

Distribution: General

English only

Report

WHO Working Group on Biological Standardization of Unfractionated Heparin

**Geneva, Switzerland
7-8 September 1999**



**WORLD HEALTH ORGANIZATION
Blood Safety and Clinical Technology**

WHO WORKING GROUP ON BIOLOGICAL STANDARDIZATION OF UNFRACTIONATED HEPARIN

WHO Headquarters, Geneva 7 – 8 September 1999

Report

1. Introduction

As recommended by the group of experts who attended the WHO Consultation on Biological Standardization of Unfractionated Heparin (WHO, Geneva, 17-18 June 1998) the remit of the Working Group (WG) is to seek a strategy that would lead to an agreed global method for the measurement of biological activity of unfractionated heparin, based on a single heparin unit, thus promoting global harmonization. The minutes of the WHO Consultation on Biological Standardization of Unfractionated Heparin held on the 17-18 June 1998 were approved by all members of the Working Group and adopted.

Dr Thomas reminded the WG that there are three major issues to be discussed in relation to biological standardization of unfractionated heparin:

- a. Harmonization of international reference preparations by use of material reflecting the characteristics of current pharmaceutical heparin preparations, particularly in relation to specific activity.
- b. Seeking agreement on an appropriate method for measurement of *in vitro* anticoagulant activity of unfractionated heparin, as a basis for assigning potency estimates.
- c. Assessing the possible clinical impact of the above changes on the potency labelling of currently licensed heparin products, and the regulatory implications of any such changes.

Since replacement of the 1st International Standard for Low Molecular Weight (LMW) Heparin would soon be necessary, Dr Thomas proposed that this topic should also be discussed as an additional item on the Agenda. The proposal was unanimously accepted by the Group.

2. Measurement of heparin biological activity

Dr Gray presented a review of the data obtained from the collaborative study performed for the calibration of the 5th International Standard (IS) for unfractionated heparin, with specific references to methodology. Seven methods had been used in the study and the European Pharmacopoeia (EP) and United States Pharmacopoeia (USP) reference preparations had been included, together with the 4th IS for unfractionated heparin, and two candidate materials. As was found in the study performed for the calibration of the 4th IS, the USP and EP standards differed from

their labelled potencies by 10.6% and 2.6% respectively, when assayed against the 5th IS by all methods. There was good agreement between methods when the 5th IS was assayed against the 4th IS and EP standard. When assayed against the USP standard, potencies from the different assay methods were more widely spread. The anti-IIa chromogenic assay gave the closest potency estimates for the 5th IS against all three standards, and thus offered the best prospect of harmonization of IU and USP units. The current generation of heparin preparations has higher mean molecular weights and narrower molecular weight distribution, as reflected by their higher specific activities (180-210 IU/mg). Better agreement of potencies by all methods was observed when these heparins were calibrated against a reference material of similar properties.

3. Methodologies considered in pharmacopoeial studies and proposals for new/alternative methodology

Dr Murano indicated that the unitage of the current USP reference Lot K4 was calibrated against Lot K3, using the USP method. Bulk material for Lots K3 and K4 was produced in the early 1970s and is no longer representative of the current generation of clinical products. The USP biological assay method uses animal-derived substrate plasma, and this is known to give variable results. The USP recognizes that there is a need to update the assay method and also to establish a reference material that reflects the characteristics of current heparin preparations.

Dr Coyne presented data on the variable heparin neutralization ability of 12 different batches of sheep substrate plasma. This variability over the preparation of substrate led to conflicting and erroneous results. A chromogenic anti-IIa method (based on thrombin inhibition), using purified and well-defined reagents, was proposed to replace the existing USP assay. A chromogenic anti-Xa assay is already in place as one of the USP tests for unfractionated heparin. It would be possible to develop a chromogenic anti-IIa method using similar buffers and reagents to the anti-Xa test, as described in the USP monograph for unfractionated heparin. Dr Coyne also gave details concerning the proposed requirements for a new USP reference standard for unfractionated heparin.

Dr Johansen proposed an anti-IIa chromogenic method based on the EP anti-IIa assay for LMW heparin, for the measurement of anticoagulant activity of unfractionated heparin pharmaceutical preparations. The method works with both crude and purified heparin materials. The modification of the current EP clotting assay for unfractionated heparin and its dependence on a well-standardized sheep plasma was discussed. The anti-IIa chromogenic assay proposed will eliminate the variability in activity estimates of plasma based assays. The 5th IS collaborative study showed good correlation of potency estimates for the EP method and the anti-IIa chromogenic assays. Therefore, the introduction of the proposed assay could facilitate global harmonization of the unitage of unfractionated heparin.

