Clot Formation, Vascular Repair and Hematoma Resolution After ICH, a Coordinating Role for Thrombin?

Richard F. Keep, G. Xi, Y. Hua, and J. Xiang

Abstract Following intracerebral hemorrhage (ICH) there is a sequential response involving activation of the coagulation cascade/platelet plug formation, vascular repair, upregulation of endogenous defense mechanisms and clot resolution. How these responses are coordinated and modified by different hematoma sizes has received little attention. This paper reviews evidence that thrombin can modulate and may coordinate the components of the endogenous response. This has potential consequences for treatment of ICH with a number of modalities.

Keywords Thrombin · Coagulation · Vascular repair · Hematoma resolution · Iron-handling proteins

Introduction

The endogenous response to an intracerebral hemorrhage (ICH) involves: (1) clot/platelet plug formation to stop the bleed, (2) vascular repair and (3) clot resolution. As clot resolution can release potentially neurotoxic chemicals (e.g., iron), there is also (4) upregulation of endogenous defense mechanisms that may counteract the effects of those chemicals (e.g., iron chelators;[1]). To avoid re-bleeding, which might occur if the clot resolved prior to completion of vascular repair or potential neurotoxicity if clot resolution occurs prior to induction of defense mechanisms, these responses must be coordinated (Fig. 1). Therapeutically, a number of approaches have been used to enhance the endogenous response. For example, Factor VIIa has been given to promote clotting [2], surgery with and without tissue plasminogen activator (tPA) has been used to accelerate clot removal [3], thiazolidinediones have been given to modulate clot phagocytosis by microglia [4], and deferoxamine has been administered to enhance iron defense [5].

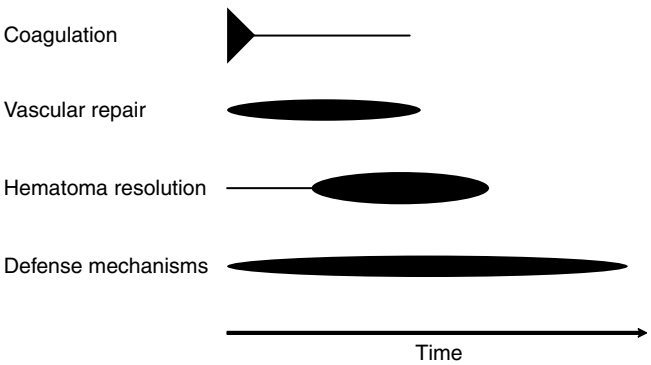

Fig. 1 The sequential endogenous response to ICH. While the coagulation cascade is activated immediately upon hemorrhage, there will be continued production of thrombin and fibrin until sufficient vascular plugging and repair have occurred to prevent further entry of fibrinogen and prothrombin. The time course of vascular repair after ICH has not been well studied. It is probably initiated immediately after ictus and is sufficient to prevent further hemorrhage by the time that hematoma resolution is complete. Fibrinolysis begins almost immediately after the hemorrhage. However, in the early stages after ICH, fibrin deposition exceeds lysis. Resolution occurs when lysis exceeds fibrin deposition (decreased thrombin cleavage of fibrinogen) and when there is clearance of cellular elements of the hematoma. The time course of resolution varies with hematoma size (*see text*). The induction of endogenous defense mechanisms involves (at least in part) the production of new protein. This involves a time delay after ictus (e.g., iron handling proteins peak 24–72 h after ICH in the rat)

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While information is available about parts of the endogenous response (e.g., the coagulation cascade and the role of microglia, leukocytes, complement and plasminogen activators in clot resolution), less is known about coordination of the endogenous responses and how they might be modulated by ICH severity. One potential coordinator of the endogenous responses is thrombin. This paper examines this potential role and highlights some therapeutic consequences.

