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Veterinary Medicines and Product Data Management

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for ProZinc (EMEA/V/C/002634)

International non-proprietary name: Insulin, human

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Boehringer Ingelheim Vetmedica GmbH submitted on 27 February 2012 an application for marketing authorisation to the European Medicines Agency (the Agency) for ProZinc, through the centralised procedure falling within Article 3(1) and point 1 of the Annex of Regulation (EC) No 726/2004 (a veterinary medicinal product developed by means of a biotechnological process). The eligibility to the centralised procedure was confirmed by the EMA/CVMP on 13-15 September 2011 as ProZinc contains an active substance which is derived by means of recombinant DNA technology.

The CVMP adopted an opinion and CVMP assessment report on 16 May 2013.

On 12 July 2013, the European Commission adopted a Commission Decision for this application.

ProZinc contains human insulin (rDNA) (INN: insulin, human), produced with recombinant DNA technology in the yeast *Pichia pastoris,* as a 40 IU/ml protamine zinc insulin suspension. The product is presented in cartons of a single 10 ml type I glass vial. The route of administration is subcutaneous. The target species is cats and the product is for use in animals with diabetes mellitus.

The product is indicated for the treatment of diabetes mellitus in cats to achieve reduction of hyperglycaemia and improvement of associated clinical signs.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC, as amended.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system was provided which fulfils the requirements of Directive 2001/82/EC, as amended. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Declarations of compliance of the manufacture of the active substance and manufacture of the product with EU GMP requirements have been provided.

The sites involved in ProZinc manufacturer are appropriately authorised and relevant GMP certificates are provided. A GMP certificate for the manufacturer of the active substance was issued following a satisfactory inspection of the site in 2012 by an EU competent authority.

ProZinc is on the market in the USA since 2009.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the finished product manufacturing and packaging sites are considered in line with legal requirements.

The active ingredient manufacturing site has been inspected by an EU competent authority, confirming the GMP status of this site.

Part 2 - Quality

Composition

ProZinc suspension for injection contains 40 IU/ml of human insulin, produced by recombinant DNA technology (rDNA) in the yeast *Pichia pastoris* as a protamine zinc insulin suspension with liquefied phenol as preservative. Excipients in the formulation are protamine sulphate, glycerol, dibasic sodium phosphate heptahydrate, zinc oxide, hydrochloric acid, sodium hydroxide and water for injections.

Container

The product is presented in 10 ml colourless glass type I vials closed with 20 mm grey butyl rubber stoppers and aluminium seals with a plastic flip-off tip. Both the vials and stoppers are compliant with the relevant European Pharmacopoeia (Ph. Eur.) monographs. The aluminium seals have no contact with the product and are considered acceptable.

Development pharmaceutics

ProZinc has been developed as an alternative to currently available veterinary medicinal products containing beef and/or pork insulins for the treatment of feline diabetes type 2.

The formulation is based on the ability for insulin to be precipitated from solution into a stable crystalline form with zinc and protamine, and these resultant insulin-zinc-protamine crystal particles confer the desired modified release properties to the insulin.

ProZinc is presented as a cloudy, white suspension for subcutaneous (SC) use in cats. A parenteral formulation was chosen because of the instability of insulin when given orally. A formulation for SC administration permits injections to be carried out by the animal owner. The ready-to-use suspension formulation offers some advantages to the user compared to lyophilised powder formulations which require reconstitution prior to use.

The concentration of insulin in the formulation (40 IU/ml) was selected so that the volumes to be administered can be measured to support accurate dosing for different cat bodyweights and insulin requirements, but are not too great a volume to be uncomfortable for the animal.

Antimicrobial preservative efficacy data are provided which support the compliance of batches containing 90% phenol with the respective Ph. Eur. monograph. This is considered acceptable to support the microbial safety of the product throughout its claimed shelf life.

Details (e.g. batch size, site of manufacture, active ingredient lot number, test results) are given for the ProZinc batches used in the clinical trials.

The choice of both the active ingredient, human insulin (rDNA), and all the excipients are considered satisfactorily justified.

Method of manufacture

The finished product manufacturing process involves the formulation of an insulin solution which includes zinc oxide and protamine sulphate solution and a separate buffer solution. The manufacturing formula for the proposed commercial batch size is given.

The product is manufactured aseptically with sterile filtration as insulin is susceptible to thermal decomposition and therefore terminal sterilisation is not a suitable means of sterilisation of the product and the use of aseptic manufacture with sterile filtration is justified.

Before sterile filtration the bioburden of the solutions are controlled and a pre-filtration bioburden limit has been defined for each solution according to the limit recommended in the CVMP note for guidance: 'Manufacture of the finished dosage form' (EMEA/CVMP/126/95) which are met for the process validation batches. The check on the integrity of the sterilising filters is performed after sterile filtration of each solution which is considered acceptable. The filtration step for sterilisation has been satisfactorily validated in accordance with the requirements of Ph. Eur. monograph 5.1.1: 'Methods of preparation of sterile products' by challenge with ≥ 1 x 10^7 cfu per cm^2 of *B. diminuta.*

The product is aseptically filled into type I colourless glass vials which are then stoppered with sterilised rubber stoppers and sealed with aluminium overseals. The filling is completed within justified maximum time periods. Satisfactory in-process controls are used.

