



Ischemic postconditioning fails to reduce infarct size in pig models of intermediate and prolonged ischemia

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ABSTRACT

Introduction and objectives: Ischemic postconditioning (iPost, coronary intermittent re-occlusion maneuvers immediately after PCI-mediated reperfusion) has been proposed to limit infarct size (IS). However, a few experimental and clinical contradictory results have been reported. We hypothesized that iPost cardioprotection is affected by the duration of ischemia. Our objective was to assess IS in the presence/absence of iPost in a pig model of myocardial infarction of variable ischemia duration.

Methods: Large white pigs (n = 38) underwent angioplasty balloon-induced coronary ischemia followed by reperfusion. Two sets of experiments were carried out: intermediate (30 min) and prolonged (40 min) ischemia. In both, pigs were allocated on a 1:1 ratio to receive iPost (4 cycles of "1 min balloon inflation followed by 1 min deflation" upon reperfusion) or control. Animals underwent contrast-enhanced multiparametric cardiac magnetic resonance scan on day 7. Primary outcome measure was cardiac magnetic resonance-based IS (% of left ventricular mass). The interaction between treatment allocation and ischemia duration was assessed using a 2-way ANOVA test.

Results: iPost was not associated with smaller IS in any of the ischemia duration protocols (intermediate ischemia: 0.3% [0.0–3.9] vs 0.9% [0.0–2.6] in iPost and control, respectively; $P = .378$; long ischemia: 31.1% [27.3–32.8] vs 27.3% [25.1–27.5]; $P = .248$). When both ischemia-duration protocols were combined, iPost was not associated with smaller IS (3.9% [0.0–30.9] vs 4.6% [0.2–25.1]; $P = .672$). T1 relaxation times were longer in animals undergoing iPost compared to controls (1306.2 ms [1190.7–1492.7] vs 1240.7 ms [1167.1–1304.5]; $P = .024$).

Conclusions: In a pig model of reperfused myocardial infarction of variable ischemia duration, iPost failed to reduce IS. T1 relaxation times were longer in animals undergoing iPost indicative of the potential harm involved in this procedure.

Keywords: Reperfusion injury. Ischemic postconditioning. Cardioprotection. Myocardial infarction.

El poscondicionamiento local no reduce el tamaño de infarto en modelos porcinos de infarto agudo de miocardio de intermedia y larga duración

RESUMEN

Introducción y objetivos: Existen resultados contradictorios sobre la eficacia del poscondicionamiento isquémico local (iPost) como intervención para reducir el tamaño del infarto (TI). Pretendemos evaluar si el efecto del iPost se ve alterado por el tiempo de isquemia en un modelo porcino de infarto de miocardio.

Métodos: Se sometió a 38 cerdos Large-White a isquemia-reperusión coronaria con balón de angioplastia. Se realizaron dos series de experimentos: isquemia de intermedia (30 minutos) y larga (40 minutos) duración. Los animales se asignaron 1:1 a iPost (4 ciclos de 1 minuto inflado/1 minuto desinflado, comenzando 1 minuto tras la reperusión) o control. Se realizó una resonancia magnética

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7 días después. El objetivo primario fue el TI medido por resonancia magnética (porcentaje de masa ventricular izquierda [%VI] con realce tardío de gadolinio). La interacción de la asignación al tratamiento y la duración de la isquemia se evaluó mediante un análisis de varianza de dos vías.

Resultados: El iPost no se asoció con un menor TI en ninguno de los grupos de duración de la isquemia (duración intermedia: 0,3% [0,0-3,9] frente a 0,9% [0,0-2,6] en iPost y control, respectivamente, $p = 0,378$; larga duración: 31,1% [27,3-32,8] frente a 27,3% [25,1-27,5] en iPost y control, respectivamente; $p = 0,248$). Al analizar conjuntamente todos los animales, el iPost tampoco se asoció con un TI más pequeño (3,9% [0,0-30,9] frente a 4,6% [0,2-25,1] en iPost y control, respectivamente, $p = 0,672$). Los tiempos de relajación en T1 fueron más largos en los animales sometidos a iPost (1.306,2 [1.190,7-1.492,7] ms frente a 1.240,7 [1.167,1-1.304,5] ms; $p = 0,024$).

Conclusiones: El iPost no logró reducir el TI en ninguna duración de la isquemia. Los tiempos de relajación T1 fueron más largos en los animales sometidos a iPost, lo que sugiere un daño potencial de esta intervención.

Palabras clave: Daño por reperfusión. Condicionamiento isquémico. Cardioprotección. Infarto de miocardio.

Abbreviations

HF: heart failure; **iPost:** ischemic post-conditioning; **IRI:** ischemia - reperfusion injury; **IS:** infarct size; **LGE:** late gadolinium enhancement; **MI:** myocardial infarction.

