WHAT THIS PAPER ADDS TO THE EXISTING LITERATURE AND FUTURE CLINICAL PRACTICE

This study reports the feasibility of minimally invasive thrombolysis using intravenously administered targeted microbubbles carrying urokinase combined with local application of ultrasound in an in-vivo model. Further development of this technique could result in the future application of minimally invasive thrombolysis in patients with peripheral arterial occlusions.
ABSTRACT

PURPOSE
Standard therapy in acute peripheral arterial occlusion consists of intra-arterial catheter guided thrombolysis. As microbubbles may be used as a carrier for fibrinolytic agents and targeted to adhere to the thrombus, we can theoretically deliver the thrombolytic medication locally following simple intravenous injection. In this intervention-controlled feasibility study, we compared intravenously administered targeted microbubbles incorporating Urokinase and locally applied ultrasound, with intravenous Urokinase and ultrasound alone.

MATERIALS AND METHODS
In 9 pigs a thrombus was created in the left external iliac artery, after which animals were assigned to either receive targeted microbubbles and Urokinase (UK+tMB-group), or Urokinase alone (UK-group). In both groups, ultrasound was applied at the site of the occlusion. Blood flow through the iliac artery and microcirculation of the affected limb were monitored and the animals were euthanized one hour after treatment. Autopsy was performed to determine the weight of the thrombus and to check for adverse effects.

RESULTS
In the UK+tMB-group (n=5), median improvement of arterial blood flow was 5ml/minute (range 0-216). Improvement was seen in 3 out of these 5 pigs at conclusion of the experiment. In the UK-group (n=4), median improvement of arterial blood flow was 0ml/minute (-10-18), with slight improvement in 1 out of 4 pigs. Thrombus weight was significantly lower in the UK+tMB-group (median 0.9383g (range 0.885-1.2809) versus 1.5399g (1.337-1.7628; P=0.017). No adverse effects were seen.

CONCLUSION
Based on this experiment, minimally invasive thrombolysis using intravenously administered targeted microbubbles carrying Urokinase combined with local application of ultrasound is feasible and might accelerate thrombolysis compared to treatment with Urokinase and ultrasound alone.
INTRODUCTION

Acute peripheral arterial thrombosis is a condition threatening both limb and life. Treatment of acute peripheral arterial thrombosis with intra-arterial catheter guided thrombolysis is successful in many cases, reducing need for (redo) surgery. However, thrombolysis takes time, while success of therapy greatly depends on duration of ischemia, i.e. time between onset of symptoms and reperfusion. And, albeit minimal, intra-arterial thrombolysis remains invasive, as a catheter is needed for local administration of the fibrinolytic agent. Due to a possible duration of ischemia when using thrombolysis compared to surgical intervention, combined with the fact that the procedure still has an invasive aspect, further optimization of thrombolytic therapy is needed.

In patients with stroke as well as patients with coronary artery disease, clot lysis can be accelerated by local application of ultrasound (i.e. sonothrombolysis) and interesting results have been found in patients with peripheral arterial occlusive disease. An even further acceleration of clot lysis can be achieved when ultrasound contrast agents (UCA's) are used. In a previous study in a porcine model, we found promising results of combining intravenously administered microbubbles with standard intra-arterial catheter delivered Urokinase and locally applied ultrasound in acute peripheral arterial occlusion. UCA's, or microbubbles, can be used as a vehicle for drug delivery: drugs can either be incorporated in the microbubble or attached to the outer layer. Furthermore, by attaching Arg-Gly-Asp-Ser (RGDS), the recognition and binding site of platelet membrane glycoprotein 2b/3a receptor (GPIIb/IIIa), microbubbles can be targeted to adhere to the surface of a thrombus (Figure 9.1).

