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**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
(CPMP)**

**POINTS TO CONSIDER DOCUMENT ON THE NON-CLINICAL
ASSESSMENT OF THE CARCINOGENIC POTENTIAL OF 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.

POINTS TO CONSIDER DOCUMENT ON THE NON-CLINICAL ASSESSMENT OF THE CARCINOGENIC POTENTIAL OF INSULIN ANALOGUES

1. INTRODUCTION

1.1 Problem statement

Native human insulin has, in addition to its metabolic actions, a weak mitogenic effect. This effect has become important for the safety assessment of insulin analogues, i.e. compounds derived from insulin with a molecular composition and/or structure that has been modified as compared to native human insulin, since structural modifications of the insulin molecule could increase the mitogenic potency, possibly resulting in growth stimulation of pre-existing neoplasms. In view of the life long exposure and large patient population involved, insulins with an increased mitogenic effect in comparison to the native human insulin in current use would constitute a major public health concern. Therefore, a thorough assessment of carcinogenic potential is indicated for all new insulin analogues.

1.2 Scope

This Points to Consider Document provides general guidance to industry and regulators on the general approach to the assessment of potential carcinogenicity of new insulin analogues. The approach should be based on current knowledge and assumes that all studies will be justified and performed according to the "state-of-the-art".

Other specific safety issues relating to insulins as embryotoxicity and possible disease- or treatment-related effects as retinal vascular proliferation is not within the scope of this document. A general guidance for the pre-clinical development of biotechnology-derived pharmaceuticals is provided in the Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (CPMP/ICH/302/95). For the timing of studies in relation to clinical trials, guidance is provided by ICH Topic M3 (CPMP/ICH/286/95), but some advice is provided in section 2.6 of this document.

1.3 Causes for concern

Pre-clinical findings

The primary example is the AspB10-substituted human insulin analogue, which was found to induce mammary tumours in rats. In subsequent *in vitro* studies AspB10 insulin was found to possess a mitogenic activity that exceeds that of normal human insulin. Although enhanced Insulin-like Growth Factor 1 (IGF-1) receptor activation and/or aberrant signalling through the insulin receptor have been implicated, the mechanism(s) responsible for the mitogenic activity remain to be clarified.

Cellular biology of growth factor

Structurally, insulin and IGF-1 as well as their receptors are related. Despite these similarities, insulin effects are mainly metabolic and IGF-1 effects mainly mitogenic and at supra-physiological concentrations the two compounds may activate each other's receptors. Furthermore heterodimers of the insulin and IGF-1 receptors (hybrid receptors) with intermediate selectivity for the corresponding hormones do occur, however their physiological significance is as yet unclear. The Post-receptor intracellular signalling pathways for insulin and growth factor are complex, and an area of active research.

2. DATA ON MITOGENICITY AND CARCINOGENICITY OF INSULIN ANALOGUES

2.1 General considerations

In the assessment of carcinogenic potential of an insulin analogue the evaluation of the total activity profile of the compound *in vitro* and *in vivo* is relevant. Deviations of the activity profile from that of native human insulin are of special interest. Therefore, the use of native human insulin as a reference compound should be considered in all studies. In addition, the inclusion of Asp B10 insulin as a positive control should be considered.

Although the exact mechanism(s) behind the increased carcinogenic and/or mitogenic potential of some insulin analogues remain to be elucidated, the insulin receptor and its intracellular signalling pathway is nevertheless sufficiently similar to that for the IGF-1 receptor to consider a thorough characterisation of possible IGF-1 like activity.

As scientific knowledge evolves, interaction with growth factor receptors and mechanisms other than those for IGF-1 need to be taken into account at successive stages during the development process.

2.2 Structure-activity relations

Information on structure-activity relations is primarily useful as a theoretical background, which may contribute to an optimal strategy for the design and evaluation of further *in vitro/in vivo* studies on pharmacodynamics and preclinical safety.

Published literature shows that a number of specific domains in the insulin and in the IGF-1 molecule are important for binding to their respective receptors. Receptor binding is only a first step in the activation of the respective metabolic and/or mitogenic pathways mediated by the relevant receptors. It is not (yet) possible to predict with any degree of certainty undesired (i.e. mitogenic) *in vivo* effects of insulin analogues from structure-activity relationships.

Those parts of the molecule known to be involved in binding of insulin to the different relevant receptors (insulin receptors, IGF1-receptor, hybrid receptors) may provide relevant information regarding potential changes in metabolic and mitogenic properties of the molecule. Characteristics other than binding, for example dissociation kinetics and receptor activation, may also be taken into consideration. In addition, consequences for mitogenicity of structural alterations resulting in changed pharmacokinetic properties or formation of potentially active fragments during degradation should be considered.

It is desirable that for each new insulin analogue a careful assessment is made considering potential consequences of each structural change in the molecule for its metabolic and mitogenic properties, due to changes in interactions on the molecular level with receptors as well as pharmacokinetic changes which may result in increased receptor activation.

2.3 In vitro assay systems to establish the receptor profile

Binding assays could be performed on human cell lines and/or recombinant expression systems, but it is recommended that cell lines are considered that could be used for *in vitro* metabolic and mitogenic functional assays. This to allow for coherence in the over-all evaluation of effect (see below).

Assay conditions need to be well characterised and attention paid to the description of details as K_i for labelled ligands, receptor numbers and occupancy.

The minimal set of ligands, apart from test compound and related analogues as detailed above, might be considered to include native human insulin; B10 Asp human insulin; IGF-1.

