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## Recombinant Activated Coagulation Factor VII and Prothrombin Complex Concentrates Are Equally Effective in Reducing Hematoma Volume in Experimental Warfarin-Associated Intracerebral Hemorrhage

Frieder Schlunk, BSc, Elizabeth M. Van Cott, MD, Kazuhide Hayakawa, PhD, Waltraud Pfeilschifter, MD, Eng H. Lo, PhD, and Christian Foerch, MD

Department of Neurology (F.S., W.P., C.F.), Goethe-University, Frankfurt am Main, Germany; the Neuroprotection Research Laboratory (F.S., K.H., E.H.L., C.F.), Massachusetts General Hospital, Charlestown, MA; and the Department of Pathology (E.M.V.C.), Massachusetts General Hospital, Boston, MA

### Abstract

**Background and Purpose**—Based on an experimental model of warfarin-associated intracerebral hemorrhage, we investigated whether the rapid reversal of anticoagulation using prothrombin complex concentrates (PCC) or recombinant activated coagulation factor VII (rFVIIa) reduces hematoma volume.

**Methods**—Mice were orally pretreated with warfarin (2 mg/kg). Intracerebral hemorrhage was induced by collagenase injection into the right striatum. Forty-five minutes later, PCC (100 IE/kg), rFVIIa (1 mg/kg), or an equal volume of saline was administered intravenously. Hematoma volume after 24 hours was quantified using a photometric hemoglobin assay.

**Results**—International normalized ratio was  $4.3 \pm 0.4$  in saline-treated mice,  $0.9 \pm 0.1$  in rFVIIa mice, and  $1.4 \pm 0.2$  in PCC mice. Intracerebral hemorrhage volume was  $29.0 \pm 19.7 \mu\text{L}$  in the saline group ( $n = 7$ ),  $8.6 \pm 4.3 \mu\text{L}$  in the rFVIIa group ( $n = 6$ ), and  $6.1 \pm 1.8 \mu\text{L}$  in the PCC group ( $n = 7$ ; analysis of variance between-group differences  $P = 0.004$ ; post hoc rFVIIa versus saline  $P = 0.021$ ; PCC versus saline  $P = 0.007$ ). No significant difference was found between PCC- and rFVIIa-treated animals.

**Conclusions**—Our results suggest that PCC and rFVIIa are equally effective in restoring coagulation and preventing excessive hematoma growth in acute warfarin-associated intracerebral hemorrhage.

### Keywords

animal models; anticoagulation; ICH; intracerebral hemorrhage; warfarin

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Intracerebral hemorrhage (ICH) occurring during warfarin anticoagulation is a severe subtype of stroke with a short-term mortality rate of approximately 50%.<sup>1</sup> Current therapeutic practice is to rapidly restore coagulation using either prothrombin complex

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Correspondence to Christian Foerch, Department of Neurology, Goethe-University, Schleusenweg 2-16, 60528 Frankfurt am Main, Germany. foerch@em.uni-frankfurt.de.

### Disclosures

None.

concentrates (PCC) or recombinant activated FVII (rFVIIa).<sup>1,2</sup> Whereas PCC substitutes all 4 vitamin K-dependent coagulation factors that are diminished by warfarin therapy,<sup>3</sup> rFVIIa boosts coagulation by directly activating factor X on the surface of activated platelets.<sup>4</sup> Despite these mechanistic differences, both substances have been shown to be effective in rapidly and completely reversing anticoagulation in clinical studies as demonstrated by correcting the prothrombin time and its derived measure, the international normalized ratio (INR).<sup>5,6</sup> However, therapeutic effectiveness of PCC and rFVIIa in terms of reducing hematoma size and improving functional outcome remains undetermined. Recently, an experimental study suggested that rFVIIa may be inferior to PCC regarding the restoration of coagulation and the prevention of hematoma growth in ICH induced during warfarin anticoagulation.<sup>7</sup> We addressed this question in our well-established mouse model of anticoagulation-associated ICH.<sup>8,9</sup>