Normal Natural History of Clot Formation and Resolution After ICH

After cerebrovascular rupture, there is almost instantaneous activation of the coagulation cascade and the start of clot formation. In about two-thirds of patients, bleeding stops soon after ictus, but in the remaining patients there is continued hematoma expansion for part of the first day [6], an expansion phase that is increased in patients on oral anticoagulant therapy [7]. In humans, the hematoma has been reported to then resolve over weeks, although this depends upon clot size [8]. It should be noted that even after the physical hematoma resolves, hematoma degradation products can still remain in the brain. Thus, high levels of iron are still found in the rat brain 28 days after ICH even though the hematoma has resolved [1].

Thrombin Generation and Coagulation After ICH

Thrombin, by converting fibrinogen to fibrin (and by stabilization of the fibrin clot via activation of Factor XIII), is central to the coagulation cascade. However, it also has other effects on the coagulation cascade, activating Factor XI, as well as cofactors VIII and V, and stimulating platelets. These effects serve to amplify the coagulation cascade. The actions of thrombin are carried out by either enzyme activity (e.g., conversion of fibrinogen to fibrin) or cell thrombin receptors (e.g., platelet activation). Three protease-activated receptors (PAR-1, -3 and -4) are thrombin receptors [9].

While thrombin is produced almost instantaneously from prothrombin at the ictus, there may be continued production until the injury site is completely plugged, preventing further entry of prothrombin into the brain. As noted above, in some patients, this may take several hours.

Thrombin and Vascular Repair

Vascular repair after ICH has received little attention. Repair entails reestablishment of the endothelial barrier, but also involves changes in other elements of the neurovascular unit (e.g., astrocytes, pericytes and vascular smooth muscle cells). Circulating endothelial progenitor cells may also be involved in re-endothelialization [10, 11]. Thrombin has profound effects on cerebral endothelial cell proliferation and shape [12, 13]. It also has marked effects on other elements of the neurovascular unit. Thus, for example, it changes astrocyte shape [14], indirectly causes pericyte contraction [15] and induces smooth muscle cell proliferation [16]. Thrombin can also cause migration of progenitor cells from the subventricular zone to sites of injury [17].

Thrombin and Clot Resolution

There is a surprising lack of definitive evidence about the relative importance of different mechanisms in clearing intracerebral hematomas. Perihematomal levels of plasminogen activators are increased after ICH [18], but the relative importance of tPA and urokinase (uPA) in fibrinolysis after ICH is uncertain. Both tPA and uPA have been used clinically to aid in clot removal, although there is, to date, no evidence that this reduces ICH-induced injury in patients [19]. Removal of the cellular constituents of the hematoma involves several processes. Erythrocyte energy failure can cause cell lysis, complement activation inserts a membrane attack complex in the cell membrane leading to lysis, and there can be phagocytosis of the erythrocytes and cell elements [4, 20, 21]. Phagocytosis can be carried out by microglia or invading macrophages from the bloodstream [4, 21].

Thrombin has the potential to impact both fibrinolysis and the clearance of cellular components of the hematoma. Thrombin indirectly impacts fibrinolysis as fibrin (and particularly partially degraded fibrin) enhances plasminogen activator activity leading to greater plasmin production [22]. Thrombin also directly induces cerebral endothelial cells to produce uPA [13]. ICH-induced inflammation is also regulated by thrombin. It upregulates inflammatory mediators, activates microglia and promotes leukocyte influx into brain [6]. Thrombin can also cause activation of the complement cascade in the brain, including membrane attack complex formation [23]. These results indicate that thrombin modulates clot resolution as well as clot formation.

Thrombin and Endogenous Defense Mechanisms

A number of mechanisms that may limit brain injury are upregulated after ICH. For example, there is a marked increase in the expression of iron-handling proteins such as ferritin, transferrin and transferrin receptor [1]. These changes

may reduce the iron toxicity that can follow hematoma resolution [6]. While expression of iron handling proteins is regulated by a number of systems, intracerebral thrombin triggers a marked increase in transferrin and the transferrin receptor expression in brain [24]. As thrombin is produced immediately upon hemorrhage, the upregulation in iron handling proteins occurs early after ICH [1], allowing the proteins to be present before the onset of erythrocyte lysis within the hematoma. Iron itself can also upregulate the expression of iron handling proteins [25], but if iron release during hematoma resolution were to be the sole signal for upregulation, there would be potential iron-induced neurotoxicity in the hours necessary for protein upregulation.

