Author's response to reviews

Title: A novel model for evaluating thrombolytic therapy in dogs with ST-elevation myocardial infarction

Authors:

Hong Zhang (zhhxw2666@163.com)
Yong-chun Cui (cuiyongchun@fuwai.com)
Yi Tian (tian_yi3000@126.com)
Wei-min Yuan (Yuanweimin_2009@126.com)
Jian-zhong Yang (yangjianzhong@fuwai.com)
Peng Peng (retchie@sina.com)
Kai Li (Likai_heart@hotmail.com)
Xiao-peng Liu (xiaopenli@yeah.net)
Dong Zhang (xiaoyuanxingyu@126.com)
Ai-li Wu (xiaoyuanxingyu@126.com)
Zhou Zhou (fwcomd@126.com)
Yue Tang (tangyue1226@vip.sina.com)

Version: 2
Date: 6 January 2016

Author's response to reviews: see over
Dear Editor,

Thank you very much for your decision letter and advice on our manuscript (No. 1694888631166473) entitled “A novel model for evaluating thrombolytic therapy in dogs with ST-elevation myocardial infarction”. We also thank the reviewers for the constructive and positive comments and suggestions. Accordingly, we have revised the manuscript. All amendments are highlighted in red in the revised manuscript. In addition, point-by-point responses to the comments are listed below this letter.

We hope that the revision is acceptable for the publication in your journal.

Look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Yue Tang

Tel: +86-10-88396321; Fax: +86-10-88398075
E-mail: tangyue1226@vip.sina.com

Deputy Director of Cardiovascular Surgery Committee
Director of Animal experimental center
Director of Beijing Key Laboratory of Pre-clinical Research and Evaluation for Cardiovascular Implant Materials,
State Key Laboratory of Cardiovascular Disease,
National Center for Cardiovascular Diseases,
Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100037, China.
First of all, we would like to express our sincere gratitude to the reviewers for their constructive and positive comments.

Replies to Reviewer 1

Major Comments

1. There are many published papers using canine model for testing thrombolytic drugs in treatment of thromboembolic diseases since 1970’s. From the abstract or Introduction, it is not clear about the novelty of this work.

Response: Thank you for your thoughtful suggestion. Several sentences have been changed in the Abstract (Page 4, Lines 55-56) and Background (Page 5, Lines 73-76) of the revised manuscript to indicate the novelty of this work. The major innovation of our study is that we applied fibrin-rich white thrombi to establish beagle model possessing features of clinically observed coronary thrombi in time window of intravenous thrombolysis of ST-elevation myocardial infarction (STEMI).

2. White embolus was produced from the supernatant after centrifugation to remove red blood cells with no anticoagulant added in the venous blood. It is not revealed until Discussion that coagulation was avoided in the process. The results would be better understood if the details of the methods were mentioned in the Methods. In addition, it is not clear why levels of fibrinogen or platelets in the supernatant were much higher than that in the whole blood after centrifugation. Is there statistic significance?

Response: The details of the methods have been added in the Methods of the revised manuscript (Page 7, Lines 97). Based on the theory of differential centrifugation, the low rotate speed (1500 rpm) can just divide the erythrocytes from supernatant. More importantly, according to our preliminary experiments, it can enrich fibrin and platelets in supernatant. The statistic results were shown in Table 2. (The order of Table 1 and 2 has been exchanged)

3. Why the components of such artificially produced white embolus are similar to that observed in human STEMI? In comparison of the current model with previous works in Discussion, it was not addressed why an artificially produced embolus is superior to that generated in vivo.
**Response:** In recent years, with the coronary thrombus suction technique coming on the scene, pathological features of intracoronary thrombi became more and more clear. Silvain J et al [a] demonstrated there were fibrin-rich thrombi in the early time (<3h) after onset of STEMI. In our study, the white thrombi were similar to that in patients with STEMI. As shown in figure 3, the white embolus mainly consisted of a dense mass of fibrin and had compacted structure under scanning electron microscope.

At present, the methods for generating embolus in vivo in large animal models mainly include electrical injury, open chest thrombosis injection, balloon occlusion and thrombin injection or copper coil-induced coronary thrombosis. The first two methods require open chest operation, with complex operations and a larger trauma. The thrombus in the last two models may have different compositions from those seen in STEMI patients [b, c]. Although the thrombus induced by electrical injury may have similar composition to human coronary artery thrombi, this method takes a long time (3.2± 0.4 hours) to complete coronary occlusion and the size of thrombus cannot be controlled [d]. The present model was a simple, efficient and inexpensive method for experiment with a smaller trauma. Furthermore, the artificially produced fibrin-rich white thrombus has uniform size and clinical features of coronary thrombi in time window of intravenous thrombolysis of STEMI. (added in the section of discussion, see lines 299-304)


4. The Method section lacks critical information. It is not clear how reperfusion time in response to rt-PA
was defined. How about if verified recanalization and reperfusion arrhythmia occurred at different time? 