INTRODUCTION

ST-segment elevation myocardial infarction (STEMI) is a life-threatening condition that affects more than 7 million people worldwide each year.¹ Despite the improved short-term survival and reduced need for repeat revascularization achieved with primary percutaneous coronary intervention (PCI), long-term survival, and the rate of heart failure (HF) have barely improved over the last few years.²

Infarct size (IS), the extent of irreversible injury after MI, is a main contributor to long-term mortality and HF in STEMI survivors.³⁻⁵ Therefore, there is a strong need for identifying interventional (invasive) and/or pharmacological strategies that can limit the extent of MI. Upon coronary occlusion, there is a time-dependent progression of irreversible injury.⁶ Therefore, timely restoration of blood flow (reperfusion) in the ischemic region is of paramount importance to reduce IS and improve the left ventricular ejection fraction (LVEF).⁷ However, reperfusion *per se* causes additional damage to the myocardium and microcirculation that contributes to the final IS,⁸ the so-called ischemia/reperfusion injury (IRI).⁹

Ischemic postconditioning (iPost) is an interventional cardiology procedure that tested extensively in experimental¹⁰ and clinical trials.¹¹⁻¹⁴ iPost is based on the idea that after index ischemia, gentle reperfusion results in less damage than abrupt straight reperfusion.¹⁴ This procedure has the great advantage of its easy implementation during primary percutaneous intervention (PCI). It consists of intermittent 1 min brief episodes of coronary flow reocclusion (ie, angioplasty balloon reinflation).

There is controversy though with some studies showing strong cardioprotection through iPost,¹¹ while others disagree.¹² One potential explanation for these controversial results is that iPost is only protective in cases where previous ischemic time has not been very prolonged.

To address whether iPost has variable cardioprotective effects depending on ischemic time, we performed a controlled experimental study in which pigs undergoing different times of coronary ischemia are allocated to iPost or control. We used state-of-the-art

technology (ie, cardiac magnetic resonance [CMR]) to accurately assess the effect of the procedure on cardioprotection.

METHODS

Studies were approved by Institutional and Regional Animal Research Committees.

Study design

The cardioprotection provided by iPost was tested in 2 different sets of experiment groups: intermediate ischemia duration (30 min) followed by reperfusion, and prolonged ischemia (40 min) followed by reperfusion. All animals underwent a multiparametric CMR scan 1 week after MI. This timing regarding the assessment of the effect of the intervention was chosen since it is the recommended one by international expert consensus.⁵ Selecting between the intermediate or prolonged ischemia time was based on our previous experiments where we saw that ischemia durations of < 30 min lead to very small IS (< 20% of the area at risk [AAR]) yielding no window for cardioprotection of established pharmacological interventions (eg, metoprolol).¹⁵ Similarly, in our experimental setting, ischemia durations > 40 min result in very large IS (> 80% of the AAR) thus reducing the window for cardioprotection.¹⁵

Following the 3Rs principle to reduce the use of animals, those on prolonged ischemia (40 min) with/without iPost (as well as the CMR data) were the ones already used in a former article with a different objective.¹⁶ In this study, the group of animals undergoing intermediate ischemia duration (30 min) with/without iPost ($n = 28$) were used *ad hoc*.

Apart from ischemia duration, the study protocol was equal for both ischemia-duration groups. For these experiments, we employed 3-month-old castrated male large white pigs weighing 30 to 40 Kg. Pigs were allocated to iPost or control before MI was induced. Myocardial AAR was assessed through multidetector computed tomography immediately after vessel occlusion following a previously published methodology.¹⁶ Seven days after the MI, all