The description of the manufacturing process and all the in process checks are well described and acceptable.

CVMP considered that sufficient process validation data have been provided.

All test results for each validation batch complied with the specifications proposed for the product.

The CVMP considered the method of manufacture to be correctly documented and sufficiently demonstrated as well as the consistency of the manufacturing process to be adequately demonstrated.

Control of starting materials

Active substance

The active substance, human insulin (rDNA), is produced by recombinant DNA technology using the methylotropic yeast strain *Pichia pastoris* as host cell and an appropriate expression vector. *Pichia pastoris* was genetically modified by the insertion of an insulin precursor (IP) gene in its genome in order to express and secret an insulin precursor (IP) protein. The IP gene was designed to code a protein with an amino acid sequence identical to the human insulin B and A chains. The gene was codon optimized for expression in *Pichia pastoris.* The recombinant clone was selected based on expression and growth characteristics.

The cloning of the expression construct, transformation of the expression construct into the host cell, clonal selection, and characterisation of the selected recombinant clone are sufficiently described in accordance with the note for guidance on quality of biotechnological products: Analysis of the expression construct in cell lines used for production of r-DNA derived protein products (CPMP/ICH/139/95) (ICH Q5B).

The genetically modified yeast cell *Pichia pastoris* expresses a single chain insulin precursor. After secretion by the yeast cells, the human insulin (rDNA) is manufactured in a process with 3 main stages involving the preparation of a laboratory seed culture, an upstream fermentation process and downstream, where the insulin precursor is subjected to enzymatic cleavage.

Batches of the insulin active ingredient are tested for compliance with Ph. Eur. monograph 838: 'Insulin, human', and details of the test methods and their validation are provided.

Data from commercial scale size batches demonstrate that the progressive removal of relevant impurities during insulin manufacturing is sufficient and is considered acceptable. The removal of host

cell DNA has been investigated in accordance with Ph. Eur. monograph 784: 'Recombinant DNA technology, products of'. It was also shown that host cell DNA is sufficiently removed to negligible levels during the manufacturing process**.**

The proposed limits for single chain precursor and host cell proteins (HCP) are appropriately justified.

The proposed residual solvents limits are in accordance with VICH GL10(R): 'Impurities: Residual solvents in new veterinary medicinal products, active substances and excipients'.

As the active ingredient will be used in a sterile dosage form, limits for microbiological quality in accordance with Ph. Eur. monograph 5.1.4. 'Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use' are applied for release of routine drug substance batches.

The processing conditions used in the extraction and purification of the enzyme used are considered suitable to reduce potential contamination with pathogenic organisms such as viral and bacterial agents taking into account Ph. Eur. monograph 5.1.7 'Viral safety' and 5.2.5: 'Substances of animal origin for the production of IVMPs'.

The tests used to investigate the primary, secondary and tertiary structural characteristics of the active ingredient human insulin (rDNA) are acceptable and support the comparability with the relevant Ph. Eur. reference standards.

The initial cloning strategy, subcloning and transformation into *Pichia pastoris* are described in sufficient detail and adequate checks to confirm construct identity and the integrity of the IP gene were carried out. The fermentation and purification processes used to manufacture the human insulin (rDNA) are adequately described. The proposed specification limits for release of the active ingredient are supported.

Excipients

All excipients comply with their respective Ph. Eur. monographs with the exception of dibasic sodium phosphate heptahydrate and liquefied phenol for which Ph. Eur. monographs are not available. However these two excipients do comply with their respective United States Pharmacopoeial Convention (USP) monographs. This is considered acceptable.

The limits for microbiological quality applied to the protamine sulphate are in accordance with the Ph. Eur. monograph 5.1.4 'Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use'.

The CVMP concluded that all starting materials are controlled according to applicable guidelines or pharmacopoeias. Controls performed on the starting materials of biological origin associated with validated treatments ensure quality of the materials and absence of risk of transmission of extraneous agents through the use of these materials.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

Only one material of animal origin used in the manufacture of the human insulin (rDNA). However it is of porcine origin and confirmation was provided that no bovine derived materials are used in the processing.

No starting materials of animal origin are used in the manufacture of the finished product.

Therefore none of the starting materials used for the active substance human insulin (rDNA) or the finished product are risk materials as defined in the current version of the note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 Rev.3).

The CVMP concluded that the risk of transmitting TSE through ProZinc has been assessed in compliance with the current regulatory texts and can be considered as negligible.

Control tests during production

In process control tests are discussed under 'Method of manufacture' above, and are all considered satisfactory to control the manufacture appropriately.

Control tests on the finished product

The finished product specification was elaborated with reference to VICH GL 40: 'Test procedures and acceptance criteria for new biotechnological / biological veterinary medicinal products' and the Ph. Eur. monographs for 'parenteral preparations', 'insulin preparations injectable', 'insulin injection, isophane' and 'insulin zinc injectable suspension'.

