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Citation for published version:

Burke, SJ, Annapragada, A, Hoffman, EA, Chen, E, Ghaghada, KB, Sieren, J & Van Beek, EJR 2007, 'Imaging of Pulmonary Embolism and t-PA Therapy Effects Using MDCT and Liposomal Iohexol Blood Pool Agent' *Academic Radiology*, vol. 14, no. 3, pp. 355-362. DOI: 10.1016/j.acra.2006.12.014

Digital Object Identifier (DOI):

[10.1016/j.acra.2006.12.014](https://doi.org/10.1016/j.acra.2006.12.014)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Academic Radiology

Publisher Rights Statement:

Published in final edited form as:
Acad Radiol. 2007 March; 14(3): 355–362.
doi: 10.1016/j.acra.2006.12.014

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Published in final edited form as:

Acad Radiol. 2007 March ; 14(3): 355–362.

Imaging of pulmonary embolism and t-PA therapy effects using MDCT and liposomal iohexol blood pool agent – preliminary results in a rabbit model

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Abstract

Hypothesis and Objectives—PEGylated liposomal blood pool contrast agents maintain contrast enhancement over several hours. This study aimed to evaluate (long-term) imaging of pulmonary arteries, comparing conventional iodinated contrast with a liposomal blood pool contrast agent. Secondly, visualization of the (real-time) therapeutic effects of tissue-Plasminogen Activator (t-PA) on pulmonary embolism (PE) was attempted.

Materials and Methods—Six rabbits (approximate 4 kg weight) had autologous blood clots injected through the superior vena cava. Imaging was performed using conventional contrast (iohexol, 350 mg I/ml, GE HealthCare, Princeton, NJ) at a dose of 1400 mgI per animal and after wash-out, animals were imaged using an iodinated liposomal blood pool agent (88 mg I/mL, dose 900 mgI/animal). Subsequently, five animals were injected with 2mg t-PA and imaging continued for up to 4 ½ hours.

Results—Both contrast agents identified PE in the pulmonary trunk and main pulmonary arteries in all rabbits. Liposomal blood pool agent yielded uniform enhancement, which remained relatively constant throughout the experiments. Conventional agents exhibited non uniform opacification and rapid clearance post injection. Three out of six rabbits had mistimed bolus injections, requiring repeat injections. Following t-PA, Pulmonary embolus volume (central to segmental) decreased in four of five treated rabbits (range 10–57%, mean 42%). One animal showed no response to t-PA.

Conclusions—Liposomal blood pool agents effectively identified acute PE without need for re-injection. PE resolution following t-PA was quantifiable over several hours. Blood pool agents offer the potential for repeated imaging procedures without need for repeated (nephrotoxic) contrast injections.

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Keywords

pulmonary embolism; blood pool; iodinated contrast agents; liposomes; CT; thrombolysis

Introduction

Patients who present with acute chest symptoms are a frequent clinical problem (1,2). This is partly due to the range of acute diagnoses that can be considered. As a result, early triage to decide on adequate management is crucial. This has led to a significant increase in CT utilization (3–6), and attempts are now focusing on reducing the number of patients requiring imaging (7,8). Essentially, the three most critical diagnoses that clinicians focus on are (1) myocardial ischemia, (2) pulmonary embolism and (3) aortic dissection or rupture. Although all three diagnoses can now be evaluated using CT, simultaneous evaluation, or a “triple rule out” CT protocol, is difficult to entertain due to competing requirements. Since conventional contrast agents are distributed and cleared within minutes (9–11), effective multifunctional imaging becomes difficult. Thus, for PE evaluation, the contrast bolus has to be timed to be optimal in the right heart and pulmonary circulation (12,13), while for assessment of the aorta and coronary arteries a greater delay from bolus to imaging is required to reach optimal contrast enhancement in the left heart.

A correctly timed contrast bolus delay is not easy to achieve, even with the aid of a test bolus or bolus detection systems in modern CT scanners, up to 10% of PE studies suffer from sub-optimal contrast in the pulmonary arteries (8,12–14). Although one could devise a combined protocol with multiple contrast bolus injections and repeated CT imaging of the relevant field of view, it is likely that the number of sub-optimal contrast studies will rise. A single study with optimal, simultaneous enhancement in multiple vascular beds would prevent repeat studies with their incremental X-ray dose with each scan and the nephrotoxicity of repeat contrast administration.

Contrary to current conventional CT contrast media, blood pool agents offer a number of theoretically interesting alternatives. (15,16). First, the contrast can be delivered as a single slowly injected dose with fewer constraints on optimization of contrast bolus (although the first pass could still take advantage of this option), thus allowing imaging of the right and left circulation within a single investigation after a single injection of contrast agent. Second, the liposomal agents used in this study have been previously shown to clear via the reticulo-endothelial system (15,16) and not via renal filtration, thus reducing the nephrotoxicity associated with conventional contrast agents. Finally, a blood pool agent could allow longitudinal studies to determine the efficacy of (potentially aggressive) treatment, such as thrombolytic therapy, in acute cardiac ischemia or major pulmonary embolism, without the need for a second contrast injection (17).