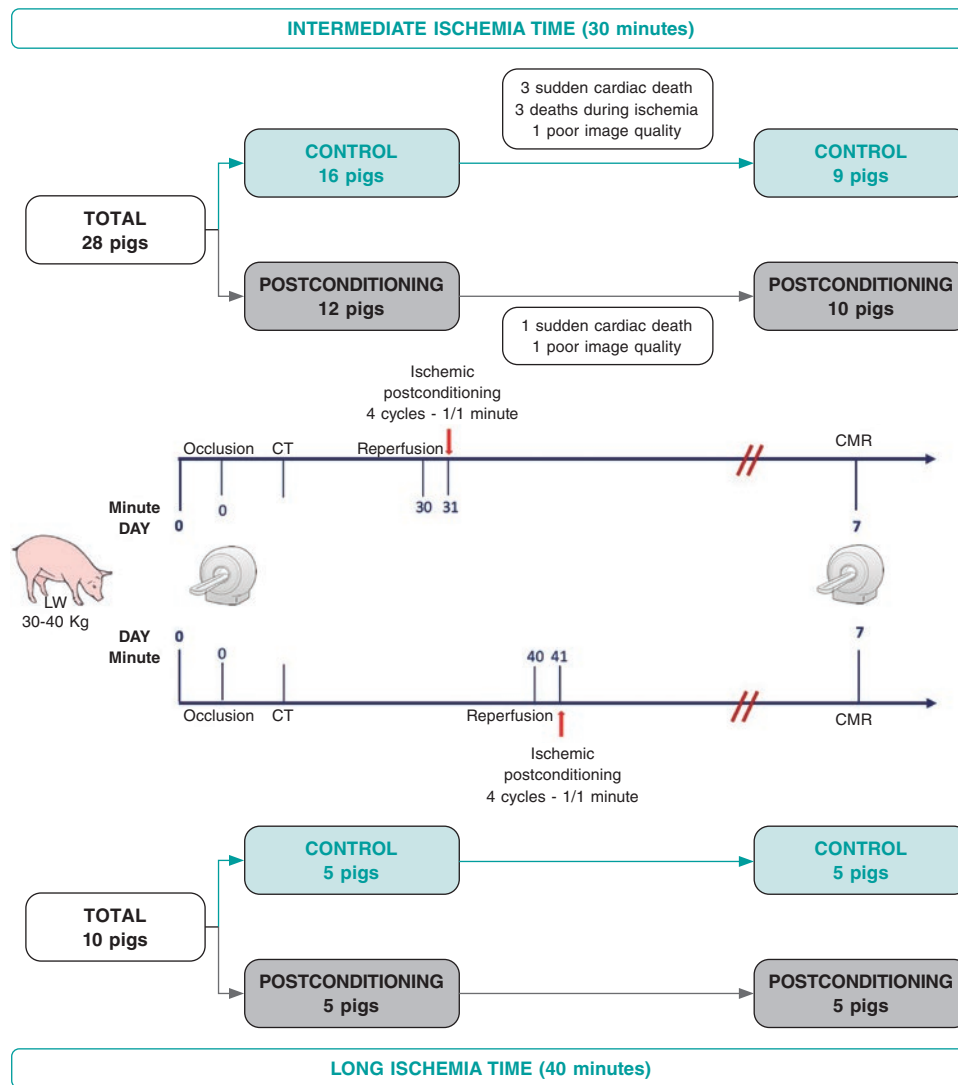


Figure 1. Central illustration. Study design and flowchart. CMR, cardiac magnetic resonance; CT, computed tomography.

animals underwent a multiparametric CMR scan to assess IS (primary outcome measure), LVEF, and T2 and T1 relaxation times both in the AAR and in the remote area (figure 1).

Anesthesia and animal care protocol

Every test or experiment was performed under deep sedation. Sedation was induced through the intramuscular injection of ketamine (20 mg/Kg), xylazine (2 mg/Kg), and midazolam (0.5 mg/Kg); and maintained through the continuous infusion of ketamine (2 mg/Kg/h), xylazine (0.2 mg/Kg/h), and midazolam (0.2 mg/Kg/h). Buprenorphine (0.03 mg/Kg) was administered immediately before the MI experiment.

Animals were intubated and received mechanical ventilatory support with volume-control synchronized intermittent mandatory ventilation (fraction of inspired oxygen = 28%).

To avoid coronary thrombosis following balloon-induced MI induction, animals received 150 mg of clopidogrel orally on the day of the procedure and 75 mg 24 and 48 hours later. All animals were euthanized immediately after the day-7 CMR scan.

Myocardial infarction and ischemic postconditioning protocol

All animals underwent the same closed-chest ischemia-reperfusion protocol¹⁶ consisting of 30-min or 40-minute left anterior descending coronary artery occlusion with a monorail angioplasty balloon inserted percutaneously through the femoral artery. Balloon was inflated at 8 atm immediately distal to the first diagonal branch. Both the location of the balloon and the status of inflation were monitored on the angiography. A single intra-arterial bolus of 300 IU/kg of unfractionated heparin was administered right before coronary occlusion. Furthermore, to reduce the rate of fatal ventricular arrhythmias, continuous infusion of amiodarone (300 mg/h, no bolus) was initiated immediately after coronary occlusion and maintained until catheters were removed.

Pigs were allocated on a 1:1 ratio to iPost or control before MI induction. After index ischemia duration (30 min or 40 min according to the protocol), animals allocated to control underwent straight chronic reperfusion (balloon deflation) while animals allocated to iPost underwent balloon deflation but 1 min after balloon was reinflated for 1 min. iPost was induced by repeating the 1 min inflation-1 min deflation cycle 4 times. Artery patency was assessed after every inflation/deflation cycle.

