



Review

Endogenous heparin activity deficiency: the ‘Missing Link’ in atherogenesis?

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Abstract

This paper reviews published studies since 1995 dealing with many atherogenic mechanisms where exogenous heparin was beneficial. In these areas endogenous heparin deficiency is likely to be harmful. Mechanisms included inflammatory factors, lower endogenous plasma heparin levels, lipoprotein lipase, chemokines, APOE e4, lipoprotein(a), among others. Demonstrated reduction of heparan sulfate proteoglycans (HSPG) and of endogenous plasma heparin was reviewed. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

This subject was discussed in an earlier paper which covered the available material through early 1996 [1]. Since then, many publications have provided further supporting evidence. Published studies since 1995 dealing with atherogenic mechanisms where exogenous heparin is beneficial will be covered. It is in such areas that a deficiency of endogenous heparin activity is more apt to be harmful. It has been estimated that the known risk factors for atherosclerosis account, at most, for only 50% of cardiovascular disease.

2. Chronic inflammatory factors

Inflammatory factors involved in atherogenesis were previously reviewed [2]. There is much further evidence. The inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin-1B (IL-1B), exert many of their pro-inflammatory effects via activation of AP-1 and NF- κ B [3]. NF- κ B is a pivotal transcription factor in chronic inflammatory disease [4]. Many factors, including AP-1, independently phosphorylate the inhibitor of NF- κ B. Inhibition of AP-1 markedly reduced the ability of TNF- α to activate inflammatory adhesion

molecules m RNA expression [5]. Casein kinase II (CKII) phosphorylates I- κ B in vitro and in vivo [6]. Lysine residues 21 and 22 are required for ubiquitin ligation which targets I- κ B for rapid degradation [7]. Thus AP-1, CK-II, and lysine residues play a role in inflammatory responses.

Exogenous heparin inhibits the activity of all the factors previously mentioned. The nuclear AP-1 complex is composed of the jun and fos proteins. There is evidence that heparin penetrates to the nucleus and that inhibition of AP-1 is a direct result of nuclear heparin [8]. Inhibition of the AP-1 pathway by heparin is ubiquitous. Heparin is the most potent CKII inhibitor thus far identified, effective at nanogram concentrations [9]. Heparin inhibits the production of the inflammatory cytokines IL-1B, IL-6, and TNF- α by stimulated human monocytes [10]. A diminution of endogenous heparin activity would weaken its restraining effect on AP-1, NF- κ B, TNF- α , and IL-1B inflammatory effects.

3. Endogenous plasma heparin

This subject is of major importance in relation to lipid abnormalities involved in atherogenesis. Thus, al-

Table 1
Average serum lipid values at varying heparin levels

	Heparin (units %)	Cholesterol (mg)	Low-density lipoproteins	
			SfO-12 (mg)	5f12-400 (mg)
Males	Up to 12	292	481	289
	12.1–15	261	501	287
	15.1–18	238	473	238
	Over 18	236	440	181
Females	Up to 12	279	569	298
	12.1–15	269	523	204
	15.1–18	267	502	148
	Over 18	226	435	115

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though previously discussed [1], it will be presented again and brought up to date. In 1955 a reproducible method for the extraction of endogenous plasma heparin was described [11]. The results showed that heparin normally is present in the blood and that it is protein bound. Thus, proteolysis of the precipitated plasma proteins is a necessary step before endogenous heparin activity can be demonstrated and quantitated. Using this method, plasma heparin levels in 153 male and 104 female nonfasting adults ranged from 1 to 2.4 mg/l [12]. The findings in relation to serum lipid values and plasma endogenous heparin levels are shown in Table 1, and the statistical analysis in Table 2. The results indicated that lower endogenous circulating heparin activity is an important determinant of serum lipid abnormalities involved in atherogenesis in individuals consuming a normal diet. The presence of endogenous circulating heparin has been confirmed by other investigators [13–15], and the levels of heparin activity demonstrated were close to the level found previously [12]. Thus, there is little reason to doubt the existence of endogenous heparin activity in human blood. However, these confirming studies did not measure serum lipid levels. This is unfortunate as the findings of the original study [12] were meaningful in atherogenesis.

