Chapter 11
Heparin Contamination and Issues Related to Raw Materials and Controls

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Abstract Heparin is a century-old polypharmacological drug critical for the practice of modern medicine. In recent years, there have been a number of issues arising in the preparation of heparin from food animal tissues. The most severe problem was the adulteration of porcine intestinal heparin with a toxic semisynthetic look-alike polysaccharide, oversulfated chondroitin sulfate, which resulted in a number of patient deaths. Since this crisis, regulatory and analytical control of heparin has been markedly improved; new challenges in securing the heparin supply chain have prompted the reintroduction of heparins from new animal sources. In future, the introduction of bioengineered heparins might offer better approaches for securing this critical drug.

Keywords Heparin · Regulatory framework · Quality control · Animal sources · Bioengineered heparin

11.1 Introduction

Heparin is probably the most widely used natural product in medicine. A polysaccharide derived from food animal tissues, heparin is an essential drug for the practice of modern medicine. The discovery of heparin in 1916, a hundred years ago this year, is credited to Jay McClean, a medical student at Johns Hopkins University, working with the physiologist William Howell [1]. While searching for substances in dog liver that caused blood to clot, McClean isolated a new substance that instead prevented blood coagulation. Howell recognizing the importance of McClean's discovery set

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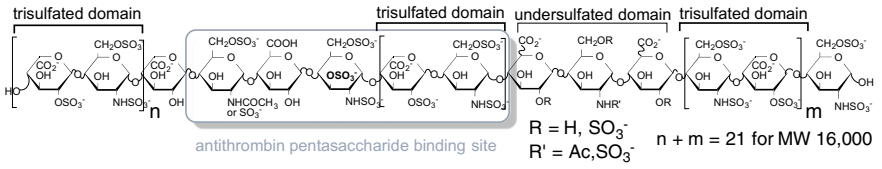

Fig. 11.1 Generalized structure of a porcine intestinal heparin chain

out to understand how this substance might be used to treat coagulation disorders [2]. Originally misidentified as a heparophosphatide, by 1928, heparin was determined to be a sulfated polysaccharide containing an uronic acid and by 1936 heparin's second saccharide unit was identified as glucosamine. In 1962, glucuronic acid was established to be present in heparin and by 1968, iduronic acid was identified using nuclear magnetic resonance (NMR) spectroscopy as the major uronic acid residue in heparin, establishing heparin's structure as a linear sulfated polysaccharide with a major repeating unit of →4) α-L-iduronic acid (IdoA) (1→4) β-D-glucosamine (GlcN) (1→. While the structural studies on heparin advanced so did its commercialization as a pharmaceutical. In Toronto, at Connaught Laboratories, heparin was produced in large scale from bovine lung tissue and a purified heparin was successfully used by 1935 as an anticoagulant drug postoperatively in surgery patients. By the time the US Food and Drug Administration (FDA) was established in 1937, heparin was already in widespread use and its approval was grandfathered in without further evaluation.

Heparin (Fig. 11.1) is a mixture of highly sulfated linear polysaccharides and is a member of family of related anionic polysaccharides called glycosaminoglycans (GAGs). GAG heparin is polydisperse (weight average molecular weight (M_W)/average molecular weight (M_N) > 1) having an M_W of 16,000 with polysaccharide chains as small as M_W of 2000 and as large as M_W of 50,000 [3]. While heparin has a major trisulfated disaccharide repeating unit, →4) α-L-IdoA2S (1→4) β-D-GlcNS6S (1→ (where S is sulfo), generally corresponding to >50% of its sequence [4], the remaining <50% of its sequence can also have significant structural heterogeneity. Uronic acid residues can include α-L-IdoA2S, unsulfated α-L-IdoA, β-D-GlcA, and in rare instances β-D-GlcA2S [5]. Glucosamine residues can also include β-D-GlcN, β-D-GlcNAc (where Ac is acetyl), β-D-GlcNS, β-D-GlcN6S, β-D-GlcNAc6S, β-D-GlcNS6S, β-D-GlcN3S, β-D-GlcNAc3S, β-D-GlcNS3S, β-D-GlcN3S6S, β-D-GlcNAc3S6S, and β-D-GlcNS3S6S. There are also very minor structures within GAG heparin that might or might not represent structural artifacts resulting from its processing, such as drug oxidation, acetylation, and epimerization [6–8].

