

Theme Issue Editorial

Heparin and its derivatives – Present and future

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Within recent years, considerable interest has arisen in the anticoagulant and non-anticoagulant functions of heparins, other glycosaminoglycans and related compounds. Orally available direct thrombin and factor Xa inhibitors are being developed as drugs for alternative treatment or prevention of thromboembolic disorders.

In this issue of *Thrombosis and Haemostasis*, readers will find selected articles dealing with these topics that were presented at the 16th Heparin Symposium organised by a group of German and Italian investigators. The meeting was held in the fall of 2008 at Villa Vigoni in Lovenno (Italy), and the guest editors appreciate the congenial atmosphere at the German-Italian Cultural Centre, created by Prof. Vogt-Spira. Contributions of selected presentations of previous symposia in this series have been published elsewhere (1–4). The current theme issue of *Thrombosis and Haemostasis* contains a compilation of reviews on (a) glycosaminoglycans and specifically modified pentasaccharides, (b) non-anticoagulant actions of glycosaminoglycans, (c) methodological aspects for analysis and determination of specific glycosaminoglycans, and (d) selected aspects of clinical topics including new direct coagulation factor inhibitors.

Anticoagulant and non-anticoagulant functions of glycosaminoglycans and their derivatives

A concise review ranging from heparins to the synthetic pentasaccharides fondaparinux and idraparinix is given by Petitou et al. (5), who provide outstanding experience in the chemistry of pentasaccharides and their derivatives. The latest addition of an anti-thrombin-dependent new anticoagulant is idraparinix bound to a biotin moiety, termed idrabiotaparinix. Thereby this anticoagulant can be neutralised by administration of avidin. Further development of idrabiotaparinix and ongoing clinical studies in patients with pulmonary embolism including the effect of antagonisation of idrabiotaparinix by avidin are the focus in the article by Harenberg et al. (6).

Non-anticoagulant functions of glycosaminoglycans appear to be relevant in tumour biology with regard to the antimetastatic

effect of heparin. Such an activity is reported by Schlesinger et al. (7) who demonstrate that the low-molecular-weight heparin (LMWH) tinzaparin interferes with integrin $\alpha 4\beta 1$ /VLA-4 binding to its counter-receptor vascular cell adhesion molecule-1 (VCAM-1). They used an acoustic wave biosensor to analyse these interactions. Whether tinzaparin may thereby also exert an antimetastatic function *in vivo* by blocking murine melanoma cell binding to the vessel wall requires further investigation.

The role of heparan sulfate proteoglycans and their modulation by heparanase in inflammatory reactions especially in regulating leukocyte extravasation is described in the paper by Li et al. (8). New mechanistic insights are provided with the help of heparanase-knockout mice.

Algal sulphated fucans, in particular fucan polysaccharides with 2,4-disulphated fucose residues, exhibit counteracting activities in blood coagulation as presented by Mourão et al. (9). While a fucosylated chondroitin sulphate promotes thrombin inhibition by heparin cofactor II, it may also activate factor XII. It was shown that in a specific animal model doses of the sulfated fucan are required to inhibit thrombosis before an increased bleeding is observed. Specific alterations in the fucan structure may alter its bimodal functions.

Collino et al. (10) describe the effects of a semi-synthetic N-O-sulphated glycosaminoglycan K5 polysaccharide derivative as protector against brain injury. The K5 derivative is a sulphated semi-synthetic glycosaminoglycan and was shown to inhibit apoptotic and inflammatory processes in a non-anticoagulant manner.

New methodological aspects for the analysis of glycosaminoglycans

Oversulfated chondroitin sulfates appear to be responsible in some heparin preparations for severe side effects during therapeutic application, including death of patients. Liverani et al. (11) have analysed the physico-chemical characteristics of heparins of various origins. They paid special attention to the contamination of the preparations by oversulfated chondroitin

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Picturesque Villa Vigoni in Lovenno (Italy) where the 16th Heparin Symposium was held in the fall of 2008.

sulfates and report on a so far unrecognised association by using enzymatic cleavage with heparanases I, II and III to detect hyper-sulfated chondroitin sulfate impurities in certain heparin preparations and fractions.

In order to identify (also deliberately added) contaminations in commercial heparin preparations, Sasisekharan et al. (12) and Guerrini et al. (13) describe the analysis of hypersulfated chondroitin sulfates by nuclear magnetic resonance (NMR), enzymatic digestion, size exclusion liquid chromatography and capillary electrophoresis techniques. While traditional screening test cannot differentiate between contaminated and non-contaminated heparin preparations, given the large array of possible impurities, mono- and bidimensional NMR spectroscopy can do so. In this manner, the quality control of heparin preparations can be improved to avoid life-threatening use of poisoned drugs.

Another approach is described by Szelke et al. (14) who developed a respective fluorescent rothenium compound for detection and antagonisation of heparins. By binding to the polycationic rothenium compound this new method can detect unfractionated heparin (UFH) and LMWHs in liquid matrices as well.

The chemical composition of LMWHs can be specified using depolymerisation of UFHs with Cu^{2+} as reagent and Fe^{2+} as catalyst, as reported by Vismara et al. (15). This work shows that the use of copper in the depolymerisation process is superior to the classical ferric-sulfate and leaves the active site on the glycosaminoglycan intact for antithrombin binding.

With the help of nuclear magnetic resonance technique Bisio et al. (16) report on new structural aspects of LMWHs and the identification of a special antithrombin-binding pentasaccharide sequence. Additional size exclusion revealed differences in the distribution of such pentasaccharides among various LMWHs.

Another method to distinguish structural components in various LMWHs and heparinoids *in vitro* is ultraviolet circular dich-

roism, as reported by Rudd et al. (17). Here, differences in the N-acetyl groups of uronic acid residues containing double-bonds in different preparations provide the basis for analysis.

Clinical applications

In their contribution Vardi et al. (18) focused on the relevance of the activated partial thromboplastin time determination for patients with acute venous thromboembolism who were treated by subcutaneous or intravenous UFH. The meta-analysis of data revealed in principle no correlation between the level of anticoagulation or the mode of heparin administration and the major clinical outcomes.

The most serious side effect of heparin therapy is heparin-induced thrombocytopenia type II (HIT II) in which autoantibodies against a complex of heparin with platelet factor 4 are present in patients' blood. Götz Nowak has used the data from three patients groups with the development of heparin-induced antibodies in order to analyse the severity of this acquired autoimmune disease (19). Measurements with an antibody-dependent platelet adhesion assay revealed high incidence of autoantibodies in patients with stroke, cardiac complications or those undergoing haemodialysis. The appropriate use of this functional platelet assay would help to prevent severe complications of HIT II.

In their contribution Laux et al. (20) summarise the clinical use of heparin and LMWHs in comparison to vitamin K antagonists. They focus on the potential indications of these conventional anticoagulants as opposed to the development of new oral direct thrombin- and factor Xa-inhibitors, and summarise ongoing studies with these drugs.

The successful use of UFH and LMWHs are limited in the therapy of arterial thrombosis associated with acute coronary syndrome (ACS). Moreover, switching from medical management to procedural intervention presents additional compli-

cations in anticoagulant therapy. A new LMWH, M118, which is designed for use in the treatment of acute coronary syndrome, is described by Kishimoto et al. (21). M118 exhibits superior phar-

macokinetics and -dynamics, can be functionally monitored and is currently under phase II evaluation in patients undergoing percutaneous coronary intervention.

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