Arterial enhanced multidetector computed tomography protocol and analysis

Multidetector computed tomography (MDCT) arterial phase studies were performed during ongoing ischemia on a 64-slice computed tomography scanner (Brilliance CT 64; Philips Healthcare, Cleveland, OH, United States) after the IV administration of iodinated contrast media. Since the MDCT scan was performed during ongoing ischemia (ie, while the balloon was inflated), non-enhanced regions accurately represent the ischemic region (ie, the AAR). MDCT images were analyzed using dedicated software (Extended MR Workspace 2.6; Philips Healthcare, The Netherlands) by 2 observers who remained blind to group allocation. Short axes orientation was obtained from volumetric computed tomography images through multiplanar reconstruction. AAR and remote areas were visually identified based on contrast enhancement differences, manually delineated, and expressed as a percentage of left ventricular (LV) area.^{15,16}

Cardiac magnetic resonance protocol

CMR scans were performed 7 days after the MI on a Philips 3-Tesla Achieva Tx whole-body scanner (Philips Healthcare, The Netherlands) equipped with a 32-element phased-array cardiac coil. The imaging protocol included a standard segmented cine imaging with a steady-state free-precession sequence to provide high-quality anatomic references and assessment of the left ventricular mass, wall thickness, and LVEF, a T1-mapping sequence (modified Look-Locker inversion recovery) to assess T1 native relaxation time, a T2 mapping based on gradient-spin-echo imaging to provide precise myocardial T2 relaxation time,¹⁷ and a T1-weighted inversion relaxation turbo field echo sequence acquired 10 min to 15 min after the administration of gadolinium contrast (late gadolinium enhancement, LGE) to assess IS. CMR images were analyzed using dedicated software (MR Extended Workspace 2.6; QMassMR 7.6; Medis, The Netherlands and IntelliSpace Portal, Philips Healthcare, The Netherlands) by 2 experienced observers in CMR analysis and blinded to group allocation.

Statistical analysis

Normal distribution of data was assessed using the Shapiro-Wilk test. Quantitative variables were expressed as median (interquartile range). Categorical variables were expressed as numbers and percentage and rounded to the nearest integer. A 2-way ANOVA test was run on the overall sample with CMR performed on day 7 (29 pigs) to examine the effect of ischemia time and iPost on primary and secondary outcomes (IS, % LV mass), IS indexed to the AAR, LVEF, T2 relaxation time, and native T1 relaxation time). Regarding variables where we found a significant interaction between the duration of ischemia and iPost, we performed a post hoc analysis (Tukey's method) to confirm the differences seen. We estimated the sample size based on our previous experiments on cardioprotection with metoprolol.¹⁵ Lost animals were replaced to achieve the required sample size.

RESULTS

Study groups

Intermediate-ischemia protocol (30 min)

As shown on [figure 1](#), 28 animals underwent MI induction after replacing the lost subjects. From the 16 pigs allocated to control, 3

(19%) died during ischemia induction, and 3 (19%) suddenly died before the day-7 CMR scan. One (10%) out of the 10 animals that completed the day-7 CMR protocol was excluded from the analysis due to poor image quality.

Zero out of the 12 pigs allocated to iPost died during MI induction (0%) while 1 (8%) suddenly died before the CMR scan. One (9%) out of the 11 animals that completed the day-7 CMR was excluded from the analysis due to poor image quality.

Therefore, the final population available for outcome assessment was 19 (9 controls, and 10 iPost, [figure 1](#)).

Prolonged ischemia protocol (40 min)

As shown on [figure 1](#), 10 animals that completed the protocol in a previously published study were included.

Baseline characteristics

Both control and iPost groups were similar in body weight and baseline CMR-based characteristics except for the indexed left ventricular mass that was larger in the control group of animals with 40 min ischemia times ([table 1](#)). A non-significant trend towards larger MDCT-based AAR (% left ventricle mass) was observed in the iPost group ([table 1](#)).

Cardiac magnetic resonance results

Effect of iPost in a model of intermediate ischemia protocol

In the intermediate-duration ischemia group, iPost did not have any effects on any of the CMR-based variables ([table 2](#); [figure 2](#), [figure 3](#) and [figure 4](#)). Both iPost and control animals present small IS with no differences being reported between the intervention groups (0.3% of LV mass [0.0 – 3.9] vs 0.9% [0.0 – 2.6] %LV in iPost and control, respectively, $P = .378$). We did not find any differences regarding the indexed IS (IS/AAR) either ([table 2](#)).

Effect of iPost on a model of prolonged ischemia protocol

The results of this experiment have already been published.¹⁶ In conclusion, iPost did not show any cardioprotective effects in terms of IS reduction (31.1% of LV mass [27.3–32.8] LV vs 27.3% [25.1– 27.5] in iPost and control respectively; $P = .248$). Regarding the previous group, we did not find any differences in indexed IS (IS/AAR). Differences in other CMR-based parameters were not observed except for a significantly longer AAR T1-relaxation time in the iPost group (1590.3 ms [1441.6 – 1591.4] vs 1309.7 ms [1248.1–1310.8] in iPost and control, respectively; $P = .002$) ([table 3](#) and [figure 2](#), [figure 3](#) and [figure 4](#)).

