Evaluation of the risks of anticoagulation therapy following experimental craniotomy in the rat

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The risk of hemorrhagic complications with anticoagulation therapy in patients following intracranial surgery has prevented investigation of the potential use of heparin in the early postoperative period. The authors have evaluated the safety of anticoagulation therapy following experimental craniotomy in male Holtzman rats. The dose and schedule of heparin administration, which elevated and maintained the activated partial thromboplastin time (APTT) within the therapeutic range of 1.5 to 3 x control APTT, was alternating doses of 400 and 500 IU/kg injected subcutaneously every 6 hours. This schedule was initiated 2, 4, 7, 10, and 14 days after craniotomy and was continued for 72 hours thereafter. The results demonstrated that the incidence of intracerebral hemorrhage declined as the postoperative interval prior to initiation of anticoagulation increased. If anticoagulation therapy was initiated during the first 7 postoperative days, the risk of intracerebral hemorrhage was high (mean 14.7%); however, if an additional 3 to 7 days elapsed prior to initiation of anticoagulation, the incidence of intracerebral hemorrhage dropped significantly (mean 0%) (p < 0.05). These results suggest that anticoagulation therapy can be safely initiated 10 to 14 days after craniotomy.

KEY WORDS anticoagulation therapy • heparin • craniotomy • intracerebral hemorrhage • rat

The treatment of thromboembolic complications (deep vein thrombosis and pulmonary emboli) in the postoperative neurosurgical patient remains a significant problem. Current therapies for these complications include inferior vena cava filtration devices, inferior vena cava plication and ligation, thromboembolectomy, and anticoagulation therapy with immediate administration of full-dose heparin followed by prolonged treatment with oral anticoagulants. Of these procedures, the most effective and least invasive is anticoagulation therapy. However, for several reasons, anticoagulation therapy has been used very conservatively by neurosurgeons. First, the consequences of intracerebral hemorrhage in a patient receiving anticoagulation agents are disastrous, and the neurosurgical patient is obviously at higher risk for these complications. Second, despite this predisposition, there are no "hard data" in the literature indicating the postoperative interval after which full-dose anticoagulation therapy can be safely initiated.

As a first step toward approaching this question, we have examined the frequency of intracerebral hemorrhage following experimental craniotomy in the rat. Our experimental protocol and results are presented here.

Materials and Methods

Animals

The subjects were 153 male Holtzman albino rats* that weighed between 250 and 500 gm at the start of experimentation. Prior to surgery, the rats were housed six to a cage in a room with controlled temperature (72°F) and period of light (14 hours of light each day); food and water were available ad libitum. After surgery each rat was housed individually under the same conditions.

Experiment I Protocol

The rats in Experiment I did not undergo surgery. To replicate previous results and to clearly demonstrate

* Holtzman albino rats obtained from Charles River Breeding Laboratories, Inc., 251 Ballardvale Street, Wilmington, Massachusetts.
adequate anticoagulation, a course involving alternating doses of 400 and 500 IU/kg of heparin administered subcutaneously every 6 hours was evaluated for a total of 72 hours. The procedure will be detailed elsewhere (LJ Smith, et al., in preparation). Briefly, it involves cannulation of the right internal jugular vein with a polyethylene catheter (PE 10) for blood collection, and determination of the activated partial thromboplastin time (APTT). Baseline APTT values were determined in 12 rats without surgery, after which they were separated into two groups of six rats. The anticoagulation course was initiated immediately in Group 1, and 12 hours later in Group 2; thereafter, the APTT was determined twice every 24 hours in each group. Blood samples for APTT assessment were drawn immediately before the next heparin dose was administered and at 6 and 12 hours after in Group 1, and at 18 and 24 hours after the heparin dose in Group 2. These schedules enabled direct comparison of potential additivity of effect over two consecutive injections within one group (that is, comparing the baseline, 6-, and 12-hour samples in Group 1 and the 18- and 24-hour samples in Group 2), as well as additivity over several days of injections.

Experiment II Protocol

Rats were separated into five experimental groups and two control groups. Animals in the experimental groups began receiving heparin on either Day 2, 4, 7, 10, or 14 after surgery. The rats in control Group 1 did not undergo craniotomy, but received the same schedule of heparin administration as the experimental groups (alternating 400 and 500 IU/kg subcutaneous heparin every 6 hours for 72 hours). Control Group 2 rats underwent craniotomy, but received saline instead of heparin. Each group consisted of 20 rats, except the group beginning heparin 2 days postoperatively, which contained 33 rats.