4. Discussion on a proposed chromogenic anti-IIa assay

Dr Shaklee favoured one global standard for unfractionated heparin and the change of the existing USP method to an anti-IIa chromogenic assay. However, he would like to see more data on the performance of anti-IIa assays in a larger study before making any final decision. Dr van Dedem would also like to see more data on the proposed anti-IIa assay. Dr Spieser stated that he is not opposed to harmonization, but like other members of the group, he would like to see more data on the anti-IIa assay, assessing products of different purity, before commitment to any changes to the EP method. He would also like to have certain points clarified by the ECBS, including whether WHO is prepared to accept assignment of potencies on the basis of only one assay method.

Dr Murano commented that the sensitivity of the regulatory authorities, pertaining to labelling issues, should be considered, but this should not hinder the harmonization process. He remained of the opinion that logical, sensible proposals, with pertinent supportive data, would be favourably embraced by the Agency(ies).

5. Proposals to promote world-wide harmonization of methodology and calibration of a new reference material for the USP

The Group strongly supported the concept of a single assay method as a major contribution towards harmonization of the unitage of unfractionated heparin. There was general consensus that the anti-IIa chromogenic method would be a good choice for such an assay, but more data are needed to ensure the robustness of the method. Dr Spieser indicated that the outcome of this collaborative exercise should not lead to a re-assignment of the potency of the current EP standard.

It was agreed that a collaborative study that combines the calibration of a new USP reference preparation and investigation of the performance of a standardized anti-IIa chromogenic assay would be desirable. The following strategy was proposed:

Materials to be included: 5th IS
EP reference preparation
USP Lot K4
Candidate material for new USP reference preparation
A low specific activity (150–170 IU/mg) heparin material

Assay methods: Proposed global anti-IIa chromogenic method
In-house anti-IIa chromogenic assays
USP anti-Xa chromogenic method
In-house anti-Xa chromogenic assays
USP method
EP method

Time scale

September 1999 – January 2000

NIBSC to send bulk heparin (same material used for the preparation of the 5th IS) to USP for filling of the candidate USP reference material.

NIBSC will procure and fill some low specific activity material. Dr Wei agreed to see if she could provide some low specific activity heparin from China. If this was not possible, a European manufacturer would be approached.

Drafting group (Dr Coyne, Dr van Dedem, Dr Charton) chaired by Dr Johansen, will prepare a standard operating procedure for the proposed global anti-IIa chromogenic method. This involves testing the “on-bench” performance of the method. The protocol for the proposed anti-IIa global method should be available by the end of 1999.

Dr Gray will draft protocol for the WHO collaborative study. This protocol will be circulated within the WG for comments.

February 2000 – May 2000

Recruitment of participants.

Samples and protocol for the WHO collaborative study sent to participants.

Results collected.

June 2000 – October 2000

Statistical analysis of data and report sent to participants and members of the WG. Progress report for the SSC meeting in Maastricht, June 2000.

November 2000

Meeting of the WG in Geneva to discuss results of the collaborative study, and consider further action.

October 2001

Report to ECBS.

The Group recognized the need to keep regulatory authorities, and especially the FDA, informed of the progress of the harmonization process. It was agreed that Drs Padilla and Murano will inform Dr Talarico (CDER/FDA) about the discussions of the Working Group. A copy of the report of the Working Group Meeting will be sent to all the participants at the WHO Consultation, June 1998.

The Group was also informed that the WHO International Pharmacopoeia (IP) monograph for unfractionated heparin is currently in the process of edition. The method included in this Pharmacopoeia is similar to the European Pharmacopoeial method and it is hoped that the revised edition will be published within the next 6 months. After consideration of the time scale of the harmonization exercise, the Group advised the WHO Secretariat to publish the IP monograph, as the proposed new global method was unlikely to be in place for at least two years.

6. Low Molecular Weight Heparin

Dr Barrowcliffe announced the need to replace the current 1st IS for Low Molecular Weight Heparin, established in 1987. The replacement standard should be representative of current clinical products. Physico-chemical and biological characterization of a panel of current low molecular weight heparin therapeutics, will be carried out by NIBSC, similar to that made for the development of the 5th IS of unfractionated heparin. This will be carried out before commencement of a large-scale international collaborative study.