## Methods

### Experimental Procedures

Male CD-1 mice (Charles River Laboratories, Wilmington, MA) aged 12 to 16 weeks were used. Warfarin (2 mg/kg body weight) was administered orally through drinking water according to a previously established protocol.<sup>8</sup> In this anticoagulation model, INR values reach the therapeutic range used in humans after a 24-hour feeding period. After warfarin withdrawal, INR values remain within the therapeutic range for 6 hours.<sup>8</sup> The plasma activity of all 4 vitamin K-dependent coagulation factors is decreased, indicating full warfarin anticoagulation.<sup>9</sup>

First, we compared the effects of PCC (n = 5), rFVIIa (n = 6), and saline (n = 3) application, respectively, on INR and coagulation factor activity in anticoagulated mice. A group of nonanticoagulated mice (n = 3) served as controls. For doing so, PCC (100 IE/kg; Beriplex; CSL Behring, Marburg, Germany), rFVIIa (1 mg/kg; NovoSeven, Novo Nordisk, Denmark), or an equal amount of saline (200  $\mu$ L) was injected into the tail vein. Fifteen minutes later, the inferior vena cava was exposed under deep anesthesia, and 0.6 mL of blood was withdrawn. Specimens were added into glass tubes containing 66.6  $\mu$ L of 3.2% citrate. INR values and plasma activity of the vitamin K-dependent coagulation factors II, VII, IX, and X were determined as described previously.<sup>8,9</sup>

Second, to verify detrimental effects of warfarin anticoagulation on hematoma volume, we induced ICH by a stereotactic injection of 0.2 U collagenase VII-s into the right striatum of anticoagulated mice (n = 7) and nonanticoagulated controls (n = 7).<sup>8</sup> Hematoma volume was determined 24 hours after ICH induction using a photometric hemoglobin assay.<sup>8</sup>

Third, we investigated the influence of anticoagulation reversal on hematoma volume in anticoagulated mice. Forty-five minutes after ICH induction, PCC (n = 7), rFVIIa (n = 6), or saline (n = 7) was injected into the tail vein in a randomized and blinded fashion. Hematoma volume was determined as described previously.

### Statistical Analysis

We used SPSS15.0 for statistical analysis. INR and hematoma volume between groups were compared using the *t* test (2 groups) or using 1-way analysis of variance with Bonferroni correction (3 groups).

## Results

Anticoagulated mice that received a saline injection had a mean INR of  $4.3 \pm 0.4$ . INR values were significantly reduced after the administration of rFVIIa ( $0.9 \pm 0.1$ ) compared

with anticoagulated mice treated with saline. PCC therapy also lowered INR values ( $1.4 \pm 0.2$ ; analysis of variance between-group differences  $P < 0.001$ ; post hoc rFVIIa versus saline  $P < 0.001$ ; PCC versus saline  $P < 0.001$ ; Figure 1). Nonanticoagulated control mice had a mean INR of  $0.9 \pm 0.1$ . The activity of all 4 vitamin K-dependent coagulation factors was found decreased after warfarin feeding, indicating full warfarin anticoagulation (Figure 2). rFVIIa application in anticoagulated mice increased the activity level of factor VII, whereas the other factors remained largely unchanged. PCC application modestly increased the activity of all 4 coagulation factors.

Warfarin-treated mice had significantly larger hematoma volumes 24 hours after ICH induction as compared with nonanticoagulated controls ( $21.7 \pm 14.3 \mu\text{L}$  versus  $8.5 \pm 2.9 \mu\text{L}$ ;  $P = 0.034$ ; Figure 3A). Mortality was 4 of 7 mice in the anticoagulated group and 0 of 7 in the nonanticoagulated control group.

