From crisis to opportunity: A perspective on the heparin crisis

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Heparin is a mixture of polysaccharide chains isolated from a biological source, porcine intestinal mucosa, and is used clinically primarily as a prophylactic agent to prevent thrombosis as well as an initial treatment of established venous thrombosis (1). Heparin has enjoyed widespread use as a medicinal agent; it and insulin are, on a unit basis, the most widely used medications in the clinic. Heparin is baseline therapeutic in a number of situations, such as kidney dialysis and medical intervention in the case of of acute coronary events. In terms of worldwide production, most of the crude, partially-purified, heparin is produced in China; in addition, China is a major locale for purification of crude heparin to form the active pharmaceutical ingredient.

In late 2007 and early 2008, clusters of serious allergic-type events were reported in patients undergoing hemodialysis who were receiving heparin (Fig. 1). The first observation was made in a paediatric patient at Children's Hospital in St. Louis (MO, USA) in mid-November, with additional cases reported in January of 2008. These initial cases were reported to the Missouri Department of Health and Senior Services which then promptly notified the Centers for Disease Control (CDC). On January 4th, the CDC alerted the US Food and Drug Administration (FDA) of the cluster of adverse events. The set of clinical findings coupled with the notion that heparin, a major pharmaceutical agent, was involved in the onset of adverse events triggered an investigation into the root cause in the hopes of quickly averting further harm to patients. The FDA, in conjunction with Baxter Laboratories (Deerfield, Illinois, USA), first withdrew from the market specific lots of heparin on January 17th, and later, on February 28th, all heparin manufactured by the company. Concomitant with the unfolding situation in the United States, suspect heparin lots were identified first in Germany and then in a number of other countries, including Canada, the Netherlands, France, Italy, Japan, China, Australia, and New Zealand.

Efforts were made to identify the source of these allergic-type reactions, and these initial investigations ruled out many obvious causes, such as the presence of adventitious viral agents or the presence of greater levels of protein impurities in suspect versus non-suspect lots of material (2). Shortly thereafter, on March 5th, the FDA released screening tests, employing nuclear magnetic resonance (NMR) and capillary electrophoresis (CE), which could be used to screen heparin lots and determine whether they were acceptable for use (3). Based on initial results from the screening tests and delineation of suspect lots from those that were clean, properties of the non-heparin material present within suspect lots could be surmised (Fig. 2). First, in the CE method, based on the migration properties of the unknown material with reference to heparin, as well as its UV- absorbance properties, the contaminant was likely to be a polysaccharide which had a higher charge density than heparin. Second, based on a distinctive proton NMR signature at 2.1–2.2 ppm, the polysaccharide contaminant was suspected to contain N-acetylgalactosamine. However, complicating the interpretation and identification of a contaminant was the fact that heparin can often be co-purified with other sulfated glycosaminoglycans, such as dermatan sulfate and chondroitin sulfate, which also contain N-acetylgalactosamine (see bottom panel of Fig. 2B). Thus, there was the additional question of whether the signatures associated with suspect heparin lots measured with these tests were simply “harmless” impurities within heparin or, conversely, an agent (or agents) which could lead to the development of allergic-type responses. An additional complicating factor was that, concurrent with the spike in serious adverse events, there was a widespread outbreak of blue-ear virus among Chinese pigs. Thus, were the signatures associated with suspect heparin the result of impurities arising from a change in manufacturing or the source material or were they the result of something else?

To solve this set of questions, address the root cause of the spike in serious adverse events, and provide a scientific foundation upon which quality control tests could be developed, the FDA brought together a multidisciplinary team from the agency, academia, and industry. In a matter of weeks, beginning in early March, this team identified the signatures from the screening tests as belonging to oversulfated chondroitin sulfate (OSCS) (4), a complex polysaccharide mixture in its own right which had never before been observed in heparin. Multiple lines of evidence converged to provide definitive identification of OSCS including multidimensional NMR, enzymatic digestion and HPLC.
Sasisekharan, Shriver: The heparin crisis analysis, and mass spectrometry. Ultimately, OSCS isolated from a suspect lot was found to match a chemically synthesised standard. Remarkably, the predominant structure of OSCS was found to have four sulfates per disaccharide unit, which is rarely, if ever, found in nature and is structurally distinct from other glycosaminoglycan impurities, such as dermatan sulfate. Consequently, it is unlikely that OSCS arose directly from a natural source, for example, virus infected pigs, or from the natural course of heparin production. From these lines of evidence, OSCS was deemed to be a contaminant within heparin. Concomitant with the structural studies, a biological investigation was begun in late March (Fig. 1) and identified one manner by which OSCS could lead to the advent of serious adverse events. Highly sulfated polysaccharides, similar to OSCS

