



3rd Workshop on the Characterization of Heparin Products

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3RD WORKSHOP ON THE CHARACTERIZATION OF HEPARIN PRODUCTS

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~ PROCEEDINGS ~

Session 1: Low Molecular Weight Heparins: Standardization and Biosimilars

Overall Summary: Presentations and discussions in this session described assays to identify and control low molecular weight heparins (LMWHs). Unlike heparin, LMWHs are obtained from UFH using different chemical or enzymatic procedures to depolymerize them; therefore, in certain respects, each product may be considered unique. As such, much of the discussion focused on the necessity of comparing “like *versus* like,” for example, regarding the need to create specific standards for each LMWH. Many of the presentations and much of the discussion focused on potency assays, but the conversation did extend to physical measurements, such as molecular weight as an identity parameter.

Dr. Elaine Gray from NIBSC initiated the session and presented on potency standards for LMWH products, the study that led to the determination of the current international heparin standard. The first low molecular weight heparin international standard (1st LMWH IS) was established in 1987. The assessment of the data in the IS studies was done using parallelism, with LMWH being compared against UFH. When assaying the candidate samples in the first LMWH IS study, there was greater variability of potency versus a UFH standard than against a LMWH standard. Thus, the concept of comparing “like *versus* like” was introduced. In this context, in the early 1990s, four major low molecular weight heparins (LMWHs) were available for clinical use and the anti-Xa to anti-IIa ratios ranged from 1.5 to 3.5. The 1st LMWH IS established in 1987 served well to calibrate these products. By 2000, eight LMWHs were licensed (mainly in Europe) as antithrombotics, with anti-Xa to anti-IIa ratios ranging from 1.5 to 10.0. An international collaborative study at that time evaluated the comparability of these eight clinical products with a view towards replacing the 1st IS. Results showed that due to non-parallelism, two out of the eight products had a tendency to give invalid assays. This indicates that not all LMWHs may be suitable potency reference standards for other LMWH preparations. The 2nd LMWH IS has been in use since 2001 and is now due for replacement. The advent of new generations of ultra-low molecular weight heparins and biosimilar LMWHs means the selection of a new IS for LMWHs needs to be carefully considered. As of September 2009 there will be a three phase study to replace the LMWH IS. The success of the study depends on the donation of materials from manufacturers to ensure testing and comparison of a broad array of LMWH samples that captures the diversity of products currently marketed.

Next, Pascal Anger (Sanofi-Aventis) addressed the limitations of common reference standards in for testing a specific LMWH, *viz.*, enoxaparin sodium. He contended that each LMWH bears

specific features that have to be characterized and thus requires appropriate reference standards. From a physical standpoint, one of the most obvious requirements is the availability of specific standards for NMR identification of a given LMWH. Another example, in the case of enoxaparin, is the percentage of oligosaccharides bearing a 1,6-anhydro structure at the reducing end of their chain. An appropriate assay for 1,6-anhydro content requires a suitability test and therefore specific standards. Additionally, this assay is directly impacted by the average molecular weight (MW) of the lot, necessitating a reliable MW assay. The current MW assay from the EP would appear, on the basis of his results, not to be reproducible enough for this purpose and more appropriate for higher MW LMWH. The proposal was made to include a specific calibration using narrowly disperse calibrants. Sanofi-Aventis has developed such a preparation of narrowly disperse LMWH fractions to use as calibrants, which in their hands leads to a method which produces less variable MW readings. The recommendation from Mr. Anger was to include at least three standards: (1) an identification reference standard (RS); (2) two RS's for MW measurement: one made of narrowly disperse MW fractions for calibration and one broader standard for suitability; and (3) a RS for bioactivity. These results would have to be confirmed in a collaborative study so that discussion on appropriate revision could be initiated.

Dr. Pilar Carrasco focused her presentation on Rovi's experience with LMWH and Ultra-LMWHs, which began in the mid 1980s. Dr. Carrasco outlined Rovi's experience with Bemiparin, a LMWH with an average molecular weight of 3,600 Daltons. Of the currently marketed LMWHs, Bemiparin has the highest Anti-IIa to Anti-Xa ratio, the longest half life and the lowest molecular weight. In addition, Rovi is developing RO-14 that has an even greater anti-IIa to anti-Xa ratio and lower molecular weight than Bemiparin. Currently the EP uses one RS for activity across the entire range of LMWHs. Rovi has adapted the EP methods for anti-IIA and anti-Xa assays slightly for suitability with their products, for example, they use a lower working range and greater incubation times in the adapted anti-IIa assay. The same results can be obtained using a number of RS, including the 2nd LMWH IS, BRP (batch 5), or an in-house standard. In addition, the adapted anti-Xa and anti-IIa activity assays can be applied to Ultra- LMWHs. For example, these modified methods allow Rovi to assign potency down to 0.3 IU/mg with no loss of parallelism. As such, according to Rovi, a specific standard for Ultra-LMWHs is not required; existing assays and standards can be adapted to provide robust, reproducible results.