Table 2
Statistical analysis of plasma heparin levels vs. serum cholesterol and standard SfO-12 and 5f12-400 low-density lipoproteins

	Heparin vs. cholesterol			Heparin vs. SfO-12 lipoproteins			Heparin vs. Sf12-400 lipoproteins		
	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>
Males	147	–0.271	<0.01	131	–0.091	>0.01	131	–0.265	<0.01
Females	113	–0.142	>0.05	77	–0.241	<0.05	77	–0.335	<0.01

Abbreviations: *n*, number of patients; *P*, probability; *r*, Pearson product moment correlation coefficient. Reprinted with permission of the publisher.

4. Lipoprotein lipase (LPL)

LPL activity, (formerly called lipemia clearing factor), is the major physiologic pathway for the removal of triglycerides from the bloodstream. Heparin is essential in lipoprotein lipase function. LPL is totally inactive when mutational changes in the enzyme prevent its binding to heparin [16]. The relation of endogenous heparin activity to LPL function in the blood and the vascular wall was reviewed (2 and references therein). Any deficiency of endogenous heparin activity would contribute to decreased LPL activity and the resultant harmful effects.

5. Hypertriglyceridemia (HTG)

An early study found that plasma LPL activity, without the prior injection of heparin, was significantly inversely related to the level of triglyceride-rich 5f12-400 lipoproteins [17]. There is much evidence that HTG is a major determinant of lower HDL levels [2]. Recent publications have added to the evidence that HTG is a risk factor for cardiovascular disease. This is independent of HDL cholesterol levels [18]. Lower endogenous heparin activity would decrease LPL activity and so contribute to HTG.

6. Chemokines

It is well known that chemokines have an important role in the infiltration of leukocytes into inflammatory sites. Chemokine production is stimulated by many factors in many cell types, both immune and non-immune. Chemokines have a critical part in both cellular inflammatory responses and in pathological processes associated with inflammation, infection, and immune-mediated diseases [19]. Chemokines are effector molecules at the initial stages of an inflammatory response [20]. Human chemokines were reviewed recently [21]. They are cationic molecules. The role of anionic

heparin in neutralizing cationic basic amino acid residues has been reviewed [22]. Decreased endogenous heparin activity would limit anti-chemokine effects.

7. Lipoprotein(a) Lpa

Lpa has an established predictive role in coronary artery disease (CAD), and it is a better discriminator than all other lipid and lipoprotein parameters [23]. Lpa promoted the proliferation of human and rat vascular smooth muscle cells (SMC) [24]. It is well known that SMC proliferation is an important pathogenic factor in atherogenesis. In both men and women less than 50 years of age, coronary angiography showed a linear relationship between plasma Lpa and the number of diseased vessels [25]. A prospective 15.4 year follow-up of 2191 healthy men aged 20–54 years demonstrated that elevated Lpa plasma levels were an independent risk factor for the development of coronary disease comparable in magnitude and prevalence to a total cholesterol level of 240 mg/dl or more, or an HDL level less than 35 mg/dl [26].

It is well known that oxidized low density lipoproteins (OXLDL) have harmful effects in arterial walls. A new finding demonstrated that OXLDL decreased matrix heparan sulfate proteoglycans (HSPG) and the matrix then retained more Lpa [27]. Leukocyte defensin, which stimulates binding of Lpa to human vascular cells, is cationic and has exposed arginines [28]. Defensin prevents the rapid degradation of Lpa. Although sparse in uninvolved vessels, defensin was found in human atherosclerotic cerebral blood vessel cells, and in the extracellular matrix (ECM). Lpa attachment to human aortic glycosaminoglycans was three-fold higher than that of LDL, resulting in increased Lpa retention in vivo in the subendothelial layer [29].

Studies have shown the importance of Lpa lysine binding sites in Lpa effects (reviewed in [30]). It was noted that Rhesus monkey Lpa binds less efficiently than human Lpa to lysine sepharose or to cultured U937 cells, that Lpa is conspicuous in the vasa vasorum of diseased coronary arteries but was not detectable in the microvasculature of normal tissues, that Lpa induces the inflammatory adhesion molecule ICAM-1 and decreases nitric oxide expression and induces EC release of the fibrinolytic inhibitor PAI-1. The review also mentioned the interesting fact that most animal species lack Lpa altogether. It is expressed only in primates and hedgehogs.