Figure 1: Timeline associated with events of the heparin crisis from the first clinical reports in November 2007 through the epidemiological findings published in December 2008.

Figure 2: Detection of a contaminant within heparin. A) Structure of heparin (top) and OSCS (bottom) shown in acidic form. Both consist of a disaccharide repeat which is repeated n or m times for heparin and OSCS, respectively. The acetyl of OSCS is shown in bold; its presence is a key structural signature for analytical tests to detect and quantify the contaminant. B) Representative CE results for a clean heparin sample (top) and a contaminated heparin sample containing OSCS (bottom). OSCS migrates through the capillary faster than does heparin, indicating it has a larger negative charge density. C) NMR results from 1.8–2.4 ppm for a clean heparin lot (top), a heparin lot containing dermatan sulfate (middle) and a contaminated heparin lot (bottom). For dermatan sulfate, the chemical shift of the methyl for the N-acetylhexosamine is between that of heparin and OSCS.
in structure, were previously known to activate the contact system (5). Activation of the contact system can lead to a rapid onset of an anaphylactoid response which agreed with the clinical findings. In addition, induction of the contact system does not require prior exposure, as is the case with an IgE-mediated response. Using multiple lots of suspect and control heparin, the authors found that activation of the contact system, as measured by kallikrein activity, occurred in suspect lots and not control lots (6). Furthermore, through a series of biochemical experiments, factor XII was found to be a key regulator of this response (Fig. 3). Finally, by screening plasma from multiple animals, the pig was found to mimic the human situation. Introduction of OSCS or OSCS-contaminated heparin into pigs at a clinically relevant dose resulted in acute changes in haemodynamic parameters consistent with activation of the contact system (6).

Importantly, in the in-vitro studies, kallikrein activation did not follow a simple dose-response but rather a more complicated bell-shaped curve where at low levels, increasing dose led to an increase in contact system activation but at higher levels, inhibition of this activation was observed. This phenomenon is also true of heparin, where depending on its concentration and “presentation”, heparin and either serve as an activator or an inhibitor of protein activity. This is certainly true for a wide variety of heparin binding proteins, such as the coagulation factors – thrombin and heparin cofactor II, for example- and growth factors, such as FGF (20). This bell-shape dose-response is likely due to the fact that, at low concentrations, the negative charge density of OSCS acts as a platform to activate factor XII, whereas at high concentrations, either through direct binding to factor XII(a) or other components of the complement/coagulation system, OSCS competes with and inhibits the activation pathway. This finding stresses two points: first, that in the investigation of structure-function relationships for this class of molecules, one needs to take care to carefully investigate dose-response relationships since the same material can have diametrically opposite effects. Secondly, only relying on an empirical observation of activation or suppression of a biological response is insufficient, development of an understanding of the underlying mechanism for activation/inhibition is critical to ensure accurate interpretation.

