Scientific letter

Cardioprotective effect of the short-acting beta-blocker esmolol in experimental ischemia/reperfusion

Efecto cardioprotector del bloqueador beta de acción ultracorta esmolol en isquemia/reperfusión experimental

To the Editor,

Intravenous administration of metoprolol during ongoing ischemia has been demonstrated to be associated with smaller infarct size (IS) in animal models[1,2] and in the METOCARD-CNIC clinical trial.[3] Clinical practice guidelines recommend (IIa-A) the use of intravenous beta-blockers at the time of presentation in patients with ST-segment elevation myocardial infarction (STEMI) who are hemodynamically stable.[4] However, in some cases, physicians do not prescribe iv beta-blockers to STEMI patients because of the fear that they can develop acute heart failure. There are beta-blockers with a very short half-life that have the theoretical advantage that, upon stopping infusion, their negative inotropic and chronotropic effects disappear within minutes. Given that not all beta-blockers are able to ameliorate ischemia/reperfusion injury,[2] there is a need to test the cardioprotective abilities of short-acting beta-blockers in a controlled experimental setting.

Esmolol is a highly $beta_1$-selective, ultra-short-acting beta-blocker with rapid onset of action (60 seconds) that reaches steady state in 6 minutes after bolus administration. These pharmacological properties make esmolol a great candidate for use in the setting of STEMI when hemodynamic instability is a potential concern. In this study, we aimed to evaluate the infarct-limiting capacity of esmolol infusion during ongoing ischemia in a pig model of anterior STEMI. The benefits of esmolol were evaluated by state-of-the-art cardiac magnetic resonance (CMR) performed at 2 different timepoints: 7 and 45 days after reperfusion. The primary endpoint of the study was day 7 indexed IS: extent of delayed gadolinium enhancement, normalized to area at risk. Area at risk (AAR) was quantified by cardiac computed tomography (CT) performed during index left anterior descending (LAD) occlusion following a previously reported methodology.[5] The main secondary endpoints were indexed IS at 45-day and left ventricular ejection fraction (LVEF) on both CMR exams.

The experimental protocol was approved by the local and Community of Madrid animal welfare committee. Experiments were performed according to current legislation. Before the actual ischemia/reperfusion experiments, a dose-response study with 5 animals was conducted to determine the most appropriate esmolol infusion rate: that achieving a 10% reduction of heart rate without sustained hemodynamic instability (250 μg/kg/min).

Fifteen male large-white pigs underwent 40 minutes of left anterior descending (LAD) coronary occlusion followed by reperfusion. Pigs were randomized to receive either esmolol (n = 8) or control (vehicle infusion, n = 7), which were initiated (without bolus) 20 minutes after LAD artery occlusion, and maintained for a total of 60 minutes (ie, it was stopped 40 minutes after reperfusion). Since the main objective of the study was to test the infarct-limiting properties of esmolol, the infusion rate was not modified during the protocol, even in those pigs developing hemodynamic instability.

Three animals (2 esmolol and 1 control) died during STEMI induction. The 2 esmolol pigs died secondary to severe hemodynamic instability caused by esmolol infusion, while the control died because of refractory ventricular fibrillation. Three additional pigs (2 esmolol and 1 control) died between day 7 and day 45 CMR.

CT-measured AAR did not differ between groups (36.4% ± 6.1% vs 33.7 ± 3.6% of left ventricular (LV) in esmolol and control, respectively; $P = .385$), figure 1A. IS was significantly smaller in the esmolol group both in the 7-day (64.4 ± 11.8% vs 84.1 ± 9.4% of AAR; $P = .01$) and in the 45-day CMR (52.9 ± 9.1% vs 71.5 ± 12.7%; $P = .04$), figure 1B. Animals in the esmolol group showed a nonsignificant trend toward higher LVEF both at 7- and 45-day CMR (day 7: 39.74.1% vs 34.0 ± 6.2%; $P = .091$; day 45: 43.4 ± 6.6% vs 35.3 ± 11.8%; $P = .264$), figure 1C. There were no differences in microvascular obstruction or in edema extension.

In this experimental setting, continuous esmolol infusion initiated during ongoing ischemia was associated with smaller IS and with a trend toward improved LVEF. The numerically higher number of deaths due to hemodynamic instability during the index STEMI can be explained by the experimental protocol aimed at addressing the infarct-limiting properties of esmolol. We speculate that, in a clinical setting, these adverse effects would not occur because, upon signs of acute heart failure, infusion can be reduced or event stopped. Whether this tailored esmolol infusion will reduce IS is unknown, but highly plausible. The main limitation of this study is the loss of 50% of the animals in the esmolol group and 29% in the control group. This experimental study complements previous clinical studies suggesting that esmolol can protect the heart during STEMI.[6] Given that not all beta-blockers exert infarct-limiting effects,[2] the effects seen with esmolol cannot be ascribed to other short-acting beta-blockers.

