Glycobiology Insights



LETTER TO EDITOR

# Challenges for Heparin Production: Artificial Synthesis or Alternative Natural Sources?

Mauro S.G. Pavão[1] and Paulo A.S. Mourão[2]

[1]Laboratório de Bioquímica e Biologia Celular de Glicoconjugados, [2]Laboratório de Tecido Conjuntivo, Programa de Glicobiologia, Instituto de Bioquímica Média and Hospital Universitário Clementino Fraga Filho—Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil. Corresponding author email: mpavao@hucff.ufrj.br

**Abstract:** The commentary emphasizes the immediate necessity to find alternative sources for the production of pharmaceutical heparin to meet the ton-amount world's demand of the glycosaminoglycan. The recent development of a synthetic approach brings considerable new technical and scientifically relevant knowledge to the field, with a strong potential for application in the future. However, the artificial approach does not offer a rapid alternative for the current world crisis affecting the production of heparin, which has to respond to an increasing worldwide demand. It is important to call attention for the availability of marine organisms that are rich sources of heparin analogs with significant anticoagulant activity, low bleeding effect that have been cultivated in very large amounts for years in different parts of the world. Additionally, alternative sources of mammalian heparin, such as bovine intestine and lung have been continually used in countries from South America, Africa and Asia, since the outbreak of the BSE without any report of prion contamination in humans. Recently, it has been shown that bovine and porcine intestinal heparins are composed by different proportions of a mixture of the same fractions that can be simply separated by anion exchange chromatography. In other words, high quality porcine heparin can be obtained from bovine tissue. We believe that alternative animal sources of heparin are currently a more realistic solution than artificial heparin to respond to the increasing demand for heparin.

**Keywords:** marine invertebrates heparins, bovine intestinal heparin, porcine intestinal heparin, artificial heparin

Video Abstract Available from http://la-press.com/t.php?i=9705

*Glycobiology Insights* 2012:3 1–6

doi: 10.4137/GBI.S9705

This article is available from http://www.la-press.com.

Heparin is the main natural anticoagulant used to treat and prevent thromboembolic disorders.[1] The increasing demand for heparin and the recent episodes involving its production called attention to the immediate need for alternative sources of the glycosaminoglycan. Currently, heparin is obtained almost exclusively from porcine intestine. A recent work by Xu et al[2] presents a new artificial alternative to replace animal heparin. However, can the artificial method respond to the increasing worldwide demand for the glycosaminoglycan? How about other known animal sources of heparin such as marine organisms or alternative mammalian tissues?

During a 75-year period of clinical usage, since the appearance of the first heparin product for intravenous use in 1936,[3] two recent incidents involving different aspects of its production have been reported. In late 2007, contamination of unopened heparin syringes with the microorganism *Serratia marcescens* caused a nationwide recall of the glycosaminoglycan in the United States. Less than one year later, in early 2008, another contamination with a non-natural oversulfated chondroitin sulfate of a heparin stock produced in China, was responsible for the death of 81 patients in the United States, according to the U.S. Food and Drug Administration. These two facts raised concerns on the reliability and safety of heparins and their low molecular weight derivatives obtained from animal sources,[4] leading to the establishment of more strict analytical methods for the testing of heparin preparations, now described in the USP Heparin stage 2 monograph.[5]

