Is Heparin Treatment the Optimal Management for Cerebral Venous Thrombosis?

Effect of Abciximab, Recombinant Tissue Plasminogen Activator, and Enoxaparin in Experimentally Induced Superior Sagittal Sinus Thrombosis

Carina Röttger, MD; Katharina Madlener, MD; Matthias Heil, PhD; Tibo Gerriets, MD; Maureen Walberer, DVM; Tiemo Wessels, MD; Georg Bachmann, MD; Manfred Kaps, MD; Erwin Stolz, MD

Background—Based on a newly developed model of reversible superior sagittal sinus (SSS) thrombosis in the rat, we investigated the effect of thrombolytic and anticoagulant treatment on recanalization, brain parenchymal changes, and motor deficits.

Methods—Thrombosis of the SSS was induced by topical application of ferric chloride. Occlusion was confirmed by magnetic resonance angiography (MRA). Six hours after operation, single treatment with 10 mg recombinant tissue plasminogen activator (rtPA)/kg and 6 mg abciximab/kg or subcutaneous injection of 450 IU/kg enoxaparin twice daily was started, each group containing 10 rats. Follow-up MRI with T2- and diffusion-weighted images was performed on the first, second, and seventh postoperative day.

Results—Control and enoxaparin-treated animals developed diffuse brain edema without infarction or intracerebral bleeding. This was indicated by an increase of T2 relaxation time and a decrease of the apparent diffusion coefficient in the parasagittal and lateral cortex. In these groups, the degree of recanalization after 7 days was comparable (48% versus 52%). Enoxaparin-treated animals showed significant amelioration of functional deficits. Clinical outcome was best in the abciximab-treated group, with a residual sinus occlusion of 36% after 1 week. Highest recanalization was achieved by lysis with rtPA (85%).

Conclusion—Enoxaparin treatment in rats with cerebral venous thrombosis significantly influences clinical outcome. However, it has no effect on recanalization. GPIIb/IIIa antagonists and rtPA accelerate thrombolysis. They may represent an alternative in treatment of cerebral venous thrombosis. (Stroke. 2005;36:841-846.)

Key Words: magnetic resonance imaging ■ rats ■ venous thrombosis

Cerebral venous thrombosis (CVT) has been considered rare. However, with improvement of imaging techniques such as MRI, CVT, is increasingly found to be the cause of brain damage. In general, the prognosis is favorable, but 8% of patients die or remain severely disabled. In this subgroup, a more aggressive treatment may be justified. Preliminary uncontrolled investigations suggest that thrombolysis may be more effective than intravenous heparin therapy, which is widely accepted for treatment of CVT. The role of platelets in the pathogenesis of CVT has not yet been studied systematically.

Based on a new rat model of superior sagittal sinus (SSS) thrombosis, this study explores the effectiveness of systemically applied recombinant tissue plasminogen activator (rtPA) and irreversible platelet aggregation inhibition by abciximab compared with weight-adapted high-dose enoxaparin therapy. The influence of these on development of brain damage and functional outcome are evaluated and compared with the natural course of disease.

Methods

Animal Preparation

In 54 male Sprague-Dawley rats (Harlan-Winkelmann, Borchen, Germany), the SSS was exposed operatively using a liquid-cooled drill with which a longitudinal canal over the whole length of the sinus was milled. The dura mater was left intact. Thrombosis was then induced by topical application of a strip of filter paper soaked in 40% ferric chloride for 4 minutes. Afterward, the field was flushed with saline and the skin wound was closed. Anesthesia for surgery...
and during the MRI was induced with 5% isoflurane and maintained with 2% to 3% isoflurane delivered in a mixture of 70% nitrous oxide and 30% oxygen through a face mask. During anesthesia, body temperature was maintained at 37°C with the help of a thermostatically controlled heating pad. All procedures were performed with approval of the authority for animal protection.

**Enoxaparin Treatment**

In 5 rats, enoxaparin (Clexane multidose; 100 mg/mL; Aventis) was injected subcutaneously in doses ranging from 100 to 1000 IU/kg body weight. Three hours after injection, blood samples were taken, and anti–factor-Xa levels were measured using a commercially available assay (IL Test TM Heparin; Instrumentation Laboratory). In this dose-finding study, 450 IU/kg was the lowest dose that markedly elevated anti–factor-Xa levels and was chosen for the following experiment.

