LEMA: Lo que importa es el camino

TITLE

Proof-of-concept methodology for studying the effect of ischemia-reperfusion injury on microvascular obstruction in myocardial samples of an in vivo model of myocardial infarction

TÍTULO

Metodología innovadora para el estudio del efecto que ejerce el daño por isquemia-reperfusión sobre la obstrucción microvascular en muestras de miocardio de un modelo in vivo de infarto de miocardio

ABSTRACT

Background. Microvascular obstruction (MVO) exerts deleterious effects after myocardial infarction (MI). We aimed to describe a new methodology to accurately evaluate MVO in an in vivo model and to elucidate the role of ischemia-reperfusion injury on the occurrence and dynamics of this process.

Methods. MI was induced in swine by means of 90-min occlusion of the mid left anterior descending coronary artery (LAD) using angioplasty balloons. Intra-coronary infusion of Thioflavin-S (T-S) was applied and compared with traditional intra-aortic or intra-ventricular instillation. LAD-perfused area (stained with T-S) and MVO (% of LAD-perfused area with a lack of T-S staining) were quantified in groups with no reperfusion (T-S administered distally through the lumen of an inflated over-the-wire balloon) and with 1-min, 1-week and 1-month reperfusion (T-S administered proximally from the intra-coronary catheter after balloon deflation).

Results. In comparison with intra-aortic and intra-ventricular administration, intra-coronary infusion of T-S permitted a much clearer assessment of LAD-perfused area and of MVO. Ischemia-reperfusion injury exerted a decisive role on the occurrence and dynamics of MVO. The no reperfusion group displayed completely preserved perfusion. With the same duration of coronary occlusion, MVO was already detected in the 1-min reperfusion group (14±7%), peaked in the 1-week reperfusion group (21±7%) and significantly decreased in the 1-month reperfusion group (4±3%, $p<0.001$).

Conclusions. The described porcine model using intra-coronary injection of T-S permits an accurate characterization of MVO after MI. We present proof-of-concept evidence on the crucial role of ischemia-reperfusion injury on the occurrence and dynamics of MVO.

RESUMEN

Antecedentes. La obstrucción microvascular (OMV) ejerce un efecto deletéreo tras un infarto de miocardio (IM). El objetivo del presente trabajo fue describir una nueva metodología para evaluar con precisión la OMV en un modelo in vivo, así como elucidar el papel del daño por isquemia-reperfusión sobre la aparición y dinámica de este proceso.

Métodos. Se utilizó un modelo porcino que fue sometido, a través de un inflado de balón introducido de forma percutánea, a una oclusión durante 90 minutos de la arteria descendente anterior (ADA). Se aplicó una infusión intra-coronaria de tioflavina-S (T-S) que se comparó con la infusión intra-aórtica y la intra-ventricular utilizadas tradicionalmente. El área perfundida por ADA (área teñida con T-S) y la OMV (% del área perfundida por ADA que no se tiñe con T-S) se cuantificaron en los grupos de no reperfusión (T-S administrada de forma distal a través de la luz de un balón de angioplastia inflado) y en los grupos de 1-min, 1-semana y 1-mes de reperfusión (T-S administrada en la parte proximal de la ADA desde el catéter intra-coronario tras el deshinchado del balón).

Resultados. La infusión de T-S intra-coronaria permitió una evaluación más precisa del área perfundida por la ADA y de la OMV en comparación con la administración intra-aórtica y la intra-ventricular. El daño por isquemia-reperfusión ejerció un papel decisivo en el desarrollo y dinámica de la OMV. El grupo de no reperfusión presentó una perfusión completamente preservada. La OMV fue detectada ya en el grupo de 1 minuto de reperfusión, tuvo su máximo incremento en el grupo de 1 semana de reperfusión y descendió significativamente en el grupo de 1 mes de reperfusión.

Conclusiones. El modelo porcino descrito, caracterizado por una inyección intra-coronaria de T-S, permite una determinación precisa de la OMV tras un IM. En este

trabajo se presenta además una prueba que evidencia el papel del daño por reperfusión en la aparición y dinámica de la OMV.

ABBREVIATIONS LIST

LAD = left anterior descending coronary artery

LV = left ventricle

MI = myocardial infarction

MVO = microvascular obstruction

RV = right ventricle

T-S = Thioflavin-S

TTZ = 2,3,5-triphenyltetrazolium chloride

1 INTRODUCTION

Timely and complete restoration of infarct vessel patency is the main goal in patients with acute myocardial infarction (MI) [1]. Nevertheless, this approach does not assure an adequate reperfusion at microvascular level and an impairment of perfusion persists in a significant number of patients [2]. This phenomenon is referred to as microvascular obstruction (MVO) and exerts a strong negative impact after MI [3-5].

In MI, ischemia-reperfusion injury has been vastly discussed [6,7] and it could exert deleterious effects on microvascular integrity [2,3]. Nevertheless, there is no definitive evidence demonstrating the direct association between reperfusion injury and the occurrence of MVO in myocardial samples obtained immediately after coronary reflow. Accurate in vivo animal models mimicking the dynamics of MVO in humans are urgently needed. This will permit a better understanding of the pathophysiology and timing of this process and, in turn, the exploration under controlled conditions of new therapeutic opportunities.

Contrasts used for studying perfusion in myocardial samples obtained from in vivo animal models have been infused in the left atrium [8], in the left ventricle [9] or intravenously [10]. In the present study we aimed to describe a new methodology based on intra-coronary contrast administration to accurately evaluate MVO in myocardial samples obtained from an in vivo swine model of anterior MI. The effect of ischemia-reperfusion injury on MVO and the dynamics of this process were also evaluated.

2 METHODS

2.1 Experimental Study

Thirty-one juvenile domestic pigs weighing 25-30 kg were used. The Animal Care and Use Committee of the University of Valencia approved the study and it conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1993).

