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REVIEW ARTICLE

Unfractionated Heparin: Multitargeted Therapy for Delayed Neurological Deficits Induced by Subarachnoid Hemorrhage

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Abstract Aneurysmal subarachnoid hemorrhage (SAH) is associated with numerous "delayed neurological deficits" (DNDs) that have been attributed to multiple pathophysiological mechanisms, including ischemia, microthrombosis, free radical damage, inflammation, and vascular remodeling. To date, effective prophylactic therapy for SAH-induced DNDs has been elusive, due perhaps to the multiplicity of mechanisms involved that render typical, single-agent therapy seemingly futile. We hypothesized that heparin, which has multiple underappreciated salutary effects, might be useful as a multitargeted prophylactic agent against SAH-induced DNDs. We performed a comprehensive review of the literature to evaluate the potential utility of heparin in targeting the multiple pathophysiological mechanisms that have been identified as contributing to SAH-induced DNDs. Our literature review revealed that unfractionated heparin can potentially antagonize essentially all of the pathophysiological mechanisms known to be activated following SAH. Heparin binds >100 proteins, including plasma proteins, proteins released from platelets, cytokines, and chemokines. Also, heparin complexes with oxyhemoglobin, blocks the activity of free radicals including reactive oxygen species, antagonizes endothelin-mediated vasoconstriction, smooth muscle depolarization, and inflammatory, growth and fibrogenic responses. Our review suggests that the use of prophylactic heparin following SAH may warrant formal study.

Keywords Subarachnoid hemorrhage · Vasospasm · Inflammation · Heparin

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Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is associated with numerous adverse sequelae [1, 2]. Patients who survive the initial hemorrhage are at high risk for delayed secondary brain injury, including cerebral infarction, neuronal cell death, white matter abnormalities, and hydrocephalus, resulting in focal neurological deficits, cortical dysfunction and long-term cognitive and psychosocial deficits. Collectively, these abnormalities are referred to as SAH-induced "delayed neurological deficits" (DNDs).

Several distinct pathophysiological mechanisms are known to play important causative roles in SAH-induced DNDs, including ischemia, microthrombosis, free radical damage, inflammation, and vascular remodeling [3–12]. Cerebral infarction is strongly associated with poor outcome after SAH, symptomatic vasospasm is the most important factor predisposing to infarction [13], and dysregulation of endothelin signaling is currently viewed as a critical antecedent to vasospasm [14–16]. However, specific block of endothelin pathways, albeit effective at reducing vasoconstriction, does not necessarily improve patient outcome [3, 17], pointing to crucial roles for pathophysiological processes other than vasoconstriction. Microthrombi may contribute to ischemic damage, but non-ischemic mechanisms also play critical roles. Free radical injury induced by blood products is particularly damaging to white matter, and the inflammatory response provoked by blood products contributes not only to vasospasm, but to edema, cell death and generalized cortical dysfunction. To date, effective prophylactic therapy for SAH-induced DNDs has been elusive [18, 19], due perhaps to the multiplicity of mechanisms involved that render typical, single-agent therapy seemingly futile.

Heparin is a member of a family of polyanionic polysaccharides called glycosaminoglycans, composed of hexuronic acid and D-glucosamine residues joined by glycosidic linkages [20]. Unfractionated heparin (UH) is a heterogeneous mixture of polysaccharide chains of varying lengths resulting in a range of molecular weights from 3 to 30 kDa, whereas low molecular weight heparin (LMWH) ranges from 3 to 6 kDa [21]. Heparin (always used here to refer to UH) has the highest negative charge density of any known biological molecule and as a result, has a high propensity to bind to positively charged proteins and surfaces. More than 100 heparin-binding proteins have been identified [22], with the growing list including numerous plasma proteins, proteins released from platelets, cytokines, chemokines, and other small biologically active molecules, as well as endothelial cells themselves [23–26]. Although clinically utilized almost exclusively for its anticoagulant properties, heparin binding can interrupt numerous other biological pathways [27]. Among its wide-ranging effects, heparin complexes with free hemoglobin itself, including oxyhemoglobin [28], it blocks the activity of free radicals (FR) including reactive oxygen species (ROS) [29], it antagonizes endothelin-mediated vasoconstriction [30–32], it binds to several cytokines and all chemokines, thereby imparting potent anti-inflammatory effects [22, 33, 34], and it binds to several growth factors, thereby imparting anti-mitogenic [35–37] and anti-fibrotic [38] effects. To our knowledge, no other compound exhibits such a broad diversity of biological effects that seem so intimately relevant to mechanisms implicated in SAH-induced DNDs. The very multiplicity of mechanisms targeted by heparin suggests that it may be particularly attractive as multitargeted prophylactic agent to ameliorate the wide-ranging abnormalities induced by SAH.

