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

This study is the first in the world to report the potential of contrast-enhanced ultrasound to improve thrombolytic therapy in a model of large peripheral arterial occlusions. This technique could result in faster revascularization and lowering of thrombolytic dose and therefore minimize complications in patients with peripheral arterial occlusions.
CHAPTER 6

ABSTRACT

INTRODUCTION
The addition of local ultrasound with contrast agents to standard intra-arterial thrombolysis has been promising in the setting of stroke and myocardial infarction. In this study, we investigated the effect of additional ultrasound and microbubbles on standard low-dose intra-arterial thrombolysis in a porcine model of extensive peripheral arterial occlusion.

METHODS
In ten pigs, extensive arterial thrombosis was induced in the external iliac artery. The ‘urokinase only’ group (UK) (n=4) received standard thrombolytic therapy: intra-arterial bolus injection of 500,000 International Units (IU) followed by continuous low-dose urokinase (50,000 IU/h) infusion via an intra-arterial catheter and local intermittent application of ultrasound to visualize vascular patency. The ‘urokinase + bubbles’ group (UK+) (n=6) received the same urokinase therapy with concomitant infusion of microbubbles (5x 5mL vials during the first hour) intravenously and local intermittent application of ultrasound. After three hours of therapy the animals were terminated. End points were thrombus weight, arterial flow and microcirculation, representing reperfusion.

RESULTS
Mean thrombus weights were 1.1 vs. 1.6 grams in the UK+ group and in the UK group, respectively (P=.01). Arterial blood flow increased in four out of six pigs in the UK+ group by a mean 61% versus one out of four in the UK group with 1%. Microcirculation and lower limb arterial pressure levels improved after start of therapy in the UK+ group, contrary to a trend of decline in the UK group. No signs of bleeding complications were observed in either group.

CONCLUSIONS
In this experimental pilot study, the addition of contrast-enhanced ultrasound improved low-dose intra-arterial thrombolytic therapy in extensive peripheral arterial occlusions. Further clinical studies are warranted.
INTRODUCTION

Acute peripheral arterial occlusions are limb- and potentially life-threatening. In western countries the incidence of patients with acute limb ischemia is around 140 per million per year, and rises every year. In spite of rapid development of new endovascular techniques, in approximately 10-30% of the patients presenting with acute lower limb ischemia amputation follows within 30 days after hospital admission. Since its introduction, intra-arterial thrombolysis has altered the treatment of acute peripheral arterial thrombosis. Randomized trials published in the 1990s showed that thrombolysis with urokinase might be a good alternative for primary surgical intervention. Nevertheless, thrombolysis using fibrinolytic agents is time consuming and patients are confined to bed for days, which increases patients’ burden. In the Netherlands we use the fibrinolytic urokinase, however, no standard protocol exists and different fibrinolytic agents with various dose protocols have been used throughout the years worldwide. Furthermore, thrombolytic therapy is accompanied by major bleeding complications in up to 9% of the cases with up to 3% intracranial bleeding, depending on dosage and length of treatment. However, low-dosed thrombolysis could be used to minimize bleeding complications. A disadvantage is that this regimen increases therapy time. Therefore improvement of thrombolytic therapy with low-dose fibrinolytics is needed to minimize bleeding complications and concomitantly reduce therapy time.

Thrombolysis can be enhanced by ultrasound (US), especially in the early phase of therapy. In turn, the thrombolytic effect of fibrinolytics combined with US can be amplified with ultrasound contrast agents. These encapsulated gas filled microbubbles (1-5µm) can pass freely through capillary systems without extravasation into the interstitial fluid. During exposure to high-intensity US, these bubbles tend to cavitate and collapse leading to formation of free radicals and microjets, causing mechanical stress, erosion of the clot and the formation of small holes in the clot surface. These mechanical effects cause destabilization of the clot structure, making it more susceptible to fibrinolytics. Nowadays second-generation contrast-agents such as SonoVue (Bracco, Switzerland) and Definity (Lantheus Medical Imaging, MA, USA) are EMEA/FDA approved and safely used in clinical practice for diagnostic purposes. For therapeutic purposes such as thrombolysis, it has been applied in patients in clinical trials for the treatment of myocardial infarction and stroke.