There have been attempts to give more homogeneous (and prolonged) contrast enhancement using macromolecular agents (10,11) and other contrast carriers (9). However, none can be defined as a true long-acting blood pool agent. Long circulating liposome-based contrast agents represent a novel class of blood pool agents for CT imaging (15). This agent consists of a non-ionic, conventional iodinated contrast agent encapsulated in polyethylene glycol coated liposomes (PEGylated liposomes). The iodinated liposomal blood pool contrast agent is cleared by the reticulo-endothelial system, thus reducing the potential for nephrotoxicity and enhancing their applications in patients who currently would be considered too great a risk for conventional iodinated contrast agents, such as those with renal impairment (15,18). A previous study using this agent showed rapid blood pool contrast attenuation in a rabbit model that remained virtually constant over a 3.5 hour period (15). To test the hypothesis that liposomal

contrast agent can yield prolonged and diagnostically sufficient enhancement of the pulmonary circulation, conventional iohexol (Omnipaque 350) was compared with iohexol-encapsulated liposomal agent (liposomal-iohexol) in a rabbit model of pulmonary embolism before and after treatment with tissue plasminogen activator (t-PA).

Materials and Methods

Rabbit in-vivo protocol

Following approval by the University of Iowa's Animal Care and Use Review Committee, male New Zealand rabbits (weight approximately 4 kg) were sedated using 18 mg of ketamine and 3mg of xylazine intramuscularly. After peripheral intravenous access was established in an ear vein, and 10 ml of blood was drawn. Further sedation was given using intravenous pentobarbital. Eight rabbits were included in this study with all eight receiving autologous blood clot to induce PE. The initial rabbit survived a 10ml clot with the subsequent two rabbits succumbing to fatal PE. The embolism protocol was then modified and the clot burden was then reduced to 1ml or until the embolism could be clearly visualized by standard contrast imaging. Of the six surviving rabbits, five received thrombolytic treatment and were monitored for regression of pulmonary embolism, while one control rabbit did not receive thrombolytic treatment

Preparation of the rabbits included anesthetizing the neck with lidocaine and an open tracheostomy with a 3Fr endotracheal tube and then placing the rabbit on a ventilator with tidal volumes of 50ml (12.5ml/kg), respiratory rate of 25/min, PEEP 10 cmH₂O, and FIO₂ of 100%. A cut down on the internal jugular vein was performed and a 6Fr central venous introducer catheter inserted.

The rabbit was then transported to the CT scanner where imaging before and after the induction of pulmonary embolism was performed. The rabbit was anesthetized during the imaging with 10 mg of pentobarbital intravenously every half hour as needed. At the end of the imaging, the rabbit was sacrificed using a lethal dose of phenobarbital and phenytoin.

Induction of pulmonary embolism

During placement of the ear vein catheter, 10ml of blood was withdrawn and allowed to clot in the syringe at room temperature. The first two rabbits in the experiment received 10ml of clot given through the central line and were successfully imaged. In two subsequent animals, the massive saddle emboli were lethal, prompting a change in our protocol to agitate the blood clot to reduce the size of clot particles and only inject 1 ml of clotted blood. This method was successfully applied in the subsequent five animals.

Thrombolytic therapy protocol

Recombinant tissue plasminogen activator (rt-PA) was chosen because of its clinical applicability and availability. The dose applied was according to clinical protocols for PE or acute coronary events, using two bolus injections of 0.5mg/kg at 30 minute intervals through the central venous catheter line. Following successful imaging of the first control rabbit, this protocol was applied in the subsequent five experiments and imaging was repeated over time without further injection of either contrast or rt-PA.

Imaging protocol

A multiple detector row CT system (Sensation 64, Siemens, Forchheim, Germany) in spiral scanning mode was used with the following parameters: keV 120, mAs 100, pitch 1, and slice collimation of 64×0.6 mm. The field of view was 10cm and images were reconstructed to fit

a 512×512 matrix. Vital signs were monitored using ECG and oxygen saturation throughout the imaging procedure.

Following a base-line non-enhanced study of chest and abdomen, the following order of imaging (see below) was applied beginning with one base-line study using conventional non-ionic low-osmolar contrast. After the injection of blood clot, CT pulmonary angiography was repeated with conventional contrast agent to confirm adequate PE had developed. After a minimum duration of 30 minutes, the liposomal blood pool agent was used for further CT pulmonary angiography at the following time points: baseline, 15, 30, 45, 60, 90, 105, 165 and 270 minutes after the initial injection of blood pool agent. Tissue-Plasminogen Activator (1 mg/animal) was administered twice (total dose 2 mg/animal) at 15 and 30 minutes after the baseline blood pool CT pulmonary angiogram.

Contrast agents

Initial, baseline imaging took place using non-ionic low-osmolar contrast (iohexol; Omnipaque 350, GE HealthCare, Princeton, NJ) at a rate of 0.5 ml/min for 8 seconds for a total dose of 1 ml/kg. The total amount of administered Iodine was 1400 mg per animal.