Several excellent reviews on the molecular and cellular mechanisms that are activated following SAH have been published recently [4, 12, 39–41], but our purpose here is different. The present review focuses specifically on heparin, and links individual cellular and molecular mechanisms activated by SAH directly to known actions of heparin. Here, we demonstrate how heparin might serve as an effective multitargeted prophylactic agent to combat SAH-induced DNDs.

Review

Oxyhemoglobin (OxyHb)

There is considerable evidence implicating a component of erythrocyte lysate (hemolysate), especially oxyhemoglobin (OxyHb) or hemoglobin degradation products, in the etiology of vasospasm [42, 43]. The release of OxyHb from lysing erythrocytes in the subarachnoid space is believed to play a key role in vasospasm. OxyHb is present in high concentrations in CSF during the time of vasospasm. Erythrocytes are the only component of blood required for vasospasm to develop, and the most vasoactive substance within erythrocytes is OxyHb. OxyHb can act by a variety of pathways over a prolonged period of time to produce arterial narrowing and ultrastructural damage to arteries resembling those encountered after SAH. OxyHb has multiple actions, including scavenging nitric oxide (NO), inhibiting endothelium-dependent relaxation, generating ROS, activating the tyrosine kinase–mitogen-activated kinase (MAPK) pathway, releasing vasoactive eicosanoids and endothelin from the arterial wall, producing bilirubin and lipid peroxides, and causing damage to perivascular nerves.

In this context, an intriguing target of heparin binding is hemoglobin itself [28]. Both OxyHb and deoxyhemoglobin are strongly bound by heparin with a stoichiometry of binding (polyanion/tetrameric hemoglobin) that is less than unity. The overall affinity of hemoglobin for different anions is greater as the charge on the anion increases, and is maximal for polyanions such as heparin, which possesses the highest negative charge density of any biological molecule. To our knowledge, the hypothesis that heparin reduces the toxicity of OxyHb (or some other component of hemolysate) in the context of SAH has not been specifically tested. The published experiment that comes closest to addressing this question involved an experimental model of SAH in which intracisternal heparin

injection was shown to significantly reduce vascular wall changes [44]. Because OxyHb as well as hemolysate are themselves toxic when injected intracisternally [45], it would be interesting to determine whether their toxicity is neutralized when they are mixed with various amounts of heparin.

Free Radicals (FR) Including Reactive Oxygen Species (ROS)

Considerable evidence suggests that FR-mediated mechanisms contribute to vasospasm after SAH [46, 47]. As noted above, OxyHb is believed to be the, or one of the, principle spasmogen(s) responsible for vasospasm. OxyHb may cause vasospasm by generating oxygen-derived FR in the subarachnoid space. Superoxide radical is formed during the auto-oxidation of OxyHb. If iron is released from the heme moiety, superoxide can generate the highly injurious hydroxyl radical. Lipid peroxides are found in elevated concentrations in CSF during vasospasm in animals and humans. There is a progressive increase in thiobarbituric acid-reactive substances with time after SAH. The prevention of vasospasm by compounds that inhibit lipid peroxidation (21-aminosteroids such as tirilazad), that scavenge FR (nicaraven) or that chelate iron (deferroxamine) supports a role for FR-mediated processes in vasospasm. One or more of several mechanisms may be downstream effectors of FR reactions. ROS have direct vasocontractile effects on cerebral arteries in vitro, but different ROS have different contractile characteristics [48]. Peroxidation of membrane lipids occurs and is proposed to be an important antecedent leading to vasospasm. Alternatively, since FR act on bilirubin, biliverdin, and possibly heme to produce BOXes (Bilirubin OXidized Products), bilirubin oxidation products may act on vascular smooth muscle cells to produce chronic vasoconstriction and vasospasm combined with a vasculopathy due to smooth muscle cell injury [7, 49].