In 10 rats, 450 IU/kg enoxaparin was applied subcutaneously every 12 hours beginning 6 hours after induction of thrombosis. Before decapitation and 3 hours after the last injection of enoxaparin, anti–factor-Xa levels were determined.

**rtPA Treatment**

In 12 animals, 10 mg/kg rtPA (Alteplase; Boehringer Ingelheim) was administered 6 hours after operation. Doses of rtPA applied in rats are much higher than those in humans because their clot lysis system is less responsive to rtPA. Ten percent of the total dose was administered as a bolus, followed by a continuous infusion of the remaining rtPA dose over 30 minutes via a tube inserted in a femoral vein.

**Abciximab Treatment**

The apparent affinity of rat platelet GPIb/IIIa receptor antigens is 24-fold lower than reported for humans. The number of binding sites is similar. Six hours after sinus thrombosis, 6 mg/kg body weight abciximab (Reopro; Lilly) was injected in a femoral vein in 10 rats, which complies with the dosage applied in rat embolic stroke model and idiopathic thrombocytopenic purpura models and is equivalent to the 24-fold dosage used in humans.

Because of the costs, drug binding of abciximab to rat platelets was demonstrated by platelet flow cytometry in 1 animal. Before and 30 minutes after intravenous application of 6 mg/kg abciximab, arterial blood samples were taken. Whole blood (20 μL) was incubated with 4 μL of Alexa Fluor (AF)–conjugated abciximab (Alexa Fluor 488 Protein Labeling Kit; Molecular Probes), and a second probe was additionally incubated with 4 μL of unconjugated abciximab. Then samples were washed with Hanks’ balanced salt solution (HBSS), and erythrocytes were lysed using ammonium chloride buffer. Afterward, cells were washed twice with HBSS and fixed by adding 2% paraformaldehyde in HBSS.

Flow cytometry was performed on a FACS Calibur (Becton Dickinson). Fluorescence was measured and examined statistically using commercially available software (Cell Quest; Becton Dickinson). In vivo binding of abciximab was demonstrated by reduced binding of AF-abciximab in vitro (see Figure 5).

**Controls**

Eleven rats served as untreated controls receiving craniotomy and induction of SSS thrombosis. An equivalent volume of isotonic saline was infused into the left femoral vein. Five rats were sham-operated, receiving only craniotomy and topical application of filter paper soaked in 0.9% saline solution.

**Physiological Variables**

In all animals treated with rtPA and abciximab, mean arterial blood pressure was monitored continuously during operation. Partial pressures of CO₂ and O₂, pH, and blood glucose were determined before and 30 minutes after craniotomy and SSS occlusion. In 5 of the untreated controls, these variables were measured in the same fashion.
T2 Relaxation Time
In the control animals, T2-RT decreased significantly in the parasagittal cortex (ROIs 1 and 2; \( P = 0.001 \)) and in the lateral cortex (ROIs 3 and 4; \( P < 0.01 \)) on day 2 after operation. The enoxaparin group showed a significant decrease of T2-RT between the second and seventh postoperative day (ROIs 1 and 2 \( P = 0.004 \); ROIs 3 and 4 \( P = 0.021 \)). The remaining treatment groups showed no statistically significant change of T2-RT over time and no difference compared with the sham-operated rats (Figure 3).

Clinical Outcome
Compared with untreated animals, all rats that received therapy improved significantly in the Rotarod test (abciximab \( P < 0.05 \); enoxaparin \( P < 0.01 \); rtPA \( P < 0.001 \)). Tolerated speed on day 7 was highest in the abciximab-treated animals with 30.0 ± 11.7 rpm (Figure 4).

Two rtPA-treated animals died from uncontrollable bleeding from the wounds. One control animal died during MRI for undetermined reason. There was no mortality in the other treatment groups. Two of the heparinized rats developed a subcutaneous hematoma. Intracranial hemorrhage was not observed in any of the groups. Abciximab caused no bleeding complications.

Physiological Variables
Mean arterial blood pressure and physiological variables were within normal ranges in animals with sinus occlusion and sham-operated rats. Application of abciximab and rtPA did not cause alterations of blood pressure during or 30 minutes after infusion.