At the same time, the necessity of alternatives to the mammalian sources of heparin, which is currently obtained almost solely from porcine intestine, became evident. Several groups focused their efforts in the artificial synthesis of heparin and developed a very expensive and laborious 50- step process, resulting in the production of a synthetic heparin analog of limited size, the expensive 1.5 kD-Arixtra pentasaccharide, which has been used since 2002 as an antithrombotic agent.[6] More recently, an alternative 12-step chemo enzymatic approach capable of synthesizing heparin oligosaccharides of defined low molecular weight, significant anticoagulant activity and with the possibility to scale-up to multi milligram quantities has been reported.[2] Undoubtedly, the development of the two synthetic approaches brought considerable new technical and scientifically relevant knowledge to the field, with a strong potential for application in the future. However, the artificial approach does not offer an immediate realistic alternative for the current world crisis affecting the production of heparin, which has to respond to a demand of approximately 100 tons/year worldwide. Several limitations are involved in the commercial production of ton quantities of artificial heparin: the technical machinery required to produce large quantities, the huge financial investment in new industrial plants, new extensive clinical trials, the extremely high price of the synthetic product compared to the very low price of unfractionated heparin (~1.5 U.S. Dollars per g).

It is clear that the world crisis of heparin has two main components: the increasing worldwide demand and the necessity to maintain the quality control during the different steps of the production. While the latter component is being realistically approached, as noted by the constant USP Heparin Study Participation calls by the U.S. Pharmacopeia, the former component, which clearly involves finding alternative sources of heparin requires the attention from the scientific community. In this regard, there are two alternative heparin sources that, when compared to the artificial synthesis, may meet the technical, logistical and economical criteria required for long-scale production in a shorter time. These souces are: marine organisms and alternative mammalian tissues, such as bovine intestine and lung.

## Heparin from Marine Organisms

Several heparin analogs obtained from different marine organisms have been described (Table 1). Some of these compounds have been extensively studied in terms of structure, biological activity and mechanism of action, and evaluated in pre-clinical experiments in rodent animals with promising results.[7]

One important aspect of heparin from marine organisms is the very low risk of contamination with pathogens, since they are evolutionally distant from mammals. Therefore, the chance of microorganism or prion infection in mammalian cells is very unlikely. Another relevant point regarding the therapeutic use of an animal-derived drug is the technical and economic possibility to obtain very large quantities in a constant and ecologically correct manner. Overall, the heparin analogs are isolated from marine invertebrate animals

**Table 1.** Main disaccharide composition and biological activity of heparinoids from marine organisms.

| Organisms (species) | GAG type (main disaccharide units) | Organ | Biological activity | Ref. |
|---|---|---|---|---|
| *Shrimp (penaeus brasiliensis)* | **Low molecular weight heparin** $[GlcA\text{-}GlcNS,6S]_n$ | *Head* | Anti-xa and anti-IIa activities; heparin cofactor II activity; antithrombotic activity; | 19,20 |
| *Shrimp (litopenaeus vannamei)* | **Heparin** $[GlcA\text{-}GlcNS,6S]_n$ and $[GlcA2S\text{-}GlcNS]_n$ | *Cephalo torax* | Anticoagulant activity | 21 |
| *Bivalve mollusk (callista chione)* | **Heparin** $[IdoA2S\text{-}GlcNS,6S]_n$ | *Internal organs* | Anticoagulant activity | 22 |
| *Bivalve mollusk (tapes philippinarum)* | **Heparin** $[IdoA2S\text{-}GlcNS,6S]_n$ | *Internal organs* | High anticoagulant activity | 23 |
| *Bivalve mollusk (mercenaria mercenaria)* | **Heparin** (3-O-sulfated GlcNAc residues) | *Internal organs* | High anticoagulant activity; anti-IIa and anti-Xa activities | 24,25 |
| *Bivalve mollusk (tivela mactroides)* | **Heparin** $[GlcA\text{-}GlcNS,6S]_n$ | *Internal organs* | High anticoagulant activity | 26,27 |
| *Bivalve mollusk (donnax striatu) s* | **Heparin** $[GlcA\text{-}GlcNS,6S]_n$ | *Internal organs* | High anticoagulant activity | 27 |
| *Bivalve mollusk (nodipecten nodosus)* | **Heparin-like** $[GlcA\text{-}GlcNAc]_n$ | *Internal organs* | Low anticoagulant activity; high antithrombotic effect | 28 |
| *Bivalve mollusk (amusium pleuronectes)* | **Low molecular weight heparin** (porcine type) | *Internal organs* | Anticoagulant activity | 29 |
| *Tunicate ascidian (styela plicata)* | **Heparin** $[IdoA2S\text{-}GlcNS,6S]_n$ | *Internal organs* | Anticoagulant and antithrombotic activities | 30,31 |
| *Echinoderm sea-cucumber (ludwigothurea grisea)* | **Fucosylated chondroitin sulfate** $[Fuc\text{-}GlcA\text{-}GalNAc6S]_n$ | *Body wall* | Anticoagulant and antithrombotic activities (oral activity) | 32 |

