Author's response to reviews

Title: Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size

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Version: 2 Date: 27 December 2007

Author's response to reviews:

Dear Editor and reviewers

Thank you for the constructive suggestions on our manuscript. Please see below the answers and changes made in response to the reviewers comments.

Reviewer 1
Reviewer's report
Title: Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size
Version: 1 Date: 11 September 2007
Reviewer: Michael Maeng
Reviewer's report:
General
The study by Dr. Gotberg and colleagues is a sequential to a previous study from the same group. Generally the study is well-designed, the results nicely presented and the manuscript is easy to read and the messages clear.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
Question: The method used for assessment of "microvascular obstruction" is not
validated. Therefore microvascular obstruction should not be used in the title, abstract, and conclusion. The authors should provide illustrations of no microvascular obstruction (pre-reperfusion hypothermia) and of microvascular obstruction (normothermia) so that the reader gets some trust to this unvalidated method.

Response: We acknowledge that we have not validated our method to quantify microvascular obstruction in ex vivo MRI. However, there is an acceptance in the literature for using the terms "microvascular obstruction" or "no reflow" to describe myocardium which exhibits hypoenhancement surrounded by hyperenhancement when imaged in vivo using delayed enhancement MRI (Albert et al, 2006, Cur Rev Cardiol; Tarantini et al, 2005, J am Coll Cardiol).

Also, histopathology has shown microvascular obstruction in human tissue which by in vivo delayed enhancement MRI was shown to exhibit hypoenhancement surrounded by hyperenhancement (Lesser et al, 2003, Circulation). Furthermore, ex vivo contrast enhanced T1 weighted MRI, as used in our study, shows excellent agreement with in vivo delayed enhancement MRI (Heiberg et al, 2007, Radiology) with regards to quantification of total infarct size. Our ex vivo method objectively quantifies regions of hypoenhancement within hyperenhancement with high spatial resolution, and we feel it is reasonable to use the term microvascular obstruction in this setting.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Response: Background section: p4, "....no therapy has yet reached clinical practice mainly because of difficulties in administration of the drug to the ischemic myocardium before reperfusion." It is this reviewers opinion that they have not reached clinical practice as the therapies have had no beneficial effects in the clinical trials. Please rephrase.

Response: We agree, the sentence has been changed to "However, no therapy has yet shown beneficial effects in clinical trials", page 4, line 14

Question: Figure 1: Based on the figure it seems that the post-reperfusion group had a
longer total intervention period. Is that true?
Response: No. The figure has been changed accordingly.
Question: The heart was removed after 4 h 22 min +/- 47 min (SEM!). Why this great variation? It should be possible to standardize in an experimental setting.
Response: The reasons for this variation in reperfusion time were logistical (transportation of the pigs) and availability of the MRI-lab. The animal-lab and the SPECT/MRI-labs were at different locations, thus the pigs had to be transported in order to acquire the in-vivo SPECT/MRI-images. Also, the MRI-lab was only available after office-hours.
Discretionary Revisions (which the author can choose to ignore)
Question: Methods: The closed chest pig model is probably the best pre-clinical model available. Temperature control is of uttermost importance and this issue is controlled perfectly. Assessment of AAR by SPECT has a higher variability than ex-vivo histomorphometry while ex-vivo MRI is reported to have an accuracy similar to histomorphometry. The combination (AAR by SPECT + IS by MRI) should thus give greater variability than histomorphometric assessment of AAR and IS. Despite this the authors find a significant difference using a relatively small number of animals.
Response: We agree with the reviewer. The closed chest pig model with careful temperature control is one of the best animal models available. As can be seen in figure 3a, variability was low even in AAR determined by SPECT. This is the reason for significant differences even in relatively small number of animals.
Reviewer 2
Reviewer's report
Title: Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size
Version: 1 Date: 25 November 2007
Reviewer: Neel Sodha
Reviewer's report:
General
Gotberg et al have performed a nice series of experiments evaluating the use of hypothermia to limit the extent of injury after myocardial ischemia reperfusion.
Key to the strength of the manuscript is the use of readily available technology and material which may facilitate its clinical utility in the future. The manuscript is well written, utilizes appropriate techniques, and draws conclusions justified by the data. The experimental intervention utilized in the study demonstrates significant reductions in myocardial infarct size after I/R, but provides little evidence of functional benefit.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Question: Additional details regarding the functional data (HR, SV, CO) is needed.
Response: The main aim of this study was to investigate the effect on hypothermia on infarct size rather than functional hemodynamics. Maeng et al (Basic Res Cardiol 2006, 101:61-68) and Dae et al (Am J Physiol Heart Circ Physiol 2002, 282:H1584-1591) have previously investigated the hemodynamic effects of hypothermia.

