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George's Marvelous Medicine: Perspectives on the Heparin Contamination Crisis

Ebaa Al-Obeidi

Abstract:

The worldwide heparin contamination that occurred in 2007 was the result of the man-made contaminant over-sulfated chondroitin sulfate (OSCS). The crisis revealed flaws in the quality control measures being taken by regulatory agencies and led to reforms of the U.S. Pharmacopeia. This case study discusses the process of heparin production as well as the drug's pharmacological profile. It proposes applying new analytical techniques and pursuing bioengineering as the two critical steps necessary to prevent a similar crisis in the future.



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Introduction:

Cardiovascular disease is a major challenge in medicine. Peripheral vein disease is one component of that challenge, often complicating an already stressed cardiovascular system. Every year, 300,000–600,000 Americans are affected by deep vein thromboses, which are blood clots that form within a deep vein [4]. One in three people can then suffer a serious complication called pulmonary embolism, wherein a clot dislodges and enters the arteries of the lung, creating a blockage [4]. Additionally, over 800,000 adults suffer a myocardial infarction annually. Also known as a heart attack, this severe blockage in the artery that perfuses heart muscle often results from a clot blockage in a plaque-narrowed artery [6].

What all these diseases have in common is their dependence on heparin, the oldest, fastest, and most well-understood anticoagulant [5]. First discovered in 1916 by Johns Hopkins medical student Jay McLean, the FDA reports that today approximately 12 million Americans use heparin each year [7]. In November 2007, hospitals began reporting that patients receiving heparin suffered from nausea, shortness of breath, and a drop in blood pressure within half an hour of treatment [2]. The reports grew until 13 states had reported cases of adverse reactions by the end of the year [2]. By January 2008, when the last case was filed, 152 adverse reactions had been reported and 81 deaths had occurred in the United States [2]. Additionally, 12 other countries also reported negative effects from tainted heparin [7]. The FDA assembled a research team of academic and industry experts, led by MIT professor Ram Sasisekharan, charging them with the task of identifying the contaminant. By March 19th, the FDA announced that they confirmed the contaminant to be man-made oversulfated chondroitin sulfate (OSCS) [9]. At \$9 a pound (compared with \$900 a pound for heparin), it was concluded that OSCS was added deliberately for monetary gain, with the lack of subsequent disclosure constituting intentional fraud [1].

Heparin Background:

Heparin is a highly sulfated glycosaminoglycan [GAG] drug that is prepared by extraction from the tissue of slaughterhouse animals, specifically porcine intestines and bovine lungs [14]. Humans also synthesize heparin but in very low, subtherapeutic concentrations [5]. Most heparin today is obtained from Chinese sources; in fact, of the 700 million pigs slaughtered each year, 400 million are slaughtered in China, the world's top consumer and producer of pig products [15]. World-wide sale of heparin is over \$3 billion and over 100 metric tons are prepared annually, requiring tissue from nearly 700 million pigs [21]. Heparin-based anticoagulants are administered in many surgical procedures, all extracorporeal therapies such as kidney dialysis and open heart surgery, and are often bound to indwelling devices including catheters and stents [21].

