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Effects of Remote Ischemic Preconditioning on the Coagulation Profile of Patients With Aneurysmal Subarachnoid Hemorrhage: A Case-Control Study

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BACKGROUND: Animal studies suggest that ischemic preconditioning prolongs coagulation times.

OBJECTIVE: Because coagulation changes could hinder the translation of preconditioning into clinical settings where hemorrhage may be an issue, such as ischemic or hemorrhagic stroke, we evaluated the effects of remote ischemic preconditioning (RIPC) on coagulation in patients undergoing RIPC after aneurysmal subarachnoid hemorrhage (SAH).

METHODS: Twenty-one patients with SAH (mean age, 56.3 years) underwent 137 RIPC sessions 2 to 12 days after SAH, each consisting of 3 to 4 cycles of 5 to 10 minutes of lower limb ischemia followed by reperfusion. Partial thromboplastin time (PTT), prothrombin time (PT), and international normalized ratio (INR) were analyzed before and after sessions. Patients were followed for hemorrhagic complications.

RESULTS: No immediate effect was identified on PTT (mean pre-RIPC, 27.62 s; post-RIPC, 27.54 s; $P = .82$), PT (pre-RIPC, 10.77 s; post-RIPC, 10.81 s; $P = .59$), or INR (pre-RIPC, 1.030; post-RIPC, 1.034; $P = .57$) after each session. However, statistically significant increases in PT and INR were identified after exposure to at least 4 sessions (mean PT pre-RIPC, 11.33 s; post-RIPC, 12.1 s; $P = .01$; INR pre-RIPC, 1.02; post-RIPC, 1.09; $P = .014$, PTT pre-RIPC, 27.4 s; post-RIPC, 27.85 s; $P = .092$) with a direct correlation between the number of sessions and the degree of increase in PT (Pearson correlation coefficient = 0.59, $P = .007$) and INR (Pearson correlation coefficient = 0.57, $P = .010$). Prolonged coagulation times were not observed in controls. No hemorrhagic complications were associated with the procedure.

CONCLUSION: RIPC by limb ischemia appears to prolong the PT and INR in human subjects with SAH after at least 4 sessions, correlating with the number of sessions. However, values remained within normal range and there were no hemorrhagic complications.

KEY WORDS: Blood coagulation, Intracranial aneurysm, Ischemic preconditioning, Subarachnoid hemorrhage

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Ischemic preconditioning is a phenomenon in which a brief period of sublethal ischemia induces endogenous protective mechanisms that increase tissue tolerance to subsequent lethal ischemia. The preconditioning ischemic stimulus can occur in distant tissue, conferring a systemic protective effect to remote organs. This phenomenon has been described as remote ischemic preconditioning (RIPC).[1]

ABBREVIATIONS: aPTT, activated partial thromboplastin time; **INR,** international normalized ratio; **MCA,** middle cerebral artery; **PT,** prothrombin time; **PTT,** partial thromboplastin time; **RIPC,** remote ischemic preconditioning; **SAH,** subarachnoid hemorrhage

In general, ischemic preconditioning stimulates endogenous anti-ischemia defense mechanisms and helps to preserve tissue energy levels during periods of decreased perfusion. The mechanisms underlying ischemic preconditioning appear to be multifactorial, and many aspects of its effects are still poorly understood. Recently, it was demonstrated that one of its

effects may be on the coagulation cascade, prolonging the clotting times of treated subjects.[2-4] Warzecha et al[4] demonstrated that preconditioning results in a significant increase in activated partial thromboplastin time (aPTT), plasma levels of D-dimers and euglobulin clot lysis time. Likewise, Stenzel-Poore et al[3] and He et al[2] both found a prolonged bleeding time in animals that underwent preconditioning by temporary occlusion of the middle cerebral artery (MCA) compared with controls.