Despite considerable evidence, it has not been fully appreciated that heparin is a potent inhibitor of FR activity [50]. Heparin decreases FR release by activated neutrophils and mitigates FR effects in vivo. It alleviates FR production, and significantly decreases hydrogen ion accumulation. Heparinase pretreatment markedly increases FR release by endothelial cells. Small amounts of heparin enhance the antioxidant activity of superoxide dismutase. Heparin sulfate proteoglycans tether antioxidant superoxide dismutase to cell surfaces and so contribute to the inhibition of FR tissue injury. Certain glycosaminoglycans, including heparin, have features that allow them to act as FR sinks. Heparin markedly reduces FR release in vivo after ischemia/reperfusion and protects endothelial cells from FR injury. Heparin scavenges FR released by the action of myeloperoxidase and it binds and sequesters Fe^{2+} ions and decreases peroxidation of unsaturated fatty acids in the presence of Fe^{2+}. Metal chelators like heparin have anti-oxidant activity and block activation of nuclear factor κB (NF-κB). Overall, considerable evidence indicates that heparin-like activity is part of the organism's anti-oxidant defenses, implying that heparin therapy may reduce the harmful actions of FR (see [50] for original citations).

Endothelin (ET)

Several highly potent vasoactive compounds have been identified in the CSF and plasma of patients after SAH. Among these, the endothelins (ETs), especially endothelin-1 (ET-1), are believed to play a critical role in SAH-induced DNDs [15, 16]. ET-1, acting by way of endothelin receptor type A on vascular smooth muscle cells (VSMC), is one of the most potent vasoconstrictors known. Acting in concert with other vasoactive factors, ETs may contribute substantially to the disturbed equilibrium between constriction and relaxation that typifies vascular responses following SAH. Endothelin concentrations in plasma or CSF correlate with delayed cerebral ischemia and vasospasm after SAH, with the highest levels of ET predicting cerebral ischemia and symptomatic vasospasm [51, 52]. The potential involvement of ET in cerebral vasospasm following SAH has triggered considerable interest in designing therapeutic strategies to inhibit biological effects of ET. Major approaches have included: (i) reducing the levels of circulating ET-1 by specific anti-ET-1 antibodies, (ii) antagonizing ET receptors, and (iii) suppressing the biosynthesis of ET-1 using inhibitors of endothelin-converting enzymes (ECEs) [14, 53]. A number of experimental studies demonstrate the preventive or therapeutic potential of ET receptor antagonists in the context of SAH [54, 55]. Importantly, it was recently found that specific block of endothelin pathways, albeit effective at reducing vasoconstriction, may not improve patient outcome post-SAH [3, 17].

ET-1 has a number of effects other than vasoconstriction. It can also cause vasodilation via release of nitric oxide (NO) and prostacyclin. More importantly, ET-1 plays a role in fibrosis, endothelial and smooth muscle proliferation, and inflammation. It increases the contractile potency of other vasoconstrictors such as norepinephrine, serotonin, and angiotensin I. It contributes to vascular inflammation by stimulating neutrophil adhesion, platelet aggregation, and smooth muscle proliferation [15, 16].

Heparin interferes with ET-1 and its downstream effects at multiple levels. Heparin downregulates transcription of the ET-1 gene in endothelial cells, with ET-1 mRNA expression significantly suppressed by heparin in a dose-dependent manner [31, 32, 56, 57].

ET-1-induced vasoconstriction, which involves a complex signaling pathway, is inhibited by heparin. Activation of the endothelin type A receptor by ET-1 results in transactivation of the epidermal growth factor receptor (EGFR), which is required for a full constrictive response to ET-1. Importantly, the specific ligand involved in EGFR transactivation has been identified as *heparin*-binding epidermal growth factor (HB-EGF) [30]. Thus, vasoconstriction induced by ET-1 is antagonized by heparin, which neutralizes activity of HB-EGF. Notably, HB-EGF−/− mice are available [30], but to our knowledge, have not been assessed for their susceptibility to SAH-induced DNDs.

Heparin also antagonizes ET-1 actions other than vasoconstriction. Heparin significantly suppresses ET-1-induced increases in intracellular calcium and inositol 1,4,5-trisphosphate levels in cultured vascular smooth muscle cells in a dose-dependent manner, and it inhibits ET-1 release from cultured endothelial cells [32]. Constitutive ET-1 overexpression (as may occur in an inflammatory environment) promotes VSMC proliferation via an external autocrine mechanism [58]. Heparin inhibits sustained activity of MAPK kinase-1 and prevents DNA synthesis induced by endothelin-1 [59].

