Ultrasound-mediated catheter delivery of tissue plasminogen activator promotes thrombolysis by altering fibrin fiber thickness and clot permeability

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Abstract

It has been proposed that low power, high frequency ultrasound can augment the ability of thrombolytic agents to dissolve clot in patients with venous thromboembolism. We created a bench model to examine what role and mechanism ultrasound may have in this process. Fibrin polymerization was analyzed through modified light-scattering experiments with the inclusion of catheter-mediated ultrasound application. We studied fibrin fiber diameters through scanning electron microscopy of ultrasound treated fibrin clots. Clot porosity was investigated using permeation tests, while fibrinolysis was analyzed through light-scattering experiments, and by changes in porosity of lysing clots under flow. Whilst application of ultrasound did not change initial fibrin polymerization, it did induce a reversible change in maximal turbidity of already formed fibrin clots. This change in turbidity was caused by a reduction in fibrin fiber diameter and was associated with an increase in clot porosity. These reversible structural changes were associated with a linear increase in fibrinolysis rates under static conditions, while an exponential increase in rates was observed under flow. The use of ultrasound augmentation of thrombolysis enhances clot dissolution through greater and more rapid fibrin degradation. This is due to conformational change created by the ultrasound in clot structure, a reversible phenomenon that may increase binding sites for lytic agent, and could potentially allow the use of lower doses and shorter infusion times of ultrasound-assisted thrombolytic to treat venous thromboembolism *in vivo*.

Introduction

Pulmonary embolism (PE) is the third commonest cause of cardiovascular death and a major cause of morbidity worldwide.[1] Treatment of the majority of PE is based on simple anticoagulation. However, some patients with PE experience hemodynamic instability and, in such circumstances, thrombolysis is considered as an adjunct to care to accelerate clot dissolution and speed up clinical recovery. Thrombolysis can be delivered systemically at high doses via a peripheral vein and is the mainstay of care for PE that results in sustained low blood pressure. Whilst doing so can prevent or reverse cardiovascular collapse, it is associated with clinically important rates of bleeding complications, in particular, a higher risk of intracranial hemorrhage,[2] that can to some degree mitigate benefits. Catheter-based delivery of lower doses of thrombolytic, given directly into the pulmonary arterial system, has been proposed as one mechanism of maintaining the therapeutic cardiovascular benefits of systemic reperfusion therapies, with the hope that local delivery could improve efficacy and/or reduce required doses, thereby potentially reducing the risk of bleeding complications.[3] One such mechanism of this is using the EKOS catheter, which utilizes ultrasound-facilitated delivery of the drug to potentiate the dose of lytic used.[4,5] In addition to PE, EKOS catheter-mediated thrombolysis is also utilized in cases of large deep vein thrombosis,[6] which can present with life-threating symptoms. However, the evidence for the adjunctive use of ultrasound to catheter-based local delivery of fibrinolysis into the pulmonary vasculature is limited.[7] Furthermore, the mechanisms underpinning ultrasound-mediated modulation of fibrinolysis are also largely unknown.

In this study, we analyzed the ability of the EKOS ultrasound catheter to affect speed and magnitude of lytic effect on a bench clot model, to quantify any benefit that might be gained. Moreover, we assessed the effects of ultrasound on fibrin clot structure, permeability and fibrin fiber architecture, to characterize underlying molecular and structural mechanisms that impact fibrinolysis rates.

Methods

EKOS system for ultrasound and catheter-mediated thrombolysis

The EKoSonic (EKOS) endovascular ultrasound delivery system and catheters were obtained from Boston Scientific (Marlborough, MA, USA). The EKOS system uses a rotating algorithm of pulses of differing wattages, and so the bench model was set up to deliver the energy levels utilized in this algorithm: 0, 9, 15, 30, and 47W ultrasound pulses. These ultrasound wattages match those of the clinical algorithm consisting of 4 pulses of 9W, 15W, 30W, and 47W. The EKOS system allowed for constant monitoring of the ultrasound power during delivery and temperature levels on the outside of the catheter. A constant temperature (average 31.7°C, range 31.0-32.9°C) was maintained within the catheter by circulating room temperature coolant at 0, 2, 2.5, 8.5, and 11 mL/minute (min) for 0, 9, 15, 30, and 47W ultrasound, respectively.

Turbidity and lysis

Clot formation and lysis was assessed by a modified turbidity assays protocol.[8] Plasma clots were formed in spectrophotometer cuvettes fitted with the EKOS catheter and ultrasonic core (*Online Supplementary Figure S1*). For turbidity assays, 4 mL of clotting mixture were prepared with plasma diluted 1:6 in tris-buffered saline (TBS; 50 mM Tris, 100 mM NaCl, pH 7.4) warmed to 32°C, 1 U/mL thrombin, and 10 mM $CaCl_2$. For lysis assays, tissue plasminogen activator (tPA) (Apollo Scientific; Stockport, UK) was added in the clotting mixture at 50 ng/mL, unless specified. In all cases, the clotting mixture was transferred into the cuvette, which was then transferred into the thermostated cuvette compartment (32°C) of a Multiskan GO spectrophotometer (ThermoFisher Scientific; Altrincham, UK). Optical density (OD) was measured at 340 nm every 12 seconds (sec) for over 75 min. Ultrasound was applied continuously at: i) 0-30 min, ii) 30-45 min, or iii) 30-75 min from the activation of clotting for turbidity, and 25-60 min from the activation of clotting for lysis (timepoints optimized with preliminary experiments), with coolant applied at the relevant speed. Experiments consisted of 6 repeats, each using a fresh catheter.