Ronda Lecky (Leo Pharma) spoke about her experience with automated anti-Xa and anti-IIa assays. To achieve and maintain reproducibility and continuity in enzymatic chromogenic assays, it is desirable to reduce the number of manually performed operations (*e.g.* handling of liquids and timing of incubations). A fully automated instrument is ideal for these assays, but calibration and maintenance of the machine are critical to generate robust data. In addition, reproducibility with automated instruments can vary for a number of reasons, and Dr. Lecky cited one specific example — uneven temperature control. With a properly running system, relative standard deviation should be low, approximately 1.1%, to ensure that the equipment can yield reproducible results. Other important areas of focus include the range and linearity of the assay, the system suitability test set-up, the stability testing of reagents, the monitoring of assay variation over time, the prompt investigation of out-of-trend responses and the acceptance testing of new lots of reagents. Performance testing of new lots of key reagents as new lots is important to ensure continuity of the assay. To control the function of reagents in the assay, one suggestion is to establish limits for the absorbance readings of the highest concentration of the RS as well as the blank solution. For example, background absorbance should be low, as close to zero as possible. The stability of the reagents is also important; in use stability and the long term

stability information are necessary. Assay variation monitoring over time allows the user to determine whether there has been a deviation from historical results for a given assay. In summary, a robust system to deal with possible sources of variation can ensure that the 95%-Confidence Interval of the enzymatic chromogenic assay results is within 97% - 103% (based on a 4+2 parallel-line assay design).

The panel discussion emphasized and revisited some of the points made during the morning presentations. Specifically, there was a focus on what is encompassed by the idea of testing like versus like and developing a reference standard to do so. There was a desire by some to move to a single LMWH standard (or two if one considers potency and chemical measurements, such as MW, separately), if the science supports this possibility: *i.e.*, it is possible to use a single standard across the spectrum of LMWH. Also noted was the fact that USP has progressed with the enoxaparin standard and monograph without a general chapter because it is the first, and so far only, LMWH to become official. However, the enoxaparin RS has been harmonized to the IS. Because the US market for LMWH is different than the European market, USP has decided to proceed with product specific monographs and reference standards. A molecular weight RS specific for enoxaparin sodium is being developed. The European representatives emphasized that the suggested approach did not fit the Ph. Eur. policy on LMWH which is based on the WHO international consensus which emphasized one standard for all already licensed LMM heparins.

Dr. Gray addressed the community again, this time focusing on an update of the biosimilar guidelines of the EMEA for LMWHs. The EMEA recommends that applicants present data demonstrating the similar nature of the proposed biosimilar to the Reference Product in terms of quality, safety, and efficacy. Accordingly, the Reference Product used in the comparison must be a medicinal product authorized in accordance with Article 8. A clinical trial is used to demonstrate comparability of the new product versus the Reference Product. Clinical safety is monitored long term because the clinical study does not give enough time to show all the potential differences between the two products. A concept paper on biosimilars recommends that non clinical studies also be employed to show comparability. *In vitro* comparisons should include a bioassay- at least anti-factor Xa and anti-factor IIa assays. *In vivo* tests should include an appropriate PD model, following at least anti-factor Xa and Anti-factor IIa assays, and other markers as well. Toxicology should also be completed.

