similar and comparable to those in healthy subjects, regardless of the timing of administration.

Study 1005 provided data regarding intra-subject variability in type I diabetes. Intra individual variability appeared similar with insulin glulisine as for insulin lispro and regular human insulin. Similar data are available in type II diabetics from Study 1006.

- Distribution and elimination

In Study 1016, the distribution of glulisine and human insulin were assessed after continuous i.v. Infusions over 2h of $0.8 mU.kg^{-1}.min^{-1}$. Distribution is similar with volumes of distribution of 13 l and 22 l and half-lives of 13 and 18 minutes, respectively.
Elimination of human insulin is rapid, and occurs by metabolic degradation and extraction by various tissues. After subcutaneous administration, insulin glulisine is eliminated more rapidly than regular human insulin with an apparent half-life of 42 minutes compared to 86 minutes. In an across study analysis of insulin glulisine in either healthy subjects or subjects with type 1 or type 2 diabetes mellitus the apparent half-life ranged from 37 to 75 minutes (interquartile range).

- Dose proportionality and time dependencies

Dose normalised results for C_{max}, $AUC_{0-2h}$and AUC_{0-end} calculated by the Applicant from a number of studies following doses of 0·1, 0·15, 0·2 and 0·3 U/kg s.c. were found to be predictable, as expected.

- Special populations

Pharmacokinetic parameters of glulisine have been examined in a number of special populations:

Subjects with renal impairment (Study 1011)
There were only minor changes in the pharmacokinetics of glulisine in patients suffering from moderate and severe renal impairment. These changes are unlikely to be of clinical relevance, and dose adjustments should only be made according to clinical effect. The SPC states however that insulin requirements may be reduced in the presence of renal impairment.

Children and adolescents with type 1 diabetes (Study 1017)
The only data available in children and adolescents is of a PD/PK nature. The study investigated children (7-11 years) and adolescents (12-16 years) with Type 1 diabetes. Insulin glulisine was rapidly absorbed in both age groups, with similar T_{max} and C_{max} as in adults. Administered immediately before a test meal, insulin glulisine provided better postprandial control than regular human insulin, as in adults. The glucose excursion (AUC_{0-6h}) was $641 mg.h.dl^{-1}$ for insulin glulisine and $801 mg.h.dl^{-1}$ for regular human insulin.
The data presented do not provide reassurance that insulin glulisine has the same effect in children and adolescents as regular insulin. Further data is required in children to provide reassurance on this point, and efficacy safety data are also desirable. The SPC states that no adequate clinical information on the use of insulin glulisine in children and adolescents is available.

The applicant intends to further evaluate efficacy and safety of insuline glulisine in children and adolescents with type 1 diabetes mellitus.

Subjects with hepatic impairment and elderly population
No studies were performed in subjects suffering from hepatic impairment. The absence of this data is not considered to be a bar to the grant of a Marketing Authorisation, however the SPC states that pharmacokinetic properties have not been investigated in patients with impaired liver function. Very Limited pharmacokinetic data is available for elderly patients with diabetes mellitus.

- Interaction studies

No clinical studies on drug-drug interactions have been performed. Based on empirical knowledge from similar medicinal products, clinically relevant pharmacokinetic drug-drug interactions are unlikely to occur

Pharmacodynamics

- Mechanism of action

Pharmacodynamics of glulisine was studied performed in part concomitantly with the pharmacokinetic studies. Euglycemic clamp technique was applied to assess the primary pharmacodynamic response to glulisine.
The mechanism of action of glulisine is mediated by the same pathways that mediate the response to human insulin. The biological effects of human insulin are mediated via the insulin receptor. The receptor binding characteristics of glulisine was examined in a number of in vitro studies. In vitro, glulisine binding to the insulin and IGF-I receptors is slightly lower than that of human insulin.

- Primary and Secondary pharmacology

Pharmacodynamically insulin glulisine appears generally equipotent to other short acting insulins such as insulin lispro and regular human insulin at low doses.

The primary pharmacology of glulisine has been reasonably well investigated. The results are consistent across the populations investigated: insulin glulisine appears similar to insulin lispro in its pharmacodynamic effect, having a very rapid onset and short duration of action. This effect is similar in normal individuals, type I diabetes type, type II diabetes, different racial groups studied and lean and obese normal individuals. Postprandial glycaemic control appears similar with insulin glulisine given immediately before a meal as with regular insulin given 30 minutes before a meal and when given just after a meal, it appears to perform similarly to regular insulin given 2 minutes before a meal.
No studies have specifically been conducted for the investigation of the secondary pharmacology of glulisine.

Clinical efficacy

The submitted data supporting the clinical efficacy of glulisine for the use in the treatment of type 1 as well as type 2 diabetes, are based on the results of three randomized active-controlled open studies (studies 3001, 3002 and 3004). The studies utilized a common core protocol that standardized most aspects of study design, inclusion/exclusion criteria, target BG values, primary and secondary efficacy variables, and safety variables.

Study 3001 was a 26-week, multinational, multicenter, controlled, open, 1:1 randomized, parallel clinical trial comparing glulisine with insulin lispro injected subcutaneously in subjects with type 1 diabetes mellitus also using insulin glargine.
Study 3002 was a 26-week, multinational, multicenter, controlled, open, 1:1 randomized, parallel clinical trial comparing glulisine with regular insulin injected subcutaneously in subjects with type 2 diabetes mellitus also using NPH insulin.
Studies 3001 and 3002 were followed by 26-week extension studies, 3011 and 3012 respectively, to assess long term safety of insulin glulisine.

Study 3004 was a 12-week, multinational, multicenter, controlled, open, 1:1:1 randomized, parallel clinical trial to assess noninferiority between pre- and postmeal administration of insulin glulisine and premeal regular human insulin in subjects with type 1 diabetes mellitus receiving insulin glargine as the basal insulin therapy.
A fourth study, Study 3006, was designed to support the claim that glulisine may be safely administered by continuous s.c. insulin infusion (CSII) via an external insulin pump to control hyperglycemia in patients with type 1 diabetes mellitus. This study was not powered to provide a formal demonstration of efficacy. Phase III clinical studies were performed between July 2001 and December 2002.

Study	Indication
3001*	Type I diabetes
3002*	Type II diabetes
3004	Post mealtime use (Type I diabetes)
3006	Continuous sc infusion using an external pump (Type I diabetes)

*** = pivotal trials**

- Dose response studies

Dose-ranging studies were not conducted. Based on the similarity of the glulisine and insulin lispro time-action profiles, the fact that insulin dose titration must be conducted on a highly individualized patient-by-patient basis and that the lack of a requirement for these studies was confirmed by scientific advice from the CPMP, it is reasonable that these studies were not performed.

- Main studies

Efficacy data comes from the three mentioned phase III studies designed to evaluate the efficacy and safety of glulisine compared with lispro or regular insulin in adult men and women (greater than 18 years of age) with type 1 or 2 diabetes (studies 3001, 3002, and 3004). The studies utilized a common core protocol that standardized most aspects of study design, inclusion/exclusion criteria, target BG values, primary and secondary efficacy variables, and safety variables.
Efficacy data are presented separately by study: pooling of data for efficacy was not considered appropriate due to differences between studies in basal insulin regimens, active comparator insulin preparations, type of DM, and the recommended timing of glulisine administration relative to meal intake.

Pivotal study 3001

Study 3001 was a 26-week, multinational, multicenter, controlled, open, 1:1 randomized, parallel clinical trial comparing glulisine with insulin lispro injected subcutaneously in subjects with type 1 diabetes mellitus also using insulin glargine.

