Author’s response to reviews

Title: Effect of ulinastatin on post-operative blood loss and allogeneic transfusion in patients receiving cardiac surgery with cardiopulmonary bypass: a prospective randomized controlled study with 10-year follow-up

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Author’s response to reviews:

Reviewer #1

Summary:

Congratulations to presenting this article for managing post-operative bleeding. The language is quite lucid and the statistics is well chartered. Going through all the details, I had few queries relating to this matter.

Critique:

1. Tranexamic Acid is well established drug for managing post-operative bleeding, and as such there is not statistical difference in outcome between use of Ulinastatin and Tranexamic Acid, so how does Ulinastatin offer any advantage for its use?

Response: We thank the reviewer for the comment and apologize for any confusion. In 2008, aprotinin, which had been used in cardiac surgery for years, was removed from the market because of its adverse effect on renal function and total mortality. Given that ulinastatin is a
protease inhibitor, which is similar to aprotinin, it was supposed that ulinastatin might have similar effect in the reduction of bleeding and transfusion. This is the background of the current study. Tranexamic acid has been used as anti-fibrinolytic agent in cardiac surgery since 1970’s and served as a positive control in the current study, while normal saline served as a negative control. The main purpose of this study is to evaluate the efficacy and safety of ulinastatin on reducing peri-operative bleeding and transfusion in cardiac surgery with cardiopulmonary bypass. Analysis showed that the hemostatic effect of ulinastatin was comparable to that of tranexamic acid. Actually, the superiority of ulinastatin on reducing bleeding and transfusion comparing with tranexamic acid was not expected in the current study. Besides the hemostatic effects of ulinastatin, the current data have proved its advantage on lung protection. Together with its reported anti-inflammation and organ protection effects,(1-8) it is reasonable to believe the advantage for the use of ulinastatin in cardiac surgery with cardiopulmonary bypass. The authors have revised the manuscript in the part of Discussion as follows. (Page 11)

Over the last ten years, growing evidence showed that tranexamic acid effectively decreased post-operative blood loss and spared blood during this trial being conducted,(9-11) tranexamic acid became the mainstay of blood conservation in cardiac surgery. Ulinastatin relieves inflammatory response induced by CPB and improves organ injuries, (1-8, 12) therefore it may provide additional advantages and become another hopeful choice for blood management.

2. Are there any documented adverse reaction for Ulinastatin, this papers doesn't mention about it ?

Response: We appreciate your question that is of great importance. The safety of ulinastatin is always our concern. Aprotinin was of great effect on reducing bleeding and transfusion in cardiac surgery and had been used for years until its adverse effect on renal function and overall mortality was disclosed by Mangano D.T. and Ferguson D.A. in 2006 and 2008 respectively(13, 14). Adverse reactions led to the removal of aprotinin, which is the background of the current study. So the safety of ulinastatin was highly emphasized in the study. That is the reason why the follow-up was as long as ten years in the current study. Given the nature of pilot study, we enrolled as many as 426 cases but it was not enough yet to disclose side effects of an agent. Actually, there were adverse events observed in the ulinastatin group, including one case of death, one case of respiratory failure, 2 cases of readmission to ICU, one case of IABP and one case of deep sternal infection. Unfortunately, the relatively small sample size of the study limited the power to tell whether these adverse reactions are associated with ulinastatin. The authors have revised the manuscript in the part of Discussion as follows. (Page 11)

Moreover, the short- and long-term safety of ulinastatin was the major concern in this study, especially in the background of aprotinin’s withdrawal from operation room. Although there were adverse events in ulinastatin group (see Table 4), the relatively small sample size of the study limited the power to tell whether these adverse reactions are associated with ulinastatin. These account for the 10 years follow-up in the current study.
3. Chest drainage beyond 12 hours is not always significant, as most of it is reactionary fluid and not bleeding.