**Note:** For complete information see reference.[7]

at reasonable yields (about 0.5%–2% of the dry weight, comparing to 0.022% from pig intestinal mucosa[8]), by procedures similar to those already employed in the preparation of pharmaceutical heparin. Several species of mollusks and sea cucumbers, including those containing high quantities of heparin analogs, have been successfully cultivated for a long time in different parts of the world. The current aquaculture technologies are capable to produce ton-quantities of starting material.[9–11] In 2001, the world's production of sea cucumber reached about 21,000 tons,[9] and that of scallops, in 1999, about 73,000 tons.[12] Possibly, major limitations for medical application of polysaccharides from marine organisms are a more profound analysis of their effects on mammalian systems and their mechanisms of action compared with heparin. Probably, these analyses could be performed in a shorter time than that required for the artificial synthesis of heparin.

## Alternative Mammalian Sources

After the outbreak, in the late 1980s, of the bovine spongiform encephalopathy, which is caused by a particular strain of prion, the commercialization of heparin from bovine tissues has ceased in Europe, Unites Stated and Japan. However, bovine heparin continued to be used clinically in countries from South America, Africa and Asia, without any reported event on prion contamination in humans.

Bovine heparins can be obtained from two main tissues: lung and intestine, and are chemically distinct from each other (Table 2). Bovine lung heparin has a higher proportion of *N*-sulfated α-glucosamine units and a lower proportion of *N*-acetylated residues than porcine mucosa heparin.[13] Bovine intestinal heparin is more heterogeneous, varying significantly in the substitutions in the α- glucosamine units: ~57% are 6-O and *N*-disulfated, as in porcine heparin, ~33% are 6-desulfated and ~10% *N*-acetylated. In recent studies,[13,14] pharmaceutical grade heparin obtained from bovine and porcine intestinal mucosa was shown to contain different proportions of the same mixture of fractions. In other words, the typical disaccharide composition of porcine intestinal heparin with high

**Table 2.** Disaccharide composition and biological activity of bovine and porcine heparins.

| Mammalian tissue | Major disaccharides | Anticoagulant activity (IU/mg) | Ref. |
|---|---|---|---|
| *Porcine intestine* | $[IdoA2S\text{-}GlcNS,6S]_n$ | 180–230 | 13,14,33 |
| *Bovine lung* | $[IdoA2S\text{-}GlcNS,6S]_n$ | ~150 | 13,14,33 |
| *Bovine intestine* | $[IdoA2S\text{-}GlcNS,6S]_n$ (57%); $[IdoA2S\text{-}GlcNS]_n$ (33%); $[IdoA2S\text{-}GlcNAc]_n$ (10%) | ~100 | 13,14 |

in vitro anticoagulant activity can be obtained from bovine intestinal heparin. Therefore, in countries with no economic limitations, bovine tissues may represent a potential alternative source of a high quality heparin provided an additional easily performed purification step is introduced in the production.

The real challenge involving the current world crisis is not to produce a few grams of an artificial heparin free of natural or non-natural contaminants, but how to produce amounts able to respond to an increasing worldwide demand, which tends to increase even more in the near future, as new therapeutic uses of the glycosaminoglycan are being revealed, for example as an anticancer drug.[15–18] We believe that alternative animal sources of heparin are an easier and faster solution than the artificial synthesis to respond to the increasing worldwide demand of the glycosaminoglycan.