METHODS

Study Participants

A total of 772 subjects entered the screening phase. Of these, 683 were randomized and 672 received study medication. Subjects were men or women, ≥18 years of age with established type 1 diabetes (onset of diabetes < age of 40 and requirements of insulin therapy since diagnosis), BMI <35 kg/m2 and HbA_{1c} range ≥6.0 to ≤11.0% and >1 year of continuous insulin treatment immediately before study entry. There were 339 subjects in the glulisine group and 333 lispro subjects randomized and treated. Of the 672 subjects treated, 23 subjects (10 glulisine; 13 lispro) were withdrawn after the start of treatment.
Exclusion criteria included active proliferative diabetic retinopathy or other unstable retinopathy, a history of non–hypoglycemia-related seizure disorders, impaired hepatic function (e.g., ALT or AST value greater than 2 × the upper limit of normal), impaired renal function (e.g., serum creatinine >177 μmol/L), previous pancreatectomy, or clinically relevant cardiovascular, hepatic, neurologic, endocrine, active cancer, or other major systemic disease that may have prevented the subject from safely completing the study.
Women of childbearing potential intending to become pregnant during the course of the study, and women who were pregnant, were not permitted in any study. Moreover, women of childbearing potential were to use a reliable contraceptive measure throughout the studies.
The ITT population was defined as all subjects randomized and treated, and consisted of 672 subjects. The PP population was defined as all ITT subjects excluding subjects with a major protocol violation. Major protocol violations occurred with 50 subjects. Subjects were included in the efficacy analyses if they had both a pretreatment and an on-treatment value available and thus, the number of subjects included in the analyses for each variable varied.
The study participants were recruited from different centers in 13 European countries and South Africa

Treatments

Subjects were randomized to receive either Glulisine or Lispro. Glulisine or lispro were dosed individually as appropriate by subcutaneous injection 0 to 15 minutes before a meal. The recommended anatomical area for s.c. injection was the abdomen. At endpoint, 85.5% of subjects were injecting their short-acting insulin into the abdomen, and there were no notable differences between treatments. In addition to the rapid-acting insulin, insulin glargine was provided by the sponsor, and was to be administered as the basal insulin once daily at bedtime. The dose of Glargine was to be titrated based on fasting BG with a goal of 5.0-6.7 mmol/l. The dose of Glulisine or Lispro was to be titrated to achieve a goal of 2 h postprandial BG of 6.7-8.9 mmol/l while avoiding hypoglycemia. The treatment was not blinded because of incompatibility of the Lispro pen and Aventis cartridges.

Objectives

Primary: to demonstrate non-inferiority of glulisine compared to lispro in the change in GHb (total glycated hemoglobin) from baseline to endpoint and to compare safety (adverse events, clinical chemistry, lipids, hematology and insulin and *E. coli* antibodies) of insulin glulisine with insulin lispro in subjects with type 1 diabetes mellitus.

Secondary: to compare glulisine with lispro in terms of changes in GHb at weeks 12 and 26, blood glucose (BG) parameters, symptomatic hypoglycemia, insulin doses, and treatment satisfaction in subjects with type 1 diabetes mellitus.

Outcomes/endpoints

Primary efficacy data were the GHb values at baseline and endpoint. Endpoint is defined as the subject's last available measurement after start of treatment.

Secondary efficacy data includes GHb values at baseline and weeks 12 and 26; self monitored BG (preprandial at breakfast, lunch, dinner; 2 hours postprandial after breakfast, lunch, dinner; at bedtime and nocturnal); dosage of both rapid-acting and basal insulin at baseline and weeks 12 and 26 and symptomatic hypoglycemia
Quality of life/Satisfaction with treatment was assessed using the Diabetes Treatment Satisfaction Questionnaire, status version (DTSQs) and the Diabetes Treatment Satisfaction Questionnaire, change version (DTSQc).

Sample size

Sample size calculation was made on the basis of the primary objective of demonstrating non.inferiority of glulisine to lispro. A sample size of 470 (235 in each group) was needed to ensure that the upper confidence limit of the two-sided 95% CI for the adjusted mean difference between groups would not exceed 0.4% GHb with 90% power and with an expected treatment difference of 0.1% GHb. The level of 0.4% GHb was chosen on the basis of previous programs of Novonorm where noninferiority margin of 0.6% was used and accepted. No definite generally accepted margin exist. However, in the light of previsously accepted limits in these types of studies, the margin of 0.4% seems appropriate.

Randomisation

Subjects were randomized to treatment and began their randomized study treatment regimen. For subjects randomized to glulisine, the starting dose of glulisine was to be the same as that of the short-acting insulin preparation at the end of the run-in phase, unless a change in dose was necessitated to meet target BG values while avoiding hypoglycemia, according to the results of the 7-point SMBG profile performed during the week before baseline. Centralised, computerized telephone randomization was used. Subjects were stratified based on whether or not they were treated with glargine or another basal insulin at visit 1.

Blinding

The study was not blinded. This can be considered as a weakness of the study, but necessary due to incompatibility of the Lispro pen and Aventis cartridges. Furthermore, considering the nature of the primary endpoint, the unblinded design is acceptable and in line with the design of previously accepted studies in this field.

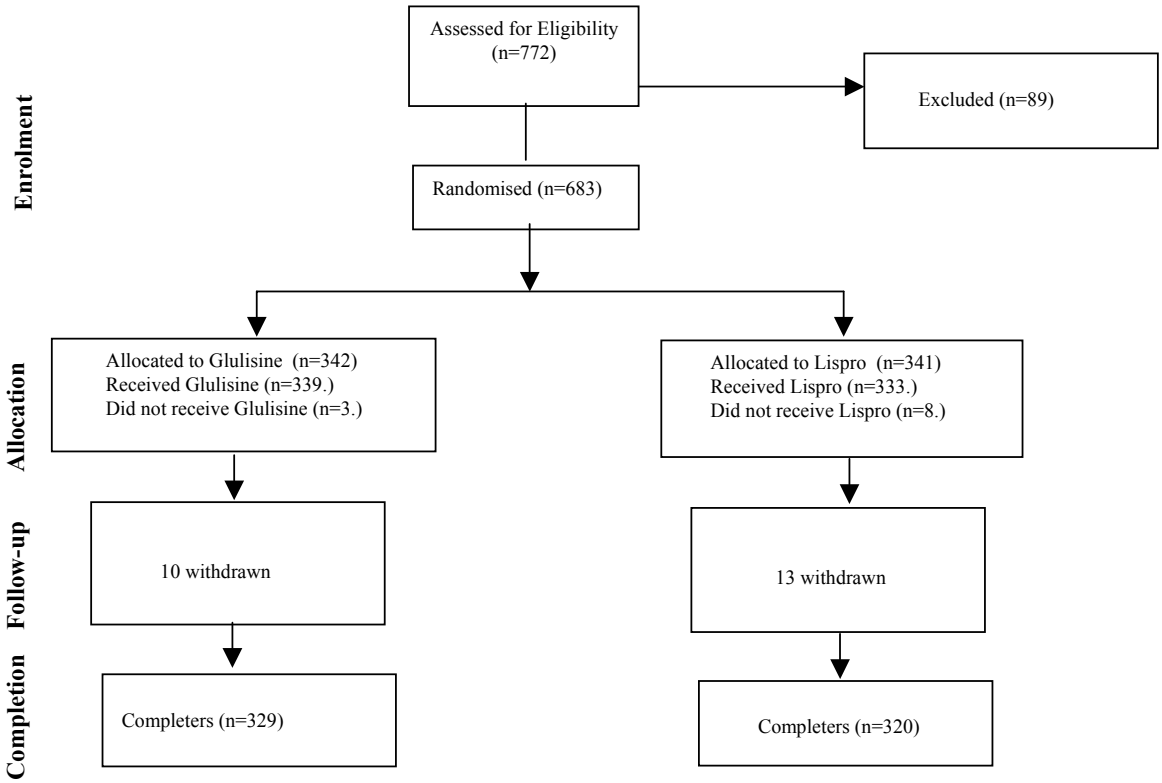
Statistical methods

The primary analysis was the analysis of change in GHb from baseline to endpoint (primary efficacy variable) using the intent-to-treat (ITT) population, defined as all randomized subjects who received study treatment. The per protocol (PP) population, defined as a subset of the ITT population including all randomized and treated subjects with no major protocol violations, was used to check for the consistency of the analyses made using the ITT population. Both the ITT and PP populations were

