Activation of complement by tissue plasminogen activator, but not acute cerebral ischemia, in a rabbit model of thromboembolic stroke

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Activation of complement has been described in a variety of autoimmune–inflammatory states. 16,19,25,28 More recently, complement activation has been demonstrated to occur in a variety of diverse pathophysiological states, including myocardial ischemia,24 adult respiratory distress syndrome,22,29 in carotid endarterectomy specimens,26 and following spinal cord injury23 and cerebral ischemia.7 However, the studies examining complement activation following central nervous system injury have been limited to the subacute or chronic state; moreover, the degree of activation demonstrated in these studies has been attributed to causes such as antiphospholipid antibodies rather than the primary insult.

Although complement activation is associated with tissue injury during inflammatory and ischemic states, complement activation in states of acute cerebral ischemia before and after administration of tissue plasminogen activator (TPA) has not yet been examined and is the focus of this investigation.

Twenty-four New Zealand White rabbits weighing 3 to 3.5 kg were used for this study. Of these, 20 were subjected to intracranial autologous clot embolization via the internal carotid artery. Three hours postembolization, rabbits received an intravenous infusion of TPA (6.3 mg/kg, 20% bolus with the remainder infused over a 2-hour interval; 12 animals) or vehicle (eight animals). All animals were observed for a total of 7 or 8 hours postembolization. These two groups were compared to a cohort undergoing sham operation with subsequent TPA infusion (four animals). Plasma samples to quantify complement component C5 hemolytic activity (C5H5O) were obtained at the following time points: 30 minutes before and after clot embolization; 1 hour before and 1 hour after the initiation of therapy with TPA or vehicle and at the completion of the protocol; 7 to 8 hours after clot embolization.

The C5 activation was not detected as the result of acute cerebral ischemia. However, animals receiving TPA with or without concomitant clot embolization exhibited C5 activation as assessed by a reduction in C5 hemolytic function, both 1 hour after initiation of TPA infusion (78.7 ± 10.3% and 77.5 ± 9.9% of baseline value, respectively; mean ± standard error of the mean [SEM]) and at the end of the protocol, 2 hours after the completion of the TPA infusion (72.5 ± 8.8% and 53.3 ± 8.1%, respectively; mean ± SEM, p < 0.05, each group). This study supports the conclusion that TPA, but not acute cerebral ischemia, may activate the complement cascade in this rabbit model of thromboembolic stroke.

KEY WORDS • complement • cerebral ischemia • neutrophil • inflammation • tissue plasminogen activator • rabbit


C O M P L E M E N T activation has been described in a variety of autoimmune–inflammatory states.16,19,25,28 More recently, complement activation has been demonstrated to occur in a variety of diverse pathophysiological states, including myocardial ischemia,24 adult respiratory distress syndrome,22,29 in carotid endarterectomy specimens,26 and following spinal cord injury23 and cerebral ischemia.7 However, the studies examining complement activation following central nervous system injury have been limited to the subacute or chronic state; moreover, the degree of activation demonstrated in these studies has been attributed to causes such as antiphospholipid antibodies rather than the primary insult.

Complement activation has also been demonstrated in states of ischemia–reperfusion injury, such as experimental models of pulmonary4 and myocardial occlusion and reperfusion.18,27 Although the significance of complement activation in states of pulmonary injury is controversial, suppression of complement activation following experimental myocardial ischemia and ischemia and reperfusion has been demonstrated to reduce the degree of injury.14,17,20

Thrombolytic therapy for acute cerebral ischemia has received considerable attention as a strategy to reduce brain injury in thromboembolic stroke.13 However, the product of tissue plasminogen activator (TPA), plasmin, has been demonstrated to activate the complement cascade.5 Indeed, a preliminary assessment of six patients receiving TPA for myocardial ischemia demonstrated complement activation. The products of complement activation such as the anaphylatoxins C3a and C5a may induce leukocyte activation, vasoconstriction, and increased vascular permeability, including alterations in blood–brain barrier permeability.3,6,9,13 Additionally, the complement membrane attack complex has been demonstrated to be cytolytic21 as well as a mediator of cardiac dysfunction.6,14

It is unknown whether acute cerebral ischemia results in complement activation nor has the ability of TPA to affect the complement cascade during cerebral ischemia been
confirmed. It was therefore the purpose of this study to examine complement activation after acute cerebral ischemia, TPA therapy, or both in an established rabbit model of thromboembolic stroke.

Materials and Methods

Twenty-four New Zealand White rabbits of either sex weighing 3 to 3.5 kg were used for this study. Twenty animals were monitored and received an autologous clot embolus to the intracranial circulation as has been previously described in detail. A reduction in cerebral blood flow to less than 15 ml/100 g/minute (hydrogen clearance technique) assured uniformity of cerebral injury. The four sham-operated animals were treated and monitored exactly as described for the embolized groups except for clot embolization.

All animals received either TPA (6.3 mg/kg, 20% bolus followed by 2-hour continuous infusion; 12 animals) or control vehicle (0.9% saline) intravenously 3 to 4 hours after autologous clot embolization (eight animals) or sham carotid isolation (four animals). All animals received ventilatory support for a total of 4 hours from the time of initiation of therapy with TPA or vehicle. That is, the experimental protocol lasted 7 or 8 hours after clot embolization. Results from the 7- and 8-hour studies were identical and therefore were pooled for this analysis.