Interaction between duration of ischemia and iPost benefits

We did not find a significant interaction between duration of ischemia time and the effect of iPost, CMR-based IS (3.9% of LV mass [0.0–30.9] vs 4.6% [0.2 – 25.1]) in iPost and control, respectively, $F [1.25] = 0.18$; $P = .672$). Therefore, iPost was not associated with smaller IS regardless of ischemia duration ([table 4](#) and [figure 2](#)). We did not find any differences in indexed IS (IS/AAR), LVEF, LV end-diastolic or end-systolic volumes either ([table 4](#), and [figure 3](#)).

Table 1. Baseline characteristics

Variable	Overall (N = 29)		Intermediate ischemia (30 min) (N = 19)		Prolonged ischemia (40 min) (N = 10)	
	Control (N = 14)	iPost (N = 15)	Control (N = 9)	iPost (N = 10)	Control (N = 5)	iPost (N = 5)
Weight, kg	36.2 (34.0-38.5)	33.5 (31.0-40.0)	36.0 (34.5-38.5)	37.5 (31.0-41.0)	36.5 (30.5-38)	32.5 (32.5-33.5)
LVEF, %	57.6 (55.2-63.0)	55.9 (52.3-59.5)	57.7 (55.9-61.3)	55.8 (52.3-65.4)	57.4 (55.2-63.0)	56.0 (55.8-59.3)
iLVEDV, mL/m ²	104.2 (93.5-105.8)	105.9 (96.1-123.6)	95.8 (87.5-105.3)	97.7 (94.5-105.9)	112.8 (105.8-123.5)	127.4 (123.6-128.4)
iLVESV, mL/m ²	43.0 (37.0-46.8)	45.6 (38.0-54.5)	40.7 (35.8-46.6)	42.8 (37.3-45.7)	46.1 (44.3-48.8)	54.4 (51.7-61.4)
Area at risk (% LV)	27.8 (26.2-27.2)	31.7 (29.2-32.3)	24.6 (23.3-27.6)	27.3 (26.0-29.2)	27.8 (26.2-27.8)	31.7 (29.2-32.3)

iLVEDV, indexed left ventricular end-diastolic volume; iLVESV, indexed left ventricular end-systolic volume; iPost, ischemic postconditioning; LV, left ventricle; LVEF, left ventricular ejection fraction.

Table 2. CMR-based parameters for intermediate ischemia group

Variable	Intermediate ischemia duration (N = 19)		
	Control (N = 9)	iPost (N = 10)	P (post hoc analysis)
IS, %LV mass	0.9 (0.0-2.6)	0.3 (0.0-3.9)	.378
Indexed IS, IS/AAR (%)	3.8 (0.0-8.5)	0.9 (0.0-15.1)	.474
LVEF, %	54.0 (50.2-55.9)	52.9 (47.1-56.0)	.521
iLVEDV, mL/m ²	98.7 (92.3-104.1)	107.3 (90.0-118.6)	.438
iLVESV, mL/m ²	45.9 (40.4-52.4)	48.5 (41.0-55.2)	.355
T2 relaxation time AAR, ms	51.3 (49.3-54.8)	57.0 (53.2-58.5)	.583
T2 relaxation time REM, ms	45.6 (42.2-46.1)	44.7 (43.7-47.4)	.881
Native T1 relaxation time AAR, ms	1179.2 (1167.1-1266.4)	1225.9 (1170.2-1306.2)	.584
Native T1 relaxation time REM, ms	1087.8 (1075.1-1109.7)	1078.4 (1051.2-1134.8)	.925

AAR, area at risk; iLVEDV, indexed left ventricular end-diastolic volume; iLVESV, indexed left ventricular end-systolic volume; iPost, ischemic postconditioning; IS, infarct size; LV, left ventricle; LVEF, left ventricular ejection fraction; REM, remote area.

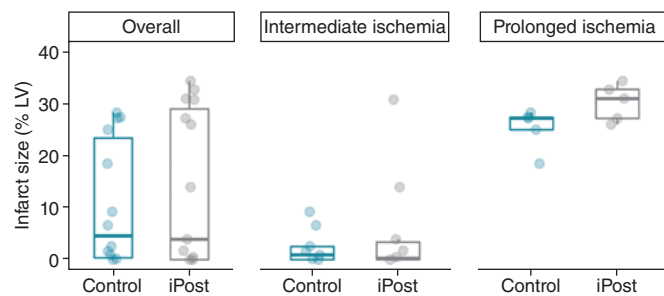


Figure 2. Differences in cardiac magnetic resonance-based infarct size (% of left ventricular mass) between control and iPost groups, in the overall population and based on ischemia duration.