Mean ICH volume in anticoagulated mice that received saline 45 minutes after ICH induction was  $29.0 \pm 19.7 \mu\text{L}$ . Both mice treated with rFVIIa and PCC showed markedly reduced hematoma volumes compared with anticoagulated mice treated with saline (rFVIIa:  $8.6 \pm 4.3 \mu\text{L}$ , PCC:  $6.1 \pm 1.8 \mu\text{L}$ ; analysis of variance between-group differences  $P = 0.004$ ; post hoc rFVIIa versus saline  $P = 0.021$ ; PCC versus saline  $P = 0.007$ ). No significant difference in terms of ICH volume was found between animals that were treated with rFVIIa or PCC ( $P = 1.000$ ; Figure 3B). In the saline-treated group, 2 of 7 animals died. There was no mortality in the rFVIIa or the PCC group.

## Discussion

Our in vivo data suggest that the coagulation activation by rFVIIa administration and the anticoagulation reversal by PCC therapy are similarly effective in reducing INR values and preventing excessive hematoma growth in a mouse model of warfarin-associated ICH.

As for PCC therapy, the substitution of concentrated amounts of the vitamin K-dependent coagulation factors appears to be a meaningful approach to reverse warfarin anticoagulation.<sup>3</sup> Clinical studies reported rapid normalization of INR values in anticoagulated patients after bolus injection of PCC.<sup>5</sup> There is also evidence that the administration of PCC is capable of limiting the expansion of warfarin-related ICH.<sup>10</sup> In a pathophysiologically different way, rFVIIa boosts coagulation by directly activating factor X on the surface of activated platelets without the need for FVIII and FIX. This leads to thrombin generation and to the formation of a stable clot at the site of injury.<sup>4</sup> rFVIIa is highly effective in rapidly reversing warfarin anticoagulation in terms of correcting the INR.<sup>4,6,11</sup>