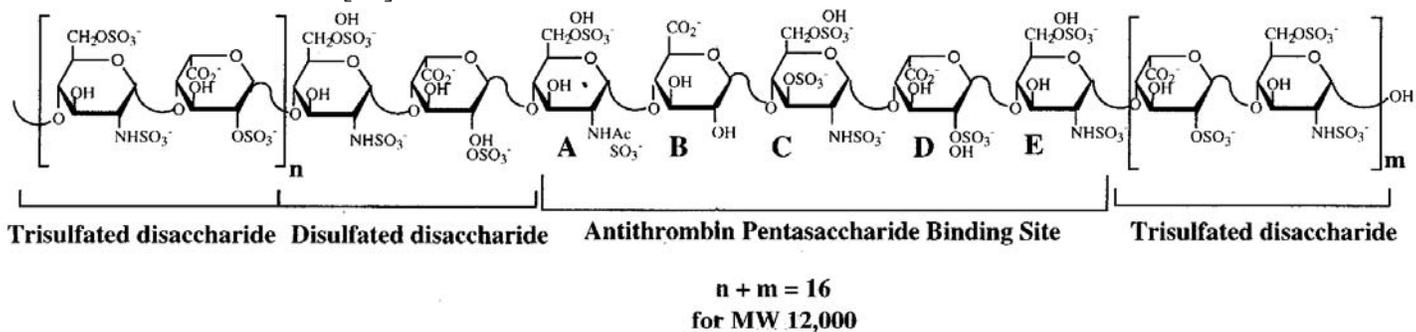


Figure 1. Chemical structure of a representative chain of pharmaceutical heparin. At positions where more than one functional group is possible, both are shown (for example, in residue A of the AT binding site, position 2 can be N-acetylated or N-sulfonated). Figure from [14].

Heparin is a disperse mixture containing a large number of polysaccharide chains of different molecular weights [14]. Its structure can be seen in figure 1, which illustrates the complexity of this anticoagulant. The process of heparin production goes through several stages, beginning with the removal of endothelial tissue from the intestinal lining [20]. It is then cleaned and ground into “hashed pork guts.” In the next step, the crude heparin is separated from the animal tissue under elevated temperature and pressure, followed by alkaline hydrolysis in presence of proteolytic enzymes [20]. At this level, the heparin is often contaminated with other impurities like peptides, nucleic acids, and lipids. The enzymes are deactivated by heating which helps sanitize the extract.

In the third step, the crude heparin extract is treated with an ion exchange resin to selectively precipitate heparin and closely related species from the solution. The resin-heparin complex is then processed to separate the heparin, which will be subjected to further purification and sanitization to remove lingering viruses. Figure 3 in the appendix includes a schematic of the process. The complexity of the heparin manufacturing process illustrates the high potential for contamination, and represents one of the major drawbacks to animal-derived drugs.

Heparin Pharmacokinetics and Pharmacodynamics:

Peak plasma levels of heparin are achieved two to four hours following subcutaneous administration, although there are considerable individual variations [5]. Log linear plots of heparin plasma concentrations with time are linear for a wide range of dose levels, which suggests the absence of zero order processes [5]. The liver and reticulo-endothelial systems are the primary sites of metabolism [25]. There is a biphasic elimination curve with a rapidly declining initial phase ($t_{1/2} = 10$ minutes) and a subsequent, slower phase which indicates uptake in organs [5]. The absence of a relationship between anticoagulant half-life and concentration half-life may reflect factors such as protein binding of heparin [5].

Heparin Mechanism of Action:

The mechanism of anticoagulation of heparin is dependent on the natural endogenous cofactor called antithrombin III, which is a glycoprotein of molecular weight 60 kD found normally in the plasma [25]. Antithrombin III functions as a suicidal inhibitor for proteases due to the presence of an Arg-Ser-peptide in its active site which, upon complexing with Ser-proteases, generates complexes unable to dissociate easily [25]. Unfractionated, or normal molecular weight, heparin chains which contain more than 18 saccharide residues near the antithrombin (AT) domain (Fig.1) possess the ability to bind to antithrombin III and thrombin simultaneously, acting as an adsorption catalyst and increasing the inactivation of the thrombin up to one thousand fold [25]. The sites of heparin inhibition of the coagulation cascade are summarized in Fig. 2.