Coagulation and Platelet Function
In the enoxaparin-treated group, anti–factor-Xa levels 3 hours after the last injection and before decapitation were 1.4 ± 0.126 IU/mL, which complies with the desired therapy effect in humans. Flow cytometry demonstrated binding of abciximab to rat platelets (Figure 5).
Discussion

Several animal models of sinus thrombosis have been developed\(^\text{10-12}\) that induce irreversible sinus occlusion. These methods are invasive and they lead to iatrogenic brain parenchymal defects. In addition, the sinus has to be opened or is permanently ligated. Thrombosis induced in this way does not permit an evaluation of the effectiveness of new therapies, especially when application of thrombogenic materials alters the physiological coagulation cascade and distorts the interpretation of possible effects. They do not replicate the course of illness in humans.

In humans, recanalization of affected sinuses occurs early and reaches 63% for the SSS within 22 days and 94% within 4 months.\(^\text{13}\) Our new model of SSS thrombosis is nonfatal, and recanalization rates in enoxaparin-treated and untreated animals comply well with those in humans. In humans, onset of thrombosis is often poorly defined by the wide range of clinical symptoms. Systematic studies of parenchymal damage in relation to recanalization and treatment are difficult to perform and interpret.\(^\text{14}\) Therefore, suitable animal models are indispensable. The current model involves a highly reproducible occlusion of the SSS with the possibility of pharmacological recanalization.

Time-of-flight MRA was chosen for evaluation of sinus recanalization because it represents the current standard imaging technique for diagnosis and follow-up of CVT in humans. It correlates well with digital subtraction angiography.

![Figure 3. Time course of T2-RT in ROIs 1 and 2 in the treatment groups and controls.](attachment:image3.png)

![Figure 4. Course of functional improvement in the Rotarod test for different treatment groups and untreated controls. Abciximab-, enoxaparin-, and rtPA-treated rats improved significantly between each observation time point.](attachment:image4.png)

![Figure 5. Binding of abciximab to rat platelets. AF-conjugated abciximab binding in vitro before (columns 1 and 2) and after (columns 3 and 4) application of 6 mg/kg body weight abciximab (nonconjugated) in vivo. Whole blood was incubated with 4 µL of AF-conjugated abciximab (dark gray). A second probe was additionally incubated with 4 µL of unconjugated abciximab (light gray). Fluorescence is reduced after in vivo application of abciximab (column 1 vs column 3). The further reduction of AF-conjugated abciximab expression after additional incubation of the probe with abciximab demonstrates that the GPIIb/IIIa receptors on the rat platelets are not completely saturated (columns 3 and 4).](attachment:image5.png)
The highest recanalization rate was reached by systemic thrombolysis with rtPA. In rat embolic stroke models, rtPA has been applied successfully. Early application reduced infarct volume and improved clinical outcome. In contrast, Gautier could show that rtPA infused 5 hours after middle cerebral artery occlusion leads to intracerebral hemorrhages and increased the size of the infarct.

Systemic thrombolysis with rtPA in cerebral sinus and cortical vein occlusion has been performed in 2 experimental studies. Röther et al induced thrombosis of the SSS in rats by ligation and injection of cephalin suspension. After treatment with rtPA, they observed partial resolution of hyperintensities in diffusion-weighted images in the parasagittal cortex. Alexander et al induced thrombosis in rabbits by dissection and compression of major dural venous sinuses. Here, systemically delivered rtPA led to total thrombolysis in 7 of 8 animals.

Case reports and several uncontrolled studies in humans have demonstrated that local or systemic thrombolysis can rapidly restore the patency of occluded sinuses and that it seems safe even in hemorrhagic infarction. Although selection for this kind of therapy was unfavorable with deteriorating and severely affected patients, they often had a better outcome after treatment compared with heparin groups. Inhibitors of platelet glycoprotein IIb/IIIa have so far not been applied in CVT. Thrombocytopenia attributable to iron deficiency anemia has long been recognized as a risk factor for CVT, although the pathomechanism is still unclear.

The data of our experiment show that abciximab may represent an attractive alternative in the treatment of CVT. It did not cause bleeding complications, and clinical outcome was best in this treatment group. In addition, this was the only substance that prolonged the phase of progressive recanalization beyond the first postoperative day.

Our animal experiment has limitations. The method to induce thrombosis by ferric chloride may not correspond to the natural pathophysiology of the disease. Species vary in the effect of anticoagulants and thrombolytics. We tried to apply adequate doses on the basis of recent literature and laboratory tests. However, we cannot ensure that our results can be transferred without restriction to the human situation. The delay between development of thrombosis and the onset of clinical symptoms in patients may impair treatment effects in the clinical situation.

References


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