Further details on our study protocol can be consulted elsewhere [11,12]. In summary, a 7F sheath was introduced into the right femoral artery to monitor blood pressure and to access the left anterior descending coronary artery (LAD). A 7F Amplatz Left 0.75 catheter was used to selectively engage the proximal LAD and a standard hydrophilic angioplasty wire was advanced and placed in the distal LAD. A 2.5 x 15 mm angioplasty balloon was inflated at 6 atm in the mid LAD distal to the first diagonal branch. Coronary artery occlusion was confirmed by contrast injection and by electrocardiographic ST-segment elevation.

Three groups of experiments with reperfusion were carried out. The balloon was deflated after 90 min of coronary occlusion and restoration of normal coronary flow was documented by angiography. In the 1-min reperfusion group (n=5), 20 mL of 4% Thioflavin-S (T-S) solution was selectively infused into the proximal LAD through the Amplatz Left 0.75 catheter 1 minute after balloon deflation and hearts were arrested with potassium chloride and excised (Figure 1). Animals in the 1-week and 1-month reperfusion groups were allowed to recover and after 1 week (n=5) or 1 month (n=5) respectively, the same study protocol was followed and 20 mL of 4% T-S solution was selectively infused into the proximal LAD through the Amplatz Left 0.75 catheter. Hearts were then arrested with potassium chloride and excised.

Afterwards, in order to evaluate the role exerted by reperfusion injury on the occurrence of MVO, the 1-min reperfusion group was compared with a no reperfusion

group (n=5) which underwent an identical 90-min period of ischemia but without reperfusion. In this group of experiments the balloon was not deflated and 20 mL of 4% T-S solution was selectively infused into the mid LAD after the first diagonal branch through the lumen of an over-the-wire balloon (Figure 1). Immediately after T-S administration, hearts were arrested using potassium chloride and then excised.

The control group was made up of 5 experiments. In this group we used the same study protocol described above, but the balloon angioplasty was not inflated and thus ischemia and infarction were not provoked. We selectively infused 20 mL of 4% T-S solution into the proximal LAD through the Amplatz Left 0.75 catheter. Hearts were then arrested with potassium chloride and excised.

A preliminary series of experiments was carried out in order to compare the trans-catheter intra-ventricular and intra-aortic instillation with the methodology used in the present study (intra-coronary infusion of T-S). MI was induced in 6 pigs following the protocol described above. Afterwards, the angioplasty balloon was withdrawn and the Amplatz Left 0.75 catheter was placed in the left ventricle (n=3) or in the aorta (n=3) where 20 mL of 4% T-S solution was infused. Hearts were then arrested with potassium chloride and excised. The precision of intra-aortic and intra-ventricular vs. intra-coronary infusion of T-S for assessing the LAD-perfused area and MVO was compared (Figure 1).

2.2 Macroscopic study of myocardial samples

Immediately after excision the whole heart was viewed under ultraviolet light and photographed. Afterwards hearts were sectioned into 5-mm thick short-axis slices. In order to assess myocardial perfusion in the left ventricle (LV), each slice was viewed under ultraviolet light and photographed (Figure 1). The LAD-perfused area was defined as the percentage of the myocardial volume showing T-S staining. MVO was

interpreted as the lack of T-S staining in the core of the LAD-perfused area (Figure 2). MVO was expressed as the percentage of the LAD-perfused area.

Thereafter, slices were incubated in 2,3,5-triphenyltetrazolium chloride (TTZ) 2% solution for 20 min at 37^{o}C. Finally, they were viewed under room light and photographed. Infarcted tissue was defined as the myocardial area that did not stain with TTZ and it was expressed as the percentage of the LAD-perfused area.

Myocardial wall thickness of the MVO, adjacent and remote areas was quantified in ultraviolet light images (before TTZ staining). The adjacent area was defined as the non-infarcted LAD-perfused area (with both T-S and TTZ staining) and the remote area as the non-LAD-perfused myocardium (without T-S staining).

A separate subanalysis of the LAD-perfused area, MVO, infarcted tissue and wall thickness of the right ventricle (RV) was performed using the same methodology exposed for these parameters in the LV.

Images were digitalized and manual quantification of all short-axis slices was carried out offline in a dedicated laboratory (Cardiac Imaging Unit, Incliva, Valencia Spain) by an experienced observer unaware of the protocol applied in each experiment. The software package MATLAB 6.5 (The Mathworks, Ink., Nattick, MA, USA) was used. A ruler was photographed beside myocardial slices in all images and it was used as a reference for measurements. This, along with the pre-defined slice thickness (5 mm), permitted the calculation of LV and RV myocardial volumes.

2.3 Endpoints

The endpoints of the present study were as follows:

First, we aimed to describe a new method based on intra-coronary contrast administration to accurately evaluate MVO in myocardial samples obtained from an in vivo swine model of anterior MI.

Secondly, we intended to contribute proof-of-concept evidence on the crucial role exerted by ischemia-reperfusion injury on the occurrence of MVO and on the dynamics of this process.

3 STATISTICAL ANALYSIS

Continuous data were expressed as the mean ± standard deviation and were compared by the unpaired Student's t-test. Percentages were compared by the chi-square statistic; the Fisher exact test was used when appropriate. Statistical significance was considered for two-tailed $p < 0.05$. SPSS 22.0 (SPSS Inc, Chicago, Illinois, USA) was used throughout.

4 RESULTS

Three experiments were not completed: 2 due to refractory ventricular fibrillation and 1 due to technical problems in the radiology equipment. Electrical ventricular defibrillation was needed in 7 pigs during LAD occlusion. No significant complications were recorded over the reperfusion period.

4.1 Methodology used for T-S infusion

Intra-coronary infusion of T-S, either from the catheter engaged in the proximal LAD (in the reperfusion groups) or from the lumen of the over-the wire balloon placed at the mid LAD (in the no reperfusion group) resulted in a much clearer definition of the LAD-perfused area and of MVO than intra-ventricular or intra-aortic injection (Figure 1).