Permeation

Clot pore size was determined using a modified permeation protocol.[9] The bottom of spectrophotometer cuvettes were perforated with 4 holes of 2 mm diameter, covered from the outside with parafilm, and fitted with the EKOS catheter and ultrasonic core (*Online Supplementary Figure S2*). 1.5 mL of clotting mixtures were prepared with plasma diluted 1:6 in TBS warmed to 32°C, 1 U/mL thrombin and 10 mM $CaCl_2$. The clotting mixture was transferred into the cuvette, which was incubated at 32°C for 20 min before a small layer of TBS (200 μL) was added, with a further incubation of 100 min. After 2 hours (hr), the parafilm was removed and TBS was added to the top of the clot up to 4 cm in height (distance from bottom of the clot to top of the liquid), which was kept constant throughout the experiment. The flow through was collected every 10 min for 1 hr and weighed. The Darcy's permeation constant, relating to average pore size, was calculated as previously described.[10] Experiments consisted of 6 repeats, each using a fresh catheter.

Details of materials, scanning electron microscopy, lysis under flow, and data analysis are provided in the *Online Supplementary Methods*.

Ethical statement

The study did not require ethical approval but respected the ethical rules of the country in which it was performed.

Results

Application of ultrasound on clot formation and structure

The effect of ultrasound delivery by EKOS on clot formation was assessed by a modified turbidity assay (*Online Supplementary Figure S1*).

First, we analyzed the effect of ultrasound on the initial step of clot formation (Figure 1A). Ultrasound was continuously applied for the first 30 min after addition of thrombin to plasma. None of the ultrasound powers tested affected the lag phase, or pre-gelation phase of turbidity (Figure 1B), and the resulting clots showed similar maximum absorbency, or fiber mass-to-length ratio (Figure 1C), indicating ultrasound does not influence fibrin clot formation.

Next, we examined the effect of ultrasound on already formed clots (Figure 1D), which is more relevant to the clinical setting of lysis of already established thrombi. Ultrasound was applied 30 min after thrombin addition, for the whole duration of the experiment. After 30 min, the clot with, and without, 9W or 15W ultrasound showed a slow and steady increase in absorbency (Figure 1E, F). However, for the first 5 min of ultrasound application, 30W power significantly diminished this increase in absorbency (by 53%), whilst power of 47W induced a plateau in absorbency (Figure 1E, F). After this period, absorbency increase rates were no longer affected by 30W and 47W ultrasound. These data suggest that higher ultrasound power alters clot structure, measurable by turbidity.

Finally, we tested whether this change in clot structure is reversible (Figure 1G). Ultrasound was applied 30 min after thrombin addition, for 15 min only, and absorbency was measured for a further 30 min. We found a similar effect at 30W and 47W immediately upon application of ultrasound, but upon stopping, absorbency rapidly and fully returned to pre-ultrasound levels (Figure 1H). Upon quantification, both 30W and 47W ultrasound significantly diminished absorbency between 30-45 min, which was followed by a rapid increase in absorbency between 45-60 min, and then matching again that of no ultrasound application between 60-75 min (Figure 1 I). The effect of ultrasound on clot structure, and its reversibility, is extremely rapid, occurring within 5 min of ultrasound application / switch-off.

Application of ultrasound reduces fiber thickness and increases clot pore size

With turbidity data indicating a significant effect on fiber mass-length ratio, especially at 47W, we next assessed clot and fiber ultrastructure by scanning electron microscopy (*Online Supplementary Figure S3*) and clot porosity by permeation assay (*Online Supplementary Figure S2*). Clot fiber thickness was significantly decreased (34%) after application of 47W ultrasound compared to control (Figure 2A-C). With changes in fiber thickness affecting overall clot density, therefore pore size, we next investigated the effect of ultrasound on clot permeability. We found that application of 47W ultrasound significantly increased (24%) average pore size compared to control (Figure 3A). Turbidity experiments