Potassium Channels

Apart from ET-1, vasoconstriction associated with vasospasm may be due to altered function of potassium channels [60]. In VSMC, cytoplasmic calcium concentration is a critical determinant of contractile state, and key mediators determining calcium influx (and hence intracellular calcium concentration) are voltage-dependent calcium channels (L-, R-, T-types). As implied by the name, "voltage-dependent" calcium channel, a critical regulator of the activity of these channels is the membrane potential of the cell, which is determined largely by potassium channels.

Several types of K^+ channels are expressed in cerebral vascular smooth muscle, including several shown to be downregulated in the context of experimental SAH [60, 61]. Germane to the present discussion, K_V channels are inhibited by heparin due to regulation by HB-EGF which, as mentioned above, is a necessary intermediate in ET-1-mediated vasoconstriction. In an elegant series of experiments, Wellman and colleagues [62, 63] showed that OxyHb suppresses K_V currents in cerebral vascular smooth muscle cells, probably due to channel endocytosis, and that this effect is mediated by HB-EGF. They showed that OxyHb activates matrix-metalloproteinase, which in turn cleaves pro-HB-EGF, releasing if from the membrane and allowing it to bind to its cognate receptor, EGFR. This complex mechanism of K^+ channel down-regulation is believed to be responsible for OxyHb-mediated membrane depolarization, calcium channel activation and vasoconstriction that are the hallmarks of the smooth muscle pathophysiology following SAH.

Growth Factors

Cerebral blood vessels affected by vasospasm exhibit structural changes consistent with the actions of vascular mitogens (growth factors) [64–66]. Smooth muscle and myofibroblast proliferation, as well as cellular necrosis and remodeling, are common features of vasospastic segments. Intimal hyperplasia, as well as collagen deposition and fibrosis, have also been extensively described. These "late" arterial wall changes are not believed to be responsible for vasospasm per se [67, 68]. However, it is possible that early phenotypic changes in VSMC that are induced by growth factors contribute to reduced cell polarization, altered response to vasodilatory agents and decreased vessel compliance after SAH [69].

There is growing appreciation that the MAPK (ERK1, ERK2) cascade may play an important role in cerebral vasospasm [70–73]. MAPK is a family of serine/threonine protein kinases involved in cell growth, transformation, and proliferation through activation of transcription factors and target genes. It also plays an important role in prolonged smooth muscle contraction by phosphorylating caldesmon, which is a thin filament-associated protein that inhibits α-actin. Tyrosine kinase and MAPK cascades play a role in hemolysate-induced contraction of rabbit cerebral arteries, independent of intracellular Ca^{2+} concentration [70].

The MAPK cascade is upregulated by several mechanisms relevant to SAH, including FR such as superoxide anion, inflammatory cytokines such as TNFα, and by one or more growth factors, including: (i) HB-EGF [74], (ii) platelet-derived growth factors [75], and (iii) thrombin [73], each of which deserves brief review.

HB-EGF, the heparin-binding member of the EGF family implicated in ET-1-mediated vasoconstriction and in K_V channel down-regulation (see above), has also been implicated in vascular pathological processes, including cerebral ischemia and VSMC hyperplasia [76–78].

Platelet-derived growth factors have also been implicated in SAH-induced vessel wall changes. As reviewed in Borel et al. [79], coagulation of subarachnoid blood activates platelets, which release potent growth factors, including platelet-derived growth factor-AB (PDGF-AB), transforming growth factor-β1 (TGF-β1), and vascular endothelial growth factor (VEGF). The PDGFs and TGF-β are potent mitogens for smooth muscle cells in the vascular media and fibroblasts in the adventitia, whereas VEGF stimulates proliferation of vascular endothelium. Previous

studies have shown that PDGFs are increased in the CSF of patients with SAH (see [79, 80] for additional citations).

Thrombin has also been implicated in SAH-induced vessel wall changes. Subarachnoid clot releases thrombin, which can act as a growth factor. As reviewed in Zhang et al. [81] and in Tsurutani et al. [82], once bleeding into the subarachnoid space occurs, thrombin is activated rapidly and remains at a high level because a firm, persistent fibrin network is produced through activation of the coagulation system in the subarachnoid space. CSF thrombin is only minimally inactivated by the antithrombin-III found in circulating blood and by the thrombomodulin found in vascular endothelial cells. Post-SAH CSF thrombin activity is correlated with the persistence of blood and development of vasospasm. After SAH, levels of thrombin-AT-III complex and prothrombin fragment F1 + 2, both molecular markers of CSF thrombin activation, are elevated and these levels correlate well with both the clinical severity at the onset of SAH and the occurrence of cerebral vasospasm (see [82] for additional citations).

