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Microvascular obstruction in the right ventricle in reperfused anterior myocardial infarction. Macroscopic and pathologic evidence in a swine model

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A B S T R A C T

Introduction: Data on right ventricular (RV) involvement in anterior myocardial infarction are scarce. The presence of RV microvascular obstruction (MVO) in this context has not been analyzed yet. The aim of the present study was to characterize the presence of MVO in the RV in a controlled experimental swine model of reperfused anterior myocardial infarction.

Materials and Methods: Left anterior descending (LAD) artery-perfused area (thioflavin-S staining after selective infusion in LAD artery), infarct size (lack of triphenyltetrazolium-chloride staining) and MVO (lack of thioflavin-S staining in the core of the infarcted area) in the RV were studied. A quantitative (% of the ventricular volume) and semiquantitative (number of segments involved) analysis was carried out both in the RV and LV in a 90-min left anterior descending balloon occlusion and 3-day reperfusion model in swine (n = 15).

Results: RV infarction and RV MVO (>1 segment) were detected in 9 (60%) and 6 (40%) cases respectively. Mean LAD-perfused area, infarct size and MVO in the RV were 33.8 ± 13%, 13.53 ± 11.7% and 3.4 ± 4.5%. Haematoxylin and eosin stains and electron microscopy of the RV-MVO areas demonstrated generalized cardiomyocyte necrosis and inflammatory infiltration along with patched hemorrhagic areas. Ex vivo nuclear magnetic resonance (T2* sequences) microimaging of RV-MVO showed, in comparison with remote non-infarcted territories, marked hypointense zones (corresponding to necrosis, inflammation and hemorrhage) in the core of hyperintense regions (corresponding to edema).

Conclusions: In reperfused anterior myocardial infarction, MVO is frequently present in the RV. It is associated with severe histologic repercussion on the RV wall. Nuclear magnetic resonance appears as a promising technique for the noninvasive detection of this phenomenon. Further studies are warranted to evaluate the pathophysiological and clinical implications.

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Introduction

Timely restoration of coronary flow represents the state-of-the-art therapy in the treatment of acute ST-segment elevation myocardial infarction (STEMI) and it has been proven to be effective in terms of systolic recovery and patient outcome [1]. Nevertheless, even after an early and complete reperfusion of the thrombotic occlusion of the epicardial coronary artery, microvascular obstruction (MVO) can occur [2]. This is a multifactorial phenomenon related to, among other factors, embolization, inflammation, hemorrhage and vasospasm. In the case of anterior myocardial infarction, it is present in the left ventricle (LV) in about 50% of patients and it is associated with more severe systolic dysfunction, left ventricular remodeling and cardiac events [2–8].

Classically, RV perfusion has been assumed to be dependent on the right coronary artery, and most studies evaluating the repercussion on the RV in STEMI have focused on LV inferior infarctions. The anterior wall of the RV, however, is supplied by left anterior descending (LAD) coronary artery branches [9]; consequently, acute LAD occlusion can cause RV infarction. We and others have recently demonstrated this finding, and preliminary data suggests its negative prognostic impact [10–17].
Nevertheless, whether MVO occurs in the right ventricle (RV) in the context of reperfused anterior myocardial infarction has not been analyzed so far. This is a relevant issue in order to open new pathophysiological pathways in the understanding of the clinical presentation and outcome of patients with large reperfused anterior STEMI.

Our aim was to characterize the presence of MVO in the RV in a controlled experimental swine model of LAD occlusion-reperfusion analyzing autopsy specimens.

Methods

Experimental Study

In our experimental study, fifteen juvenile domestic female 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 of the swine model can be found elsewhere [17]. In summary, pigs were sedated, anesthetized and mechanically ventilated. A 6-F sheath was introduced into the right femoral artery to measure blood pressure and to access the LAD. An Amplatz Left 0.75 catheter and a standard hydrophilic angioplasty wire were used. Ischemia was induced by inflating a 2.5x10 mm balloon (Abbot Vascular, Santa Clara, CA, USA) at four atmospheres in LAD after the first diagonal branch. Coronary occlusion was confirmed by contrast injection and by electrocardiographic ST-segment elevation. After 90 min, the LAD balloon was deflated and restoration of normal coronary flow was documented by angiography. The animals were allowed to recover. No LAD dissection or sustained coronary closure was detected at reperfusion or at 72 h angiography.