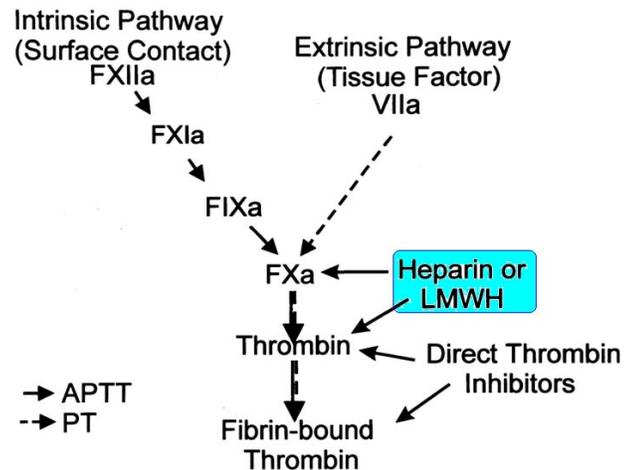


Figure 2. Heparin inhibition of coagulation cascade from extrinsic and intrinsic sources [25].

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OSCS Background:

In order to identify the contaminant, the investigative team relied heavily on proton NMR and capillary electrophoresis, and confirmed it to be OSCS in early March 2008 [9]. Chondroitin sulfate is a natural substance extracted from animal cartilage that can be used in supplements to treat arthritic joints; oversulfation, however, arises from processing of the substance, and so it was concluded that the contaminant had been added intentionally [2]. The structure can be seen in figure 3.

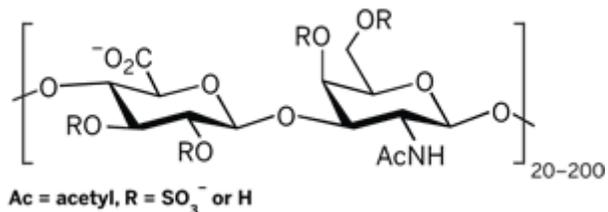


Figure 3. Structure of OSCS from [17].

OSCS Mechanism of Toxicity:

The precise biochemistry behind the observed allergic reactions is still being investigated, but what is known currently is that OSCS binds tightly to antithrombin III, but, in contrast to heparin, it does not induce antithrombin III to undergo the conformational change required for its inactivation of thrombin and factor Xa [9].

Discussion:

The administration of contaminated heparin resulted in over 200 deaths worldwide – half of which were here in the US – due to hypotension triggered by anaphylactic shock [9]. In response to this crisis, the FDA has taken two important steps. The first has been to issue import alerts to stop shipments of tainted heparin from suspect Chinese suppliers. So far, 22 companies have been added to the list as potential sources of contaminated drug [10]. The FDA's second step entailed requesting that the U.S. Pharmacopeial Convention (USP) revise the heparin monograph, which in 2009 was updated to include two new techniques for the analysis of heparin purity: proton NMR spectroscopy and capillary electrophoresis, both of which had been used in the identification of the contaminant during the investigation in 2008 [2]. These joined the previously mandated techniques of chromatographic identification via HPLC, and assays for organic and inorganic impurities [11].

These measures succeeded in reducing the number of adverse reactions to background levels, but also had the unintended consequence of causing a heparin shortage which continues to harm care in the U.S. today [12, 13]. The combination of limited suppliers and added lengthy quality control tests has strained the manufacturing and delivery process and caused major suppliers of heparin such as Baxter, B. Braun, and Hospira to struggle to meet demands [12].

The current state of heparin production also suffers from the lack of a clean, well-regulated process. In China, the pig intestines are often cooked in unregulated family workshops that lack sanitary production conditions and are not certified (see figures 5 and 6 in the appendix for snapshots of typical raw heparin family workshops) [15,19]. Large slaughterhouses, however, are not necessarily cleaner; for instance, a local Chinese newspaper reported that a sausage factory that was making heparin on the side had dirty water on the floor populated by ten-centimeter-long worms [20]. These factories are not regularly inspected, and only poorly documented records are kept about where the pigs processed originated from [20]. This makes tracing the source of contamination tremendously difficult and time consuming, and thus the FDA is still identifying sources of contaminated heparin even four years after the initial outbreak. These serious regulatory gaps stem from the globalization of trading, and were major contributors to the contamination crisis.