In combining incorporation of a fibrinolytic agent in targeted microbubbles (tMB) and destroying the tMB with high-intensity ultrasound once attached to a thrombus, we theoretically have a less invasive way of applying (local) intra-arterial thrombolysis, as placement of an intra-arterial catheter is no longer necessary. Hua et al showed promising results of targeted tPA loaded microbubbles in regards to efficacy and dosage of tPA needed in a rabbit model of small arterial occlusion. This model resembled small occluded arteries in ischemic stroke. To objectively assess the feasibility of this less invasive treatment in large occluded arteries, as is the case in acute peripheral arterial occlusion in humans, we designed an intervention-controlled study using a porcine model. The hypothesis of the study was that systemically administered tMB with incorporated urokinase and locally applied ultrasound will enhance thrombolysis compared to systemic sonothrombolysis alone. To our knowledge, no previous studies have been published exploring the combination of intravenously administered, thrombus targeted and urokinase loaded microbubbles with ultrasound therapy in an in-vivo model of acute peripheral arterial thrombosis.
FIGURE 9.1
SCHEMATIC OF TARGETED MICROBUBBLE

SonoVue microbubbles containing urokinase. The RGDS on the surface of the targeted microbubble binds to the glycoprotein 2b/3a receptor of the platelet membrane, keeping the microbubble at the site of the occlusion until it is destroyed with a high pressure ultrasonic wave and releases its contents locally.

SF6 = Sulphur hexafluoride, UK= Urokinase, RGDS = Arg-Gly-Asp-Ser, GPIIb/IIIa = glycoprotein 2b/3a receptor
MATERIALS AND METHODS

Approval of the Animal Ethics Committee was obtained before the start of the study and all procedures were done in accordance with the Dutch national guideline for humane animal treatment (Code Of Practice Welzijnsbewaking van proefdieren, 2004) as well as the European Directive 2010/63/EU on protection of animals used for scientific purposes.

Nine female Yorkshire pigs, 6 to 8 months old and with a median weight of 68kg (range 63-75), were housed at the research facility during one week before initiation of the protocol to allow for quarantine and acclimatization. Animals were randomly assigned to either the intervention group: intravenously administered RGDS targeted microbubbles (tMB) combined with urokinase and local ultrasound (UK+tMB-group; n=5) or the control group: intravenously administered urokinase and local ultrasound (UK-group; n=4).

Before start of the procedure, all pigs received premedication with 40 milligrams (mg) of methylprednisolone and 500mg of indomethacin to prevent potential allergic reactions to the microbubbles. Pigs can be allergic to the lipids of the microbubble shell. Therefore, this protocol was developed in an earlier study, when 4 pigs showed severe allergic reactions to the SonoVue microbubbles. In the same study, our protocol for inducing and maintaining anesthesia was described. In short, the pigs were sedated with an intramuscular injection of 28 mg per kg body weight ketamine (Alfasan, Woerden, The Netherlands), 0.5 mg per kg body weight midazolam (Actavis bv, Baarn, The Netherlands) and 1 mg of atropine (Pharmachemie, Haarlem, The Netherlands). Induction of anesthesia followed with an intravenous (i.v.) injection of 20 mg etomidate (B. Braun, Melsungen, Germany), followed by airway cannulation. During the procedure, we maintained anesthesia with endotracheally administered isoﬂurane, 1.5–2.0% (Pharmachemie, Haarlem, The Netherlands) and with 50 µg/h fentanyl (Hameln Pharmaceuticals, Hameln, Germany), followed by airway cannulation. The vessel was then clamped proximally and distally and 100 units of bovine thrombin (Calbiochem) were injected intraluminally. A catheter was placed in the right carotid artery to allow for regular arterial blood gas determination and to continuously monitor systemic blood pressure. Furthermore, heart rate, oxygenation and body temperature were measured during the entire procedure. Thermometers were placed between the toes of both hind legs and the left femoral artery was cannulated to monitor blood pressure of the affected limb. A laser Doppler probe (periflux 4001 master, Perimed, Järfälla, Sweden) was placed transcutaneously on the affected limb to measure microcirculation. As described previously, the left external iliac artery was identified via a midline laparotomy and the circumflex iliac artery was ligated. This to provide an adequate vessel length of at least 4 cm for our experiment. Blood flow in the iliac artery was measured using an ultrasonic flow probe (Transonic Systems Inc, Ithaca, USA). A stenosis was created in the distal external iliac artery to more closely mimic a clinical situation, as well as to prevent dislocation of a fabricated clot. The diameter of the vessel was reduced with a vascular tourniquet, decreasing the flow in the iliac artery by 40-60%. To promote adhesion of thrombus to the vessel wall, the endothelium was damaged over four centimeters by clamping and declamping the iliac artery. The vessel was then clamped proximally and distally and 100 units of bovine thrombin (Calbiochem) were injected intraluminally. The proximal clamp was removed after 60 minutes, the distal clamp after 90 minutes. In case of persistent flow in the iliac artery, the vessel once again was occluded and another 100 units of thrombin were administered.

