Peer-Review Record

Characterization of atrial and ventricular remodeling in an improved minimally invasive mouse model of transverse aortic constriction

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by Jose Alberto Navarro-Garcia, Satadru K. Lahiri, Yuriana Aguilar-Sanchez, Anilkumar K. Reddy, Xander H. T. Wehrens

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Academic Editor: Ali J. Marian

Reviewer 1: Anonymous

Reviewer 2: Anonymous

Reviewer 3: Anonymous

Reviewer 4: Anonymous

Round 1

Reviewer 1 Report

In this manuscript, the authors monitored atrial and ventricular remodeling progression in an improved minimally invasive mouse transverse aortic constriction model (mTAC). While several mTAC models have been reported with various modifications mentioned by the authors, this study further optimized TAC surgical procedures, leading to a lower mortality rate with high reproducibility. Applying this mTAC, the author assessed cardiac remodeling and functions in both atria and ventricles at multiple time points.

Here are several suggestions for the improvement of the manuscript.

1. Systolic function assessment by long-axis with B-mode will be more accurate.
2. The legends of Figure 4 and Figure 5 seem mixed up with each other.
3. A wave was still displayed (shown in Figure 5C) while the heart failed at the end of the 8-week TAC, inconsistent with the stage of heart failure.

Author Response

In this manuscript, the authors monitored atrial and ventricular remodeling progression in an improved minimally invasive mouse transverse aortic constriction model (mTAC). While several mTAC models have been reported with various modifications mentioned by

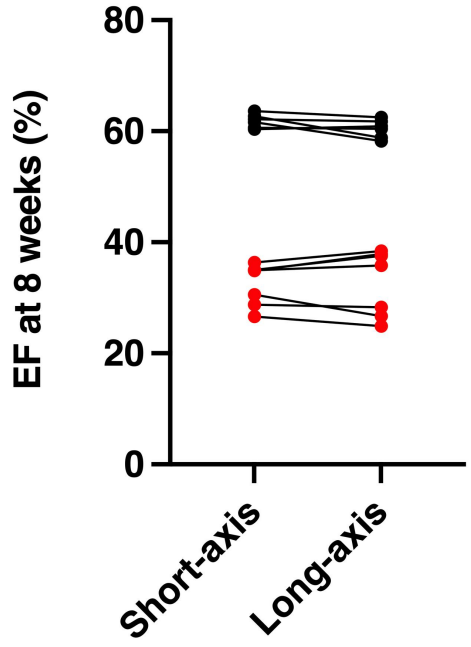
the authors, this study further optimized TAC surgical procedures, leading to a lower mortality rate with high reproducibility. Applying this mTAC, the author assessed cardiac remodeling and functions in both atria and ventricles at multiple time points.

Here are several suggestions for the improvement of the manuscript.

1. Systolic function assessment by long-axis with B-mode will be more accurate.

We only acquired long-axis images for the endpoint. We did process the long-axis B mode images to obtain M-mode images. We further analyzed cardiac function from these long axis M mode images at 8 weeks and compared those with the cardiac function data obtained from the short axis M mode images at the same point. Our finding clearly showed that cardiac function data obtained from either long-axis or short-axis images were comparable.

Please see the reviewer only figure and table below showing the EF from both axis:



Surgery	EF (%) (Short Axis)	EF (%) (Long Axis)
mSham	62.14	61.79
mSham	61.63	58.23
mSham	63.65	62.51
mSham	62.69	58.82
mSham	60.65	60.45
mSham	60.35	60.93
mTAC	26.60	24.89
mTAC	30.56	26.69
mTAC	35.02	37.56
mTAC	36.35	38.41
mTAC	34.87	37.92
mTAC	34.97	35.85
mTAC	28.79	28.28

2. The legends of Figure 4 and Figure 5 seem mixed up with each other.

The figure order and references have been corrected.

3. A wave was still displayed (shown in Figure 5C) while the heart failed at the end of the 8-week TAC, inconsistent with the stage of heart failure.

Thank you for this comment. In this figure, the mitral inflow (measured as E/A ratio) is presenting both waves A and E even during diastolic dysfunction. It has been described that grade 3 and 4 diastolic dysfunction is characterized by high E-wave amplitude and low A-wide, with an E/A ratio bigger than 1.5 (PMID: 16534017).