Dr. Gillian Woollett (Engel & Novitt, LLP) reviewed the background to the on-going legislative biosimilars debate in the US. She outlined the dichotomy of regulatory pathways for biological products: one considering them as drugs, as regulated by the Food, Drug & Cosmetic Act and the other as biologics, regulated by the Public Health Service Act. This situation is unique to the US as no other country has two such distinct regulatory pathways. In this situation, for example, the LMWHs are regulated as drugs, not biologics. Given that the Hatch-Waxman Act in 1984 enabled subsequent products to reference previously approved FD&C Act products, but did not consider PHS Act biologics, the present debate is about creating a statutory authority for FDA to implement a regulatory pathway for subsequent entry biologics. Dr. Woollett stated her view that legislation can support scientific progress and a regulatory framework for the approval of biologics. In her opinion, the FDA already has the authority through existing pathways to decide whether follow-on products are interchangeable. Going forward we should learn from generic drugs, though not become weighed down in an excessive patent process. The generic market must work together to increase competition and access. The comparability framework needs to be obtainable so that access and innovation can increase. The progress in Europe on biosimilars is noteworthy as is the

initiative lead by WHO, a non-regulatory authority, to create a guideline on biosimilars that allows for some level of global consistency. The role of reference standards, and how these may fit within a global development program for biosimilars was also raised. Finally, Dr. Woollett gave a summary of the legislation that is currently being debated by the US Congress – then in the form of two bills being attached to the larger Health Care Reform efforts in both the House and Senate. Finally, Dr. Wollett noted that market access will be critical as biosimilars are used globally and patients require access everywhere. Manufacturers produce for the largest markets and will be approved for those markets, if the same data set cannot be used for other markets than there are hurdles to accessing the market and patients cannot access the biosimilars.

Mr. Li Li (Hepalink Pharmaceutical Co., Ltd) presented the heparin program at Hepalink. The quality of heparin is carefully controlled for LMWH production. Mr. Li asserted that Hepalink has a traceable heparin supply and Hepalink is the only Chinese manufacturer that has been approved by the FDA, EDQM, the German GMP inspection, and Australian inspection. In terms of impurities, removal of proteins from heparin ensures more stable color and fewer allergic reactions. Hepalink recommends that the protein limit be set at 0.25% due to their allergic potential and that USP standards should be improved in this area. Furthermore, Hepalink suggests that generic LMWH should not only comply with pharmacopeial standards, but also complete a comparison with the innovator product. To better control impurities in heparin, Hepalink recommended that USP should implement and/or improve assays for the potency of heparin, as well as residual protein and nucleic acid content. In discussions afterward, Mr. Li noted that Hepalink uses traceability instead of testing to ensure the absence of cross-species contamination. In this case, the slaughterhouses are all located very close to the workshops and pig intestines are not purchased from small farm operations.

Dr. Jeanine Walenga (Loyola University) presented *in* and *ex vivo* comparisons of a number of LMWHs. Since the molecular profiles and composition of branded (and potential biosimilar) LMWHs vary, it was hypothesized that the immunogenic potential of each LMWH differs. A study was done to test whether antibodies in patients on different LMWHs differed depending on the product administered. Antibodies varied by subtype within each type of LMWH used, and between each LMWH administered. The prevalence of HIT Abs ranged from 12-18% in patients treated with different branded LMWHs. Notably, patients with HIT showed a higher proportion of anti-heparin IgG Abs. Differences were found in the ability of each LMWH to release PF₄ and serotonin. In *in vitro* platelet aggregation assays, each of LMWHs produced a response to HIT antibodies, but the response differed between them. This difference was also observed for different versions of the same product, for example enoxaparin sodium. In the platelet aggregation and ¹⁴C-SRA HIT assays, different versions of enoxaparin available in different countries all showed a response but to a different extent from one another. Based on these comparisons, Dr. Walenga hypothesized that differences in LMWH composition are determinants of immunogenicity and that the immunogenic profile of a LMWH can be used to define bioequivalence.

Dr. G. Nadamuni (Gland Pharma) gave a presentation on the experience that he and his company in India have producing heparin and LMWH. Gland began manufacturing heparin sodium decades before adding LMWH to their product line. Dr. Nadamuni noted in the quality of control of enoxaparin, NMR is used to determine 1,6-anhydro content. In addition, Gland uses TMA-SAX instead of CTA-SAX for separation of heparin and LMWH oligosaccharides due to better column stability and better resolution.

Session 2: Characterization and Standardization

Overall Summary: Presentations and discussions in this session examined the role of other analytical tests, including NMR, HPLC, and AT-III affinity in characterizing and controlling LMWH.