Liposomal-iohexol formulations were fabricated by methods similar to those described previously (16). Briefly, a lipid mixture consisting of 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Genzyme, MA), Cholesterol (Sigma, St Louis, MO) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(poly(ethylene glycol))-2000] (mPEG2000-DSPE) (Genzyme, MA) in the ratio 55:40:5 was dissolved in ethanol at 60°C. The ethanol solution was then hydrated with iohexol solution (350 mg I/mL) and stirred for 1½ hr at 70°C. The resulting liposomal solution was sequentially extruded at 70°C on Lipex Thermoline extruder (Northern Lipids, Vancouver, Canada) with three passes through 0.4 µm Nuclepore membrane (Waterman, Newton, MA), five passes through 0.2 µm Nuclepore membrane and eight passes through 0.1 µm Nuclepore membrane. The external phase was then cleaned and the liposomes simultaneously concentrated by diafiltration using MicroKros modules (Spectrum Laboratories, CA) of 50 nm cutoff. The size of the resultant liposomal formulations obtained was determined by dynamic light scattering (DLS) using a Brookhaven Instruments BI-9000AT Digital Autocorrelator, a BI-200SM goniometer (JDS Uniphase; San Jose, CA) and a Hamamatsu photomultiplier (supplied by Brookhaven, Long Island, NY). The resultant size of liposomes in the formulation as determined using DLS was 105 nm with a polydispersity index of 0.107. The iodine content of the formulation was determined using a UV spectrophotometer (Libra S32 PC, Biochrom) at 245 nm. The size of the resultant liposomal formulations obtained was determined by Dynamic Light Scattering using a Brookhaven Instruments BI-9000AT Digital Autocorrelator, a BI-200SM goniometer (JDS Uniphase; San Jose, CA) and a Hamamatsu photomultiplier (supplied by Brookhaven, Long Island, NY).

Mean size of liposomes as determined by dynamic light scattering was 93 ± 0.1 nm. The polydispersity index for the formulation was 0.118. The iodine concentration of the final formulation was determined as 88 mgI/ml.

The liposomal formulation was transported under refrigerated condition from Texas to the imaging site in Iowa. Prior to starting the study, the formulation was brought to room temperature and subsequently administered by hand held injection through the central line with a total Iodine dose of 900 mg per animal.

Image analysis

CT images were evaluated for level of contrast opacification of the pulmonary artery using standard measurements of mean attenuation (Hounsfield Units; HU) over time. Time-attenuation curves were assessed for contrast enhancement dynamics.

Quantitative analysis of pulmonary embolus burden with both contrast agents and subsequent response to thrombolytic therapy was performed using an in house developed software system (Volumetric Imaging Display and Analysis: VIDA) (19), which has a region of interest module that adjusts hand drawn regions of interest based upon the half-maximum principle. This method has been used and validated for identifying airway wall borders (20). From a hand drawn region, the software identifies the ROI's center of mass and sends a set of rays out from the center point over a user-determined distance. The voxel values are evaluated along each ray, a minimum (in this case the clot) and a maximum value (either enhanced contrast around the clot or the vessel wall) are determined and the user-drawn edge is adjusted to fall at the half-way point along the maximum and minimum locations. ROIs were drawn at identical slice positions, with identical window levels and magnification settings. However, the half-max adjustment is calculated on the original 16 bit image data and thus is not affected by the window and level settings used to display the images. The edge adjusted ROI was smoothed with a three point smoothing algorithm and a final volumetric value was calculated by the software, adding each of the individual 2D ROIs identified on the axial sections spanning the extent of the clot. Response to thrombolytic treatment was reported as percentage changes in volumetric measurements from the initial clot burden as measured with the initial liposomal contrast.

Results

Contrast agent comparison

CT image density analysis showed good opacification of the pulmonary artery with both conventional iohexol and liposomal-iohexol and allowed detection of pulmonary embolism in all six rabbits that survived the initial PE induction. Time attenuation curves using the liposomal contrast agent confirmed a stable plateau phase greater than 100HU that remained stable over several hours. Figure 1 shows a typical time-attenuation curve for the last rabbit imaged initially with liposomal contrast agent one hour after conventional contrast to avoid early contamination. Previous rabbits showed mild, early contamination with conventional contrast with early peaks in the time-attenuation curves, which then showed a uniformly stable attenuation above 100 HU for several hours.

When comparing conspicuity of pulmonary embolism, there was very close correlation between conventional iohexol and liposomal iohexol. Pulmonary emboli were demonstrated ranging from saddle embolus in the pulmonary trunk down to subsegmental pulmonary arteries. For measurements, we opted to evaluate the central to segmental branches only. Figures 2 and 3 depict filling defects within the contrast column on two comparable slices through the pulmonary trunk and main pulmonary arteries for both contrast agents. The liposomal agent clearly shows better uniformity of contrast enhancement throughout the thoracic vessels, while the emboli are equally well visualized using both agents.