Thrombin, the end product of the coagulation cascade, is known to play a role in various aspects of brain injury. For example, after intracerebral hemorrhage, thrombin is known to contribute to the early formation of brain edema by directly disrupting the blood-brain barrier. Furthermore, it contributes to infiltration of inflammatory cells, vascular damage, and cellular toxicity.[5,6] Therefore, longer blood clotting times after preconditioning could have protective effects by decreasing perihematomal edema and tissue toxicity after intracerebral hemorrhage. Thrombin also obviously contributes to the thrombus formation underlying ischemic stroke, and its reduction could limit thrombus formation after ischemic stroke.[3,6,7]

These effects of thrombin and the fact that preconditioning reduces coagulation are potentially somewhat of a double-edged sword. On the one hand, the effect of preconditioning effect on coagulation may mitigate some of the negative effects of thrombin formation in the setting of hemorrhagic and/or ischemic stroke. On the other hand, abnormal coagulation profiles could increase the risk of hemorrhagic transformation and hematoma expansion after both hemorrhagic and ischemic stroke, which could potentially limit the translation of preconditioning techniques into clinical practice.

Because there has been increasing interest in finding a role for ischemic preconditioning for cerebrovascular conditions, further evaluation of its effects on coagulation in humans is warranted. To this end, we set out to evaluate the immediate and late effects of RIPC on coagulation in patients enrolled in 2 trials of RIPC after aneurysmal subarachnoid hemorrhage (SAH).[8-10]

PATIENTS AND METHODS

After approval from the local institutional review boards, we studied the 21 subjects of the ongoing phase I clinical trials of RIPC after SAH enrolled to date at the University of California Los Angeles and the University of Miami. Individuals 18 to 80 years of age with aneurysmal SAH confirmed by computed tomography (CT) or lumbar puncture, with aneurysms protected by clipping or coiling, were included. Patients with a history of peripheral vascular arterial or venous disease or peripheral nerve disease were excluded. None of the patients had any previous medical conditions (eg, liver disease, nutritional deficiencies) that would affect coagulation. All SAH patients, including patients undergoing RIPC and controls, received subcutaneous low molecular weight heparin, which does not affect prothrombin time (PT), partial thromboplastin time (PTT), or international normalized ratio (INR), for deep venous thrombosis prophylaxis while in the intensive care unit. The dose or type of low molecular weight heparin given did not vary over time. No other anticoagulants were administered to any of the patients. All patients or their legally authorized representatives provided signed informed consent. Additionally, 21 age- and sex-matched controls with SAH but not receiving RIPC were included for comparison.

Patients underwent RIPC sessions every 24 to 48 hours between days 2 and 12 after aneurysm rupture. The RIPC consisted of 3 to 4 cycles of 5 to 10 minutes of inflation of an adult, large lower extremity blood pressure cuff, to a pressure of 200 mm Hg or 30 mm Hg above the systolic blood pressure baseline value for each patient. The absence of distal pulse was confirmed by Doppler evaluation or palpation of the dorsalis pedis artery. If Doppler pulse signals or a palpable pulse were detected, the cuff was further inflated until they disappeared. After 5 to 10 minutes of ischemia time, the cuff was deflated and distal pulse recovery was evaluated. After 5 minutes of reperfusion, the cuff was inflated again using the protocol described. A total of 3 or 4 inflations followed by reperfusion were performed in each session.

Patients were monitored before, during, and after the RIPC sessions, as described previously.[8,10] In summary, systemic hemodynamic monitoring was conducted by recording systolic, diastolic, and mean arterial pressure, heart rate, and central venous pressure. Pain scale scores, temperature, and oxygen saturation were also recorded. The patients were followed with hourly neurological examinations and CT and brain magnetic resonance imaging scans were performed as indicated by changes in the neurological examination findings, placement or removal of external ventricular catheters, and before transfer out of the intensive care unit.

To evaluate the effects of RIPC on blood coagulation, we analyzed venous blood samples obtained throughout the course of hospitalization from patients undergoing RIPC and control patients with SAH who did not receive RIPC. In the RIPC group, samples were taken starting the day of admission to the neurointensive care unit and before and after preconditioning sessions. In the control group, the first postadmission coagulation samples collected were compared with samples from posthemorrhage days 8 through 10, the same average posthemorrhage time that RIPC patients had their last RIPC session. From the fresh plasma sample, PTT and PT were determined using the Sysmex CA7000. The INR was calculated as follows: $INR = [(\text{patient PT})/(\text{mean of reference range PT})]^{ISI}$, where ISI = international sensitivity index determined for each PT reagent.