Dr. Peter Jongen (RIVM) gave a presentation outlining the current characteristic tests for LMWH in Europe. Because of their origin (isolation from animal tissue), LMWHs are viewed as biological medicinal products by the EMEA and as such, the extent of analytical characterization could be a key factor for their regulation. Physico-chemical characterization of LMWHs is complicated by the fact that they are *per se* a heterogeneous mixture of many different structures. Nevertheless, characterization is required to verify the nature of the substance, consistency in production, comparability of clinical material, and stability during shelf life. The European Pharmacopeia tests for LMWH include identification by ^{13}C NMR, anti-factor Xa/anti-factor IIa activity, mean molecular mass and dispersion, and sodium or calcium content, where appropriate. The European Pharmacopeia monograph provides a good basis for release testing. Notably, purity specifications for LMWHs with respect to artificial contaminants, such as OSCS, will be covered by the PhEur monograph for UFH. In the future, more detailed analytical testing may allow for the development of manufacturing variations and new license applications.

Dr. Ian McEwen (MPA) presented on NMR analysis of UFH and LMWHs. In the European Pharmacopeia, ^{13}C -NMR is an identification test for LMWH. While no S/N ratio is specified in the monograph, to obtain a decent ^{13}C spectrum takes many hours, thus raising the concern that the test might be cumbersome. To reduce time of analysis, a better choice for the identity test of LMWH is to use ^1H -NMR or two dimensional ^1H - ^{13}C correlated NMR. In Dr. McEwen's estimation, either NMR experiment will take no more than 10-20 minutes. Purity can also be assessed by proton NMR, with OSCS yielding a small signal at approximately 2.15 ppm. By comparing over 350 spectra of UFH sodium, it was concluded that there is a characteristic "spectrum" of heparin sodium with some minor variations. However, this is not the case for LMW heparins because the degradation methods employed for the production of the different LMW heparins give different ^1H -NMR signal patterns. Still, the major signals are recognizable in the different types of LMW heparins and can be used for a common identification test. If this approach is used, a two dimensional ^1H - ^{13}C correlated NMR experiment like HSQC or HMQC would be the best choice. Identification by 2D NMR can be done using six signals. If on the other hand ^1H -NMR is used, a standard for each type of LMWH must be used.

Dr. Christian Viskov (Sanofi-Aventis) spoke on advances in analytical and separation methods that have enabled the isolation and sequencing of a number of "active" process-dependent components of LMWHs. Sanofi-Aventis has used this technology to map the ATIII binding components of LMWH. These studies demonstrate that the classical pentasaccharidic sequences are located at different sites along the oligosaccharide chains. For example, it has recently been reported that different octasaccharides isolated from enoxaparin that contain ATIII-binding sequences possess different binding affinity and potency. These differences were found to be due to structural differences of the flanking sugars surrounding the pentasaccharide sequence (J Biol Chem 2008; 283: 26662-26675). For example, one finding was that 1,6 anhydro moieties at the reducing end decreased affinity, even though this modification was not present within the canonical ATIII-binding pentasaccharide sequence. Furthermore, there seemed to be a poor correlation between the binding strength and the anti-factor Xa activity of isolated oligosaccharides. As such, the link between antithrombin affinity and the anti-factor Xa activity of an oligosaccharide is likely more complex than previously envisioned. According to Dr. Viskov,

these results demonstrate that the process can modulate the activity of a LMWH oligosaccharide mixture.

Dr. Ishan Capila (Momenta Pharmaceuticals) presented chromatographic methods related to the analysis and control of the saccharide composition of enoxaparin sodium, and the analysis and control of impurities and contaminants in enoxaparin sodium. In compositional analysis, three fundamental steps have to be optimized and controlled to ensure an accurate assessment of composition. These include — (1) digestion of the oligosaccharide mixture into its saccharide components which necessitates control of the purity and catalytic activity of enzymes used for the digestion along with optimization of enzymatic digestion conditions; (2) separation of the saccharide components using a high resolution, robust and reproducible procedure; and (3) accurate quantification, including utilizing pure saccharide standards to determine a response factor and therefore a mole % of saccharide components. This approach is considered useful in achieving correct mass balance and providing assurance that all saccharide components are measured. The second part of Dr. Capila's talk was focused on the ensuring control of the starting UFH used to manufacture enoxaparin sodium, particularly as it relates to the detection of OSCS. To this end, a Strong-Anion-Exchange (SAX) HPLC method that was developed at Momenta Pharmaceuticals was presented and compared to the proposed USP SAX-HPLC method. The Momenta method has an LOD of ~ 0.05% and a shorter run time. Dr. Capila asserted that using such sensitive analytical methodologies to screen starting UFH or even crude heparin can help ensure the integrity of the supply chain. Finally, the talk also briefly discussed how a multi-level approach is required to ensure control of starting UFH used for the manufacture of enoxaparin sodium. This involves traceability of material, an understanding of the appropriate process design to manufacture the starting UFH, a strong Quality Assurance program and sensitive analytical methodologies.