In light of these shortcomings, I believe that two consecutive steps must be taken in order to create a more reliable supply of heparin. First, quality control standards must be updated to include robust methods that have been recently developed. These new assays will facilitate more rigorous testing of imported heparin, but they represent only an interim measure in the plan to prevent another heparin crisis. The long term solution lies in the second step: using biological engineering to synthesize heparin and thereby move towards a more reliable, sustainable, and sanitary synthetic source.

The major challenge in the analysis of heparin impurities is the detection of structurally related impurities [9]. Thus the implementation of novel and reliable analytical methods is essential for assessing the purity of unfractionated heparin. Physical methods such as attenuated total reflection-infrared spectroscopy and Raman spectroscopy have shown sensitivity in detecting OSCS when tested against samples from the 2008 outbreak [26]. These techniques allow for the detailed identification of both heparin and OSCS fingerprints, and have been effective against other common contaminants. Additionally, biological assays that focus on testing coagulation time are particularly attractive because they typically yield faster results. In particular, activated partial thromboplastin time (aPTT) and prothrombin time (PT) can be utilized to verify whether heparin is efficiently inhibiting coagulation [9]. These two techniques have the added unique advantage of using human instead of sheep plasma (which is more common of current methods), and this improves the sensitivity and reliability of the assays. Two additional purity assays have recently been developed in which incubation of heparin samples with heparinase I followed by either a fluorescence measurement or chromatogenic factor Xa-assay adds insights into whether the heparin samples under evaluation possess the characteristic $\alpha(1,4)$ glycosidic linkages of heparin which heparinase I selectively cleaves [26].

Future work in the field of quality assessment is aiming towards developing quick and cheap thin-layer chromatography (TLC) methods [9]. Thus far, researchers have succeeded in applying TLC “to determine the size and purity of GAG-derived oligosaccharides, to analyze the activities of polysaccharide lyases acting on GAGs, and to monitor the preparation of GAG-derived oligosaccharides.” [9] Currently, the method is only capable of separating oligosaccharides with low net charges and work must be done to accommodate heparins, which have the highest negative charge density of any known biological molecule [27]. Nonetheless, this new assay has the potential to improve costs and accelerate screening for contaminants such as OSCS.

Man-made contaminants aside, there are a plethora of risks inherent in animal-derived drugs. According to Beni et al, “viruses, bacterial endotoxins, transmissible spongiform encephalopathy agents, lipids, proteins, and DNA,” are all within the range of potential biological contaminants that plague animal-based pharmaceuticals [9]. Moreover, the preparation of pharmaceutical-grade heparin as described earlier introduces additional impurities from solvents, heavy metals and extraneous cations [9]. The methods included in the USP monograph for heparin help minimize these contaminants, but carrying out the necessary assays is a tremendously time consuming process that does not eliminate all safety concerns. For example, the U.S. and Europe do not allow for the import of bovine-based heparin due to fears of transmitting mad cow disease [20]. Ultimately, the tougher regulations resulting from the OSCS crisis have led to drug shortages which have caused a 10-fold price increase in the cost of the drug [15]. Thus, in light of the serious shortcomings of current heparin manufacturing and quality control, and given the present sophistication of bioengineering, I believe that research

into synthetic alternatives has the potential to provide a robust, reliable, cost-effective, and safer source of heparin for the future.

The major challenge to creating synthetic heparin is the complexity of its structure; its polydisperse, microheterogeneous structure is difficult to mimic. In fact, the currently marketed synthetic heparin pentasaccharide, fondaparinux, has had stifled success in part for this reason; its rapid urinary excretion precludes its use in dialysis patients, and it is only effective against deep vein thrombosis [18]. The solution, I believe, is in a chemo-enzymatic approach based on the use of a host organism which naturally synthesizes necessary intermediates for heparin that can be further chemically altered to create the fully sulfonated heparin.