Statistical Analysis

Statistical analysis was performed with SPSS software version 19.0 (SPSS Inc, Chicago, Illinois), applying a full factorial general linear model for repeated measurements.

RESULTS

PTT, PT, and INR were analyzed in a total of 21 patients enrolled in 2 trials of RIPC in SAH subjects at the University of California Los Angeles and the University of Miami. The patients included 14 females and 7 males with a mean age of 56.3 years (range, 38-81 years; SD, 13.3). Hunt and Hess scores were as follows: 4 (19%) grade 1, 4 (19%) grade 2, 6 (28.6%) grade 3, 4 (19%) grade 4, and 3 (14.3%) grade 5. On the presenting CT scan, 1 patient (4.7%) had Fisher grade 1, 5 (23.8%) had grade 2, 5 (23.8%) had grade 3, and 10 (47.5%) had grade 4. Patients were followed between 11 and 90 days. By the end of follow-up, clinical or imaging evidence of hemorrhagic complications had developed in none of the patients.

The immediate effect of RIPC on the coagulation profile was evaluated in 9 patients who had blood samples taken immediately before and immediately after each preconditioning session. The

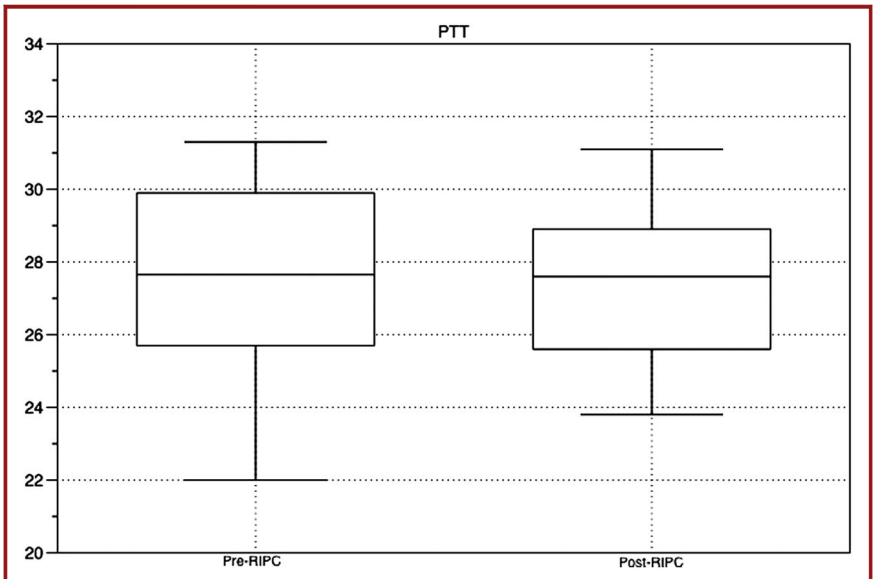

FIGURE 1. *Immediate effect of remote ischemic preconditioning (RIPC) on partial thromboplastin time (PTT). Mean pre-RIPC, 27.62 s; post-RIPC, 27.54 s;* P = .82.

patients included 6 female and 3 male patients with a mean age of 52 years (range, 38-73 years; SD, 11.74). No significant immediate changes were observed on PTT (mean pre-RIPC, 27.62 s vs post-RIPC 27.54 s, *P* = .82), PT (mean pre-RIPC 10.77 s vs post-RIPC 10.81 s, *P* = .59) or INR (mean pre-RIPC 1.030 vs post-RIPC 1.034, *P* = .57) after each session (Figures 1-3).