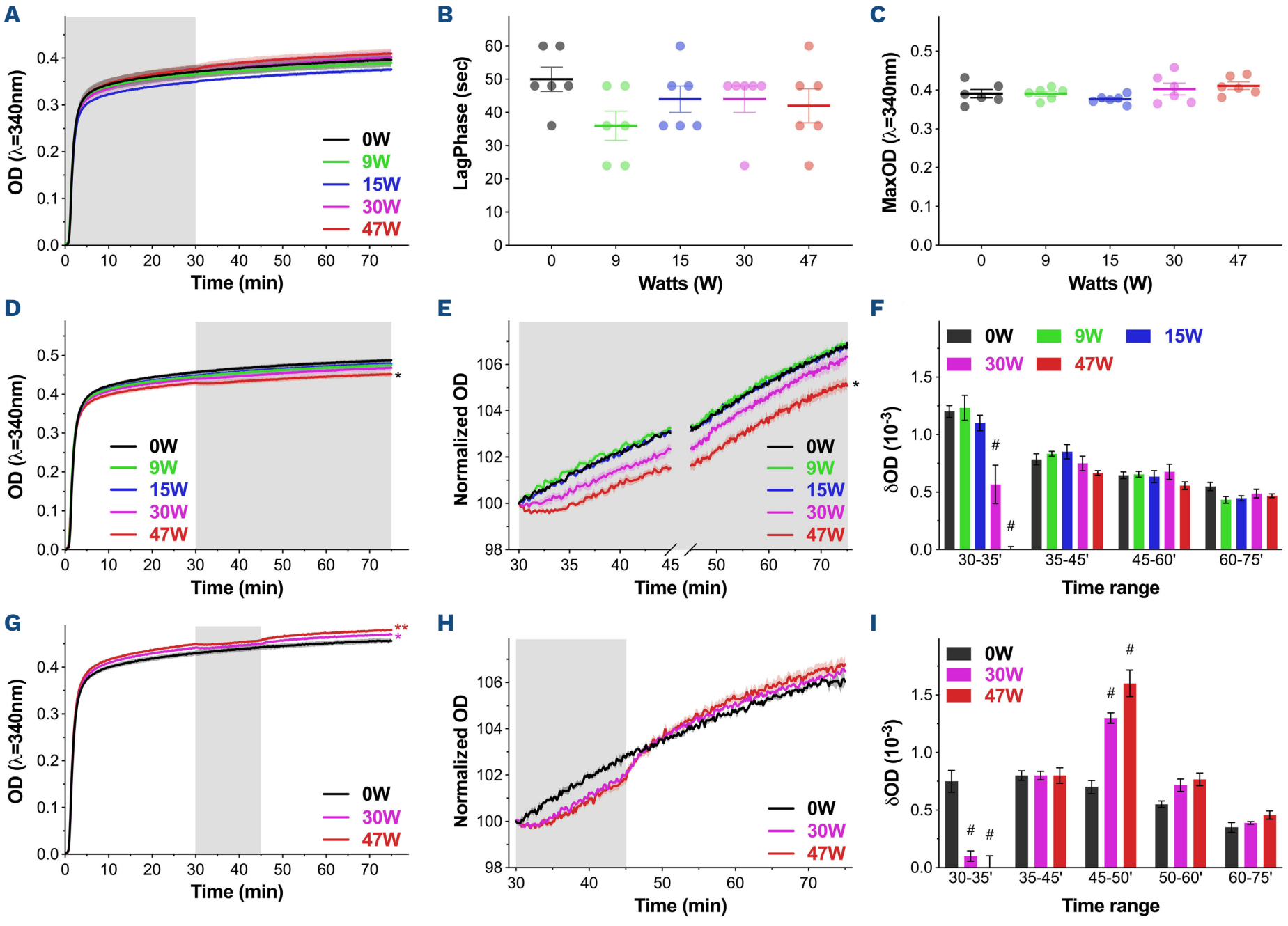

Figure 1. EKOS does not affect clot formation, but does alter clot structure. Plasma clot formation was measured by turbidity experiments, in the absence or presence of ultrasound (gray-shaded areas) at 9, 15, 30 and 47W. Application of ultrasound during the first 30 minutes (min) of clotting (A) did not affect the lag phase (B) or maximum optical density (MaxOD) (C). Application of ultrasound after 30 min of clotting (D) resulted in a decrease in MaxOD that was significant at 47W (E). When comparing the change in OD between various timepoints (F), the reduction in δOD was significant for 30W and 47W after the first 15 min, whilst remaining unchanged across all ultrasound powers between 45-75 min. Transient application of ultrasound between 30-45 min (G,H) resulted in a significantly decreased δOD at 30W and 40W (I), that was reversible once the ultrasound was turned off (H,I). Interestingly, the transient application of ultrasound resulted in a significantly increased MaxOD at 47W (G-I). Group comparisons were analyzed using two-way ANOVA with Dunnett's multiple comparison test. Data are represented as mean ± standard deviation (N=6). **P*<0.05, ***P*<0.01, #*P*<0.0001. sec: seconds.