used for the analyses of all efficacy variables. Analyses of demographic baseline data and safety variables were conducted using the ITT population.
The primary analysis assessed non-inferiority using the upper bound of the 95% confidence interval (CI) for the difference in the adjusted mean change in GHb from baseline to endpoint which was compared with the predefined non-inferiority margin of 0.4% GHb.
Both the ITT and PP populations were used for the analyses of all efficacy variables. Analyses of demographic baseline data and safety variables were conducted using the ITT population.
Noninferiority would be demonstrated if the upper bound of the CI was ≤0.4%. If non-inferiority was demonstrated, then a corresponding check of statistical superiority (i.e. that the upper bound of the CI is <0.0%) would be performed without an alpha penalty since this is a closed procedure.
Continuous variables (e.g. GHb) were analyzed by an analysis of covariance (ANCOVA) with treatment and (pooled) center as fixed effects and the baseline value of the variable as a covariate. The assumptions of the model such as parallelism were tested and the distribution of the residuals from the model was examined. To assess the comparability at baseline between treatments, continuous baseline variables were analyzed by an analysis of variance (ANOVA) with treatment and (pooled) center as fixed effects. As this study was stratified at randomization, a further exploratory analysis of the primary efficacy variable was performed which had the stratum variable (use of glargine vs. use of other basal insulin at study entry) included in the model as an additional effect.
Categorical variables (e.g. frequency of hypoglycemic events) are presented by frequency distributions. Treatment groups were compared using the Cochran-Mantel-Haenszel test stratified by (pooled) center.

RESULTS

Participant flow



A total of 772 subjects entered the screening phase, of which 683 were randomized and 672 received study medication. There were 339 glulisine subjects and 333 lispro subjects randomized and treated. Of the 672 subjects treated, 23 subjects (10 glulisine; 13 lispro) were withdrawn after the start of treatment. The ITT population was defined as all subjects randomized and treated, and consisted of 672 subjects.

Recruitment and conduct of the study

Subjects with type 1 diabetes were recruited from centers in 13 European countries and South Africa. This completed Phase III efficacy study was conducted between 5 July 2001 to 5 August 2002. After a screening and 4-week run-in phase, during which all subjects received glargine as the basal insulin and lispro, subjects were randomized and entered a 26-week treatment phase. Subjects who completed the 26 weeks of treatment were intended to be enrolled in an extension study (Study 3011).

Baseline data

With respect to most baseline data the two groups were comparable (ITT populations). Subjects randomized to glulisine had a longer time since diagnosis of diabetes, and a longer duration of previous insulin therapy by approximately 2 years. These differences were statistically significant. The types of basal and short-acting insulin preparations at study entry were similar between treatments.

Numbers analysed

In the ITT population a total of 672 patients distributed on the glulisine group (N = 339) and the lispro group (N = 333) were analysed. In the PP population 622 patients distributed on the glulisine group (N = 315) and the lispro group (N = 307).

Outcomes and estimation

Primary efficacy variable: change from baseline to endpoint in GHb:

Population/ Timepoint	Glulisine		Lispro		Glulisine – lispro		
	N	Mean [a]	N	Mean [a]	Difference in adjusted mean	95% CI	p-value
ITT population (N=653)							
Baseline	331	7.60	322	7.58			
Endpoint	331	7.46	322	7.45			
Mean change from baseline at endpoint	331	–0.14	322	–0.13			
Adjusted mean change from baseline at endpoint	331	–0.14	322	–0.14	0.00	(–0.090.10)	0.9329
PP population (N=622)							
Baseline	315	7.58	307	7.58			
Endpoint	315	7.44	307	7.45			
Mean change from baseline at endpoint	315	–0.14	307	–0.13			
Adjusted mean change from baseline at endpoint	315	–0.14	307	–0.15	0.01	(–0.09; 0.11)	0.8591

[a] Adjusted means and differences from ANCOVA model. P-values from a 2-sided test for difference.

It appears from the table that treatment groups had similar GHb levels at baseline. The two groups in the ITT as well as the PP population showed a similar reduction in mean GHb over the course of the study, with an adjusted mean change from baseline at endpoint of –0.14% in both groups in the ITT population and -0.14% in the glulisine group and –0.15% in the lispro group in the PP population.
Because noninferiority was demonstrated, a corresponding check of statistical superiority (i.e. that the upper bound of the CI is <0.0%) was performed. Based on this analysis, glulisine was not found to be superior to lispro because the upper bound of the 95% CI was above zero for the ITT population as well as the PP population.

With regard to GHb over time, the comparison between treatments for the change from baseline to each visit was consistent with the results of the primary analysis at endpoint in both the ITT and PP populations.
Overall, SMBG profiles were similar in both treatment groups at baseline and throughout the treatment period for preprandial or postprandial, bedtime, and nocturnal SMBG levels.

In the initial part of the study, subjects in the glulisine group displayed a higher frequency and rate of all symptomatic hypoglycemia. These differences diminished towards the latter part of the study. From month 4 to treatment end, the frequency and rate of all categories of symptomatic hypoglycemia were similar between treatments.

Pivotal study 3002

Study 3002 was a 26-week, multinational, multicenter, controlled, open, 1:1 randomized, parallel clinical trial comparing glulisine with regular insulin injected subcutaneously in subjects with type 2 diabetes mellitus also using NPH insulin.

METHODS

Study Participants

A total of 1186 subjects entered the screening phase. Of these 878 was randomized and 876 received study medication. Subjects were men or women, ≥18 years of age with type 2 diabetes mellitus as established in the medical history and not requiring continuous insulin therapy since diagnosis and >6 months of continuous insulin treatment immediately before study entry.
The ITT population was defined as all subjects randomized and treated, and consisted of 876 subjects. The PP population was defined as all ITT subjects excluding subjects with a major protocol violation. Major protocol violations occurred with 178 subjects.
Subjects were included in the efficacy analyses if they had both a pretreatment and an on-treatment value available and thus, the number of subjects included in the analyses for each variable varied.
Exclusion criteria were identical to study 3001. The study participants were recruited from different centers in North America and Australia

Treatments

Subjects were randomized to receive either glulisine or regular insulin. Glulisine or regular insulin, individually dosed as appropriate by s.c. injection. Glulisine was to be injected 0 to 15 minutes prior to the meal and regular insulin 30 to 45 minutes prior to the meal. The short acting insulins should be administered before at least two meals a day (breakfast and dinner). The recommended anatomical area for s.c. injection was the abdomen. At endpoint 95.0% were injecting their short-acting insulin into the abdomen, and there were no notable differences between treatments.
Glulisine or regular insulin were to be taken in combination with NPH insulin twice daily (individually titrated by s.c. injection) as part of a basal/bolus insulin regimen.
Subjects were allowed to mix their short-acting insulin with NPH immediately prior to injection (i.e. not later than 2 minutes before injection) and allowed to continue on oral hypoglycemic agents (OHA) during the treatment phase.

Objectives

Primary**:** to demonstrate noninferiority of glulisine compared to regular insulin in the change in GHb from baseline to endpoint and to compare safety (in terms of adverse events, clinical chemistry, lipids, hematology, insulin antibodies and E. coli protein antibodies) of glulisine with regular insulin in subjects with type 2 diabetes mellitus.
Secondary: to compare glulisine with regular insulin in terms of changes in GHb from baseline to weeks 12 and 26, self-monitored blood glucose (BG) parameters, laboratory-measured fasting plasma glucose (FPG) and postmeal plasma measurements made in association with the in-clinic test meal at endpoint, symptomatic hypoglycemia, insulin doses and Treatment Satisfaction in subjects with type 2 diabetes.