Response: We thank for the reviewer’s suggestion. Post-operative bleeding (chest drainage) is the primary outcome of the current study and is shown in the manuscript in detail. The authors agree that the chest drainage within early hours postoperatively is of more significance. Given the current data, the chest drainage within 24 hours is analysed. The manuscript has been revised in the Abstract, the Results and Table 3 accordingly.

In Abstract:

Compared with placebo, ulinastatin significantly reduced the volume of post-operative blood loss within 24 hours (688.39±393.55ml vs 854.33±434.03ml MD -165.95ml 95%CI -262.88ml to -69.01ml, p<0.001) and the volume of allogeneic erythrocyte transfusion (2.57±3.15 unit vs 3.73±4.21 unit, MD-1.16 unit 95%CI -2.06 units to -0.26 units, p=0.002).

In Results: (Page 9)

In post hoc analyses (Table 3), postoperative blood loss within 24 hours (404.87±253.58ml vs. 527.73±300.4ml, MD -122.86ml, 95% CI -195.87ml to -49.86ml, p<0.001, for the first 8 hours; 183.94±151.83ml vs. 205.57±129.57ml, MD -21.63ml, 95% CI -55.49ml to 12.22ml, p=0.016, for the second 8 hours; 99.58±94.75ml vs. 121.03±101.62ml, MD -21.45ml, 95% CI -44.58ml to 1.68ml, p=0.029, for the third 8 hours; 688.39±393.55ml vs 854.33±434.03ml, MD -165.95ml, 95% CI -262.88ml to -69.01ml, p<0.001, for the wholly first 24 hours) and total blood loss (801.7±460.14ml vs. 1016.67±529.08, MD -214.98ml, 95% CI -338.60ml to -91.36ml, p<0.001) were significantly reduced in patients receiving ulinastatin compared with placebo.

4. There is statistical significant difference in RBC transfusion rated between Tranexamic Acid and Placebo, and not difference between Ulinastatin and Placebo.

Response: We thank the reviewer’s comment. The objective of the study is to evaluate the efficacy and safety of the ulinastatin in reducing bleeding and transfusion in cardiac surgery with cardiopulmonary bypass. Allogeneic RBC transfusion is a key measurement including both the volume and the rate transfused. Compared with placebo, ulinastatin reduced the average volume of allogeneic erythrocyte transfusion (2.57±3.15U vs 3.73±4.21U, MD -1.16U) with statistical significance (p=0.002) and the rate of allogeneic erythrocyte transfusion (58.45% vs 69.50%) with a marginal statistical significance (p=0.053). The potential explanation lies in the relatively small sample size (142 patients for ulinastatin group and 141 patients for placebo group). Further clinical trial with larger scale is expected to reinforce.
5. You mentioned that Tranexamic Acid has shown increased thrombotic rates, but it’s not reflected in your study.

Response: We thank the reviewer’s comment and apologize for any confusing expression in the manuscript. Evidences have demonstrated that tranexamic acid is effective in blood conservation and does not increase thrombotic events such as myocardial infarction, renal dysfunction and brain infarction.(9, 11, 15) However, tranexamic acid is associated with increased incidence of seizure. In the ATACAS study, the incidence of seizure in the tranexamic acid group and the placebo group is 0.7% and 0.1% respectively with a statistical significance (p=0.002). In the current study, the incidence of seizure in the ulinastatin group, the tranexamic acid group and the placebo group is 0.00%, 1.40% and 0.71% respectively without statistical significance (p=0.369). Especially, analysis between the tranexamic acid group and the placebo group does not reveal a statistical significance either (p=0.5699). The potential explanation lies in the relatively small sample size. To avoid any misleading, we deleted the related expression and the Discussion part of manuscript has been modified as follows. (Page 11)

This trial was mainly designed to evaluate the blood conservation effect of ulinastatin on heart surgery with CPB, with tranexamic acid as positive control and normal saline as negative control. Over the last ten years, growing evidence showed that tranexamic acid effectively decreased post-operative blood loss and spared blood during this trial being conducted,(9-11) tranexamic acid became the mainstay of blood conservation in cardiac surgery. Ulinastatin relieves inflammatory response induced by CPB and improves organ injuries,(1-8, 12) therefore it may provide additional advantages and become another hopeful choice for blood management.