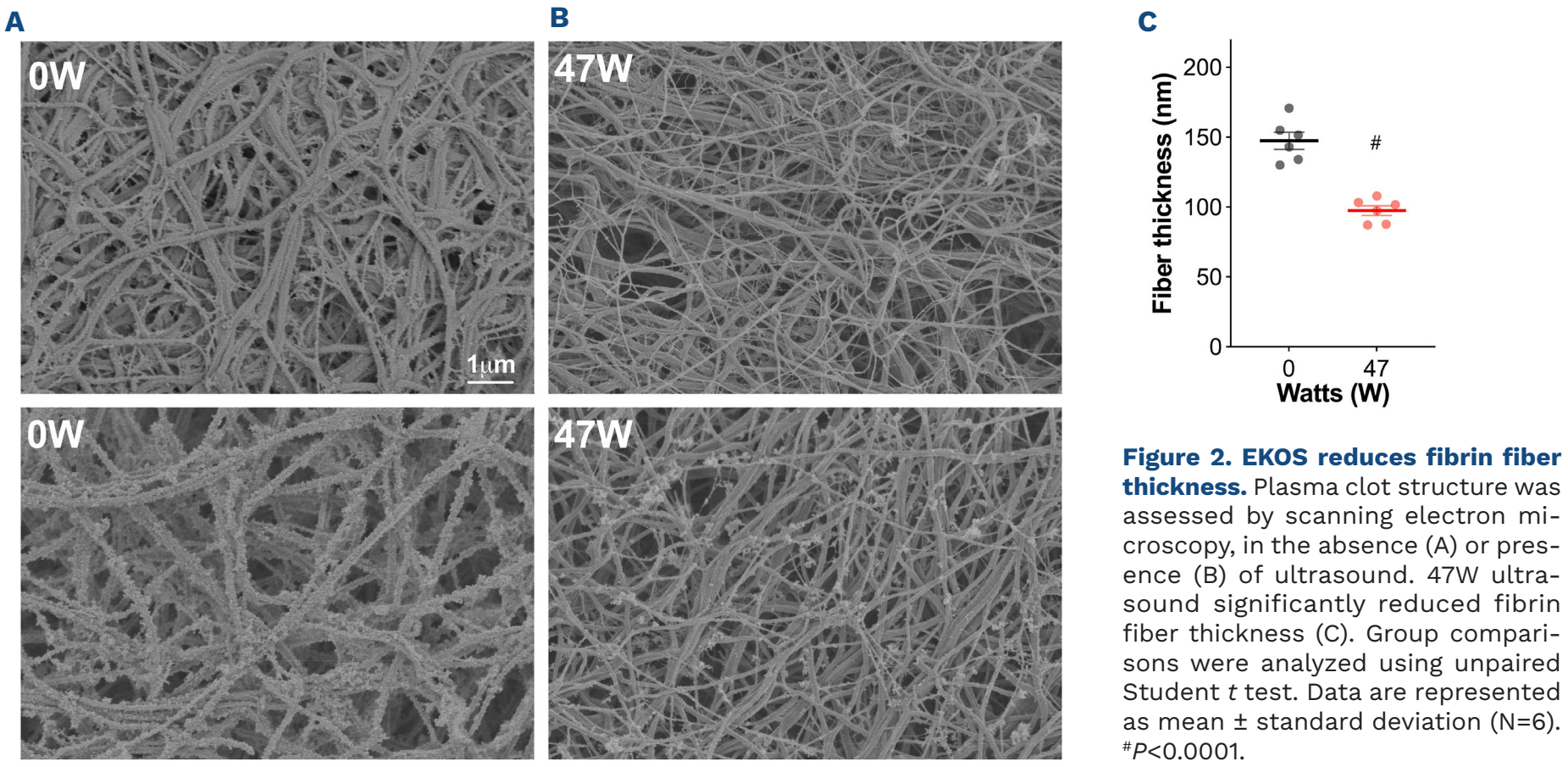

Figure 2. EKOS reduces fibrin fiber thickness. Plasma clot structure was assessed by scanning electron microscopy, in the absence (A) or presence (B) of ultrasound. 47W ultrasound significantly reduced fibrin fiber thickness (C). Group comparisons were analyzed using unpaired Student *t* test. Data are represented as mean ± standard deviation (N=6). #*P*<0.0001.

showed a transient effect on clot structure, so we investigated the effect of stopping ultrasound application on pore size. In agreement with the reversible effect observed by turbidity, the increase in pore size upon application of 47W ultrasound was fully reversible (Figure 3B), with the pore size returning to its original pre-ultrasound value.

Application of ultrasound increases the rate of internal and external clot lysis

To confirm the effect of ultrasound on clot lysis, its main target for clinical application, we first used our modified turbidity set-up (*Online Supplementary Figure S1*) in the presence of tPA. Ultrasound was applied 30 min after addition of thrombin and tPA, for a further 30 min. We found that 30W and 47W increased the rate of lysis, whilst 9W and 15W had no effect on clot lysis (Figure 4A). Times to 25%, 50%, 75%, and 100% lysis were all decreased at 30W, and especially at 47W (Figure 4B). 47W ultrasound significantly reduced all lysis time parameters, whilst significance was achieved at 15W and 30W for time-to-100%-lysis only (Figure 4C). These data indicate that 47W ultrasound induced an immediate speeding-up of lysis, whilst lower powers (15W and 30W) appeared to only affect lysis after longer application time.

Next, we applied a modified permeation assay (*Online Supplementary Figure S4*) to assess the effect of ultrasound on clot lysis under flow, when tPA is added to a formed clot. 0.4 mg/mL tPA was applied to the clot during ultrasound delivery, and permeation was measured whilst lysis was quantified by measuring D-dimers released into the flow-through. We found that permeation increased exponentially and significantly after 30 min of

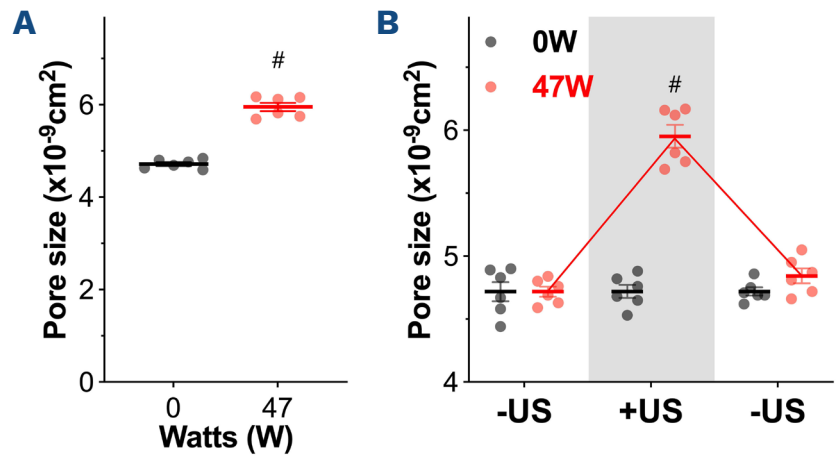

Figure 3. EKOS increases clot permeability. Plasma clot pore size was assessed by permeation experiments, in the absence or presence of ultrasound (US). 47W US significantly increased the pore size within the clots (A), an effect that was reversible once US (gray-shaded area) was stopped (B). Group comparisons were analyzed using one-way ANOVA with Tukey multiple comparison test. Data are represented as mean ± standard deviation (N=6). #*P*<0.0001.

47W ultrasound application, demonstrated by increased flow-through over time, whilst remaining consistent in the absence of ultrasound (Figure 5A). The start of the exponential increase in pore size matched that of the appearance of D-dimer in the flow-through at 30 min (Figure 5B). We also found that, after 50 min, D-dimer amount exponentially and significantly increased in the presence of 47W ultrasound, whilst remaining steady in the absence of ultrasound (Figure 5B). Of note, all clots collapsed between 80-90 min in the presence of 47W ultrasound, whilst remaining stable up to the 90 min endpoint in the absence of ultrasound.

Application of ultrasound decreases the amount of tissue plasminogen activator required to achieve similar lytic efficiency to higher dose

To assess whether ultrasound decreases the amount of tPA required to achieve the same lytic efficiency as higher concentrations, we performed lysis assays in our modified turbidity set-up (*Online Supplementary Figure S1*) in the presence of varying concentrations of tPA combined with the absence or presence of tPA. We found that a decrease in tPA concentration from 50 ng/mL to 37.5 ng/mL, in the absence of ultrasound, significantly prolonged the time to half-lysis from 28.3 to 75.3 min (Figure 6A, B). In the presence of ultrasound, the time to half-lysis was significantly reduced to 18.0 min (50 ng/mL tPA) and 32.37 min (37.5 ng/mL tPA), with the latter value similar to that of 50 ng/mL tPA without ultrasound (28.3 min). We, therefore, showed that, compared to a standard dose of tPA (50 ng/mL), we achieve a similar lytic efficiency with 25% less tPA (37.5 ng/mL) when ultrasound is applied. These data indicate the potential for ultrasound to diminish lytic dose *in vivo* whilst still achieving the same thrombolytic action.

