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## Guideline on non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight-heparins

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*Low molecular weight heparins, low molecular mass heparins, similar biological medicinal products, biosimilar, comparability, quality, non-clinical studies, clinical studies, extrapolation*

# Guideline on non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight-heparins

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## Executive summary

This guideline lays down the non-clinical and clinical requirements for low molecular weight heparins (= low molecular mass heparins, LMWHs) containing medicinal products claimed to be biosimilar to another one already marketed. The quality section addresses some aspects specific to LMWH, the non-clinical section addresses the pharmaco-toxicological requirements and the clinical section the requirements for pharmacokinetic, pharmacodynamic and, where needed, safety/immunogenicity studies as well as pharmacovigilance aspects. Whereas the parent guideline required a comparative clinical trial by default, the revised guideline focusses on demonstration of biosimilarity based on a strong and convincing physicochemical and functional data package and comparable pharmacodynamic profiles. Pre-marketing clinical immunogenicity data may not be necessary if the immunogenic potential can be adequately characterized in suitable and sensitive *in vitro* tests. In addition, the non-clinical section has been amended to follow a risk-based approach.

### 1. Introduction

Heparin is a highly sulphated and heterogeneous member of the glycosaminoglycan family of carbohydrates consisting of various disaccharide units. The most common disaccharide unit is composed of a 2-O-sulfated  $\alpha$ -L-iduronic acid and 6-O-sulfated, N-sulfated  $\alpha$ -D-glucosamine, IdoA(2S)-GlcNS(6S). Endogenous heparin is synthesised in the granules of mast cells and possesses the highest negative charge density of all known biological molecules.

Heparin catalyzes the inhibition of several serine proteases of the plasmatic coagulation system by antithrombin. For the binding of heparin to antithrombin (ATIII), a pentasaccharide sequence, which contains a 3-O-sulphated glucosamine residue, is important. Upon binding to the enzyme inhibitor antithrombin, heparin causes a conformational change in the antithrombin molecule which results in its active site being exposed for inhibition of activated coagulation factors. Furthermore, heparin acts as a catalytic template to which the inhibitor and activated serine proteases such as thrombin (factor(F)II, FIIa), FIXa and FXIa bind. This effect depends essentially on the number of monosaccharides and the position of the sulphate groups in the heparin molecule. Heparin molecules containing fewer than 18 monosaccharides do not catalyze inhibition of thrombin but still inactivate FXa. Heparin enhances the rate of thrombin–antithrombin reaction at least a thousand-fold resulting in a stable 1:1 complex after the serine-protease attacks a specific Arg-Ser peptide bond at the reactive site of antithrombin. In addition, other actions, which are independent from antithrombin, contribute to the antithrombotic effects of heparin. Such mechanisms include the release by the vascular endothelium of tissue factor pathway inhibitor (TFPI), a major natural inhibitor of the coagulation system, the interaction with heparin cofactor II, the inhibition of procoagulant effects of leukocytes, the promotion of fibrinolysis, and effects on vascular endothelium (receptor mediated and receptor independent).

Low molecular weight heparins (LMWHs) are prepared from unfractionated heparin by various chemical or enzymatic depolymerisation processes. As a result of the depolymerisation process, LMWHs are mainly enriched in molecules with less than 18 monosaccharide units. This reduction in molecular size is associated with a loss of thrombin inhibitory activity and an increase in FXa inhibition compared to unfractionated heparin.

All currently licensed LMWHs are derived from porcine intestinal mucosa. The observed heterogeneity of these LMWH results from the nature of unfractionated heparin and the depolymerisation process (chemical, enzymatic).

Currently approved originator LMWHs differ in their pharmacokinetic/pharmacodynamic properties. Due to difficulties in the physical detection of LMWH, conventional pharmacokinetic studies cannot be performed. Instead, the absorption and elimination of LMWHs are studied by using pharmacodynamic tests, most importantly the measurement of anti-FXa and anti-FIIa activity.

All originator LMWHs have indications in the treatment of venous thrombosis (treatment and prophylaxis of venous thromboembolism (VTE) and some have additional indications related to arterial thrombosis, i.e. acute coronary syndrome and myocardial infarction.