While sulfated GAGs, such as heparan sulfate (HS), chondroitin and dermatan sulfates, and keratan sulfates are biosynthesized in the endoplasmic reticulum and Golgi of all animal cells, heparin is biosynthesized only in granulated cells, such as mast cells [9]. Heparin is biosynthesized as a proteoglycan (PG) in mast cells, linked at multiple sites to its core protein called serglycin. Heparin biosynthesis (Fig. 11.2) involves glycosyltransferases acting in concert to initiate chain growth on the core

protein through a →β-D-GlcA (1→3) β-D-galactose (Gal) (1→3) β-D-Gal (1→4) β-D-xylose (Xyl) (1→*O*-serine (Ser) tetrasaccharide linkage region and to extend the →4) β-D-GlcA (1→4) β-D-GlcNAc (1→ heparosan polysaccharide chain, and *N*-decetylase/*N*-sulfotransferase isoforms that modify selected GlcNAc residues giving rise to GlcN and GlcNS residues and a C5-epimerase acting at selected GlcA residues giving rise to IdoA residues, and finally 2-*O*-, 6-*O*- and 3-*O*-sulfotransferases giving rise to a large number of different sulfation patterns found within the heparin chains [10]. Heparin is believed to be further processed, through the action of proteases and β-endoglucuronidase (heparanase) as it is packed into the mast granules, to afford chains consisting of both peptidoglycosaminoglycan and GAG chains.

The heparin isolated from mast-cell-rich tissues, such a liver, lung or intestine, is called crude heparin or raw heparin (Fig. 11.3). Raw heparin is isolated by grinding the tissue, salting out, proteolysis, and anion-exchange resin capture and has similar molecular weight properties as pharmaceutical heparins [11]. The animal tissues used to prepare heparin comes from slaughterhouses that are not current good manufacturing process (cGMP)-compliant. Raw heparin, prepared from the intestinal or lung of food animals including cow, sheep, and pigs, has been used to manufacture pharmaceutical heparin [11]. In China (and possibly other countries), before 2008, this raw heparin was recovered from intestinal mucosa in non-cGMP-compliant workshops and then consolidated before being shipped to a cGMP-compliant pharmaceutical manufacturing facility. At these manufacturing facilities raw heparin is bleached to remove protein, subjected to cation exchange to replace unwanted metals with sodium, alcohol precipitated to enrich and to remove nucleotidic impurities, desalted, dried, and analyzed to afford heparin active pharmaceutical ingredient (API).

The biological and pharmacological activities of heparin have been extensively studied [12–14]. Heparin is primarily known for its anticoagulant activity and primarily acts on the soluble coagulation cascade (Fig. 11.4) through the inhibition of both thrombin generation and the action of thrombin on the soluble blood protein fibrinogen to for an insoluble fibrin clot [15]. Heparin can act to inhibit the coagulation factor Xa (FXa), a serine protease, which converts prothrombin (FII) to thrombin (FIIa), thus inhibiting thrombin generation. Heparin can also inhibit the serine protease FIIa. The inhibition of both FXa and FIIa results from heparin binding to antithrombin III (AT) a weak serine protease inhibitor (Serpin). Heparin binds AT through a small number of pentasaccharide sequences each having a critical 3-*O*-sulfo group, called heparin's AT binding site β-D-GlcNS/Ac6S/6OH (1→4) β-D-GlcA (1→4) β-D-GlcNS**3S**6S/6OH (1→4) α-L-IdoA2S (1→4) β-D-GlcNS6S [12]. Heparin also exerts anticoagulant activity through AT-independent mechanisms [16] in the soluble coagulation cascade through heparin cofactor II and other proteins, in platelets and at the endothelium. In addition to heparins anticoagulant activity, there are many other biological and pharmacological activities associated with heparin [12, 17, 18]. Heparin's major biological role is believed to be its anti-infective and antiparasitic activities closely related to the mast-cell-rich tissues in which heparin is found [12, 19]. Pharmacologically, heparin is known to exhibit antimetastatic and other beneficial activities [18, 20].