Heparin activity would, at least partially, inhibit many of the pro-atherosclerotic effects of Lpa just reviewed, via inhibition of the key role of lysine binding sites in Lpa effects. Again, this implies that a decrease

of endogenous heparin activity would enhance harmful Lpa effects.

8. Angiotensin-II (ANII), angiotensin converting enzyme (ACE)

Drugs that inhibit an overactive renin-angiotensin (RA) system in vascular walls have proven their clinical value. In the last 4 years, various studies have further clarified the pathogenic mechanisms involved in ANII and ACE effects. There is an increased accumulation of tissue ACE in human atherosclerotic artery disease [31]. ANII is present in coronary atherosclerotic lesions in humans and in monkeys, and decreases as lesions regress [32]. ACE causes endothelial dysfunction [33]. ACE upregulates tissue factor (TF) synthesis in monocytes, thus enhancing prothrombotic tendencies [34]. The catalytic sites of ACE contain histidine 361–365, 959, and 963 as essential residues [35].

The actions of heparin which minimize the atherogenic effects of ANII and ACE were reviewed earlier [2]. Anionic heparin inhibition of the catalytically essential cationic histidine residues of ACE provides further evidence. All these beneficial effects of heparin on angiotensin damage would be diminished by a decrease of endogenous heparin activity.

9. Phospholipase A₂ (PLA₂)

Many new studies give additional information about the harmful role of PLA₂ activity in atherogenesis. There is high expression of secretory PLA₂ type II throughout the media in both normal and atherosclerotic arteries [36]. PLA₂ is very basic (pH 10.5), remarkably stable, and has three heparin-binding sequences [37]. In the human abdominal aorta and coronary arteries there was stronger PLA₂ activity in atherosclerotic lesion areas [38]. PLA₂ enhances LDL and Lpa binding [39].

An inhibitor of PLA₂ suppresses inflammatory responses [40]. Inhibitors of cytosolic or secretory PLA₂ block TNF-induced activation of transcription factor NF-κB and expression of adhesion molecule ICAM-1 [41]. PLA₂ is the rate-limiting enzyme in the formation of platelet-activating factor (PAF), an very potent inflammatory mediator.

PLA₂ binds to glycosaminoglycans secreted by human arterial SMC, including chondroitin sulfate (CS). CS stimulates PLA₂, a reaction inhibited by heparin [42]. Heparin prevents the binding of PLA₂ to phospholipid micelles [43]. There are five mammalian secretory PLA₂ and all have an essential basic histidine residue in their catalytic mechanism [44], which would be inhibited by heparin.

10. Viruses

Viruses are among the many factors that can injure endothelium and so contribute to atherogenesis. This has been reviewed to date [2]. Herpes simplex virus-1 (HSV-1) is the best studied and has many harmful pro-inflammatory and pro-coagulant effects. Cytomegalovirus (CMV), a herpes virus family member, caused EC damage and early atherosclerotic lesion in normocholesterolemic rats [44]. The injection of fragments of HSV-1 and HSV-2 genomes into isolated nonhyperlipidemic rabbit arterial segments induced intimal lesions proving that viruses can initiate atherosclerotic lesions [45]. Increasing evidence indicates that viruses are frequently involved in the etiology of atherosclerotic disease ([46] and references therein).

Several recent studies have provided additional information. Using carotid endarterectomy specimens from 76 patients, chlamydia was found in 71%, CMV in 35.5% and HSV-1 in 10.5%. None of these were present in normal carotid tissue [47], and serum antibodies did not predict the presence of the organisms in tissue. A recent review discussed a vast array of circumstantial evidence indicating that herpes viruses are involved in the development of atherosclerosis, restenosis after angioplasty, accelerated atherosclerosis in heart transplants, and induction of a prothrombotic phenotype in vascular EC [48]. In bovine kidney cells heparin had, by far, the highest inhibitory effect on HSV-1, HSV-2, and pseudorabies virus (PRV) as compared to other sulfated polyelectrolytes [49]. PRV is a herpes virus and the binding domain of PRV glycoprotein C is composed of three clusters of basic amino acid residues [50], which explains its binding to and inhibition by heparin.

