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A pilot trial of microplasmin in patients with long-term venous access catheter thrombosis

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Abstract *Background* Microplasmin, a truncated form of plasmin, degrades fibrin and reacts with the circulating inhibitor α_2-antiplasmin. We investigated the safety and efficacy of intra-catheter microplasmin bolus administration for the restoration of catheter function in long-term venous access catheter thrombosis. *Methods* This open-label, ascending-dose, pilot study enrolled 31 subjects. Two doses of microplasmin were evaluated, (5 mg and 8 mg) administered via a 2 ml intra-catheter bolus injection in 10 and 21 patients respectively. Catheter function was evaluated 30 min after the first bolus administration. In case of incomplete catheter function restoration, a second bolus was administered with reassessment of catheter function 30 min thereafter. *Results* After the first bolus, complete restoration of catheter withdrawal function was observed in 5 out of 10 (50%) and 14 of out 21 (66%) subjects treated with 5 mg and 8 mg respectively and in 8 out of 10 (80%) and 18 out of 21 (86%) subjects after a second administration of microplasmin. No bleeding complications nor other adverse events were related to microplasmin. *Conclusions* In this pilot trial, microplasmin restored catheter function in a safe and effective way.

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Keywords Microplasmin · Central venous access device · Thrombolysis · Catheter function

Abbreviations

CVAD	Central venous access device
tPA	Tissue-plasminogen activator
α_2-AP	α_2-Antiplasmin

Introduction

Long-term venous access catheters, also referred to as long-term central venous access devices (CVADs) are indispensable for many patients. Catheter malfunction, primarily due to thrombotic occlusion, is a common complication requiring intervention in up to 25% of long-term catheters [1, 2]. Because of the risks and costs associated with the removal and replacement of the CVAD, salvage of occluded catheters via thrombolysis is a preferred treatment option. Several thrombolytics have been investigated in this setting, including urokinase, recombinant tissue plasminogen activator (tPA), staphylokinase and alfimeprase [3–8].

Microplasmin is a truncated form of plasmin that lacks the 5 kringle domains [9]. Like plasmin microplasmin is a serine protease that degrades fibrin and reacts with the circulating inhibitor α_2-antiplasmin (α_2-AP).

Preclinical experiments have demonstrated the efficacy of microplasmin in various thrombosis models. In a rabbit extracorporeal loop thrombosis model and a dog coronary thrombosis model, microplasmin was an effective fibrinolyticum when administered at the site of thrombosis [9].

Although bleeding complications are rare with the small doses of thrombolytics that are used for CVAD thrombotic

occlusion, all available thrombolytic agents are plasminogen activators and are associated with an increased risk of bleeding due to the systemic effect of the thrombolytic drug. Plasmin or derivatives thereof, which are fibrinolytic when administered at the site of thrombosis but rapidly neutralized in the systemic circulation by α_2-AP, would be expected to be devoid of such systemic bleeding complications. Furthermore, direct fibrinolytics may potentially speed up the clot lysis since these agents act independently of the endogenous plasminogen. Patients with occluded CVADs may benefit from a faster restoration of catheter patency.

This pilot trial was set op to investigate the safety and efficacy of the direct lytic agent microplasmin when administered directly at the site of a clot in patients with a thrombotic CVAD occlusion.

Patients and methods

The study protocol was approved by an Independent Ethics Committee. All patients gave written informed consent. EUDRA-CT 2005-003224-19.

Inclusion and exclusion criteria

Patients aged 18–80 years with a withdrawal occlusion of a long-term venous access catheter previously functioning properly were included. Patients presenting with catheters unable to inject into, with a known allergy to radiographic contrast dye, with recent active bleeding, with recent major open surgery or major trauma, with recent stroke or a neurosurgical procedure or with evidence of a non-thrombotic cause of catheter occlusion were excluded. Patients currently being treated with vitamin K antagonists resulting in an international normalized ratio >2.0 at most recent measure or with heparins in a therapeutic dosage were also excluded, as were patients with renal insufficiency (plasma creatinin level >2.0 mg/dl), with abnormal liver function tests (AST or ALT > 3x ULN), with significant hypertension (SBP $\geq$ 180 mmHg and/or DBP $\geq$ 110 mmHg) or patients who had previously been randomized into this trial.

Administration of microplasmin

The study was a single-arm, open-label, dose-escalating trial. Microplasmin (Thrombogenics NV, Leuven, Belgium) was administered via direct intra-catheter 2 ml bolus injection, followed 30 min later by a catheter function assessment. Two concentrations of microplasmin were investigated, 5 mg/2 ml and 8 mg/2 ml. Only if the catheter function was not restored, a second identical dose was administered and catheter function was reassessed 30 min thereafter. If the withdrawal occlusion persisted after the second administration of microplasmin, the patient was treated according to best medical practice at the investigator's discretion. A cathetergram was required before study drug administration in the first 5 patients of both cohorts and repeated in these patients after catheter function was restored or after the second bolus injection of microplasmin in case of non-restoration of the catheter function. Thrombus resolution was assessed based on the radiologist's review of the cathetergram prior to the treatment allocation and again after the treatment. A cathetergram was optional for the other patients in both cohorts. The investigators together with an independent study safety committee reviewed safety and efficacy data and could decide to modify treatment dose or cohort.

