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EDITORIAL

A tale of pigs, beta-blockers and genetic variants

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The extent of irreversible loss after an acute myocardial infarction (MI) is still a major determinant of long-term outcomes. Therefore, strategies to limit MI size are needed [1]. Despite many decades of intense experimental research, besides early reperfusion, there are no interventions unequivocally associated with MI size reduction.

In most disciplines, new therapeutic targets are identified in experimental settings, ideally by performing in vivo experiments closely resembling the given human condition [19]. For the case of reperfused MI, the experimental setting mimicking the human scenario is in theory simple: generate a temporary complete occlusion of a coronary artery in an experimental animal, and measure the extent of irreversible loss (i.e., MI size) with any of the well validated techniques (ex vivo or in vivo). Large animals (pigs, dogs, etc.) are better suited as a translational trampoline since their anatomy and physiology is closer to humans than that of murine species [17, 24]. Despite the use of fine experimental protocols in large animals, validation of positive results in clinical trials is disappointingly low. The research community has intensively thought of the reasons for this poor translation reaching some valid conclusions [3, 10]. One of the most frequently claimed reasons for the poor translation is that patients presenting with MI are usually middle-age or old, opposed to most experimental animals used, which are generally juvenile. Another relevant claim is that patients usually have co-morbidities and take medications, something not happening in animals used for experiments [7]. Finally, animals used for research are most of the times inbred strains. The latter is extremely different from humans, where inbred is anecdotic. The selected animal strain used for an experiment might have a gene variant making them more prone or resistant to any condition or intervention. A paradigmatic example of the impact of the selected strain on cardiovascular traits and responses to interventions is the C57BL/6 mouse strain. The C57BL/6J substrain has a mutation in the nicotinamide nucleotide transhydrogenase (Nnt) gene that affects protein expression in the mitochondria and in consequence many different metabolic traits and responses to mitochondria-targeted interventions [23]. Such is the impact of this gene mutation that C57BL6 substrains need to be chosen according to the purpose of the study because phenotypic differences are known to affect the experimental results.

In the present issue of the journal, Gerd Heusch's team reports the results of experiments performed in a pig model of MI (ischemia/reperfusion, I/R) testing the cardioprotective effects of metoprolol [18]. Their main interest was to use a pharmacological intervention with consistent cardioprotective results in another laboratory (ours) in several different articles to eventually identify a drug that could be used as a positive control in the search for novel cardioprotective interventions. All the previous studies with positive results in our laboratory were performed in the pig model of I/R by different operators [4, 8, 9, 14, 22]. Surprisingly, the results reported in the editorialized article [18] do not show a consistent MI size reduction with metoprolol. While MI size and microvascular obstruction were numerically smaller in metoprolol, they did not reach statistical significance and thus should be considered as neutral [18]. Although they used a power analysis-based prospective study design, one possible argument would be that the study was still underpowered and an adaptive design (i.e., calculate final sample size based on a first group of animals and use observed effect size as the expected result) could have demonstrated that MI sizes were significantly different. Performing a very simple calculation based on the results of the present study (MI size

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in placebo 46 ± 8%, and in metoprolol 42 ± 8) [18] with an alpha 0.05 and power of 80%), shows that a sample size of 126 pigs (63 in each arm) would be needed. Of course, this is out of any judicious mind and thus the argument of an underpowered study does not stand. The second argument to explain differences is that the experimental settings were different: open chest in the present study vs closed chest in the others, different anesthesia protocols, different reperfusion times (few hours here vs several days in the other studies), and different techniques to measure MI size (pathology here vs. magnetic resonance imaging in the previous studies) among others. The authors have explained in detail these differences and it is not useful to repeat them here, I refer readers to the discussion section of the editorialized article [18]. From these, I will only mention the duration of reperfusion as an important one for the specific case of metoprolol, since we have observed that this drug abrogated neutrophil-mediated exacerbated inflammation [4, 6, 8] and this mechanism of injury persists during the first 24 h [13, 16]. The effect of metoprolol on abrogating exacerbated inflammation has been demonstrated in other conditions, such as COVID-related respiratory distress syndrome [5] or ischemic stroke [6]. Generally speaking, the experience of a laboratory in performing such complex studies could have an impact on the lack of reproducibility, but we are talking about studies performed in 2 of the laboratories with greatest expertise in pig MI models worldwide and the proficiency and unbiased execution of the studies is out of discussion.