Outcomes/endpoints

As in study 3001 the primary efficacy data was GHb values recorded at baseline and at endpoint for each subject. The primary efficacy measure was the change from baseline to endpoint in the blood level of GHb.
Secondary efficacy data includes GHb values at baseline and weeks 12 and 26; SMBG profiles recorded using home BG monitors (preprandial at breakfast, lunch, dinner; 2 hours postprandial after breakfast, lunch, dinner; and at bedtime); in-clinic FPG and plasma glucose at 1 hour and 2 hours after a test meal at the endpoint visit; symptomatic hypoglycemia; dosage of both short-acting and basal insulin preparations at baseline and weeks 12 and 26 and endpoint and quality of life using the Diabetes Treatment Satisfaction Questionnaire status.

Sample size

Sample size calculation was made on the basis of the primary objective of demonstrating noninferiority of glulisine to regular insulin. A sample size of 676 (338 in each group) was needed to ensure that the upper confidence limit of the two-sided 95% CI for the adjusted mean difference between groups would not exceed 0.4% GHb with 90% power and with an expected treatment difference of 0.1% GHb. With an expected rate of 20% of subjects not evaluable due to early withdrawal or protocol violation, it was thus planned to randomise 423 subjects in each treatment groups.

Randomisation

After the run-in phase, in which subjects received the same standard regimens of basal and short acting insulin preparations, eligibility for entry into the study was confirmed and subjects were to be randomized to study treatment as soon as possible after reaching the end of the 4-week run-in phase, irrespective of whether they had met target BG values. Centralised computerized telephone randomization was used.

Blinding

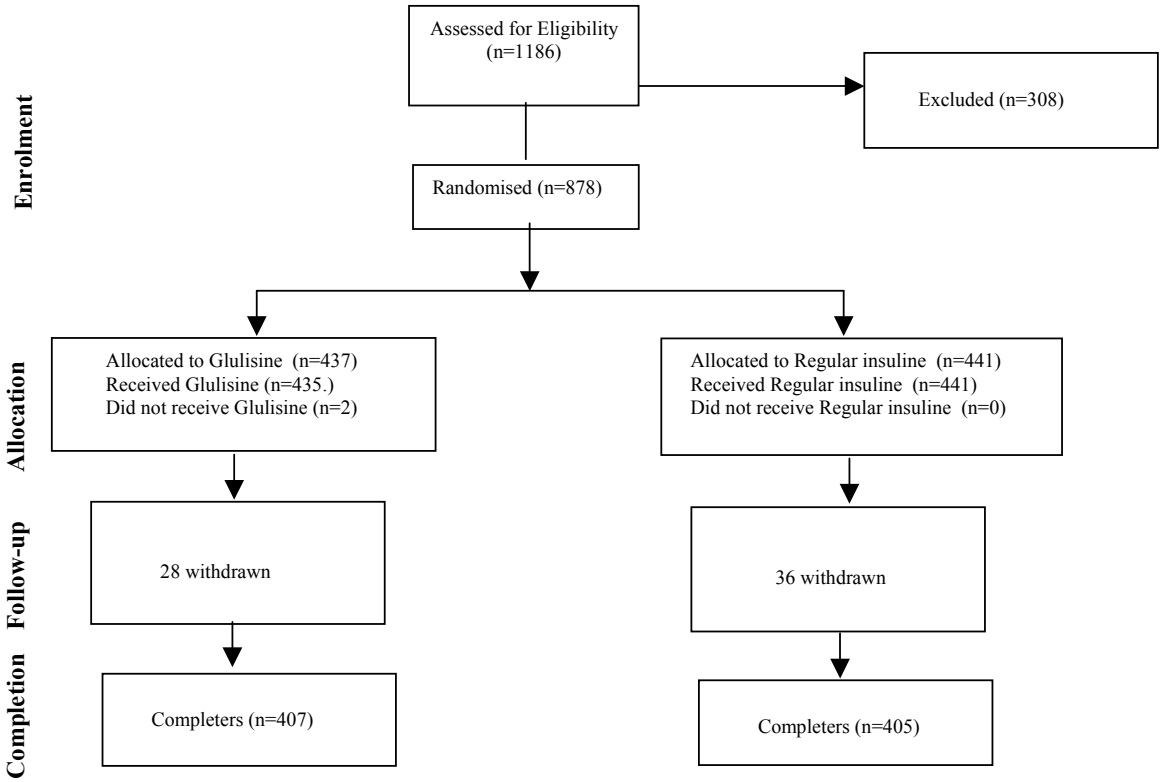
As for study 3001, the study was not blinded.

Statistical methods

Statistical methods used are similar to those for Study 3001.

RESULTS

Participant flow



A total of 1186 subjects entered the screening phase, of which 878 were randomized and 876 received study medication. There were 437 glulisine subjects and 441 subjects in the regular insulin group randomized and treated. Of the 876 subjects treated, 64 subjects (28 glulisine; 36 regular insulin) were withdrawn after the start of treatment. The ITT population was defined as all subjects randomized and treated, and consisted of 876 subjects.

Recruitment and conduct of the study

The study was a multinational, multicenter study carried out from 2 July 2001 to last patient out: 18 October 2002. Subjects with type 2 diabetes were recruited from centers in North America and Australia. After a screening and 4-week run-in phase, during which all subjects received NPH insulin as the basal insulin and regular insulin as the short-acting insulin, subjects were randomized and entered a 26-week treatment phase. Subjects who completed the 26 weeks of treatment were intended to be enrolled in a safety extension study (Study 3012)

Baseline data

With respect to most baseline data the two groups were comparable (ITT populations).
The types of basal and short-acting insulin preparations at study entry were similar between treatments.

Numbers analysed

In the ITT population a total of 876 patients distributed on the glulisine group (N = 435) and the regular insulin group (N = 441) were analysed. In the PP population 798 patients distributed on the

glulisine group (N = 342) and the regular insulin group (N = 356). Thes numbers exceed the calculated number of subjects needed to demonstrate non-inferiority of glulisine to regular insulin.

Outcomes and estimation

Primary efficacy variable: change from baseline to endpoint in GHb:

	Glulisine		Regular insulin		Glulisine – regular		
Population/ Timepoint	**N**	**Mean** [a]	**N**	**Mean** [a]	**Difference in adjusted mean**	**95% CI**	**p-value** [b]
ITT population (N=807)							
Baseline	404	7.57	403	7.50			
Endpoint	404	7.11	403	7.22			
Mean change from baseline at endpoint	404	–0.46	403	–0.29			
Adjusted mean change from baseline at endpoint	404	–0.46	403	–0.30	–0.16	(–0.26; –0.05)	0.0029
PP population (N=698)							
Baseline	342	7.53	356	7.47			
Endpoint	342	7.09	356	7.22			
Mean change from baseline at endpoint	342	–0.44	356	–0.25			
Adjusted mean change from baseline at endpoint	342	–0.44	356	–0.28	–0.16	(–0.27; –0.05)	0.0049

[a] Adjusted means from ANCOVA model (ANOVA at baseline).

[b] P-values from a 2-sided test for difference.

It appears from the table that treatment groups had similar GHb levels at baseline and both treatment groups in the ITT population showed reductions in mean GHb over the course of the study, with an adjusted mean change from baseline at endpoint of -0.46% in the glulisine group and -0.30% in the regular insulin group. There were similar reductions in GHb levels in the PP population in both treatment groups.
Based on the predefined non-inferiority margin of 0.4%, the non-inferiority of glulisine compared with regular insulin was shown by the fact that the upper bound of the 95% CI was below 0.4%. The upper limit of the 95% CI was below zero (-0.05%), which established statistical superiority (p=0.0029, ITT population). The results of the PP analyses confirmed these findings. Although statistical superiority was demonstrated it is questioned whether this absolute difference between treatment groups is of clinically relevance.