The Panel discussed how existing methods need to be optimized, as demonstrated by the 1D NMR change to 2D NMR, which provides more information. A harmonized approach is also necessary to move forward in the global market of heparins. There was also discussion about what tests should be used to release of heparin lots *versus* what may be needed in a regulatory sense. Finally, in terms of harmonization, USP and EDQM are working differently on the issues that surround the standardization of heparin. There was a void in the specifications relating to UFH that needed to be addressed in light of the heparin crisis; however, this void been addressed. There was general agreement that harmonization as the pharmacopeias move forward is of the utmost importance due to the global nature of the heparin market. Due to regulatory differences there will likely be different specifications in different regions, however, methods can be harmonized to best allow manufacturers to access more markets.

Session 3: Unfractionated Heparin Issues

This session covering the 2nd day of the workshop dealt with the responses to the contamination of unfractionated heparin (UFH), resulting in actions of licensing authorities, the development of new analytical tools, monograph updates and industry experience with the updated monograph and methods.

Replacement of UFH International Standard for Potency

The first two presentations were about the potency standardization of UFH. In her presentation Dr. Elaine Gray from NIBSC summarized the collaborative study conducted to value assign the 6th International Standard (IS) for UFH and the USP heparin potency reference standard (RS), USP Heparin Sodium for Assays RS: Thirty-three laboratories from 18 countries contributed data obtained from 12 different assay methods to value assign the 6th IS for UFH against the 5th IS for UFH. Based on the lowest range of intra-laboratory variation (%GCV: 2.2 – 6.4) for the different assay methods and the lowest inter-laboratory variation by all methods (%GCV = 3.6), candidate W has been recommended to become the WHO 6th IS for UFH with an assigned value of 2145 IU/ampoule by all methods. A new United States Pharmacopeia (USP) RS Lot F for potency of Heparin Sodium has also been selected and value assigned by sub-group analysis of data obtained using the proposed new USP monograph potency method (anti-factor IIa assay). Nine participants carried out this assay. Both candidates W and X gave comparably lowest intra- and inter-laboratory variation for the USP anti-factor IIa chromogenic assay, but candidate X is marginally better than candidate W in that it gave the best agreement of potency estimates by the three present and future USP monograph methods. Candidate X was accepted as the USP RS Lot F for potency of Heparin Sodium, with an assigned value of 2144 USP unit/ampoule. As both the 6th IS for UFH and the USP RS Lot F are traceable and value assigned against the 5th IS for UFH, the long-standing disparity between the USP unit and the International Unit for UFH is resolved.

In his presentation Dr. Job Harenberg (Ruprecht–Karl’s University, Heidelberg) gave an overview of how the UFH therapy is monitored by aPTT. The dose is subsequently adjusted according to the aPTT value measured. The difficulties of the standardization of aPTT measurements were addressed. Furthermore, the clinical routine management by the use of Protamine for antagonization of UFH was reviewed. Protamine is given at a defined ratio compared to UFH. This ratio changes by changing the USP method.

Contamination of Heparin—Progress Report from the Licensing Authorities

In the next three presentations the licensing authorities gave a status update of the contamination issue for unfractionated heparin in their respective area of jurisdiction.

Dr. Ali Al-Hakim (FDA) gave an update of the current situation in the US. FDA proposes to further update the USP heparin sodium monograph with respect to increasing the sensitivity of the NMR test and to improve impurity tests in terms of sensitivity for the detection proteins and nucleic acids. An impurity method for the detection of lipids was also proposed along with a molecular weight determination method.

Dr. Shinobu Uzu (Ministry of Health, Labor and Welfare (MHLW), Japan) presented the situation in Japan. Marketing authorization holders (MAHs) were instructed to screen their API using the CE and NMR methods as published by the FDA in 2008 and some products had to be recalled. However, no adulteration related adverse events have been reported.

A new ¹H-NMR method has been introduced for the detection of OSCS and there are requirements for the impurities Barium, total nitrogen and proteins. A test for dermatan sulfate (DS) and nucleotides is under development along with an update of the protein testing. As further safety measures, MHLW instructed MAHs to validate heparin products and their raw materials in

terms of quality control and GMP and to verify the purity of refined heparin as raw materials in each lot based on relevant monographs.