It is considered that none of the Ph. Eur. monographs are applicable in their entirety because of the insulin/protamine sulphate ratio and/or the zinc content in ProZinc. The tests and limits meet the relevant requirements of the monographs for 'insulin preparations injectable' and 'insulin injection, isophane'. The analytical methods are those of the Ph. Eur. monographs or are based on them and are well described in the dossier. The analytical validation is considered satisfactory and acceptable. The product specification is considered satisfactory.

Stability

Stability studies on the active substance were conducted under long term conditions at -20 ºC ± 5 ºC and under accelerated conditions (6 months at 5 ºC ± 3 ºC) at process scale–The CVMP considered a 48 month storage period for the active substance as acceptable.

Stability data for the finished product are available for commercial scale batches under long-term testing for up to 24 months (5 °C ± 3 °C) and under accelerated conditions (25 °C ± 2 °C/60%RH ± 5%RH) for up to 6 months. The samples were stored in both the upright and inverted positions. The stability studies are still on-going covering up to 36 months storage under long-term conditions. Different batches of the active substance were used in the different finished product batches applicant will place batches on stability annually.

A freeze-thaw study was conducted on one pilot scale batch. The parameters tested were all those listed on the specification. However, the precaution 'Do not freeze' is considered prudent and acceptable.

An in-use stability study was conducted on commercial batches according to the respective VICH guideline. Results are presented for up to 28 days and no adverse trends are observed, with all results within the specification.

A photostability study was conducted in line with the respective VICH guideline. Lower, out-of-specification results for insulin and a corresponding increase in high molecular weight proteins were observed for the product in the primary container (clear glass vial), whereas all results were within specification before and after exposure for the vial kept stored inside the carton. The outer packaging has therefore been demonstrated to provide appropriate protection from light and an appropriate

warning is included on the SPC and labelling. However, the use of a clear glass vial for this photosensitive product is justified as the visual control of the proper re-suspension of the product prior to use is paramount.

The stability data are considered suitable and acceptable to support the proposed shelf life of 24 months, the in-use shelf life of 28 days and all the storage precautions for the product.

Overall conclusions on quality

Overall, the quality part of the dossier is satisfactorily detailed and complies with the relevant directives, guidelines and monographs.

The initial cloning strategy, subcloning and transformation into *Pichia pastoris* are described in sufficient detail and adequate checks to confirm construct identity and the integrity of the IP gene were carried out. The fermentation and purification processes used to manufacture the human insulin (rDNA) are adequately described. The proposed specification limits for release of the active ingredient are supported.

The manufacture of ProZinc finished product is sufficiently detailed. The proposed specifications and tests for release of ProZinc batches are satisfactory.

Appropriate controls are in place to support the proposed 48 month storage period for the active substance.

The proposed 24-month shelf life, the 28-day in-use shelf life and all the storage precautions are considered appropriate and fully supported by the stability studies. Appropriate advice on storage conditions to ensure the stability of the product has been included in the SPC.

The applicant will perform stability tests on commercial batches annually.

Part 3 – Safety

ProZinc is a suspension for subcutaneous injection for cats. The formulation contains human insulin produced by recombinant DNA technology using the yeast *Pichia pastoris* as a protamine zinc suspension in strength of 40 IU per ml. The initial recommended initial dose is 0.2 to 0.4 IU/kg bw every 12 hours. For cats previously controlled on insulin, a higher starting dose up to 0.7 IU/kg bodyweight may be appropriate.

Safety documentation

Pharmacodynamics

See Part 4.

Pharmacokinetics

No pharmacokinetic studies using the final formulation of ProZinc have been conducted in the target species. However, publically available literature data on protamine zinc insulin (PZI) and other human and animal–origin insulins can be extrapolated to ProZinc.

Absorption:

The time action profile of insulin is determined by its absorption characteristics. The extent and duration of effect can be prolonged by complexing insulin with other substances. Protamine zinc human

insulin (rDNA) is insulin with a delayed absorption and onset of action due to the addition of protamine and zinc leading to crystal formation. After subcutaneous injection, proteolytic tissue enzymes degrade protamine to permit the absorption of insulin. In addition, interstitial fluid will dilute and break down the formed zinc insulin hexamer complexes and result in a delayed absorption from the subcutaneous depot.

Published data on the absorption kinetics of regular, isophane and protamine zinc (PZI) insulin in non-diabetic cats show that PZI has a slower rate of absorption and a longer time above baseline of insulin concentrations following subcutaneous administration of a fixed single dose (0.5 IU/kg) than regular or isophane insulin: mean times to peak insulin concentration were 30, 90 and 240 minutes and mean time to return to baseline insulin concentration were 6, 8 and 16 hours for regular insulin, isophane insulin and PZI, respectively.

Distribution:

Once absorbed from the subcutaneous site, insulin will enter the circulation and diffuse into tissues, where it binds to insulin receptors found on most tissues. Target tissue organs are liver, muscle and adipose tissue.

Metabolism:

Following the binding of insulin with the insulin receptor and the subsequent action, insulin is released back into the extracellular environment. It may then be degraded to small peptides and individual amino acids on passage through the liver or by the kidney. Degradation normally involves endocytosis of the insulin-receptor complex, followed by the action of insulin-degrading enzyme.