The therapeutic application of microbubbles in thrombolysis has been investigated in animal models of myocardial infarction and stroke. However, it has never been investigated in large peripheral arterial occlusions. We aim to improve the thrombolytic therapy of patients with peripheral arterial occlusions by reducing bleeding complications using low-dose thrombolysis with urokinase and concomitantly shorten the therapy time with the use of microbubbles. Therefore in the present study we investigated the in-vivo application of this thrombolysis protocol in a porcine model of large peripheral arterial occlusion. End points were thrombus weight, arterial flow and microcirculation. We hypothesized that contrast-enhanced ultrasound can enhance low-dose thrombolysis in-vivo.
CHAPTER 6

METHODS

GENERAL PROTOCOL AND ANESTHESIA

Approval of the Animal Ethics Committee was obtained before initiation of the study. Ten female adolescent Yorkshire pigs were housed at the research facility for a minimum of at least one week before initiation of the experiment to allow for quarantine and acclimatization. Animals were randomized (random choice of an animal by the animal caretaker) to either the control group (intra-arterial UK, designated as the UK group, n=4) or the intervention group (intra-arterial UK with intravenous microbubbles and local application of ultrasound, designated as UK+ group, n=6). The pigs are numbered in chronological order.

At the start of procedure, the animals were sedated with an intramuscular injection of ketamine 28mg/kg, midazolam 0,5mg/kg and atropine 1mg. Anesthesia was induced with 20mg of etomidate intravenously, after which intubation followed. Where necessary, the administration of etomidate was repeated to allow for cannulation of the airway. During the procedure, anesthesia was maintained with isoflurane 1,5-2,0% endotracheally, and fentanyl 50µg/h, midazolam 50mg/h and pancuronium 20mg/h intravenously. Furthermore 5 ml/kg/h of NaCl 0.9% were administered intravenously. Tidal volumes were set at 10ml/kg with a frequency of 15-18/min and adjusted depending on capnography, maintaining the CO2 concentration between 35-40mmHg. These initial parameters were modified after serial blood gas measurements to keep the pCO2 between 25 and 35 mmHg and the pH within normal limits.

All pigs (control group and intervention group) received 40mg methylprednisolone and 500mg indomethacin premedication and 2500mg acetylsalicylic acid in three doses during the procedure to prevent allergic reaction to the microbubbles.13

An overview of the experimental protocol can be found in Figure 6.1A catheter was placed in both the right carotid and femoral artery to measure blood pressure. Thermometers were placed in the esophagus and between the toes of both hind legs to measure core- and limb temperature. The electrocardiogram, blood pressure, blood gases, urine output, transcutaneous oxygen saturation, and body temperature were monitored throughout the entire procedure.

EXPERIMENTAL SURGICAL PROTOCOL

Via a midline laparotomy, the left common and external iliac arteries were identified and the left internal iliac artery was ligated. A stenosis was created in the left external iliac artery by reducing the diameter of the artery with a ligature, decreasing the flow in the iliac artery by 50%±10%. Proximal from the stenosis the endothelium of the arterial wall was mechanically damaged by clamping and declamping over a length of 4 centimeters of the external iliac artery in order to damage the endothelium and promote thrombus formation. Subsequently this artery was then clamped proximally and distally over a total length of 4 cm and 100 units (U) of bovine thrombin (Calbiochem, EMD/Merck, Germany) was injected intraluminally in order to create a thrombus. The proximal clamp was removed after one hour and the distal clamp was removed 30 minutes thereafter (Figure 6.1B). In case of persistent flow in the external iliac artery, the thrombus induction procedure was repeated by reclamping and additional injection of bovine thrombin.
FIGURE 6.1
OVERVIEW OF EXPERIMENTAL PROTOCOL

A

Diagram of experimental setting with pig lying supine with site of occlusion highlighted. Location of microcirculation-, limb pressure and flow measurements shown in figure.