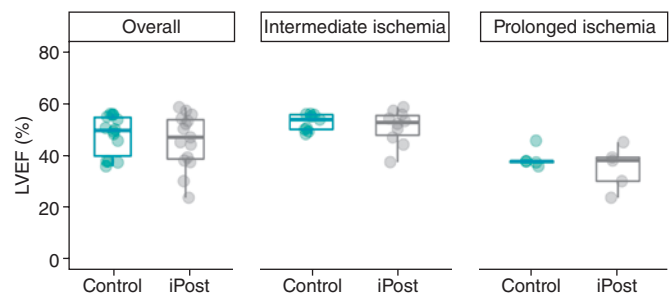


Figure 3. Differences in cardiac magnetic resonance-based LVEF (%) between control and iPost groups in the overall population and based on ischemia duration.

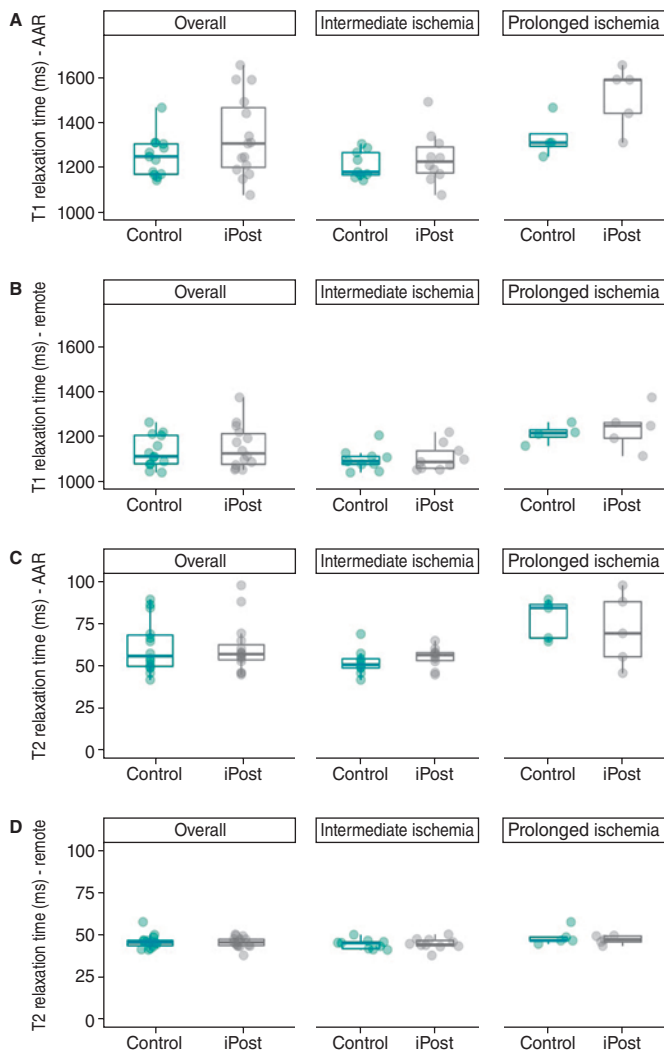


Figure 4. Differences in cardiac magnetic resonance native T1 and T2 relaxation times (ms) between control and iPost groups, in the overall population and based on ischemia duration. **A:** native T1 relaxation time at the area at risk. **B:** native T1 relaxation time at remote area. **C:** native T2 relaxation time at the area at risk. **D:** native T2 relaxation time at remote area. AAR, area at risk.

Conversely, animals treated with iPost presented longer native-T1 relaxation times in the AAR (1306.2ms [1190.7–1492.7] vs 1240.7ms [1167.1–1304.5] in iPost and control, respectively ($F [1.25] = 5.79$, $P = .024$) without any differences being reported in the remote area or in T2 relaxation time (figure 4).

DISCUSSION

In this study, we tested the potential cardioprotective effect of iPost in a large animal model of reperfused MI with intermediate (30 min) and prolonged (40 min) ischemia times.¹⁵ In our pig model of ischemia/reperfusion, iPost failed to reduce IS in any of the ischemia duration protocols as seen on the state-of-the-art CMR 7days after MI. A non-significant sign of damage (trend towards larger IS and lower LVEF, as well as significantly longer T1 relaxation times in the ischemic region) associated with iPost was observed in animals in the prolonged ischemia protocol. Our data do not support iPost as an intervention capable of improving outcomes in the IRI setting.

iPost is a very attractive intervention to reduce IRI since it can be applied in the cath lab at the time of reperfusion.² Technically, it is a straightforward intervention that does not require any additional material to that already used during primary PCI.