Heparin suppresses phenotypic changes of VSMC associated with proliferation in vitro, prevents intimal hyperplasia after arterial injury in vivo [59, 83], and inhibits growth factors involved in vessel wall changes post-SAH. As implied by its name, heparin is a potent inhibitor of HB-EGF and thus blocks HB-EGF-mediated VSMC hyperplasia [74, 84]. Moreover, heparin blockade of thrombin-induced VSMC migration involves inhibition of EGFR transactivation by HB-EGF-like growth factor [37, 85, 86]. Heparin is a potent modulator of receptor binding of growth factors including fibroblast growth factor (FGF) VEGF and PDGF [87–89], and heparin inhibits thrombin-induced mitogen-activated protein kinase signaling in VSMC [59]. Localized release of perivascular heparin inhibits intimal proliferation after endothelial injury without systemic anticoagulation [86]. Heparin reduces proliferative angiopathy following subarachnoid hemorrhage in cats [90]. The preventive effect of intracisternal heparin regarding proliferative angiopathy after experimental SAH in rats [44] was mentioned above.

Chemokines, Cytokines

As detailed in the excellent reviews by Dhar and Diringer [91] and by Provencio and Vora [5], activation of a systemic immune response after SAH is frequently manifested by elevated levels of circulating cytokines, the major effectors of systemic inflammation. The clinical manifestations of this process have been termed the Systemic Inflammatory Response Syndrome (SIRS), a constellation of findings originally described in association with sepsis. SIRS is characterized by elevated heart rate, respiratory rate, temperature, and leukocyte count. It reflects a systemic process associated with endothelial activation and dysfunction that predisposes to altered tissue perfusion, organ failure, and worse outcome. This host response also includes activation of complement and coagulation cascades, with the potential for thrombosis and impaired microcirculatory flow. High levels of catecholamines are released after SAH and are known to correlate with extra-cerebral organ dysfunction including myocardial stunning, neurogenic pulmonary edema and activation of a systemic immune response. SIRS is seen in most patients with SAH, and is associated with extra-cerebral organ dysfunction and worse outcome. Its components, such as fever and leukocytosis, have long been associated with adverse events after SAH.

SIRS is associated with vasospasm [92]. There is growing recognition that inflammation, both local (neuroinflammation) and systemic, plays an important role in the pathogenesis of vasospasm after SAH [93–98]. Infiltrates of inflammatory cells are seen in the walls of vasospastic arteries. Levels of several important proinflammatory proteins such as ICAM-1, VCAM-1, L-selectin, and E-selectin are elevated after SAH compared with control patients. Levels and time course of E-selectin, a protein involved in leukocyte rolling prior to diapedesis, is associated with moderate to severe vasospasm. In addition, IL-6, IL-β, and TNFα are increased in SAH and are associated with poor outcome. Agents that block these inflammatory cascades, such as corticosteroids and non-steroidal anti-inflammatory agents, may reduce experimental vasospasm. Overall, it is likely that inflammatory activation places patients at higher risk for development of DNDs, both ischemic and otherwise (see [5, 91] for original citations).

Apart from vasospasm, neuroinflammation is also closely linked to deterioration of higher cortical function including memory [99–102]. Cognitive dysfunction and memory deficits are observed following many inflammatory states, including infection, traumatic brain injury, normal aging, and Alzheimer's disease. Similarly, cognitive dysfunction and memory deficits are very common following SAH, leading to life-altering psychosocial deficits in patients with otherwise "favorable" outcomes [103–105]. Although frequently attributed to ischemia, other mechanisms, especially neuroinflammation, are likely to play an important role, since even SAH patients without vasospasm frequently suffer from these sequelae.

Heparin possesses potent anti-inflammatory and immunomodulatory activities [22, 33, 34]. In various clinical settings involving high-risk patients, heparin can reduce the inflammatory response, lower oxidative stress, and help to ameliorate clinical outcome [106–109]. In patients as well as in experimental models of ischemia, heparin reduces inflammatory responses and is positively associated with early recovery [110, 111].

Anti-inflammatory effects of heparin are mediated through one or more of several mechanisms (Table 1).