B Experimental protocol over time (mins). Each small vertical line on the x-axis represents a measurement point. The arrow corresponds to t=0 i.e. the moment of initiation of therapy after which immediate measurement followed.

Abbreviations: B = Baseline, S = Stabilization period (10 minutes).

- Creating stenosis
- Thrombus induction
- Release of the proximal clamp
- Release of the distal clamp
If necessary, small side branches were coagulated and large side branches were ligated. Blood flow in the external iliac artery distal to the occlusion was measured using an ultrasonic perivascular flow probe (T106, Transonic Systems Inc). A Laser Doppler probe (PeriFlux 4001 Master, Perimed Instruments) was placed transcutaneous on the affected limb in order to measure subcutaneous microcirculation.

After thrombus induction (see above) and 10 minutes of thrombus stabilization, a thrombolysis catheter (Royal Flush High-Flow, Cook Medical®, Amsterdam, the Netherlands) was placed intra-arterially just proximal to the occlusion and thrombolysis was initiated. The catheter was placed using an antegrade approach via the common iliac artery in analogy to the standard intra-arterial thrombolytic treatment of peripheral arterial occlusions in patients. The control group, i.e. ‘the UK group’ received an intra-arterial bolus injection of 500,000 International Units (IU) of UK via the catheter, followed by the continuous low-dose infusion of 50,000 IU UK per hour. A diagnostic ultrasound probe (Philips Sonos 7500, 1.6 Mhz., focus: 3cm, MI: 1.2, S3 transducer, Philips, Best, the Netherlands) was directed at the external iliac artery at the site of the thrombus to visualize vascular patency during the procedure. To mimic transcutaneous application, the probe was placed on a balloon filled with saline resulting in a distance between the probe and the treatment artery of 3cm. In the ‘UK+ group’, the same UK infusion regimen was administered as in the UK group and the ultrasound probe was placed following the same protocol. However, in addition 5 vials (25mL) of microbubbles (SonoVue 5mL, Bracco, Switzerland, prepared following the manufacturer’s manual) were infused via the ear vein during the first hour of thrombolysis. One vial was infused gradually during 10 minutes, after an additional 5 minutes a new vial was infused. To ensure replenishment of the microbubbles in the treatment area, ultrasound impulses were applied intermittently (5 seconds off, 1 second on) until all microbubbles were destroyed at the site of the occlusion.

After 3 hours of thrombolytic therapy the pigs were terminated and autopsy was performed. Brains, kidneys, liver, lungs, heart and spleen were cut in thin slices and macroscopically inspected for potential (hemorrhagic) adverse events and tissue samples were taken. The left external iliac artery was excised and the persisting thrombus and 4 cm surrounding external iliac artery to which it adhered were weighed together. As control, four centimeters of the untreated right external iliac artery were excised and weighed.

The data were analyzed with SPSS (IBM Statistics v20, Chicago, IL, USA). A Mann-Whitney-U test or an unpaired Student’s t-test was used to compare continuous variables with (non-) parametric distributions. A Chi-square test was used to compare proportions between groups. A P-value of 0.05 or less was considered statistically significant.
RESULTS

BASELINE AND THROMBUS INDUCTION BEFORE START OF THERAPY

Baseline parameters at the start of the experiments of all pigs and per subgroup are presented in Table 6.1. Thrombus induction varied between pigs in duration and amounts of thrombin needed (Table 6.2).