4.2 Dynamics of MVO

MVO was detected in LV myocardial samples of all reperfused experiments. MVO was already detected 1 min after reperfusion, peaked at 1 week and decreased at 1 month. The extent of MVO detected in the 1-month reperfusion group was significantly lesser than that observed in the 1-week reperfusion group (Figure 3, Table 1). Similar results regarding the dynamics of MVO occurred in the RV (Supplementary Figure 1, Supplementary Table 1).

4.3 Proof-of-concept evidence of the effect of ischemia-reperfusion injury on MVO

In experiments performed under the same controlled conditions and duration of coronary occlusion, MVO only occurred in myocardial samples obtained from reperfused swine. Figure 2 depicts the crucial role of reperfusion injury on the occurrence of MVO. Whereas myocardial perfusion was completely preserved in myocardial samples obtained from all experiments in the no reperfusion group, MVO was detected in all experiments of the 1-min reperfusion group.

4.4 Structural consequences of MVO

Infarct tissue (as derived from TTZ staining) was detected in all cases in the 1-week and 1-month reperfusion groups (Table 1).

LV myocardial wall thinned in the MVO area in the 1-month reperfusion group compared with the adjacent and remote areas at the same time point (Table 1) and compared with the MVO area in the 1-week reperfusion group (Figure 4).

A similar tendency regarding the extent of the infarcted area and the association of MVO with progressive wall thinning occurred in the RV (Supplementary Figure 2, Supplementary Table 1).

5 DISCUSSION

The main contribution of the present study is the description of an experimental model of anterior MI that, by means of a novel method based on intra-coronary infusion of T-S, permits a precise characterization of MVO in myocardial samples. This methodology allowed us to consistently demonstrate the crucial effect exerted by ischemia-reperfusion injury on the dynamics of MVO.

5.1 Methodology used for T-S infusion

Animal models recreate human disease and constitute an essential tool for a better understanding of the underlying mechanisms. Currently, due to the similar size and cardiac physiology of swine and human heart, pigs represent the preferred specie for experimental studies in cardiovascular diseases [13]. Although swine models of acute MI have been well defined and widely used, methodology specifically focused on the characterization of MVO after MI has not been developed.

T-S, a dye that stains perfused endothelium, was used to quantitatively measure MVO. Intra-atrial [8], intra-ventricular [9] and intra-venous [10] infusion of a variety of contrasts for different purposes such as for analysis of perfusion, area at risk or MVO have been previously reported (Supplementary Table 2). However, as far as we know, a specific methodological description of the contribution of intra-coronary dye administration has not been explored. From our previous experience using intra-coronary contrast echocardiography in humans, we learnt that this route offered the highest definition of myocardial perfusion [14]. This background inspired us to undertake the present study. In the described swine model of anterior MI, intra-coronary infusion of T-S permitted an excellent delineation of the LAD-perfused area and of MVO in myocardial samples obtained immediately after sacrifice. Conversely, T-S infusion from intra-aortic and intra-ventricular routes resulted in a much poorer definition of myocardial perfusion.

The intra-coronary infusion of T-S was carried out either from the catheter engaged in the proximal LAD (once the angioplasty balloon had been deflated and removed) or through the lumen of an over-the wire balloon placed at the mid LAD (which was maintained inflated during the entire experiment). The first strategy was helpful to evaluate the dynamics of MVO at different time points of the ischemia-reperfusion process. The second model allowed us to explore the state of microvasculature after a long period of ischemia but immediately before the potentially deleterious effect exerted by reperfusion injury.

Thus, this novel strategy using intra-coronary infusion of T-S appears as a simple and reliable approach that permits an accurate characterization of myocardial perfusion and MVO in myocardial samples obtained from an in vivo swine model of anterior MI. This methodology may be helpful in the future for achieving further progresses in the comprehension of the mechanisms underlying MVO and for exploring the effects of new therapeutic opportunities addressed to prevent or reverse this process.

5.2 Dynamics of MVO

Over the early hours and days after reperfusion, changes occur in the state of microvasculature under the influence of multiple pathogenic components [2,3]. We and others have demonstrated, using imaging techniques in patients, that there is a tendency towards spontaneous resolution of MVO during the weeks and months following reperfused MI [2,3,15,16].

The in vivo model presented here represents an ideal platform to characterize the dynamics of this process. For this purpose we undertook experiments at different post-reperfusion times: 1 minute, 1 week and 1 month after reperfusion. Important changes in the extent of MVO were observed: it was already detected immediately

after reperfusion, reached its biggest extent at 1 week and almost completely resolved at 1 month. Our results have potential implications in terms of diagnosis and therapy.

Firstly, there is no agreement regarding the most appropriate moment for evaluating MVO in patients with MI. On the basis of our results, it could be suggested that analysis by imaging techniques at pre-discharge (around 1 week after MI) can provide an approximate estimation of the entire microvascular injury. Secondly, the dynamic behavior of MVO offers a therapeutic target beyond myocardial salvage. Until the moment, apart from timely reperfusion within the very few hours following coronary occlusion, approaches tested for reducing infarct size have shown to be unsuccessful [17]. Therefore, acting upon the damaged myocardium remains difficult. However, attending to its more progressive development, MVO offers a hypothetically longer therapeutic window and thus appears as a novel and realistic objective. However, though the deleterious effects of a larger extent of MVO on patients' outcome and LV remodeling have been well documented, so far the efficacy of therapies addressed to reduce MVO have failed and future endeavors must prove their value in addition to timely coronary reperfusion in rigorous randomized trials [18-20].

As expected, infarcted tissue was not detected in controls, in the no reperfusion and the 1-min reperfusion groups. The maximum extent of infarct area occurred 1 week after reperfusion. At 1 month, as a consequence of the shrinking process, infarct size diminished.