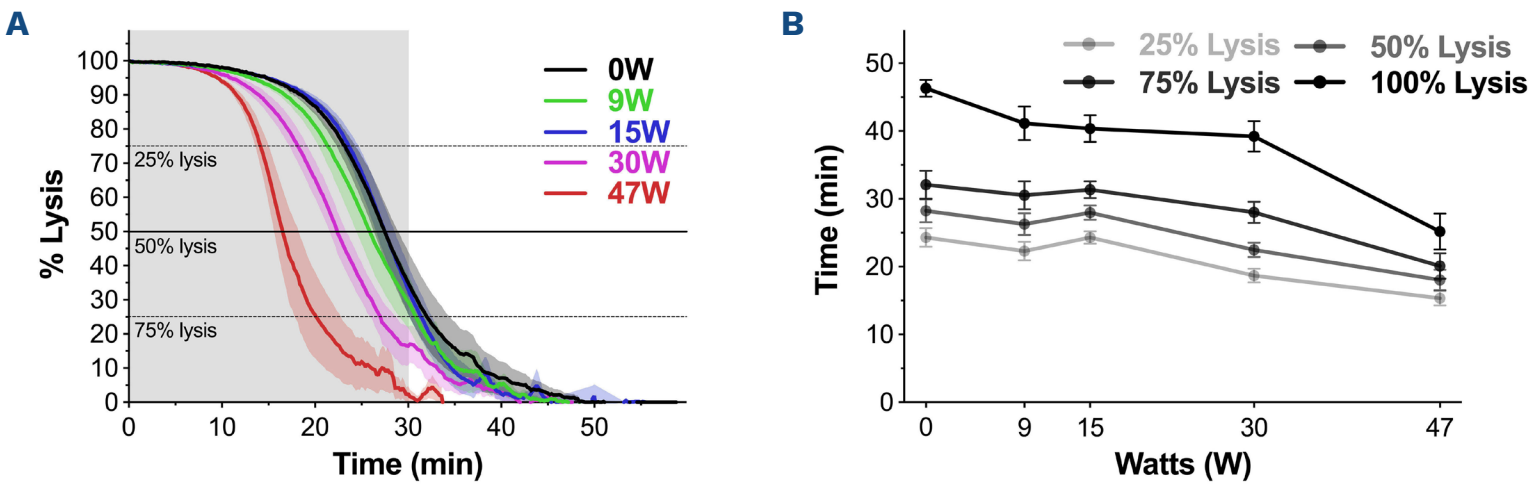

Figure 4. EKOS increases clot lysis rates. Plasma clot lysis was measured by turbidity experiments, in the absence or presence of ultrasound (US). US (gray-shaded area) at 9, 15, 30 and 47W was applied after 30 minutes (min) of clotting for 30 min. Turbidity curves normalized to 100% optical density (OD) at 30 min (A) show that 30W and 47W US sped up clot lysis. 30W and 47W US reduced the time to reach 25%, 50%, 75%, and 100% lysis (B,C). Group comparisons were analyzed using two-way ANOVA with Dunnett's multiple comparison test. Data are represented as mean ± standard deviation (N=6). **P*<0.05, ***P*<0.01, $^{\$}$*P*<0.001, $^{\#}$*P*<0.0001.

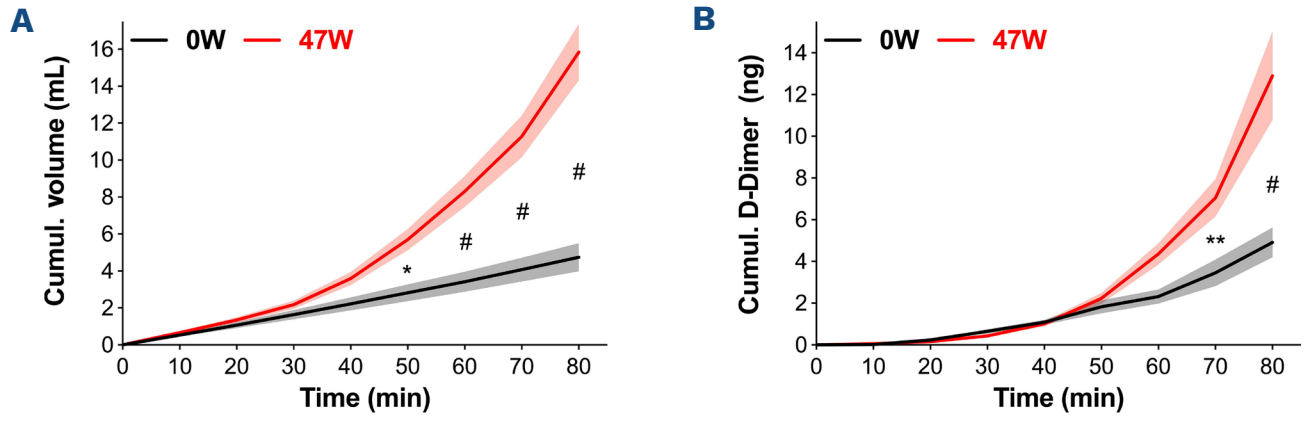

Figure 5. EKOS increases clot lysis under flow. Permeation experiments were performed on plasma clot, with application of tissue plasminogen activator (tPA) once the clot has formed, in the absence or presence of ultrasound (US). 47W US significantly and exponentially increased the rate of permeation as tPA flowed through the clot, compared to the absence of US (A). The amount of D-Dimer in the flow-through also significantly and exponentially increased with 47W US, compared to the absence of US (B). Of note, all clots exposed to 47W US collapsed between the 80-90 minutes (min) timepoints, whilst those without US remain structurally intact during the 90 min measurement period. Group comparisons were analyzed using one-way ANOVA with Tukey multiple comparison test. Data are represented as mean ± standard deviation (N=6). **P*<0.05, ***P*<0.01, $^{\#}$*P*<0.0001. Cumul.: cumulative.