TABLE 6.1

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TOTAL GROUP OF SUBJECTS (N=10)</th>
<th>UROKINASE (N=4)</th>
<th>UROKINASE + BUBBLES (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>60 (58-90)</td>
<td>60 (59-90)</td>
<td>61 (58-84)</td>
</tr>
<tr>
<td>MAP systemic (mmHg)</td>
<td>71 (51-100)</td>
<td>70 (59-98)</td>
<td>76 (51-100)</td>
</tr>
<tr>
<td>Systemic systolic pressure (mmHg)</td>
<td>94 (72-129)</td>
<td>94 (79-114)</td>
<td>93 (72-129)</td>
</tr>
<tr>
<td>Systemic diastolic pressure (mmHg)</td>
<td>62 (40-90)</td>
<td>58 (49-90)</td>
<td>68 (40-86)</td>
</tr>
<tr>
<td>Pulse frequency (pulses per minute)</td>
<td>64 (55-95)</td>
<td>64 (57-70)</td>
<td>64 (55-95)</td>
</tr>
<tr>
<td>Systemic T (degrees Celsius)</td>
<td>37.6 (35.5-38.5)</td>
<td>37.6 (37.0-38.2)</td>
<td>37.6 (35.3-38.5)</td>
</tr>
<tr>
<td>Flow in iliac artery (ml/min)</td>
<td>100 (73-289)</td>
<td>107 (74-250)</td>
<td>100 (73-289)</td>
</tr>
<tr>
<td>Microcirculation (PU)</td>
<td>44 (28-63)</td>
<td>38 (28-47)</td>
<td>51 (29-63)</td>
</tr>
<tr>
<td>T affected limb (degrees Celsius)</td>
<td>35.0 (31.2-37.0)</td>
<td>35.0 (34.3-35.7)</td>
<td>35.2 (31.2-37.0)</td>
</tr>
<tr>
<td>T control limb (degrees Celsius)</td>
<td>35.1 (28.3-36.4)</td>
<td>33.6 (33.3-35.6)</td>
<td>35.7 (28.3-36.4)</td>
</tr>
<tr>
<td>MAP affected limb (mmHg)</td>
<td>64 (45-83)</td>
<td>64 (45-83)</td>
<td>65 (53-80)</td>
</tr>
<tr>
<td>Systolic pressure affected limb (mmHg)</td>
<td>67 (50-95)</td>
<td>64 (50-93)</td>
<td>72 (64-95)</td>
</tr>
<tr>
<td>Diastolic pressure affected limb (mmHg)</td>
<td>57 (43-78)</td>
<td>57 (43-78)</td>
<td>60 (48-72)</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. Values presented are medians (range). Baseline parameters were measured after creation of the stenosis (50% flow reduction).
TABLE 6.2
THROMBUS-INDUCTION, CHANGES IN THE LIMB WITH TIME AND THROMBUS WEIGHT POST-MORTEM

<table>
<thead>
<tr>
<th></th>
<th>UROKINASE</th>
<th>UROKINASE + BUBBLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>THROMBUS INDUCTION DURATION (MIN)</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>AMOUNTS OF THROMBIN (U)</td>
<td>225</td>
<td>100</td>
</tr>
<tr>
<td>CHANGES IN FLOW</td>
<td></td>
<td>mL/min</td>
</tr>
<tr>
<td>Microcirculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP Limb</td>
<td></td>
<td>mmHg</td>
</tr>
<tr>
<td>T affected limb</td>
<td>°C</td>
<td>0.3</td>
</tr>
<tr>
<td>T control limb</td>
<td>°C</td>
<td>0.8</td>
</tr>
<tr>
<td>THROMBUS WEIGHT</td>
<td>g</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Change in various parameters due to therapy in individual pigs, i.e. t=180 vs. value after stabilization thrombus; designated in text as ∆. %B (B=Baseline) is defined as the following ratio: change in flow / baseline flow (flow after creating stenosis) *100%. Abbreviations: ml/min = milliliters per minute, MAP = Mean Arterial Pressure, mmHg = millimeter of mercury, U = units, PU = Perfusion Units, T = Temperature