After 72 h, 20 mL of 4% thioflavien-S (T-S) solution was selectively infused into the LAD using a 2.8 F microcatheter (Progreat, Terumo. Clara, CA, USA) at four atmospheres in LAD after the balloon deflation. Restoration of normal coronary flow was documented by angiography. The animals were allowed to recover.

Endpoints

The primary endpoint was to determine and quantify the presence of MVO in the RV in the setting of an experimentally induced anterior STEMI caused by transitory LAD occlusion.

Secondary endpoints were to characterize the pathological features of MVO in the RV with optical and electron microscopy and to carry out a preliminary characterization of ex-vivo MVO in the RV using nuclear magnetic resonance micro-imaging.

LAD-Perfused Area, Infarct Size and Microvascular Obstruction

The LV and RV were sectioned into 5-mm thick short-axis slices. In order to detect T-S stained areas, each slice was viewed from the apical side under ultraviolet light and photographed. Afterwards, slices were incubated in 2.5x3,5-triphenyltetrazolium chloride (TTZ) 2% solution for 20 min at 37 °C. Finally, they were viewed under room light and photographed (Fig. 1a). Images were digitalized and manual definition of endocardial and epicardial borders of all short-axis slices were carried out offline by an experienced independent investigator using the software package MATLAB 6.5 (The Mathworks, Inc., Natick, MA, USA). 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 volumes (Fig. 1b).

MVO was interpreted as the lack of T-S staining in the core of the infarcted area (namely myocardial area that did not stain with either TTZ or with T-S) (Figs. 2 and 3). We considered the presence of MVO if >1 segment was altered, and we quantified it both quantitatively as the percentage of the ventricular volume and semiquantitatively as the number of involved segments. For that purpose, we considered the 17-segment model of the LV and the 9-segment model of the RV [9].

The LAD-perfused area, both in the RV and LV, was defined as the percentage of the myocardial volume showing T-S staining. Infarcted tissue was defined as the myocardial area that failed to stain with TTZ. We considered infarction to be present if more than 1 segment was altered. Infarct extent was quantified as the number of involved segments (following the same model as that for MVO quantification), and as the percentage of myocardial volume.

The adjacent area was defined as the non-infarcted LAD perfused area (both T-S and TTZ staining) and the remote area as the non-LAD perfused myocardium (without T-S staining) (Figs. 2 and 3).

Histological Analysis

Biopsy specimens were taken from the 5-mm thick short-axis slices immediately after the extraction and slicing of the heart and were processed with paraffin. Afterwards, 2 μ-thick myocardial samples from paraffin-embedded RV and LV biopsy specimens were extracted to histologically characterize MVO, infarcted, adjacent and remote areas (Fig. 3).

All histological samples underwent hematoxylin and eosin stain analysis using optical microscopy. For visualization with electron microscopy, both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) samples were fixed in 2.5% glutaraldehyde and then fixed in osmium tetroxide. The presence of hemorrhage, nécrosis, inflammation (defined as mononuclear infiltration), edema and congestion in the sampled areas were semi-quantitatively determined (using a scale from - to ++: 0 = absence, +++ = extensive).

Nuclear Magnetic Resonance Micro-Imaging Analyses

Biopsy specimens were taken from the MVO, infarcted, adjacent and remote areas. After slicing the myocardial tissue in a suitable size (1 cm x 1.5 cm), samples were frozen with liquid nitrogen and stored in a -80 °C freezer until ex-vivo imaging measurement was carried out.

Nuclear magnetic resonance images were acquired at room temperature (25 °C). High spatial resolution images were obtained in a 14-Tesla vertical axis imager (Bruker-AVANCE 600 system, 600 MHz proton frequency, Bruker Biospin GmbH, Rheinstetten, Germany) equipped with a 10 mm micro-imaging 1H coil tuned to the appropriate proton frequency.