Reviewer 2 Report

Heart failure (HF) is the leading cause of death worldwide. A common surgical technique to induce HF is transverse aortic constriction (TAC), which induces pressure overload. The conventional TAC (cTAC) procedure is a highly invasive surgery which is associated with severe inflammation and excessive perioperative deaths. Dr Wehrens's team established an improved, minimally invasive TAC (mTAC) procedure that does not require thoracotomy. This model was associated with low peri-operative mortality and a highly reproducible constriction.

This research is well written and clearly organized, except the following concern need to be addressed. It would be better to set another cTAC group to show the difference between mTAC and cTAC.

Author Response

Heart failure (HF) is the leading cause of death worldwide. A common surgical technique to induce HF is transverse aortic constriction (TAC), which induces pressure overload. The conventional TAC (cTAC) procedure is a highly invasive surgery which is associated with severe inflammation and excessive perioperative deaths. Dr Wehrens's team established an improved, minimally invasive TAC (mTAC) procedure that does not require thoracotomy. This model was associated with low peri-operative mortality and a highly reproducible constriction.

This research is well written and clearly organized, except the following concern need to be addressed. It would be better to set another cTAC group to show the difference between mTAC and cTAC.

Thank for your comment. We agree that it should be interesting to compare and show both kind of surgeries together. However, we are not performing conventional TAC surgeries currently in the lab, so the comparison could not be done. We added the absence of a cTAC in the manuscript as a limitation of the study.

Reviewer 3 Report

This is a well-written surgical procedural paper that aims to reduce surgical field exposure and complications related to TAC surgery for induction of pressure-overload induced hypertrophy and heart failure in rodents. The surgical technique is well-described and should be reproducible.

Author Response

This is a well-written surgical procedural paper that aims to reduce surgical field exposure and complications related to TAC surgery for induction of pressure-overload induced

hypertrophy and heart failure in rodents. The surgical technique is well-described and should be reproducible.

We thank the reviewer for his/her positive evaluation of our manuscript.

Reviewer 4 Report

Here, the authors established minimally invasive TAC (mTAC) that does not require thoracotomy, in order to improve upon the conventional (cTAC) method, which is associated with inflammation and perioperative deaths. mTAC was associated with low mortality and a reproducible constriction, led to LV remodeling, left atrial enlargement and dysfunction, and LV fibrosis. The authors did not find changes in the right ventricle (RV) or the right atrium (RA). The reasons for and approaches used to find no changes in RA and RV may need to be expanded. Specific concerns related to that and additional concerns can be found below. The authors characterized a very exciting and valuable model of cardiac hypertrophy and failure that should replace cTAC. The manuscript is written exceptionally well and is a pleasure to read.

Major concerns:

1. At what time point was RV function assessed by pulmonary vein flow doppler? This should be indicated.

2. Related to the above comment, pulmonary arterial remodeling and right-sided pathology were previously seen in cTAC, where LV and RV remodeling followed different timelines. Would it be possible for the authors to assess Doppler traces of pulmonary vein flow at different time points in their 8-week time course, if this data already exists in the echos they performed? Or perhaps they may be able to analyze RV dimensions and function from short axis views of the ventricles, although those would have to have a clear view of the RV, which is challenging to obtain if the short axis was mainly focused on LV.

3. Would it be possible to show the data for no changes in the right atria?

4. Lines 267-269 state that "…mTAC is a highly reproducible surgical model to study HF and its progression, including the secondary development of AF." Since AF is not assessed in this study, can this wording be softened?

5. If there are samples saved for biochemistry, it would be helpful if the authors show changes in fetal and fibrosis associated gene expression in mTAC, which are classically associated with pathological hypertrophy in the cTAC model.

6. It would enhance the claim that mTAC results in minimal activation of inflammation, if the authors were to examine inflammation associated gene expression in mTAC. It would

be very interesting if that were to be compared with inflammation in cTAC, to stake the claim that there is less inflammation in cTAC.

7. Myocyte cross-sectional area data should be added.

8. Would it be possible that atrial fibrosis be assessed in the histology already performed? That would be very valuable for characterization of the atria.