**Response:** The definition of reperfusion time has been stated in the Methods of the revised manuscript (Page 9, Lines 146-147).

5. The description of Results is often over-simplified. How many animals were studied in each group was not clear. For instance, transient premature ventricular fibrillation occurred in two cases in the red embolus group and four cases in the rt-PA group (line 179). How many dogs are in each group? Was the defibrillated dog also included in the group? When LAD occlusion took place, the recanalization occurred in two dogs of the red embolus group (line 184). Are those two dogs anything to do with the two with ventricular fibrillation?

**Response:** We have described more details in the Results. A total of twenty-one dogs were used in the experiment. One animal died of ventricular fibrillation due to the extended duration of the catheter in the LAD. Two animals had diagonal branch embolisms. These three animals were excluded from the study. Finally, 18 male beagles were included and divided into three groups: red embolus group (n=6), white embolus group (n=6) and white embolus+ rt-PA group (n=6). Transient premature ventricular fibrillation occurred in two cases in the red embolus group and four cases in the rt-PA group and was reversed to sinus rhythm after defibrillation. Furthermore, these six animals present coronary recanalization (reperfusion) according to coronary angiogram (Page 12, Lines 179-188).

It is not stated whether the results are from the white or red embolus group in the legend of Figure 2. In addition, there is no supporting data under the section of estimation of bleeding risk (line 206-207). It may be necessary to add “data not shown” if no information is provided.

**Response:** We have clarified that the results were from the white embolus group in legend of Figure 2 (Page 25, Lines 440-441). 

We did not collect the data about bleeding risk assessment after observation. This is a limitation of our study. We have added “data not shown” to the statement of estimation of bleeding risk in the Results (Page 13, Line 209).

In figure 4, representative figures demonstrating infarct size in treated vs.untreated groups may be more convincing in illustrating thrombolysis effects.
Response: Representative figures demonstrating infarct size was added in figure 4.

Minor concerns:

1. Terminology should be consistent, either platelets (line 59) or thrombocytes (Table 1) may be used in the manuscript.
   Response: Correction has been made in Table 2 of revised manuscript. (The order of Table 1 and 2 has been exchanged)

2. Representative angiogram from three rats are presented in figure 1; however, labeling of the figures and figure legends are not efficient for convey of the ideas. It would be more clear to label the three panels on the left as red embolus, white embolus, and white embolus + rt-PA, with basal, + embolus, and 120 or 180 min on top of the upper panel. Then, the figure legend can be much shortened.
   Response: Correction has been made in figure 2. And the figure legend has been shortened (Page 25, Lines 446-451). (The order of figure 1 and 2 has been exchanged)

3. Line 172- In statistical analysis, a P value less than 0.05 is considered as significant, not less or equal to 0.05.
   Response: Correction has been made in the revised manuscript (Page 11, Line 173).

Replies to Reviewer 2

Major Compulsory Revisions:

1. The experiment design is confusedly written and difficult to follow. Needs major revision.
   Response: Thanks for raising this critical issue. The statement of experiment design has been regulated in Methods of the revised manuscript.

2. In “Model evaluation” It is stated that recanalization was verified either by angiography or reperfusion arrhythmias. In experimental models all recanalization needs to be verified by angiography.
   Response: Thanks for the comments. We have made correction on Page 9, Lines 146-147. Angiography was performed at the moment of injection and every 30 min for three hours after injection to evaluate the degree of occlusion and/or autolysis. Coronary angiogram was also carried out at 10, 20, 30, 60, 90 and
120 min after using rt-PA or at the time of occurrence of arrhythmia or electrocardiographic changes to evaluate the thrombolytic effects. Reperfusion time was defined as the time when recanalization was verified by coronary angiogram.

The arrhythmias in the presence of acute myocardial infarction can occur as result of ischemia and it is not specific indicator for recanalization (reperfusion).

**Response:** Thanks for your helpful comment. We corrected the statement by removing “reperfusion” from “reperfusion arrhythmias”.

**Minor Essential Revisions:**

1. In “Thrombolysis protocol” please cite reference for the rt-PA dose and infusion protocol.


2. In the “Results / pathological studies” please explain the method of left ventricle infarct area measurement.

   **Response:** The method of left ventricle infarct area measurement has been addressed in the Results of revised manuscript (Page 13, Lines 210-212).

---

**Replies to Reviewer 3**

The manuscript entitled "A novel model for evaluating thrombolytic therapy in dogs with ST-elevation myocardial infarction" presents a new experimental method to evaluate new thrombolytic drugs. The authors give detailed view on their experimental protocol supporting the relevance of this model in preclinical and experimental fields. The main restriction of this article that due to primary percutaneous coronary intervention is widely available the thrombolytic therapy has limited indication in the real world, however guidelines gives recommendation is special circumstances for this therapy. The English of the text is of good quality, clear and easy to follow. Figures are clear and self-explaining. The
discussion of findings is well balanced.

Response: Thanks for your positive comment on the present study.