Comparative Effectiveness of Hemostatic Therapy in Experimental Warfarin-Associated Intracerebral Hemorrhage

Sergio Illanes, MD; Wei Zhou, MD; Sönke Schwarting, MD; Sabine Heiland, PhD; Roland Veltkamp, MD

Background and Purpose—Intracerebral hemorrhage associated with oral anticoagulants has a poor prognosis. Current treatment guidelines are based on case series and plausibility only, and a common consensus on effective hemostatic therapy is missing. We compared the effectiveness of diverse hemostatic approaches in a mouse model of warfarin-associated intracerebral hemorrhage.

Methods—Male C57BL/6 mice received anticoagulant treatment with warfarin (0.4 mg/kg for 3 days). Intracerebral hemorrhage was induced by striatal injection of collagenase, and 30 minutes later, mice received an intravenous injection of saline (200 μL, n = 15), prothrombin complex concentrate (100 U/kg, n = 10), fresh-frozen plasma (200 μL, n = 13), recombinant human Factor VII activated (3.5 mg/kg, n = 8 and 10 mg/kg, n = 8), or tranexamic acid (400 mg/kg, n = 12). Intracerebral hemorrhage volume was quantified on T2-weighted images after 24 hours.

Results—Mean hematoma volumes were 7.4 ± 1.8 mm³ in the nonwarfarin controls and 21.9 ± 5.0 mm³ in the warfarin group receiving saline. Prothrombin complex concentrate (7.5 ± 2.3 mm³) and fresh-frozen plasma (8.7 ± 2.1) treatment resulted in significantly smaller hematoma volume compared with saline. Recombinant human Factor VII activated (10 mg/kg: 14.7 ± 3.4; 3.5 mg/kg: 15.0 ± 6.8 mm³) and tranexamic acid (16.2 ± 4.1 mm³) were less effective. Water content in the hemorrhagic hemisphere was similar in all groups except for tranexamic acid in which it was significantly increased.

Conclusions—Prothrombin complex concentrate and fresh-frozen plasma effectively prevent hematoma growth in murine warfarin-associated intracerebral hemorrhage, whereas Factor VIIa was less effective. Tranexamic acid exacerbates perihematomal edema in this mouse warfarin-associated intracerebral hemorrhage model. (Stroke. 2011;42:00-00.)

Key Words: experimental stroke ■ Factor VII activated ■ fresh-frozen plasma ■ intracerebral hemorrhage ■ prothrombin complex concentrate

Approximately 15% of all strokes in industrialized countries are caused by intracerebral hemorrhage (ICH), and currently approximately 10% to 18% of all ICHs are associated with prior use of oral anticoagulants. The incidence of oral anticoagulant ICH is expected to further increase in the near future in parallel with the prevalence of atrial fibrillation, the most frequent indication for long-term therapy with vitamin K antagonists. With higher strength of anticoagulation, expressed by the international normalized ratio (INR) for prolongation of the prothrombin time at the time of admission, correlates with initial hematoma size,2 progressive hematoma enlargement after admission,3 functional outcome,4 and mortality.5 Hematoma growth is an important predictor of poor outcome after ICH.6

Current treatment guidelines for warfarin-associated ICH (W-ICH) recommend the administration of coagulation factors and vitamin K.7 However, so far these guidelines are mainly based on clinical case series and expert opinion, and it is unknown which strategy is best to stop hematoma enlargement.8 Because ICH in patients on warfarin is related to a bigger hematoma size and a worse prognosis, the primary aim of acute oral anticoagulant ICH management is to reverse the anticoagulatory effect of vitamin K antagonists.9 In addition to the replacement of coagulation factors such as prothrombin complex concentrate10 (PCC) and fresh-frozen plasma10 (FFP), the administration of hemostatic agents such as recombinant Factor VII activated11 (FVIIa), and tranexamic acid12 (TA) may be beneficial. Unfortunately, clinical and experimental studies comparing these regimens in W-ICH do not exist to date. Recently, a murine model of W-ICH has been established13 and further characterized,14 so it allows preclinical testing of various therapeutic approaches.