The most common adverse reaction of heparins is bleeding, whilst the most serious is the rarely observed heparin-induced thrombocytopenia type II (HIT II), which is caused by the induction of antibodies directed against neoantigens of heparin-platelet factor 4 (HPF4) complexes. Binding of these antibody-HPF4 complexes may activate platelets and generate thrombogenic platelet microaggregates. Patients developing immune-mediated heparin-induced thrombocytopenia are at risk of potentially life-threatening arterial and venous thromboembolic complications (heparin-induced thrombocytopenia and thrombosis, HITT). Although, compared to unfractionated heparin, the incidence of HPF4-antibodies and HIT II appears to be reduced with LMWH, it is obligatory to monitor the platelet count regularly in all patients using LMWH and to test for HPF4-antibodies in those who develop thrombocytopenia or thromboembolic complications.

## 2. Scope

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

This product specific guideline complements the above guideline and presents the current view of the CHMP on the non-clinical and clinical requirements for demonstration of comparability of two LMWH-containing medicinal products.

This Guideline should be read in conjunction with the requirements laid down in the EU Pharmaceutical legislation and with relevant CHMP guidelines (see 3. Legal Basis and relevant guidelines).

## 3. Legal basis and relevant guidelines

- Directive 2001/83/EC, as amended, in particular in Directive 2001/83/EC Art 10(4) and Part II of the Annex I of Directive 2001/83/EC, as amended.
- Guideline on similar biological medicinal products (CHMP/437/04 Rev. 1)
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMA/CHMP/BMWP/42832/2005 Rev. 1).
- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: Quality issues (EMA/CHMP/BWP/247713/2012)
- Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products (EMA/CHMP/BWP/429241/2013)
- ICH guideline S 6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998)

- Guideline on Immunogenicity Assessment of Biotechnology-derived Therapeutic Proteins (EMA/CHMP/BMWP/14327/2006)
- Guidelines on good pharmacovigilance practices

#### 4. Specific aspects of the quality comparison

Information on the biological source of the biosimilar LMWH should be available as well as on the manufacturing process of unfractionated heparin, its mode of depolymerisation and the respective process conditions. Comprehensive characterisation and comparison of the biosimilar and reference LMWH is essential and should be performed using state of the art methods. Compliance with the requirements of European Pharmacopoeia (Ph.Eur.) is considered a minimum standard only.

The comparative analyses of physicochemical and biological attributes of the biosimilar and the reference LMWH should demonstrate high similarity with respect to:

- molecular weight distribution and overall chemical composition
- starting material (tissue type and species) and mode of depolymerisation
- disaccharide building blocks, fragment mapping profiles and sequences of selected unfragmented oligosaccharides
- biological and biochemical assays.

#### 5. Non-clinical studies

Non-clinical studies should be performed before initiating clinical trials. The studies should be comparative in nature and should be designed to detect differences in the response between the biosimilar and the reference LMWH and not just assess the response *per se*. The approach taken will need to be fully justified in the non-clinical overview.

##### Pharmacodynamic studies

*In vitro* studies:

In order to compare the pharmacodynamic activity of the biosimilar and the reference LMWH, data from a number of comparative bioassays (based on state of the art knowledge about clinically relevant pharmacodynamic effects of LMWH and including, at least, evaluations of anti-FXa and anti-FIIa activity) should be provided. Standardised assays in accordance with the Ph.Eur should be used to measure activity. Such data may already be available from bioassays submitted as part of the quality dossier.

*In vivo* studies:

If physicochemical and biological characterisation of the biosimilar and the reference LMWH performed using sensitive state-of-the-art methods convincingly demonstrates close similarity, *in vivo* studies are not required as part of the comparability exercise.

Otherwise, the *in vivo* pharmacodynamic activity of the biosimilar and the reference LMWH should be quantitatively compared in:

- An appropriate *in vivo* pharmacodynamic model, which takes into account state of the art knowledge about clinically relevant pharmacodynamic effects of LMWH and includes at least an evaluation of anti-FXa, and anti-FIIa activity and of release of tissue factor pathway inhibitor.

and/or

- A suitable animal venous or arterial thrombosis model can be used which appropriately reflects the intended clinical effects of LMWH.

### **Toxicological studies**

Generally, separate repeated dose toxicity studies are not required.

