Contaminants in Heparins Continue to be Unfolded

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September 4, 2008; 1:00 p.m. (EST): Earlier this year several batches of heparins were initially recalled due to the increased prevalence of adverse reactions and deaths. Initially the US FDA recalled specific batches of heparin resulting in a total recall of Baxter’s unfractionated heparin. Since then Baxter has not reintroduced this product despite the fact that it had 50% of the total market for unfractionated heparin in the US. The recall impacted on extensive investigations resulting in inspections and warning letters to different suppliers of heparin. Subsequently, the identification of a heparin-like contaminant resulted in a more widespread global recall of heparin. Because low molecular weight (LMW) heparins are produced from unfractionated heparin, several batches of LMW heparin were also recalled in Europe and Southeast Asia. Subsequently, the main contaminant was identified to be over-sulfated chondroitin sulfate (OSCS). However, the presence of other contaminants was also identified. The contaminants vary in heparins and may be partly due to carry over of the manufacturing process. However, the presence of OSCS in significant amounts (5-30%) was apparently intentional. The US FDA eventually required additional analysis to determine the purity of imported heparins. The US Pharmacopeia (USP) also addressed this issue and is in the process of revising the heparin monograph including additional analytical approaches to assure purity of heparin. Despite this progress, the biological impact of the contaminant has been minimally addressed. Very limited information on the influence of the contaminant’s presence in heparin on its anticoagulant and other pharmacological effects have become available. Since the contaminant represents an unnatural hyper-sulfated glycosaminoglycan, and since it has been used in a large number of patients for a span of over one year, its residual and sustained pharmacotoxic effects should be clearly addressed. Moreover, its immunogenic profile should be clearly understood in terms of the type of antibodies and their biological functions. The two major publications appearing in April, 2008, addressed the basic chemical structure of OSCS and one of its biological actions, i.e., contact activation which is commonly seen with almost all of the sulfated glycosaminoglycans.

In the first published article, “Contaminated Heparin Associated with Adverse Clinical Events and Activation of the Contact System,” Kishimoto et al., New England Journal of Medicine, 2008, 358:2457-67 discussed the role of OSCS in contaminated heparin in reference to adverse clinical events and the activation of the contact system. (1) The authors concluded that this manuscript provided a scientific rationale for the potential biologic link between OSCS and observed clinical events. Moreover, the authors suggested that a simple non-specific amidolytic method for the generation of kallikrein could be used for the screening of OSCS and other highly sulfated polysaccharide components in heparin. This manuscript
implied that contact activation leading to the generation of kallikrein and subsequent formation of bradykinin is responsible for the anaphylactic responses in patient’s anticoagulated with heparin.

This manuscript primarily addressed the hypothesis that the presence of a semi-synthetic contaminant, namely OSCS is primarily responsible for the reported adverse events and deaths from the use of contaminated heparin. However, the reported adverse reactions and deaths from the use of contaminated heparin represent complex pathophysiologic responses with multi-factorial etiologies where OSCS mediated contact activation may or may not have played a role. Some of these recalled products also contained high molecular weight dermatan sulfate and other glycosaminoglycans.

The presence of OSCS and its chemical characterization in some of the batches of recalled heparins was also published about the same time in an article by Guerrini et al. (2) This article only addressed the chemical analysis of the isolated contaminant and did not provide any data on the potential biological effects or the interaction of this contaminant with plasma proteins. Moreover, this article focused on one contaminant isolated from a specific batch of recalled heparin. No information on the molecular weight and other properties of this contaminant was provided. To date there is no follow-up on this article and no information on the molecular heterogeneity of the different types of OSCS, which may be present in other batches of heparins and LMW heparins. The impact of the contaminant in LMW heparins is also not addressed, despite the fact that OSCS has been found in various batches of LMW heparins.

The synthesis and initial biological activities of OSCS was first reported by Murayama, et al. in 1998. (3) This report only discussed the synthesis and some of the anticoagulant activities of the synthetic OSCS. This report clearly pointed to the fact the OSCS can be easily synthesized from chondroitin sulfate obtained from different sources. While OSCS represented one of the main measurable contaminants, the recalled heparin preparations may also have contained several other heparin-like and non-heparin substances, which have not been fully investigated and their association with the reported adverse events has not been established at this time. The contaminated heparins also contained high molecular weight dermatan sulfate which itself may have complex interactions with heparin and OSCS.