After identification of the contaminant within specific heparin preparations and elucidation of its potential biological role in eliciting an anaphylactoid response, several additional developments have occurred. First, the analytical studies published in April 2008 pointed to OSCS as the culprit of the anaphylactoid response. However, critically, a link needed to be established between the clinical presentation and the heparin lots administered to patients. This was not an easy task, especially given the fact that for many patients the particular lot of heparin administered was not recorded, nor in some cases, were the lots of heparin known within the facility at the time of an adverse event. Nevertheless, even with this set of challenges, a study led by the CDC demonstrated convincing epidemiological evidence that correlated confirmed and probable cases of an allergic-type response with OSCS-contaminated heparin (8). Taken together with the initial biological findings, this data strongly suggests that OSCS contamination can lead to serious adverse events in humans. Second, additional biological roles for the contaminant have been identified, including potentially eliciting heparin-induced thrombocytopenia (9). Additionally, several reports have identified other biological or analytical techniques that can separate contaminated lots from uncontaminated lots. In terms of analytical techniques, electrochemical detection (10), infrared and Raman spectroscopy (11) and strong anion exchange chromatography (12) have all been demonstrated to detect OSCS in heparin, and others have and are sure to be developed (13). These techniques, and others that are in development, hold much promise as rapid screening tools and/or methods to monitor supply chain.

In addition to screening assays for OSCS, to adequately control heparin requires a robust assessment of lot potency. It is clear that the current compendial assay, relying on gross inhibition of clot formation is neither specific for heparin (many agents, including OSCS have activity in this assay) nor does it possess the requisite sensitivity to control the quality attributes of heparin. Measuring aspects of anticoagulant activity that are at least somewhat specific to heparin, such as activated partial thromboplastin time (aPTT) (14) or the ratio of anti-factor Xa to anti-factor IIa activity, can be used to differentiate heparin from contaminated heparin and clearly provide a better mechanism for control. Especially in the case of the anti-Xa/anti-IIa ratio, a ratio of ~1 is clearly characteristic of heparin, and differentiates it from low-molecular-weight heparins (LMWHs) (with anti-Xa/anti-IIa ratios of >2) as well as other polysulfated polysaccharides, which lack the requisite pentasaccharide sequence required for anti-Xa activity and thus elicit anticoagulant activity through other mechanisms, such as heparin co-factor II.
Finally, the issue of contaminated heparin has spread to encompass other heparin-based products, including heparin-coated medical devices, such as stents, and the LMWHs. Indeed, lots of enoxaparin sodium, the most widely prescribed LMWH, have been reported to contain OSCS. Furthermore, a recent study has demonstrated that OSCS is not removed by any of the processes used to make LMWH from heparin (15). Given the importance of LMWH in the prophylaxis and treatment of deep venous thrombosis, adaptation of screening methods and better measurement of potency of the heparin starting material is critical towards ensuring the safety of these agents.

In the context of the “heparin crisis” and the scientific investigation that facilitated its resolution, the question remains—are there lessons to be learned to avoid a similar situation in the future? While the loss of a single life is a tragedy, in many ways, the heparin crisis highlights some of the best aspects of collaborative science. The coordination of activities across the FDA, the CDC, academic physicians and scientists, and industry allowed this mystery to be solved expeditiously using thorough and rigorous science. Over the course of only four months (from January until April of 2008), clusters of allergic-type reactions were identified within patient populations, heparin was attributed as the source of the allergic-type reactions, the contaminant within heparin preparations was identified, a potential role for the contaminant in the advent of adverse reactions was determined, and screening methods were put into place to ensure the safety of the remaining heparin supply. The scientific and managerial leadership taken by the FDA is to be commended and is certainly one of the primary reasons that there was such a rapid response and that a wider crisis was averted. This model may hold promise to address other unmet medical and public health needs.

A second important “lesson learned” from the heparin crisis is the ongoing need for medical surveillance of approved biological medicines (16). One of the primary reasons that the U.S. was the first to report serious adverse events associated with heparin therapy was the reporting system that was in place. Because of the speed with which a cluster was identified, starting with the initial astute observations at the Children’s Hospital in St. Louis, its source—OSCS—and methods to screen for OSCS could be rapidly developed. Of the more than seven million single-dose units and three million multi-dose vials of heparin supplied by Baxter between November 2007 and January 2008 (an estimate of total doses used [8]), the number of reported adverse events to date is probably in the low hundreds, or approximately 0.1–0.01% of total heparin doses administered. Thus, the ability to pick the proverbial “needle in the haystack” ultimately led to the recognition that there was a problem and minimising, as much as possible, the public health impact. Unfortunately, it is likely that the medical surveillance net of the U.S. and Europe will be repeatedly tested in the coming years, whether it is the importation of adulterated drugs or the introduction of adventitious disease agents (16, 17). In the former category, there are existing examples including the ongoing situation with the introduction of melamine into pet food and infant formula to yield artificially high-protein content; in the later category is the potential humanisation of avian flu which could lead to a pandemic. Thus, given the increasing rate of globalisation, the development of additional, and multinational, health surveillance tools is essential.