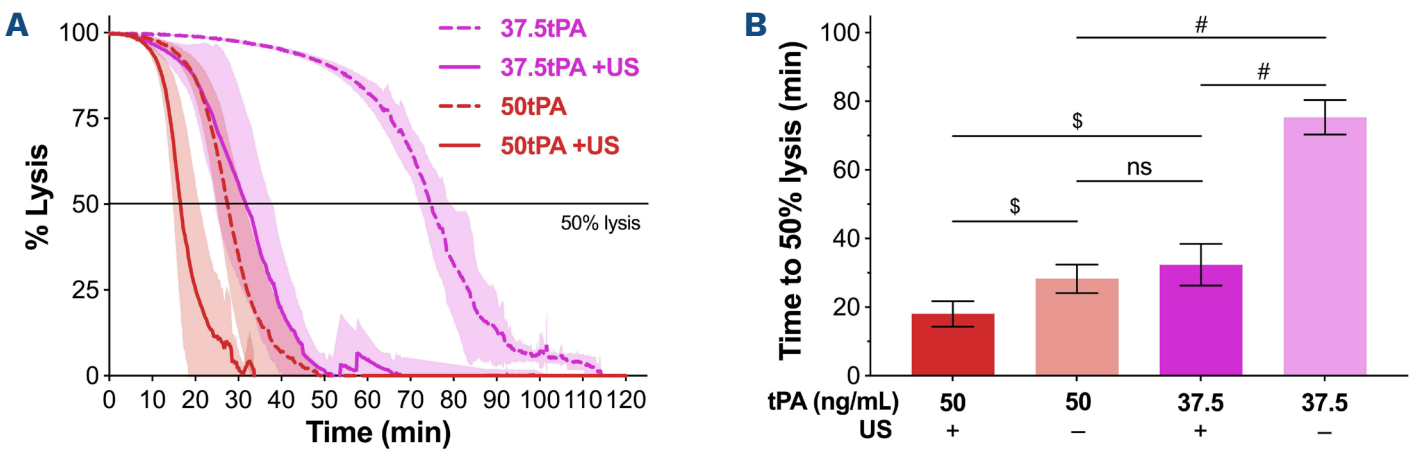

Figure 6. Lower tissue plasminogen activator doses are required for similar efficacy when ultrasound is applied. Plasma clot lysis was measured by turbidity experiments, in the absence or presence of ultrasound (US) with various doses of tissue plasminogen activator (tPA). US was applied after 30 minutes (min) of clotting, for the duration of the lysis, at 47W. Turbidity curves normalized to 100% optical density at 30 min (A). At 50 ng/mL tPA, 47W US significantly reduced lysis time, whilst lysis with 37.5 ng/mL tPA alone showed a significant reduction in lysis time (A,B). However, application of US in the presence of 37.5 ng/mL tPA increased lysis efficiency to similar level to 50 ng/mL tPA without US (A,B). Group comparisons were analyzed using one-way ANOVA with Tukey multiple comparison test. Data are represented as mean ± standard deviation (N=6). $^{\$}P<0.001$, $^{\#}P<0.0001$.

Discussion

Our study shows that *in vitro* application of ultrasound through a catheter placed close to a plasma clot accelerates fibrinolysis rates, and that these effects are associated with changes in clot structure, based on thinning of fibrin fibers and increased porosity. These effects on clot structure provide a possible mechanism for the effects of ultrasound on fibrinolysis, since increased porosity and thinning of fibrin fibers increase the access of fibrinolytic enzymes to the core of the clot and its constituent fibrin fibers. The mechanistic effects were mainly present at ultrasound outputs of 30W and 47W in the context of this model system. Two key findings are of particular interest. Firstly, the effects of ultrasound on clot structure were completely reversible, with onset of effects and return to baseline occurring within minutes of application and discontinuation of ultrasound, respectively. Secondly, the increase in fibrinolysis was measurable under static conditions but enhanced exponentially under flow conditions. These data suggest that ultrasound provides provide a fully reversible effect on fibrin fiber architecture that increases lysis, particularly when blood flow has been (partially) restored.

Reversibility of ultrasound-mediated effects on fibrin structure provides evidence that there are no long-lasting changes in clot structure induced by the application of ultrasound. We further showed that ultrasound did not change the clot formation stage, but only had an effect after clots were fully formed. We observed a reversible thinning of fibrin fibers, which could be caused by disbanding or dispersing of internal fibrin structures. Ultrasound-induced fiber dispersion is then reflected by smaller fiber diameters after dehydration and fixation in SEM. A previous study showed that fibrin fibers are remodeled by reversibility of binding interactions that underpin fiber formation, as shown by re-arrangements of fluorescently labeled fibrin molecules in fully assembled fibers.[11] However, this type of fibrin remodeling was abrogated after fibrin crosslinking by activated Factor XIII.[11] Since our experiments are performed with blood plasma, the clots of which are fully crosslinked by Factor XIII, the reversibility observed here is likely due to another process. A possible mechanism for the reversible fiber dispersing by ultrasound is based on increased thermodynamic structural kinesis. We have shown that clot structure is reversibly altered by ultrasound shortly after formation; however, PE occlusion results from a breakdown of preformed 'older' deep vein thrombi. Our current experimental set-up did not allow for these effects to be assessed on 'older' thrombi, which will need further investigation.

