Twenty-five years of preconditioning: are we ready for ischaemia? From coronary occlusion to systems biology and back

David Garcia-Dorado*, Ignasi Barba, and Javier Inserte

Laboratory of Experimental Cardiology, Heart Department, Vall d’Hebron University Hospital and Research Institute, Universitat Autònoma de Barcelona, Passeig Vall d’Hebron 119-129, 08035 Barcelona, Spain

Received 2 May 2011; revised 16 May 2011; accepted 18 May 2011; online publish-ahead-of-print 9 June 2011

It was 25 years ago that the phenomenon of ischaemic preconditioning was first described. The protection afforded by preconditioning was found to be exceptionally robust and aroused immediate interest amongst the scientific community. During the last quarter century, a large research effort has been made to elucidate its molecular mechanisms with the final aim of using this knowledge to develop new cardioprotective treatments. The scientific impact of the discovery of ischaemic preconditioning has been huge—it has allowed a change of paradigm in the understanding of ischaemia–reperfusion injury, from being a mere consequence of energy deprivation to being a complex, active process taking place to a large extent during the reperfusion phase. However, the clinical impact has been small, and some have anticipated a loss of interest in preconditioning unless this changes in the near future. We propose that the failure to develop clinical applications from ischaemic preconditioning is due in part to the incomplete understanding of its mechanisms and that a new integrative scientific approach should be used to resolve the complex networks of preconditioning protection signalling.

Keywords
Myocardial infarction • Reperfusion • Cardioprotection

1. Introduction

In 1986, Murry et al. described that the application of brief periods of coronary occlusion reduced the extent of the necrosis produced by a more prolonged occlusion period applied thereafter. This phenomenon was labelled ‘ischaemic preconditioning’ and immediately engendered enormous interest, opening a new field of research that has produced more than 5790 papers to date. One of the reasons for this immediate success has been the robustness of the phenomenon, which has been consistently reproduced in any model and species tested. However, research on ischaemic preconditioning has not been free of criticism, and it is not exaggerating to say that at this moment, it is rapidly losing ground. There is a steep decline in the number of publications on ischaemic preconditioning and an even more marked drop in the number of sessions dedicated to it in scientific meetings. A growing number of cardiovascular scientists find it discouraging that more than two decades of preconditioning research have failed to generate results applicable to patients. Has research on ischaemic preconditioning helped us to treat patients suffering acute myocardial ischaemia? Is it an exhausted, outdated area of research? Here, we propose a justified answer to these questions.

2. The original description

Twenty-five years is a long time in biomedical research, and most of today’s cardiovascular scientists were at university or high school when ischaemic preconditioning was first described. Many of the papers published in those days were difficult to access in electronic format until recently, and it is quite possible that the original paper by Murry et al., cited more than 3800 times in these 25 years, has been read far less often. We recommend this article to the reader and summarize here its main findings. The article described that four episodes of 5 min occlusion in the circumflex coronary artery of dogs reduced infarct size, measured 4 days later, caused by 40 min of occlusion applied immediately thereafter. The protection was not related to changes in collateral flow and was not observed when the prolonged ischaemic period was extended to 180 min. The authors provided extremely detailed information on all the procedures and results and a thorough and clear discussion of the potential mechanisms, but they acknowledged that its mechanism was unknown. In fact, the study exemplifies how a good, descriptive study may be of more interest and impact than many reports that investigate mechanisms (see below). Finally, the authors had a vision of the potential clinical implications of its discovery that deserves to
be literally quoted: ‘It is possible, therefore, that patients who experience repeated episodes of angina may similarly precondition their myocardium, and in so doing, alter the time course of cell death after the onset of a sustained coronary occlusion. If this is true, then the onset and early progression of cell death may be slower in many patients than the results of animal studies have suggested may be the case. A slower progression of cell death implies a longer window of time in which it might be possible to salvage myocardium via reperfusion, e.g., with thrombolytic therapy or coronary angioplasty.’

After this original report, the term preconditioning was used in many different contexts, including different kinds of stimuli able to elicit protection when applied before ischaemia (pharmacological, thermal, hypoxic, electrical, etc.) and different forms of protection (infarct size, arrhythmias, contractile failure, etc.), but at present, it is generally used in its original meaning of protection against cell death. In addition, it was described that although the duration of preconditioning protection is limited, disappearing a few hours after the preconditioning stimulus, it reappeared 24 h later and lasted several more hours, a phenomenon known as second window or delayed protection.