Local ischemic preconditioning (repetitive cycles of brief coronary artery occlusion/blood flow restoration before prolonged ischemia) has consistently shown to be a very strong cardioprotective intervention¹⁸ to reduce IRI. In most (if not all) experimental settings this strategy is consistently associated with a massive reduction of IS. However, local ischemic preconditioning is not feasible to be applied at the centers where patients already have initiated coronary artery occlusion. To overcome this limitation, Vinten-Johansen's group tested whether the same ischemic conditioning maneuver started right after reperfusion (iPost) could also be associated with smaller IS.¹⁰ This group reported, in the dog model of IRI (60 min ischemia followed by blood flow restoration), that 3 cycles of "30 sec re-occlusion/30 sec reperfusion" applied 1 min after reperfusion were associated with a significant reduction of IS.¹⁰ Due to its easiness of implementation, iPost was translated very fast to a pilot clinical trial. Ovize's group reported that in a small group of patients with STEMI, iPost (in this case 4 cycles of 1 min occlusion/1 min reperfusion) started right after PCI-mediated reperfusion was associated with smaller IS.¹¹ In another small trial of 79 patients with STEMI, Freixa et al. reported that iPost not only did not reduce IS, but was associated with significantly less myocardial salvage.¹⁹ Two larger clinical trials, POST²⁰ (N = 700), and DANAMI-3-iPOST¹² (N = 1234) failed to prove the benefits of iPost.

There are some potential explanations for the divergent results. It has been speculated that there can be an interaction between the cardioprotection provided by iPost and the duration of preceding ischemia.²¹ However, this has not been tested in an *ad hoc* designed study. With this in mind, we conducted this study, where we did not find any cardioprotection provided by iPost regardless of the ischemia duration.

Although it seems that 30 min and 40 min of ischemia duration are not very different, we have previous reported in the pig model that occlusion times < 30 min are associated with a very small IS while occlusions > 40 min are associated with transmural infarction.^{15,22,23} Overall, in our study, 4 cycles of iPost (1 minute occlusion/1 minute reperfusion) did not have any cardioprotective effects regarding IS reduction neither expressed as % of LV mass nor as % of AAR (table 2). This was the case in both ischemia duration protocols. Although no formal interaction between ischemia duration and iPost effects on IS was found some findings suggest a possible deleterious effect of iPost in the longer ischemia duration protocol (trend towards a higher IS and a lower LVEF and, especially a significantly longer T1 relaxation time in the AAR). In addition, even in the absence of any significant differences in the intermediate ischemia group, when visualizing individual data (figure 2), asymmetry towards larger IS is seen in the iPost group including the 2 subjects with the highest IS of the entire 30 minute occlusion cohort. Furthermore, differences in secondary CMR-based outcomes suggest a potential deleterious effect of iPost in our ischemia-reperfusion model: pigs in the iPost group had significantly longer native T1 relaxation times, a surrogate marker of increased interstitial fibrosis.⁵ In addition, we found a non-significant trend towards poorer LVEF in animals undergoing iPost both in the intermediate and prolonged ischemia groups. Nevertheless, this finding can be due to the non-significant trend towards larger AAR in the iPost group (table 1).

One possible explanation for these results is the delayed start of the iPost protocol since animal experiments have shown that the

Table 3. CMR-based parameters for the prolonged ischemia group

Variable	Long ischemia duration (N = 10)		
	Control (N = 5)	iPost (N = 5)	P (post-hoc analysis)
IS, %LV mass	27.3 (25.1-27.5)	31.1 (27.3-32.8)	.248
Indexed IS, IS/AAR (%)	98.2 (70.7-98.8)	96.2 (93.4-100)	.640
LVEF, %	37.9 (37.4-38.0)	38.2 (30.2-39.4)	.323
iLVEDV, mL/m ²	150.8 (150.4-153.1)	150.9 (148.3-159.8)	.644
iLVESV, mL/m ²	95.1 (94.1-96.6)	105.4 (88.8-109.8)	.234
T2 relaxation time AAR, ms	85.0 (67.0-87.0)	69.8 (55.9-88.7)	.342
T2 relaxation time REM, ms	47.0 (47.0-49.0)	47.6 (46.2-49.6)	.420
Native T1 relaxation time AAR, ms	1309.7 (1248.1-1310.8)	1590.3 (1441.6-1591.4)	.002
Native T1 relaxation time REM, ms	1209.7 (1156.8-1217.3)	1246.8 (1191.2-1260.9)	.101

AAR, area at risk; iLVEDV, indexed left ventricular end-diastolic volume; iLVESV, indexed left ventricular end-systolic volume; iPost, ischemic postconditioning; IS, infarct size; LV, left ventricle; LVEF, left ventricular ejection fraction; REM, remote area.