We have previously demonstrated that RV structural damage is not exclusive of inferior MI. In anterior MI both infarct tissue and MVO can be detected in the anterior territory of the RV [11]. The present study confirms the structural consequences of anterior MI on the RV. The course of MVO in the RV paralleled that observed in the LV.

5.3 Proof-of-concept evidence of the effect of reperfusion on MVO

The role exerted by ischemia-reperfusion injury in terms of myocardial damage has been largely debated. MVO has a multifactorial pathogenesis that include the combination of a variety of components [2,3]. The significance of ischemia-reperfusion injury, if any, on this puzzle has not been totally understood. Controversy on the significance of ischemia-reperfusion injury has existed for years [6,7], but this mechanism has been suggested to play a role on MVO appearance [2,3].

In this study, beyond theoretical statements, we aimed to contribute proof-of-concept data on the decisive influence of ischemia-reperfusion injury on MVO. In order to specifically address this issue, we designed 2 series of experiments in which anterior MI was provoked using identical conditions. The only differing factor was reperfusion. In the first series, at the end of the 90-min LAD occlusion, T-S was injected through the lumen of the balloon that was maintained inflated; thus reperfusion was not allowed. In the second group, at the end of the 90-min LAD occlusion, the balloon was deflated and removed and T-S was infused through the catheter engaged in the proximal LAD after a 1-min reperfusion period. Striking differences in terms of MVO were observed when myocardial samples of both series were compared. Whereas myocardial perfusion was completely preserved in the no reperfusion group, MVO was observed in all cases that underwent as short as 1-min reperfusion.

In our view the presented data contribute convincing evidence on the decisive role of ischemia-reperfusion injury on MVO. Taking into account the almost immediate onset of this mechanism (within the first minute after coronary reflow), our results highlight the utmost importance of an exquisite management of patients during this short period of time. A number of medical or invasive approaches aimed at reducing MVO in reperfused MI have been controversial or unsuccessful [18,19]. Therefore, beyond the well-established effectiveness of timely primary percutaneous intervention, great efforts are still needed in order to better understand and handle the crucial peri-reperfusion minutes.

5.4 Study limitations

A number of pathogenic components might share a role in the pathophysiology of ischemia-reperfusion injury and MVO: distal embolization, inflammation, vasospasm, ischemia, edema, hemorrhage, endothelial injury, mitochondrial damage or the rapid re-alkalization of the ischemic territory among others. Further advances in order to clarify the relative weight of these factors on the occurrence of MVO are needed, but this objective was out of the scope of the present study.

As always in basic research, translation to clinical practice needs caution. Data obtained in the present work could inspire studies in humans both to replicate our results using imaging techniques and to encourage the design of trials aimed to obtain novel therapeutic tools for minimizing the deleterious effect of reperfusion injury on microvascular perfusion.

6 CONCLUSIONS

The in vivo experimental model presented here can be helpful as a platform for further translational studies focused on a better understanding of MVO and on exploring alternative therapeutic opportunities under highly controlled conditions. The immediate onset of MVO following balloon deflation demonstrates the decisive role of ischemia-reperfusion injury on the occurrence of this process and demands awareness on the management of this short but critical period of time in the revascularization of MI patients.

7 REFERENCES

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TABLES

Table 1. LAD-perfused area, microvascular obstruction, infarct area and myocardial wall thickness in the left ventricle in the 3 series of experiments.

LEFT VENTRICLE	**1 min**	**1 week**	**1 month**
LAD-perfused area (% of LV)	65±6	76±8	66±13
MVO (% of LAD-perfused area)	14±7[a]	21±7[b]	4±3[c]
Infarct area (% of LAD-perfused area)	0±0	34±13[b]	26±11[b]
Myocardial wall thickness:			
MVO area (mm)	10±2.3	9±2.7	7±2.7[d]
Adjacent area (mm)	11±2.4	11±2.7	11±3.8
Remote area (mm)	12±2.6	11±3.0	13±4.2

[a] $p<0.01$ vs. controls

[b] $p<0.001$ vs. controls

[c] $p<0.001$ vs. 1 week

[d] $p<0.001$ vs. adjacent and remote areas

Abbreviations: LAD = left anterior descending coronary artery; LV= left ventricle; MVO = microvascular obstruction.

FIGURE LEGENDS

Figure 1. Methodology used for Thioflavin-S infusion. Left: Diagrams summarize the different methods used for Thioflavin-S infusion. Right: Pictures obtained under blue ultraviolet light.

A. Infusion in aorta or in the LV resulted in a poor definition of LAD-perfused area and MVO.

B. Infusion into the catheter engaged in the proximal LAD resulted in a perfect definition of the LAD-perfused area and MVO. This method was used in the control group and in the 1-min, 1-week and 1-month reperfusion groups. Light blue points represent the site of T-S infusion.

C. Infusion into the mid LAD through the lumen of an over-the-wire angioplasty balloon that was maintained inflated throughout the entire experiment resulted in a perfect definition of LAD-perfused area. This method was used in the no reperfusion group.

Abbreviations: LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; LM = left main stem; LV = left ventricle; MVO = microvascular obstruction; RV = right ventricle; T-S = Thioflavin-S.

Figure 2. Effect of ischemia-reperfusion injury on the occurrence of microvascular obstruction. Images illustrate that under the same conditions and duration of coronary occlusion, perfusion was preserved in the no reperfusion group whereas MVO was detected as soon as 1 minute after reperfusion. White asterisks indicate the MVO area. White points delimit LAD-perfused area.

A. Example of an entire heart and slices of the no reperfusion group.

B. Example of an entire heart and slices of the 1-min reperfusion group.

Abbreviations: LAD = left anterior descending coronary artery; MVO = microvascular obstruction.

Figure 3. Dynamics of microvascular obstruction in the left ventricle. The extent of MVO is represented as percentage of LAD-perfused area. MVO was already detected in the 1-min, peaked in the 1-week and partly resolved in the 1-month reperfusion group.