Once a thrombus was created, it was left to stabilize for another 10 minutes. After stabilization, an ultrasound probe (Philips Sonos 7500) with a diagnostic S3 transducer (Philips, Best, the Netherlands) was directed at the site of the thrombus in the iliac artery. In a human
setting, the ultrasound probe would be placed on the skin directly over the target vessel. As closure of the abdominal wound would prevent adequate flow measurements through the iliac vessel, we simulated the clinical setting by placing a balloon filled with saline over the occlusion site. The ultrasound probe was placed on the balloon, resulting in a distance between the probe and the treatment vessel of 3 cm. The mechanical index (MI) was set to 1.1 with a focus of 3 cm and a frequency of 1.6 MHz.

The pigs in the UK+tMB-group (intervention group) now received an intravenous bolus injection of 500,000 units of urokinase combined with 2 vials of targeted microbubbles (10 cc all together, containing 8 µl of microbubbles per cc), followed by three repeated gifts of 50,000 units urokinase combined with 1 vial of targeted microbubbles every fifteen minutes (5 cc), leading to a total treatment period of 1 hour. The ultrasound contrast agent used was SonoVue (Bracco Diagnostics Inc, Milan, Italy), which contains sulphur hexafluoride (SF6) encapsulated by phospholipids (Macrogol 4000, DSPC, DPPG, Palmitic acid). As described by Mu et al, RGDS adheres to components of the SonoVue microbubble outer layer via ionic bonds or physical adsorption. To create the targeted microbubbles, we followed the same direct conjugation method, wherein the urokinase and RGDS peptide (Tocris bioscience, Bristol, UK) were added to 5 ml of normal saline. The SonoVue powder was diffused in this solution and shaken for 1 minute to create the urokinase loaded targeted microbubbles. Before each injection, heart rate (BPM), blood pressure (mmHg) and temperature of the pig (°C) were measured, as well as blood flow through the vessel (ml/min), microcirculation in the affected limb (Perfusion Units; PU) and temperature of both limbs (°C). For the UK-group (control group), the same protocol was adhered to, with exclusion of the microbubbles. As UCAs are visible on ultrasound, the investigators were not blinded to the type of treatment given.

Ultrasound treatment was initiated during the start of infusion of the urokinase. In order for the microbubbles in the UK+tMB-group to satiate the treatment area, ultrasound impulses were applied intermittently (5 seconds off, 1 second on) until all microbubbles were destroyed. With a pulse duration of 3 µs, the pulse repetition frequency was 24 kHz, with a total exposure time of less than 10 minutes. One hour after last injection of urokinase, the pigs were euthanized and autopsy was performed. All organs were macroscopically inspected for possible (hemorrhagic) adverse events and tissue samples were taken. The weight of persisting thrombus in the iliac artery was measured. Primary endpoints were flow through the affected vessel and weight of thrombus at termination of the procedure. Secondary endpoints were microcirculation, blood pressure and temperature of affected limb as well as any adverse events.

Data were analyzed with SPSS (IBM Statistics v20, Chicago, IL, USA). An unpaired Student’s t-test or a Mann-Whitney-U test was used for comparison of continuous variables with (non-)parametric distribution and a Chi-square test was used to compare proportions between groups. A P-value of 0.05 or less was defined as statistically significant.
RESULTS

Baseline characteristics were similar in both groups with regard to weight, core temperature, blood pressure both systemic and in the affected limb, flow through the iliac artery after creation of stenosis (= baseline flow) and percentage of flow reduction due to creation of the stenosis (Table 9.1). Median time to creation of thrombus was 170 minutes (range 93-200) in the UK+tMB-group versus 120 minutes (89-220) in the UK-group (P=0.524) and median units of thrombin needed for creation of the thrombus was 200 (range 100-225) in the UK+tMB-group and 163 (100-350) in the UK-group and (Table 9.2).