The single case report described by the authors on the patient developing hypotension and other adverse reactions during dialysis represent an incidental finding and the authors fail to describe the nature of the anaphylactoid reaction. Moreover, none of the plasma markers and other workups for anaphylaxis was reported. In addition, the authors did not provide information on the amount of contaminant in the heparin preparation to which this patient was exposed. This single case report does not prove cause-effect for OSCS in producing anaphylaxis. More recently, the FDA has asserted that three cases of deaths are linked with heparin preparations containing OSCS. No detail on relevant data is available. In a news article in the Chicago Tribune, Wednesday, July 30, 2008/ Section 3, the FDA said “it completed its review of 93 deaths reports related to heparin that the agency reviewed from Jan. 1st to March 31st, when reports of heparin adverse reactions peaked.” Of the ten cases of
severe anaphylaxis and hypotension, only three of those were reported traced to the lot
numbers of heparins that tested positive for OSCS. The cause for the other seven
deaths was not determined. Of the remaining 83 reports, the causes of deaths were complex
which also included bleeding. The details of the 3 reported deaths, which were linked to OSCS
containing heparin, were not available. It is important that supportive data on the levels of
contact mediated activation products such as bradykinin, anaphylatoxins, and other mediators
should be provided to prove cause and effect. Interestingly, even after the reported
recall of contaminated heparin earlier this year, the number of reported deaths and the
reported deaths with one or more allergic/hypotensive symptoms remained significantly high
(March 2008). Because of the recall of the contaminated heparin, one would have expected a
proportionate decrease during this period of time. Therefore, the recent assertions that 3
deaths associated with contaminated heparins provide proof for definitive cause and effect
maybe premature.

The reported amidolytic activity of generated kallikrein represents a non-specific method
which should have been evaluated in conjunction with pre-kallikrein and high molecular
weight kininogen levels in various systems studied. Moreover, the amount of kallikrein
generated varied widely and the relevance to bradykinin was not established. Heparin itself is
capable of activating the contact system and the extent of this activation varies largely in both
normal individuals and patients. Other heparin related agents are also capable of causing
activation of the contact system, in addition to the complex contribution of anti-hypertensive
drugs and extracorporealal devices, which may up regulate various mediators such as
bradykinin and anaphylatoxins.

Chondroitin sulfate and related derivatives produce complex responses on plasmat and
cellular target sites. Moreover, sulfated chondroitin sulfates exhibit much stronger binding to
plasma proteins and are resistant to endogenous GAG degrading enzymes such as
heparanase. In a recent article, Hamad et al. concluded that platelets are capable of
triggering complement activation in the plasmat phase by releasing endogenous chondroitin
sulfate which may lead to inflammatory signals mediated by anaphylatoxins. These
authors pointed to a different mechanism of the complement activation by chondroitin sulfate
release from activated platelets, eventually leading to the generation of C5b-9. This activation
leads to the generation of C5a which up-regulates CD11b on both polymorphonuclear
leukocytes and monocytes. This proposed mechanism may have a more important
implication on the pathogenesis of OSCS-mediated processes than contact activation. Such
a mechanism may also explain the observed resistance to anticoagulation and thrombosis in
patients treated with contaminated heparin. OSCS itself is capable of activating platelets
which may lead to an increased risk of thrombosis. The findings reported in this publication
points to the complexities of the interactions of chondroitin sulfate and related agents which
not only act at plasmat but also cellular sites involving both blood and endothelial cells.
Therefore, additional studies to address this pathway may be helpful in the understanding of
the pathogenesis of OSCS mediated anaphylactic and hypotensive effects. This study clearly
suggests that the chondroitin sulfates and related products have complex biological
interactions which are not fully understood at this time. The article by Kishimoto et al.
provides an over-simplified approach to a complex and serious problem.

The reported in vivo studies utilizing a pig model represent only a few animals per group with
wide variations. (1) For example, of the six pigs treated with OSCS contaminated heparin,
only two showed a clear drop in blood pressure. It is not surprising that of the three pigs
treated with OSCS a profound drop on blood pressure was observed. This material has never
been tested for these observed effects, which may be related to several additional factors
such as the molecular weight, degree of sulfation and the release of other mediators such as
nitric oxide. Supportive data on the generation of hypotensive mediators in conjunction with
pre-kallikrein and high molecular weight kininogen should have been included. Moreover, the
generation of kallikrein in the pig treated with contaminated heparin and OSCS does not
 correspond with the observed hypotensive effects in these studies. Furthermore, the
concentration of heparin and related agents were rather high in these studies.

From a statistical standpoint, the interpretations on the relevance of contact activation
mediated generation of bradykinin in the in vitro studies and pig model may not be relevant to
the adverse reactions and deaths. (1) The behavior of OSCS when injected in combination
with heparin may be different when compared with OSCS administered alone. The authors
have stated that 5mg/kg of OSCS - contaminated heparin was given to six pigs in a single
intravenous dose. The amount of OSCS in this preparation was not stated. Moreover, a dose-
dependent effect of OSCS was not discussed to explain the anaphylactoid response.