The long-term, global effect of RIPC on the coagulation profile was analyzed in the whole cohort of 21 patients. For the entire cohort, a statistically significant increase in PT and INR but not PTT was identified after exposure to a complete series of at least 4 sessions (mean PT pre-RIPC 11.33 s vs post-RIPC 12.1 s, *P* = .01; mean INR pre-RIPC 1.02 vs post-RIPC 1.09, *P* = .014, mean PTT pre-RIPC 27.4 s vs post-RIPC 27.85 s, *P* = .092) (Figures 4 and 5). There was also a significant direct correlation between the number of RIPC sessions and the increase in PT (Pearson = 0.59, *P* = .007) and INR (Pearson = 0.57, *P* = .010) (Figures 6-8). An average

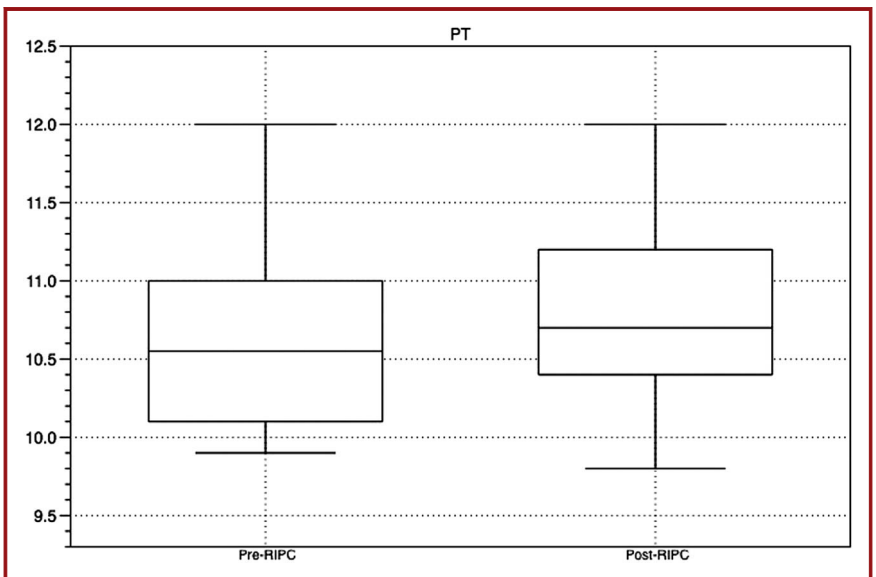

FIGURE 2. *Immediate effect of remote ischemic preconditioning (RIPC) on prothrombin time (PT). Mean pre-RIPC, 10.77 s; post-RIPC, 10.81 s;* P = .59.

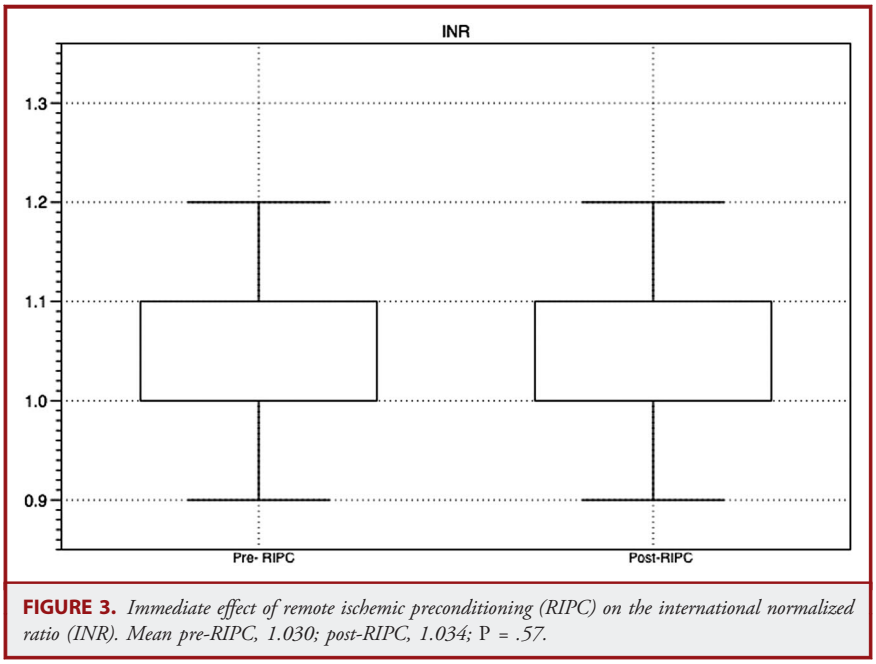

FIGURE 3. *Immediate effect of remote ischemic preconditioning (RIPC) on the international normalized ratio (INR). Mean pre-RIPC, 1.030; post-RIPC, 1.034;* P = *.57.*

follow-up of 19.4 days demonstrated no hemorrhagic complications associated with the procedure.