While bacteria are both easy and inexpensive to engineer and grow, they lack a Golgi apparatus which is the major site of carbohydrate synthesis, most notably GAGs [21]. Thus they are incapable of making all but the simplest glycoproteins, and do not represent a viable option. Yeast, on the other hand, are eukaryotes, and therefore have a Golgi. However, they do not naturally produce GAGs and are missing the necessary ability to introduce sulfates into polysaccharides [27]. Cell culture is another common option for biosynthesis, and appears attractive since heparin is biosynthesized in mast cells [28]. However, mast cells cannot be grown in culture and even when converted to mastocytoma cell lines, they grow very poorly and thus are not good candidates for metabolic engineering [21]. Ultimately, attention has been turned to Chinese hamster ovary (CHO) cells which have all the appropriate structures and pathways to make necessary intermediates, and are already in use for the commercial production of other glycoprotein drugs [25]. Thus, I believe that the future of safe heparin production lies in glycoengineering, which presents a viable alternative for producing one of the most critical anticoagulants in a safe and reliable manner.

Conclusion:

Unfortunately, only hindsight is 20/20: while it is easy to develop assays to detect a known contaminant *following* a crisis, it is nearly impossible to implement quality control measures stringent enough to find all possibly harmful foreign substances. Indeed, the heparin crisis illustrates a critical point: safety monitoring of animal-derived drugs is necessarily reactive, and thus it is vital that we pursue a bioengineered heparin in order to prevent many of the inherent disadvantages of animal-base therapies and prevent patient endangerment and death. The heparin crisis of 2007 demonstrates that regulatory agencies are hard-pressed to oversee the safety of every medicine in a globalized marketplace where supply chains are long and opaque, often involving many manufacturers in many locations [18]. In this age of multinational medicines, it is essential that we invest more funding in bioengineering to create safer heparin treatments. While heparin itself is inexpensive, its human cost was far too high.

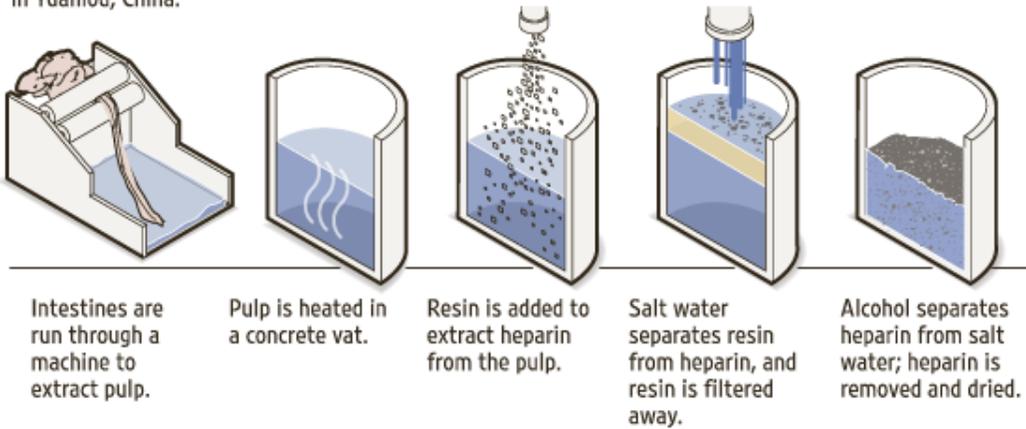
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Appendix:

The Birth of a Medicine

Heparin, a type of sugar that can help prevent blood clots, has its origins in slaughterhouses and sausage-casing factories in China. How the process unfolds at the Yuan Intestine and Casing Factory in Yuanlou, China:



Note: Illustration is schematic.

Source: Yuan Intestine and Casing Factory

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Figure 4. From [20].



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Figure 5 A family-owned crude heparin manufacturing facility. Image from [20].



Figure 6 The owner of a raw heparin manufacturing facility sorts through pig intestines. Image from [22].

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