Author’s response to reviews

Title: Levosimendan Combined with Epinephrine Improves Rescue Outcomes in a Rat Model of Lipid-based Resuscitation from Bupivacaine-induced Cardiac Arrest

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Author’s response to reviews:

Dear Editor,

Thank you very much for giving me the opportunity to revise my article entitled “Levosimendan Combined with Epinephrine Improves Rescue Outcomes in a Rat Model of Lipid-Based Resuscitation from Bupivacaine-Induced Cardiac Arrest” (BANE-D-17-00170).

We have revised the manuscript carefully according to the reviewers’ insightful suggestions and advice. We would also thank the two reviewers for their excellent comments and suggestions regarding my paper, and we truly appreciate the considerable time and effort expended on their part to improve the paper. We called American Journal Experts (http://bit.ly/AJE_BS) for help with English usage and they were a big help. The manuscript was proofed for English grammar, spelling, typos and word choice. I believe the paper has been substantially improved.
Here are my specific responses to the reviewers as follows:

Review1:

1. About the first sentence: The guideline of advanced cardiac life support developed by the American Heart Association in 2015 increased emphasized that chest compression and respiratory support were much more important than any resuscitation drugs, including epinephrine. (Page 5 line 5-8)

   Thank you for your suggestion. We have revised this sentence to “The guidelines for advanced cardiac life support that were developed by the American Heart Association in 2015 increased the emphasis on the importance of chest compressions and respiratory support. Nevertheless, in the clinical cardiac arrest scenario, epinephrine is also widely used.”

2. In abstract it is not completely clear which groups did better in the secondary analyses (ROSC, return to first heart beat, etc.), just that there were differences. Please make improve this (Page4 line1-15)

   Thank you for your suggestion. We have changed this part to “The rates of ROSC in LiEL and LiE groups were higher than LiL group (P < 0.001; LiEL vs LiL, P = 0.001; LiE vs LiL, P = 0.007). The survival rate in LiEL group was higher than LiE group (P = 0.003; LiEL vs LiE, P = 0.008; LiEL vs LiL, P = 0.001). The time to first heart beat in LiEL group was shorter than LiE, LiL groups. (P < 0.001; LiE vs LiEL, P = 0.001; LiL vs LiEL, P < 0.001). The time to ROSC in LiEL group was shorter than LiE, LiL groups (P < 0.001; LiEL vs LiE, P <0.001; LiEL vs LiL, P < 0.001). The result was similar for the bupivacaine concentration of cardiac tissue and plasma (cardiac tissue: P = 0.002; plasma: P = 0.011). Furthermore, there were significant differences in the blood-gas values at 40 minutes, wet-to-dry lung weight ratio, and ratio of damaged alveoli among groups. The LiEL group had the best result for all parameters (P < 0.01, P = 0.008, P < 0.001, respectively). Additionally, significantly less epinephrine was used in the LiEL group (P < 0.001).”

3. "The amount of epinephrine…” should be changed to something like, "Significantly less epinephrine was used in…” (Page4 line 14-15)

   Thank you for your suggestion. We have changed “The amount of epinephrine” to “significantly less epinephrine was used in the LiEL group”.
4. In the conclusion, please change "less" to "decreased" (Page 4 line 20)

Thank you for your suggestion. We have changed “less” to "decreased".

5. How was the bupivacaine dose (15 mg/kg) determined? Is treatment effective at higher doses? In the referenced paper (Ref 11) 20 mg/kg was the dosing. Is the treatment ineffective at this dose?

Firstly, The literature (Weinberg GL, VadeBoncouer T, Ramaraju GA, Garcia-Amaro MF, Cwik MJ: Pretreatment or resuscitation with a lipid infusion shifts the dose-response to bupivacaine-induced asystole in rats. ANESTHESIOLOGY1998; 88:1071–5) indicates that 15mg/kg bupivacaine can cause induce cardiac arrest in all rats and cannot be reversed by saline. It was determined that the dose of bupivacaine in 15mg/kg was sufficient to establish a cardiac arrest model for the rat.