These long-term changes in coagulation parameters were compared with age- and sex-matched controls with SAH but without RIPC over the same time periods. The demographic and clinical characteristics of the controls compared with the patients undergoing RIPC are presented in Table 1. In the control group, there were no statistically significant changes in PT, PTT, or INR over a period of 8 to 10 days, comparable to the treatment period of patients receiving RIPC. The mean PT baseline was 10.7 s vs 10.9 s post, $P = .38$; the mean PTT baseline was 25.1 s vs 26.3 s post, $P = .094$; and the mean INR baseline was 1.03 vs 1.06 post-RIPC, $P = .19$.

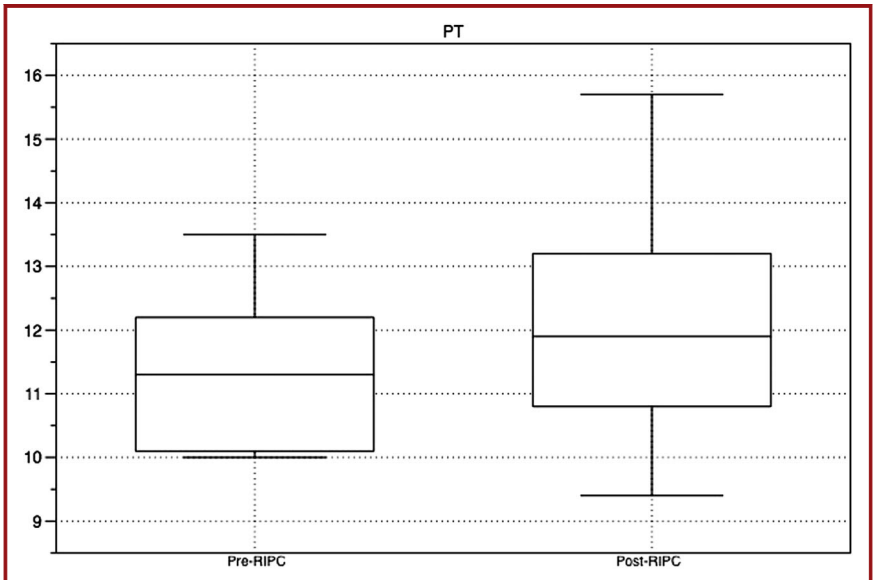

FIGURE 4. *Late effect of remote ischemic preconditioning (RIPC) on prothrombin time (PT). Mean PT pre-RIPC, 11.33 s; post-RIPC, 12.1;* P = *.01.*

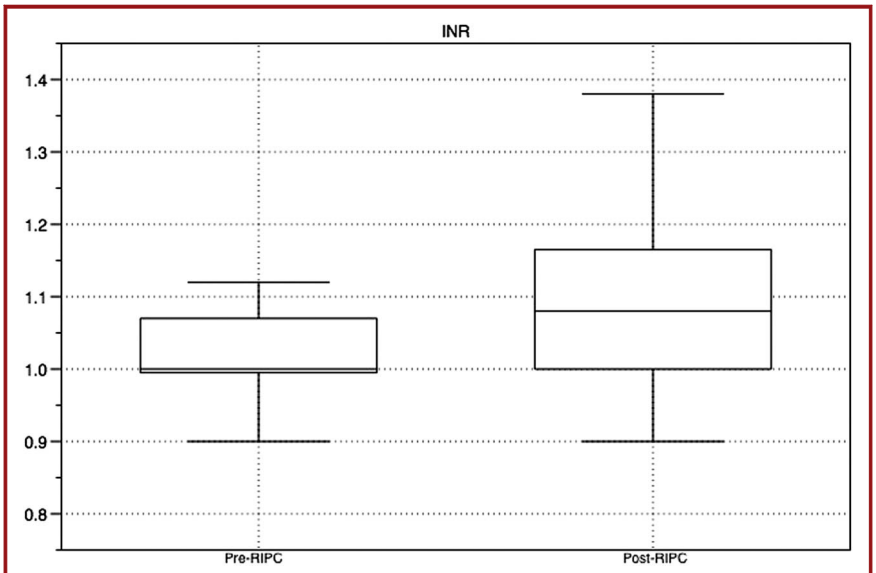

FIGURE 5. *Late effect of remote ischemic preconditioning (RIPC) on the international normalized ratio (INR). Mean INR pre-RIPC, 1.02; post-RIPC, 1.09;* P = *.014.*