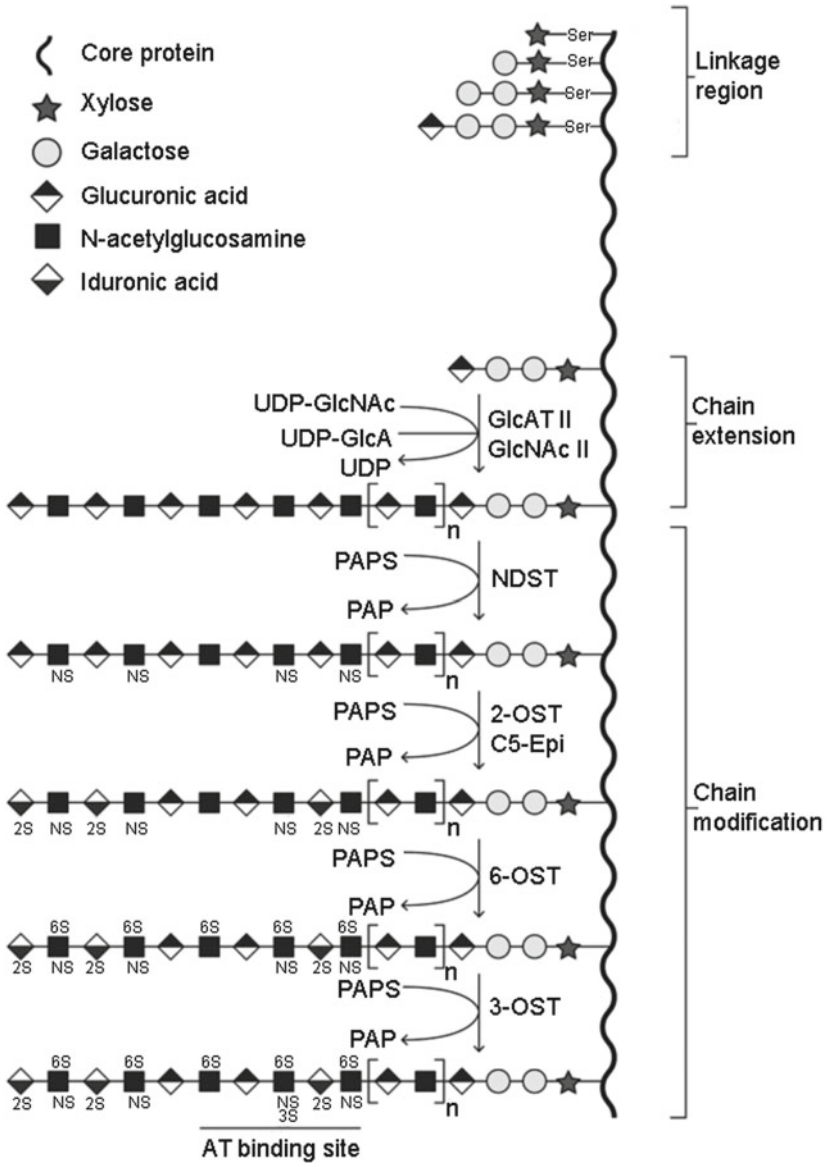

Fig. 11.2 Biosynthesis of heparin. Synthesis begins with the stepwise addition of four monosaccharides (Xyl, Gal, Gal, and GlcA) to a serine residue of the serglycin core protein. Chain extension occurs through the alternate addition of GlcNAc and GlcA. The chain is modified through the action of epimerase and sulfotransferase enzymes to produce a variable structure with and antithrombin binding sites

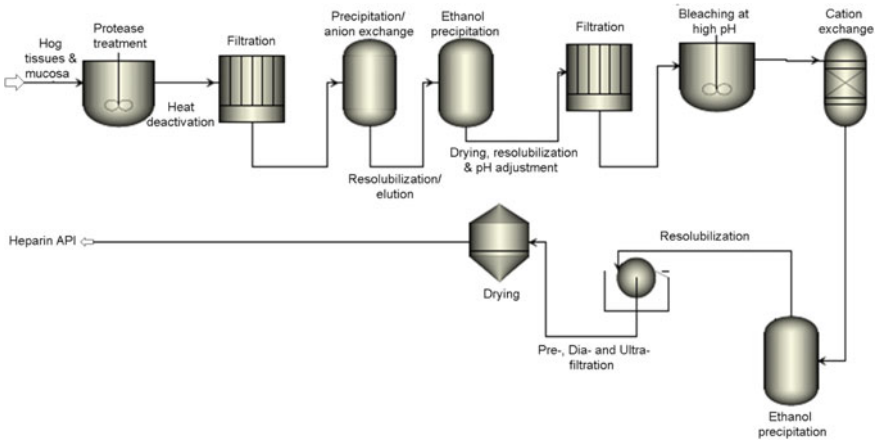

Fig. 11.3 Process flow diagram for production of heparin

11.2 Heparin's Application as a Therapeutic

Since its introduction as a pharmaceutical for the postoperative anticoagulation of surgery patients in 1935, the applications for heparin and the types of heparin have dramatically increased [12]. The advent of modern medicine now includes the use of heparin in extracorporeal therapy, such as kidney dialysis and heart lung oxygenation. Heparin is used to treat deep vein thrombosis and is widely used in the coating of indwelling medical devices such as catheters and stents. Today, the total annual worldwide heparin market is ~$8 billion corresponding to 52% of the anticoagulant market [15] and ~100 ton of API.

There are several different types of heparin products [15, 12]. Unfractionated heparin, derived primarily from porcine intestinal mucosa, is polydisperse and has an average molecular weight of 16,000, and it is generally administered *intravenously* requiring careful monitoring and hospital administration. Heparin shows similar activity against thrombin generation (anti-FXa activity) and thrombin inhibitory activity (anti-FIIa activity). It is protamine reversible is cleared through both the liver and kidney, so that it can be administered to renal impaired patients, and it is not lost in renal dialysis [21]. Low-molecular heparins (LMWHs) are prepared from unfractionated heparin through its controlled chemical or enzymatic depolymerization and are used primarily for treating deep vein thrombosis (DVT). LMHs are also polydisperse and have an average molecular weight of 4000–8000 and selectively show anti-Xa activity with weak anti-IIa activity. In many ways, these LMWHs can be more structurally complex than heparin as they often contain process artifacts arising from the chemical or enzymatic depolymerization reactions used in their preparation [22]. These process artifacts represent chemical characteristics of each type of LMWH (LMWHs prepared using different processes are considered different chemical entities and each require separate new drug approval and separate