With regard to secondary parameters, short-acting and basal insulin doses were comparable between treatments at baseline. At endpoint, subjects treated with glulisine had significantly lower BG values at 2-hour postprandial breakfast and dinner than did those treated with regular insulin
No consistent differences between treatments with respect to blood glucose excursions and blood glucose variability were found.
Plasma glucose levels during the in clinic test meal were lower in the glulisine group compared with the regular insulin group. However, because this test was not performed at baseline a proper evaluation is not possible.
The rate of hypoglycemia decreased during the course of the study to a similar extent in both treatment groups.

Pivotal study 3004

Study 3004 was a 12-week, multinational, multicenter, controlled, open, 1:1:1 randomized, parallel clinical trial to assess noninferiority between pre- and postmeal administration of insulin glulisine and premeal regular human insulin in subjects with type 1 diabetes mellitus receiving insulin glargine as the basal insulin therapy.

METHODS

Study Participants

A total of 1138 subjects entered the screening phase. Of these 866 were randomized and 860 received study medication. Subjects were of similar characteristics to those in study 3001. There were 286 subjects in the premeal glulisine group, 296 postmeal glulisine and 278 regular insulin subjects randomized and treated. Of the 860 subjects treated, 63 subjects were withdrawn after the start of treatment (19 premeal insulin glulisine subjects, 18 postmeal insulin glulisine subjects and 26 regular insulin subjects).
Exclusion criteria was similar to study 3001.
The ITT population was defined as all subjects randomized and treated, and consisted of 860 subjects.
The PP population was defined as all ITT subjects excluding subjects with a major protocol violation.
Major protocol violations occurred with 116 subjects. Subjects were included in the efficacy analyses if they had both a pretreatment and an on-treatment value available and thus, the number of subjects included in the analyses for each variable varied.

Treatments

Subjects were randomized to receive Insulin glulisine or regular human insulin, individually dosed as appropriate by subcutaneous (s.c.) injection. Glulisine was to be injected either 0 to 15 minutes prior to the meal or whichever of the following occurred first: immediately after completing a meal or 20 minutes after starting a meal. Regular insulin was to be injected 30 to 45 minutes prior to the meal. Glulisine or regular insulin were not to be mixed with insulin glargine.
Either glulisine or regular insulin was to be taken in combination with insulin glargine once daily at bedtime as the basal insulin.
The dose of Glargine was to be titrated based on fasting BG with a goal of 5.0-6.7 mmol/l. The dose of Glulisine or Lispro was to be titrated to achieve a goal of 2 h postprandial BG of 6.7- 8.9 mmol/l while avoiding hypoglycemia.

Objectives

Primary: to evaluate noninferiority of premeal glulisine or postmeal glulisine compared to premeal regular insulin and postmeal glulisine compared to premeal glulisine in terms of change in GHb from baseline to endpoint and safety (in terms of adverse events, clinical chemistry, lipids and hematology) in subjects with type 1 diabetes mellitus.
Secondary: to assess changes in GHb at weeks 8 and 12, BG parameters, symptomatic hypoglycemia, and insulin doses in subjects with type 1 diabetes mellitus administered premeal glulisine or postmeal glulisine or premeal regular insulin

Outcomes/endpoints

As in study 3001 and 3002 the primary efficacy data was GHb values recorded at baseline and at endpoint for each subject. The primary efficacy measure was the change from baseline to endpoint in the blood level of GHb.

Secondary efficacy data includes GHb values at baseline and weeks 8 and 12; SMBG profiles recorded using home BG monitors (reprandial glucose and 2-hour postprandial glucose values as well as bedtime glucose values at baseline and weeks 8 and 12 and endpoint) Dosage of short-acting and basal insulins at baseline and weeks 8 and 12 and endpoint, and reports of symptomatic hypoglycemia from the time of informed consent to the end of the treatment phase.

Sample size

Sample size calculation was made on the basis of the primary objective of demonstrating noninferiority of premeal glulisine or postmeal glulisine to premeal regular insulin and postmeal glulisine to premeal glulisine. A sample size of 744 (248 in each group) was needed to ensure that the upper confidence limit of the two-sided 98.33% CI (type I error adjusted for multiplicity =0.008) for the adjusted mean difference between groups would not exceed 0.4% GHb with 90% power and with an expected treatment difference of 0.1% GHb.

Randomisation

Randomisation and stratification was done as for previous pivotal studies. In this case, two schedules were used: subjects prior on glargine and subjects who were not.

Blinding

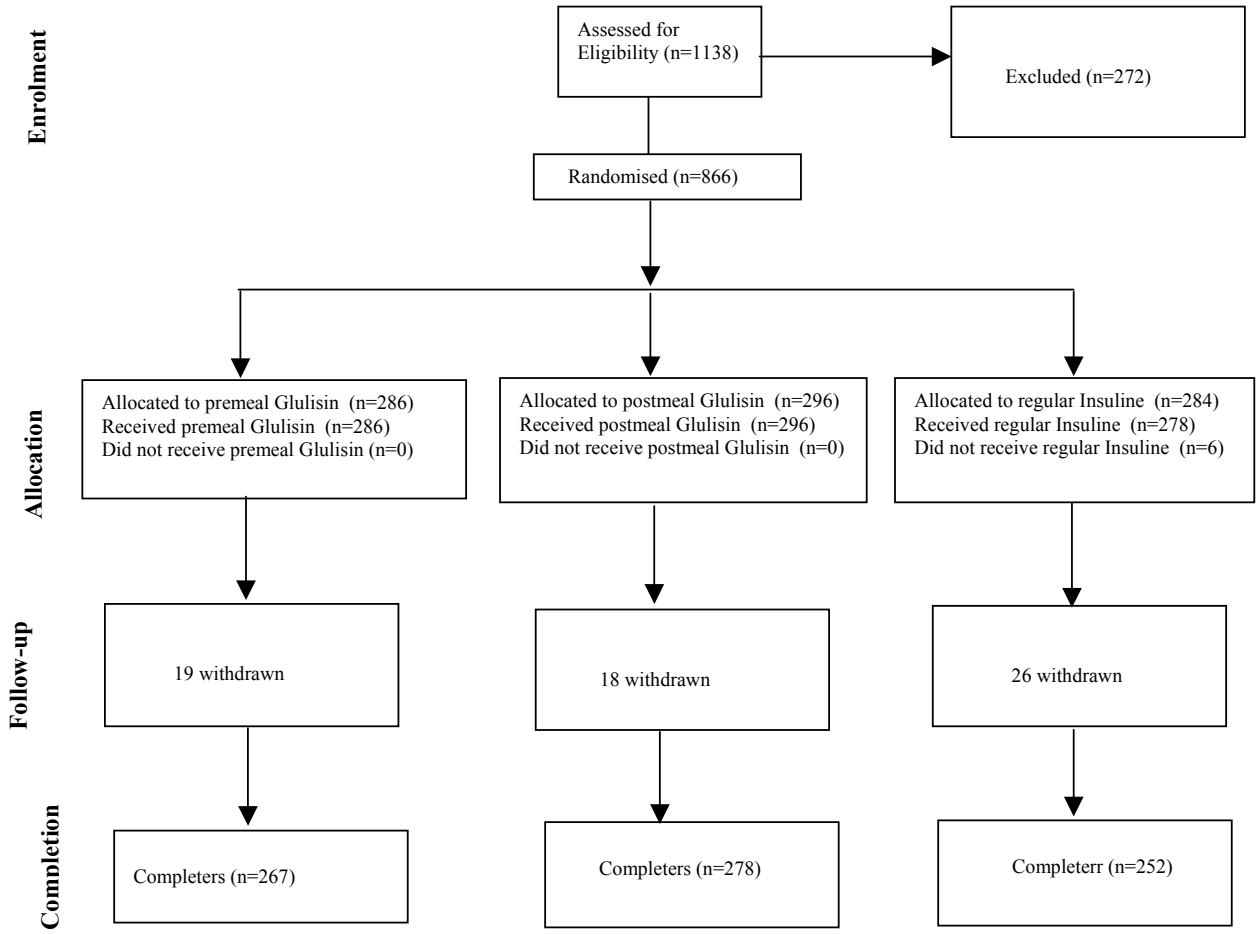
As for studies 3001 and 3002, the study was not blinded. The objective of this study, premeal compared to postmeal glulisin, makes a blinding design impossible.