In conclusion, this study shows that esmolol infusion is able to reduce indexed IS in an experimental acute myocardial infarction model and shows a trend toward LVEF improvement. However, there are safety concerns regarding hemodynamic instability that require further evaluation.

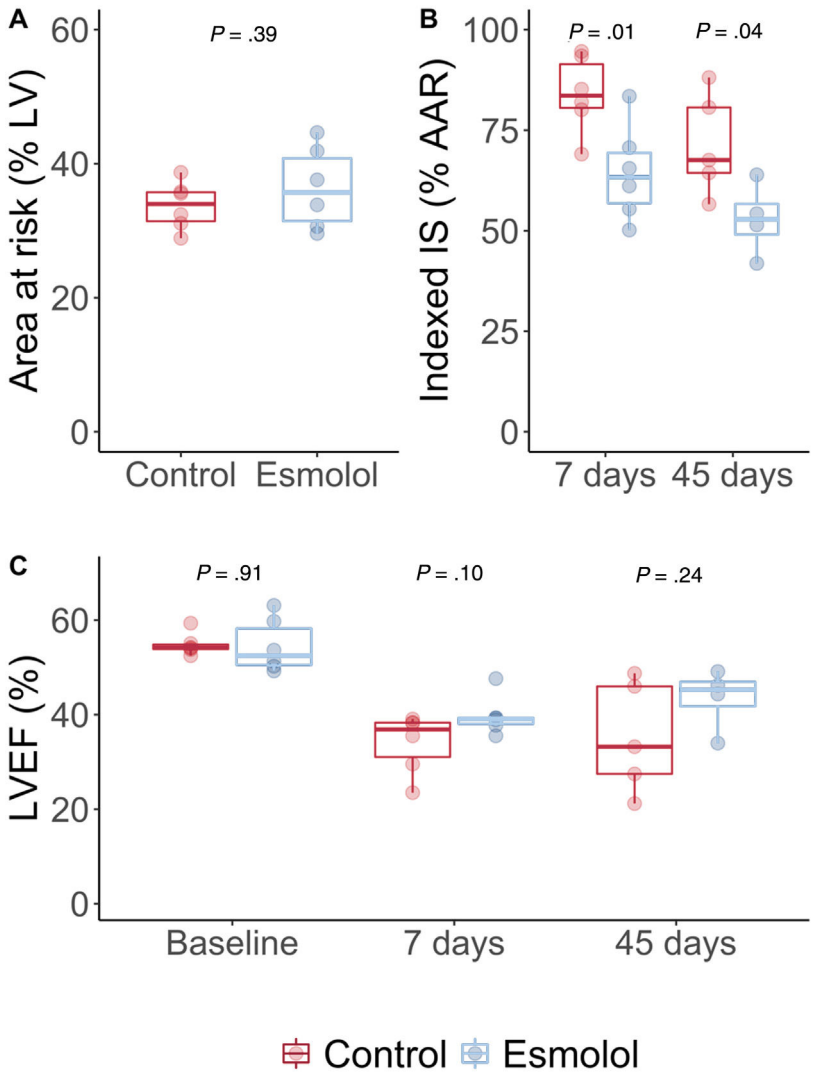

Figure 1. A: area at risk (% of LV mass) on computed tomography exam during index coronary occlusion. B: indexed infarct size (% of AAR with delayed gadolinium enhancement) on 7- and 45-day CMR. C: left ventricular (LV) ejection fraction (LVEF) at different timepoints. Boxplots represent median and interquartile range. Circles represent individual data. AAR, area at risk.

FUNDING

This study was funded by a grant from the Spanish Society of Cardiology *Investigación Traslacional 2017*. J. Nuche is a recipient of a predoctoral grant *Jordi Soler Soler* from the CIBERCV. The CNIC is supported by the *Instituto de Salud Carlos III* (ISCIII), the *Ministerio de Ciencia e Innovación* and the Pro CNIC Foundation, and is a Severo Ochoa Center of Excellence.

AUTHORS' CONTRIBUTIONS

J. Nuche: design of the protocol, performance of experiments, data analysis, and drafting of the manuscript. S. Huertas: performance of experiments. C. Galán-Arriola: performance of experiments. Analized data. P. López-Ayala: performance of experiments. Critical revision of the manuscript. M. Lobo: performance of experiments. B. Ibáñez: conceptual design of the study. Handling of funding. Critical revision of the manuscript.

CONFLICTS OF INTEREST

None.