While the pathogenesis of OSCS contaminated heparins is primarily linked with contact
activation, the authors have totally ignored other pathophysiologic mechanisms, such as the
generation of antibodies in patients who have been repeatedly exposed to these products.
Over-sulfated GAGs bind much more strongly to heparin binding proteins such as platelet
factor 4 and related polycationic proteins. Moreover, OSCS may interact with various
endothelial, blood cellular and plasmat proteins. It should be stressed that repeated exposure
to OSCS containing heparin could easily lead to the generation of a wide array of antibodies,
which may mediate complex pathophysiologic responses. Moreover, such responses may be
population dependent, resulting in variable pathophysiologic effects. To date, neither the
article by Kishimoto, et al. nor the regulatory statements from different regulatory agencies
have considered the immunogenic implications and their relevance to the pathogenesis of
anaphylactic responses in those patients who suffered severe reactions. It is important to
take into consideration the immunogenic effects of OSCS and other contaminants while
investigating a cause and effect relationship of the reported deaths and adverse events.
Simply pointing to the activation of the contact system as the sole cause for these complex
events in those patients who suffered from these catastrophic adverse events is not realistic.

The authors also did not discuss the effects of OSCS on blood coagulation and platelet count
in the animals treated with this agent. Moreover, the interaction of OSCS with heparin in
terms of anticoagulant responses was not discussed. OSCS may potentially exhibit several
pharmacodynamic interactions with heparin. Such interactions may be equally important to
explain the pathogenesis related to the use of contaminated heparin. The recalled heparin
preparations also contain dermatan sulfate, which may cause complex interactions with OSCS and heparin. Both the OSCS and dermatan sulfate produce anticoagulant effect and exhibits varying degrees of interactions with heparin. Therefore, it is not surprising that some of the patients treated with contaminated heparin also exhibited bleeding complications.

The contaminated heparins contain OSCS of different molecular and structural features with marked differences in their biologic activities. Neither the paper from Kishimoto (1) nor the report on the chemical structure by Guerrini (2) identified the source of OSCS in heparins. The OSCS can be derived from several sources including bovine, porcine, murine and other natural sources. These differ in molecular weight and anticoagulant properties. Moreover, these OSCS exhibit a spectrum of biochemical interactions, which are not fully understood at this time. Each of these has a different profile in terms of their interaction with heparin and because of the different interaction with platelet factor 4 and other basic proteins, each may have a different immunogenic profile. It is important to profile these utilizing the following approaches:

1. Contaminants interactions with HIT antibodies
2. Contaminated-mediated activation of platelets
3. Contaminant effects on other cells (endothelial)
4. Contaminated neutralization by protamine sulfate and polybrene
5. Molecular heterogeneity of the contaminants
6. Contaminant interactions with serpins
7. Enhancement of bleeding by contaminants
8. Pharmacodynamics of the contaminants with or without heparin
9. Protein binding of the contaminants
10. Inhibition of heparanase and related enzymes

The progressive inclusion of OSCS to unfractionated heparin dates back to early 2007. While the data on the relative levels of contaminants in the earlier batches (July 2007 to December 2008) is not available, some of the products introduced after January 1, 2008 apparently contained varying measured levels of OSCS. Thus, it is likely that some heparin products with smaller amounts (<15%) OSCS may also have been used without any sizeable adverse effects. For this reason it would have been reasonable to report the relative levels of OSCS in those recalled heparins which were associated with severe adverse effects and deaths. Be that as it may, it is likely that a large number of patients have been exposed to varying levels of the contaminant and the reported adverse events and deaths may only represent a fraction of this population. To what extent the long-term effects of the OSCS will impact the overall physiologic and immunologic systems in the exposed population remains unknown. Therefore, fundamental work on the pharmacokinetics and pharmacodynamics of OSCS is imperative to understand the long-term effect of OSCS. This should be done in carefully designed studies and in translational research by recalling those patients who have been
exposed to OSCS over a period of time. The long-term health implication of the contamination in heparin remains unknown at this time.

Several editorials have also addressed the issues related to contaminants in coagulation products such as the plasma proteins, relative purity of heparin, and the issues related to the multi-factorial pathogenesis associated with OSCS. (5-7) Unlike plasma proteins the heparins represent a complex mixture of polymers obtained from a heterogeneous group of animals of diverse geographical origin. The quality of heparin is largely determined by the type of raw material used and the manufacturing process. However, the pharmacopeial methods used to assay these agents have remained primitive for some time. Therefore, the use of newer technology to investigate complex drugs such as heparin should be facilitated at all levels. Regardless of this the pharmacology of poly=therapeutic agents such as heparin is much more complex and should be addressed separately. The routine recommendations for biologics, including proteins, may not be applicable to complex carbohydrates. With proper guidelines for the pharmacopeial characterization and regulatory stipulations, safer heparins should become available.

In conclusion, the nature of the reported adverse events related to the use of contaminated heparin is complex and multifactorial, which is further compounded by varying patient responses. While this article by Kishimoto et al. provides preliminary data on the generation of kallikrein and anaphylatoxins by OSCS, these results are at best preliminary and should not be considered the etiology for the catastrophic hypotensive syndrome leading to death in over 100 patients. Therefore, until further observations are made in well-designed and statistically valid studies, this report should be considered preliminary. It should also be stressed that the effect of contaminants, in particular OSCS on the hemostatic system, in particular blood coagulation and platelet activation, with the immunogenic potential to generate of antibodies represents additional potential pathogenic mechanisms which may contribute to the observed adverse events and deaths.

REFERENCES