Minor concerns:

1. This reviewer is curious, is mTAC more or less tedious for an experienced surgeon to perform. Could the authors comment on how long it takes to do mTAC compared to cTAC?

2. What defines moderate-to-severe aortic constriction. Please indicate how this is defined.

3. Figures 4 and 5 seem to be switched.

4. Line 206 should say Figure 3 instead of Figures. Line 208 should say Figure 3B not Figures 3B.

5. Line 215 should refer to Fig 3E and line 216 should refer to Fig 3F.

6. Line 276 should say reduced not reduce.

7. What are the units for 0.3-0.6 on line 303? mm?

8. Line 364 the word “of ” should be removed.

Author Response

Here, the authors established minimally invasive TAC (mTAC) that does not require thoracotomy, in order to improve upon the conventional (cTAC) method, which is associated with inflammation and perioperative deaths. mTAC was associated with low mortality and a reproducible constriction, led to LV remodeling, left atrial enlargement and dysfunction, and LV fibrosis. The authors did not find changes in the right ventricle (RV) or the right atrium (RA). The reasons for and approaches used to find no changes in RA and RV may need to be expanded. Specific concerns related to that and additional concerns can be found below. The authors characterized a very exciting and valuable model of cardiac hypertrophy and failure that should replace cTAC. The manuscript is written exceptionally well and is a pleasure to read.

Major concerns:

1. At what time point was RV function assessed by pulmonary vein flow doppler? This should be indicated.

RV function has been assessed at the endpoint of the study (8 weeks after surgery) and registered by echocardiogram.

The following information has been included in this paragraph:
"Next, atrial function and structure were assessed using echocardiographic studies. Representative long-axis echocardiography revealed clear images of the aortic root (Ao) and left atrium (LA) at 8-week post-surgery in mTAC and Sham mice (Figure 5A). The left atrial (LA) size was significantly larger in mTAC mice compared to Sham mice (Figure 5B, $P < 0.01$). No changes were observed in the right atria (RA) size in mTAC mice (not shown). The atrial contractile function was studied using echo Doppler in mTAC mice at 8 weeks after surgery (Figure 5C). Representative color Doppler images of the mitral valve's early and late flow peaks revealed an increased mitral early to after waves (E/A) ratio in mTAC versus Sham mice (Figure 5D, $P < 0.01$), indicating abnormal ventricular filling probably because of decreased atrial contractility."

2. Related to the above comment, pulmonary arterial remodeling and right-sided pathology were previously seen in cTAC, where LV and RV remodeling followed different timelines. Would it be possible for the authors to assess Doppler traces of pulmonary vein flow at different time points in their 8-week time course, if this data already exists in the echos they performed? Or perhaps they may be able to analyze RV dimensions and function from short axis views of the ventricles, although those would have to have a clear view of the RV, which is challenging to obtain if the short axis was mainly focused on LV.

Thank for your comment. The echocardiogram studies have been recorded in short-axis mode, so we don't have a clear view of the RV in our short-axis echo images.

3. Would it be possible to show the data for no changes in the right atria?

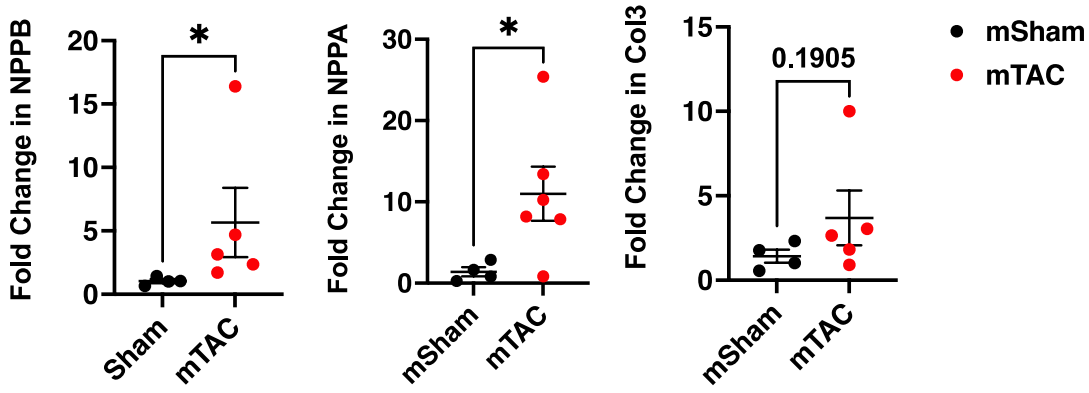
A deeper analysis of RA would be beneficial in the present manuscript, however, as indicated in the previous question, we don't have a clear view of the RA in our long-axis echo images.