In agreement with the hypothesis of thermodynamic structural kinesis, our data indicate that catheter-mediated ultrasound application increases access of fibrinolytic factors to the interior of fibrin fibers. Moreover, the rates of fibrinolysis accelerated exponentially when we applied flow. A key mechanism underpinning this accelerated lysis under flow is that there is a constant supply of fresh tPA applied to the clot, whereas a fixed concentration of tPA was used in the static system. Furthermore, as the fibrin clot was degraded by tPA, flow increased, resulting in delivery of growing quantities of tPA to the lysing clot and an exponential increase in clot breakdown.

The reversible changes in fibrin structure underpinning increased fibrinolysis prompted us to study whether similar fibrinolysis rates could be achieved with lower doses of tPA in the presence of ultrasound. Indeed, when ultrasound was applied, we could lower the dose of tPA by approximately 25% to achieve the same rate of clot breakdown under static conditions. It is likely that an even greater reduction in dose may be achieved when flow has been partially restored. Note that the concentrations of tPA in these *in vitro* experiments (ng/mL) are different from those applied clinically (mg/kg). Our findings have several important clinical implications. Catheter-directed throm-

bolysis is selectively used in two principal scenarios for the treatment of pulmonary embolism. The first is in patients in whom there are signs of right ventricular dysfunction, and catheter lytic is considered as a preferential strategy to the use of anticoagulation alone, with the aim of accelerating recovery in PE patients with right ventricular dysfunction and/or hypoxia. The second is in patients with hemodynamic instability caused by PE, in whom concerns exist over the bleeding risks associated with high-dose systemic thrombolysis.[12] Our findings suggest a potential role for ultrasound facilitation of thrombolysis with respect to both of these groups, albeit with potentially important caveats.

Firstly, in patients with PE and hemodynamic disturbance, the risk of death or destabilization is most pronounced within the first 48-72 hr of presentation.[13] This is due to a 'spiral of shock' whereby the longer the right ventricle is under strain, the worse the hemodynamic disturbance becomes.[3] A pharmacomechanical strategy of reperfusion, using both ultrasound and thrombolytic, could, therefore, accelerate clot dissolution and, as a consequence, increase recovery of the failing right ventricle when compared to a strategy of catheter or peripheral thrombolysis alone, both in terms of speed and volume of clot dissolution, as evidenced by D-dimer and time to dissolution.[14,15] The second advantage of a combined strategy with ultrasound is the facilitation of lower doses of thrombolytic to dissolve a given amount of clot. In the case of patients with high bleeding risk, the advantages are clear – the circulating volume of lytic would be lower and, therefore, in elderly patients or cancer patients the bleeding risk might also be lower.

This concept is supported by several human clinical trials of EKOS. Initial published studies of catheter lysis with the EKOS system had utilized 20 mg of alteplase over 15 hr,[4] or 24 mg over 12 hr.[16] However, the OPTALYSE study randomized patients to differing doses of alteplase (from 8 mg to 24 mg for bilateral PE) with shorter durations of delivery (from 2 to 6 hr). Interestingly, just 8 mg (over 2 hr or 4 hr) and 12 mg (over 6 hr) of alteplase with EKOS were all able to successfully offload the right ventricle (RV) on computed tomography at 48 hr, with durable clinical benefit at one year,[17] although the absence of an anticoagulation control arm prevented comparison to lytic-free treatment in that trial. In the ULTIMA study, however, there were no changes at 24 hr in the degree of echocardiographic RV ventricular dilatation in patients treated with anticoagulation alone, suggesting that resolution of RV strain is a relatively slow process when treated with anticoagulation alone, when compared to EKOS with alteplase.[13]

In KNOCOUT PE, 489 patients were enrolled in a real-world prospective observational study, reporting data on outcomes and bleeding from real-world use of EKOS.[18] The majority of patients were enrolled after publication of OPTALYSE, and so lower doses of lytic given over shorter time periods were increasingly used in clinical practice, with a median dosage across the study of 18 mg of alteplase. Major bleeding rates across the full cohort at 72 hr were 1.6%, markedly lower than those observed in the earlier SEATTLE II and OPTALYSE studies, which the authors speculate may have been due to lower dose protocols in higher bleeding risk patients. The 8 mg and 12 mg protocols tested within OPTALYSE were utilized in 31% of patients in the KNOCOUT study.[18]

Combining bench data from the current manuscript with the available prior *in vivo* clinical data on the use of ultrasound-assisted catheter-based thrombolysis, therefore, raises the possibility that ultrasound could accelerate clot dissolution and/or reduce the dose of lytic. A single head-to-head comparison of catheter lysis *versus* ultrasound-assisted catheter lysis showed no difference in clot burden reduction on CT scanning performed at 48 hr, but the study was underpowered (N=81) and infusion times were not standardized.[7] Larger studies are needed to test either clinical outcome and/or benefit on RV strain resolution or bleeding risk reduction. The largest trial to date of ultrasound-assisted catheter lysis (a randomized outcome trial of EKOS plus anticoagulation *vs*. anticoagulation alone) is currently ongoing, aiming to recruit 544 patients.[19] However, this trial is not modulating lytic dosing, and will not compare ultrasound-facilitated lysis against either passive catheter lysis or systemic lysis. Nevertheless, it will test the role of ultrasound facilitation of lytic against current standard of care in intermediate-high risk PE, and we await the results with interest.