Secondly, in our previous study (Li Z, Xia Y, Dong X, et al. Lipid resuscitation of bupivacaine toxicity: long-chain triglyceride emulsion provides benefits over long- and medium-chain triglyceride emulsion. Anesthesiology 2011, 115(6):1219-1228.), the rate of ROSC was 80% and the survival rate was 76.7% when the dose of bupivacaine in 20mg/kg was used. In our other study (Jin Z, Xia Y, Xia F, et al. Epinephrine administration in lipid-based resuscitation in a rat model of bupivacaine-induced cardiac arrest: optimal timing. Reg Anesth Pain Med 2015, 40(3):223-231.) which 15mg/kg dose was used, the rate of ROSC and the survival rate were both 100%. Therefore, we used bupivacaine 15mg/kg in order to reduce differences between the groups.

In our early study (M Luo,X Yun ,C Chen ,N Bao ,X Feng ,et al. Giving priority to lipid administration can reduce lung injury caused by epinephrine in bupivacaine-induced cardiac depression. Regional Anesthesia & Pain Medicine, 2016 , 41 (4) :469 ), we found that epinephrine increased pulmonary artery pressure and lung damage. Levosimendan can properly diastolic pulmonary artery and peripheral blood vessels. So the heart function can be improved by reducing the posterior load of the heart. The rate of ROSC was 80% when bupivacaine in 20mg/kg and adrenalin was used alone for resuscitation. So we deduce that the dose of bupivacaine 20mg/kg is effective when levosimendan which can reduce side effects of adrenaline is added.
6. Were chest compressions on non-responders really continued for 40 minutes? How fast were the compressions? In the referenced paper (Ref 11) chest compressions were done at 200/minute. This time they were at 300/minute?

How was this measured and why was this changed?

Yes, we continued for 40 mins if a rat was not ROSC.

The frequency of compression was 300/minute this time, because the baseline values of heart rate were all above 300/minute (table 1), thus, we increased the speed of chest compression to have a better effect. And in our previous study, according the article “Yeh ST1, Lee HL, Aune SE, Chen CL, Chen YR, Angelos MG. Preservation of mitochondrial function with cardiopulmonary resuscitation in prolonged cardiac arrest in rats. J Mol Cell Cardiol. 2009 Dec;47(6):789-797”, we just compressed at 200/min.

We observed our compression frequency through medlab monitoring system as we administered CPR. We maintained a frequency above 300 per minute. The depth of compression was not precisely monitored, but we could observe the arterial blood pressure (through the medlab system monitor) and we were able to maintain the blood pressure greater than 30 mmHg / 20 mmHg. There was extensive training of the laboratory techniques in how to administer CPR before experiment.

7. Why didn't all groups get the same dosing (including of saline)? If it was to standardize total volume this point should be made explicit. (Page 9 line 2-7)

Thank you for your suggestion. We have changed “to standardize total volume, all groups got the same dosing. Group LiEL received epinephrine 2μg•kg-1 and levosimendan 150μg•kg-1; Group LiE received epinephrine 2μg•kg-1 and saline 6 ml•kg-1; Group LiL received levosimendan 150μg•kg-1 and saline 1 ml•kg-1.” to “To standardize the total volume, all groups received the same volume. Group LiEL received epinephrine 2μg•kg-1 (1ml•kg-1) and levosimendan 150μg•kg-1 (6ml•kg-1); Group LiE received epinephrine 2μg•kg-1 (1ml•kg-1) and saline 6 ml•kg-1; Group LiL received saline 1 ml•kg-1 and levosimendan 150μg•kg-1 (6ml•kg-1).”

8. At the end of the 40 minute period after the blood gas measurements were collected, I assume the animals were euthanized before collecting heart and lung samples. This should be indicated for completeness. (Page 10 line 4)

Thank you for your suggestion. we have added “The rats were euthanized by air injection,”
9. BE in Table 3, please verify sign (-) (Table 3)

Thank you for your suggestion. we have revised the sign(-).