4. Lines 267-269 state that "…mTAC is a highly reproducible surgical model to study HF and its progression, including the secondary development of AF." Since AF is not assessed in this study, can this wording be softened?

Thank you for this suggestion, we have modified this sentence.

5. If there are samples saved for biochemistry, it would be helpful if the authors show changes in fetal and fibrosis associated gene expression in mTAC, which are classically associated with pathological hypertrophy in the cTAC model.

Thank you for the suggestion. We have run PCRs for some hypertrophy and fibrosis markers. We found increased levels of NPPB and NPPA, and a tendency to increased Col3 expression in mTAC hearts versus Sham. The significant data have been included in the manuscript (see Figure 6B-C).



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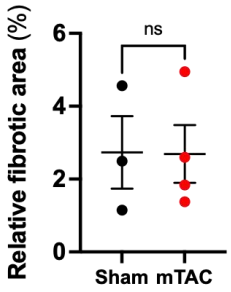
Thank you for the suggestion. It would be useful to compare inflammatory markers between both kind of TAC surgeries, however, our lab is not performing cTAC surgeries currently. We added the lack of a cTAC group as a limitation of the present work.

7. Myocyte cross-sectional area data should be added.

Thanks for the suggestion. Our mTAC hearts were sectioned longitudinally (four-chamber) as we assessed both ventricular and atrial fibrosis. Therefore, we didn't have short axis sections from these mTAC hearts to evaluate the myocyte cross sectional area with WGA staining.

8. Would it be possible that atrial fibrosis be assessed in the histology already performed? That would be very valuable for characterization of the atria.

Thanks for the comment. We have measured the fibrotic area in the atria from mTAC versus Sham mice. However, no significant differences have been found between both groups. We included this data in the result section as followed at line 254:
"However, no significant differences in atrial fibrosis were found when compared both groups (data not shown)."



Minor concerns:

1. This reviewer is curious, is mTAC more or less tedious for an experienced surgeon to perform. Could the authors comment on how long it takes to do mTAC compared to cTAC?

Based on our experienced surgeon, the minimally invasive TAC model is a less tedious surgery that the conventional TAC for several reasons. Firstly, the intubation of the animal could be avoided (even though we don't necessarily recommended it) what would make the surgery faster. Secondly, the manipulation of the aortic arch is much easier with mTAC compared to cTAC, demonstrated by smaller intra-operative mortality. Thirdly, when closing the surgery area in mTAC surgery as we are closing above the lungs, not directly over the lungs as happens in cTAC, decreasing this way the risk of pinching the heart or the lungs with the needle of the suture.

2. What defines moderate-to-severe aortic constriction. Please indicate how this is defined.

The severity of the constriction is defined based on the ratio between both carotids blood flow obtained from doppler studies. Thus, a ratio between 5 to 8 is considered a moderate-to-severe constriction, while ratios above 8 are considered severe constriction as the differences between both flow are too big.

3. Figures 4 and 5 seem to be switched.

The figure order and references have been corrected.

4. Line 206 should say Figure 3 instead of Figures. Line 208 should say Figure 3B not Figures 3B.

Thank you for these comments, we have corrected this.

5. Line 215 should refer to Fig 3E and line 216 should refer to Fig 3F.

Thank you for these comments, we have corrected this.

6. Line 276 should say reduced not reduce.

The typo has been corrected.

7. What are the units for 0.3-0.6 on line 303? mm?

Thank you for the comment. The units used for the aortic stenosis are mm. We have corrected the manuscript in order to avoid this doubt to the readers.

8. Line 364 the word “of ” should be removed.

The typo has been corrected.