Statistical methods

The 3 primary comparisons performed in this study were premeal glulisine compared to regular insulin, postmeal glulisine compared to regular insulin, and postmeal glulisine compared to premeal glulisine. The primary analysis was the analysis of change in GHb from baseline to endpoint (primary efficacy variable) using the ITT population. The PP population was used to check for the consistency of the analyses made using the ITT population. Both the ITT and PP populations were used for the analyses of all efficacy variables. Analyses of demographic baseline data and safety variables were conducted using the ITT population.
The primary analysis assessed noninferiority using the upper bound of the 98.33% confidence interval (CI) for the difference in the adjusted mean change in GHb from baseline to endpoint which was compared with the predefined noninferiority margin of 0.4% GHb.
Noninferiority would be demonstrated if the upper bound of the CI was ≤0.4% If noninferiority were demonstrated, then a corresponding check of statistical superiority (i.e. that the upper bound of the CI is <0.0%) would be performed without an alpha penalty since this is a closed procedure.
Continuous variables (e.g. GHb) were analyzed by an analysis of covariance (ANCOVA) with treatment and (pooled) center as fixed effects and the baseline value of the variable as a covariate. The assumptions of the model such as parallelism was tested and the distribution of the residuals from the model was examined. To assess the comparability at baseline between treatments, continuous baseline variables were analyzed by an analysis of variance (ANOVA) with treatment and (pooled) center as fixed effects. As this study was stratified at randomization, a further exploratory analysis of the primary efficacy variable was performed which had the stratum variable (use of glargine vs. use of other basal insulin at study entry) included in the model as an additional effect.
Categorical variables (e.g. frequency of hypoglycemic events) are presented by frequency distributions. Treatment groups were compared using the Cochran-Mantel-Haenszel test stratified by (pooled) center.

RESULTS

Participant flow



A total of 1138 subjects entered the screening phase, of which 866 were randomized and 860 received study medication. There were 286 premeal glulisine subjects, 296 postmeal glulisine and 278 regular insulin subjects randomized and treated. Of the 860 subjects treated, 63 subjects (19 premeal glulisine; 18 postmeal and 26 regular insulin) were withdrawn after the start of treatment. The ITT population was defined as all subjects randomized and treated, and consisted of 860 subjects.

Recruitment and conduct of the study

The study was a multinational, multicenter, open, controlled parallel group 1:1:1 study carried out from 25 september 2001 to last patient out: 6 september 2002. Subjects with type 1 diabetes were recruited from 94 centers in US (65 sites), Canada (16 sites) and Australia (13 sites). After a screening and 4-week run-in phase, during which all subjects received glargine as the basal insulin and regular insulin as the short-acting insulin, subjects were randomized and entered a 12-week treatment phase.

Baseline data

In general baseline data were fairly balanced across the three treatment groups.

Numbers analysed

In the ITT population a total of 860 patients distributed on the premeal glulisine group (N = 286), postmeal glulisine group (N=296) and the regular insulin group (N= 278) were analysed. In the PP population 741 patients distributed on the premeal glulisine group (N = 249), postmeal glulisine group (N=260) and the regular insulin group (N= 232) were analysed.

Outcomes and estimation

Primary efficacy variable: Change from baseline to endpoint in GHb: (ITT population)

Treatment	**Premeal glulisine**	**Postmeal glulisine**	**Regular insulin**	**Difference** [a]		
	(N=268)	**(N=276)**	**(N=257)**	**Adjusted mean**	**98.33% CI**	**p-value**
Postmeal glulisine vs. regular						
Mean baseline		7.70	7.64			
Mean endpoint		7.58	7.52			
Mean change from baseline at endpoint		–0.12	–0.12			
Adjusted mean change from baseline at endpoint		–0.11	–0.13	0.02	(–0.11; 0.16)	0.6698
Premeal glulisine vs. regular						
Mean baseline	7.73		7.64			
Mean endpoint	7.46		7.52			
Mean change from baseline at endpoint	–0.27		–0.12			
Adjusted mean change from baseline at endpoint	–0.26		–0.13	–0.13	(–0.26; 0.01)	0.0234
Postmeal vs. premeal glulisine						
Mean baseline	7.73	7.70				
Mean endpoint	7.46	7.58				
Mean change from baseline at endpoint	–0.27	–0.12				
Adjusted mean change from baseline at endpoint	–0.26	–0.11		0.15	(0.02; 0.29)	0.0062

[a] Adjusted means from ANCOVA model. P-values from a 2-sided test for difference.

At baseline subjects in all treatment groups had similar GHb levels.
All treatment groups in the ITT population showed statistically significant within-group reduction in mean GHb over the course of the study, with an adjusted mean change from baseline at endpoint of -0.26% in the premeal glulisine group, –0.11% in the postmeal glulisine group, and -0.13% in the regular insulin group

Postmeal vs regular. During the study reductions in GHb were similar for the postmeal glulisine group compared to regular insulin group (mean difference.0.02%, 98.33% CI=[-0.11%, 0.16%], p=0.6698).
Postmeal vs. premeal. There was a smaller observed reduction in GHb for the postmeal glulisine group compared with the premeal glulisine group (mean difference 0.15%, [0.02%, 0.29%], p=0.0062, after adjustment for multiplicity.
Premeal vs. regular. There was a larger observed reduction in GHb for the premeal glulisine group compared with the regular insulin group (mean difference –0.13%, [-0.26%; 0.01%]; p=0.0234, which was not statistically significant after adjustment for multiplicity).

With regard to secondary endpoints, a the decrease in mean GHb levels in all treatment groups was achieved by week 8, with the most pronounced decrease being in the premeal glulisine group.
At endpoint, there was a similar decrease in the adjusted mean short-acting insulin dose in the premeal glulisine (–0.88 U) and postmeal glulisine groups (–0.47 U, p=0.5451; ITT population). In contrast, the regular insulin dose increased at endpoint by an adjusted mean of +1.75 IU.
The total daily insulin dose remained at approximately baseline levels in the premeal glulisine group (adjusted mean change at endpoint: +0.04 U) and postmeal glulisine group (adjusted mean change: -0.22 U) (ITT population). In contrast, the total insulin dose increased from baseline to endpoint by an adjusted mean of +2.35 IU in the regular insulin group (p=0.0014 compared with postmeal glulisine; p=0.0042 compared with premeal glulisine). Findings were similar in the PP population.
Throughout the treatment phase, the premeal glulisine BG values were statistically significantly lower than those in the postmeal glulisine or regular insulin groups for the 2-hour post-breakfast and 2-hour post-dinner measurement. Otherwise the SMBG profiles were similar in all treatment groups during the study.

Overall, there were no statistically significant differences between treatments in the rate of any category of symptomatic hypoglycemia during any phase or time period throughout the study.

- Discussion on clinical efficacy

Efficacy of glulisine in the treatment of DM was shown in adult subjects with type 1 and type 2 DM, in basal-bolus regimens using either glargine or NPH as the basal insulin and in type 2 diabetics in combination with OHA treatment.
In all three pivotal studies glulisine was shown to be non-inferior to either insulin lispro (3001) or regular insulin (3002 and 3004) in terms of glycemic control, i.e. change in total glycated hemoglobin (GHb) from baseline to endpoint. In study 3001(type 1 diabetics) Glulisine demonstrates similar efficacy in terms of change in GHb and self-monitored BG values from 7-point profiles, compared to lispro without requiring an increase in basal or total daily insulin dose, which was seen in the lispro group.
In study 3002 in subjects with type 2 diabetes statistical superiority of glulisine to regular insulin was shown. Glulisine treated subjects had a larger reduction in the adjusted mean change from baseline to endpoint in GHb (-0.46%) than did those subjects treated with regular insulin (-0.30%). Although this reduction was statistical significant, it is questioned whether the absolute difference of 0.16% GHb between groups is of clinically relevance
Furthermore, subjects treated with glulisine had lower BG values at all times during the 7-point self-monitored BG profiles at endpoint than did those treated with regular insulin, with the 2-hour postprandial breakfast and dinner differences reaching statistical significance. A statistically significantly lower FPG as well as 1-hour and 2-hour postprandial plasma glucose measured during the in-clinic test meal were also observed in the glulisine group.