Thrombin Inhibition After ICH

Understanding the temporal effects of thrombin in ICH not only requires knowledge of its production and targets, it also requires information about what factors inhibit thrombin's action and how these change after ICH. Protease nexin-1 (PN-1) is the main thrombin inhibitor present in brain. It is highly expressed in astrocytes, but there is also evidence for neuronal expression [9]. PN-1 (mRNA and protein) is upregulated in the brain after rat ICH [26]. Plasminogen activator inhibitor (PAI)-1 can also inhibit thrombin, and it too is upregulated after ICH [27]. However, although these inhibitors may inhibit free thrombin, a substantial amount of thrombin remains bound within the hematoma where it is protected from inactivation [28]. Thus, as the hematoma resolves there is continual release of thrombin from the hematoma. The action of that thrombin will depend on access to targets (for example, fibrinogen and platelets).

Thrombin as a Coordinator of the Endogenous Responses and Therapeutic Consequences

A temporally coordinated endogenous response to ICH involving coagulation/platelet plug formation, vascular repair, hematoma resolution and upregulation of defense mechanisms necessitates some crosstalk between these different events. The pleiotropic effects of thrombin suggest that it may be involved in such coordination (Fig. 2). As described above, it is central to the coagulation cascade and platelet plug formation, it affects vascular repair and hematoma resolution, and it can induce some of the endogenous defense mechanisms. Thrombin is initially formed at the onset of hemorrhage, and this is important for coagulation and platelet plug formation. This early production of thrombin may also be important for initiating vascular repair and providing the signal for upregulation of defense mechanisms, such as iron handling proteins.

The release of clot-bound thrombin may also provide a way of coordinating hematoma resolution with vascular