6. What was the way of randomisation as the numbers look quite similar for type of cardiac surgery in different groups ? Where the numbers kept similar intentionally for different groups or it was incidentational ?

Response: We appreciate your question. This is a randomized and double-blind study. Blocked randomization was applied. The randomization sequence was generated by computer in permuted blocks by a 1:1:1 ratio and was masked in sealed, sequentially numbered opaque envelopes. The balance in distribution of surgical procedures among groups was naturally formed. This distribution was in accord with the institutional practice in 2008, given that over 8000 cases of open heart surgery was carried out in Fuwai Hospital in 2008. The text in Methods has been revised as below. (Page 5)

Patients were randomly assigned into three groups for the use of ulinastatin (group U), tranexamic acid (group T) or placebo (group C). The randomization sequence was generated by computer in permuted blocks by a 1:1:1 ratio and was masked in sealed, sequentially numbered opaque envelopes. Patient enrollment, randomization, and blinding were conducted and supervised by an independent committee. The participants, medical staff, and investigators were unaware of the treatment allocation until the end of the study.
Reviewer #2

Critique:

Personally, I had only one question regarding the design of the study itself. The Material and Methods section indicates that "Study medication in all three groups of patients was pumped intravenously over 10 minutes at two time points, after anaesthesia induction and after administration of protamine". Of course, the clinical effect of the prescribed drugs can only be evaluated in the earliest postoperative period. In fact, the work itself on evaluating the effectiveness of ulinastatin on post-operative blood loss and allogeneic transfusion in patients receiving cardiac surgery with cardiopulmonary bypass is devoted to this. Surely, even 1 day after the introduction of these drugs in the blood, even traces of the injected drugs will not be detected. In that case, for what purpose a prospective randomized controlled study with 10-year follow-up?

Response: We appreciate the reviewer’s question. The current study stands on the background that aprotinin, which had been used in heart surgery for years, was removed from the market because of its adverse effect on renal function and total mortality. The study was designed to evaluate the efficacy and safety of ulinastatin as a potential substitute for aprotinin. The safety of the agent, especially its influence on long-term outcome, was one of the key points of the study. To our knowledge, there was not any study on ulinastatin with follow-up for more than 1 year. It is expected to evaluate the effect of ulinastatin on long-term outcome in cardiac surgery with cardiopulmonary bypass. A prolonged follow-up helps to evaluate not only any possible side effects but also the potential advantages of agent. The reduction of blood loss and allogenic transfusion itself could improve long-term outcomes. Furthermore, the anti-inflammatory and organ protective effects of ulinastatin on long-term outcome has not been systematically evaluated. This is the rationale of the prolonged follow-up in the study. The manuscript has been revised accordingly, as shown below.

In page 11:

Since reports (16-18) showed that blood transfusions during or after cardiac surgery were associated with increased long-term mortality, whether the blood conservation effect of ulinastatin can be translated into the improvement of long-term outcome became intriguing. Moreover, the short- and long-term safety of ulinastatin was the major concern in this study, especially in the background of aprotinin’s withdrawal from operation room. Although there were adverse events in ulinastatin group (see Table 4), the relatively small sample size of the study limited the power to tell whether these adverse reactions are associated with ulinastatin. These account for the 10 years follow-up in the current study.

In page 13:

The population in this trial was medium- or low-risked, which resulted in a much lower observed in-hospital mortality (0.47%) in accordance with the low average in-hospital mortality in this
hospital, than would normally be expected with CPB alone (3.2% to 12.8%).(19) In this situation, it was difficult to compare in-hospital and long-term mortality among groups. This is another reason why the follow-up was prolonged, from one year originally to 10 years, to observe the time-magnified effect of different treatments on long-term survival and morbidities.

