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POINTS TO CONSIDER ON THE NEED FOR ASSESSMENT OF REPRODUCTIVE TOXICITY OF HUMAN INSULIN ANALOGUES

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Points to consider have been developed to provide advice on selected areas relevant to the development of medicinal products in specific therapeutic fields.

This document will be revised in accordance with the scientific advances made in this area.

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POINTS TO CONSIDER DOCUMENT ON THE NEED FOR ASSESSMENT OF REPRODUCTIVE TOXICITY OF HUMAN INSULIN ANALOGUES

1. INTRODUCTION

1.1 Problem statement

Women of childbearing potential with Diabetes Mellitus need special and careful control of their glucose level if they wish to become pregnant. The treatment of choice in pregnant diabetic women is the administration of human insulin, even in Type 2 diabetes. This is because the use of insulin allows a more precise control of the glucose level than can be obtained with antiglycemics

New insulin analogues are being developed with the aim of modifying the duration of activity and improving the ease of administration. Some of these new compounds act rapidly and others have a prolonged duration of action giving patients overnight glycaemic cover. It may be difficult to model the impact of such modifications in animals, in view of the differences in pharmacokinetics in the different species and the use of supratherapeutic doses in safety studies.

The current models for reproductive toxicity employ healthy animals. However, the administration of insulin to non-diabetic animals affects glucose homeostasis, which in itself has teratogenic effects. Therefore the question has been raised whether reproductive toxicity studies should be mandatory for newly developed insulin analogues and if not, how the safety of these analogues could be established when used during pregnancy.

1.2 Scope

This document provides guidance to the industry on the general approach for assessment of reproductive toxicity of new insulin analogues. The selected approach should be based on current knowledge and all studies should be scientifically justified and performed according to the "state-of-the-art" in accordance with CPMP/ICH guidelines.

2. INSULINS DURING PREGNANCY

2.1 Glucose regulation during pregnancy

From epidemiological data in diabetic pregnant women treated with human insulin, it is well known that the mortality and morbidity of their offspring can be significantly reduced by careful control of the maternal plasma glucose level (starting prior to conception).

Furthermore, the effects of loss of control of glucose level are different depending on the phase of pregnancy. Lack of glucose control around conception is associated with a high risk of spontaneous abortion and fetal cardiovascular and respiratory changes, whereas glucose imbalance later in pregnancy increases the risk of macrosomia.

While insulin administration at therapeutic doses in pregnant diabetic women has been shown to decrease birth defects, the opposite may be true in normal healthy animals. Administration of insulins at high dosages (or hypoglycemic agents in general) to normal non-diabetic animals during pregnancy, is documented in a number of published reports to cause a teratogenic response, which is generally accepted to be related to the hypoglycemia induced by the insulin and not by the hormone as such. However, in more recent unpublished studies

with insulin analogues and human insulin positive controls, the incidence of fetal abnormalities in animals was essentially unaffected at doses at the high end of the effect span without causing undue mortality due to hypoglycemia.

2.2 The Insulin Growth Factor system during embryonic development

Structural changes by amino-acid replacement may influence the pattern of activity of insulin analogues and change the ratio between the metabolic and mitogenic activity. In this respect, the knowledge of the fetal development of insulin and insulin-like growth factors (IGF) is important. Insulin receptors (IR) are known to exist early in embryonic life; as early as in an 8-cell stage in mice and in a 2-cell stage in rats and humans.

Data from mouse mutants demonstrate that the interaction between IGF-I/IGF-1R and IGF-II/IGF-1R is of major importance to embryonic growth with a predominant influence of the IGF-II/IGF-1R interaction. This interaction is, however, not constant, and with increased foetal age, the IR-mediated action of IGF-II on growth increases. Further, the interaction of insulin analogues with the two IR isoforms (IR-A and IR-B) is important to determine. IR-A is predominant in foetal tissues and binds IGF-II with similar affinity as insulin does. Activation of IR-A by insulin leads primarily to metabolic effects, while activation of IR-A by IGF-II leads primarily to mitogenic effects.

Overexpression of insulin in transgenic mice embryos results in an increased foetal beta-cell proliferation, while mice overexpressing IGF-I and IGF-II have an increased weight due to organomeglay without an apparent increase in skeletal growth. In humans, the similar can be seen as organ visceromegaly and beta-cell hyperplasia of the infant of the diabetic mother, where hyperglycaemia results in foetal hyperinsulinaemia.