CHANGES DURING THE EXPERIMENT AFTER START OF THERAPY

FLOW

After induction of the stenosis the median (baseline) flow was 100 ml/min (range 73–289). After execution of the occlusion protocol complete occlusion of the external iliac artery was reached in 7 out of 10 pigs. In three pigs some flow persisted, i.e. 29% (=84/289; UK+ group), 26% (=27/102; UK+ group) and 54% (=135/250; UK group) of their baseline flows. After 3 hours of treatment, revascularization was regained in the UK+ group in 4 out of 6 pigs, with a median of 27% (range 0–96) of baseline flow and a mean increase of 61% (±42%). However, two of them already regained some flow during the stabilization period, as can be observed in Figure 6.2, right panel. Nevertheless, despite incomplete occlusion at the moment of initiation of therapy, arterial flow levels in these pigs showed marked increases during the rest of the procedure (up to 42% (=121/289) and 96% (=98/102) of baseline flow respectively). Of the 4 pigs with no arterial flow at the start of therapy, one (b2) showed higher flow, one showed temporary increase of flow (b6, reocclusion by embolization) while the other two (b4,
b5) did not. Changes in flow are presented per pig in Table 6.2. In the UK group, no increase in flow was observed in three out of 4 pigs within 3 hours of therapy (Figure 6.2; left panel). One pig had little increase in flow, although the occlusion of this pig’s external iliac artery was partial at the time of release of the distal clamp (from 54% (=135/250) to 55% (=138/250) of baseline flow).

**FIGURE 6.2**
**ARTERIAL BLOOD FLOW**

Arterial blood flow of individual pigs. On top of the graph group mean systemic- and limb arterial pressures are shown. See table 2 for symbols corresponding to pig ID's. Unfilled symbols depict partial occlusion at the moment of initiation of therapy; filled symbols depict complete occlusion. The arrow corresponds to t=0 i.e. the moment of initiation of therapy after which immediate measurement followed. S = stabilization period. Baseline flow (flow after creating a stenosis) not shown here.

= Administration of 1 vial of microbubbles

**MICROCIRCULATION**

At the start of the experiments the median (baseline) microcirculatory flow was 44 Perfusion Units (PU, range 28-63). After execution of the occlusion protocol the median microcirculatory flow was 21 PU (range 11-31). The microcirculation partly remains because of the presence of existing collateral circulation.

In Figure 6.3 microcirculation levels are depicted for the different treatments and stratified for total- or partial occlusion at the moment of initiation of therapy. Microcirculation levels reflect a trend towards increase in pigs treated with urokinase + bubbles. Changes in microcirculation due to therapy (∆MC, microcirculation at t=180 vs. microcirculation after stabilization thrombus) per pig are presented in Table 6.2. They show an increase in micro-
circulation in 3 out of 6 pigs in the UK+ group, median change = 23% (-24 – 146), and only a slight increase in 2 out of 4 pigs in the UK group (5% and 12%), median change = -22% (-52 – 12) in the whole UK group.

**FIGURE 6.3**

MICROCIRCULATION

Mean microcirculation curves. The two main groups, i.e. based on therapy, are each divided in 2 subgroups depending on whether there was total or partial occlusion at the moment of initiation of therapy. Unfilled symbols depict pigs treated with urokinase; filled symbols depict pigs treated with urokinase + bubbles. The large arrow corresponds to the moment of initiation of therapy. Continuous line depicts pigs with total occlusion, dashed line depicts pigs with partial occlusion at the moment of initiation of therapy.

**PRESSURES AND TEMPERATURE**

Mean arterial limb pressures fluctuated amongst groups during the procedure showing a trend to increased pressures in the UK+ group (Figure 6.2, values at the top). Changes in mean arterial limb pressures (Δ limb pressure) per pig as presented in Table 6.2 show increased limb pressures in 4 out of 6 pigs in the UK+ group, median increase of 46% (-6–104), and decreased limb pressures in 3 out of 4 pigs in the UK group, median change in limb pressures of -13% (-19–0) in the whole UK group.

Systemic temperature of nearly all pigs (except b1) rose during the procedures (overall median increase in systemic temperature: 0.6°C (-0.1–2.2). There were slight temperature changes during the procedure in the affected limbs (Δ T affected limb) of all pigs (Table 6.2), however none significant. Temperature of the control limbs (Δ T control limb) of all pigs
remained the same (Table 6.2).

In one animal of the UK+ group the pig was terminated before the experiment was ended after 135 minutes of therapy because of cardiac arrhythmias.