* $p<0.01$ vs. control group; ** $p<0.001$ vs. control group; *** $p<0.001$ vs. 1-week reperfusion group.

Abbreviations: LAD = left anterior descending coronary artery; LV = left ventricle; MVO = microvascular obstruction.

Figure 4. Consequences of microvascular obstruction on the left ventricular myocardial wall thickness. Slices of no reperfusion and 1-min, 1-week and 1-month reperfusion groups stained with TTZ (left panels) and T-S (right panels). White asterisks indicate the MVO area in the reperfused groups. White points delimit LV wall thickness in the MVO area. A significant thinning of the LV wall in the MVO area took place 1 month after reperfusion.

Abbreviations: LV = left ventricle; MVO = microvascular obstruction; T-S = Thioflavin-S; TTZ = 2,3,5-triphenyltetrazolium chloride.

FIGURE 1

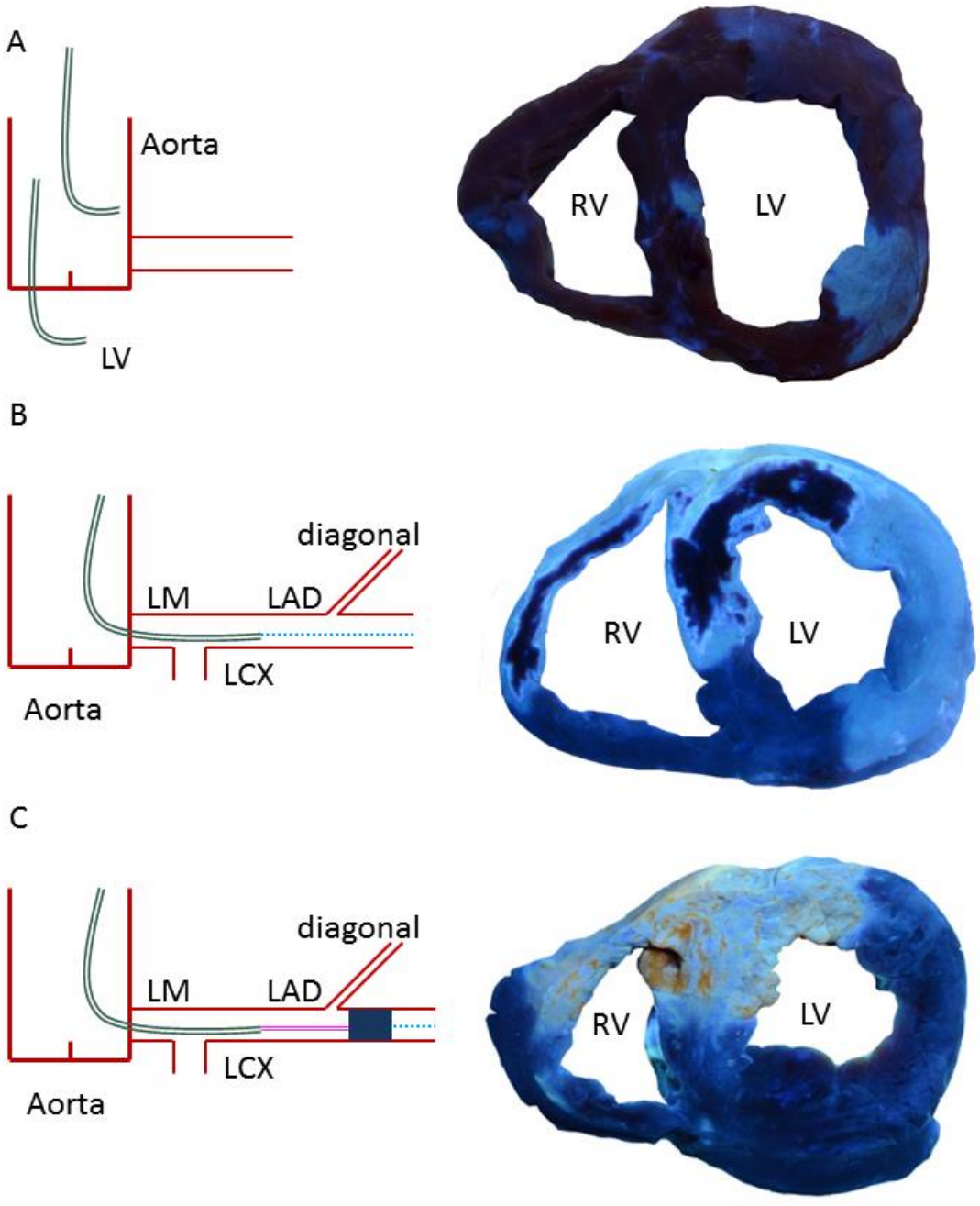
A
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FIGURE 2