The neurosurgical procedure was as follows. Rats were anesthetized with 38 mg/kg pentobarbital (Nembutal), and a midline scalp incision was made. A burr hole 2.5 mm in diameter was placed with a dental drill 1.5 mm anterior to the lambda and 2.0 mm lateral to the longitudinal suture. Following cauterization of the dura, a wound to the brain was created by inserting a No. 18 needle (1.3 mm in diameter, flat tipped, with a bone wax-filled cannula) perpendicular to the skull to a depth of 6 mm. The needle was then removed and the cortex was suction-aspirated to the edge of the burr hole. Hemostasis at the wound site was achieved, after which the burr hole was closed with sterile bone wax, and the scalp was closed with 3-0 silk in interrupted sutures.

At the termination of heparin (or saline) administration, the rats were sacrificed with Nembutal and the brains perfused with 75 ml of physiological saline followed by 75 ml of 10% formalin via a left ventricular cardiac puncture. Skin, skull, and meningeal wounds were examined for evidence of hemorrhage, after which the brains were removed from the skull and placed in 10% formalin. After fixation, each brain was first examined grossly for evidence of hemorrhage. Next, each brain was cut into 2-mm coronal sections, and further examined for evidence of hemorrhage. The hemorrhage was categorized as follows: 1) skin wound hemorrhage; 2) epidural hemorrhage; 3) subdural hemorrhage; 4) intracerebral hemorrhage (intracerebral hematoma greater than the width of the wound lesion (1.3 mm) in its greatest diameter); and 5) intraventricular hemorrhage. An autopsy examining the major soft-tissue organs was also performed on each rat. Statistical analysis of the data employed the Scheffe and the chi-square tests.

Results

Experiment I

An effect of repeated heparin administration on APTT over 72 hours was found in the Experiment I rats. In general, alternating 400 and 500 IU/kg every 6 hours produced and maintained a level of anticoagulation within the therapeutic range of 1/2 to 3 x the control APTT of 16.5 ± 0.7 sec (mean ± standard error of the mean) for the entire 72 hours of the experiment. Previous work indicated that the level of anticoagulation 2 hours after subcutaneous injections of heparin can be as much as two to three times that seen at 6 hours (LJ Smith, et al., in preparation). Therefore, there undoubtedly were periods during which these rats received hyperanticoagulation doses during this experiment. The APTT’s observed at 36, 60, 66, and 72 hours were slightly lower than those at other intervals, but were still within the therapeutic range. Moreover, these differences were not statistically different by the Scheffe test. The consistently lower APTT’s at 60, 66, and 72 hours, however, could be interpreted to suggest that higher doses of heparin would be required to maintain adequate anticoagulation beyond 72 hours.

Experiment II

Using the Experiment II procedure, the incidence of external and/or subdural hemorrhage was between 8% and 10% for all groups investigated. This incidence was independent of both the postoperative interval and anticoagulation therapy, suggesting that another factor was responsible for these hematomas. The most likely reason for the hemorrhage was scratching at the wound site by the rat. No epidural, subdural, or purely intraventricular hematomas were observed in any of the 153 rats involved in this study. Moreover, on gross examination, no brain showed any evidence of an intracerebral hematoma, but when the brain was sectioned, the presence or absence of an intracerebral hemorrhage was very obvious. Figure 1 demonstrates the typical appearance of the brain wound in rats with and without intracerebral hematoma. Although more detailed quan-
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FIG. 1. Coronal sections of rat brains. *Left:* Typical appearance of a control rat brain. The depth of cortical matter removed was 1.5 to 2.0 mm and the depth of the brain injury was approximately 5.0 mm from the outer cortical surface. The width of the intracerebral hematoma was less than 1.3 mm (the diameter of a No. 18 needle). *Right:* Typical appearance of an intracerebral hemorrhage from the group with a 2-day interval between surgery and initiation of anticoagulant therapy.

tification of the size of the intracerebral hematoma was attempted, we found that each animal that exhibited hemorrhage developed a hematoma greater than 3 mm and less than 5 mm in diameter, and that all but one of these (4.5 mm diameter) dissected intraventricularly. As the postoperative interval before initiation of anticoagulation therapy increased, the incidence of intracerebral hemorrhage decreased (Fig. 2). The 2-day interval had the highest incidence of intracerebral hemorrhage (18%), followed by the 4- and 7-day intervals, each with an incidence of 10%. The incidence dropped to 0% for both the 10- and 14-day intervals. Thus, when anticoagulation therapy was initiated during the first 7 days after intracranial surgery, a high incidence of intracerebral hemorrhage (14.7%) was noted. However, after an additional 3 to 7 days postoperatively, the incidence dropped significantly to 0% (p < 0.05, chi-square with correction for continuity).