Elimination:

Insulin is mainly eliminated via liver (40%) and kidney (60%).

Under clinical field conditions in diabetic cats the maximal action on blood glucose concentrations (i.e. blood glucose nadir) after subcutaneous administration is observed at a mean range of 6 hours (range 3 to 9 hours). In the majority of cats the glucose lowering effect lasted for a minimum of 9 hours after first insulin injection. However, available blood glucose data indicate that for most cats with diabetes mellitus injection of PZI insulin twice daily is needed to adequately control the diabetic state.

Given that the most important pharmacological effect of insulin is its blood glucose lowering effect, it is accepted that insulin pharmacokinetic profiles do not provide further clinically relevant details for the establishment of a safe and effective dosing regimen in diabetic cats.

Toxicological studies

No conventional toxicity studies in the target species cats were performed with the active substance of ProZinc, as numerous toxicity reports are available in the scientific literature relating to human insulin. Human insulin (rDNA) is structurally identical to endogenous human insulin and is not expected to produce toxic responses other than those associated with its physiological or pharmacological properties.

It is accepted that the standard tests on oral toxicology would be meaningless given that insulin is a polypeptide which is subject to degradation in the gastrointestinal tract by digestive enzymes and therefore is not active following oral administration.

Based on the data presented the following is accepted:

- The main adverse effect of insulin arising from overdose is hypoglycaemia. The consequences of hypoglycaemia are well documented, in severe cases resulting in coma and death.
- Insulin is unlikely to directly induce reproductive toxicity and is an unlikely teratogen. Whilst effects on organogenesis and embryo development have been reported in pregnant diabetic women in publically available literature, these effects are considered being the result of the physiological state of hypo- or hyperglycaemia rather than to exposure to insulin itself.
- Human insulin is not considered genotoxic or carcinogenic.

While the toxicological data package does not include the conventional battery of studies, it is accepted that the toxicological profile of the active substance insulin human has been adequately characterised.

The safety and efficacy of ProZinc in breeding, pregnant and lactating cats has not been evaluated. Therefore, an appropriate statement is included in section 4.7 of the SPC.

Tolerance in the target species is addressed in Part 4 of this report.

Studies of other effects

Immunogenic potential

A clinical problem associated with insulin administration in humans is insulin allergy which has been attributed to the use of bovine and porcine insulin is reported to occur in about 2% of patients. There is a general assumption that these reactions are largely due to reactions to animal proteins or polypeptides. However, the allergic reactions against insulin are also considered to be mainly caused by insulin degradation products, minor contaminants or due to sensitivity to one of the formulation components. It was assumed that the occurrence of such reactions would cease with the introduction of human insulin. However, although the incidence of allergic reactions was reduced, there are still reported cases of allergy in humans associated with the clinical use of human insulin (rDNA), which may present as urticaria, generalized erythema or anaphylaxis. It is considered extremely unlikely that such events could be triggered by skin contamination with insulin products as these events are related to the parenteral administration of therapeutic quantities. Furthermore, the entry of insulin through the outer layers of skin will be prevented by its high molecular size.

The development of anti-insulin antibodies in the target species cat has been described but is uncommon. Importantly, in cats with anti-insulin antibodies no effect on glycaemic control is evident. It can be concluded that the long-term administration of ProZinc is unlikely to lead to significant formation of circulating antibodies and, where anti-insulin antibodies develop, they are not expected to be of any clinical relevance. This is supported by the fact that systemic allergic reactions were not observed in the field study.

Based on the information presented, the immunogenic potential of the product appears to be of limited clinical relevance. Available US pharmacovigilance data indicate that the potential for hypersensitivity-type reactions in the field associated with use of the product is low. Appropriately, section 4.3 of the SPC includes the following statement: 'Do not use in case of hypersensitivity to the active substance or to any of the excipients.'

Local effect studies

No studies with the final formulation have been conducted to evaluate the potential for skin irritation and sensitisation or ocular irritation. However, it is accepted that the active substance and the excipients each have limited irritation or skin sensitisation potential. All excipients are well established and commonly used in other medicinal preparations such as authorised human insulin products.

Additionally, the available pharmacovigilance data in the US show that no cases of skin irritation, eye irritation or skin sensitization in owners regularly injecting twice daily their diabetic cats were reported. In conclusion, the absence of local effect studies with the final formulation is considered justified and acceptable.

Observations in humans

It is considered that human insulin produced by recombinant DNA technology has been safely used in humans since the early 1980s; since then many insulin formulations containing human insulin (rDNA) have been marketed. Aside from the physiological hypoglycaemic effect of insulin, common adverse events reported in humans include headache, upper respiratory tract infections, influenza-like symptoms, injection site pain and reactions, back pain, allergic reactions, rash and pruritus, nausea and diarrhoea.