Thrombolytic effect measurements

Pulmonary emboli were observed in all six animals. One animal remained untreated (control), while five received t-PA per protocol and clot volumes were measured and plotted over time (Figure 4). Volumetric analysis showed no change in pulmonary embolism size for the single untreated (control) rabbit, which died relatively soon following PE induction (curve not shown). In the five treated animals, one animal showed no significant response, and the volume measurements were stable (within the measurement error of 8%; rabbit #2). The other four

rabbits showed clot volume reduction, ranging from 10 – 57%, mean 42% (Figure 4). Figures 5–6 show examples of the observed reduction in the thrombus burden in the main and branch pulmonary arteries in this rabbit model utilizing the liposomal blood pool agent.

Discussion

The aim of this study was to test the concept of prolonged imaging of the pulmonary arterial tree using the long circulating liposomal CT contrast agent, and compare the diagnostic quality to the reference standard of an optimally timed bolus of low-osmolar contrast agent (iohexol). In particular, we were interested in showing the feasibility of imaging treatment effects in a thrombolytic pulmonary embolism rabbit model. It appears that the results were positive both in PE identification and for demonstration of treatment effects. However, several issues need further discussion.

First, the evaluation of PE partly depends on the size of emboli. In this rabbit model, central clot is already fairly small when compared to the human pulmonary arteries. Nevertheless, as the study demonstrated, both conventional and liposomal contrast enhanced CT pulmonary angiography were successful in outlining filling defects within the pulmonary artery compatible with embolus. This is along the line of expectations, as previous work has demonstrated the feasibility of blood pool agents in a rabbit model (10,15).

Second, it was possible to repeat the imaging procedure without the additional need for contrast agent injection for up to 4 hours. The findings demonstrate a biphasic enhancement pattern with an equilibration and a plateau phase of the contrast agent, similar to other tested long circulating and blood pool agents (9,10,18,21). However, our experiment demonstrated a much longer maintenance of contrast enhancement in the pulmonary arteries, while the contrast density (in the range of 130 HU) remained at higher values than other agents described thus far (9–11,18,21). These two essential components enable visualization of the effects of thrombolytic therapy of the central and lobar pulmonary emboli. In fact, the volume reduction in the central clot of 40% could be measured, whereas the clots in the lobar vessels improved significantly (but were too small to measure with confidence).

One of the five treated rabbits showed no significant response to t-PA and the clot size remained relatively stable. The literature also shows that some patients don't respond to fibrinolytic therapy, both in cardiac (22), arterial (23) and venous thromboembolic applications (24). Most of such studies don't show effect measurements until 6 – 24 hours following the initial therapy application. Thus, the fact that we have demonstrated similar findings in this rabbit model within minutes/hours of drug administration may yield a model that could be applied for novel drug development and help in gaining more insight into dynamic therapeutic effects within minutes to hours of drug delivery.

The results of this study have important potential applications for future clinical use. The prolonged density increase in the vascular space allows for repeated CT imaging without the need for repeated contrast injections. Combined with the altered clearance mechanism via the liver and spleen (organs of the reticulo-endothelial system), this is an important break-through in the pharmacokinetics of iohexol which is not thought to be particularly hepato-toxic compared to chemotherapeutic liposomes (doxorubicin), however needs to be explicitly investigated. (25) Altered clearance by liposomal contrast agents may allow contrast studies in patients with impaired renal function, as well as, multiple combined use of CT in acute settings, such as for “triple rule out” in the context of patients presenting with acute chest pain.

Another important potential application relates to the use of thrombolytic therapy in myocardial infarction/acute ischemia and in patients with massive (and potentially submassive) pulmonary embolism. The application of blood pool agents could determine the effects of such treatment

over a period of several hours (possibly days), without the risk of nephrotoxicity. Thus, earlier management decisions would be feasible based on the visualization of treatment response or lack thereof. This practice has already been evaluated for patients with pulmonary embolism (17), but these patients required repeated contrast injections and imaging was repeated at approximately 24 hours post treatment. The use of blood pool agents will allow greater flexibility, as the nephrotoxic time limitations will no longer be an issue.

Currently, the production of this blood pool agent is limited in capacity to small animals, but scaling up of this production is currently under-development. Furthermore, the potential toxicity and clinical safety of this agent is yet to be proven. Nevertheless, these initial in vivo studies demonstrate the feasibility of performing repeated imaging and the potential applications are of huge interest. Thus, studies using larger animals will be the next logical step in the development of this agent for clinical use.