As to receptor types, at a minimum insulin- and IGF-1 receptors might be considered for investigation in man and intended toxicology species.

Studies on receptor kinetics and down-stream signalling could be valuable to the understanding of a compound's action profile in general. Due to incomplete knowledge in this area, interpretation of such data with respect to potential carcinogenicity should be done with caution and in accordance with the current state of scientific knowledge.

2.4 In vitro /ex vivo cell culture systems to establish the mitogenic potential

Mitogenic potency of a new insulin analogue should be examined in suitable *in vitro* systems under conditions facilitating coherent comparison of results on its mitogenic- relative to its metabolic potential as compared to that obtained with normal human insulin. It is desirable that *in vitro* mitogenicity is studied in systems allowing not only detection and discrimination of increased mitogenicity mediated by the insulin receptor and (if applicable) the IGF-1 receptor, and hybrids of these receptors, but also the measurement of receptor affinity (see section 2.3). Although assays with the highest sensitivity to specific mitogenic effects may not be the most specific and sensitive tests to measure metabolic effects and vice versa, it is desirable that in addition to the use of such specific assays also assays are used in which both effects are measured in similar systems. Since there is evidence that receptors in neoplastic tissues may react differently from those in normal tissues, it is desirable that the choice of test systems will cover testing of mitogenicity in non-neoplastic as well as in neoplastic tissues.

The possibility that in future new pathways mediating mitogenic effects of insulin might be found should not be overlooked. Therefore, other assay systems aimed at the detection of mitogenic potential might be considered in the context of current scientific understanding of cellular biology. The rationale of the choice of a particular system and its characteristics should be justified.

Note that data that allow for a comparison of relative mitogenicity among species are important for the justification of species and strain selection in chronic repeated dose toxicity studies that are used for the evaluation of cellular proliferation (see also section 2.5).

To facilitate the assessment of the data, the justification of the choice of any test system needs to be accompanied by data on its validity (e.g. sensitivity, specificity, reproducibility, etc) for the detection of mitogenic effects of insulin, its analogues and, if applicable, IGF-1.

The results of the *in vitro* tests should be evaluated for changes in mitogenic as compared to metabolic potency in comparison to normal human insulin and relevant positive controls. A comprehensive interpretation in view of current science of the whole package of data, including assessment of mechanisms underlying any differences from normal human insulin should be added. The relevance of the findings from the *in vitro* tests for the design and interpretation of results of *in vivo* carcinogenicity studies should be discussed.

In addition to mitogenicity, other *in vitro* assays for adverse changes as cell transformation and oncogen expression might become of use in risk assessment when scientifically justified.

2.5 In vivo bioassays to investigate any concern for a carcinogenic potential

Species/strain selection

Due to substantial background data on spontaneous tumour incidence the rat may be considered a suitable species and in view of known responsiveness to Asp B10 human insulin at present the Sprague-Dawley rat may be thought of as a first-hand choice. If the rat is found unsuitable, other species or models, like the promotion of established human tumour cell lines grafted on immunodeficient animals might be considered. However, the choice of animal species and strains should be carefully considered on the basis the *in vitro* profile from these species. The suitability of an assay is dependent on that the test compound has a relative mitogenic/ metabolic *in vitro* potency ratio in the selected species comparable to man.

Incorporation of sensitive indices of cellular proliferation into the study design in repeated dose toxicity studies/carcinogenicity studies

Organs with known spontaneous tumour incidence in the selected species and strain may, in addition to routine examination including histopathology, be processed for cellular proliferation markers, as, for example, BrdU and/or post-staining for nuclear cell cycle antigens.

Route of administration and dose levels

For a general guidance the reader is referred to the Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (CPMP/ICH/302/95). For the high dose group, the attainment of a primary, glucose lowering effect, at the high end of the effect span obtained with the test compound is recommended, ensuring a high exposure without causing undue mortality due to hypoglycemia. However, the pharmacokinetic properties should be taken into consideration, e.g. short-acting insulins may require more frequent dosing to mimic the clinical exposure.

The dose level for a reference insulin group should produce an equivalent glucose lowering effect to that achieved in the high dose group for the test substance.

If additional formulations or clinical routes are intended, their qualification by long-term studies to assess possible carcinogenic potential through high local concentration/deposits overflow on other growth factor receptors might be considered. Increased cellular proliferation at the administration site could be included in such studies.

Potential effects of change in kinetics due to molecular modification

Molecular modifications in insulin analogues which do not increase the *in vitro* mitogenic or metabolic potency of the molecule as such, but which change the tissue distribution of the compound, could still induce increased mitogenicity *in vivo* due to increased local effector concentrations at receptor level. Therefore, also potential effects of changes in kinetics due to molecular changes in the analogues should be taken into account, when relevant.

Study duration of long term repeated dose studies

Repeat dose study of 6 months duration should normally be scheduled in the toxicology program. Incorporation of sensitive indices of cellular proliferation into the study design (see above) should be considered.

Further investigations of carcinogenicity

If *in vitro* tests and/or repeated dose toxicity studies reveal evidence for increased mitogenicity or other effects which are cause for concern (like *in vitro* transformation capacity), but repeated dose toxicity studies do not reveal actual evidence of increased tumour

formation, further *in vivo* carcinogenicity testing should be considered for the assessment of increased tumourigenic potential.

2.6 Timing of studies

In analogy with ICH Topic M3 it is suggested that *in vitro* mitogenicity studies are performed before first human use (phase I); chronic studies with extended histological investigation for increased proliferation are performed before clinical trials in man exceeding 6 months of duration; any carcinogenicity studies are performed before marketing authorisation application.