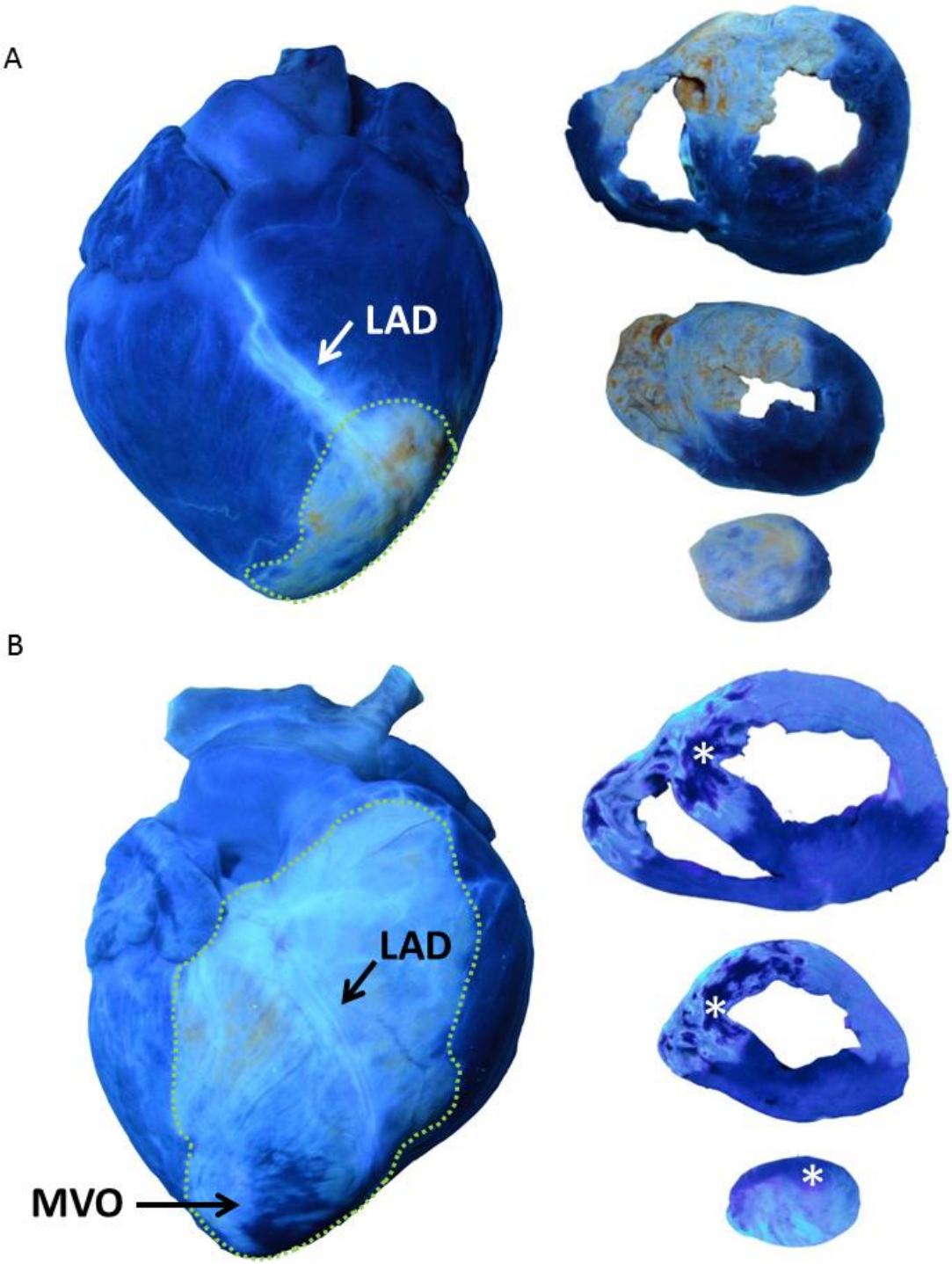


FIGURE 3

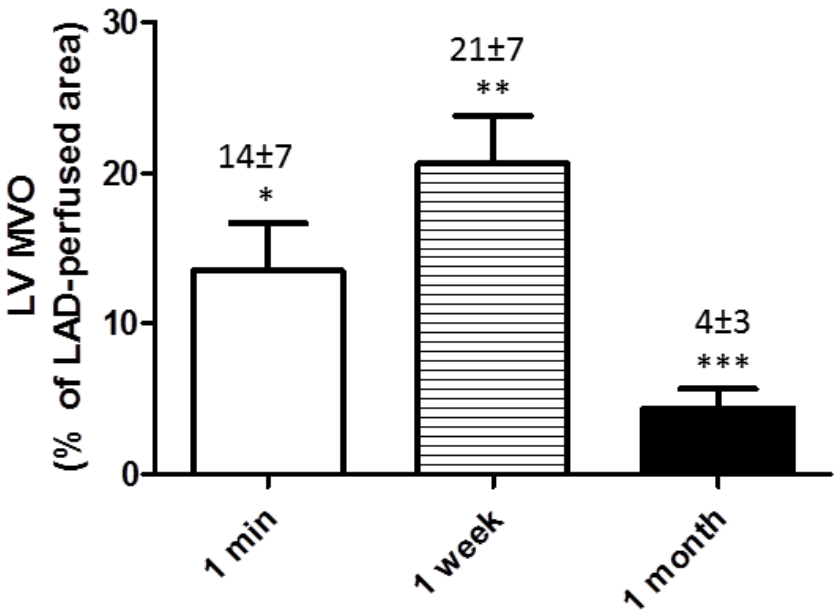
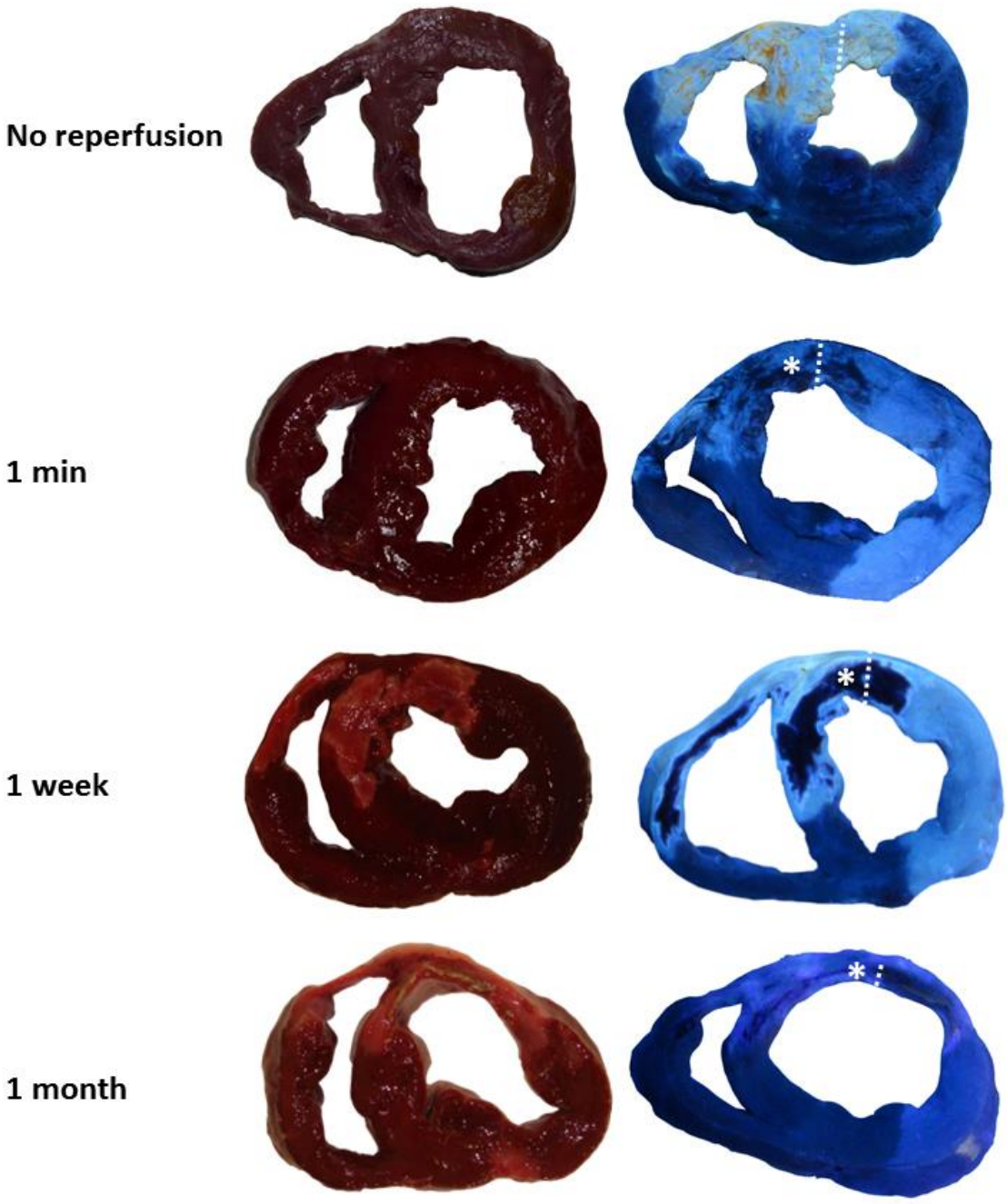