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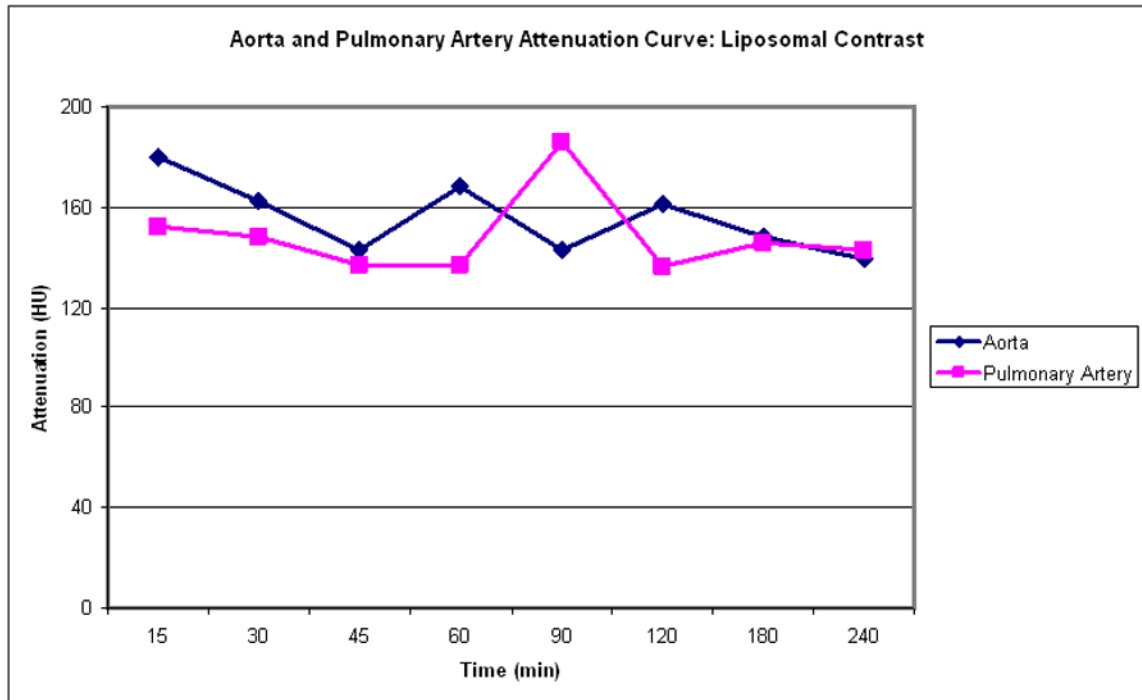


Figure 1. This graph depicts a typical time-attenuation curve in the pulmonary artery and aorta over time with stable attenuation above 100 HU for four hours.