According to pharmacovigilance data available since the product ProZinc was first marketed in the US in 2009, five cases of human exposure were reported in the period of 2009 to 2011. Two of the five cases showed no clinical symptoms. In two other cases, the owners were diabetic themselves and accidentally self-administered the wrong insulin formulation. The last case was poorly reported and suggested dermal irritation and pain following accidental self-injection.

User safety

A user risk assessment in line with the relevant guideline (EMEA/CVMP/543/2003-Rev.1) was provided. It is accepted that the principal route of exposure of concern is inadvertent self-injection and the potential risks for the user are hypoglycaemia and allergic reaction. Even though the probability of such exposure is low, it is considered appropriate to include following user warnings in the SPC and package leaflet:

'Accidental self-injection can provoke clinical signs of hypoglycaemia and there is a low possibility of an allergic reaction in sensitised individuals'.

The product is subject to prescription. As such, it is expected that the prescribing veterinary practitioner will advise the animal owner/user of correct administration technique.

The product labelling will carry the statement 'Keep out of sight and reach of children'.

Environmental risk assessment

The applicant provided an environmental risk assessment in line with the Guideline on Environmental Impact Assessment for Veterinary Medicinal Products – Phase I (CVMP/VICH/592/98-FINAL).

Given that the product is:

- for individual animal treatment subject to veterinary prescription,
- the product is indicated for non-food animals,

the environmental risk assessment can stop at Phase I.

ProZinc is not expected to pose a risk to the environment when used according to the SPC recommendations.

Overall conclusions on the safety documentation

The pharmacokinetic properties of the active substance have been adequately described. By adding protamine and zinc to the product, the onset and duration of effect has been extended.

No conventional toxicity studies were performed with ProZinc in the target species as numerous toxicity reports relating to human insulin are available in the scientific literature. Human insulin (rDNA) is structurally identical to endogenous human insulin and is not expected to produce toxic responses other than those associated with physiological/pharmacological properties, i.e. hypoglycaemia and associated clinical signs.

While the toxicological data package does not include the conventional battery of studies, it is accepted that the toxicological profile of the active substance has been adequately characterised.

As the safety and efficacy of ProZinc in breeding, pregnant and lactating cats has not been evaluated, an appropriate statement is included in section 4.7 of the SPC.

A user risk assessment in line with the relevant CVMP guideline EMEA/CVMP/543/2003 Rev. 1 has been provided. The principal route of exposure of concern is inadvertent injection and the principal risks associated with user exposure to this product are hypoglycaemia and allergic reaction. Appropriate user safety statements to mitigate these potential risks are included in the SPC.

Based on the data provided the ERA can stop at Phase I. ProZinc is not expected to pose a risk for the environment when used according to the SPC recommendations.

Residues documentation

Not applicable.

Part 4 – Efficacy

Pharmacodynamics

Information to characterise the pharmacodynamic properties of insulin was presented in the form of publically available literature and proprietary data, including data on: the blood glucose lowering effects of protamine zinc human insulin (PZIR) and insulin of different origin in humans; the effect of PZIR and protamine zinc beef/porcine insulin (PZI) in rats; and, the blood glucose lowering effect of different insulins, including PZIR, in the target species cats. Based on the information presented, it is accepted that the pharmacodynamic properties of the active substance have been adequately described. In summary:

- Insulin is a potent mediator of carbohydrate and fat metabolism.
- In insulin responsive tissues, insulin facilitates cellular uptake and metabolism of glucose.
- The main effect of insulin is the reduction in circulating blood glucose concentrations.
- In humans, the potency of human insulin (rDNA), semisynthetic human insulin, pancreatic human insulin and purified porcine insulin is similar in vitro (biologic activity and receptor binding assays)
- ProZinc contains human insulin produced by recombinant DNA technology as opposed to insulin of animal origin. In humans, no significant differences regarding the glucose lowering effect have been reported between human insulin (rDNA) and insulin of animal origin (porcine or beef).

- In cats, the pharmacological effect of PZI in terms of lowering of blood glucose has been demonstrated in diabetic cats when administered twice daily subcutaneously. In one study, the starting dose for PZI insulin was between 0.2 and 0.6 IU/kg every 12 hours.
- In rats, the serum glucose profile of PZIR is broadly similar to that of PZI when administered subcutaneously to diabetic rats. According to these data protamine zinc human insulin (rDNA) and beef/pork origin can be considered to have an equal pharmacological effect, similar to the observation in humans.

Based on the above, it is accepted that further laboratory studies investigating the pharmacological effect of human insulin (rDNA) in healthy cats are not necessary.

Data from the pivotal field study (IPI-PZI-0106) were used for characterising the pharmacological effects of ProZinc in diabetic cats based on individual blood glucose curves. The maximal action on blood glucose concentrations (e.g. blood glucose nadir) after subcutaneous administration was observed at a mean of 6 hours (range 3 to 9 hours). In the majority of cats the duration of action lasts for a minimum of 9 hours (the effect on blood glucose was evaluated up to 9 hours following injection only). This indicates that ProZinc is suitable for twice daily treatment of diabetic cats. However, it is generally accepted that the blood glucose lowering effect of an insulin product should be determined for the individual patient and the insulin dose should be adjusted according to clinical response to achieve adequate glycaemic control.