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https://doi.org/10.1016/j.rec.2021.11.009
1885-5857/

New KCNQ1 c.604 + 1G >C variant associated with Jervell-Lange Nielsen syndrome in homozygosity and compound heterozygosity

Nueva variante KCNQ1 c.604 + 1G >C asociada con síndrome de Jervell-Lange Nielsen en homocigosis y heterocigosis compuesta

To the Editor,

Congenital long QT syndrome (LQTS) is a hereditary disease characterized by abnormal cardiac repolarization (QT interval prolongation) and susceptibility to sudden cardiac death due to *torsade de pointes*. Its most severe form, Jervell and Lange-Nielsen syndrome (JLNS), is associated with sensorineural deafness and is caused by homozygous or compound heterozygous mutations in 2 possible genes (*KCNQ1* and *KCNE1*). Its prevalence is very low, affecting 1 to 4 million children worldwide (less than 1% of patients with LQTS), and limited numbers of cases have been reported in Spain.[1] We present 2 families with JLNS linked to the same previously undescribed variant (KCNQ1 c.604 + 1G>C) found in homozygosity in 1 case and in compound heterozygosity in the other, together with a molecular analysis of the changes caused by this variant.

Family 1: the index case (III.9, figure 1A) is a girl with JLNS diagnosed at birth (QTc > 550 ms) who was asymptomatic with propranolol treatment and whose first manifestation was sudden cardiac death at 12 years old while swimming. She was a homozygous carrier of the KCNQ1 c.604 + 1G>C variant, as well as heterozygous for the KCNH2 c.38C>A and AKAP9 c.7010A>G variants, none previously described. Her 5-year-old brother (III.10) is a carrier of the same variants, also has JLNS (QTc > 500 ms), and is asymptomatic with nadolol and prophylactic defibrillator implantation at 7 years old. The family has a history of consanguinity in distant ancestors, as well as of sudden cardiac death. A family study was conducted according to current recommendations.[2] All family members underwent cardiac stress testing to assess their QT interval response to exercise. Their phenotype was considered normal if the baseline QTc interval was < 450 ms in male relatives or < 460 ms in female relatives and the exercise response was normal. Several family members are heterozygous carriers of the variants and exhibit incomplete penetrance and variable expressivity; none had severe manifestations.

Family 2: the index case (III.2, figure 1B) is a newborn who was examined due to severe bradycardia; he had a QTc > 500 ms and sensorineural deafness (JLNS). A next-generation sequencing study revealed the heterozygous variant KCNQ1 c.604 + 1G>C, which is associated with the *KCNQ1* c.1513_1514delCA variant, previously identified to be pathogenic (ClinVar, ID52985). He was asymptomatic with nadolol treatment. The parents and other relatives are heterozygous carriers of the variant, with variable phenotypic expression concerning the QTc interval, and remain asymptomatic.

The intronic variant *KCNQ1* c.604 + 1G>C is not included in any public database and is of uncertain significance. To determine its effect on the protein (Kv7.1 channel), a molecular study was performed, with the approval of the hospital ethics committee. The patients gave informed consent for the performance and publication of the work. Given its location in the first base of intron 3 to 4, we suspected that the mutation would affect mRNA splicing. For this reason, using RNA extracted from peripheral blood mononuclear cells, RT-PCR was performed to amplify the sequence around the variant. In homozygosity (proband, III.10), the amplified band was smaller than that from a healthy person, whereas heterozygous patients (II.5, II.7, and II.8) showed 2 bands (figure 2A). Sequence analysis of the bands indicated that the c.604 + 1G>C variant caused a loss of the splicing donor site at the start of intron 3 to 4. Consequently, the cell machinery appeared to use a cryptic splicing site located in exon 3, resulting in the loss of a section, as can be seen in figure 2B and in line with the descriptions of intronic mutations in other genes.[3] This led to the generation of a truncated and nonfunctional protein (p.Y184Pfs*82; with 264 amino acids vs the 676 in the wildtype channel) (figure 2C). Accordingly, homozygous patients do not generate the I_{KS} current and show a more severe phenotype (JLNS). In the case of family 2, the c.1513_1514delCA variant introduces a change in the reading frame and also produces a truncated Kv7.1 channel (p.Q505Afs*10; figure 2C) with 513 amino acids. This channel lacks a large part of the carboxy terminus, including 3 amphipathic helices (B, C, and D), which would alter the tetramerization of the channel subunits and their trafficking to the plasma membrane.[4] Consequently, the index patient also lacked functional Kv7.1 channels and could not generate I_{KS} current. In contrast to this severe condition, it is common to find family members who are heterozygous for *KCNQ1* and have a much more benign phenotype. The variants associated with JLNS (typically frameshift or truncation) do not usually exert a dominant-negative effect under heterozygous conditions, as was seen in both families. The additional effects of the other variants of uncertain significance on the phenotype of family 1 is the subject of another investigation, although it is suspected that an additive polygenic effect explains the observed phenotypic heterogeneity.

Our opportunity to study 2 families with JLNS caused by a new variant expressed in 2 different forms (homozygous and compound heterozygous) has enhanced our understanding of the spectrum of presentations typically detected in families with JLNS, from asymptomatic carriers to individuals with different degrees