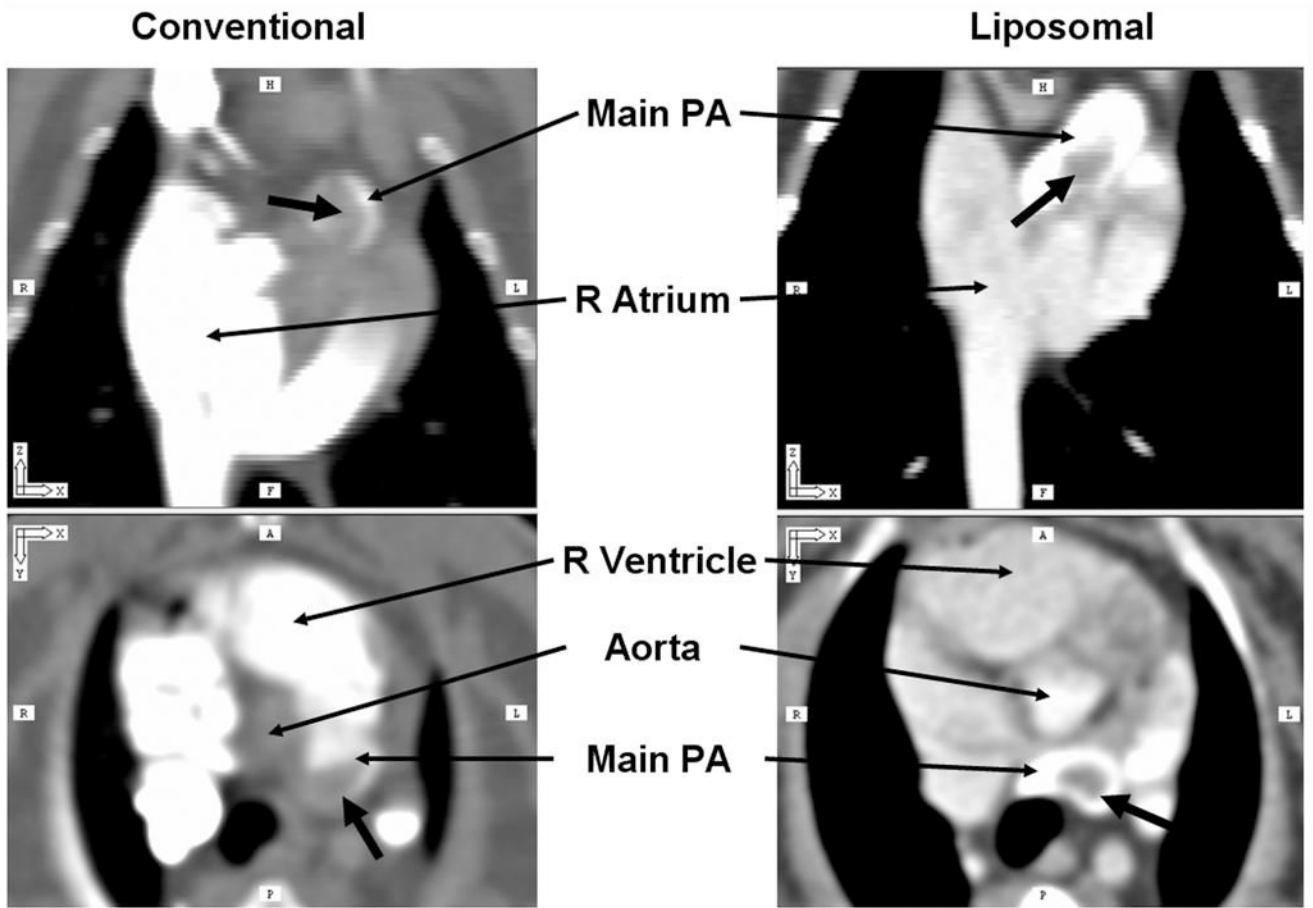


Figure 2. These images demonstrate side-by-side comparison of the images in the same rabbit using conventional versus liposomal iohexol contrast agent. A central pulmonary embolus at the bifurcation is present (bold arrow) and the key structures are identified. Notice that the liposomal agent gives almost homogeneous contrast in both the right and left sided heart, whereas the conventional contrast necessitates bolus timing for right sided enhancement.

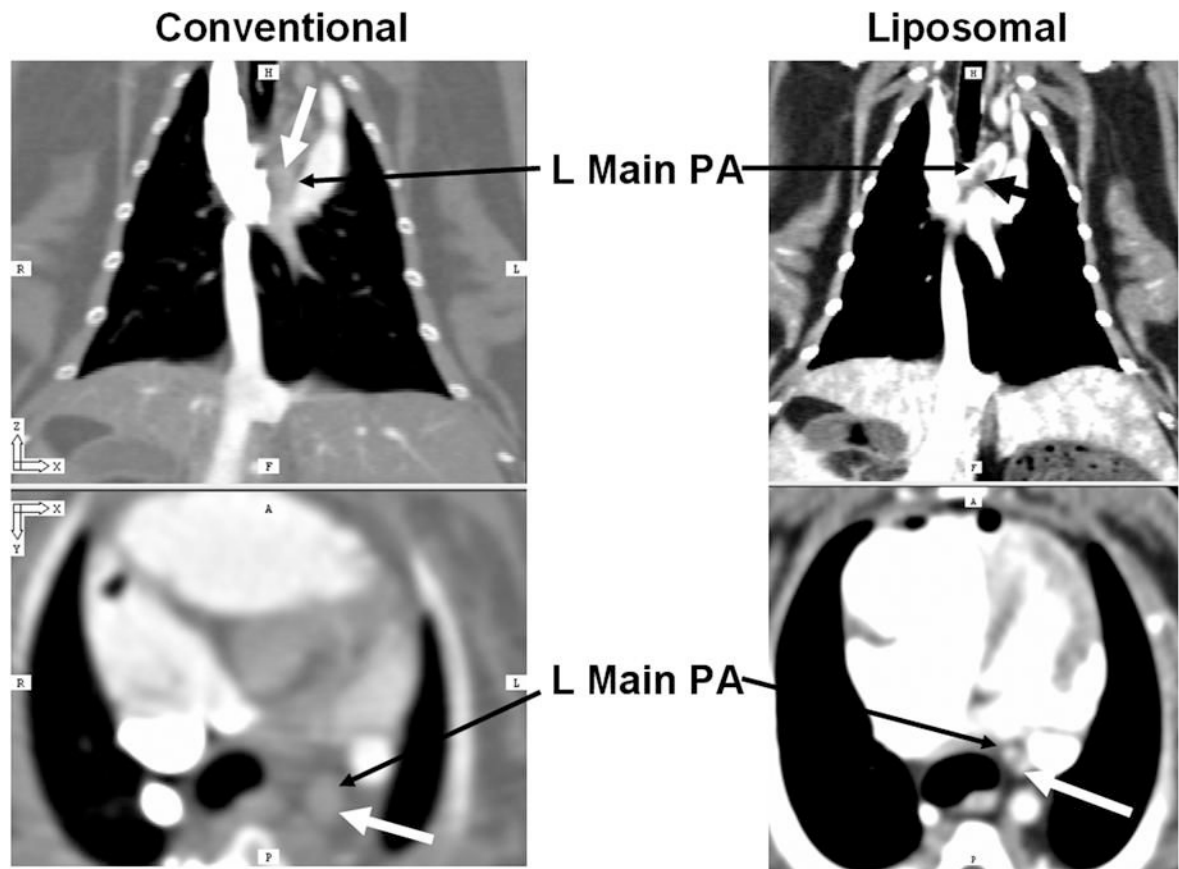


Figure 3. Side-by-side comparison of images in a rabbit following central PE injection, demonstrating the clot in the left main/lower lobe branch using both conventional and liposomal iohexol contrast agents (bold arrows). There is a slight difference in slice position, but otherwise there is excellent correlation both in axial and reconstructed coronal planes.