Table 1 Molecular targets of heparin and their potential impact on inflammatory responses

Cellular target	Physiological effect
Cytokines/interleukins	Decrease TNF production and effect
	Decrease IL-1, IL-6, and IL-8 release
	Decrease TNF and IL-6
	Increase IL-10
Complement	Inhibit both the classic and alternative pathways
	Reduce with assembly of C5b-C9 (MAC)
	Decrease complement levels
Lymphocytes	Decrease cytotoxic T-cell activity
	Impair T-cell adhesion and migration
	Suppress natural killer cell activity
Neutrophils, PMN	Inhibit neutrophil superoxide production
	Reduce granulocyte activity and inhibit production of lactoferrin and myeloperoxidase
	Attenuate neutrophil infiltration
	Decrease neutrophil phagocytosis
	Attenuate CD-11b dependent adherent mechanism
Monocytes, TF, and TFPI	Increase TFPI; prolonged infusions decrease TFPI
	Bind to monocytes, inhibits TF production and gene expression
	May indirectly inhibit MTF by increasing IL-10 and decreasing IL-6 and IL-8 production

Adapted from Elsayed and Becker [33]

Heparin may exert its anti-inflammatory effects through the transcription factor, NF-κB [112]. Once bound and/or internalized into the cytoplasm, heparin can bind electrostatically to the positively charged nuclear localization sequence of NF-κB and prevent it from translocating to the nucleus. Inflammation is associated with the coordinated action of a series of cytokine and adhesion molecule genes. Regulation of these genes involves NF-κB, a ubiquitous inducible transcription factor. NF-κB is activated by a vast number of agents including cytokines, growth factors, and FR including ROS generated after SAH. The genes regulated by NF-κB are diverse but include those that transcriptionally promote expression of many inflammatory and immune response genes including ICAM-1, L- and P-selectins, and interleukin-6 and 8. In addition, the pro-inflammatory cytokine best recognized for activating NF-κB, TNFα, induces profound alterations in endothelial cell phenotype, including upregulation of expression of ET-1 [113, 114], which as reviewed above, has been strongly implicated in SAH-induced DNDs.

Anti-inflammatory effects of heparin also result from its ability to compete for binding with numerous pro-inflammatory cytokines and chemokines, and thereby inhibit their function [115–117]. Chemokines are a superfamily of small, heparin-binding cytokines that induce directed migration of various types of leukocytes through interactions with a group of seven-transmembrane G protein-coupled receptors. At present, over 40 members have been identified in humans. Chemokines are potent attractants for leukocytes such as neutrophils and monocytes, and are thus important mediators of acute and chronic inflammatory responses [116, 117]. The continuous expression of chemokines is associated with chronic inflammation [115, 118] of the sort seen after SAH.

The underlying mechanism for the anti-inflammatory effect of heparin involving cytokines and chemokines is that heparin, a soluble glycosaminoglycan (GAG), has a structure and biological activities that are similar to those of cell-surface GAG such as heparan sulfate, integral components of the extracellular matrix, cell and basement membranes. GAGs play an important role in immune and inflammatory responses. During inflammation, the electrostatic interactions of membrane-associated GAG to cytokines and chemokines generally enhance their functions and stability, promoting localization of these molecules onto the extracellular matrix or cell membranes at specific anatomical sites. Binding of cytokine to GAGs is important for augmentation of specific receptor activity and protection from proteolytic inactivation. Additionally, because they immobilize and oligomerize chemokines on endothelial surfaces, GAGs facilitate the formation of concentration gradients and thereby promote leukocyte migration. Binding of cytokine or chemokine to GAG also may cause oligomerization or conformational changes of the ligand, which influence interactions with the specific receptor. Notably, ligands bound to soluble GAG such as heparin may not be accessible for binding to their cognate receptors because of electrostatic repulsion between

mutually acidic molecules. These complex interactions between membrane-bound and circulating factors determine the ability of heparin to modulate or inhibit immune responses (see [119] for original citations).

Heparin in Patients with SAH

Having reviewed the various pathophysiological mechanisms induced by SAH that may be susceptible to blockade by heparin, we now examine the few reports available that address the potential value of heparin in managing patients with SAH. Unfortunately, the available data are too sparse and too diverse in nature to permit a proper meta-analysis.