DISCUSSION

In this study, we evaluated the potential effects on coagulation induced by application of RIPC after SAH. Tests of coagulation, commonly used in clinical practice, were assessed in the patients undergoing RIPC and the control group. PTT measures the activity of the factors that belong to the intrinsic (factors XI, IX, and VIII) and common (factors X and II and fibrinogen) coagulation pathways.[11,12] PT and INR evaluate the common coagulation pathway with the addition of the extrinsic (factor VII) pathway. Considering that bleeding time has poor sensitivity and specificity and is no longer a recommended test for bleeding disorders, it was not performed in this study.[13,14]

Identifying any potential effects of RIPC on coagulation profiles is a fundamental step in the safe clinical application of RIPC in patients with potential risks of hemorrhage such as ischemic

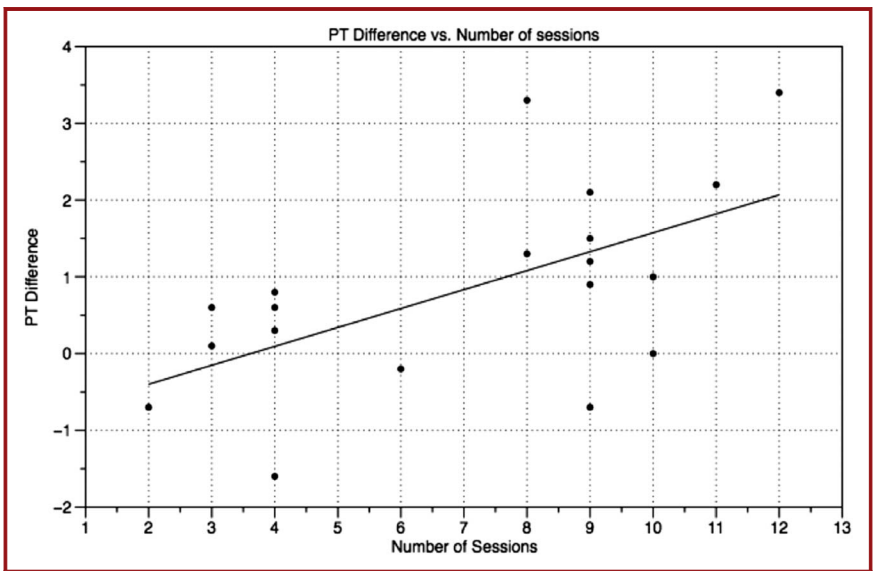

FIGURE 6. *Scatterplot showing a positive correlation between the number of remote ischemic preconditioning sessions and the increase in prothrombin time (PT).*

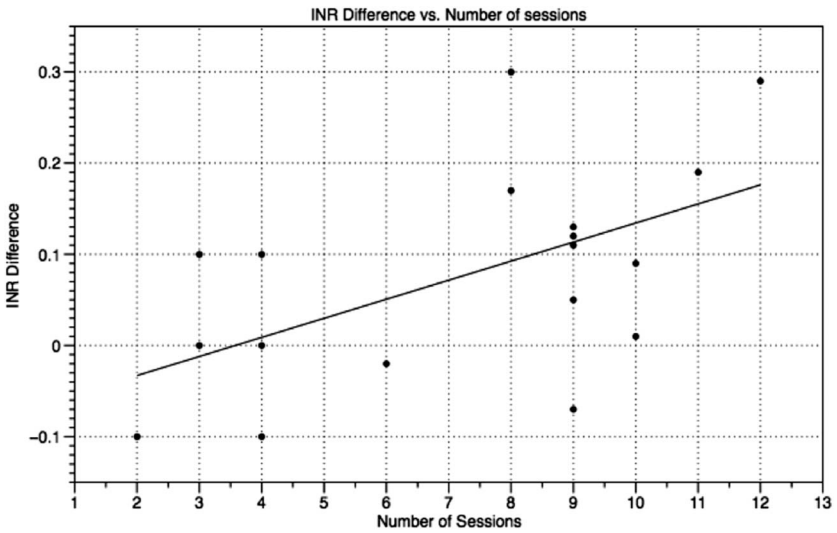

FIGURE 7. *Scatterplot showing positive correlation between the number of remote ischemic preconditioning sessions and the increase in the international normalized ratio (INR).*