3. Scientific implications

The results of the original study were paradoxical and unexpected, as adding ischaemia to ischaemia resulted in less ischaemic injury. This refuted the prevalent view of ischaemic injury as the mere manifestation of energy deprivation, and the authors had to work out an explanation to reconcile their results with this prevalent view. They hypothesized that protection could be due to slowed ATP depletion during ischaemia, based on their measurements of high-energy phosphates in the myocardium subjected to repeated episodes of ischaemia of 10 min of duration that they had submitted a few weeks before and that was published 1 year later in the American Journal of Physiology. Interestingly, this hypothesis, which fit into the paradigm of ischaemia—reperfusion injury accepted at the time, received support from several subsequent studies but is now recognized as unable to explain the phenomenon.

To fully appreciate the scientific implications of the discovery of preconditioning protection, we must be aware of the scientific understanding of myocardial cell death secondary to ischaemia at the time it was described. The group of Jennings and Reimer had published a series of fundamental articles describing the relationship between the duration of ischaemia and the extent of necrosis in the dog papillary muscle and in the whole ventricle subjected to transient occlusions of the left circumflex artery. These and other authors had described the importance of collateral flow and the progression of infarct from the subendocardial to the subepicardial layers as the wavefront phenomenon. Pathological and ultrastructural studies demonstrated minor or no changes during ischaemic periods prolonged enough to cause large infarcts. However, the ATP content in ischaemic myocardium was found to closely correlate with the extent of myocardial necrosis observed after reperfusion. The scientific paradigm at the time was that ischaemic damage progression during ischaemia was mainly due to energy depletion and secondary catabolite accumulation and that if a certain level was reached, cardiomyocytes entered a phase of ‘irreversible ischaemic injury’. According to this paradigm, cells irreversibly damaged could exhibit a largely preserved structure but were bound to suffer dramatic changes, including sarcolemmal rupture, if subjected to reperfusion, or progressive oedema and rupture (oncotic death), if ischaemia was maintained. The main determinant of the ability of cells to survive during reperfusion was considered to be the severity of energy depletion reached during ischaemia. Damage caused by transient ischaemia was thus seen as a mere consequence of energy deprivation. It then followed that the only way to limit damage should be to limit deprivation. The rapid and prominent changes occurring during reperfusion were thus interpreted as the manifestation of damage that had already occurred during the ischaemic phase, brought out by a prominent burst of free radical species. Consistent with this view, a number of well-conducted studies that tested the effect of anti-oxidant strategies applied at the time of reperfusion on infarct size were negative, although there were a few studies that found that pharmacological interventions aimed at other mechanisms of injury could salvage myocardium when applied at the time of reperfusion.

In this context, the discovery of preconditioning protection had a huge scientific impact because very soon after its description, it was proven that its mechanism was independent of energy preservation during ischaemia: different studies demonstrated that the protection was preserved in models in which preconditioned myocardium showed a more profound energy depletion during ischaemia than non-preconditioned tissue. Ischaemic preconditioning was a solid evidence that myocardial necrosis induced by transient ischaemia cannot be explained as a passive energy starvation phenomenon, but is rather the consequence, to some extent, of an active process. The phenomenon of ischaemic preconditioning refuted the energy deprivation theory and was consistent with the notion—the minority view at the time—that infarct size could be limited by interventions not restricting the duration or severity of ischaemia. This cleared the way for the research into treatments to be applied at the time of reperfusion.

The mechanism of the protection induced by ischaemic preconditioning has been studied in thousands of articles and discussed in excellent review articles. Here, we will only consider some aspects of those findings that had a major impact on the field. After an initial period of characterization of the phenomenon regarding its time-course response and universal reproducibility in many different organs, species, and conditions, a very important step in the elucidation of preconditioning protection was the identification of different elements of the response: receptors, signalling pathways, and effectors of a signal. Another important step was the recognition that preconditioning, although applied before ischaemia, exerted its protection at the time of reperfusion by interfering with the effectors of cell death.

However, despite a huge amount of data, there are many obscure zones in our understanding of preconditioning protection. Mitochondrial ATP-dependent K+ channels proposed to play an important role are not essential, at least in some species, and their molecular identity remains unknown. The generation of reactive oxygen species is another important signalling element, but their exact role and site of production has not been completely defined. Attenuated mitochondrial permeability transition appears to be a key element, but its relationship to sarcolemmal rupture observed during early reperfusion injury and the molecular nature of the pore remains unknown. Other mitochondrial elements, such as connexin 43, have been shown to participate in preconditioning protection, but the molecular mechanism of this participation has not yet been defined. The main limitation in our knowledge of ischaemic...
preconditioning is the lack of understanding of the relationship between the entangled bunch of signalling pathways that have been described to be involved in protection. Stimulation of any of them appears to be sufficient to elicit protection, suggesting that they operate in parallel and are redundant, while the individual inhibition of any of them does not prevent protection, indicating that they act in series or are additive. We must admit that the molecular mechanisms of ischaemic preconditioning remain largely unknown.