In specific cases, e.g. when novel or less well studied excipients are introduced, the need for additional toxicology studies should be considered.

The conduct of toxicity studies to assess unspecific toxicity only is not recommended. Impurities, such as proteins should be kept at a minimum in accordance with pharmacopoeial monographs, which is the best strategy to minimise any associated risk.

If immunogenicity is not evaluated in a clinical trial, the immunogenic potential of the biosimilar and the reference LMWH needs to be compared in appropriate non-clinical tests. The predictivity of animal studies for evaluation of immunogenicity in humans is usually considered low. However, *in vitro* tests may be used to characterise the physicochemical properties of the HPF4 complexes. Available methods allow the determination of the binding affinity of LMWH to PF4, the stoichiometry, charge and size of the resulting complexes and changes in the occurrence of secondary structure elements ( $\alpha$ -helices and  $\beta$ -sheets) in the PF4 protein. These characteristics of the HPF4 complex should be determined as a function of concentration and ratio of LMWH and PF4. In addition, consideration should be given to investigating the ability of the HPF4 complexes to bind to previously formed antibodies against HPF4 and to activate thrombocytes by using sera from HIT II patients.

The suitability of any employed test needs to be appropriately justified. In order to demonstrate sufficient sensitivity of these assays, unfractionated heparin (which is more immunogenic than LMWH) may serve as positive control.

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

## **6. Clinical studies**

### **Pharmacokinetic/Pharmacodynamic studies**

Due to the heterogeneity of LMWHs, conventional pharmacokinetic studies cannot be performed. Instead, pharmacodynamic activities, most importantly anti-FXa and anti-FIIa activity, should be compared between the biosimilar and the reference LMWH. In addition, the ratio of anti-FXa and anti-FIIa activity should be compared as well as Tissue Factor Pathway Inhibitor (TFPI) activity.

These pharmacodynamic properties should be investigated in a randomized, single-dose, two-way crossover and preferably double-blind study in healthy volunteers using subcutaneous administration. Since subcutaneous administration covers both absorption and elimination of LMWH, additional pharmacology studies for intravenous or intra-arterial use, if applicable, are not required.

The selected dose should be in the sensitive (steep) part of the dose-response curve.

Equivalence margins should be pre-specified and appropriately justified.

## **Clinical efficacy**

Pivotal evidence for similar efficacy will be derived from the similarity demonstrated in physicochemical, functional and pharmacodynamic comparisons. A dedicated comparative efficacy trial is therefore not considered necessary.

## **Clinical safety**

Biosimilar and reference LMWH should exhibit convincingly similar physicochemical and functional characteristics and pharmacodynamic profiles. Under this premise, adverse effects that are related to exaggerated pharmacological effects (e.g. bleeding) can be expected at similar frequencies. If, in addition, the impurity profile and the nature of excipients of the biosimilar do not create uncertainties with regard to their impact on safety/ immunogenicity, a safety/immunogenicity study may not be needed. In this case, further exploration of the immunogenic potential as suggested in section 4 (*Non-clinical studies*) should be performed.

Otherwise, comparative safety/immunogenicity data in patients should be generated pre-marketing. In such a clinical study, immunogenicity assessment should include the determination of HPF4- antibodies and the obligatory monitoring of platelet counts for the detection of HIT II events. In addition, major bleeding events and clinically relevant non-major bleeding events should be carefully assessed and documented. A consistent and clinically relevant classification of bleedings should be used. Preferably, the adjudication of bleeding events should be performed by a central independent and blinded committee of experts.

## **7. Pharmacovigilance plan**

Within the authorisation procedure, the applicant should present a risk management plan (RMP) in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should take into account identified and potential risks associated with the use of the reference product and how these safety concerns will be addressed in post-marketing follow-up. Monitoring of rare serious adverse events known to be associated with LMWHs such as Heparin-induced Thrombocytopenia Type II (HIT II, HITT) as well as anaphylactoid and anaphylactic reactions should specifically be addressed in the RMP.

## **8. Extrapolation of indication**

Demonstration of biosimilarity, based on physicochemical and functional characterisation, pharmacodynamic profiles, and, where needed, safety/immunogenicity trial, will allow extrapolation to other routes of administration and indications licensed for the reference medicinal product, if applicable and appropriately justified.