THROMBUS WEIGHTS AND ABSENCE OF BLEEDING COMPLICATIONS AT THE END OF THE EXPERIMENT

Thrombus weights were significantly lower in the pigs of the UK+ group when compared to the UK group, median 1.1g (0.8-1.3) vs. 1.6g (1.3-1.9) (P=.01; Figure 6.4). The weights of the 4 cm excised right external arteries varied with a maximum of 0.03g. Note that all the pigs in the UK+ group had lower thrombus weights post-mortem if compared to the pigs in the UK group; the exception in the latter group (pig a1) that showed already high flow at the moment of initiation of therapy. Importantly, no signs of hemorrhagic complications were observed during the procedures or in any of the organs investigated at autopsy.

FIGURE 6.4
THROMBUS WEIGHTS POST-MORTEM

Thrombus weights post-mortem grouped and of all individual pigs. Asterisks mark pigs in which only partial occlusion was reached before initiation of therapy. NB: note the difference in y-axis between grouped (A) and individual (B) figures. Dark columns: UK group, light columns: UK+ group.
DISCUSSION

In this study we investigated the therapeutic application of contrast-enhanced ultrasound with low-dose urokinase to enhance thrombolysis in order to lower the required doses of urokinase and to shorten therapy duration, both aiming at reducing complications. From a clinical perspective, thrombolytic therapy with urokinase for peripheral arterial occlusion takes one or more days at average to regain vascularization and ensure relief of symptoms.\(^5\) In the present study we observed that in 3 hours of therapy-time, thrombus weights were on average 30% lower in animals treated with urokinase + bubbles. Furthermore we observed increases in arterial flow, microcirculation and limb arterial pressures in the experimental group, whereas in the UK group an increase in arterial flow was observed in only one out of four pigs and deterioration of microcirculation and limb arterial pressures. This raises the potential for contrast-enhanced ultrasound to enhance thrombolysis and shorten therapy duration in humans.

The most feared adverse events during thrombolytic therapy are bleeding complications, especially the occurrence of intracranial bleeding. During the performed procedures with low-dose urokinase, no signs of bleeding complications were observed. Although no conclusions regarding the incidence of (intracranial) bleedings can be drawn from this small number of subjects, it is likely that a lower dose of urokinase lowers the risk of bleeding complications. Furthermore, shorter therapy duration could lower the risk of occurrence of other complications as well and most importantly lowers patient burden. In the present study the total therapy time was unfortunately limited to a maximum of 3 hours, due to ethical and practical reasons. However, in 3 hours marked improvement of reperfusion was observed in the group with microbubbles without any hemorrhagic complications.

In this study we used pigs for their resemblance to human cardiovascular anatomy and coagulation parameters. However, pigs are known to be allergic to nanoparticles’ lipid shell, so we needed to provide medication to prevent allergic reactions.\(^13,14\) To the best of our knowledge, no previous studies have been performed regarding thrombolysis with additional contrast-enhanced ultrasound in large animal models of peripheral arterial occlusion. In the setting of myocardial infarction Xie et al. showed improvement in epicardial recanalization rates as well as improvement in microvascular flow to the risk area with contrast-enhanced ultrasound and pro-urokinase-induced thrombolysis after acute coronary thrombotic occlusion in pigs\(^12\). This supports the results of our feasibility study. Importantly, treatment with contrast-enhanced ultrasound in addition to the administration of fibrinolytics could benefit the microcirculation as well. Clinically this could indicate potential improvement in the disease management of a patient’s leg microcirculation. A potential explanation for the beneficial effects of contrast-enhanced ultrasound on the microcirculation could be a NO-dependent mechanism, as opted in the coronary setting.\(^15\)