Development of resistance

The spontaneous form of diabetes mellitus seen in cats closely resembles that of human type 2 diabetes which is a heterogeneous disease characterised by a combination of impaired insulin action (insulin resistance) and β-cell failure. However, immunological reactions or failure of treatment as a result of insulin resistance due to ProZinc therapy is considered to be negligible in diabetic cats, and not regarded as potential risk factor for lack of efficacy.

Pharmacokinetics

See Part 3.

Target animal tolerance

The physiologic effect of both, endogenous or exogenous insulin is to lower blood glucose concentrations. The amount of insulin required to regulate blood glucose concentrations within a normal range varies considerably over time both within and between individuals with diabetes mellitus. An overdose of insulin results in hypoglycaemia.

Hypoglycaemia can occur with changes in insulin dosage, an overlap of insulin activity, or with a well-established dose in an individual with changes in physiological status. Hypoglycaemia may be associated with clinical signs that range from mild (e.g. lethargy, weakness, or ataxia) to severe (e.g. seizures, coma, or death), and is a common adverse effect of insulin administration in cats. There is extensive literature documenting the physiologic effects of insulin, the general safety of insulin, as well as the common adverse events associated with insulin therapy.

Specific target animal safety studies other than field studies were not provided in support of this application. It is accepted that for the active substance insulin, such a study design is not appropriate due to the potential for harm resulting from hypoglycaemia. Therefore, data generated in the context of the field studies are considered pivotal in assessing the safety of the product.

The safety of ProZinc was evaluated in the pivotal field study and a second, extended field study as reported and commented on below. The most common adverse events seen in the extended use study were (in order of decreasing frequency): vomiting decreased appetite or not eating, lethargy/sluggish, diarrhoea/soft stool/fluid filled bowels, hypoglycaemia, unsteady/wobbly/trembling/glassy-eyed/spacey/depressed etc., abnormal integument, inappropriate elimination.

In further support of the safety of the product, it was demonstrated that the product has been authorised and marketed in the USA since October 2009 demonstrating the post authorisation safety experience. The most commonly reported adverse events (polyuria, polydipsia and lethargy) are typical clinical signs related to the underlying diabetic disease. Other commonly reported adverse events such as vomiting, diarrhoea and decreased appetite are clinical signs often found (especially in elderly cats) due to other underlying conditions.

Potential hypersensitivity to the active substance has been addressed adequately with a contra-indication in SPC point 4.3.

In conclusion, it is accepted that adverse events other than hypoglycaemia and local injection site reactions are unlikely to be related to ProZinc administration.

Hypoglycaemia is the most significant potential adverse event and clinical signs of overdose are included in section 4.6 and 4.10 of the SPC.

Dose determination/justification

No dose determination studies in cats were provided but reference was made to published literature on pharmacokinetic/pharmacodynamic data in laboratory animals, in the target species cat and in humans.

It is considered that the safety and efficacy of the appropriate dose regimen is mainly influenced by the pharmacodynamic (blood glucose lowering) effect. An appropriate dose for an individual cat is dependent upon a number of factors including bodyweight, diet, exercise level and concurrent disease. The starting dose should be sufficient to reduce blood glucose levels with a low risk of undesirable hypoglycaemia with subsequent insulin doses individually adjusted based upon response (clinical signs, blood glucose concentration and fructosamine concentration).

The proposed starting dose of 0.2 to 0.7 IU/kg, subcutaneously, every 12 hours was based on the established dose for PZI VET in cats available from peer-reviewed literature, and on the results of two pilot field studies conducted in a total of 50 diabetic cats (see below). Given that ProZinc and PZI VET are identical apart from the origin of the insulin (human insulin (rDNA) in ProZinc and beef/pork insulin in PZI VET) and that human insulin (rDNA) and beef/pork insulin are considered to have the same potency, it is acceptable to extrapolate the dose from PZI VET to ProZinc. For cats not previously treated with insulin, a lower starting dose of 0.2 to 0.4 IU/kg is recommended.

Field trials

Four field studies in total were performed – two pilot field studies, one pivotal field study and one 'extended duration' field study which was an extension to the pivotal field study and focused on safety issues.

Both pilot studies were non-GCP studies and involved a switch of treatment from beef/porcine insulin in PZI VET to protamine zinc human insulin (rDNA) in the proposed new formulation for a 30-day period. The proposed new test formulation was administered twice daily to all cats with a mean insulin dose of 3.0 to 4.6 IU per animal (0.4 – 0.7 IU/kg). Whilst conclusions from these pilot studies can only

be considered limited, it is accepted that human insulin protamine zinc in the final formulation appeared to behave similarly to PZI VET in diabetic cats and to provide similar glycaemic control over the 30 day study period. These studies can be considered supportive for the proposed dosing regimen.

Based on the published data and the pilot field data, a starting dose of 0.2 to 0.7 IU/kg was chosen for use in the pivotal field study.