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TOTAL GROUP OF SUBJECTS (N=9)</th>
<th>UROKINASE + TARGETED MICRO-BUBBLES (N=5)</th>
<th>UROKINASE (N=4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>68 (63-75)</td>
<td>71 (63-75)</td>
<td>68 (66-73)</td>
<td>ns</td>
</tr>
<tr>
<td>MAP systemic (mmHg)</td>
<td>105 (65-118)</td>
<td>105(75-105)</td>
<td>98.5 (65-118)</td>
<td>ns</td>
</tr>
<tr>
<td>Systemic systolic pressure (mmHg)</td>
<td>123 (82-145)</td>
<td>123(106-126)</td>
<td>125 (82-145)</td>
<td>ns</td>
</tr>
<tr>
<td>Systemic diastolic pressure (mmHg)</td>
<td>95 (57-105)</td>
<td>95 (60-96)</td>
<td>85 (57-105)</td>
<td>ns</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>78 (50-117)</td>
<td>75 (50-117)</td>
<td>80 (63-88)</td>
<td>ns</td>
</tr>
<tr>
<td>Systemic T (degrees Celsius)</td>
<td>37.2 (36.2-38)</td>
<td>36.8 (36.4-37.9)</td>
<td>37.6 (36.2-38)</td>
<td>ns</td>
</tr>
<tr>
<td>Flow in iliac artery (ml/min)</td>
<td>127 (104-213)</td>
<td>113 (104-207)</td>
<td>129 (111-213)</td>
<td>ns</td>
</tr>
<tr>
<td>Degree stenosis iliac artery (%)</td>
<td>46 (28-55)</td>
<td>45 (28-55)</td>
<td>51 (42-52)</td>
<td>ns</td>
</tr>
<tr>
<td>Microcirculation (PU)</td>
<td>36 (20-149)</td>
<td>39 (22-149)</td>
<td>31 (20-109)</td>
<td>ns</td>
</tr>
<tr>
<td>T affected limb (degrees Celsius)</td>
<td>34.5 (26.3-36)</td>
<td>34.2 (26.3-36)</td>
<td>34.9 (33.6-35.6)</td>
<td>ns</td>
</tr>
<tr>
<td>T control limb (degrees Celsius)</td>
<td>34.6 (30.1-35.3)</td>
<td>33.3 (30.1-34.6)</td>
<td>34.8 (34.6-35.3)</td>
<td>ns</td>
</tr>
<tr>
<td>MAP affected limb (mmHg)</td>
<td>74 (55-108)</td>
<td>70 (60-108)</td>
<td>78 (55-95)</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic pressure affected limb (mmHg)</td>
<td>103 (70-133)</td>
<td>101 (74-133)</td>
<td>103 (70-124)</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic pressure affected limb (mmHg)</td>
<td>68 (47-97)</td>
<td>68 (53-97)</td>
<td>65.5 (47-81)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Abbreviations: n= number, kg = kilograms, MAP= Mean Arterial Pressure, ml/min = milliliters per minute, mmHg = millimeter of mercury, PU = Perfusion Units, T = Temperature, ns = not significant. Values presented are medians (range)

**ARTERIAL BLOOD FLOW**

A complete occlusion was reached in 6 out of 9 pigs. There was some residual flow through the iliac artery in 3 pigs, respectively 49% (51/104ml/min; UK+tMB-group), 38% (=50/131ml/min; UK-group) and 4% (9/213ml/min; UK-group) of baseline flow.

In the UK+tMB-group, 4 out of 5 pigs showed increase in arterial blood flow during
### TABLE 9.2
THROMBUS-INDUCTION, CHANGES IN THE LIMB WITH TIME AND THROMBUS WEIGHT POST-MORTEM.

<table>
<thead>
<tr>
<th></th>
<th>UROKINASE + TARGETED MICROBUBBLES</th>
<th>UROKINASE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>THROMBUS INDUCTION DURATION (MIN)</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>AMOUNTS OF THROMBIN (U)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CHANGES IN ml/min</td>
<td>216</td>
<td>97</td>
</tr>
<tr>
<td>Flow %B</td>
<td>104</td>
<td>86</td>
</tr>
<tr>
<td>Microcirculation PU %B</td>
<td>29</td>
<td>139</td>
</tr>
<tr>
<td>MAP Limb mmHg %B</td>
<td>30</td>
<td>-17</td>
</tr>
<tr>
<td>T affected limb °C %B</td>
<td>4.3</td>
<td>7.3</td>
</tr>
<tr>
<td>T control limb °C %B</td>
<td>1.4</td>
<td>4</td>
</tr>
<tr>
<td>THROMBUS WEIGHT g</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Change in various parameters due to therapy in individual pigs, i.e. t=135 vs. value after stabilization thrombus; designated in text as Δ. %B (B=Baseline) is defined as the following ratio: change in flow / baseline flow*100%
Abbreviations: ml/min = milliliters per minute, MAP = Mean Arterial Pressure, mmHg = millimeter of mercury, U = units, PU = Perfusion Units, T = Temperature, ns = not significant
Arterial blood flow of individual pigs in the intervention group (A) versus the control group (B). Group mean systemic- and limb arterial pressures are shown at the top of both graphs. Unfilled symbols depict partial occlusion at start of therapy; filled symbols depict complete occlusion. The arrow corresponds to the moment of initiation of therapy, i.e. t=0. S = thrombus stabilization period.