Catheter function assessment

Withdrawal occlusion was defined as the inability to withdraw blood from the CVAD though infusion of fluids was possible. Catheter function was assessed prior to treatment allocation and 30 min after study drug administration. Function was assessed by attempting to withdraw blood with an empty 10 ml vacuum tube (Becton Dickinson Vacutainer System) via negative vacuum pressure as previously described [1, 7]. Blood was withdrawn from the catheter until the 10 ml tube was filled, and the time to fill the 10 ml tube was documented with a maximum of 120 s. Complete restoration of catheter function was defined as the ability to fill the 10 ml tube via the negative vacuum pressure within 25 s. In addition, a categorical assessment of catheter function (normal, slow, or no withdrawal) was made. Slow withdrawal was defined as a hindrance to efficient or timely subject care, and no withdrawal as no or virtually no blood withdrawal (<1 ml) in a 2 min period.

In addition to these catheter function assessments, any catheter-related problems requiring intervention that occurred from the time of study drug administration until 4 weeks after study drug administration were documented.

Laboratory assessments

Routine coagulation parameters (PT, aPTT) and markers of systemic lysis (plasma plasminogen, fibrinogen, α_2-AP, D-dimer) were measured prior to study drug treatment and again 30 min after the last administration of study drug. Coagulation testing was obligatory in the first 5 patients of every cohort, thereafter all laboratory tests were determined only at the investigator's discretion.

Statistical methods

Based on results seen previously with other thrombolytic agents, a useful dose of microplasmin should be successful

(i.e. complete restoration of catheter withdrawal function) in more than 60% of cases. Frequency distributions and corresponding percentages are used to describe categorical variables. Coagulation parameters are described as mean ± standard deviation. Paired t-testing was used to compare laboratory parameters before and after treatment.

Results

Demographic and other baseline characteristics

Thirty-one subjects were enrolled in this pilot trial. Ten subjects were included in the cohort with 5 mg/2 ml intraluminal bolus administration, and 21 subjects in the cohort with 8 mg/2 ml bolus administration. Table 1 provides the demographic data. After review of the efficacy of the first 10 patients in the 8 mg cohort, an additional 10 patients were to be included into this cohort to confirm the efficacy and an additional 11 patients were actually recruited. Twenty-nine totally implanted venous port catheters (Bard Accesses, Salt Lake City, US; B.Braun, Boulogne Cedex, France; Smiths Medical, Deltec, St.Paul, US) and 2 tunneled Hickman catheters (Bard Accesses, Salt Lake City, US) were treated.

Efficacy

The efficacy end-points were the restoration of the catheter withdrawal function after the first and second administration of microplasmin. The results are summarized in Table 2.

With a 5 mg bolus, 5 out of 10 subjects (50%) had a complete restoration of the catheter withdrawal function after the first bolus. With an 8 mg bolus, 15 out of 21 subjects (67%) had a complete restoration of function of the catheter withdrawal function after the first bolus.

In the first and the second cohort, 5 out of 10 and 7 out of 21 subjects respectively received a second bolus. Catheter function was completely restored in an additional 3 patients in cohort 1 and in 4 patients in cohort 2.

Two patients in cohort 1 had no restoration of the catheter function after 2 administrations of microplasmin. In 1 subject withdrawal function was restored 2 h after supplementary urokinase administration. One patient was treated with urokinase without restoration of catheter function.

In cohort 2, administration of 2 boluses of microplasmin failed to restore catheter function in 3 patients. In 1 subject, catheter function restored after 120 min, before the administration of alternative thrombolytic therapy. One subject underwent interventional removal of a fibrin sleeve [10]. All further attempts to restore catheter patency were stopped in a third patient without catheter function restoration after 2 boluses of microplasmin.

Cathetergram, when performed, documented complete or partial thrombus resolution in all patients assessed.

During follow-up up to 30 days, 6 out of 8 catheters in cohort 1 were still functional without additional intervention. Two patients needed an additional intervention. In cohort 2, 1 catheter was removed because of suspected catheter-related infection, 2 patients received intra-luminal urokinase, and 2 patients were lost from follow-up.

Table 1 Demographic data of the enrolled subjects

Baseline characteristics	Cohort 1	Cohort 2
Number of subjects (M/F)	10 (4/6)	21 (5/16)
Age, mean (SD), years	61 (11)	59 (8)
Time since CVAD insertion, months (SD)	16 (17)	13 (13)

SD Standard Deviation; *CVAD* Central Venous Access Device

Clinical laboratory evaluation and safety evaluation

There were no bleeding complications nor other adverse events during the trial that were considered to be possibly related to the trial medication.

Markers of systemic lysis documented no change in fibrinogen, α_2-AP, and plasminogen levels in both groups when compared to baseline. D-Dimers increased, as shown in Table 3, consistent with the intended clot lysis.

