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

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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

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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 GPIIb/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 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 FACSort 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.

**Functional Assessment**

Motor impairment was assessed using the Rotarod test on the first, second, and seventh day after operation according to an established protocol.

**Magnetic Resonance Imaging**

In each animal, MRI was performed on a 7-T unit after sinus occlusion. It was repeated on the first, second, and seventh postoperative day. In the sham-operated and untreated control group, MRI was also performed preoperatively.

The MRI protocol (PharmaScan; Bruker), animals were fixed in a head and body holder. The imaging protocol included a diffusion-weighted, T2-weighted, and a venous 2D time-of-flight MRA sequence. Diffusion-weighted images were acquired with a fat-suppressed echo-planar imaging sequence. The apparent diffusion coefficient (ADC) was calculated by a least-squares fit and composed onto an ADC map. The T2 relaxation time (T2-RT) was mapped using a Carr Purcell Meiboom Gill spin echo sequence. The T2 maps were calculated by a least squares fit. MRA was performed with a 2D time-of-flight sequence. A caudal presaturation of the neck arteries was applied.

**Data Evaluation and Statistical Analysis**

ADC and T2-RT time were measured in regions of interest (ROIs). On each of 5 slices, 2 ROIs were positioned cortically adjacent to the SSS (ROI 1 and 2), and 2 further ROIs were placed symmetrically in the lateral cortex (ROI 3 and 4).

On MRA source images of 0.3-mm thickness, the confluens sinuum and the rostral end of the frontal cortex were identified. Sinus occlusion was determined as percentage of slices without visible flow in the SSS.

Physiological variables were compared with a 2-tailed Student t test. Changes of ADC, T2-RT, and degrees of sinus occlusion were evaluated using the Friedman repeated-measures ANOVA on ranks. Recanalization rates, ADC, and T2-RT between the groups were compared with the 1-way ANOVA test. Correlation between Rotarod test and degree of recanalization was investigated with the Spearman rank correlation test. Values of P<0.05 were considered statistically significant.

**Results**

**Recanalization**

An average occlusion of the SSS of 93.7±6.6% was achieved after operation. Abciximab and rtPA lead to an improved recanalization already on day 1 (P<0.001), whereas in heparinized rats, recanalization beyond the spontaneous course of untreated controls was observed not before day 2 (P<0.001; Figure 1). After 7 days, untreated controls and heparinized rats showed a residual occlusion of 47.6±30.1% and 51.5±33.6%, respectively, which was significantly higher (P=0.025) than in the rtPA group (14.5±16.3%). The corresponding value for the abciximab group was 35.5±29.3%.

**Apparent Diffusion Coefficient**

The ADC increased significantly on the first postoperative day in the parasagittal cortex (ROIs 1 and 2), in the controls (P<0.001), and the enoxaparin-treated group (P<0.001). In the lateral cortex (ROIs 3 and 4), this change was observed in all animals with sinus thrombosis (P<0.001) independent of the type of treatment. Compared with the sham-operated rats, the ADC was significantly lower in all but the abciximab-treated rats after operation in ROI 1 and 2. At the remaining imaging time points, no significant differences between sham-operated and treated animals were found (Figure 2).
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\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.
phy. However, it is subject to certain artifacts.\textsuperscript{15} Signal loss can be observed in the region of the posterior aspect of the SSS and transverse sinuses because these segments gradually become coplanar with the imaging plane.\textsuperscript{16} Although we cannot exclude inline artifacts, they are unlikely to be a major problem in the rat brain because of the less convex course of the SSS.

We interpret the significant decrease of ADC in the animals of our experiment as diffuse brain edema especially pronounced in the parasagittal parenchym. The subsequent decrease of T2-RT could be attributed to a normalization of vasogenic brain edema. ADC and T2-RT normalized with recanalization of the thrombosed SSS. This could explain the delayed decrease of T2-RT in the enoxaparin-rats, for which recanalization became significant as late as day 2. T2-RT changes were avoided in animals treated with rtPA or abiciximab. In our experiment, definitive infarction or intracerebral bleeding spontaneously or under therapy were not detected on MRI. Therefore, we assume that thrombosis of the SSS induced by ferric chloride has no tendency to propagate into cortical veins.\textsuperscript{17}

In contrast to our findings, Tsai et al made a different observation in humans with isolated sinus thrombosis: they described a correlation between increased intradural sinus pressure and brain swelling, sulcal effacement, and mass effect. These structural changes were completely reversible up to a certain degree if thrombolytic treatment was performed. But signal changes on MRI in the sense of alterations made to derive information on the time of onset of the CVT\textsuperscript{20} and to predict the extent of ultimate infarction.\textsuperscript{21} Explanations for these tissue changes, which differ markedly from arterial infarcts and are amenable to complete restitution,\textsuperscript{22} remain hypothetical. Some authors attribute them to mild hypoperfusion\textsuperscript{23} and the breakdown of blood–brain barrier attributable to high venous pressure.\textsuperscript{18}

A widely accepted treatment for CVT consists of dose-adjusted intravenous unfractioned heparin. In a controlled trial, it reduced the risk of severe disability and fatal outcome without promoting intracerebral hemorrhage.\textsuperscript{24} However, it is unclear whether subcutaneously applied low–molecular weight heparin is equally effective.\textsuperscript{25}

By determining anti–factor-Xa activity, we could show that the animals were effectively anticoagulated. Starting anticoagulation 6 hours after induction of thrombosis did not accelerate recanalization. Changes of ADC and T2-RT were not prevented. But animals in this treatment group showed a significant improvement of motor abilities compared with untreated controls. This observation is in line with the clinical amelioration in heparinized patients with CVT.\textsuperscript{24} In a permanent sinus occlusion model in the rat, Ferriechs et al could achieve a normalization of pathological brain tissue impedance with a singular application of heparin after induction of thrombosis.\textsuperscript{26}

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.\textsuperscript{27} 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.\textsuperscript{28}

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.\textsuperscript{11} 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.\textsuperscript{29}

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.\textsuperscript{2}

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.\textsuperscript{30}

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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