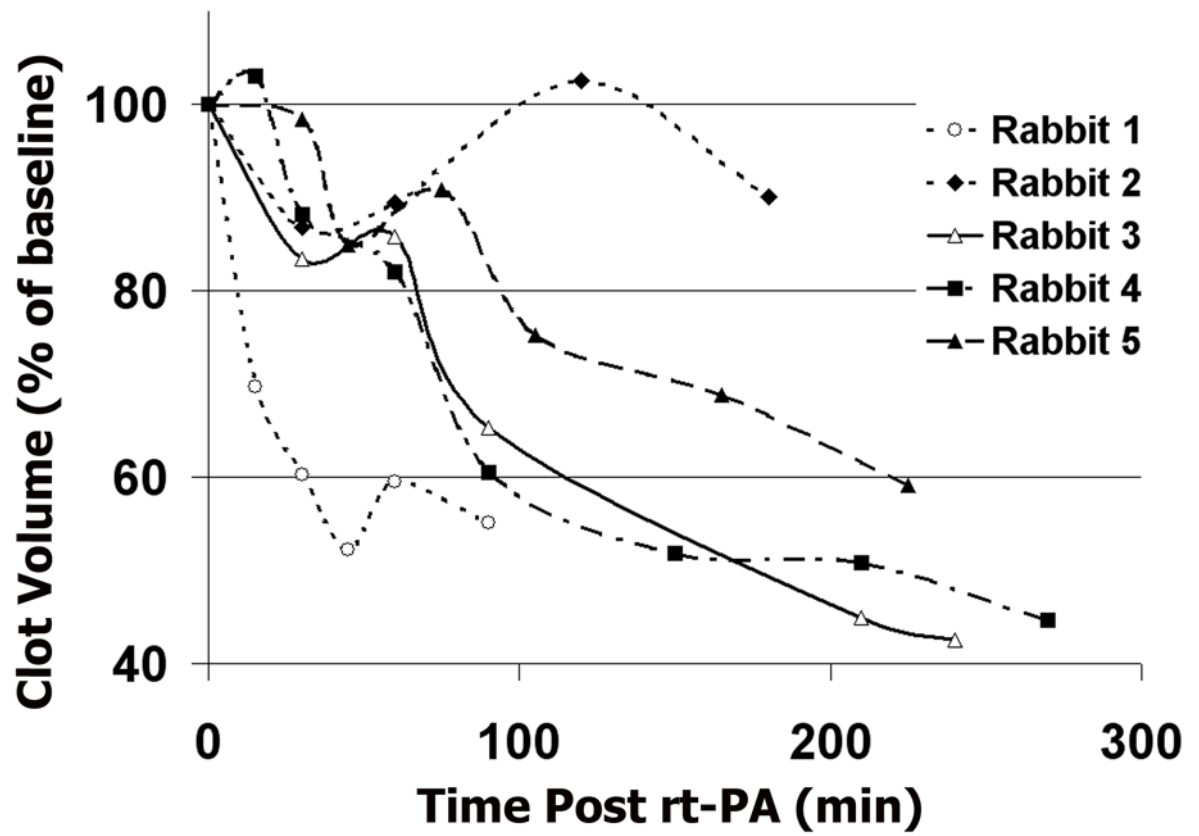


Figure 4. This graph demonstrates the clot size measurements (% volume from baseline) over time following injection of t-PA. Rabbit 2 shows no significant response, but the other 4 treated rabbits show a clot volume decrease in the range of 40–60%.