Gradient echo images were acquired to obtain T2*-weighted images (repetition time/echo time 1500 ms/15 ms).

Results

LAD occlusion was conducted in sixteen pigs; one of them died during balloon inflation due to refractory ventricular fibrillation. Experiments were successfully finished in the other fifteen cases. Electrical ventricular defibrillation was needed in four of them during LAD occlusion. No significant complications were recorded over the 72 h reperfusion period.

Macroscopic Analysis

LAD-perfused area, infarct size and microvascular obstruction

All specimens showed LAD perfused area in the RV. A large area of the anterior wall of the RV (33; SD 13%) was perfused by LAD. Macroscopic evidence of RV infarction was present in nine cases (60%). Mean infarct size in RV was 13.5 segments (SD 11.7%), and an average of 2.27 segments (SD 1.4) were involved (Fig. 2). MVO was detected in the core of the infarcted areas in six cases (40%) with a mean of 1.4 segments (SD 1) per swine. Expressed as percentage of RV volume, MVO accounted for 3.4 (SD 4.5%).

Regarding LV, it displayed a LAD-perfused area of 69.8 (SD 16.4%) and infarcted tissue was present in all cases, with a mean infarct size
Fig. 1. Title: Macroscopic analyses. 1a) Title: Examples of experiments with and without microvascular obstruction. Caption: Example of 2 of the analyzed swine hearts with anterior myocardial infarction. Left images show thioflavin-S (TS) staining of the LAD perfused area (light blue). Right images show infarcted area as non-stained with 2,3,5-triphenyltetrazolium-chloride (TTZ) myocardium. A: Example of a swine heart with a large LAD perfused area (arrow), RV infarction (lack of TTZ staining – arrow-), and microvascular obstruction (lack of both TS and TTZ staining – asterisk). B: Swine heart with RV infarction (arrow), but with minimal MVO. Abbreviations: a = anterior; LV = left ventricle; p = posterior; RV = right ventricle. 1b) Title: Calculation of volumes. Caption: Slices were photographed, digitalized and measured offline by an independent observer. Manual definition of endocardial and epicardial borders of all short-axis slices permitted the calculation of volume of each determined area. Basal, mid and apical slices of an experiment with extensive right and left ventricular microvascular obstruction (left) and infarct (right) are displayed.
of 23.8 (SD 7.8%), LV infarction and MVO (>1 segment) were detected in all cases. Infarct extent was 5.4 segments (SD 1.6), and the average number of MVO segments was 3.8 (SD 1.5) (8.4; SD 4.2% of LV volume). Table 1 summarizes RV and LV findings.

**Histological Analysis**

Both in RV and LV, optical microscopy in the MVO regions showed cardiomyocyte necrosis, patched areas of hemorrhage, congestion of red blood cells and severe mononuclear infiltration. In the infarcted region, generalized cardiomyocyte necrosis, edema and mononuclear infiltration were detected. Adjacent areas showed slight mononuclear infiltration, edema and contraction bands. Structure was much more preserved in the latter regions. No significant structural alterations were observed in remote areas (Table 2, Fig. 3).

In the TEM and SEM captions, in parallel with optical microscopy, red blood cells and mononuclear cell pluggings were visualized in the MVO regions. In the infarcted region, inflammatory cell infiltration, myofibril necrosis and swollen mitochondria were present. Adjacent areas appeared as a transition territory with both normal cardiomyocytes and necrotic myofibrils. Consistent with the optical microscopy, remote areas were preserved (Fig. 3).

Table 2 summarizes the results of the semiquantitative analysis in optical microscopy of the pathological findings in each area studied regarding hemorrhage, necrosis, inflammation, edema and congestion.

**Nuclear Magnetic Resonance Micro-Imaging Analyses**

Fig. 4 illustrates typical findings in Nuclear magnetic resonance micro-imaging of the infarcted and remote areas in the RV.

In the infarcted area, hyperintense regions mainly corresponded to the presence of severe edema in histological samples. Dark zones in the core of the infarcted area correlated with the presence of severe necrosis, massive inflammatory infiltration and patched hemorrhage. Regions with intermediate intensity corresponded to inflammatory infiltration and necrosis. Remote areas showed bright intensity, and in optical microscopy corresponded to normal myocardium.