Reviewer #3

Summary:

It is a good article but in my view needs certain clarifications:

Critique:

1. In the "Intervention" heading it is mentioned as double blind study and study material was prepared by the hospital pharmacy. It is mentioned that in Group U the whole medicine was transfused after induction and only normal saline was infused after giving protamine. If they were blind about the medicine then how come they changed the protocol in Group U.

Response: We thank for the reviewer’s question and apologize for any confusing or misleading expression in the manuscript. Yes, the current study was double-blinded, i.e. the participants, medical staff, and investigators were unaware of the treatment allocation until the end of the study. To fulfill the concealment, identical syringes of 50 mL labeled with the randomization number were prepared by the hospital pharmacy. There were two syringes prepared for each patient labeled ‘#1’ and ‘#2’, which was delivered after anaesthesia and after neutralization respectively. In the tranexamic acid group, both the syringes contained 15 mg/kg tranexamic acid to fulfill a total dosage of 30 mg/kg. In the ulinastatin group, the syringe #1 contained 1,000,000 U ulinastatin and the syringe #2 contained normal saline. In the control group, both the syringes contained normal saline. The manuscript has been revised as follows. (Page 6)

Study and placebo medication were prepared by the hospital pharmacy. Identical syringes of 50 mL labeled with the randomization number contained transparent solution, 30 mg/Kg body weight of tranexamic acid (Jie Ning®; Changchun Tiancheng Pharmaceutical Co., Changchun, China), 1,000,000 U ulinastatin (Tian Pu Luo An®; Guangdong Tianpu Biochemistry Pharmaceutical Co., Guangzhou, China) or normal saline. There were two syringes prepared for each patient labeled ‘#1’ and ‘#2’. In the tranexamic acid group, both the syringes contained 15 mg/kg tranexamic acid to fulfill a total dosage of 30 mg/kg. In the ulinastatin group, the syringe #1 contained 1,000,000 U ulinastatin and the syringe #2 contained normal saline. In the control group, both the syringes contained normal saline. The syringe #1 and #2 were pumped intravenously after anaesthetic induction and after the administration of protamine respectively.

2. Blood loss in first 24 hour is more significant as after that mostly it is serous loss.
Response: We thank for the reviewer’s suggestion. The primary outcome of the current study is postoperative bleeding (chest drainage), which is analyzed in detail in the manuscript. The authors agree that the chest drainage in the first 24 hours is of more significance. The blood loss in the first 24 hours is analyzed and highlighted in the Abstract, the Results and Table 3.

In Abstract:

Compared with placebo, ulinastatin significantly reduced the volume of post-operative blood loss within 24 hours (688.39±393.55ml vs 854.33±434.03ml MD -165.95ml 95%CI -262.88ml to -69.01ml, p&lt;0.001) and the volume of allogeneic erythrocyte transfusion (2.57±3.15 unit vs 3.73±4.21 unit, MD-1.16 unit 95%CI -2.06 units to -0.26 units, p=0.002).

In Results: (Page 9)

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3. Looking into the group Trenexamic group had better results.

Response: We thank the reviewer for the comment and apologize for any confusion in the manuscript. The current study stands on the background that aprotinin, which had been used in heart surgery for years, was removed from the market in 2008 because of its adverse effect on renal function and total mortality. Given that ulinastatin is a protease inhibitor, which is similar to aprotinin, it was supposed that ulinastatin might have similar effect on peri-operative bleeding. The purpose of this study is to evaluate the efficacy and safety of ulinastatin on reducing peri-operative bleeding and transfusion in cardiac surgery with cardiopulmonary bypass. Tranexamic acid has been used as anti-fibrinolytic agent in cardiac surgery since 1970’s and served as a positive control in the current study, while normal saline served as a negative control. The main finding of the study is that ulinastatin could reduce blood loss and allogeneic transfusion in this specific population. As for the comparison of the effect of ulinastatin and tranexamic acid, reduced mean value of postoperative bleeding, RBC and plasma transfusion as well as reduced incidence of major bleeding, reoperation and the exposure to allogeneic RBC and plasma were observed in the tranexamic acid group with however no statistical significance. Namely, the effect of ulinastatin and tranexamic acid on postoperative bleeding and allogeneic transfusion is similar. The manuscript has been updated accordingly as follows. (Page 11)
Over the last ten years, growing evidence showed that tranexamic acid effectively decreased post-operative blood loss and spared blood during this trial being conducted.(9-11) Tranexamic acid became the mainstay of blood conservation in cardiac surgery. Ulinastatin relieves inflammatory response induced by CPB and improves organ injuries, (1-8, 12) therefore it may provide additional advantages and become another hopeful choice for blood management.