Dr Murano welcomed the opportunity to work with WHO on the issue of standardization of low molecular weight heparin. USP has proposed but has not yet established USP monograph methods and reference material in this area and it would be helpful to establish a USP Low Molecular Weight Heparin standard in harmony with the International Standard. The USP will work with the WHO and EP to achieve this aim.

Dr Thomas reminded the Group that some manufacturers and academic institutes are of the opinion that each low molecular weight heparin needs a separate standard and requested the opinion of the members in the Group on this issue. Dr van Dedem was of the view that there is no need for separate standards for measurement of anticoagulant activity of each low molecular weight heparin. Although each product has its own EP monograph, the anticoagulant activity still complies with the general monograph on the anticoagulant activity of low molecular weight heparin. Dr Spieser also endorsed the concept of one single standard. The EP collaborative study carried out approximately 3 years ago, included 5 different low molecular weight heparins, and this showed these products compared well against each other.

The Group agreed that, while it is clear that all these products are indeed different in many respects (molecular mass, physicochemical properties, Anti Xa/Anti IIa ratio etc.), one single standard is probably sufficient to give reliable and reproducible potency estimates for all low molecular weight heparins. However, a pilot collaborative study is required to investigate the comparability of current low molecular weight heparins before appropriate candidates could be chosen for the main collaborative study to establish the replacement for the 1st International Standard. NIBSC will co-ordinate the study in late 1999, involving approximately 10 laboratories, examining a panel of current low molecular weight heparins, and using anti-IIa and anti-Xa chromogenic methods based on the EP monograph for Low Molecular Weight Heparin. A meeting will be convened to decide on the candidate

materials. As the pilot study will result in a delay in starting the main study, a shortage of the current International Standard for Low Molecular Weight Heparin may result as it would take longer than originally anticipated to establish the replacement. Dr Spieser offered the use of the EP reference material, which was calibrated against the 1st IS, if a shortage develops.

7. Any Other Business

The Minutes of this meeting will be presented to the 50th Meeting of the WHO Expert Committee on Biological Standardization, to be held on 25-29 October 1999. Progress report will also be given at the Scientific Standardization Committee (SSC) of the International Society of Thrombosis and Hemostasis (ISTH), at its 46th Annual Meeting, Maastricht, June 2000.

**WHO WORKING GROUP ON BIOLOGICAL STANDARDIZATION
OF UNFRACTIONATED HEPARIN**

WHO Headquarters, Geneva 7-8 September 1999

Dr Trevor W. Barrowcliffe

National Institute for Biological
Standards
and Control (NIBSC)
South Mimms, Potters Bar
Hertfordshire EN6 3QG
UK

Dr Kristian Johansen

Director, Biological Development
Leo Pharmaceutical Products Ltd.
55 Industriparken
DK-2750 Ballerup
Denmark

Dr E. Charton

Scientific Officer
EDQM
European Pharmacopoeia
Council of Europe
226 Avenue de Colmar B.P. 907
67029 Strasbourg Cedex 1
France

Professor Leng Wei

Director, Division of Hormones
National Institute for the Control of
Pharmaceutical & Biological
Products
Ministry of Public Health
The Temple of Heaven
Beijing 100050
People's Republic of China

Dr E. Coyne

3470 North Lake Shore Drive #27B
Chicago
IL 60657
USA

Dr G.W.K. van Dedem

Diosynth bv
P.O. Box 20
NL 5340 BH Oss
The Netherlands

Dr Elaine Gray (Rapporteur)

National Institute for Biological
Standardization and Control
(NIBSC)
Blanche Lane
South Mimms, Potters Bar
Hertfordshire EN6 3QG
UK
Tel.: 44 1707 654 753
Fax.: 44 1 707 646 730
email: egray@nibsc.ac.uk

Dr Gene Murano

Director
Division of Biologics and Biotechnology
US Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20852
USA

Dr Patrick Shaklee

Scientific Protein Laboratories Inc.
700 E Main Street
P.O. Box 158, Waunakee
Wisconsin 53597-0158
USA

Dr J.-M. Spieser

Head of Division IV
Biological Standardization and OMCL
Network
European Pharmacopoeia Commission
Council of Europe
226 Avenue de Colmar B.P. 907
67029 Strasbourg Cedex 1
France

Dr D. Thomas (Chairman)

The Old Barn
North Green
Kirtlington
Oxford OX5 3JZ
UK

WHO SECRETARIAT:

Dr J.C. Emmanuel, Director, BCT
Dr A. Padilla, BCT/QSD (**Secretary**)
Dr C. Heuck, BCT/DIL
Dr E. Griffiths, V&B/QSB