The potential role of contrast-enhanced ultrasound in thrombolysis has also been shown in smaller animal models (rabbit iliofemoral arteries): Nishioka et al.\(^16\) and Birnbaum et al.\(^17\) showed dissolution of in-vivo thrombus after treatment with contrast-enhanced ultrasound solely, thus without the use of a thrombolytic drug. The (non-targeted) microbubbles were administered intra-arterially (Nishioka et al.) and intravenously (Birnbaum et al.), the latter importantly without loss of effectiveness compared to intra-arterial infusion. A drawback of treating thrombotic occlusions without fibrinolytics is the possible occurrence and persistence of distal emboli. The rabbit iliofemoral arteries treated with contrast-enhanced ultrasound showed no (0 out of 17, Nishioka et al.) or few cases (1 out of 10, Birnbaum et al.) of distal embolization. Distal emboli can occur during standard urokinase therapy, however, the
continuous infusion of fibrinolytics dissolves them. In our study we also used continuous infusion of low-dose urokinase as part of the experimental treatment with microbubbles, which likely dissolves any distal emboli.

The therapeutic application of contrast-enhanced ultrasound and thrombolysis in humans is still in pilot stage. In a clinical pilot setting microbubbles in combination with ultrasound have shown a trend toward higher early recanalization and clinical recovery rates in acute stroke patients when used as an adjunct to standard intravenous tPA therapy.\(^9\) Despite limitations regarding sample size and deployment of operator-dependent techniques this trial shows promising results and the authors warrant continuation of clinical trials with contrast-enhanced thrombolysis. However, in the setting of acute stroke the safety in terms of intracranial hemorrhage and micro-embolization of this therapy needs to be established first, before evaluating its efficacy in a phase-II clinical trial setting. In the acute cardiac care setting a thrombolysis protocol with microbubbles was feasible and safe during treatment and follow-up.\(^10\) In the latter, a trend toward a higher epicardial recanalization rate in patients treated with ultrasound and microbubbles seems present, although group sizes were too small in that pilot study to provide conclusions regarding patency rates. Patient inclusion of this trial is still ongoing. Contrast enhanced ultrasound combined with thrombolysis has not been applied in patients with critical limb ischemia.

For acute peripheral arterial occlusions in the limbs we recommend more studies on the optimization of contrast-enhanced ultrasound techniques for therapeutic thrombolytic use: different microbubbles and fibrinolytics as well as different infusion protocols could be used to enhance thrombolysis and minimize bleeding complications. In this study we were limited to our own low-dose urokinase thrombolysis protocol for translational purposes and we used a contrast agent that is clinically available and used in our current practice. However, utilization of other fibrinolytic agents such as tPA and other market-approved lipid-based contrast agents such as Definity (Lantheus Medical Imaging, MA, USA) have also proven feasible.\(^9,11\) Furthermore we used freshly formed thrombus in our model, whilst the duration of ‘acute’ formed thrombus in patients at the moment of presentation with symptoms can vary from a few hours- to a few days. In addition we applied acute damage and induction of thrombotic occlusion in an otherwise ‘normal’ artery without atherosclerosis. Although we used a standardized thrombus induction protocol, in three out of 10 pigs limited flow persisted after release of the distal clamps. This variation can most likely be attributed to individual differences in coagulation profile. However, resembles the clinical situation of patients: a major reduction in flow only could lead to clinical symptoms and requirement of thrombolysis. Furthermore, changes in parameters due to therapy remain relevant (Table 6.2).
CONCLUSION

In conclusion, we observed beneficial effects of contrast-enhanced ultrasound on thrombolysis with urokinase in extensive peripheral arterial occlusions: a significant reduction in thrombus weight was reached in the pigs receiving additional contrast-enhanced ultrasound. Moreover, iliac blood flow, microcirculation and limb arterial pressures tended to improve within 3 hours of therapy. Therefore, it seems that time to thrombus resolution could be likely shortened. No hemorrhagic complications occurred during these experiments. Contrast-enhanced ultrasound has the potential to improve thrombolytic therapy in large peripheral arterial occlusions: this technique could result in faster revascularization and lowering of thrombolytic dose and therefore minimize complications. Our data warrant prospective studies in patients with peripheral arterial occlusions.

ACKNOWLEDGEMENTS

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REFERENCES