Received June 14, 2010; final revision received August 11, 2010; accepted August 23, 2010.
From the Departments of Neurology (S.I., W.Z., S.S., R.V.) and Neuroradiology (S.H.), University Heidelberg, Heidelberg, Germany; UTAC (S.I.), Hospital Clínico Universidad de Chile, Santiago, Chile; and Neuroradiomedico Clínica Dávila (S.I.), Santiago, Chile.
Correspondence to Roland Veltkamp, MD, Department of Neurology, University of Heidelberg, Im Neuenheimer Feld 400, Heidelberg 69120, Germany. E-mail: roland.veltkamp@med.uni-heidelberg.de
© 2010 American Heart Association, Inc.
Stroke is available at http://stroke.ahajournals.org
DOI: 10.1161/STROKEAHA.110.593541
In this preclinical model, the total hematoma volume is achieved 6 hours after ICH induction without further hematoma enlargement.14

The main purpose of our translational study was the comparative effectiveness of different hemostatic regimens on hematoma growth after collagenase-induced ICH in mice on prior treatment with warfarin. Additionally, the impact of the hemostatics on the developing cerebral edema was compared.

Materials and Methods

The study was conducted in accordance with national guidelines for the use of experimental animals. The protocols were approved by the local and governmental committees for animal care and use (Regierungspräsidium Karlsruhe). In all experiments, sexually mature male mice were used (C57BL/6, Charles River Laboratories [Kisslegg, Germany], 10 to 12 weeks of age, body weight 20 to 27 g, n = 76).

Warfarin Administration

Warfarin is a water-soluble molecule and it is completely absorbed after oral administration.13 A total of 2.5 mg warfarin (Coumadin; Bristol-Myers-Squibb) was dissolved in 800 mL of drinking water for 72 hours as reported previously,14 assuming that the normal water intake of a C57BL/6 mouse weighing 25 g is 3.25 g/24 hours.16 Accordingly, mice received approximately 0.01 mg warfarin per day (0.4 mg/kg). The actual effect of warfarin on the coagulation was checked in each mouse by a point-of care coagulometer before ICH induction as described earlier.14 Collagenase was injected only in mice in which the INR was within the predefined target anticoagulation (PoC INR 3 to 6).

Induction of ICH

Spontaneously breathing mice were anesthetized with halothane (1.5% to 2%) in an oxygen/air mixture. Animals were placed in a stereotactic frame (Model 51650; Stoelting) with a mouse adaptor (Model 51625; Stoelting). A bore hole was drilled (0.5 mm anterior and 2 mm lateral to bregma), and a 10-µL needle (SGE) was placed into the left striatum at 3.5-mm depth from the scull. Then, 0.3 µL of saline containing 0.045 U collagenase Type VII (Sigma) was injected. After 5 minutes, the needle was withdrawn, the bore hole was sealed with bone wax, and the scalp was sutured. The surgical procedure lasted 15 to 20 minutes.

Experimental Design

After the striatal collagenase injection, the left femoral vein was exposed. Beginning 30 minutes after collagenase injection, 200 µL of saline (control) or the dissolved coagulation factors (see subsequently) was injected slowly over 5 minutes. After removal of the needle, the vein was compressed for 10 minutes and the femoral wound was sutured. After discontinuation of inhalative anesthesia, the animal was allowed to awaken and kept under a heating lamp with free access to food and water. Four different agents (each injection of 200 µL volume) were studied: (1) PCC (Beriplex 500; CSL Behring; dose 100 U/kg); (2) murine FFP (produced by centrifugation of fresh mouse blood in a EDTA-coated tube for 10 minutes at 1500 rpm as described17; (3a) FVIIa (Novoseven; NovoNordisk; dose 3.5 mg/kg), and (3b) FVIIa, at a dose of 10 mg/kg, and TA (Cyklokapron; Pfizer) at a dose of 400 mg/kg. Effect of each treatment regimen on INR was measured 30 minutes after injection using the previously established PoC coagulometry.14

MRI Hematoma Volumetry

We have previously shown that MRI T2* volume of hypointensity is an excellent surrogate of intracerebral hematoma volume.14 Mice were examined in a 2.35-T scanner (Biospec 24/40; Bruker Medizintechnik). A resonator cage with a 40-mm inner diameter was used as the radiofrequency coil. Mice were deeply anesthetized with halothane 1.5% to 2% and placed into the MRI scanner. T2*-weighted imaging was achieved using the MGE 8 echo sequence (TR 1500 ms, TE 16.5 ms, field of view 5.5×5.5 cm, matrix 256×256, 6 slices).