Review2

1. please clarify the method by which chest compressions were delivered was this an automated compression device? if so in what manner was it timed to ventilation?

This was not an automated compression device, we did it by ourselves. Chest compressions were on the lower-middle sternum at 300 compressions per minute by our index finger and middle finger. Resuscitation was performed according to the following teamwork algorithm: provider 1 was responsible for the infusion of the lipid emulsion and resuscitation drugs; provider 2 implemented external chest compressions; and provider 3 maintained the airway and assessed the native RPP during resuscitation. Provider 2 and provider 3 was replaced each other for every 10 mins.

2. How were animals secured to ensure appropriate compressions were consistently delivered to each animal?

Firstly, we can monitor the compression times, the blood pressure and the electrocardiogram in real time through a device called Medlab.

Secondly, our group based on previous experiments (Ref 4, Ref 11, Ref 13), we had rich experience.

Thirdly, the chest compressions were implemented by a special provider who was trained severely.

Fourthly, all the people were blinded to the group assignments.

3. survival was calculated at 40 minutes; why this time as opposed to 60 minutes or perhaps 1 day?

In our previous study (Li Z, Xia Y, Dong X, et al. Lipid resuscitation of bupivacaine toxicity: long-chain triglyceride emulsion provides benefits over long- and medium-chain triglyceride
emulsion. Anesthesiology 2011, 115(6):1219-1228.), the time of ROSC was almost in 30 minutes. So in this study, we recorded ROSC at 40 minutes without missing a very few rats that recovered more than 30 minutes.

Here are our reply to the editor comments:

Q1: One major concern for this study noted by both reviewers is the need for more clarification regarding CPR management, and how it was provided. Was the quality of CPR the same in both groups? can this be proven? Were compressions performed by an automated device? if not, how was quality of compressions ensured to be same for both groups? Were those managing CPR blinded to animal groups? etc.

This was not an automated compression device, we did it by ourselves. Chest compressions were on the lower-middle sternum at 300 compressions per minute by our index finger and middle finger. Resuscitation was performed according to the following teamwork algorithm: provider 1 was responsible for the infusion of the lipid emulsion and resuscitation drugs; provider 2 implemented external chest compressions; and provider 3 maintained the airway and assessed the native RPP during resuscitation. Provider 2 and provider 3 was replaced each other for every 10 mins.

The quality of CPR was almost the same of all animals, because: Firstly, we monitor the compression times, the blood pressure and the electrocardiogram in real time through a device called Medlab. Secondly, our group based on previous experiments (Ref4, Ref 11, Ref 13), we had rich experience. Thirdly, the chest compressions were implemented by a special provider who was trained severely. Fourthly, all the people were blinded to the group assignments.

Q2: Another major concern is the lack of consistency in doses and CPR management from previous studies and between the two groups as pointed out by reviewer 1.

The frequence of compression was 300/minute this time, because the baseline values of heart rate were all above 300/minute (table 1), thus, we increased the speed of chest compression to have a better effect. And in our previous study, according the article “Yeh ST1, Lee HL, Aune SE, Chen CL, Chen YR, Angelos MG. Preservation of mitochondrial function with cardiopulmonary resuscitation in prolonged cardiac arrest in rats. J Mol Cell Cardiol. 2009 Dec;47(6):789-797”, we just compressed at 200/min.
We observed our compression frequency through medlab monitoring system as we administered CPR. We maintained a frequency above 300 per minute. The depth of compression was not precisely monitored, but we could observe the arterial blood pressure (through the medlab system monitor) and we were able to maintain the blood pressure greater than 30 mmHg / 20 mmHg. There was extensive training of the laboratory techniques in how to administer CPR before experiment.

In our previous study (Li Z, Xia Y, Dong X, et al. Lipid resuscitation of bupivacaine toxicity: long-chain triglyceride emulsion provides benefits over long- and medium-chain triglyceride emulsion. Anesthesiology 2011, 115(6):1219-1228.), the time of ROSC was almost in 30 minutes. So in this study, we recorded ROSC at 40 minutes without missing a very few rats that recovered more than 30 minutes.