Then, what are we left with to explain these striking differences? All point to the strain of pigs and their potential variable response to insults and interventions. Here, I will present a brief historical perspective of our experience with metoprolol in I/R in the pig model since it might help in understanding these apparent discrepancies. Our first study in the saga (in a lab in USA) was performed in Yorskshire pigs under volatile anesthesia and testing metoprolol 7.5 mg injected half way ischemia duration [14]. At that time we chose metoprolol since it was the beta-blocker available in our laboratory. Serendipity played a role here since if atenolol had been the beta-blocker available, we would have never demonstrated any benefits since today we know they have a different cardioprotective effect [4, 11]. When I launched my own group in Madrid, the first experiments performed by my team to set up protocols were to replicate the described metoprolol results [14]. These new experiments were performed in a different pig strain (Large White) and injecting the same metoprolol 7.5 mg dose. To our surprise, we did not observe strong cardioprotection. We scrutinized all possible reasons, including differences between Yorkshire and Large White pigs (which appear to come from a common ancestor and have separated more than 200 years ago). To rule out that different strains had differential responses to metoprolol, we did a simple dose response study with heart rate as the primary outcome measure in 5 pig strains: Yorkshire, Large White, Landrace, Landrace x Large White, and Pietrain. To our surprise, the effect of metoprolol was almost negligible in the 3 latter strains with different doses. It was maximal in Yorkshire, and in Large White it was only significant at a higher dose. We chose to perform our experiments in the Large White strain and based on these dose response studies, increased the dose of metoprolol to 0.75 mg/kg (3 times higher than the 7.5 mg dose used in the past). With this metoprolol dose, we have consistently demonstrated a highly consistent MI size reduction [9, 22]. It is noteworthy that Heusch and colleagues have found an almost negligible effect of metoprolol on heart rate, since they discuss "*somewhat surprisingly, metoprolol in our present study reduced heart rate only slightly as compared to the placebo group at some time points*…." [18]. This mild effect on heart rate is consistent with our dose response study in other pig strains.

It is very interesting to note that the density of beta adrenergic receptors in several tissues from different pig strains is variable [2]. Notably, the quality of pig meat is affected by the administration of beta-blockers differentially in different strains [25], and the natural selection of pigs used for food might have been unbiasedly done based on the adrenergic responses.

There is a very important piece of information in Heusch's paper that clearly suggests that the Göttingen pigs they used in this study had a very different response to metoprolol administration than the Large White strain we use. This relates to the antiarrhythmic effect of metoprolol. In Heusch's paper, metoprolol administration did not reduce at all the incidence of ventricular fibrillation (VF) episodes [18]. In their study, ≈40% of placebo- and ≈60% of metoprolol-treated pigs developed ≥ 1 VF events during ischemia (i.e., higher incidence in metoprolol animals). Conversely, in our studies, ≈70% of placebo- and ≈40% of metoprolol-treated pigs developed ≥ 1 VF events [22] (i.e., much lower incidence in metoprolol animals). Noteworthy, while we used metoprolol on top of amiodarone, Heusch's team did not use amiodarone. The capacity of metoprolol to prevent VF in the context of ongoing MI has been consistently reported in multiple clinical trials [12], serving as the basis for the strong recommendation of beta-blockers for this purpose by clinical practice guidelines.

In summary, the lack of effect of metoprolol in Heusch's study could be secondary to the strain of animals they used, which seem to be resistant to some effects of this specific drug. For other interventions targeting different pathways, it might be the case that Göttingen pigs are responsive and Large White not. These results highlight the complexity of translation and the need to consider genetic basis of animals used in experimental settings. In this regard, it is well known that clinical trials testing the same intervention sometimes have different results. Despite the case might not be as strong

as with inbred animals, genetic differences might explain the variable response to some cardioprotective interventions in humans. In fact, polymorphisms in the gene encoding for β1 adrenergic receptor (the target of metoprolol) have a well-known impact on the response of heart failure patients to beta-blockers [20, 21]. Unpublished data from our group show that polymorphisms in this gene had a significant impact on the cardioprotection afforded by metoprolol in our METOCARD-CNIC trial [15].

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Declarations

Conflict of interest Nothing to declare.

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