LV MVO
(% of LAD-perfused area)
30
20
10
0
14±7
*
21±7
**
4±3
***
1 min
1 week
1 month

FIGURE 4



SUPPLEMENTARY MATERIAL

Supplementary Table 1. LAD-perfused area, microvascular obstruction, infarct area and myocardial wall thickness in the right ventricle in the 3 series of experiments.

RIGHT VENTRICLE	**1 min**	**1 week**	**1 month**
LAD-perfused area (% of RV)	44±13	45±9	37±12
MVO (% of LAD-perfused area)	11±6[a]	12±10[a]	5±5
Infarct area (% of LAD-perfused area)	0±0	31±19[b]	21±20
Myocardial wall thickness:			
MVO area	5±0.6	5±0.9	4±0.8[c]
Adjacent area	6±1.7	5±1.4	6±1.8
Remote area	6±1.7	5±1.9	7±2.4

[a]$p<0.05$ vs. control

[b]$p<0.01$ vs. control

[c]$p<0.05$ vs. remote

Abbreviations: LAD = left anterior descending coronary artery; MVO = microvascular obstruction; RV = right ventricle.

Supplementary Table 2. Routes, animal models and parameters evaluated in experimental studies focused on the analysis of myocardial perfusion after myocardial infarction.

Thioflavin-S	**Animal model**	**Parameter evaluated**	**References**
Intra-atrial	Mongrel dogs	Microvascular obstruction	[1]
Intra-atrial	White rabbits	Microvascular obstruction	[2]
Intra-atrial	White rabbits	Microvascular obstruction	[3]
Intra-atrial	Mongrel dogs	Microvascular obstruction	[4]
Intra-ventricular	Mongrel dogs	Microvascular obstruction	[5]
Intra-ventricular	Mongrel dogs	Microvascular obstruction	[6]
Intra-venous	Dogs	Flow distribution	[7]
Tail veins	Rats	Microvascular obstruction	[8]
Microspheres			
Intra-atrial	Mongrel dogs	Myocardial blood flow	[9]
Intra-atrial	Pigs	Myocardial blood flow	[10]
Intra-ventricular	Mongrel dogs	Myocardial blood flow	[6]
Intra-ventricular	Mongrel dogs	Myocardial blood flow	[11]
Intra-ventricular	Mongrel dogs	Myocardial blood flow	[12]
Carbon black			
Intra-atrial	Dogs	Flow distribution	[7]
Evans Blue			
Aortic root	Pigs	Area at risk	[13]
Aortic root	Pigs	Area at risk	[10]
Aorta	Green monkeys	Area at risk	[14]
Intra-atrial	Mongrel dogs	Area at risk	[15]
Intra-atrial	Pigs	Area at risk	[16]
Tail veins	Rats	Area at risk	[8]

References of Supplementary Table 2

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Supplementary Figure 1. Dynamics of microvascular obstruction in the right ventricle. MVO was already detected in the 1-min, peaked in the 1-week and partly resolved in the 1-month reperfusion group.

A. Quantification of MVO (represented as percentage of LAD perfused area).

* $p<0.05$ vs. control.

B. Illustrative slices of the 1-min, 1-week and 1-month reperfusion groups. Arrows indicate the MVO area.

Abbreviations: LAD = left anterior descending coronary artery; MVO = microvascular obstruction; RV = right ventricle.

Supplementary Figure 2. Consequences of microvascular obstruction on the right ventricular myocardial wall thickness. A significant thinning of the RV myocardial wall in the MVO area took place 1 month after reperfusion.