= injection of  = 500,000 IU Urokinase  = 50,000 IU Urokinase  = 1 vial of microbubbles
the experiment. One pig showed a complete return to baseline flow (216/207ml/min =104%) at the end of the procedure. Of the other 4 pigs in the UK+tMB-group, 2 more showed improvement in arterial flow at conclusion of the experiment. One of these had a partial occlusion at start of therapy and showed an increase in arterial flow of 5ml/min. The second had complete occlusion at initiation of therapy and showed an increase of 97ml/min, returning to 86% of baseline flow. Of the last 2 pigs in the UK+tMB-group, 1 showed a temporary increase in flow (49/130ml/min = 38% of baseline flow) followed by re-occlusion, most likely due to peripheral embolization (Figure 9.2A).

In the UK-group, 2 out of 4 pigs had a partial occlusion at start of therapy. One of these showed slight improvement of arterial flow at the end of the experiment (18ml/min) and one showed a decrease in blood flow of 10ml/min; the 2 pigs with a complete occlusion at the start of treatment did not show any improvement with UK therapy (Figure 9.2B).

MICROCIRCULATION

Median baseline microcirculatory flow was 36 PU (range 20-149), 39 in the UK+tMB-group and 32 in the UK-group (Table 9.1). After stabilization of thrombus, median microcirculation in the entire group decreased to 27 PU (range 9-35). In Table 9.2, differences in microcirculation at initiation of therapy versus at the end of the experiment are shown per individual pig. Out of the 5 pigs in the UK+tMB-group, 4 showed increase in microcirculation (range -13-139 PU); median difference 43%. In the UK-group, 2 out of 4 pigs show a slight increase in microcirculation (range -4-3 PU) with a median difference of -4%. In Figure 9.3, mean microcirculation curves of the 2 treatment groups are shown, divided in 2 subgroups depending on complete or partial occlusion at start of therapy (Figure 9.3).

BLOOD PRESSURE AND TEMPERATURE

Mean systemic arterial pressures dropped 15% in both groups during the experiment. In both the UK+tMB-group and UK-group mean limb arterial pressures varied only slightly. There was no significant difference between mean limb arterial pressures at the start of therapy versus at the end of the experiment (ΔMAP limb) in either group, nor was there a difference noticeable between the groups (Table 9.2/Figure 9.2).

During the procedure, systemic temperatures of all but 1 pig (b1) rose, with a median increase of 1.2°C (range -0.7-2.9). All pigs showed slight temperature changes in the affected limb from baseline temperature to end of procedure (ΔT affected limb), but there were no significant differences between both groups. The same was true for temperatures of the control limb (Table 9.2).

THROMBUS WEIGHT

The median thrombus weight at the end of the procedure was 0.9383g (0.885-1.2809) in the UK+tMB-group and 1.5399g (1.337-1.7628) in the UK-group (P=0.017) (Figure 4). All pigs in the UK+tMB-group had a lower thrombus weight at autopsy than pigs in the UK-group. In the UK+tMB-group, thrombus weighed between 0.89 and 0.98g in 4 out of 5 pigs. The last pig in the UK+tMB-group (a5) however, had a markedly higher thrombus weight of 1.28g.
ADVERSE EVENTS

Apart from possible embolization in 1 pig (a5), no adverse events were encountered during the experiments and no signs of adverse effects were found at autopsy, especially no signs of hemorrhage.