4. Translation

The main argument against the relevance of research on ischaemic preconditioning is that it needs to be applied before ischaemia, which is not feasible in most clinical situations, including acute coronary syndrome. Although it can be used during procedures involving myocardial ischaemia, these cause in general relatively little damage whose further reduction with preconditioning is expected to have minor if any effect on clinical prognosis. It must be acknowledged, however, that the scientific impact of preconditioning and the momentum that it gave to the development of the reperfusion injury paradigm resulted in the development of therapeutic strategies applied at the time of reperfusion, in particular ischaemic post-conditioning and pharmacological protection. Interestingly, there is evidence that pre- and postconditioning-induced protection share some signalling mechanisms, although other mechanisms appear to be specific to postconditioning.

A second limitation for the translation of ischaemic preconditioning is that it requires generation of brief episodes of myocardial ischaemia. Relatively, recent studies show that intermittent ischaemia at a distance may induce myocardial protection as well, either when applied before, during, or immediately after the prolonged ischaemic episode (remote pre-, per-, and postconditioning). The mechanisms of this protection are more obscure than that induced by classical ischaemic preconditioning, but again, it has been described that they may share some signalling pathways. Thus, it probably makes sense to conceive of a continuum of myocardial endogenous protection states that can be elicited by different triggers at different times of ischaemia–reperfusion, that share a number of signalling pathways, and whose main result is to limit myocardial reperfusion injury. Particular cardioprotection interventions can thus be placed in a three-dimensional space whose dimensions are time of application, type of stimulus, and site of delivery.

A potential and very desirable spin-off of research on ischaemic preconditioning may be the development of pharmacological interventions that could mimic or induce myocardial endogenous states and could be applied at the time of reperfusion. This development was not included as a potential clinical implication in the original report by Murry et al., which is fully consistent with the prevalent view at the time that ischaemic injury was an energy deprivation process that could only be attenuated by slowing energy depletion. However, it should be considered a research objective now, when cell death secondary to ischaemia/reperfusion is seen as a consequence of active signalling processes that can be interfered with.

This objective has been achieved only to a very limited extent to date. As has been reviewed elsewhere, the experience with pharmacological protection against reperfusion injury in patients is limited to very few proof-of-concept studies that were based on the application of pre-existing drugs such as cyclosporine A, adenosine, or atrial natriuretic peptide.

5. The future

The reason for this failure to translate basic research on preconditioning into clinical practice is probably related to a large extent to the incomplete understanding of the mechanisms involved in preconditioning protection. Some researchers might say that if we have not been able to translate preconditioning protection to patients in 25 years, then maybe it is time to admit that the phenomenon is not so relevant and that further investigation may be of little social impact. However, it could also be that the mechanisms of the phenomenon are far more complex than anticipated or that our approach to resolve them has not been well oriented. Both reasons could come into play. In this case, more research using new and powerful scientific approaches would be needed to elucidate the mechanisms of ischaemic preconditioning protection in a way that allows the development of useful clinical applications. In fact, the reductionist approach used to investigate cardioprotection has provided only fragmented information about signalling pathways whose interdependence and connections with cellular systems are largely unknown, and this is a critical limitation in the process of identifying new targets and treatments. To overcome this limitation, network and context analysis of data provided by high-throughput methods needs to be performed, and the results have to be integrated into meaningful, predictive models.

Twenty-five years may be a long period to wait for translation of a treatment to patients, but it would not be logical or fair to conclude that translation is impossible. There are many examples in other areas of research such as cancer or immune and infectious diseases in which translation of knowledge into practical applications has taken longer. Should we have dismissed gene therapy as a useless approach after more than 20 years without significant clinical application?

6. Conclusion

The discovery of ischaemic preconditioning had a large scientific impact and was a big step forward in the understanding of the pathophysiological and clinical manifestations of acute coronary syndrome. We must confront the fact that 25 years after its description, its molecular mechanism has not been completely resolved and that this has thus far limited translation to patients. We are not yet ready to treat ischaemia–reperfusion injury in routine clinical practice. But the answer to this cannot be other than a really renewed research effort.

Conflict of interest: none declared.

Funding

This study was supported by the Spanish Ministry of Science (RETICS-RECAVA RD06/0014/0025; CICYT SAF/2008-03067, FIS-P1080238, and PS09/02034). I.B. is a recipient of a Ramon y Cajal grant contract.

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