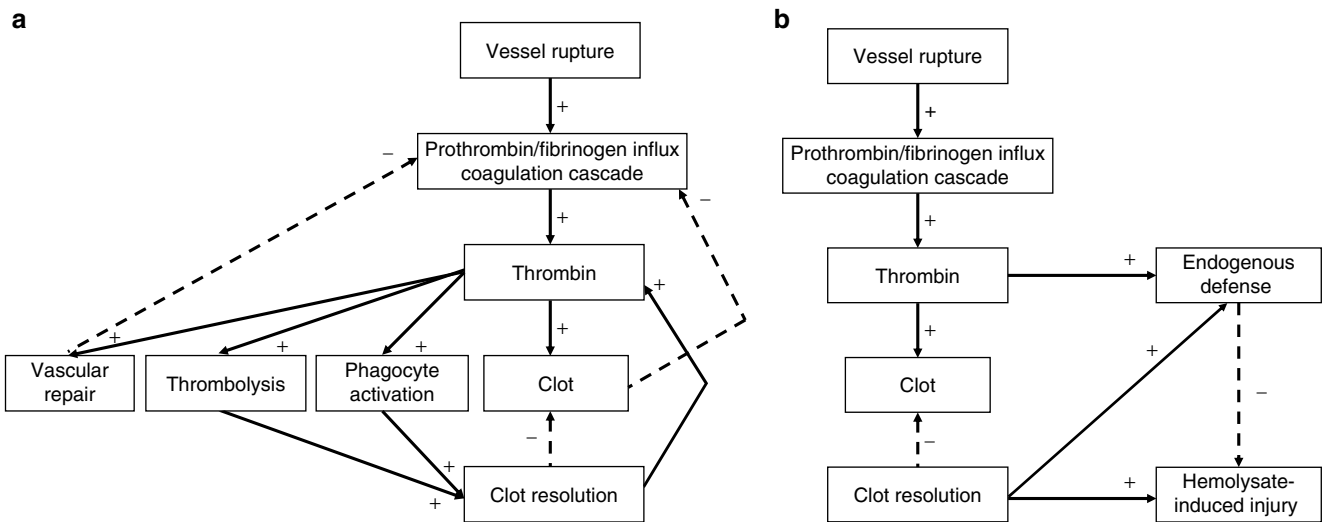

Fig. 2 Thrombin impacts many elements of the endogenous response to ICH. (**a**) The production of thrombin serves to seal the site of initial rupture via production of a clot/platelet plug, and it is also involved in regulating vascular repair. Thrombin is also involved in clot resolution. By sealing the rupture, clotting and vascular repair can stop further entry of prothrombin and fibrinogen into the brain, preventing further fibrin deposition. In addition, thrombin activates microglia, promotes macrophage entry into the brain (both cell types are involved in hematoma phagocytosis) and can accelerate fibrinolysis, aiding hematoma resolution. Because thrombin is present within the clot, hematoma resolution may cause its release, which may serve to produce more fibrin if the vasculature has not been fully repaired. (**b**) Thrombin is also involved in upregulating defense mechanisms in the brain (e.g., iron-handling proteins), which may reduce clot-derived neurotoxicity. While clot-derived factors may also upregulate defense mechanisms, thrombin upregulation may occur, earlier providing greater protection

repair. If hematoma degradation occurs before vascular repair is fully achieved, leakage of fibrinogen from the damaged vessel may be cleaved by thrombin from the degrading hematoma to form a new fibrin clot. This might provide a quicker response than activation of the coagulation cascade and creation of thrombin from prothrombin leaking from the bloodstream. In addition, the continual release of clot-bound thrombin from the resolving hematoma may provide a signal to maintain upregulation of phagocytosis and defense mechanisms until the clot has finally resolved. If this is the case, thrombin may also participate in ending the endogenous response to ICH. As the vessel is repaired and the hematoma resolves, there will be a decline in extravascular thrombin, and this may be a signal for the cessation of the inflammatory response and the return of defense mechanisms to normal levels. This potential role of thrombin requires further examination.

ICH severity varies from microbleeds to hematomas of over 100 mL. Presumably, the extent of the endogenous response needs to vary with the size of the hematoma. As the amount of prothrombin entering the brain with the hemorrhage varies with the size of the hematoma, thrombin production would reflect the severity of the hemorrhage and may regulate the endogenous response to match ICH size.

A role for thrombin in coordinating the endogenous response would have several therapeutic consequences. Thrombin has been the target in clinical and preclinical ICH trials. Increasing thrombin production to prevent hematoma expansion was the basis of the Factor VIIa trial [2]. While that trial showed that Factor VIIa could reduce hematoma growth, it did not improve outcome. In contrast, there is preclinical evidence that thrombin can participate in ICH-induced injury [29], and delayed thrombin inhibition with argatroban improved outcome in a rat ICH model [30]. These results reflect the differential time-dependent roles of thrombin in ICH.

Clot removal has been examined extensively as a method of reducing ICH-induced injury without, as yet, significant clinical benefit [19]. The poor outcome in patients that had ultra-early clot removal because of rebleeds [31] reflects the importance of ensuring that vascular repair has occurred prior to clot removal/resolution. Whether more delayed clot removal may have unanticipated effects on ICH-induced injury by affecting the endogenous response to ICH by removing clot-bound thrombin has not been examined (e.g., will it affect the upregulation of endogenous defense mechanisms?).

Summary

Thrombin has pleiotropic effects on the response of the brain to ICH, including roles in coagulation/platelet plug formation, vascular repair, clot resolution and upregulation of endogenous defense mechanisms, and may act as a coordinator of the endogenous response. Understanding the integration of the response of the brain to ICH may give insight into how better to modulate that response to reduce ICH-induced injury.

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Conflict of interest statement We declare that we have no conflict of interest.

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