**Discussion**

The main finding of the present study is that MVO occurs in the RV in an experimental swine model of reperfused anterior myocardial infarction.

**RV Necrosis in Anterior Infarctions**

Traditionally, RV necrosis has been thought to occur exclusively in inferior LV infarctions. The association between anterior LV infarction and RV involvement has been previously described in anatomopathologic studies, but evidence is scarce. Using scintigraphy, Tobinick et al. [14] did not detect RV systolic dysfunction in 24 anterior infarction patients. Marmor et al. [15] studied RV and LV systolic function with radionuclide ventriculography in 22 patients with anterior infarction and reported a persistent impairment of LV function, but only transient systolic dysfunction of the RV. Jensen et al. recently showed, however, that RV infarction is frequent in anterior STEMI (65% vs. 47% in inferior STEMI) and commonly not extensive [16]. We have previously reported that RV...
infarction is present in 50% of the cases in a swine experimental model of anterior myocardial infarction and in 40% of patients who underwent cardiac magnetic resonance after spontaneous anterior myocardial infarction [17].

Our results strengthen current evidence of RV involvement in anterior myocardial infarction. The present study shows in a highly controlled experimental model of anterior LV myocardial infarction that an important part of the RV perfusion depends on the LAD artery (33 ± 13%), although LAD occlusion results in small infarct size (13.5 ± 11.7%). The low metabolic requirements of the RV, its ability to increase oxygen extraction along with the presence of collateral flow might be possible mechanisms involved in the relative infarct protection observed in the RV [18–20].

MVO in the RV in Anterior Infarctions

In STEMI, despite successful restoration of epicardial blood flow, perfusion at the microvascular level may remain impaired [1,2]. This is a multifactorial phenomenon referred to as MVO, which occurs, among other factors, as a consequence of embolization, inflammation, vasospasm and oxidative stress. Several studies have shown that the

Table 1
Characteristics of the study group.

<table>
<thead>
<tr>
<th>Number of swine</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right ventricle</td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
</tr>
<tr>
<td>Ventricular mass (g/m²)</td>
<td>6.2 SD 1.9</td>
</tr>
<tr>
<td>LAD perfused area (% of ventricular volume)</td>
<td>33.9 SD 13.0</td>
</tr>
<tr>
<td>Infarct presence (n,%)</td>
<td>9/15 (60%)</td>
</tr>
<tr>
<td>Infarct size (% of ventricular volume)</td>
<td>13.5 SD 11.7</td>
</tr>
<tr>
<td>Infarct size (number of segments)</td>
<td>2.3 SD 1.4</td>
</tr>
<tr>
<td>MVO presence (n,%)</td>
<td>6/15 (40%)</td>
</tr>
<tr>
<td>MVO (number of segments)</td>
<td>1.4 SD 1.1</td>
</tr>
<tr>
<td>MVO (% of ventricular volume)</td>
<td>3.4 SD 4.5</td>
</tr>
</tbody>
</table>

Abbreviations: LAD: Left anterior descending artery; MVO: microvascular obstruction.

Table 2
Histological findings.

<table>
<thead>
<tr>
<th></th>
<th>MVO</th>
<th>Infarct</th>
<th>Adjacent</th>
<th>Remote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>RV</td>
<td>LV</td>
<td>RV</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Necrosis</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Inflammation</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Edema</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Congestion</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: LV: left ventricle; RV: right ventricle; MVO: microvascular obstruction.
The presence of MVO is associated with worse outcome and left ventricular remodeling compared to non-MVO infarctions [2-8]. We have previously demonstrated the importance of MVO in terms of systolic recovery and patient outcome. In a group of 304 patients, the presence of MVO early (1 week) after STEMI predicted a higher probability of depressed ejection fraction 6 months later [7]. Moreover, in a series of 206 patients followed up for a median of 4 years we have recently reported that the presence of MVO is strongly associated with a higher rate of adverse cardiac events (33% vs. 12%) [21]. Timely detection of MVO is desirable since it potentially allows for early risk stratification, and it opens new pathophysiological and therapeutic pathways for a better management of patients.