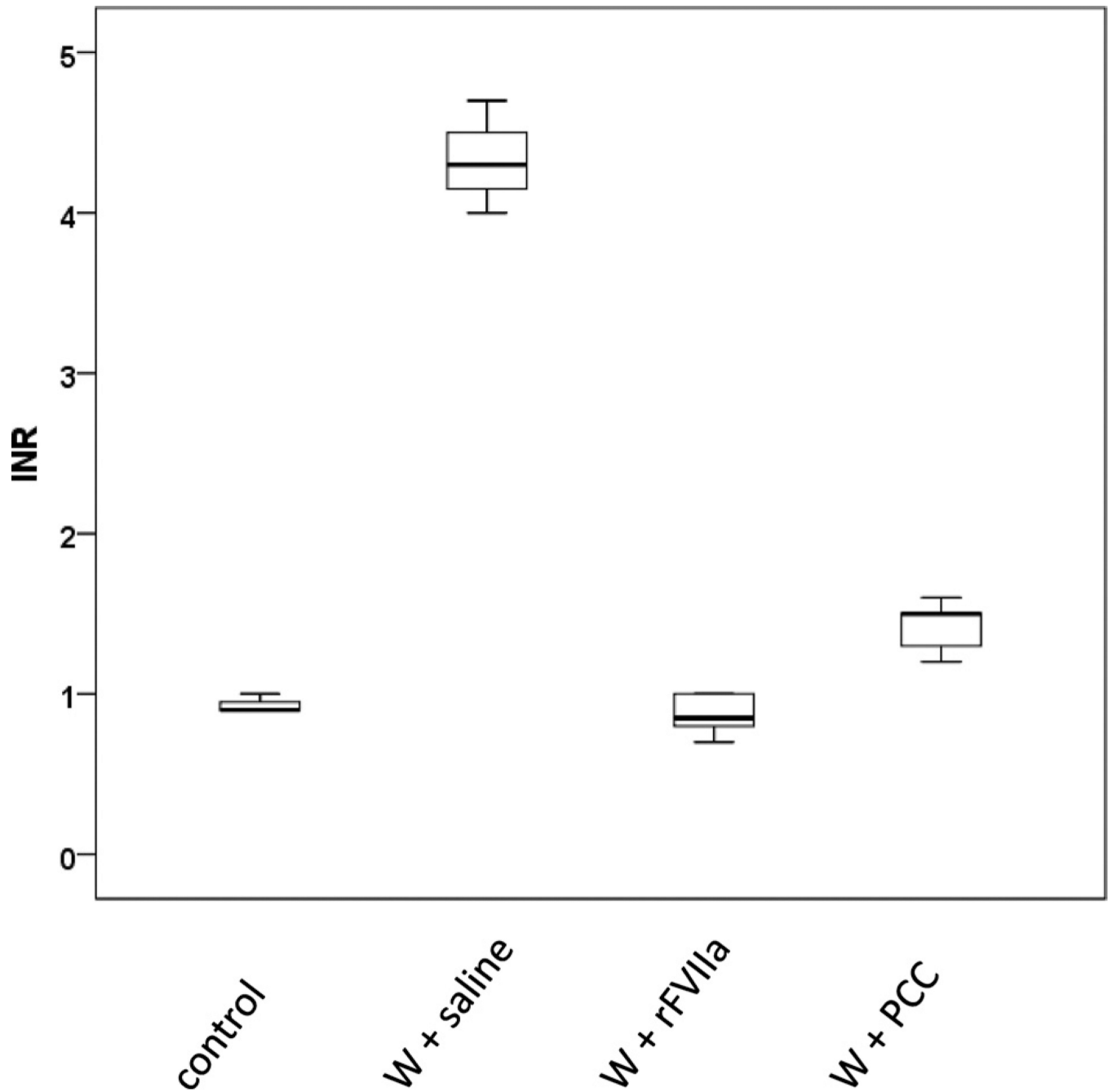
In contrast, a recently published experimental study reported rFVIIa to be not capable of normalizing INR values or preventing extensive hematoma growth as compared with PCC and fresh-frozen plasma.<sup>7</sup> In our experimental study, however, we identified rFVIIa to be highly effective in reversing warfarin anticoagulation and reducing ICH volume. Our findings are in line with other studies performed in rodents indicating normalization of INR values after rFVII therapy.<sup>12</sup> The reasons for the divergent results are not entirely clear but are most likely related to medication, dosing, or application issues. The INR with some prothrombin time reagents does not correct as much as others after administration of rFVIIa<sup>13</sup>; thus, one possibility is that the point-of-care INR device used in the other study was not as responsive to rFVIIa as the laboratory INR used in our study. However, this would not entirely explain the different effectiveness of rFVII in terms of reducing hematoma growth. In our study, INR values were determined in a reference group and not in the actual mice that underwent ICH induction due to the lethal amount of blood that had to

be collected for INR determination in the clinical laboratory. This makes a direct comparison of INR values and ICH volume impossible. Nevertheless, our model has been shown to reliably establish effective warfarin anticoagulation.<sup>7–9</sup> Another limitation is the restricted reproducibility of the collagenase ICH model, because the digestive activity of the enzyme varies between product lot numbers. This hampers the comparability of the results between different experimental studies.