Question: Did all animals receive equivalent amounts of intravenous fluids?
Response: The animals in the control group did not receive any additional intravenous fluids except for the fluids given with the anesthesia. (buffered glucose solution). We considered using normothermic saline for the control group. However, we refrained from doing so since the primary aim of this study was to achieve rapid cooling and observe any reduction in infarct size. We were also afraid that a large bolus of warm saline would be considered artificial as a control group since this is never used in the clinic.

Question: Why were baseline MR studies for HR, CO, and SV performed in only seven animals?
Response: The MRI and the animal lab were at different locations, thereby limiting our capacity to perform in-vivo MRI in all pigs. As part of the protocol we intended to do baseline hemodynamic measurements in a limited number of pigs for comparison with after myocardial infarction.

Question: Were core temperatures similar between groups during acquisition of post I/R functional data?
Response: We did not have the ability to measure core temperature during the in-vivo MRI acquisition. All equipment with metal had to be removed before MRI.

Question: Please provide additional discussion as to why no significant differences were observed between groups for CO given the marked differences in infarct size.
Response: We believe that the area at risk is still stunned during MRI measurements. Furthermore, the remaining myocardium (lateral and inferior) is healthy in the pigs and has the ability to compensate. The stroke volume remained unchanged, thus any difference in cardiac output would be attributed to a difference in heart rate (known to be reduced during hypothermia). Additional comments have been added on page 21, under hemodynamic measurements.

Question: The data presented demonstrate a trend towards worse CO in animals subject to hypothermia than normothermia. Additional commentary regarding these findings would be worthwhile given cardiac output / index are followed much more commonly in the clinical setting than infarct size.
Response: See the response above.

Minor Essential Revisions

Question: Given that VF/VT is a significant problem after myocardial I/R injury, data regarding the incidence of VF/VT would add to the paper. Please include the incidence of VF/VT during the experimental protocol and provide discussion if there were significant differences between groups.
Response: Data regarding the incidence of VT/VF have been added on page 12, line 10-13 and on. There were no significant differences (chi-square test).

Question: Please provide additional discussion regarding why there was lack uniform timing for the duration of I/R between animals.
Response: See answer to question by Reviewer 1.

Reviewer 3
Reviewer's report
Title: Rapid short-duration hypothermia with cold saline and endovascular cooling before reperfusion reduces microvascular obstruction and myocardial infarct size
Version: 1 Date: 30 October 2007
Reviewer: Daniel Sessler
Reviewer's report:
General
Overall, this is an excellent study that uses sophisticated methodology to answer an important question. The methodology appears to be technically appropriate
(although I am no expert in cardiology).

It would be helpful if the authors estimated the relative contributions of the cold intravenous fluid and the intravascular cooling catheter. In minor detail, but it would be helpful if the authors wrote the text into more paragraphs. Currently, some paragraphs go on for pages!

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

Comments: Page 2, paragraph 2: It would be helpful if the authors specified exactly when hypothermia was induced in each of the hypothermia groups.

Page 2, paragraph 3: The first sentence of the results is unclear. It would be easier for readers if you simply listed the ischemic area divided by the area at risk for each of the three groups.

Page 5, paragraph 1: The authors might consider referencing a study by Rajek et al. that quantifies the affect of cold fluid administration on core temperature: Anesthesiology: 93: 629, 2000.

Page 6: Please replace ¿dinitrous¿ with ¿nitrous.¿

Page 7, paragraph 3: Please specify how the randomization was conducted.

Page 8, paragraph 1: Please specify where core temperature was measured, and how.

Page 11, paragraph 4: Throughout the manuscript, please replace ¿central temperature¿ with ¿core temperature.¿ The reason is that central implies central nervous system.

Page 12: This is the results section rather than the measurement section. It would thus be best if you deleted ¿measurement of¿ on the first header and ¿assessment of¿ from the second header. Throughout the manuscript, please replace ¿between the groups¿ with ¿among the groups.¿

Page 13: The fourth sentence on this page, beginning ¿pre-reperfusion hypothermia¿ is unclear. There are too many percentages floating around. I suggest simply listing the ischemic area divided by the area at risk for each of the
three groups.

Figure 3: I suggest deleting this figure. It basically only contains three numbers and is not a result.

Response: Good suggestions. We have changed the manuscript according to most of the discretionary suggestions made by the reviewer.

We hope that the manuscript now will be accepted for publication in BMC cardiovascular disorders.

I hereby declare that the manuscript in its whole or part of it neither has been published nor is under consideration for publication by any other journal.

I also declare that the co-authors have read the manuscript and approved its submission to Basic Research in Cardiology.

Any cost for printing colour figures will be paid for upon final acceptance of the manuscript

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