Administration of heparin has been advocated to reduce complications of SAH [120, 121], based on the hypothesis that microemboli are an important cause of ischemic injury following SAH [8, 9]. In support of this hypothesis, an anticoagulating dose of heparin was reported to reduce ischemic events in patients with SAH who were undergoing gradual carotid ligation [122, 123]. In a related finding, the anticoagulation of patients with SAH using low molecular weight heparin (LMWH) (Enoxaparin) was found in one study to significantly reduce vasospasm [124], although this was not confirmed by a second group of investigators [125, 126]. Discrepant findings with LMWH may reflect the possibility that some other mechanism reviewed above that is targeted by unfractionated heparin (and not by LMWH), may be more important that microemboli.

Unfractionated vs. Low Molecular Weight Heparin

There is considerable evidence to support the concept that the biological effects of unfractionated heparin are not reproduced by LMWH. Different molecular weight fractions have different effects on various biological systems. Heparin inhibition of endothelial cell proliferation and organization is dependent on molecular weight in a manner that differs from that required for anticoagulant activity [127]. The binding of unfractionated heparin to thrombin-activated endothelial cells is significantly greater than the binding of LMWH [128], suggesting that unfractionated heparin would be more effective in limiting inflammatory responses involving endothelial surfaces [33]. P- and L-selectin binding is inhibited by unfractionated heparin at concentrations 12–50-fold lower than those recommended for effective anticoagulation in vivo, with LMWHs being much poorer inhibitors [129]. Similarly, the antiproliferative activity of heparin on smooth muscle is maximal in its high molecular weight component [130]. LMWHs have lower affinities than unfractionated heparin for all cell receptors studied and are less likely to exert their effects through cellular interactions [131, 132]. Rats subjected to temporary focal cerebral ischemia and that received unfractionated heparin show significantly better outcomes compared to animals receiving an equivalent dose of LMWH [133]. Such data suggest that the mixed reports of efficacy of LMWH on SAH-induced DNDs [124–126] may be related to the absence of crucial high molecular weight components that are present only in unfractionated heparin.

Manner of Administration of Heparin

The manner of administration of heparin may play an important role in efficacy. Evidence gathered in clinical trials with LMWH or UH given subcutaneously at low or moderate doses to patients with acute stroke is believed to be a poor predictor of potentially beneficial effects of UH administered IV on a weight-adjusted basis [134]. (This opinion is reinforced by the fact that the primary reason that most clinicians use early intravenous anticoagulation is to prevent early stroke recurrence, not to improve outcome after stroke [135]). Animal studies indicate that, when heparin is used to target growth factors (intimal hyperplasia), its effects are actually adverse when it is administered twice daily subcutaneously, whereas greater beneficial effects are observed when it is administered by continuous intravenous infusion [136]. This may be the reason that standard prophylaxis against deep vein thrombosis (heparin, 5000 IU subcutaneously, 2–3 times daily) has not been associated with any salutary effect regarding SAH-induced DNDs.

Is There a Role for Unfractionated Heparin in Patients at Risk for DNDs?

The use of heparin in the context of SAH has not gained popular acceptance among clinicians, due possibly to a lack of widespread appreciation of its pleiotropic properties, and to well justified fears of complications including heparin-induced thrombocytopenia (HIT) [137] and hemorrhage [55]. However, the information compiled here suggests that a non-anticoagulating dose could potentially benefit patients with SAH who are at risk for DNDs. Indeed, the authors have used the following approach for some time with encouraging results: in patients whose aneurysm is confirmed to be excluded from the circulation, unfractionated heparin (8 IU/kg/h by constant IV infusion) is started 12 h after craniotomy, with the dose being increased by 1 IU/kg/h every 12 h to a final dose of 10 IU/kg/h, and is maintained for 10–14 days. Although a review of these patients is in progress and it is too early to draw conclusions, our experience to date suggests that this regimen is safe, results in no elevation of partial thromboplastin times, and confers significant protection from early and late DNDs.

Summary

Experimental and clinical studies have shown numerous beneficial effects of heparin in remarkably divergent conditions, from cancer [138] to a variety of inflammatory conditions, including but not limited to allergic reactions, burns, inflammatory bowel disease, and vascular inflammation [33, 34, 107, 108, 111, 112, 139–142]. As reviewed above, heparin exhibits a broad diversity of biological effects, many of which can be directly tied to pathophysiological mechanisms that have been implicated in SAH-induced DNDs. As a result, heparin may well serve as a long-sought multitargeted prophylactic agent against SAH-induced DNDs. Based on the review presented here, we believe that further study of the safety and efficacy of prophylactic unfractionated heparin in patients with SAH is warranted.

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