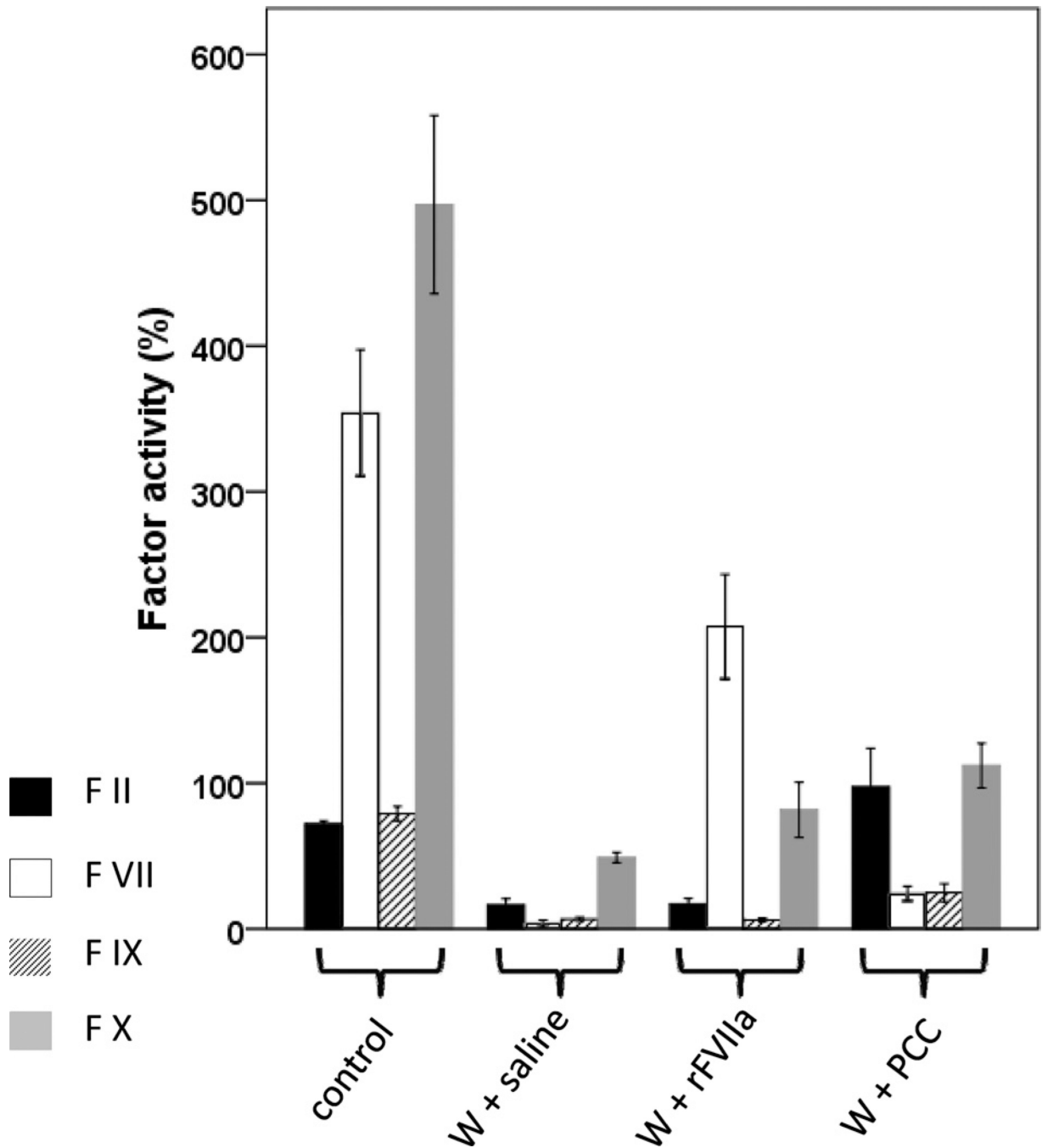
In summary, our results suggest that both PCC and rFVIIa are potent treatment options for anticoagulated patients with ICH, but human studies would be needed to confirm these findings.