Finally, there are lessons to be learned about control of supply chain and development of appropriate levels of testing to ensure product uniformity and quality (18). Clearly, one of the issues is lack of worldwide harmonisation with regards to quality control tests, both the types of tests employed as well as the pass/fail criteria. Prior to the advent of contaminated heparin, there were significant differences between the tests performed and the criteria employed, including the use of impurity tests (i.e. DNA, proteins, etc.) Fortunately, the United States and European Pharmacopeias are moving towards harmonisation, most significantly with regards to the definition of heparin activity and its measurement. Combining these efforts with those of regulators from other countries will be important to ensure systematic testing and vigilance of the heparin supply chain. However, more fundamentally, it is often the case that complex drugs, such as heparin, are not described at molecular level, but rather are controlled by a set of attributes, such as activity. In turn, these attributes, lack much descriptive power of the mixture itself, even though manufacturers often apply a (lengthy) list of tests. For example, in the case of heparin, despite the fact that the United States Pharmacopeia monograph for heparin contains more than 10 tests, including an activity (clotting) test, the presence of OSCS could not be detected in heparin even though in some cases, OSCS approached ~30% of the mixture! This inability to detect OSCS is because the material structurally resembles heparin, possessing similar charge density as well as some level of anticoagulant activity. Finer measurement of both the activity of heparin (such as measuring the anti-Xa: anti-IIa ratio) as well as definition of molecular level attributes through techniques such as NMR were and are clearly needed. Thus, with regards to complex pharmaceutical agents, such as heparin, what is required is to introduce a more rigorous framework towards testing and quality control, ensuring the absence of contamination. To this end, there must be a recognition, description, and quantification of the fact that individual analytical or biological tests have a certain amount of information associated with them, and that some tests present more information than others. In this context, for complex pharmaceutical agents, the development of a framework to handle the information content of individual assays is required. This framework can be developed outside of the context of a quality-control (QC) environment but is entirely consistent with QC-related tests and indeed provides the underlying logic for why certain tests are necessary.

With the advent and penetration of powerful analytical techniques, such as mass spectrometry and NMR, there are techniques that can provide molecular-level information. For example, in the case of heparin, enzymatic digestion followed by HPLC or CE analysis can be used to obtain compositional information; similarly, NMR can also be used to obtain compositional information. The two methods in combination can be used to confirm each other and provide greater understanding than either alone (19). Importantly, there are different ways by which one can obtain pertinent information and it is not always necessary to “build a better mousetrap”. Within this framework even “low-tech” techniques, such as measurement of the color content, provide valuable information. Rather than the individual analytical or biological assays, it is the framework that is important, including recognition (and minimisation) of one’s so-called “blind spots”.

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Importantly, the heparin lesson holds true for other complex mixtures used as pharmaceutical agents, whether they are proteins, peptides, polysaccharides, nucleic acids or other classes of molecules. Ever advancing analytical technology and structure-function understanding should be incorporated into aspects of control and comparability. As such, the framework proposed here can play a wider role than providing a process to control existing pharmaceutical agents; it can also play a role in the evolving regulatory framework around issues such as follow-on biologics.

The heparin crisis has been called the proverbial “canary in the coalmine” (18) in that, in this era of globalisation, we should change our thinking to avoid such tragedies. Thus, in this context, the heparin crisis should not be thought of as a one-off occurrence. The “lessons learned” from the crisis should be built upon. Similarly, the shortcomings that the heparin crisis exposed in the quality control of complex pharmaceutical agents must be resolved, including developing more integrated testing regimens as opposed to exclusively end product testing. In this manner, we can increase the safety of not only existing complex pharmaceutical agents but also ones that may come in the future. From crisis comes opportunity, in this case an opportunity to transform the control of complex pharmaceutical agents.

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References