## Funding

CNPq, FAPERJ. MSGP and PASM are research fellows from CNPq and FAPERJ.

## Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

## References

1. Spyropoulos AC. Insights from the 7th Southwest symposium on thrombosis and hemostasis. *Thrombosis Research*. 2008;123 Suppl 1:S1.
2. Xu Y, Masuko S, Takieddin M, et al. Chemoenzymatic synthesis of homogeneous ultralow molecular weight heparins. *Science*. Oct 28, 2011; 334(6055):498–501.
3. Best CH. Preparation of heparin and its use in the first clinical cases. *Circulation*. Jan 1959;19(1):79–86.
4. Liu H, Zhang Z, Linhardt RJ. Lessons learned from the contamination of heparin. *Natural product reports*. Mar 2009;26(3):313–21.
5. Heparin Sodium. *Interin Revision Annoucement*. In: U.S. Pharmacopeia, USP; 35:2009.
6. Petitou M, van Boeckel CA. A synthetic antithrombin III binding pentasaccharide is now a drug! What comes next? *Angew Chem Int Ed Engl*. Jun 14, 2004;43(24):3118–33.
7. Kozlowski EO, Gomes AM, Silva CS, Pereira MS, Vilela-Silva ACES, Pavao MSG. Structure and biological activities of glycosaminoglycan analogs from marine invertebrates: new therapeutic agents? In: Pavão MSG, editor. *Glycans in Diseases and Therapeutics*. Berlin, Springer; 2011: 159–84.
8. Linhardt RJ, Ampofo SA, Fareed J, Hoppensteadt D, Mulliken JB, Folkman J. Isolation and characterization of human heparin. *Biochemistry*. Dec 15, 1992;31(49):12441–5.
9. Conand C. In: *Advances in Sea Cucumber Aquaculture and Management*. Rome: FAO; 2004.
10. Helm MM, Bourne N. The hatchery culture of bivalves. In: *A Practical Manual Hatchery Culture of Bivalves*. Rome: FAO; 2004.
11. Lem A. International trade in aquaculture products. In: *Topics Fact Sheets*. FFaA ed; 2005.
12. Bourne NF. In: *Aquaculture International*, Vol. 1, Springer 2000.
13. Aquino RS, Pereira MS, Vairo BC, et al. Heparins from porcine and bovine intestinal mucosa: Are they similar drugs? *Thrombosis and Haemostasis*. May 2010;103(5):1005–15.
14. Tovar AM, Capille NV, Santos GR, et al. Heparin from bovine intestinal mucosa: Glycans with multiple sulfation patterns and anticoagulant effects. *Thrombosis and Haemostasis*. Mar 22, 2012;107(5).
15. Castelli R, Porro F, Tarsia P. The heparins and cancer: review of clinical trials and biological properties. *Vasc Med*. May 2004;9(3):205–13.
16. Lee DY, Lee SW, Kim SK, et al. Antiangiogenic activity of orally absorbable heparin derivative in different types of cancer cells. *Pharmaceutical Research*. Dec 2009;26(12):2667–76.
17. Laubli H, Borsig L. Heparins attenuate cancer metastasis: are selectins the link? *Cancer Investigation*. Jun 2009;27(5):474–81.
18. Casu B, Vlodavsky I, Sanderson RD. Non-anticoagulant heparins and inhibition of cancer. *Pathophysiology of Haemostasis and Thrombosis*. 2008;36(3–4):195–203.
19. Dietrich CP, Paiva JF, Castro RA, et al. Structural features and anticoagulant activities of a novel natural low molecular weight heparin from the shrimp Penaeus brasiliensis. *Biochimica et Biophysica Acta*. Aug 5, 1999;1428(2–3): 273–83.