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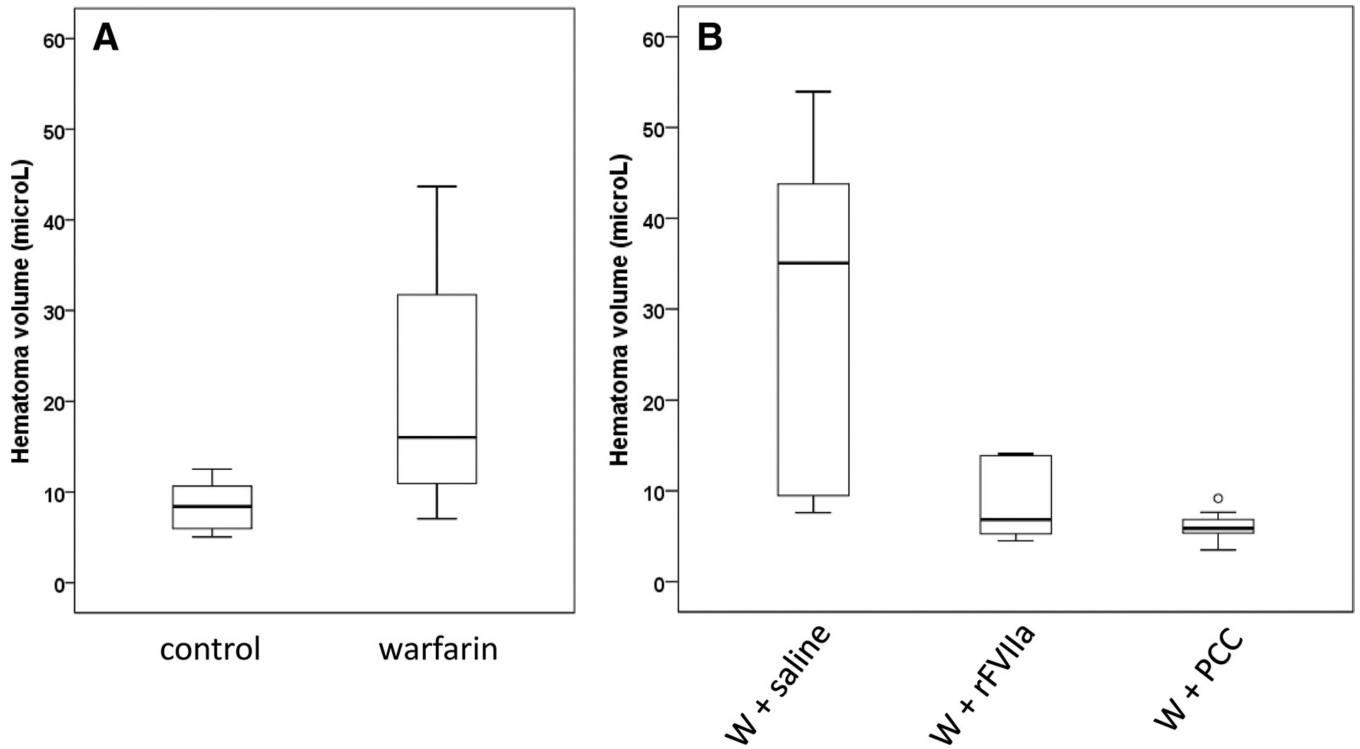


**Figure 1.** INR values in untreated controls (control; n = 3) and in anticoagulated mice that received saline (W + saline; n = 3), rFVIIa (W + rFVIIa; n = 6), or PCC (W + PCC; n = 5), respectively. INR values are represented as box and whiskers plots. By definition, the box indicates the 25th and 75th percentile of the distribution and the whiskers the 10th and 90th percentile, respectively. INR indicates international normalized ratio; rFVIIa, recombinant activated coagulation factor VII; PCC, prothrombin complex concentrates.



**Figure 2.**

Activity of the vitamin K-dependent coagulation factors II, VII, IX, and X, measured with human factor deficient plasma. As compared with untreated controls, warfarin anticoagulation led to a drop in activity of all 4 factors. rFVIIa therapy resulted in an increase in factor VII activity. PCC application modestly increased the activity of all 4 coagulation factors. Data are presented as mean  $\pm$  SEM. rFVIIa indicates recombinant activated coagulation factor VII; PCC, prothrombin complex concentrates.



**Figure 3.**

**A**, Hematoma volume 24 hours after ICH induction was larger in warfarin-treated mice ( $n = 7$ ) as compared with nonanticoagulated controls ( $n = 7$ ;  $P = 0.034$ ). **B**, Hematoma volume 24 hours after ICH induction in anticoagulated mice receiving saline (W + saline;  $n = 7$ ). Both rFVIIa- (W + rFVIIa;  $n = 6$ ) and PCC- (W + PCC;  $n = 7$ ) treated groups showed significantly smaller hematoma volumes. ICH indicates intracerebral hemorrhage; rFVIIa, recombinant activated coagulation factor VII; PCC, prothrombin complex concentrates.