Finally, the question of an ultrasound power algorithm remains open. In the current study, 30W and 47W demonstrated highly reversible changes in clot structure. Lower wattages were less effective. The only available commercial system to utilize this pharmacomechanical strategy, the EKOS system, deploys a rotating algorithm of wattage, cycling through low wattages, below the threshold of 30W, up to a maximum of 47W.[5] Further studies of whether a rotating algorithm offers improved safety or efficacy over and above a fixed algorithm at 30W to 47W would be of interest. The manufacturers of the EKOS system are currently conducting clinical trials with a new algorithm deploying higher wattages, designed to further shorten time and dose of ultrasound-assisted lytic delivery in PE (clinicaltrials.gov 06310018). Additional studies would be required to assess the impact of this new protocol.

In conclusion, in our bench model, low-power ultrasound reversibly altered clot configuration and increased the rate of clot dissolution in the presence of thrombolytic agent. These findings support the concept of ultrasound-facilitated pharmacomechanical treatment of venous thromboembolism, although the optimal algorithm of ultrasound wattage for safety or efficacy and the optimal dosage of adjunctive lytic to be used in patients to gain the greatest net clinical benefit require further investigation.

Disclosures

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Ultrasound System®, but all the work was performed independently by the investigators. ASPS is a consultant to Medtronic, Boston Scientific, Philips, Angiodynamics, Penumbra, and Recor Medical, and has stock options in Althea Medical. All of the other authors have no conflicts of interest to disclose.

Contributions

CD and RASA designed the study, performed the research, interpreted the data, performed the statistical analysis, and wrote the manuscript. ASPS interpreted the data and wrote the manuscript. All authors critically reviewed and approved the final manuscript for publication.

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Data-sharing statement

Data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Cohen AT, Agnelli G, Anderson FA, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. Thromb Haemost. 2007;98(4):756-764.
2. Abraham P, Arroyo DA, Giraud R, Bounameaux H, Bendjelid K. Understanding haemorrhagic risk following thrombolytic therapy in patients with intermediate-risk and high-risk pulmonary embolism: a hypothesis paper. Open Heart. 2018;5(1):e000735.
3. Konstantinides SV, Meyer G, Becattini C, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J. 2020;41(4):543-603.
4. Kucher N, Boekstegers P, Muller OJ, et al. Randomized, controlled trial of ultrasound-assisted catheter-directed thrombolysis for acute intermediate-risk pulmonary embolism. Circulation. 2014;129(4):479-786.
5. Soltani A, Volz KR, Hansmann DR. Effect of modulated ultrasound parameters on ultrasound-induced thrombolysis. Phys Med Biol. 2008;53(23):6837-6347.
6. Shi Y, Shi W, Chen L, Gu J. A systematic review of ultrasound-accelerated catheter-directed thrombolysis in the treatment of deep vein thrombosis. J Thromb Thrombolysis. 2018;45(3):440-451.
7. Avgerinos ED, Jaber W, Lacomis J, et al. Randomized trial comparing standard versus ultrasound-assisted thrombolysis for submassive pulmonary embolism: the SUNSET sPE trial. JACC Cardiovasc Interv. 2021;14(12):1364-1373.
8. Duval C, Allan P, Connell SD, Ridger VC, Philippou H, Ariens RA. Roles of fibrin alpha- and gamma-chain specific cross-linking by FXIIIa in fibrin structure and function. Thromb Haemost. 2014;111(5):842-850.
9. Gauer JS, Duval C, Xu RG, et al. Fibrin-glycoprotein VI interaction increases platelet procoagulant activity and impacts clot structure. J Thromb Haemost. 2023;21(3):667-681.
10. Pieters M, Undas A, Marchi R, et al. An international study on the standardization of fibrin clot permeability measurement: methodological considerations and implications for healthy control values. J Thromb Haemost. 2012;10(10):2179-2181.
11. Chernysh IN, Nagaswami C, Purohit PK, Weisel JW. Fibrin clots are equilibrium polymers that can be remodeled without proteolytic digestion. Sci Rep. 2012;2:879.
12. Pruszczyk P, Klok FA, Kucher N, et al. Percutaneous treatment options for acute pulmonary embolism: a clinical consensus statement by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function and the European Association of Percutaneous Cardiovascular Interventions. EuroIntervention. 2022;18(8):e623-e638.
13. Meyer G, Vicaut E, Danays T, et al. Fibrinolysis for patients with intermediate-risk pulmonary embolism. N Engl J Med. 2014;370(15):1402-1411.
14. Nadjiri J, Kierse A, Sendlbeck M, et al. Efficacy of ultrasound assisted catheter-directed thrombolysis compared to catheter-directed thrombolysis in vitro. Acta Radiol. 2023;64(1):119-124.
15. Engelberger RP, Schroeder V, Nagler M, et al. Enhanced thrombolysis by ultrasound-assisted catheter-directed thrombolysis and microbubbles in an in vitro model of iliofemoral deep vein thrombosis. Thromb Haemost. 2019;119(7):1094-1101.
16. Piazza G, Hohlfelder B, Jaff MR, et al. A prospective, single-arm, multicenter trial of ultrasound-facilitated, catheter-directed, low-dose fibrinolysis for acute massive and submassive pulmonary embolism: the SEATTLE II study. JACC Cardiovasc Interv. 2015;8(10):1382-1392.
17. Piazza G, Sterling KM, Tapson VF, et al. One-year echocardiographic, functional, and quality of life outcomes after ultrasound-facilitated catheter-based fibrinolysis for pulmonary embolism. Circ Cardiovasc Interv. 2020;13(8):e009012.
18. Sterling KM, Goldhaber SZ, Sharp ASP, et al. Prospective multicenter international registry of ultrasound-facilitated catheter-directed thrombolysis in intermediate-high and high-risk pulmonary embolism (KNOCOUT PE). Circ Cardiovasc Interv. 2024;17(3):e013448.
19. Klok FA, Piazza G, Sharp ASP, et al. Ultrasound-facilitated, catheter-directed thrombolysis vs anticoagulation alone for acute intermediate-high-risk pulmonary embolism: rationale and design of the HI-PEITHO study. Am Heart J. 2022;251:43-53.