4. Cost effectiveness of Ulinastatin vs Tranexamic acid was not discussed.

Response: We appreciate the reviewer’s comment. Cost effectiveness is an important topic in health economics and is a concern to both the health provider and the patients. Hemostatic effect of ulinastatin is rarely investigated before. As a pilot exploration, the current study focused on the efficacy and safety of the agent however, with the relatively small sample size, cost effectiveness was not evaluated quantitatively in the study. The cost of ulinastatin is nearly eight folds to tranexamic acid with a similar hemostatic effect to the latter. However, given the anti-inflammatory and organ protective effect of the agent, the exact cost effectiveness of ulinastatin deserves further evaluation by well-designed clinical trial with larger scale in the future. The discussion of cost effectiveness of ulinastatin is updated in the revised manuscript as follows. (Page 13)

Cost effectiveness is an important topic in health economics and is a concern to both the health providers and the patients. Hemostatic effect of ulinastatin was rarely investigated before. As a pilot exploration, the current study focused on the efficacy and safety of the agent and, especially, with the relatively small sample size, cost effectiveness was not evaluated quantitatively in the study.

5. More than 15% patients were lost in follow-up which is a significant number looking into small sample size.

Response: We appreciate the reviewer’s comment and fully agree with that. Actually, it was a real challenge to achieve a follow up as long as ten years. We have two mountains to climb. The first one was the compliance of the participants and the second one was the large map and the unbalanced development of the country. Fuwai hospital is the National Center for Cardiovascular Disease with nationwide patients. Some participants were not well educated and some were embarrassed in life quality, leading to an adverse adherence to the follow up. In the past ten years, the investigators managed to achieve a convincing rate of follow-up by face-to-face interview, email, letters, telephone call or Wechat according to different regions, educational levels and social status. The authors has revised the limitation of the study in the manuscript as below. (Page 14)

There were 14.7% patients lost in follow-up. It was a challenge to achieve a follow-up as long as ten years. It could be attributed to the compliance of the participants and the large map and the unbalanced development of the country. Fuwai hospital is the National Center for Cardiovascular Disease with nationwide patients. Some participants were not well-educated and some were embarrassed in life quality, leading to an adverse adherence to the follow-up. In the past ten years, the investigators managed to achieve a convincing rate of follow-up by face-to-
face interview, email, letters, telephone call or Wechat according to different regions, educational levels and social status.

Reviewer #4

Summary:

Carefully Reviewing the manuscript, I think that this trial will play a pivotal role in researching the area of post-operative bleeding management in Cardiac Surgery on Cardiopulmonary Bypass Machines. However, further investigations are required to establish the superiority or inferiority of ulinastatin over tranexamic acid; in terms of cost-effectiveness, complications and indications.

Response: We thank the reviewer’s inspiring comment. Ulinastatin has been used in cardiac surgery for decades with its hemostatic effects rarely reported. The current investigation is a pilot study evaluating the efficacy and the safety of the agent on postoperative bleeding and allogeneic transfusion. A well designed randomized and controlled trial is expected to reinforce the superiority or inferiority of the agent with evaluation of the cost-effectiveness.