Discussion

We performed a pilot trial to assess the safety and efficacy of microplasmin intraluminal bolus administration in patients with catheter withdrawal dysfunction. Microplasmin at a dose of 5 mg and 8 mg completely restored catheter function after 30 min in 50% and 66% of patients, respectively. After a second administration in patients without complete catheter function restoration after the first bolus administration, the successful restoration of catheter function was 80% and 86%, respectively.

The results of this small pilot trial demonstrate that the doses tested were safe and rapidly restored catheter function.

Current thrombolytic agents for CVAD occlusion include urokinase and recombinant t-PA. Rapid restoration of catheter patency is important for the timely administration of live-saving therapies. Limitations of the study are the small sample size and the lack of comparator therapies. In the pooled analysis of trials that investigated the efficacy of 1 or 2 administrations of 2 mg tPA with an evaluation of efficacy 2 h after the administration of tPA, catheter

Table 2 Catheter withdrawal function after microplasmin intra-luminal bolus administration

Restoration of catheter withdrawal function *N*	Cohort 1(5 mg) 10	Cohort 2 (8 mg) 21
Complete restoration after first bolus	5/10 (50%)	14/21 (66%)
Complete restoration after second bolus	3/5	4/7
Complete restoration after first or second bolus	8/10 (80%)	18/21 (86%)

N = number of subjects assessed

Catheter withdrawal function is assessed with withdrawal speed. Time in seconds needed to fill a 10 ml vacuum tube is reported (s/10 ml). Catheter withdrawal function is considered completely restored if the filling time is below or equal to 25 s, and partial if above 25 s. Catheter function is assessed 30 min after the first and second bolus administration

Table 3 Evaluation of coagulation parameters before (pre) and after (post) administration of microplasmin

Coagulation parameter (reference range)	Cohort 1 (microplasmin 5 mg/2 ml)				Cohort 2 (microplasmin 8 mg/2 ml)			
	Pre	*SD*	Post	*SD*	Pre	*SD*	Post	*SD*
PT (9.0–12 s)	10	*0,1*	10	*0,10*	12	*1,8*	11	*1,3*
APTT (24–31 s)	27	*2,4*	26	*1,78*	30	*4,3*	30	*4,5*
Fibrinogen (2,0–3,8 g/l)	4,2	*1,4*	3,9	*1,27*	5,2	*0,1*	4,7	*0,1*
D-Dimer (<500 µg/l)	810	*598*	1600*	*1443*	1271	*444*	3201*	*1255*
Alfa2-Antiplasmin (50–150%)	96	*20*	90	*6,16*	107	*18*	98	*5,7*
Plasminogen (50–150%)	86	*29*	87	*16,46*	81	*6*	78	*2,8*

* $P < 0.05$, post vs. pre; SD refers to standard deviation of pre and post values of the coagulation parameters

function was restored in 75% of patients after 1 dose and in 85% after 2 doses [5]. Future trials will need to address the potential efficacy benefit of microplasmin compared to urokinase and t-PA. Patients may benefit mainly from faster restoration of catheter function.

The results of this pilot trial also demonstrate the ability of a relatively small trial in subjects with thrombotic CVAD occlusion to provide proof of principal for a direct thrombolytic agent administered in the vicinity of the clot. Unlike commonly used thrombolytics (urokinase, t-PA, tenecteplase) that act as plasminogen activators, microplasmin is a serine protease that directly cleaves fibrin and is independent of plasminogen activation. Microplasmin is a truncated form of human plasmin that lacks the 5 kringle domains of plasmin. This recombinant DNA-derived protein degrades fibrin but also reacts with the circulating inhibitor α_2-AP. All available thrombolytic agents are associated with an increased risk of bleeding due to their systemic effect, even when administered locally at the site of thrombosis. Microplasmin is rapidly neutralised in the systemic circulation by α_2-AP and is expected to be devoid of such systemic bleeding complications. This hypothesis is supported by preclinical experiments with both plasmin and microplasmin [9, 11–13]. Microplasmin was an effective fibrinolytic agent in a rabbit arteriovenous shunt thrombosis model and in different stroke models.

One of the main drawbacks of catheter-directed thrombolysis of acute peripheral arterial thrombosis and ileofemoral deep vein thrombosis is the long length of infusion time and the bleeding risk of these procedures. Direct lytic agents such as plasmin or derivatives thereof could be useful for local catheter-delivered thrombolytic therapy of peripheral arterial or venous thromboembolic disease. Alfimeprase is another recombinant direct fibrinolytic that has been investigated in catheter occlusion [8, 14]. Because of its unique interaction with fibrin and α_2-AP, microplasmin may provide rapid clot lysis and reduce the bleeding risk through diminished systemic fibrinolytic effects for catheter-directed thrombolysis. This pilot study supports the concept of catheter-directed thrombolysis with microplasmin.

Conflict of interest Peter Verhamme is a consultant for Thrombogenics NV and has received research grant support from Thrombogenics NV. The other authors have no conflict of interest.

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