The pivotal field study was a multicentre baseline controlled study (i.e. it did not include a 'parallel' control group), conducted in the USA which is reported as being in compliance with GCP standards. The absence of an EU study is not considered to be of concern given that geography is not expected to impact on the manifestation and treatment of diabetes mellitus. While the absence of a control group may be considered a weakness, the decision to conduct a single arm study was justified on the fact that at the time of study initiation no control product was available in the USA. In addition, given the nature of the disease condition under treatment, the use of a placebo control group was considered unethical.

One hundred eighty-nine (189) diabetic cats were enrolled in the study, of which 176 received PZIR. All cats that received PZIR were included in the safety evaluation and one hundred thirty-nine (139) cats were evaluated for efficacy. The cats were of various breeds and gender, aged between 3 and 19 years, and representing a heterogeneous group potentially with differing management (diet etc.). It is accepted that the chosen study population can be considered as representative of the general feline diabetic population. To be included in the study cats were required to be either treatment naïve (which was the majority of cats) or poorly regulated on insulins other than PZI VET (blood glucose >250 mg/dl, glucosuria) and to show clinical signs of polyuria and polydipsia and/or weight loss. Cats with the following conditions were excluded from the study: hyperthyroidism, or life-threatening illnesses, i.e. Feline Leucaemia Virus (FeLV), Feline Infectious Peritonitis (FIP), neoplastic processes. Furthermore, cats receiving glucocorticoids with in the past 30 days or megestrol acetate with the past 6 months were excluded.

Cats were examined on days 7, 14, 30 and 45 after initiation of insulin treatment. For treatment-naïve diabetic cats, one blood glucose value was obtained and designated as both the glucose mean and glucose nadir. For cats poorly-regulated on insulin other than PZI VET, a 9-hour glucose curve was conducted to determine glucose mean and glucose nadir.

Efficacy evaluation was based on the per-protocol (PP) analysis. Primary efficacy variable was the significant improvement at Day 45 as compared to Day 0 in the control of diabetes, in at least one blood variable (fructosamine, glucose nadir or glucose mean) and in at least 1 out of 3 clinical parameters (body weight, polyuria or polydipsia). In treated cats, mean blood glucose, mean glucose nadir and mean fructosamine values decreased from day 0 to day 45 and at least a change in one of the clinical parameters, e.g. mean increase of body weight, was achieved in 116 out of 139 cats. Based on the pivotal primary efficacy variable, 116 cases out of 139 (83.5%) were considered treated successfully.

Based on the individual blood glucose curves derived in this study the pharmacological effects of ProZinc were characterised. The peak effect of ProZinc is at about 5 to 7 hours and the duration of action lasts for a minimum of 9 hours indicating that ProZinc is suitable for twice daily treatment of diabetic cats. However, it remains the case that the blood glucose lowering effect of an insulin product should be determined for the individual patient and that the insulin dose should be adjusted accordingly to achieve adequate glycaemic control.

In the pivotal field study, it was shown that ProZinc reduces hyperglycaemia (blood glucose concentration) and associated clinical signs in diabetic cats, with the majority of treated cats experiencing an improvement in glycaemic control. It can be accepted that the main treatment goal of

diabetes mellitus in cats is to improve clinical signs and blood parameters and that this treatment goal has been proven in the pivotal field study conducted with ProZinc.

The mean initial insulin dose used in the field study was 0.44 IU/kg every 12 hours (range 0.22 – 1.06 IU/kg) and was only slightly increased to 0.66 IU/kg (range 0.09 – 2.2 IU/kg) on day 30. The dose was adjusted according to clinical signs and blood glucose nadirs on days 7, 14 and 30. A lower starting dose of 0.2 to 0.4 IU/kg is recommended for cats not previously administered insulin: based upon the results of the pivotal field study, 95% of cats were administered an initial dose between 0.2 and 0.4 IU/kg.

From all data presented, it is accepted that the proposed starting dose of 0.2 to 0.4 IU/kg every 12 hours is appropriate with a higher starting dose up to 0.7 IU/kg for cats previously controlled on insulin. Treatment is initiated with a relatively low starting dose to avoid hypoglycaemia. Dose adjustments are performed after several days (5 to 7 days). Individual differences in the response to insulin require close monitoring until the appropriate insulin dose is established. Recommendations on the magnitude of dose adjustment are included in the SPC.

Subsequent to the pivotal field study an 'extended duration' field study was performed to further investigate safety. This included 145 of the cats from the previous pivotal field study (as reported above) which were maintained on PZIR. This extended field study followed on directly from the end (day 45) of the pivotal field study and continued for a further 136 days (total study duration for both studies was 181 days). It was designed to demonstrate the safety of long-term administration of the product for a period of 136 days (examined on study days 0, 34, 68, 102 and 136). While efficacy evaluation during the extended study is descriptive in nature only, it was adequate to show efficacy of the product in managing hyperglycaemia and associated clinical signs in diabetic cats over a period of 6 months.