Measuring Cerebral Edema

Immediately after the MRI, anesthetized mice were euthanized. The brain was removed and the 2 hemispheres were divided. Each hemisphere was weighed using an electronic analytic balance (Sartorius 2001 MP2) to determine the wet weight. Then, both hemispheres were dried in an oven at 70°C for 48 hours and weighed again to obtain the dry weight. To correct the effect of the hematoma size over water content, the blood wet and dry weights were calculated apart and the hemisphere wet weight was corrected subtracting the hematoma wet weight (WWc), and the dry weight...
was corrected subtracting the hematoma dry weight (DWc). The formula \((\text{WWc}/\text{H11002})/\text{WWc}/\text{H11003} - 100\) was used to calculate the water content expressed as percentage of wet weight.\(^{18}\)

**Statistical Analysis**

All values are expressed as mean\(\pm\)SD. Comparison of mean values was performed by analysis of variance for multiple comparisons with post hoc Tukey test using SPSS 13.0 for Mac analysis software. Comparison of ordinal variables was performed by chi\(^2\). A probability value \(<0.05\) was considered statistically significant.

**Results**

**Coagulation Status**

The baseline anticoagulatory effect as measured by PoC INR did not differ among groups except for the nonanticoagulated control group (0.96\(\pm\)0.05; Figure 1). Thirty minutes after hemostatic therapy (ie, 60 minutes after ICH induction), the INRs were differently affected in the respective treatment groups: 4.6\(\pm\)0.4 in the W-saline group, 4.0\(\pm\)1.1 in the W-TA group, 3.0\(\pm\)1.1 in the FVIIa 3.5 mg/kg group, 2.7\(\pm\)0.3 in the FVIIa 10 mg/kg group, 1.8\(\pm\)0.2 in the W-FFP group, 0.9\(\pm\)0.1 in the W-PCC group, and 0.9\(\pm\)0.1 in the nonanticoagulated group (Figure 1). Thus, significant differences regarding the effectiveness of INR reversal were observed between the hemostatic approaches (analysis of variance \(P<0.0001\)).

**Hematoma Volume**

The hematoma volumes 24 hours after the ICH induction differed substantially among groups: 21.9\(\pm\)5.0 mm\(^3\) in the W-Saline group, 16.2\(\pm\)4.1 mm\(^3\) in the W-TA group, 15.0\(\pm\)6.8 mm\(^3\) in the W-FVIIa 3.5 mg/kg group, 14.7\(\pm\)3.4 in the W-FVIIa 10 mg/kg group, 8.7\(\pm\)2.1 mm\(^3\) in the W-FFP group, 7.5\(\pm\)2.3 mm\(^3\) in the W-PCC group, and 7.4\(\pm\)1.8 mm\(^3\) in the nonanticoagulated mice group (analysis of variance \(P<0.0001\); Figure 2). There was a good correlation between final hematoma size and PoC INR 30 minutes after warfarin antagonization (\(R=0.74, P<0.001\); Figure 3).

**Cerebral Edema**

Water content in the hemorrhagic hemisphere did not differ among treatment groups except for TA, which led to a significant increase of edema. (analysis of variance \(P=0.0009\); Figure 4).

**Discussion**

The major new findings of our study are: (1) PCC and FFP are equally effective in preventing hematoma growth in experimental W-ICH, whereas FVIIa is less potent; (2) effective reversal of the anticoagulatory effect of warfarin as measured by INR is a good predictor of final hematoma size; and (3) TA also has an intermediate hemostatic effect, but it substantially increases edema formation.