stroke, intracerebral hemorrhage, and SAH. Warzecha et al[4] evaluated the effects of ischemic preconditioning on aPTT, plasma level of D-dimer, and euglobulin clot lysis time when used on a rat cerulein-induced pancreatitis model. It was found that ischemic preconditioning prolonged aPTT, increased the plasma level of D-dimer, and significantly decreased euglobulin clot lysis time, suggesting suppression of the intrinsic and common pathways of coagulation and activation of fibrinolysis. He et al[2] demonstrated prolongation of bleeding and thrombin clotting time in rats 72 hours after 15 minutes of transient MCA preconditioning, before induction of experimental intracerebral hemorrhage. Furthermore, Stenzel-Poore et al[3] found that preconditioning suppresses blood coagulation, specifically platelet aggregation, by assessing real-time polymerase chain reaction, Western blots, and bleeding times in mice who underwent either occlusion of the MCA for preconditioning (15 minutes of MCA

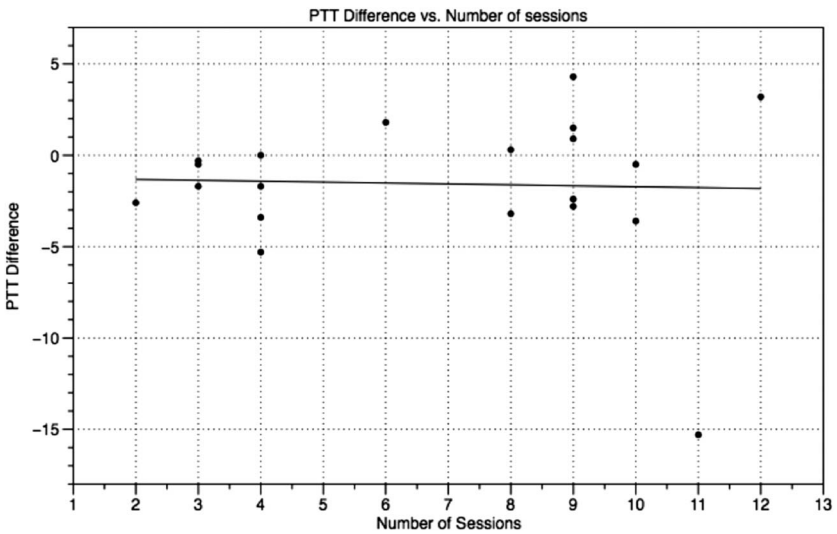

FIGURE 8. *Scatterplot showing no relationship between the number of remote ischemic preconditioning sessions and partial thromboplastin time (PTT).*

TABLE 1. Demographic and Clinical Characteristics of Remote Ischemic Preconditioning Session Subjects and Controls[a]

	Average Age (Range), y	Sex	Hunt and Hess Grade		Fisher CT Grade	
RIPC subjects	56.3 (38-81)	14 female	1	n = 4	1	n = 1
		7 male	2	n = 4	2	n = 5
			3	n = 6	3	n = 5
			4	n = 4	4	n = 10
			5	n = 3		
Control subjects	56.3 (35-76)	14 female	1	n = 1	1	n = 1
		7 male	2	n = 5	2	n = 2
			3	n = 8	3	n = 9
			4	n = 4	4	n = 9
			5	n = 3		

[a]RIPC, remote ischemic preconditioning; CT, computed tomography.

occlusion) or injurious ischemia (60 minutes of MCA occlusion). In that study, bleeding times were significantly longer 72 hours after preconditioning than in untreated controls due to overexpression of cyclo-oxygenase 1 and prostacyclin synthase that suggested increased prostacyclin synthesis, which could explain a decrease in platelet aggregation.

