Rev Esp Cardiol. 2015;68(11):928–934

Original article

Intracoronary Infusion of Thioflavin-S to Study Microvascular Obstruction in a Model of Myocardial Infarction

Arantxa Hervas,[a] Elena de Dios,[a] Maria J. Forteza,[a] Gema Miñana,[a] Julio Nuñez,[b] Amparo Ruiz-Sauri,[c] Clara Bonanad,[b] Nerea Perez-Sole,[a] Francisco J. Chorro,[b] and Vicente Bodi[b,*]

[a] Instituto de Investigación Sanitaria, INCLIVA, Valencia, Spain
[b] Servicio de Cardiología, Hospital Clínico Universitario, Universitat de València, INCLIVA, Valencia, Spain
[c] Universitat de València, INCLIVA, Valencia, Spain

Article history:
Received 23 January 2015
Accepted 21 April 2015
Available online 5 August 2015

Keywords:
Myocardial infarction
Microcirculation
Swine model

ABSTRACT

Introduction and objectives: Microvascular obstruction exerts deleterious effects after myocardial infarction. To elucidate the role of ischemia-reperfusion injury on the occurrence and dynamics of microvascular obstruction, we performed a preliminary methodological study to accurately define this process in an in vivo model.

Methods: Myocardial infarction was induced in swine by means of 90-min of occlusion of the mid left anterior descending coronary artery using angioplasty balloons. Intracoronary infusion of thioflavin-S was applied and compared with traditional intra-aortic or intraventricular instillation. The left anterior descending coronary artery perfused area and microvascular obstruction were quantified in groups with no reperfusion (thioflavin-S administered through the lumen of an inflated over-the-wire balloon) and with 1-min, 1-week, and 1-month reperfusion (thioflavin-S administered from the intracoronary catheter after balloon deflation).

Results: In comparison with intra-aortic and intraventricular administration, intracoronary infusion of thioflavin-S permitted a much clearer assessment of the left anterior descending coronary artery perfused area and of microvascular obstruction. Ischemia-reperfusion injury exerted a decisive role on the occurrence and dynamics of microvascular obstruction. The no-reperfusion group displayed completely preserved perfusion. With the same duration of coronary occlusion, microvascular obstruction 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%; $P < .001$).

Conclusions: We present proof-of-concept evidence on the crucial role of ischemia-reperfusion injury on the occurrence and dynamics of microvascular obstruction. The described porcine model using intracoronary injection of thioflavin-S permits accurate characterization of microvascular obstruction after myocardial infarction.

Infusión intracoronaria de tioflavina-S para el estudio de la obstrucción microvascular en un modelo de infarto de miocardio

Palabras clave:
Infarto de miocardio
Microcirculación
Modelo porcino

RESUMEN

Introducción y objetivos: La obstrucción microvascular produce efectos nocivos después del infarto de miocardio. Con objeto de esclarecer el papel de la lesión por isquemia-reperfusión en la aparición y la dinámica de la obstrucción microvascular, se llevó a cabo un estudio metodológico preliminar para definir con exactitud este proceso en un modelo *in vivo*.

Métodos: Se indujo un infarto de miocardio en cerdos mediante una oclusión de 90 min en la parte media de la arteria coronaria descendente anterior izquierda empleando balones de angioplastia. Se aplicó una infusión intracoronaria de tioflavina-S y se comparó con la instilación tradicional intraaórtica o intraventricular. Se cuantificó el área perfundida por la arteria coronaria descendente anterior izquierda y la obstrucción microvascular en los grupos sin reperfusión (administración de tioflavina-S a través de la luz de un balón hinchado montado sobre la guía) y con reperfusión de 1 min, 1 semana y 1 mes (administración de tioflavina-S mediante el catéter intracoronario después de deshinchar el balón).

Resultados: En comparación con la administración intraaórtica e intraventricular, la infusión intracoronaria de tioflavina-S permitió una evaluación mucho más clara del área perfundida por la arteria coronaria descendente anterior izquierda y de la obstrucción microvascular. La lesión por

SEE RELATED ARTICLE:
http://dx.doi.org/10.1016/j.rec.2015.06.022, Rev Esp Cardiol. 2015;68:919–20.

* Corresponding author: Servicio de Cardiología, Hospital Clínico Universitario, Universitat de València, INCLIVA, Blasco Ibáñez 17, 46010 Valencia, Spain.
E-mail address: vicente.bodi@uv.es (V. Bodi).

http://dx.doi.org/10.1016/j.rec.2015.04.016
1885-5857/

isquemia-reperfusión tuvo un papel decisivo en la aparición y la dinámica de la obstrucción microvascular. El grupo sin reperfusión presentó una perfusión completamente preservada. Con la misma duración de la oclusión coronaria, la obstrucción microvascular se detectó ya en el grupo de reperfusión de 1 min (14 ± 7%), alcanzó un máximo en el grupo de reperfusión de 1 semana (21 ± 7%) y se redujo significativamente en el grupo de reperfusión de 1 mes (4 ± 3%; p < 0,001).

Conclusiones: Se presenta una prueba de concepto del papel crucial que desempeña la lesión por isquemia-reperfusión en la aparición y la dinámica de la obstrucción microvascular. El modelo de cerdo descrito, que emplea inyección intracoronaria de tioflavina-S, permite una caracterización exacta de la obstrucción microvascular después del infarto de miocardio.

Abbreviations

AMI: acute myocardial infarction
LAD: left anterior descending coronary artery
LV: left ventricle
MVO: microvascular obstruction
RV: right ventricle
T-S: thioflavin-S

INTRODUCTION

Timely and complete restoration of infarct vessel patency is the main goal in patients with acute myocardial infarction (AMI).[1] Nevertheless, this approach does not ensure adequate reperfusion at the microvascular level, and 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 AMI.[3–5]

Ischemia-reperfusion injury has been extensively discussed in AMI[6,7] and it could exert deleterious effects on microvascular integrity.[2,3] Nevertheless, there is no definitive evidence demonstrating a 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. Such models would permit a better understanding of the pathophysiology and timing of this process and, in turn, the exploration of new therapeutic opportunities under controlled conditions.

In the present study, we aimed 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. Up to now, 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 (LV)[9] or intravenously.[10] To effectively study MVO, we performed a preliminary methodological study, which consisted of investigating the best route to administer thioflavin-S (T-S) to accurately define MVO.

METHODS

Experimental Study

Thirty-one juvenile domestic pigs weighing 25 kg to 30 kg were used. The study protocol was approved by the local animal care and use committee and conforms to the current Spanish regulations (Royal Decree 53/2013, of February 1) and European Directive 2010/63/EC.

Further details on our study protocol can be consulted elsewhere.[11,12] In summary, pigs were pretreated with intravenous amiodarone (300 mg) and lidocaine (30 mg) to reduce life-threatening arrhythmias. A 7 Fr sheath was introduced into the right femoral artery to monitor blood pressure and to access the left anterior descending coronary artery (LAD). A 7 Fr 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 mm 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% T-S solution was selectively infused into the proximal LAD through the Amplatz Left 0.75 catheter 1 min 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, 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 angioplasty balloon 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 to compare the transcatheter intraventricular and intra-aortic instillation with the methodology used in the present study (intracoronary infusion of T-S). The protocol described above was used to induce AMI in 6 pigs. Afterward, the angioplasty balloon was withdrawn and the pigs were allowed to recover. One week after infarction, the Amplatz Left 0.75 catheter was placed in the LV (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 intraventricular vs intracoronary infusion of T-S for assessing the LAD-perfused area and MVO was compared (Figure 1).