The improved glycemic control in glulisine subjects was not associated with an increase in insulin dose as basal, short-acting and total daily insulin doses displayed similar increases from baseline in both treatment groups. There were no statistically significant differences between the two treatment groups in any of these insulin dose changes or in the number of injections of short-acting insulin per day. Finally no noteworthy differences in the reporting of all categories of symptomatic hypoglycemia were noted between the treatment groups.
In the study 3004 in type 1 diabetics postmeal administration of Glulisine was shown to be non-inferior to premeal administrered glulisine in terms of changes from baseline to endpoint in GHb. Both regimens was shown to be non-inferior to regular insulin administered 30 to 45 minutes before a meal. Throughout the treatment phase the premeal glulisine BG profile values were consistently lower than the postmeal glulisine or the regular insulin groups for the 2-hour post-breakfast and 2-hour post-dinner measurements and these differences between the treatment groups were statistically significant.
Regarding insulin dose the total daily insulin dose remained near baseline levels in both the premeal glulisine group and postmeal glulisine group but increased by an adjusted mean of 2.35 IU in the regular insulin group.
The efficacy of glulisine appears to be maintained irrespective of BMI, age, sex, race, baseline degree of glycemic control, or in the absence or presence of concomitant OHAs.
In conclusion the applicant have submitted data that demonstrates evidence of efficacy for the use of glulisine in type 1 as well as type 2 diabetes patients and data clearly demonstrates non-inferiority of glulisine to other short acting insulins on the market.

Clinical safety

Safety parameters are mainly based on four completed active-controlled studies in the Phase III clinical program for glulisine, each conducted in adult patients with type 1 (study 3001, 3004 and 3006) or 2 (study 3002) diabetes. These studies were performed between July 2001 and December 2002.
Study 3006 was a 12-week, multinational, multicenter, controlled, open, 1:1 randomized, parallel clinical trial comparing the safety of insulin glulisine and insulin aspart used in continuous subcutaneous insulin infusion (CSII) in subjects with type 1 diabetes mellitus. The rationale for performing Study 3006 was to support the claim that glulisine may be safely administered by CSII via an external pump to control hyperglycemia in patients with type 1 DM. Because this study was designed to assess if the physicochemical properties of glulisine were compatible with pump use, and not to formally demonstrate efficacy, a limited number of subjects was studied: 29 glulisine and 30 aspart.
The safety-evaluable population was all subjects enrolled, who received at least one dose of study treatment, and with information in the database after the entry visit.

- Patient exposure

The total safety database comprises 2715 patients from a total of 18 clinical trials. Of these 1617 received insulin glulisine and 1295 received a comparator insulin product.
728 adults with type I diabetes received the product for a period of $\geq$ 12 weeks and 416 patients with type II diabetes received it for at least a similar time period.

- Adverse events

Data concerning adverse events is available from the pooled clinical pharmacology studies, where, in general, patients received one or a maximum of 2 doses of test medication and also from the phase III studies in type I diabetes (3001, 3004 and 3006) and in type II diabetes (3002).
In clinical pharmacology studies, the commonest reported adverse events in the glulisine group were headache (14.5%), hypoglycaemia (2.8%) nausea (1.6%) and vomiting 1.2%. Similar frequencies of these events were observed with comparator medications. Overall, 23.8% of subjects treated with insulin glulisine suffered an adverse event in the clinical pharmacology trials compared with 21.6% of those who received a comparator.

Over all pooled phase III studies, 69.4% of subjects in both the glulisine and pooled comparator groups experienced 1 or more treatment-emergent adverse event (TEAE)

Phase III studies (Type I diabetes)

The comparators used against insulin glulisine in these studies were insulin lispro in study 3001, regular insulin in study 3004 and insulin aspart in 3006.

The commonest reported adverse events in insulin glulisine treated patients were:

Nasopharyngitis	9.9%
Hypoglycaemia	6.3%
Upper Respiratory Infection	6.3%
Influenza	3.6%
Hypoglycaemic Coma	3.5%
Pharyngitis	3.2%
Headache	2.8%
Sinusitis	2.2%

The frequency of these events was similar in insulin glulisine and comparator treatments both in terms of frequency and quality.

Phase III studies Type II diabetes

Only one phase III study is presented (Study 3002). Data is available for insulin glulisine compared to regular human insulin. The commonest reported adverse events in insulin glulisine treated patients were:

Upper Respiratory Infection	13.8%
Oedema	8.3%
Arthralgia	7.4%
Diarrhoea	6.4%
Nasopharyngitis	6.2%
Headache	5.5%
Influenza	5.3%
Back pain	4.1%
Sinusitis	3.4%
Urinary Tract Infection	3.4%
Cough	3.2%
Nausea	2.8%
Pharyngitis	2.3%

A similar frequency and quality of adverse events was recorded in the comparator group.
In general, the common adverse events reported are considered to be those to be expected in a diabetic population. Adverse events worthy of discussion are hypoglycaemia, cardiac disorders, eye disorders, possible hypersensitivity disorders, neoplasms and injection site abnormalities.

Hypoglycaemia
During the clinical trial program hypoglycaemic events including severe and/or nocturnal events were seen in patients treated with insulin glulisine at a comparable frequency with the control group.

Cardiac Disorders: the numbers of events reported and considered to be treatment related are very few. In type I diabetes a single event was recorded in study 3004 in an insulin glulisine treated patient - a case of sinus tachycardia. No other treatment related event was recorded from any other trial.

In type II diabetes no treatment related event was recorded.

Eye disorders: despite the data available is limited on this regard, it does not give rise to concern that insulin glulisine is associated with an increased severity or frequency of ocular adverse events.

Injection site disorders: the data regarding injection site reactions are reassuring. Insulin glulisine use is not associated with an increase in frequency or severity of injection site reactions.

Neoplasms: there is nothing to suppose that insulin glulisine is likely to be associated with an increased frequency of neoplasia, compared to other insulins. The issue should however be re-reviewed when further long-term safety data are available and during the life of the product.

- Serious adverse event/deaths/other significant events

Apart from hypoglycaemic events, serious adverse events have been compared between insulin glulisine and comparator treatments. The frequencies of all serious adverse events are remarkably similar between insulin glulisine and comparators (Type I diabetes insulin glulisine 12.3%, comparator 12.6%; Type II diabetes insulin glulisine 12.6%, comparator 11.6%. The nature and frequency of the individual events is similar between insulin glulisine and comparator.
Keto-Acidosis was a rare event. Six cases occurred in the clinical trial database; 3 in each of insulin glulisine and comparator cohorts.

3 deaths were recorded in these studies (3001, 3002, 3004 and 3006) all during the treament phase of study 3002 (type 2 diabetes): one in patients who received insulin glulisine compared with 2 in patients who received regular insulin.
The death in the glulisine arm was attributed to gastro intestinal hemorrhagia with shock and subsequent MI. The deaths in the regular arm were attributed to cardiac arrest.

- Laboratory findings

Trends in laboratory data were analysed. No trends were observed except that absolute eosinophils increase was rather more common in those treated with comparator (1.4%) than in those treated on insulin glulisine (0.6%).