Dr. Anita Szajek (USP) gave a brief update heparin contamination as provided by the TGA, Australia. OSCS was detected in 2 batches of blood collection tubes. Beginning July 1, 2009 Australia adopted EP and USP standards for heparin. Because the monographs between the pharmacopeias are not harmonized they are having difficulty reconciling the requirements. The USP approach is challenging because USP units are required and Australian regulators require units to be expressed in international units.

Physico-Chemical Characterization of UFH

In four subsequent presentations methods for physico-chemical characterization of UFH by the use of SAX-HPLC, CE, NMR and molecular weight profiling were presented.

Mr. Pascal Anger (Sanofi-Aventis) presented a SAX HPLC method coupled with depolymerization for monitoring of potential new contaminants. The method was described as a quick, QC friendly method complementary to other methods like NMR and bioassays for the detection of contaminants of polysaccharidic nature with negative charge e.g. sulfated polysaccharides. The broad heparin peak can mask part of the chromatogram. Unmasking this part of the chromatogram was achieved by the use of H_2NO_3 . Finally, it was demonstrated how heparinase depolymerization followed by disaccharide analysis by SAX HPLC can be used as partial assessment of species origin.

Dr. Todd Wielgos (Baxter) presented an optimization of the CE method for detection of OSCS. Optimization of the method included an increase in buffer molarity, reduction of the internal diameter of the capillary, change in buffer counter ion, change in buffer pH and shortening of the capillary length. As a result of these changes, the migration time dropped from 20 minutes to under five minutes, the resolution of OSCS from heparin was improved and the LOD for OSCS reduced. Furthermore it was shown that the optimized CE method was robust and could be transferred to different instruments in different labs.

Dr. Ian McEwen (MPAgency, Sweden) presented on identity and purity testing by NMR. It was shown from a study of more than 350 heparin samples that 1H NMR spectra of UFH have very similar signal patterns, forming the basis for the ID test for UFH by 1H NMR. Two dimensional (2D) NMR instead of 1H -NMR was proposed for the identification of heparin. The proposal was based on a common list of 6 proton carbon correlated signals. Furthermore, it was demonstrated how 1H -NMR can be used for the identification and quantification of OSCS and dermatan sulfate (DS) and it was also mentioned that 1H -NMR or 2D-NMR can be used for the control of other unknown impurities.

In her presentation, Dr. Barbara Mulloy (NIBSC) pointed out that currently the molecular weight profile is not a defining characteristic for UFH. Pharmacopeial monographs do not make reference to molecular weight for UFH nor do compendial methods exist and there is no easily available calibrant. However, a universally accepted method for the determination of molecular weights for UFH might be useful. It was concluded that the most feasible method for the determination of molecular weight distribution for heparin is to use gel permeation chromatography and a broad standard for calibration: A polydisperse heparin sample with a table listing the proportion of the

sample above a series of defined molecular weights. This system is already in use for low molecular weight heparin, using the 2nd International Standard (IS) Low Molecular Weight Heparin for Molecular Weight Calibration as calibrant. A suitable sample of heparin, one of the candidates for the 6th IS UFH, is available and will be characterized in a two-stage collaborative study.

Pharmacopeias—Heparin Monograph Revisions & Global Harmonization

In the following presentations a status of the updates of the USP, EP and JP monographs were given.

In her presentation Dr. Anita Szajek (USP) gave a summary of the revision history: A stage 1 was published in June 2008 as revision bulletin introducing ¹H NMR and CE methods for identification and absence of OSCS. The stage 2 revision proposal was published in Feb 2009 as an Interim Revision Announcement (IRA) with an anticipated official date of October 2009. The fast revision process was triggered by the urgency of the crises. After the stage 2 revision the monograph for heparin sodium has three specific, complementary ID methods: ¹H NMR, SAX HPLC and Anti-factor Xa/Anti-factor IIa ratio.

It also includes a new, specific potency assay with a new reference standard in USP Heparin Units and higher potency specification: NLT 180 USP Heparin U/mg. The limit for related substances from raw material: chondroitin sulfate (CS) and DS is determined via a galactosamine impurity method. The revision further adds a limit of nucleotidic impurities and residual solvents and updates the limit of protein impurities. An absence of OSCS specification is included in ¹H NMR and SAX HPLC.

