Reviewer A

Comment 1:

Line 179: More specific about the ultrasound insonation should be included. What was the pulse duration of the Doppler signal? What are the approximate dimensions of the acoustic field? What are the specifications of the Doppler probe (128 elements? Linear or curvelinear probe?) Does the MI correspond to the B-mode or Doppler pulse?

Reply 1: Pulse Duration: 2ms

Acoustic field: Approximate size of the colour box: 4,9cm x 8,1cm

Doppler Probe: Phased Array; 80 Elements

The MI corresponds to the doppler pulse. Those information were added to the manuscript.

Changes in the text: See Line 198: A 3-Mhz Doppler diagnostic ultrasound probe (Sonos 7500; Philips Ultrasound, USA; probe: diagnostic phased array probe with 80 elements) was placed above the skull, and the distance between the skull and ultrasonic probe was bridged with ultrasound gel (Transatlantic Handelsgesellschaft Stolpe & Co. mbH, Neu-Anspach, DE). Transcranial colour-coded duplex ultrasound (TCCD) was applied continuously for the duration of the treatment (B-mode, colour Doppler functions switched on, approximate size of the colour box: 4,9cm x 8,1cm). The Doppler beam was aligned to expose the entire brain incorporating the circle of Willis and the occluded MCA. The spectral Doppler sample volume (57 mm) was placed in the midbrain (pulse duration 2ms, maximum output [mechanical index of the Doppler of 1.7]). *

Comment 2:

Figure 7: What is the infarct size percentage correspond to? Legend does not reflect the data.

Reply 2: The percentage corresponds to the ipsilateral hemispheric volume.

Changes in the text: The informations were added on the appropriate parts: "Mean value with standard deviation of the infarct size as percentage of ipsilateral hemispheric volume 24 hours after thrombus induction." This was added to line 327, line 342 and line 351

Comment 3:

Line 328: Interesting that the ultrasound group showed some actual treatment. Prior studies with moderate ultrasound intensities found no influence of ultrasound and microbubbles, particularly for well formed clots akin to what is used here (see, for

instance, Bader Ultrasound Med Biol 2013).

Reply 3: As stated in the manuscript, heterogeneity of the results is large in the different trials. Unfortunately, our results lead to further heterogeneity. The paper of Bader et al. refers to different in vitro studies. However, in an in vivo model, an effect of mmSTL might be present due to intrinsic t-PA. This was further discussed in our manuscript.

Changes in the text: Line 455: The following was added to the discussion section of the manuscript: We were able to show an effect of mmSTL without use of rt-PA. Lu et al. and Re et al. demonstrated an effect of mmSTL without rt-PA in rats, but used a different clot model. Brown et al. were also able to show an effect of mmSTL alone, but in rabbits . In vitro-studies have suggested, that moderate ultrasound intensities have a limited effect on thrombolysis without use of rt-PA. However, in the in vivo situation, intrinsic t-PA is present that might facilitate a thrombolytic effect.

Comment 4:

Line 401: The study does not show significance when it comes to improvements when including ultrasound, though. Based on these data, sonothrombolysis shows no benefit over rt-PA alone (so why do it if it won't help?)

Reply 4: Of course, the reviewer is right. Just like neuroprotection, STL failed to show an effect on functional outcome in studies in humans. Therefore, STL can not be recommended.

Changes in the text: Line 483: We added the following sentences to the manuscript for clarification: Nevertheless, sonothrombolysis might share the fate of neuroprotection, as it might be effective in animal models but shows no effect on functional outcome of stroke patients.   Therefore, sonothrombolysis will still play no role in clinical routine.

Comment 5:

Also Line 401: Some comments on ways to improve the model would be appropriate. There were a number of non-responding animals who did not stroke or who died, which makes for a difficult study.

Reply: To improve the model, it should be possible to produce a thrombus that always has the same constitutions and comparable properties to humans. Thus, the scatter of infarct sizes would no longer exist and the non-responsive or dead animals can be eliminated in advance. The intraoperative mortality might be lower when using continuous pulse oximetry and orotracheal intubation. By this approach, lower dosages of isoflurane can be used which might lead to a lower rate of complications and the person performing the surgery can react faster to changes in SpO2 and the heart rate.
We added this to the manuscrips

Changes in the text: Line 497

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be used which might lead to a lower rate of complications and the person performing the surgery can react faster to changes in SpO2 and the heart rate. By this approach, mortality and rate of model failure might be reduced.

Reviewer B

Major:

Comment 6:

Why the absolute infarct size did not differ among the 4 groups 90 minutes after surgery. If the sonothrombolysis will provide the therapeutic effect, early recanalization should be achieved.

If the sonothrombolysis can increase the rate of delayed recanalization, hemorrhagic transformation should be accompanied.

Reply 6: The MRI after 90min was used to exclude model failure. At this point, there might be some penumbral tissue, that is still vital via collaterals that deteriorate over the time. There is also secondary infarct growth due to compressive effects on vessels via cerebral oedema. Vasogenic oedema begins to manifest as early as 20 minutes after vessel occlusion and is responsible for up to 50% of the definitive infarct volume. Another effect is that T2-imaging does not show definitive infarct size after 90 minutes. This will take some hours. Therefore, the missing differences of the groups is somewhat expected and no effect of early recanalization can be deduced. The reviewer offers a valuable notion when discussing a delayed recanalization. As we saw no differences of intracerebral bleeding between the groups a delayed recanalization seems unlikely as a mechanism of action for STL. For clarification, we will discuss this in the manuscript.

Changes in the text:

Line 385: As we saw no differences of intracerebral haemorrhage between the groups a delayed recanalization seems unlikely as a mechanism of action for STL. A delayed recanalization is associated with reperfusion injury and a higher rate of intracerebral haemorrhage (28).

90 minutes after thrombus induction, we saw no differences between the treatment groups. This might be mainly due to the circumstance, that T2-imaging, that reflects cerebral vasogenic oedema, needs time to show an ischemic lesion. 90 minutes after thrombus induction, there might remain some penumbral tissue that is still vital via collaterals that deteriorate over the course of time, resulting in infarct growth. The vasogenic oedema leads to secondary infarct growth due to compressive effects on cerebral vessels, but needs time to develop. It is responsible for up to 50% of the definite infarct volume (29).