A. Quantification of the right ventricular myocardial wall thickness in the MVO, adjacent and remote areas in the 1-week and 1-month reperfusion groups.

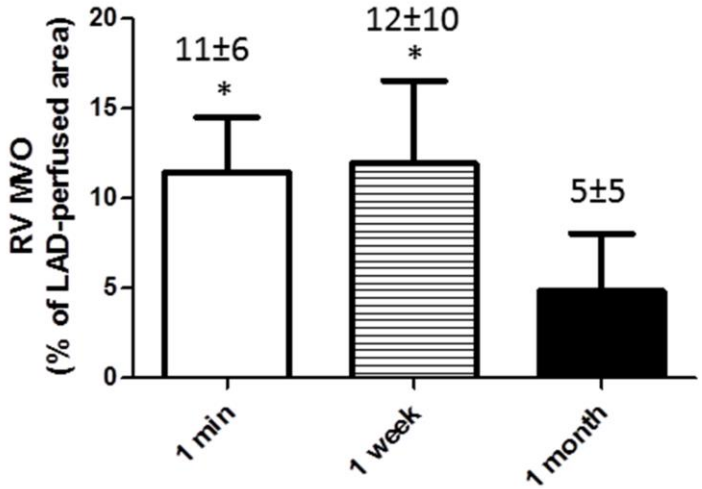
* $p<0.05$ vs. remote area.

B. Illustrative slices of the 1-week and 1-month reperfusion groups. The white points line indicates the right ventricular myocardial wall thickness in the MVO area.

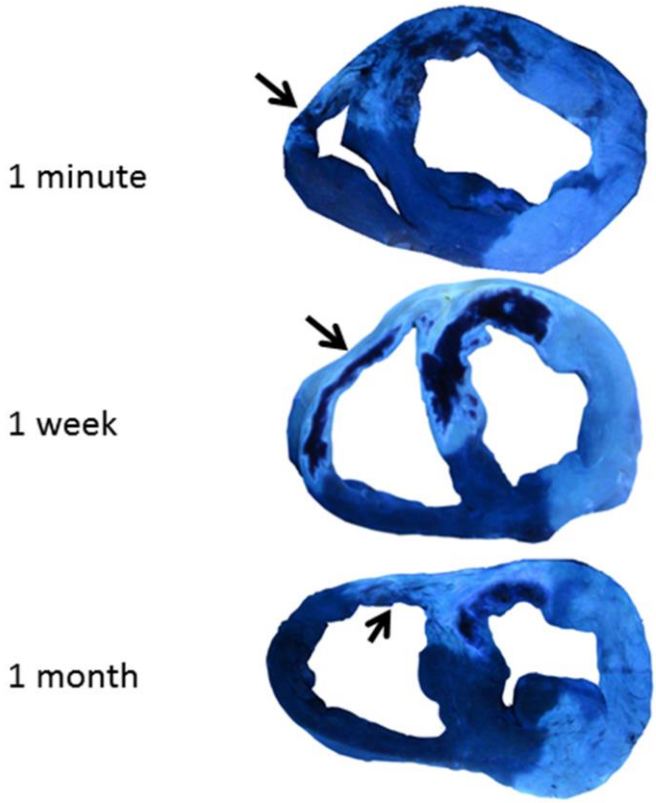
Abbreviations: LV = left ventricle; MVO = microvascular obstruction; RV = right ventricle.

SUPPLEMENTARY FIGURE 1

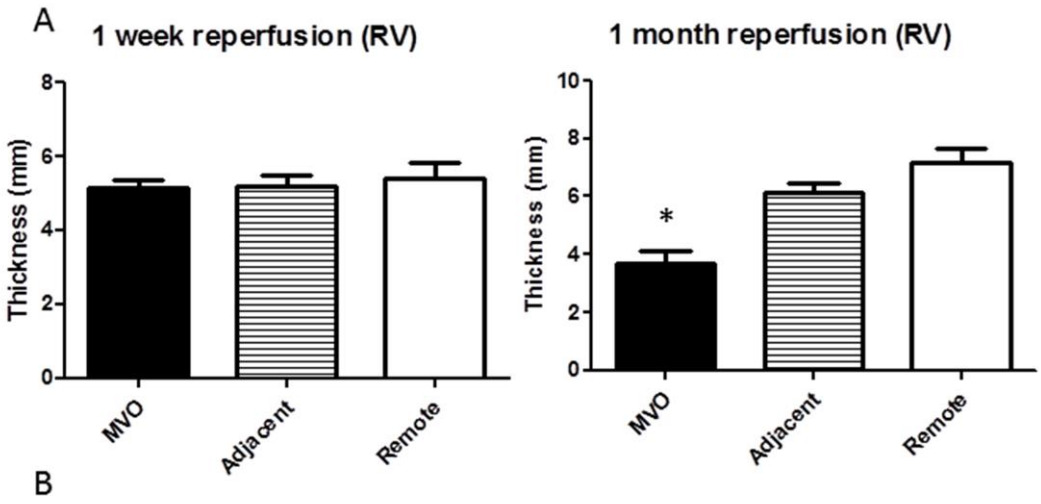
A



B

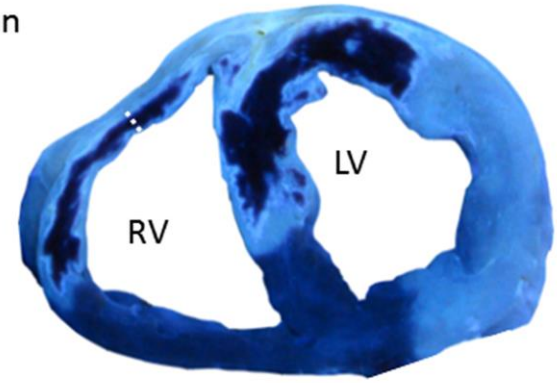


SUPPLEMENTARY FIGURE 2



B

1 week reperfusion



1 month reperfusion