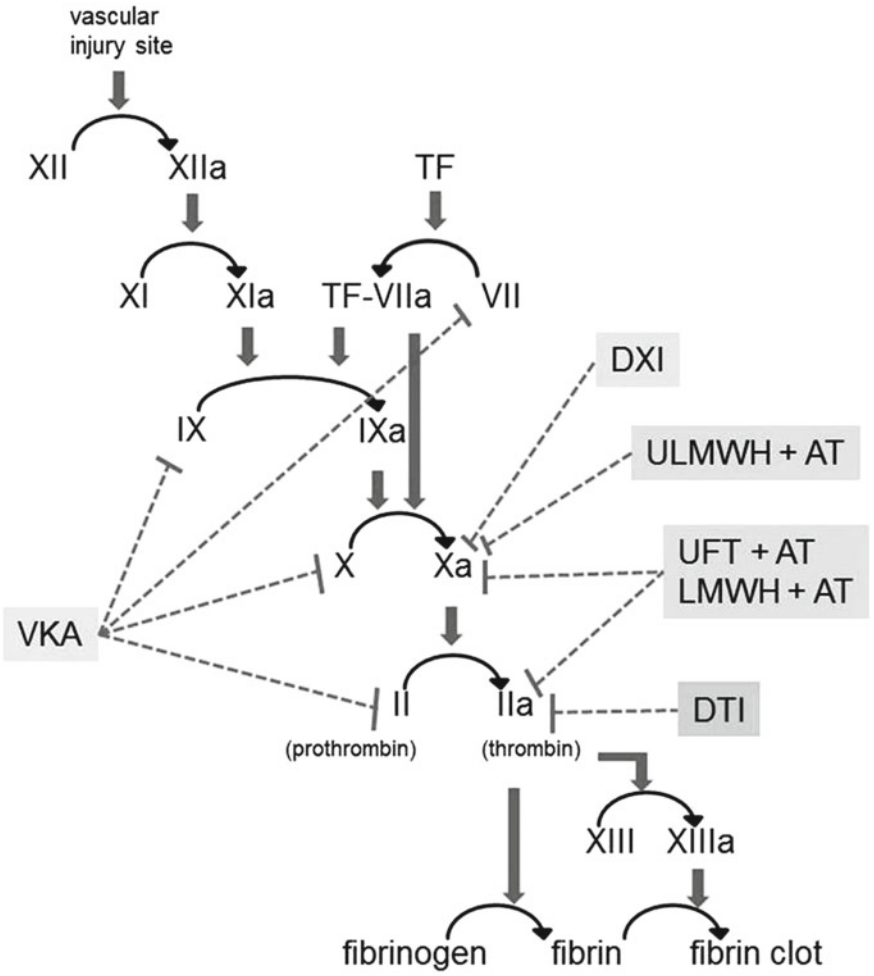

Fig. 11.4 Coagulation cascade. Main reactions of the coagulation cascade after activation through vascular injury or by tissue factor (TF) and major clinical anticoagulants including direct factor Xa inhibitor (DXI), direct thrombin inhibitor, and vitamin K antagonists (VKAs)

monographs) and used in their analytical identification. They are *subcutaneously* bioavailable and do not require monitoring allowing their home use and show partial protamine reversibility [21]. Currently, there is only one ultra-low-molecular weight heparin (ULMWH), a synthetic pentasaccharide called Arixtra® (fondaparinux). This chemically synthesized product is a monodisperse pure chemical entity that is *subcutaneously* bioavailable and is not protamine reversible. Since it is expensive and only shows renal clearance, it has limited clinical applications [21]. LMWH represents ~70%, unfractionated heparin ~25% and ULMWH ~5% of today's US heparin anticoagulant market [15].

11.3 The Heparin Contamination Crisis

Approximately 1.2 billion hogs are slaughtered for food annually worldwide [4]. The intestinal mucosa of most of these hogs are recovered and processed into the 100 ton of heparin used each year. Typically, one hog affords three doses of heparin or a single dose of LMWH. Nearly 60% of the hogs slaughtered each year come from China with 20% coming from the European Union, 14% coming from the USA, and 6% from the rest of the world.