Generally, data obtained from rodents are consistent with data in humans, but there are differences in expression of the IGF-system. In contrast to human foetuses, which show a severe intra-uterine growth retardation due to insulin deprivation, lack of insulin does not seem to have any detrimental consequences for the mouse embryo since the maturation of this hormonal system seems to occur just prior to birth. In humans, insulin is involved in foetal development during the entire last trimester of pregnancy.

Based on this important role of insulin-like growth factors during development, it is important to have knowledge of the potential affinity and activity of the insulin analogue with respect to the IGF-I and possibly IGF-II receptors.

2.3 Placental transfer

The human placental barrier becomes functional after around 8 weeks of implantation. Before that, the conceptus is thought to be nurtured via the yolk sac and by diffusion. In this early period, there is no known regulatory mechanism of insulin and glucose availability for the conceptus, other than maternal homeostasis. The placenta is restrictive to free unmodified human insulin. Although the mechanism of this blockade is not fully known, it should not be expected that free insulin analogues with small amino acid replacements as well as other modifications would cross the placenta. However, modifications might increase the possibility of antibody formation and thus enhance the risk of placental transfer. Yet, the possible consequences for the foetus in such a scenario are not known. Furthermore, changes of the lipophilicity of insulin might change its diffusion properties over the placenta barrier.

2.4 *In vitro* embryotoxicity

The use of *in vitro* embryo culture studies may be considered to study the direct effects of insulins in a relevant part of the development, namely during organogenesis. Rat embryos can

be studied in culture until day 10-12 of gestation. During this period, the embryo develops from the early somite stage to a situation where the neural tube is closed, the heart is functional, and many other organ "anlagen" have been formed. In culture, the glucose concentration can be controlled, enabling to study the effects of insulin analogues separately.

The endpoints that can be considered are primarily the binding characteristics of embryonal insulin receptors (affinity and density) and the functionality of these receptors together with morphological and immunohistochemical analysis. However, since the pharmacokinetic properties of the insulins are of crucial relevance, a final assessment cannot be derived from *in vitro* data only.

2.5 *In vivo* studies

Traditionally, reproduction toxicity studies are performed in healthy animals, usually the rat and the rabbit. Therefore, a careful choice of doses is important. From studies with insulin analogues performed so far, it appears that an appropriate criterion might be that the maximum dose should be at the high end of the effect span, and hence, just maximally affecting the glucose concentration. Exceeding this 'minimum' (probably receptor-saturating) dose runs the risk of causing non-physiological prolonged hypoglycaemia. In other words the maximum dose should be chosen on the basis of a pharmacodynamic endpoint.

However, the pharmacokinetic properties should be taken into consideration, e.g. short-acting insulin analogues may require more frequent dosing to mimic the clinical exposure.

The reproductive toxicity of insulin analogues could possibly be tested by inducing pregnancy in diabetic animals in which the glucose level is normalized by the compound under study. This would mimic the situation of human insulin-controlled diabetic pregnancy. Diabetic rat models are available and may be useful tools in this respect. However, due to the lack of experience with the application of such models for safety evaluation/risk assessment, their usefulness is uncertain at this point in time.

3. CONCLUSIONS

With the present knowledge, animal studies performed in healthy animals seem to be appropriate to investigate a possible teratogenic potential of insulin analogues. However, hypoglycemia might lead to teratogenicity and therefore, the doses should be carefully selected to distinguish the teratogenic effects of the test compound from those caused by pronounced hypoglycemia. From available studies with insulin analogues, it appears that the highest dose should be determined at the high end of the effect span, and hence, maximally affecting the plasma glucose concentration. Exceeding this minimum receptor-saturating dose runs the risk of causing non-physiological prolonged hypoglycaemia. In other words the highest dose in embryotoxicity studies should be chosen on the basis of a pharmacodynamic endpoint.

Furthermore, the direct influence of insulin analogues on embryonic development could be studied with the use of *in vitro* embryo cultures. Another approach would be the use of the diabetic rat, although the relevance of this model for human risk assessment is at present not fully evaluated.

Consequently, alternative test systems like the diabetic rat model need to be further developed. In addition, it is important to consider the influence of the pharmacokinetic

properties as regards the development of the embryo/foetus together with the use of specific immunohistochemical endpoints.

Related Guidelines:

ICH S5A Note for Guidance on Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products(CPMP adopted September 93)

ICH S5B Note for Guidance on Reproductive Toxicology: Toxicity on Male Fertility (CPMP/ICH/136/95 - adopted Dec. 95)