To date, the effectiveness of PCC and FFP has not been tested face to face in experimental W-ICH. There are several ongoing trials comparing these hemostatic approaches in patients with ICH\(^{19}\) (International Normalized Ratio Normalization in Coumadin Associated Intracerebral Haemorrhage trial) and other conditions.\(^{20}\) Retrospective clinical series suggest a relative similar effect with a slower INR reduction in FFP than PCC.\(^{21}\) In the only previous interventional experimental study in W-ICH examining the effectiveness of PCC, it restored the plasma levels of the coagulation Factors II, VII, IX, and X and reduced the hematoma volumes by approximately 58% compared with untreated animals.\(^9\) This is consistent with our findings showing a reduction of...
mean hematoma volume of 65% by PCC. Also, PCC injection normalized the INR within 30 minutes. Administration of FFP is another standard approach for patients with W-ICH.7,8 However, a limitation of reversal of warfarin-induced anticoagulation by FFP is that a large volume is required, which can cause fluid overload.22 In this study, we administered 200 µL of murine FFP, which volumewise would correspond to 6 U of FFP in humans (1 to 1.5 L). Intriguingly, hemorrhage volume was reduced as effectively by FFP as by PCC. However, translation of these findings into the clinical setting should take into consideration that PCC has advantages over FFP, including immediate availability, more rapid administration, and a smaller volume load. A disadvantage of PCC compared with FFP is its higher cost. However, using a simple point-of-care coagulometer allows rapid bedside dosing adjustments of PCC in patients with coumarin-associated intracerebral hemorrhage.23 An important difference between the protocol for injection of FFP in the present study and in clinical practice is that FFP was injected as a bolus in our model, whereas administration of FFP is frequently administered over several hours in patients.

Some case reports suggest that FVIIa may be an effective agent in hemorrhage associated with oral anticoagulants including W-ICH.24 Rapid correction of the INR was already demonstrated in a small clinical series.25 In 2 Phase III trials examining FVIIa (Novo7) in spontaneous ICH, hemostatic therapy reduced the rate and extent of early secondary hematoma growth. However, a beneficial effect on clinical outcome found in the earlier study was not confirmed in the subsequent study.26 In the present study, both a lower and a higher dose of human FVIIa failed to reduce intracerebral hematoma growth as effectively as PCC and FFP. Moreover, FVIIa only induced a partial reversal of the anticoagulatory effect of warfarin as measured by the INR. Although similar doses of human FVIIa (Novo7) have been shown to reverse the anticoagulatory effect of warfarin in other models, there is also evidence that it only transiently corrects the warfarin-induced deficiency of FVII and fails to replace the other deficient coagulation factors associated with oral vitamin K antagonist therapy.27 An alternative explanation for the only intermediate effectiveness may be that we used human factor instead of murine VIIa. No differences in the final hematoma size or coagulation defect correction were seen between both FVIIa doses. Previous studies have established that 8 mg/kg is needed to achieve the maximal effect of human FVIIa in murine bleeding studies.10 We further tried 20 mg/kg in a single mouse without a good INR correction and a big hematoma final size (data not shown); nevertheless, mice treated with FVIIa at 10 mg/kg had 49% less volume than W-Saline control mice and 50.3% more than nonwarfarin controls (Figure 5).

TA is a comparatively inexpensive antifibrinolytic that competitively inhibits the cleavage of plasminogen to plasmin. This agent was approved by the US Food and Drug Association in 2009 for treatment of menorrhagia.29 Moreover, it is currently studied in a Phase III trial for traumatic bleeding.30 In murine coagulation models, the dose used in our study had a similar effect on thrombus formation as the maximal dose used for humans (100 mg/kg). Remarkably, mice treated with TA had a significant decrease of mean hematoma volume by 26% compared with controls. Similar to FVIIa, injection of TA only slightly decreased the INR in warfarin-treated mice. Importantly, TA was the only agent that significantly increased edema formation in our study. The mechanisms underlying this side effect are unknown. Potentially, TA may accelerate fibrin clot stabilization, which is a necessary step in the early phase of perihematomal edema formation after ICH.32,33 This is a major limitation for TA use in ICH.

A limitation of our study is that no behavioral tests were performed. Future studies should examine whether the reduction of the hematoma size is associated with a better functional outcome.

In summary, our findings reveal important differences regarding the effectiveness and side effects of common hemostatic agents in experimental W-ICH. PCC and FFP appear to reverse the anticoagulatory effect of warfarin most effectively and thereby prevent secondary hematoma enlargement.

Sources of Funding
S.I. is supported by a DAAD-CONICYT Scholarship. R.V. is supported by an Else-Kröner-Memorial Scholarship. PCC (Beriplex) was a kind gift from CSL Behring.

Disclosures
None.

References


Comparative Effectiveness of Hemostatic Therapy in Experimental Warfarin-Associated Intracerebral Hemorrhage
Sergio Illanes, Wei Zhou, Sönke Schwarting, Sabine Heiland and Roland Veltkamp

Stroke. published online December 2, 2010;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2010/12/02/STROKEAHA.110.593541.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/