The rationale for retaining and expanding ¹H NMR method along with the rationale for replacing CE method with SAX method when advancing from stage 1 to stage 2 was given and the reasoning behind the introduction of a Limit of Galactosamine in Total Hexosamine method was discussed. The presentation concluded with USP/FDA's recommendations regarding future Heparin monograph revisions: ¹H NMR spectrum with higher sensitivity, chromatographic identity with improved resolution and robustness, nucleotide impurities with tighter spec., protein impurities with tighter spec., lipid content and molecular weight method.

Mr. Jean-Marc Spieser (EDQM) gave a status on the update of the heparin monograph in the European Pharmacopeia. A stepwise revision approach is being followed: A short term revision has been implemented to include ¹H NMR and CE in the manufacturing section of the monograph. According to the flexible approach concept no specific procedures are suggested and acceptance criteria are at the discretion of the competent authority.

The plans for longer term revision were presented. These would consider: A higher specific activity of NLT 180 IU/mg and species verification testing, updating of ID tests by discussing the merits of one versus two dimensional NMR and deletion of optical rotation and zone electrophoresis, updating of impurity specifications and testing for nucleotide and protein impurities, tightening of Nitrogen specification. Various methods for testing of related substances are being considered including a limit 2% for DS by SAX HPLC. The chromogenic anti-factor IIa method is under consideration as potency assay.

In her presentation Dr. Nana Kawasaki (JP) presented the plans for the update of the heparin sodium and heparin calcium monographs. For heparin sodium the update plans include two identification tests by ^1H -NMR and weak anion-exchange HPLC (WAX-HPLC) respectively, a modified OSCS purity test by ^1H -NMR and a galactosamine purity test by ABEE-derivatization followed by HPLC with fluorescent detection. No specific limit for the content of galactosamine was disclosed.

For heparin calcium the ^1H NMR ID test and the galactosamine impurity test are proposed to be introduced at a later stage. Purity tests for proteins and nucleotides are expected to be further updated at a later stage. It should be noted that both heparin calcium and heparin sodium utilize an anti-factor Xa based assay as a potency assay.

Industry Implementation of the Proposed USP Heparin Monographs

In the subsequent three presentations feedback from industry stakeholders to some of the new methods and limits in the proposed USP heparin monograph were given.

In his presentation Dr. Erwin Kellenbach (Schering-Plough) described the evolution of the regulatory requirements in the heparin field triggered by the heparin crises starting with the implementation of CE and NMR requirements to the current stage 2 revision of the USP. The participation of his company in the various monograph revisions in Europe and in the US was described. When using the old USP potency RS, Schering-Plough experienced problems meeting the revised potency limit of NLT 180 IU/mg. This will be further evaluated when the new potency RS is available. Also the updated proton NMR method causes Schering-Plough's heparin to fail and requires further investigation.

Dr. Edwin Moore (Baxter) described in his presentation the thorough evaluation of three of the new USP monograph tests: the chromatographic purity, the galactosamine impurity procedure and protein impurity procedure introduced with the stage 2 revision of the USP. Specific experience based proposals for optimizing especially *the* protein impurity and *the* heparin chromatographic purity tests were given.

In his talk Dr. Christian Viskov (Sanofi-Aventis) presented a case where a signal at 2.18 ppm in the ^1H spectrum from a large number of batches of heparin sodium from a particular supplier was seen. According to the proposed USP monograph no signal should be found here. After extensive investigations of the heparin structure (use of enzymatic sequencing, HPLC-MS, preparative orthogonal HPLC methods and NMR) it was found that this extra signal was a heparin process fingerprint. Pure oligomers bearing the particular structural moieties were isolated and characterized. It was concluded that the impurity was at about 0.3% in heparin the backbone.

Conclusions

Worldwide regulatory expectations regarding the quality of unfractionated heparin have increased since the global crisis of 2008. At the workshop, USP and FDA jointly announced their intent to further revise heparin monographs to ensure the continued supply of safe and effective heparin for patients. Workshop participants strongly agreed that modernization of heparin monographs was necessary but grappled with the questions of methods, specifications, and speed of implementation. In general, industry

concluded with and supported the modernization efforts, but manufacturers are concerned about the lack of harmonization among pharmacopeias. Moving forward, it is highly desirable for regulators and pharmacopeias to continue fostering an international harmonization dialog for heparin and low molecular weight heparin products.