In November 2007 through January 2008, a number of severe anaphylactoid reactions in the USA, resulting in severe hypotension and in some cases death, were associated with the *intravenous* administration of heparin sold by Baxter Healthcare [23]. This suspect heparin was processed by Scientific Protein Laboratories (SPL) from raw heparin originating in China. By mid-January 2008, Baxter voluntarily recalled nine lots of tainted heparin. After ruling out microbial contamination of heparin, attention focused on chemical impurities. A group of scientists from the US FDA, the pharmaceutical industry, and academic laboratories immediately opened an investigation into the cause of these severe reactions. Careful examination of the problem batches of heparin using capillary electrophoresis (CE) and NMR revealed the presence of an impurity. By March 2008, the FDA released NMR and CE methods for screening heparin and LMWH API for the presence of impurity. Approximately, half of the heparin sold in the USA and half of the LMWH sold in Europe was found to contain this impurity and was quarantined. The carryover of OSCS by the process used to make LMWH was also elucidated [24]. By April 2008, the structure of this impurity was identified as an oversulfated chondroitin sulfate (OSCS) [25]. OSCS, an inexpensive semisynthetic GAG, was not an impurity but rather a contaminant that was ultimately viewed as an adulterant that had been illicitly added to heparin, presumably for financial motives. Subsequent animal studies demonstrated that OSCS caused hypotension when administered to pigs [26] and biochemical studies suggested that this was the result of cross-talk between the coagulation, kinin–kallikrein, and complement cascades (Fig. 11.5) resulting in the factor XIIa-based enhancement of vasoactive bradykinin production [27].

At the height of the heparin contamination crisis, there were concerns that such a large part of the worlds' heparin supply had been adulterated. The withdrawal of all heparin from the marketplace could reduce or eliminate many modern medical practices including hemodialysis, blood oxygenation required in open heart, many major surgical procedure requiring heparin, the production and use of heparin-coated medical devices, and the treatment of DVTs. There were brief concerns that the Surgeon General might need to take control of the US heparin supply, preventing its export and rationing its use, to ensure the safety of the American people. Fortunately, in the USA, there were sufficient quantities of uncontaminated heparin to supply modern medicine until the withdrawn supplies of adulterated heparin could be replaced. Despite having an adequate supply of this life-saving drug in the USA, many elective surgical procedure had to be canceled in 2008 to conserve these short supplies. Indeed, in Europe where most of the LMWH had been contaminated some patients

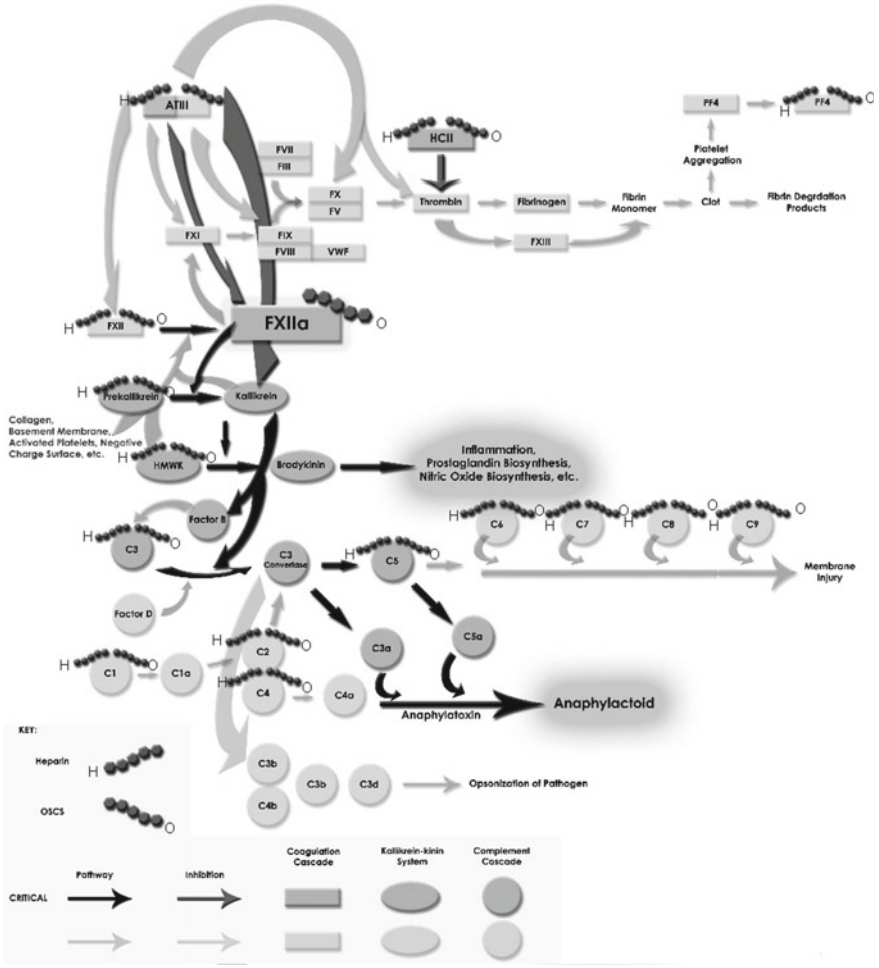

Fig. 11.5 Composite figure of the coagulation cascade (rectangles), the kallikrein–kinin system (ovals), and the complement cascade (circles). The points where heparin (binding on left) and OSCS (binding on right) show interactions in these pathways are shown

were given LMWH that was known to contain OSCS because of an insufficient supply of safe, uncontaminated drug.