The safety of the final formulation was evaluated in the field study and the extended use field study. The most common adverse events seen in the extended use study were (in order of decreasing frequency): vomiting, decreased appetite or not eating, lethargy/sluggish, diarrhoea/soft stool/fluid filled bowels, hypoglycaemia, unsteady/wobbly/trembling/glassy-eyed/spacey/depressed etc., abnormal integument, inappropriate elimination.Other reported adverse events (polyuria, polydipsia and lethargy) are typical clinical signs related to the underlying diabetic disease. Other commonly reported adverse events such as vomiting, diarrhoea and decreased appetite are clinical signs often found (especially in elderly cats) due to other underlying conditions.

In conclusion, it is accepted that adverse events other than hypoglycaemia and local injection site reactions are unlikely to be related to ProZinc administration.

Pharmacovigilance data from the USA were provided supporting the safety of the product since authorisation and marketing in the USA in 2009.

Overall conclusion on efficacy

The results of the pivotal field study show that ProZinc reduces hyperglycaemia (blood glucose concentration) and associated clinical signs in diabetic cats, with the majority of treated cats experiencing an improvement in glycaemic control. It can be accepted that the main treatment goal of diabetes mellitus in cats is to improve clinical signs and blood parameters and that this treatment goal has been proven in the pivotal field study conducted with ProZinc.

The proposed starting dose for insulin treatment of 0.2 to 0.4 IU/kg with a higher starting dose up to 0.7 IU/kg for cats previously controlled on insulin is considered appropriate. Dose adjustments are performed after several days (5 to 7 days). The blood glucose lowering effect of the product should be

determined for the individual patient and the insulin dose should be adjusted accordingly to achieve adequate glycaemic control.

Based on the safety assessment in the pivotal field study and the extended field study it is accepted that adverse events other than hypoglycaemia and local injection site reactions are unlikely to be related to ProZinc administration.

Part 5 – Benefit-risk assessment

Introduction

The veterinary medicinal product ProZinc 40 IU/ml is a suspension for injection for cats, containing protamine zinc human insulin produced by means of recombinant DNA technology in yeast (*Pichia pastoris*) as active substance. Protamine zinc human insulin (rDNA) is analogous to human insulin, however with a delayed absorption and onset of action due to the addition of protamine and zinc leading to crystal formation. It is intended for subcutaneous use in cats with diabetes mellitus.

The product is presented in packs of a single 10 ml type I glass vial.

The recommended and approved indication is 'For the treatment of diabetes mellitus in cats to achieve reduction of hyperglycaemia and improvement of associated clinical signs'.

Benefit assessment

Direct therapeutic benefit

ProZinc is effective for the treatment of diabetes mellitus in cats to achieve reduction of hyperglycaemia and improvement of associated clinical signs in diabetic cats, when injected subcutaneously with a starting dose of 0.2 to 0.4 IU/kg twice daily with a higher starting dose up to 0.7 IU/kg twice daily for cats previously controlled on insulin. This was shown in the pivotal efficacy study.

Additional benefits

ProZinc increases the range of available treatment possibilities.

Risk assessment

The formulation and manufacture of ProZinc is well described and specifications set will ensure that a product of consistent quality will be produced.

The physiologic effect of both endogenous and exogenous insulins is to lower blood glucose concentrations. An overdose of insulin results in hypoglycaemia. Hypoglycaemia can occur with changes in insulin dosage, an overlap of insulin activity, or with a well-established dose in an individual with changes in physiologic status. Hypoglycaemia may be associated with clinical signs that range from mild (e.g. lethargy, weakness, or ataxia) to severe (e.g. seizures, coma, or death), and is a common adverse effect of an overdose of insulin in cats. A possible risk is therefore associated with a relative overdose resulting in hypoglycaemia in the treated animal. Appropriate text has been included in the SPC.

The safety and efficacy of ProZinc in breeding, pregnant and lactating cats has not been evaluated. An appropriate text has been included in the SPC.

The risk for the user is generally low. The principal route of exposure of concern is considered to be inadvertent injection and the potential risks for the user are hypoglycaemia and allergic reactions. Appropriate user safety statements are included in the SPC to mitigate these potential risks.

ProZinc is not expected to pose a risk to the environment when used according to the SPC.

Risk management or mitigation measures

Given the possible influence of very stressful events, concomitant treatment with gestagens and corticosteroids or other concomitant diseases on the insulin effectiveness, the SPC includes a special warning that the insulin dose may need to be adjusted in these cases.

No studies investigating the use of ProZinc in breeding, pregnant and lactating cats have been conducted. Given that in general, insulin requirements during pregnancy and lactation might be different due to a change in the metabolic state, the SPC includes a statement that close glucose monitoring and veterinary supervision are advised.

Evaluation of the benefit-risk balance

The formulation and manufacture of ProZinc is well described and specifications set will ensure that product of consistent quality will be produced.

Following initial and regular monitoring, ProZinc is generally well tolerated and presents a low risk for users and the environment and appropriate warnings have been included in the SPC and product literature.

The product has been shown to be efficacious for the indication 'For the treatment of diabetes mellitus in cats to achieve reduction of hyperglycaemia and improvement of associated clinical signs'.

The CVMP therefore concluded that the product has been shown to have a positive benefit-risk balance overall.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of ProZinc were considered to be in accordance with the requirements of Directive 2001/82/EC, as amended, and that the benefit-risk balance was favourable.