FIGURE 9.3
MICROCIRCULATION

Mean microcirculation curves of the two groups, divided in 2 subgroups depending on total or partial occlusion at the start of therapy. Unfilled symbols depict partial occlusion at start of therapy; filled symbols depict complete occlusion. The arrow corresponds to the moment of initiation of therapy, i.e. t=0. S = thrombus stabilization period.

= Administration of UK with or without microbubbles
Thrombus weights post-mortem grouped (A) and of individual pigs (B). NB: there is a difference in y-axis between grouped and individual figures.

UK = Urokinase, tMB = targeted Microbubbles

- UK = UK group, complete occlusion
- UK+ = UK + tMB group, complete occlusion
- = UK group, partial occlusion at start of therapy
- = UK + tMB group, partial occlusion at start of therapy
DISCUSSION

Outcome of acute peripheral arterial thrombosis varies from complete recovery to amputation and greatly depends on duration of ischemia. Optimization of therapy is needed to increase limb survival. The combination of fibrinolytic therapy, ultrasound and UCA’s (ultrasound contrast enhanced sonothrombolysis) shows promising results regarding time to reperfusion in both cerebral and myocardial infarction, as well as in our earlier study in a porcine model of acute peripheral arterial occlusion. Further optimization of thrombolysis might be achieved by avoiding use of intra-arterial catheters and by accelerating lysis.

UCA’s can be loaded with a drug and targeted to adhere to a specific site. Once attached to this site, they can be destroyed with high-intensity ultrasound, releasing the preloaded drug at the designated location. In this study, we used UCA’s as a vehicle for the fibrinolytic agent, thus avoiding placement of an intra-arterial catheter. The use of the carrier function of thrombus targeted microbubbles to create a form of minimally invasive local intra-arterial thrombolysis has, to our knowledge, not been described before in an in vivo model of acute peripheral arterial occlusion.

At the end of this short experiment, we observed an increase in arterial blood flow in 3 out of 5 pigs in the UK+tMB-group, whereas only 1 out of 4 pigs in the UK-group had a slight increase in arterial blood flow. Only partial occlusion was reached in 3 out of 9 pigs at start of therapy. Two of the pigs with only partial occlusion at start of therapy were treated in the UK-group. One of these showed further deterioration during the experiment and the other showed only minor improvement. Therefore, reaching only partial occlusion in 3 out of the 9 pigs does not bias our experiment in favor of the UK+tMB-group. Thrombus weights at autopsy were significantly lower in the UK+tMB-group versus the UK-group. Interestingly, one pig in the UK+tMB-group (a5) had a markedly higher thrombus weight of 1,28g compared to other pigs in this group. This was the same pig that had a temporary increase in arterial blood flow during the experiment, after which the iliac artery re-occluded. The temporary increase in blood flow as well as the higher thrombus weight might be explained by embolization occurring during therapy. We did not find sufficient evidence to prove or disprove this theory at autopsy. Apart from possible distal embolization in this one case, there were no signs of adverse events during the experiments or at autopsy. Based on this experiment, a less invasive manner of sonothrombolysis seems feasible for acute peripheral arterial occlusion.

We realize there are some limitations to this study. Duration of therapy was shorter than is usual in a clinical setting and we did not examine possible long term effects of the tMB combined with urokinase and ultrasound on local tissue. However, based on ethical considerations of animal welfare, longer duration of the experiment was not justified. Study population was kept small based on the same ethical principles.

Although some promising results were found in this experiment, further research on biomechanical properties of the microbubbles is necessary. More information is needed towards the exact concentration of incorporated Urokinase in the microbubble, the concentration of RGDS on the outer layer of the microbubble and the therapeutic effect of the RGDS, before this therapy can be investigated in patients. We will conduct in-vitro studies and investigate the targeted microbubbles on their stability, dose response- and temporal efficacy in order to assess their optimal configuration.
CONCLUSION

This study showed that minimal invasive thrombolysis is feasible using intravenously administered targeted microbubbles carrying urokinase combined with local application of ultrasound. This technique is effective and might accelerate thrombolysis, which is potentially beneficial in patients with acute peripheral arterial thrombosis. Further research regarding stability, dose response- and temporal efficacy is needed to assess optimal configuration of the microbubbles.

ACKNOWLEDGEMENTS

We would like to acknowledge the Amsterdam Animal Research Center of the VU University medical center for their animal care and their support of these experiments.
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