11.4 Response to the Heparin Contamination Crisis

The immediate response to the heparin crisis in 2007–2008 was an international one led by the US FDA and involved the US Centers for Disease Control (CDC), simi-

lar international agencies, the pharmaceutical industry, and academic scientists and physicians having a deep understanding of heparin chemistry, biology, and pharmacology [23, 25]. The CDC first associated unexpected deaths with certain batches of heparin marketed by Baxter. Testing by Baxter of sterility quickly ruled out microbial contamination of these batches. Initial theories on the association of suspect batches of porcine intestinal heparin with anaphylactyoid-like reactions and hypotension included the possible presence of porcine virus or protein, a mistake in the processing of raw heparin leading to the introduction of process impurities, or a mistake in processing leading to the inadvertent introduction of a contaminant. It should be noted that an impurity is a substance that can be present in a drug product (from starting material or from the manufacturing process) but at only very low levels and a contaminant is a substance that should never be present in a drug product and can be inadvertently introduced through an error or purposely (criminally) introduced as an adulterant. When detailed analysis by NMR, CE, and other methods suggested the presence of a chemical substance in heparin that did not belong in this drug, the FDA released guidelines, and the pharmaceutical industry adopted these, to test heparin already in the USA and to quarantine heparin entering the USA until such testing was performed. The elucidation of the structure of the unnatural OSCS [25] and no other unnatural substances [28, 29] and the discovery of its hypotensive effect in biochemical and animal studies [27, 26] confirmed that the suspect lots of Baxter heparin were adulterated with the contaminant OSCS.

The secondary response after the immediate crisis had been resolved, included investigative (law enforcement and congressional), pharmacopeial (i.e., USP), analytical investigations from academia and industry, and regulatory (FDA) (Table 11.1). Investigation by the US Federal Government and suggested that the introduction of the inexpensive unnatural OSCS into batches of raw heparin was a deliberate act of adulteration motivated by financial gain. To date, no one has been charged with a crime in this case. Compendial methods for the bioanalysis of heparin were already underway in 2007 with the goal of updating of global (less specific) clotting-based assays with more specific, biochemically defined amidolytic assays [30]. Post-crisis a decision was made at the USP in collaboration the Pharmacopeia of other nations to reevaluate and strengthen the entire heparin monograph [31]. Analytical investigations were intensified to develop orthogonal methods [32] to secure the safety of complex drug products like heparin and LMWHs. The regulatory response of the US FDA has made a comprehensive response to the 2008 heparin crisis taking a much more active role in the post-approval review of complex drug products. The control of heparin raw materials has been enhanced, the limits for additional process impurities further tightened, and heparin's polydispersity has been tightened. The FDA has enhanced its research program for discovering new methods to monitor heparin products and this research team now advises the agency how to respond to quality issues of heparin products entering the supply chain. The FDA requires manufacturers to provide analytical data for every batch of heparin entering the US market and reviews these data before accepting shipments. The FDA has increased the number of random tests on heparin and has taken on the mission to integrate review, inspection, surveillance, and research across a drug product's life cycle. Since 2008, the

Table 11.1 Lessons learned and solutions proposed

Develop an improved understanding of heparin and glycosaminoglycan biology/pharmacology
Update analytical and QC methods
Enhance Pharmacopeial/compendial methods
Control supply chain
Diversify suppliers
Re-consider process equivalence in developing generics
Consider non-animal sourcing of heparin

FDA has established offices in Asia and elsewhere and increased collaboration with foreign regulatory agencies in inspecting manufacture sites and production records. In 2015, the US Congress passed the Drug Supply Chain Security Act requiring an electronic identifier be present on the package of prescription drugs sold in the USA, facilitating traceability in the drug supply chain [31].

11.5 Control of Heparin Production

The increased control on heparin production takes several forms. There is now additional regulatory control that allows traceability between drug product (API batch) and the herd of animals (farms an slaughterhouse) the API is derived from. The consolidator step, in which batches of raw heparin coming from workshops, is also now more highly regulated. Much of this control is in the form of record keeping and facility inspections. The compendial control of pharmaceutical heparin API has also been enhanced, and the current USP heparin monograph is considered one of the most up-to-date and elaborate drug monograph utilizing orthogonal methods for determining drug identity and maintaining drug quality. Furthermore, the use of modern technological methods such as ^{1}H-NMR for the characterization of drug purity is new to drug monographs. Additional analytical methods, including multi-dimensional NMR, disaccharide and oligosaccharide analysis, liquid chromatography-mass spectrometry, Fourier transform infrared spectroscopy, and principal component analysis, are now being applied by academia, industry, and regulatory agencies, providing continual surveillance of raw heparins, heparin API, heparin products, and LMWHs. Techniques like polymerase chain reaction (PCR) [33], NMR [34, 35], and stable isotope analysis [36] are being used to monitor the species (porcine, bovine, ovine, etc.,) source of raw heparins. The variability of porcine intestinal heparins and various low-molecular weight heparin innovator products and generic counterparts, while in the past rigorously examined by the FDA, are now being rigorously examined in the open scientific literature [37–40].