- Immunogenicity

The analogue nature of insulin glulisine requires that immunogenicity is studied in some detail and the clinical implications of antibody development assessed.
Antibody development was assessed in studies 3001 and 3002. 4 types of antibody were assayed – cross-reactive to human insulin, glulisine specific and lispro specific (in the patient subsets receiving these analogues) as well as human insulin specific antibodies (measured in those who received lispro or glulisine).
In general, no significant elevation of antibody level from baseline values was observed in insulin glulisine treated patients as a whole. Those with a >95% increase from baseline in cross-reacting antibodies were evaluated further. There were more of these receiving insulin glulisine than comparator insulin (study 3001; glulisine 7.8%, lispro 1.9%), (study 3002; glulisine 6.8%, regular insulin 3.2%). In view of the applicant, no trend was observed in these individuals for clinical events such as change in GHb, change in insulin dose, injection site reactions or hypoglycaemia. 2 glulisine and 2 comparator patients from this cohort experienced events likely to be allergic in nature and 1 glulisine and 3 comparator subjects from this group experienced an injection site reaction.

- Discontinuation due to adverse events

In the clinical trials in type I diabetes, 5.1% of patients in the insulin glulisine cohort withdrew from the study compared with 6.2% in the comparator arms. The reasons for these withdrawals were similar in those treated with insulin glulisine or comparator. 1.1% of each group withdrew due to adverse events. 0.1% of patients in the insulin glulisine arm withdrew due to lack of efficacy; in the comparator cohort it was 0.6%.
In the study in type II diabetes 6.4% of those treated with insulin glulisine withdrew compared with 8.2% in the comparator arm. Adverse events were causal in 1.1% of glulisine patients and 1.4% in the comparator arm. Lack of efficacy was responsible in no patients treated with glulisine and 0.1% of those who received the comparator.

- Post marketing experience

There is no post-marketing experience.

- Discussion on clinical safety

The database is sufficient in size to provide information regarding the safety of insulin glulisine in the short term (i.e over 6 months). . Long term extension studies for 3001 and 3002 up to 12-month treatment duration were provided and overall safety was assessed upon receipt of these data. There was no change in the safety profile of glulisine.

Data provided are in general reassuring with regard to allergy, injection site reactions. In the short term, antibody formation to insulin glulisine is not associated with clinically important sequelae.
Hypoglycemia appears as the most frequent undesirable effect of insulin therapy. During the clinical trial program hypoglycaemic events including severe and/or nocturnal events were seen in patients treated with insulin glulisine at a frequency comparable to the control group.

The CHMP concluded that the general data indicate that glulisine is well tolerated and has a safety profile similar to other compared insulins.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The information provided in the application demonstrated consistent batch-to-batch production of Apidra achieving a well-defined quality for the active substance and the medicinal product. The fermentation, basic down-stream processing and purification of the active substance, insulin glulisine, are adequately controlled and validated. Appropriate active substance specifications have been set. The active substance has been well-characterised using state-of the-art methods with regard to its physicochemical characteristics. The manufacturing process of the medicinal product has been described and validated in sufficient detail. The quality of the medicinal product is controlled by adequate test methods and specifications. No excipients of human or animal origin are used in the product manufacture and therefore there is no risk of contamination with viral or TSE agents by these ingredients. No animal ingredients are used for the cell culture process, the basic downstream processing and purification with the exception of porcine enzymes. Sufficient virus validation data were provided for the manufacturing processes of porcine enzymes and, as well, for the manufacturing process of insulin glulisine to consider that Apidra is virologically safe.

Non-clinical pharmacology and toxicology

Overall, the primary pharmacodynamic studies provided adequate evidence that insulin glulisine behaved similarly to human insulin with respect to insulin receptor binding, insulin receptor autophosphorylation and single-cell carrier-mediated glucose transport and lipogenesis, although generally with a slight shift to the right of the dose-response curve. A slightly more pronounced hypoglycaemia than human insulin in rats, and an earlier onset of action in dogs were observed in *in vivo* studies.
Studies on secondary pharmacodynamics, in particular effects on IGF-1 and mitogenic activity, did not raise any concern at the expected concentration in human therapy.
The general pharmacology studies appropriately support the non-clinical pharmacology profile of insuline glulisine, which is considered to be favourable.
From the pharmacokinetic point of view, rats and dogs were the most relevant species for non-clinical efficacy and safety studies. The non-clinical pharmacokinetic properties for insulin glulisine have been appropriately described. A number of studies concerning the absorption, distribution, metabolism and excretion of the product in rats and dogs were performed. Insulin glulisine is well and rapidly absorbed in these species, and showed no major differences in the distribution of human insulin. It is mainly excreted in the urine.
Overall, the toxicology programme, performed in mice, rats, dogs and rabbits, showed exaggerated pharmacodynamic effects in the repeat-dose toxicity tests. The toxicity of insulin glulisine is considered similar to that of human regular insulin

Efficacy

The efficacy of insulin glulisine in the treatment of DM was shown for adult subjects with type 1 and type 2 DM, in basal-bolus regimen using either glargine or NPH as the basal insulin and in type 2 diabetics in combination with OHA treatment.
In three studies, insulin glulisine was shown to be non-inferior to either insulin lispro (3001) or regular insulin (3002 and 3004) in terms of glycemic control, i.e. change in total glycated hemoglobin (GHb) from baseline to endpoint.
In study 3002 in subjects with type 2 diabetes statistical superiority of glulisine to regular insulin was shown. Although this reduction was statistically significant, it is questioned whether the absolute difference (0.16% GHb) between groups is of clinically relevance.
In the study 3004 in type 1 diabetics postmeal administration of insulin glulisine was shown to be non-inferior to premeal administrered glulisine in terms of changes from baseline to endpoint in GHb. Both regimens were shown to be non-inferior to regular insulin administered 30 to 45 minutes before a meal.
The efficacy of glulisine appears to be maintained irrespective of BMI, age, sex, race, baseline degree of glycemic control, or in the absence or presence of concomitant OHAs.
It can be concluded that the data submitted demonstrates evidence of efficacy for the use of glulisine in type 1 as well as type 2 diabetes patients, and that non-inferiority of glulisine to other short acting insulins on the market has been adequately demonstrated.

Safety

The safety database from different short-term studies included 1617 insulin glulisine-treated patients and 1295 receiving a comparator insulin product. One year extension studies for studies 3001 and 3002 have been provided. In general the analysis shows that the frequency of adverse events was similar in insuline glulisine and in comparator treatments both in terms of frequency and quality. The common adverse events reported were those expected in the diabetic population. Hypoglycemia appears as the most frequent undesirable effect of insulin therapy. During the clinical trial program hypoglycaemic events including severe and/or nocturnal events were seen in patients treated with insulin glulisine at a comparable frequency with the control group.

Because of the analogue nature of insuline glulisine, immunogenicity studies were performed, and the results showed no concern.
It can be concluded that the overall general data indicate that insuline glulisine is well tolerated and has a safety profile similar to lispro and regular insulin.

Benefit/risk assessment

Diabetes Mellitus is a chronic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Insulin glulisine (3^{B}Lys-29^{B}Glu-human insulin) is a recombinant rapid-acting analogue of human insulin produced using *Escherichia coli*. The time-concentration and the time-action profiles of glulisine, define it as a member of the rapid-acting insulin subfamily of short acting insulin preparations. The Applicant claims that due to insulin glulisine's rapid onset and short duration of action, an adequate control can be achieved even when administered within 15 minutes before meals or immediately after meals.
Insulin glulisine has shown a similar effect to other rapid-acting insulin preparations in terms of glycemic control (change in total glycated hemoglobin from baseline to endpoint).
Postmeal administration of insulin glulisine was shown to be non-inferior to premeal administrered glulisine in terms of changes from baseline to endpoint in glycated hemoglobin, and to regular insulin administered 30 to 45 minutes before a meal.
It is concluded that insulin glulisine is well tolerated and has a safety profile similar to other compared insulins. Therefore, insulin glulisine may play a role in the treatment of adult patients with diabetes mellitus. A positive benefit/risk ratio can be concluded.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Apidra in the treatment of adult patients with diabetes mellitus was favourable and therefore recommended the granting of the marketing authorisation.