20. Demir M, Iqbal O, Dietrich CP, et al. Anticoagulant and antiprotease effects of a novel heparinlike compound from shrimp (Penaeus brasiliensis) and its neutralization by heparinase I. *Clinical and Applied Thrombosis/Hemostasis: Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*. Jan 2001;7(1): 44–52.
21. Brito AS, Arimateia DS, Souza LR, et al. Anti-inflammatory properties of a heparin-like glycosaminoglycan with reduced anti-coagulant activity isolated from a marine shrimp. *Bioorganic and Medicinal Chemistry*. Nov 1, 2008;16(21):9588–95.
22. Luppi E, Cesaretti M, Volpi N. Purification and characterization of heparin from the Italian clam Callista chione. *Biomacromolecules*. May–Jun 2005; 6(3):1672–8.
23. Cesaretti M, Luppi E, Maccari F, Volpi N. Isolation and characterization of a heparin with high anticoagulant activity from the clam Tapes phylippinarum: evidence for the presence of a high content of antithrombin III binding site. *Glycobiology*. Dec 2004;14(12):1275–84.
24. Jordan RE, Marcum JA. Anticoagulantly active heparin from clam (Mercenaria mercenaria). *Archives of Biochemistry and Biophysics*. Aug 1, 1986;248(2):690–5.
25. Ulrich PN, Boon JK. The histological localization of heparin in the northern quahog clam, Mercenaria mercenaria. *Journal of Invertebrate Pathology*. Oct 2001;78(3):155–9.
26. Pejler G, Danielsson A, Bjork I, Lindahl U, Nader HB, Dietrich CP. Structure and antithrombin-binding properties of heparin isolated from the clams Anomalocardia brasiliana and Tivela mactroides. *The Journal of Biological Chemistry*. Aug 25, 1987;262(24):11413–21.
27. Dietrich CP, Nader HB, de Paiva JF, Santos EA, Holme KR, Perlin AS. Heparin in molluscs: chemical, enzymatic degradation and 13C and 1H n.m.r. spectroscopical evidence for the maintenance of the structure through evolution. *International Journal of Biological Macromolecules*. Dec 1989; 11(6):361–6.
28. Gomes AM, Kozlowski EO, Pomin VH, de Barros CM, Zaganeli JL, Pavao MS. Unique extracellular matrix heparan sulfate from the bivalve Nodipecten nodosus (Linnaeus, 1758) safely inhibits arterial thrombosis after photochemically induced endothelial lesion. *The Journal of Biological Chemistry*. Mar 5, 2010;285(10):7312–23.
29. Saravanan R, Shanmugam A. Isolation and characterization of low molecular weight glycosaminoglycans from marine mollusc Amussium pleuronectus (linne) using chromatography. *Applied Biochemistry and Biotechnology*. Mar 2010;160(3):791–9.
30. Gandra M, Cavalcante M, Pavao M. Anticoagulant sulfated glycosaminoglycans in the tissues of the primitive chordate Styela plicata (Tunicata). *Glycobiology*. Dec 2000;10(12):1333–40.
31. Santos JC, Mesquita JM, Belmiro CL, et al. Isolation and characterization of a heparin with low antithrombin activity from the body of Styela plicata (Chordata-Tunicata). Distinct effects on venous and arterial models of thrombosis. *Thrombosis Research*. 2007;121(2):213–23.
32. Mourao PA, Pereira MS, Pavao MS, et al. Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm. Sulfated fucose branches on the polysaccharide account for its high anticoagulant action. *The Journal of Biological Chemistry*. Sep 27, 1996;271(39):23973–84.
33. Mulloy B, Gray E, Barrowcliffe TW. Characterization of unfractionated heparin: comparison of materials from the last 50 years. *Thrombosis and Haemostasis*. Dec 2000;84(6):1052–6.

## Supplementary Data

A video abstract by the authors of this paper is available. video-abstract9705.mov