11.6 New Animal Sources for Heparin

In July 2015, the US FDA held and open meeting examining the reintroduction of bovine heparin on the US market. The rationale for this meeting was to improve the security of the heparin supply [41]. First, heparin used in the USA comes from a single species and any disease impacting that species might jeopardize the stable supply of this medically critical drug. Second, over half of the heparin used in the USA comes from China and any disruption of trade between the USA and China could result in a serious crisis. Third, with the advance of medicine in second and third world countries, the demand for heparin may soon exceed the supply from pigs as nearly all the pig intestine produced worldwide is currently going toward the production of heparin. Fourth, there are religious groups in which the use of pig-derived products are either forbidden or discouraged. The current European Pharmacopeia requires that heparin come from the intestine of food animals such as pig, cow, and sheep, and all three sources have been used in the past to prepare heparin API in various countries. The FDA focused on the bovine lung heparin as this product was marketed in the USA with a good safety record until the 1990s when it was voluntarily withdraw out of concerns about an outbreak of bovine spongiform encephalopathy (BSE or 'mad cow disease) in Europe. In response to the urging of the FDA, a USP panel was constituted to begin to draft a monograph for bovine heparin.

There are several issues to be resolved regarding the introduction of heparins from other animal sources into the US market. Bovine heparin is not identical to porcine heparin and has different structural, biological, and pharmacological properties. One fundamental concern is that bovine heparin has considerably lower anticoagulant activity as measured by the current anti-FIIa and anti-FXa assays than porcine intestinal heparin. While higher gravimetric amounts of bovine heparin might be given to get the same units/kg body weight, heparin neutralization by protamine (a heparin antagonist or antidote), which is based on gravimetric dose would be different, potentially leading to confusion among clinicians. Moreover, bovine lung heparin, which had been previously used in the USA, appears not to be commercially viable and instead bovine intestinal heparin, which never has been used in the USA, appears the economically best choice. While any bovine heparin carries the additional risk of BSE, precautions must be taken to source the raw bovine heparin from countries with negligible incidence of BSE or to use closed herds for its production. Finally, the reintroduction of heparin from a new species lead to the potential blending of heparins from two species, violating labeling laws, complicating traceability, and compromising the integrity of both porcine and bovine heparins. Indeed, while it is possible to use sophisticated methods to detect minor levels of blending 90% from one source with 10% from another, using routine analytical methods, it is difficult to detect even 75:25 or 50:50 blending of pure API from different species. The potential introduction of ovine mucosal heparin or porcine lung heparin into the US market further confounds such analysis as blending of heparin API from multiple organs and species would be very difficult to detect analytically and could only rely regulatory and record keeping detection.

11.7 Improved Analytical and Regulatory Framework

The quality and robustness of analytical methods and regulatory framework have improved remarkably since 2007–2008. Today, inspections of foreign manufacturing partners and increased record keeping requirements make it difficult to imagine another crisis resulting from the adulteration of a heparin product. Changes to the Pharmacopeia utilize orthogonal assays to ensure that all API has a very low level of impurities and no contaminants. Moreover, even more sophisticated analytical techniques are being developed that look more deeply into the composition and structure of heparins and LMWHs. The introduction of heparins from new animal tissue sources will provide new challenges to the FDA, USP, pharmaceutical industry, academic scientists, and clinicians. Among these challenges include: ensuring that a bottle contains what it says on the label; maintaining the potency and batch-to-batch variability of each heparin product; understanding the structural differences between products and working from multiple and often very complex monographs; and finally understanding the pharmacological and clinical differences between different heparin products. For example, in the case of monograph differences, it is still unclear whether or not there will be a need for a reference standard (RS) for each type of heparin on the market (i.e., porcine intestinal heparin RS, bovine lung heparin RS, bovine intestinal heparin RS, ovine intestinal heparin RS, etc.,) greatly complicating the task of clinical laboratories. In another example, if the potency of different heparins (measured as units/mg) is very different, will it become difficult for the clinician to adjust the dosage of protamine antidote following procedures involving heparin leading to failure to reverse or protamine overdosing. Finally, clinicians are very conservative, and rightly so, in their practice of medicine particularly in the use of a drug like heparin that can cause bleeding. The price of a dose of porcine intestinal heparin is quite low, and it appears that bovine intestinal heparin might, at best, be identical in cost. What motivation would a physician have to adopt a different drug of nearly equal cost without any additional benefits to their patients. This might be the biggest challenge in introducing heparins from new animal sources.