As far as we know, this is the first report focused on analyzing MVO in the RV in the context of anterior LV infarction. This finding was detected in a small but significant region of the RV (1.4 ± 1 segments).

As the macroscopic and histologic samples clearly showed, it associates with severe damage of the RV structure, including massive necrosis and inflammatory infiltration. Further studies are needed to investigate the clinical implications of this phenomenon.

There is no ideal animal model that both perfectly imitates human myocardial infarction and is technically feasible in the laboratory [22,23]. The animal model used in the present study reproduces two of the contributing mechanisms of MVO in STEMI: the epicardial artery occlusion and the reperfusion injury. Other factors involved in the pathophysiology of MVO, such as atherosclerosis, plaque rupture and microembolization did not participate in our swine model [24]. However, in humans, the main factor underlying the severe myocardial damage and MVO that takes place in STEMI is a sudden occlusion of the epicardial artery in absence of collateral circulation. Thus, with certain limitations, the swine model applied in the present could be useful to...
analyze under highly controlled conditions the pathophysiology of STEMI in patients.

**Diagnostic, Prognostic and Therapeutic Implications**

In terms of diagnosis, an accurate characterization of the RV using imaging techniques is a challenge. Cardiovascular magnetic resonance has become the gold standard technique for an accurate evaluation of the structural consequences of STEMI. We have previously reported the usefulness of this technique for detecting RV infarction [17]. However, as a result of insufficient spatial resolution, the high mobility and the small RV wall thickness, detection of MVO in the anterior RV wall is, to the present date, a challenge. For this reason we undertook a preliminary study to assess the nuclear magnetic resonance micro-imaging characteristics of ex-vivo samples selectively sliced from the core of RV MVO regions. For this purpose, we used T2* sequences which have been demonstrated to be highly useful for the identification of edema and hemorrhage. In summary, in this controlled scenario we observed that in the infarcted area, hyperintense regions corresponded mainly to the presence of severe edema whereas dark zones correlated to the presence of severe necrosis and massive inflammatory infiltration. Moreover, as recent data suggest, patched hemorrhage areas were also present in regions with these characteristics. These preliminary data illustrate the potential of nuclear magnetic resonance for characterizing MVO in the RV, but undoubtedly, further improvements are needed in order to transfer these results to clinical practice.

Regarding the prognostic implications, Miszalski-Jamka T, et al., in a cohort of ninety-nine patients who underwent an early cardiac magnetic resonance after STEMI treated with primary angioplasty, showed that the extent of RV infarction and RV dysfunction were independent prognostic predictors, and the evaluation of the RV may be particularly useful for the risk stratification in patients with depressed LV function after STEMI [10]. Taken into consideration that both, RV dysfunction and MVO have emerged as potent prognostic factors in the case of LV anterior STEMI, it could be speculated that the presence of MVO in the RV might bring about deleterious consequences.

Finally, it could be speculated that several clinical signs (such as hypotension or right heart failure) or events (such as ventricular arrhythmias) in the case of extensive anterior LV infarctions may, at least in part, depend on RV MVO and necrosis. Therefore, data reported in the present study could be useful in the future to explore new therapeutic opportunities in the management of STEMI patients. Further studies will be needed, however, to investigate the clinical implications of the findings reported in the present study.

**Limitations**

Extrapolation of our experimental model of STEMI to humans has to be viewed with caution. The severe RV myocardial damage and MVO detected in the swine could be due not only to prolonged ischemia but also to reperfusion injury and the variety of factors associated with this complex process. Further studies will be needed to clarify the exact mechanisms underlying the structural damage detected in the RV as well as its clinical and pathophysiological implications.

**Conclusions**

In reperfused anterior myocardial infarction, MVO is frequently present in the RV. It is associated with severe histologic reperfusion on the RV wall. Nuclear magnetic resonance appears as a promising technique for the noninvasive detection of this phenomenon. Further studies are warranted to evaluate the pathophysiological and clinical implications.

**Conflict of Interest Statement**

No conflicts of interest exist in the study.

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