Serum and plasma samples were collected on ice, the former were allowed to clot and the latter were added to ethylenediamine tetraacetic acid. All samples were then centrifuged at 4˚C and the serum and plasma samples were stored immediately at −270˚C until analyzed for complement activity.

Serum and plasma samples were collected at the following time points: 30 minutes before (baseline) and after autologous clot embolization or sham carotid isolation (eight animals) or sham procedure (four animals). All animals received ventilatory support for a total of 4 hours from the time of initiation of therapy with TPA or vehicle. That is, the experimental protocol lasted 7 or 8 hours after clot embolization. Results from the 7- and 8-hour studies were identical and therefore were pooled for this analysis.

When complement activation occurs, C3 and C5 are cleaved and inactivated, and the generation of C3 and C5 fragments (C3a and C5a, C5b and C5b) is proportional to the extent of activation. Because there are no readily available commercial antibodies or assays for rabbit complement fragments (C3a and C5a), we chose to evaluate complement activation by looking at decreases in the hemolytic activity of C5, which is inversely proportional to the extent of activation.

During the experimental protocol, mean arterial pressure (50–60 mm Hg), core and mean temperature, and arterial blood gas levels were all maintained within physiological ranges. At the end of the protocol, the rabbits were killed with an overdose of sodium pentobarbital (150 mg/kg) in accordance with procedures outlined by the University of Vermont Institutional Animal Care and Utilization Committee.

Results

No significant activation of C5 could be detected up to 8 hours after autologous clot embolization (Fig. 1). At the final time point, 7 to 8 hours after autologous clot embolization, C5H5O levels were 90.5 ± 15% of baseline values (mean ± standard error of the mean, eight animals). In the cohort undergoing embolization and receiving TPA, significant C5 activation was noted following the initiation of the TPA (78.7 ± 10.3% baseline). Further activation of C5 was detected at the completion of the protocol, with a value of 72.5 ± 8.8% of baseline levels noted (Fig. 1; p < 0.05). A similar degree of C5 activation was demonstrated in the group receiving TPA without subsequent clot embolization (77.5 ± 9.9% and 53.3 ± 8.1% baseline, 1 hour after initiation of TPA, and at protocol completion, respectively; p < 0.05).

Discussion

The current study demonstrates that, in a rabbit model of thromboembolic stroke, acute cerebral ischemia does not result in significant complement activation as assessed by C5H5O levels. However, the administration of TPA 3 to 4 hours after acute cerebral ischemia did result in sig-
Activation of complement by TPA

Significant activation of C5. Similar changes in C3 activation (1 hour after initiation of TPA therapy and at protocol completion, respectively) were seen in two small cohorts (three rabbits each) treated with TPA without (80.8 ± 10.6% and 52.9 ± 9.5% baseline) or with subsequent clot embolization (47.3 ± 9.1% and 42 ± 7% baseline).

Local complement activation has been noted previously after myocardial ischemia and ischemia and reperfusion. Moreover, although suppression of complement activity in these models has significantly reduced tissue injury, the role of the complement cascade in acute cerebral ischemia has been essentially uninvestigated.

It is well established that the anaphylotoxins C3a and C5a, as well as the terminal membrane attack complex C5b-9 may result in tissue injury directly or indirectly via the release of mediators and/or secondary to leukocyte activation. The demonstration of complement activation during ischemic states and with TPA administration, as well as the established role of these complement fragments in tissue injury, indicated that it would be of great interest to examine complement activity following thromboembolic stroke, thrombolytic therapy, or both.

The complement fragment C5a is a chemotaxin and is a known activator of leukocytes. Indeed, early neutrophil activation and accumulation in states of ischemia and ischemia and reperfusion, as well as the previous identification of complement deposition within ischemic myocardium have suggested the hypothesis that complement activation in myocardial ischemia may result in further injury, possibly secondary to neutrophil activation. However, our investigation indicates that acute cerebral ischemia does not result in systemic complement activation, although the administration of TPA in acute cerebral ischemia did activate the complement cascade. It is currently unknown whether activation of the complement cascade by TPA may limit its efficacy directly by causing tissue injury or indirectly via leukocyte activation within ischemic brain regions, particularly after reperfusion. Preliminary evidence in this model suggests that TPA administration does not immediately result in neutrophil activation, as assessed by systemic neutrophil counts or by ex vivo neutrophil aggregation and oxygen free radical release. However, it is possible that a greater degree of complement activation may occur at time points later than 8 hours after clot embolization, perhaps resulting in significant effects on leukocyte function. Additionally, as noted, it is possible that the degree of complement activation noted in the current study may affect subpopulation(s) of leukocytes that are not available for sampling or that have been removed from the systemic circulation because of their activation.

An approximate 25% activation of complement was noted in this study. This represents the generation of 3000 ng of C5a per milliliter of serum, enough to create biological effects at the local level as well as systemically.

Conclusions

Acute cerebral ischemia does not result in systemic complement activation in a rabbit model of thromboembolic stroke. However, significant complement activation with TPA administration was demonstrated regardless of whether this was associated with subsequent clot embolization. This is the first study to examine complement activation in acute cerebral ischemia as well as the related effects of TPA administration in this pathophysiological state. Further studies are necessary to determine the mechanism by which TPA activates the complement cascade and to examine the significance of this activation as a possible contributor to ischemic brain injury.

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