11.8 The Future of Heparin and Heparin-Based Products

Heparin was discovered 100 years ago, and its use predates the establishment of the US FDA. Thus, it is important to ask how long this old drug will remain in routine use. The answer from virtually all heparin scientists, clinicians, and the pharmaceutical industry and regulatory bodies is that heparin will be routinely used throughout the foreseeable future. Despite the recent introduction of specific and orally active FXa inhibitors and anticoagulants acting on platelets, heparin is still the only drug used in hemodialysis and open-heart surgery and remains the drug of choice in postoperative surgical patients and in treating DVTs [15]. Heparin and LMWH currently commands over half of the anticoagulant market and will probably

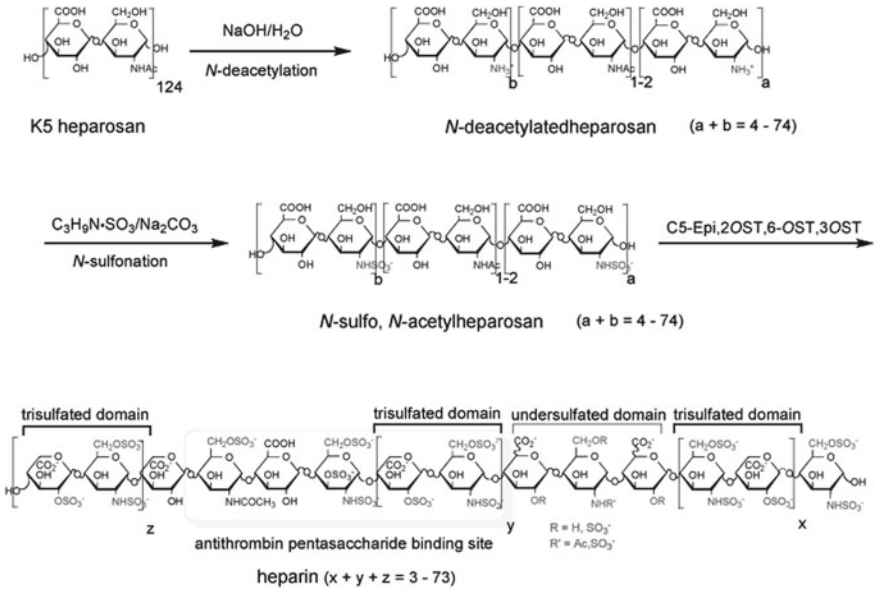

Fig. 11.6 Method for the preparation of a non-animal-sourced bioengineered heparin. Heparosan is produced by fermentation and is chemically de-*N*-acetylated and *N*-sulfonated, and its molecular weight is trimmed. Enzymatic modification involves the use of C5-epimerase (C5-pi), 2-*O*-sulfotransferase (2-OST), 6-*O*-sulfotransferase (6-OST), and 3-*O*-sulfotransferase (3-OST)

remain in this dominant position for years to come. Part of the success of this drug is its polypharmacological nature [42]. In addition to regulating coagulation, it has favorable anti-inflammatory activity, anti-infective properties, and even has favorable effects on wound healing [19, 43, 44]. Selective homogeneous agents often just do not have this wide array of favorable off-target effects.

The application of modern biotechnological approaches to the preparation of heparin and LMWHs offers one of the best opportunities to improve quality, safety, and availability without sacrificing the benefits of these critical polypharmacological agents. The preparation of non-animal-sourced bioengineered heparins was first described a decade ago [45, 46]. Today, the heparosan backbone →4) β-D-GlcA (1→4) β-D-GlcNAc (1→ can be chemoenzymatically converted to mixture of heparin chains having sequences, molecular weight properties and anticoagulant activity comparable to porcine intestinal heparin (Fig. 11.6) [4]. The major challenges that remain in making bioengineered heparin a viable replacement for animal-sourced heparins are the ability to scale these processes, to produce a cost-competitive product and to secure regulatory approval. Ultimately, it might even be possible to use metabolic engineering to prepare heparin [14, 47]. The challenges here are even greater since heparin is only biosynthesized by animal cells [10], and the fermentation of animal cells can only be used to prepare kilogram quantities of high-value pharmaceuticals, like recombinant glycoprotein drugs [48]. Microbial cells can be

used to prepare low-cost and high-volume products, but the metabolic engineering of the entire heparin biosynthetic pathway is daunting.

11.9 Conclusions

In summary, heparin is a 100-year old polypharmacological drug critical in the practice of modern medicine. In recent years, there have been a number of issues arising in the preparation of heparin from food animal tissues. The most severe problem was the adulteration of porcine intestinal heparin with a toxic semisynthetic look-alike polysaccharide that resulted in a number of patient deaths. Since this crisis regulatory and analytical control of heparin has been markedly improved. New challenges in securing the heparin supply chain have prompted the reintroduction of heparins from new animal sources. In the future the introduction of bioengineered heparins might offer better approaches for securing this critical drug.

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