11. Selectins, integrins (MAC-1)

P and E-selectins play an important role in both early and late stages of atherosclerotic lesion development [51]. The sequential activation of P-selectin and the B₂ integrin MAC-1 supports the firm adhesion of neutrophils in flow [52]. MAC-1 appears to play a unique role in the signaling required for diverse adhesion-dependent functions of activated polymorphonuclear leukocytes (PMNL). MAC-1 is the major integrin of phagocytic cells, essential in many areas. MAC-1 has an important role in the transient activation of IL-8 and IL-1B genes of PMNL during the inflammatory response [53]. An antibody to MAC-1 reduced intimal thickening after angioplasty or stent implantation in rabbits [54]. Heparin inhibits ligand binding to MAC-1 [55].

12. Advanced glycation end products (AGE)

The many harmful effects of AGE contributing to atherogenesis have been reviewed [2,56]. Age modifications are irreversible, except perhaps in the early stages. Because of their frequently higher blood glucose levels, AGE form at an accelerated rate in diabetic patients. This may be a decisive factor in the well-known earlier development of atherosclerosis in diabetics. In all types of human atherosclerotic lesions, a low-moderate extracellular AGE deposition was present [57].

The initial formation of AGE involves the condensation of glucose with the E-amino groups of lysine [58]. Anionic heparin binds to and inhibits positively charged lysine [22], an action which would prevent the production of AGE. The AGE receptor (RAGE) contains a lactoferrin-like peptide highly homologous to, or identical with, lactoferrin [59]. Heparin binds to lactoferrin with mutual inhibition [60]. Although it has not been directly investigated, the existing data suggest that heparin impairs AGE formation and its receptor binding.

13. CD40, CD40 Ligand (CD40-L)

There is considerable evidence that CD40 and its ligand CD40-L are involved in the inflammatory process and in atherogenesis. They trigger production of the pro-inflammatory cytokines IL-6, IL-8, IL-1B and TNF- α by human endothelial cells (EC) and monocytes [61]. Interaction of CD40–CD40-L leads to endothelial activation and leukocyte adhesion, at least partly mediated via expression of E-selectin, ICAM-1, and increased TNF- α -induced VCAM-1 expression [62]. Activated T lymphocytes within the atherosclerotic vessel wall express the CD40-L, as do human EC, smooth muscle cells, and macrophages in human atherosclerotic lesions [63]. CD40-L signals production of active IL-1B by stimulating cleavage by interleukin converting enzyme (ICE), thus processing the IL-1B precursor [64]. This is a new pathway of ICE activation which could participate in the inflammatory aspects of atherogenesis. Inhibition of CD40 signaling reduces atherosclerosis in hyperlipidemic mice. Aortic lesion size decreased by 59%, their lipid content by 79%, and atheroma contained significantly fewer macrophages and T lymphocytes [65]. CD40 ligation induces tissue factor (TF) expression in human vascular SMC in vitro and in vivo [66]. Thus, CD40–CD40-L interaction may play a pathogenic role in both long-term atherogenesis and in the triggering and propagation of acute coronary syndromes. Data which indicate the importance of CD-40-CD40-L signaling in late atherosclerotic changes suggest that interruption of this pathway may decrease the acute manifestations of atherosclerosis [67].

As already noted, the ligation of CD40 activates IL-1 β -converting enzyme (ICE) in vascular EC and SMC thus generating active IL-1 β . The reaction of the inactive precursors of IL-1A, IL-1B and TNF- α on specific lysines activates these potent inflammatory cytokines [68,69]. Heparin inhibition of lysines probably would substantially interfere with CD40/CD40-L inflammatory effects.