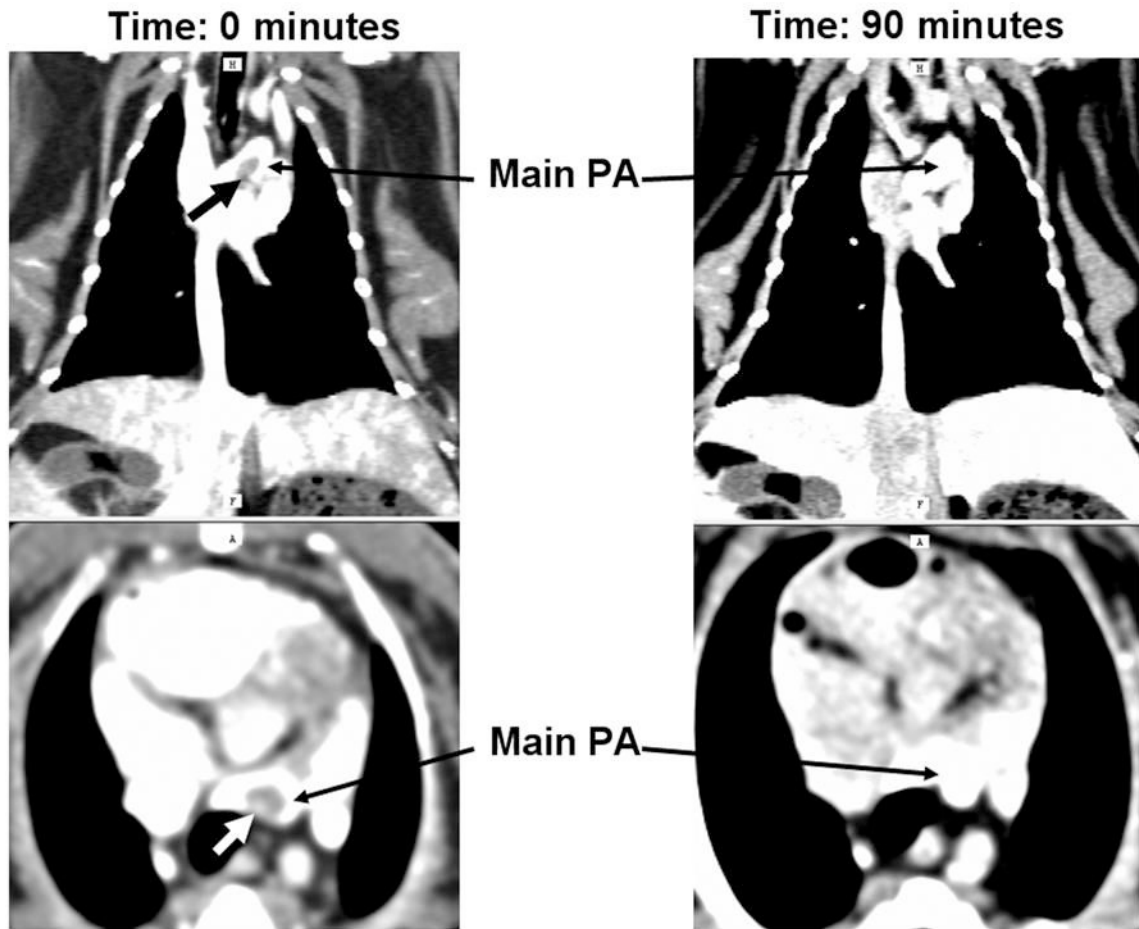


Figure 5. These image series demonstrate clot resolution of a central embolus (bold arrow) in response to t-PA during a 90-minute interval using liposomal iohexol contrast.

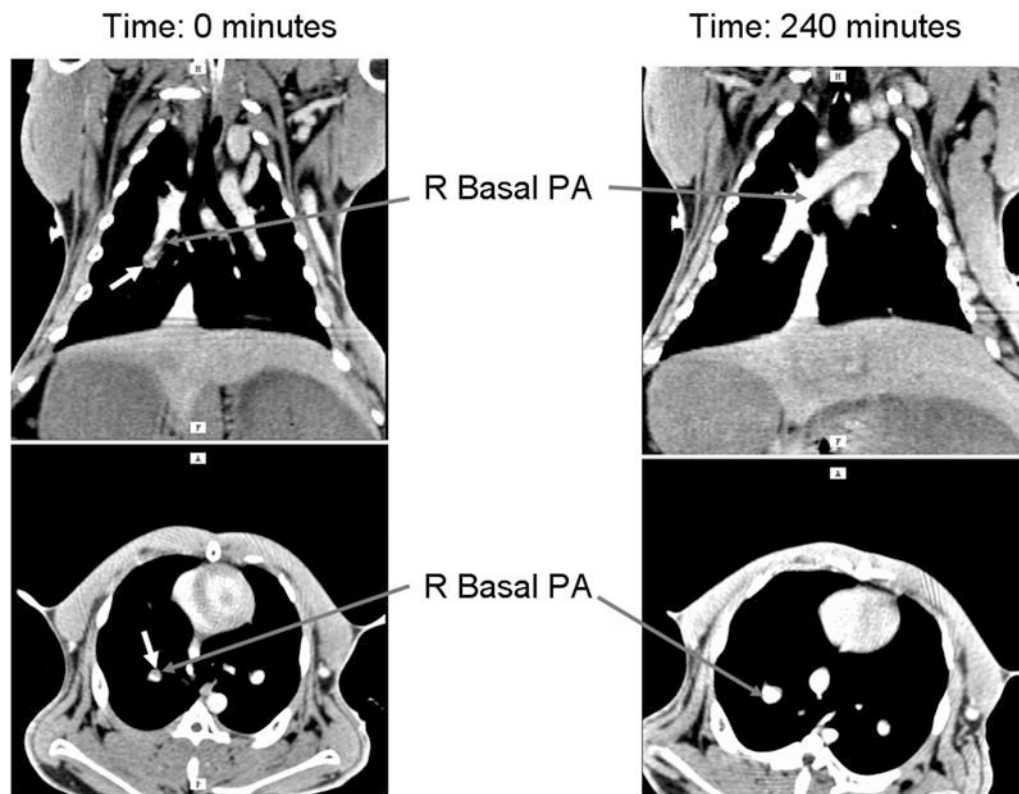


Figure 6. These series of images demonstrate near-complete clot resolution of a right lower lobe embolus (bold arrow) in response to t-PA during a 240-minute interval using liposomal iohexol contrast. It should be noted that no additional contrast was administered during this time period.