Table 4. CMR-based parameters for the overall population including intermediate and prolonged ischemia times

Variable	Overall population (N = 29)		
	Control (N = 14)	iPost (N = 15)	P (2-way ANOVA)
IS, %LV mass	4.6 (0.2-25.1)	3.9 (0.0-30.9)	.672
Indexed IS, IS/AAR (%)	15.7 (0.7-70.7)	15.1 (0.0-96.2)	.965
LVEF, %	49.8 (37.9-55.1)	47.1 (38.2-54.4)	.738
iLVEDV, mL/m ²	104.9 (98.1-150.4)	118.6 (90.3-148.3)	.751
iLVESV, mL/m ²	53.5 (45.4-94.1)	55.2 (43.8-88.8)	.628
T2 relaxation time AAR, ms	56.4 (49.9-69.4)	57.5 (53.2-65.4)	.531
T2 relaxation time REM, ms	45.9 (43.6-47.0)	46.0 (43.8-47.8)	.892
Native T1 relaxation time AAR, ms	1240.7 (1167.1-1304.5)	1306.2 (1190.7-1492.7)	.024
Native T1 relaxation time REM, ms	1107.5 (1075.1-1203.9)	1111.4 (1057.8-1218.2)	.164

AAR, area at risk; iLVEDV, indexed left ventricular end-diastolic volume; iLVESV, indexed left ventricular end-systolic volume; iPost, ischemic postconditioning; IS, infarct size; LV, left ventricle; LVEF, left ventricular ejection fraction; REM, remote area.

cardioprotective effect of iPost is restricted to the first minute after reperfusion with no effect observed if the maneuver delays for another 60 seconds.^{24,25} In fact, in clinical trials in which iPost proved to be effective, the inflation/deflation protocol was started immediately after reperfusion.¹¹

Study limitations

This study has some limitations that must be acknowledged. Despite being one of the most translatable, the present pig model has some differences with human IRI: tolerance to ischemia is species-dependent, and duration of ischemia in pigs is not equivalent to humans; similarly, the time-dependent progression of irreversible injury is much faster in pigs compared to humans as seen by the transmural progression of infarction between 30 min and 40 min of ischemia. Another limitation of the study is that animal allocation to iPost or control was not entirely random, but rather based on an alternative assignment; however, the person responsible for the CMR analysis

validation was blind to the subjects' allocation group. In addition, as previously presented, data on the prolonged ischemia group correspond to experiments previously conducted at our center by a different operator and published elsewhere. The use of animals of different breeds, different anesthesia protocols, different material or any other environmental factors could explain, at least partially, the great differences seen on IS between the intermediate and the prolonged ischemia groups. Nevertheless, the decision to use these previously reported data was based on the principle of reducing animal use in animal research.^{26,27}

CONCLUSIONS

In a pig model of ischemia/reperfusion, iPost (4 cycles of 1 min balloon inflation/deflation) initiated immediately after reperfusion ineffectively reduced IS. The lack of benefits was consistent across different ischemia duration protocols, which ruled out an interaction between duration of coronary occlusion and iPost benefits.

Overall, we observed a sign of harm due to iPost (significantly longer T1 relaxation times) mainly driven by an effect in the prolonged ischemia protocol.

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AUTHORS' CONTRIBUTION

J. Nucho was responsible for conducting experiments and CMR analysis; also, for data analysis and manuscript drafting. C. Galán-Arriola was responsible for the blind CMR analysis and offered support for statistical analysis and figure design. R. Fernández-Jiménez was responsible for the experiments and analysis of the prolonged ischemia group. M. I. Higuero Verdejo, R. Vazirani, M. Anguita-Gámez, and A. Lanaspá gave support in the experimental setting. G. J. López Martín was responsible for CT and CMR acquisition. J. Sánchez-González designed the CMR protocol. B. Ibáñez was responsible for the project design, results supervision, and final approval of the manuscript draft.

CONFLICTS OF INTEREST

J. Sánchez-González is an employee of Philips Healthcare. The authors have no conflicts of interest to declare. All co-authors have seen and agreed on the contents of the manuscript and there is no financial interest to report.

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WHAT IS KNOWN ABOUT THE TOPIC?

- There is a strong need to identify interventional or pharmacological interventions that can reduce infarct size (IS), a main contributor to long-term heart failure in STEMI survivors.
- There is controversy surrounding the cardioprotection associated with local iPost in experimental models and clinical trials.
- A possible explanation to this controversy is the potential interaction between the duration of ischemia and the benefits of iPost.

WHAT DOES THIS STUDY ADD?

- It shows that iPost is not associated with IS reduction.
- The lack of benefits is consistent across different ischemia duration protocols, which rules out a possible interaction between duration of coronary occlusion and iPost benefits.
- iPost can be associated with damage especially when applied after long ischemia duration.

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