Author’s response to reviews

Title: Heart rate variability after radiofrequency ablation of epicardial ganglionated plexuses on the ovine left atrium

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Version: 2 Date: 22 Oct 2017

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Lianjun Gao (Reviewer 1):

Question: A group of sheep just experienced pericardial space opened and dorsal LA area revealed is necessary as a control group, or at least to illustrating the phenomenon that thoracotomy and opening of pericardial sack alone would affect HRV in short term, most likely days until healing.

Reply: We are grateful for your insights. They are really valuable and correspond with our considerations about the design of the study. We considered the option of the control group to evaluate the effects of thoracotomy and pericardial sac opening on heart rate variability at the start of the study. Our assumption was similar to yours. Likely, a surgical intervention of such
extent could have produced a short termed autonomic response of a sizable amount. But on the other hand, as pericardial nerves do not have connection with myocardial plexus we had doubts about that. Unfortunately, we decided to avoid controls for thoracotomy. But we still hope that our findings in this study are valuable even without the controls. We added a note in discussion concerning this matter: …<We recognize the limitation of our study as we did not control for possible acute effects of thoracotomy and pericardial sack opening on the HRV. But as thoracic and pericardial nerves have no direct interconnections with intracardiac nerves we did not expect a strong HRV response to the procedure.>…

Question: Histopathological examination of the left atrium should be performed in the study to prove the elimination or reduce of the nerve.

Reply: We accepted the recommendation and added our findings from histological examinations. Both histochemical and immunohistochemical data prove extensive damage of the nerves on the left atrium that actually continue to the left ventricle. In spite of fact that histochemical staining for AChE demonstrates that majority of epicardial nerves and ganglia in the left dorsal neural subplexus were detectable in both the control and the ablated hearts, the epicardial nerves and ganglia on the dorsal left ventricle and coronary sinus from the control animal group were more intensively stained for AChE in comparison to the ablated hearts as staining of intrinsic neural structures of the latter hearts was manifestly obscure at the place below the RFA sites (please, look into the added figure 3). Similarly, immunohistochemical labeling for general neural marker PGP 9.5 and adrenergic marker to tyrosine hydroxylase demonstrates staining both in the control and experimental animal groups, but nerve fibers from the control epicardial nerves of the left dorsal neural subplexus in all examined animals were more densely, abundantly and evenly distributed in the transverse sections of the nerves in comparison with the ablated ones, in which a sharp decrease in number and the patchy distribution of axons positive for both markers (PGP 9.5 and TH). These observations strongly confirm the effective RFA of the dorsal left atrial neural structures in the group of experimental animals.

Question: The discussion of the results about the change of HRV needs to be more detailed, eg. the role of vagus and sympathetic nerve and change according to the results of HRV.

Reply: Following the recommendation of the reviewer, we updated and revised the discussion about sympathetic and parasympathetic effects in our study: …<It is important to acknowledge that HRV reflects the fluctuations of ANS without a quantitative ability [5, 6] and we could not
speculate which component of ANS was modulated more in our study. On the other hand, ganglionated plexuses in the left atrium are of very intricate composition of sympathetic, parasympathetic and mixed nerves [2]. This makes the selective ablation almost impossible. So isolated effect on one of the systems could not be expected. We could interpret that according to the increase in LF/HF ratio ablation affected more sympathetic nerve fibres. Though, as both frequencies decreased significantly it would be difficult to prove such a fact.>…

Agnieszka Noszczyk - Nowak (Reviewer 2):

Question: Still figures are unreadable. Number of animals and standard deviation are missing.

Reply: Thank you for a good advice. We remade the figures and they look much better now. Added standard deviations in graphs, and number of study animals is noted in legends.

Question: Please add the results of pre-study experiments about effectiveness of ablation (material and methods section). In my opinion, the results must be supplemented with the results histopathological examination.

Reply: We accepted the recommendation and added the histological data. For detailed information please look up to the reply to Lianjun Gao’s question.