Both control groups (craniotomy with no heparin and no craniotomy with heparin) had no intracerebral hemorrhage. No deaths or gross neurological or behavioral changes were observed following craniotomy or during anticoagulation drug administration. Peak and trough values of APTT for each of the control experimental groups were determined over the 72 hours of anticoagulation and compared by the Scheffe test: no significant differences were demonstrated. Mean APTT’s for each group were then calculated over the 72 hours of anticoagulation therapy and again compared by the Scheffe test: once more, no significant differences were demonstrated.

Autopsy revealed extracranial hemorrhage in two of the 153 rats. One rat suffered a gastrointestinal hemorrhage upon heparinization, and a second was found to have had a unilateral renal intraparenchymal hemorrhage. Closer examination of this kidney revealed underlying chronic hydronephrosis.

Discussion

The dangers of thromboembolic complications during the postoperative period in surgical patients are well known. These complications need to be detected early and treated effectively. Several possible treatments are available but are related to significant morbidity and mortality. Inferior vena cava ligation is associated with a 14% operative mortality, a 7% incidence of recurrent emboli (1.9% fatal), and a 23% incidence of postphlebitic sequelae (edema, recurrent thrombophlebitis, leg ulcers, stasis dermatitis, acute massive venous thrombosis, and venous claudication). Similarly, inferior vena cava plication is followed by a 10% operative mortality, a 6% incidence of recurrent emboli (1.9% fatal), and a 15% incidence of postphlebitic sequelae. The treatment that is least invasive, fastest to implement, and most efficient is anticoagulation ther-

![Incidence of Intracerebral Hemorrhage](image-url)
therapy. However, due to the potentially serious consequences of hemorrhagic complications associated with anticoagulant drugs in the neurosurgical patient, clinical studies of the therapeutic potential compared with the limitations of this form of treatment have not been carried out in a consistent fashion. Using the rat as an experimental model, we have evaluated the safety of anticoagulation therapy during the postoperative period following intracranial surgery. Current work suggests that the rat may be an adequate model for this investigation (LJ Smith, et al., in preparation).

The dosage of heparin that produced and maintained a level of anticoagulation within the therapeutic range of 1.5 to 3 x the control APTT (16.5 ± 0.7 sec) for 72 hours was 400 IU/kg alternated with 500 IU/kg, injected subcutaneously every 6 hours. With this regimen, the APTT remained elevated to at least 1.5 x control APTT for the 72 hours, but was not sufficiently elevated, despite periodic hyperanticoagulation, to induce spontaneous soft-tissue or intracranial hemorrhage. However, intracerebral hemorrhage was observed in some animals that had previously undergone a craniotomy. As expected, as the postoperative interval before initiation of anticoagulation therapy increased, the incidence of intracerebral hemorrhage decreased. The observed 18% incidence of hemorrhage at the 2-day interval, 10% at both the 4-day and 7-day intervals, and 0% at both the 10-day and 14-day intervals suggests that anticoagulation therapy for thromboembolic complications carries a substantial risk of intracerebral hemorrhage (approximately a 15% incidence) when treatment is initiated in the first 7 days following intracranial surgery. If, however, an additional 3 days or more have elapsed prior to initiation of anticoagulation therapy, the risk of intracerebral hemorrhage decreases significantly. These results could not be explained by different levels of anticoagulation between the groups where heparin was begun at different postoperative times.

The nature of the thromboembolic complication or the status of the surgical patient may suggest anticoagulation as the preferred choice of antithromboembolic treatment. This investigation may provide a first step toward evaluating the advisability of anticoagulation therapy during the postoperative period in the neurosurgical patient. Considering the risk associated with alternative antithromboembolic therapies and our ability to establish and maintain any given level of anticoagulation with intravenous infusion, anticoagulation therapy could be a very attractive antithromboembolic option. However, further validation of this model in larger animals, analyzing both the time course and the minimum APTT's required to achieve antithrombotic effectiveness, is necessary before the jump to human neurosurgical patients is possible.

References


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