Our results suggest that, although over time RIPC contributes to a statistically significant prolongation in coagulation parameters, the magnitude of this change is small and does not reach levels outside the range clinically considered normal for humans. Specifically, there were no significant immediate variations in PTT, PT, and INR before and after each RIPC session. However, a statistically significant increase in PT and INR, but not PTT, was identified after exposure to the complete series of at least 4 sessions, with a direct correlation between the number of sessions and the magnitude of increase in PT and INR. Despite the prolongation of these tests, the changes were subtle and the values remained within normal limits. Control patients without RIPC did not demonstrate coagulation time prolongation. None of the patients receiving RIPC had complications that could be attributed to coagulation dysfunction such as rebleeding and increase or appearance of new parenchymal hematomas. Our results also suggest that RIPC has a direct effect on the extrinsic pathway of blood coagulation in humans with SAH and points to either an effect on the factor VII activity or an effect on tissue factor–factor VII interaction. In general, the results of this study provide evidence of the safe use of RIPC in clinical practice from the coagulation perspective.

There is also evidence to suggest that the subtle change in coagulation times toward a more anticoagulated state, although small, may contribute to the protective effects of RIPC in the setting of SAH. Previous studies, both in human SAH and in a rat model of SAH, demonstrated that SAH leads to a slightly hypercoagulable state.[15,16] Although these studies did not evaluate all the same coagulation parameters as those investigated in this study, they support the conclusion that the changes in coagulation that we observed after SAH are not due to the underlying pathology alone and can be attributed to the RIPC procedures. Furthermore, the slight anticoagulant effect produced by RIPC may be protective in the setting of SAH in that it counters the slightly hypercoagulable state demonstrated in those studies.

Contrary to the animal studies discussed earlier, we did not observe a prolongation of PTT, even after multiple RIPC sessions. The reason for this finding is not clear, although the direct clamping of arterial vessels used in the animal models may have produced a slight activation of intravascular coagulation and stimulated fibrinolysis to decrease endovascular thrombi formation and increase blood flow, as previously suggested.[4]

CONCLUSION

RIPC by limb ischemia appears to minimally, but statistically significantly, prolong the PT and INR in human subjects with SAH after at least 4 sessions, a change that correlates with the number of sessions and was not seen in age- and sex-matched controls with SAH. However, coagulation times remained within normal clinical ranges, and there were no hemorrhagic complications. No significant effect on PTT was identified. These findings provide evidence of the safety of RIPC use in clinical practice from the perspective of coagulation.

Disclosure

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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COMMENT

The authors provide the first human evidence that remote ischemic preconditioning produces a small but significant anticoagulant effect that seems to primarily involve the extrinsic pathway of the coagulation cascade. This result corroborates recent animal studies investigating the same preconditioning strategy, although the underlying mechanism of the observed anticoagulant effect appears different between species. Importantly, the authors note that the magnitude of the anticoagulant effect is very mild (prothrombin time and international normalized ratio values remained within normal limits) and was not associated with any hemorrhagic complications. The latter has obvious importance when one considers applying an ischemic remote preconditioning therapeutic strategy to a hemorrhagic stroke patient population. Definitive conclusions regarding the presence and safety of this anticoagulant effect in subarachnoid hemorrhage patients, however, will require study of a much larger group of subjects. This paper is a welcome addition to the growing literature describing the concept of applying preconditioning-based therapeutics to subarachnoid hemorrhage patients in an effort to reduce delayed cerebral ischemia (DCI) and the morbidity and mortality that it causes. In many ways, subarachnoid hemorrhage is the ideal clinical scenario for such approaches given the high incidence of DCI in this patient population, the significant morbidity that DCI causes, and the stereotypical delay between subarachnoid hemorrhage and DCI onset (typically 4-10 days). Whether a peripherally acting preconditioning strategy such as that used in this human study or a more centrally acting preconditioning strategy such as that used in our recent proof-of-concept animal study[1] ultimately proves efficacious against DCI remains to be determined.

Gregory J. Zipfel
St. Louis, Missouri

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