14. Apolipoprotein E, e4 (APOE, e4)

A meta-analysis of 14 published studies revealed that the e4 allele of apolipoprotein E was associated with an increased incidence of coronary heart disease [70], and with the highest blood cholesterol levels [71]. The clearance of triglyceride-rich lipoproteins from the blood is impaired in individuals expressing an e4 allele [72]. An autopsy study of 700 men showed that the APOE e4 allele was a significant risk factor for coronary and aortic atherosclerosis at early middle age [73]. The single amino acid substitution of arginine replacing cysteine at residue 12 in the e4 allele of apolipoprotein E results in a unique conformation and functional ability that distinguishes APO E4 from E3 and E2. The APOE e2 allele codes for cysteine at positions 112 and 158; the e3 allele codes for cysteine at position 112 and for arginine at 158; the e4 allele codes for arginine at both positions [74]. Anionic heparin binds to and inhibits arginine basic residues [22], and would markedly inhibit e4 effects.

15. Myeloperoxidase (MPO)

A specific marker of MPO-catalyzed oxidation is markedly increased in LDL isolated from human atherosclerotic intima [75]. MPO-generated reactive nitrogen species convert LDL into an atherogenic form in vitro [76]. MPO plays an important role in protein and lipid oxidation in human atherogenesis [77] and oxidizes proteins and lipids independently of metal ions. There is no MPO in normal arteries. Heparin and dextran sulfate inhibit MPO activity [78].

16. Endothelial dysfunction

Abnormal endothelium, or normal endothelium exposed to physical or biochemical stresses adversely affecting its function, is believed to play a central role in the development of atherosclerotic lesions. Recently, it was stated that 'the natural conclusion to draw from many observations is that endothelial injury or dysfunction, if not the root cause, is the primary accelerant of the atherosclerotic process and that treatment directed

to restoring normal endothelial function will slow disease progression' [79]. Years ago it was demonstrated that a decrease in endothelial surface negative charge enhanced the uptake of LDL and fibrinogen by arterial walls [80].

The many known factors that harm endothelial function now will be discussed. Published evidence in this subject prior to 1990 was reviewed previously [81]. Normal endothelium has a negative electrical charge [82], primarily resulting from endothelial glyocalyx heparin and heparan sulfate (HSPG) activity [83]. HSV-1, widely distributed in humans, inhibited up to 85% of heparan sulfate synthesis in EC in vitro [84]. Activated human T lymphocytes release intact at least 50% of porcine aortic EC-HSPG [85]. Platelet activating factor (PAF) decreases sulfated glycans of the EC glyocalyx [86]. There is a high susceptibility of HSPG to oxidant injury. IL-1 β and TNF- α mediate suppression of heparin-like compounds on cultured porcine aortic EC [87]. They decrease HSPG synthesis to less than 60% resulting in almost complete loss of endothelial negative surface charge [88]. Heparanases which degrade HSPG are expressed by macrophages and platelets in atherosclerotic lesions [89].

It is apparent that various pathogenic factors contribute to a reduction of human endothelial glyocalyx heparin activity. Heparin has an inhibitory effect on many of them. Also, heparin and low molecular weight heparin stimulated HSPG synthesis by rabbit aortic EC 2–3-fold [90].

17. Animal and human HSPG decrease in atherosclerosis

Atherosclerosis-susceptible pigeons aortic SMC had approximately 50% of cell surface HSPG as compared to atherosclerosis-resistant pigeons [91]. In diet-induced atherosclerosis of monkeys, heparan sulfate decreased with increasing severity of the lesions, and increased as lesions regressed [92].

The HSPG findings in humans are similar to those in experimental animals. In human coronary arteries a large decrease of heparin and heparan sulfate content, and a proportional increase of chondroitin-6-sulfate and dermatan sulfate, was associated with increasing severity of atherosclerosis [93]. The content of HSPG and its proportion to the total proteoglycan content was also markedly less in atherosclerotic human cerebral arteries [94]. In human aortas, the proportion of heparan sulfate relative to the total proteoglycan content is 41% in normal and 20% in atherosclerotic areas [95]. Heparan sulfate in human atherosclerotic lesions decreased as lesion severity increased [96]. The divergent biologic properties of HSPG and chondroitin proteoglycans play a crucial role in atherogenesis [97].

After a thorough review it was concluded that ‘HSPG may be considered as an antagonist to lesion development’ [98].

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