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Themed Section: Conditioning the Heart – Pathways to Translation

EDITORIAL

'Conditioning the heart' – lessons we have learned from the past and future perspectives for new and old conditioning 'drugs'

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Introduction

Almost a year ago my 6-year-old son asked me: 'Mom, what is it you are doing every day in your laboratory?' First of all I started explaining in children's terms what the noble gas helium is, what it can do, and why we think that it is not only good to blow up balloons but might also be good for the heart of some patients.

However, he was not satisfied by this answer and proceeded asking things like: '. . . and why are you doing this? What will you do next? How long have you been doing this?'

Finally, I ended up saying something that I had already realized for quite a while: 'Actually, I do not know why I am still doing this "conditioning" research.' After having said so, I was quite upset as I have never been honest enough to myself to admit that most of the research others and we do is still not translated to the clinical situation. Do we really help patients to get better?

Thus, despite several decades of research on preconditioning, postconditioning and pharmacological conditioning of the heart, we have yet to see therapeutic realization of the potential powerful protective effects of conditioning on infarction, mechanical dysfunction and arrhythmias associated with acute myocardial ischaemia and reperfusion.

Eventually, I was searching for supportive views to get back on track with my own research, and I must admit that the overwhelming participation of excellent researchers in this themed issue gave me a lot of motivation to hang on to the pathway of getting cardioprotective strategies by conditioning translated from the experimental laboratory to the patient.

This is where the idea was born to bring together researchers from all over the world to share their thoughts about this question in the themed issue: 'Conditioning the heart-Pathways to translation-scope for drug discovery'.

Summary of content

Cohen and Downey are pioneers in the area of 'conditioning' the heart and their review takes us through a brilliant historical journey going back to 1971 when Maroko and co-workers suggested that ST-segment shifts might be used as a marker for infarct size reduction, and that infarct size reduction would be a therapeutic option to prevent cardiac damage by ischaemia/reperfusion (I/R) injury. Four decades later, passing pioneers like Hearse and Murry, Cohen *et al*. review several pathways of pre- and post-conditioning that have been elucidated (Cohen & Downey, 2015). The endogenous mediators of the trigger phase include adenosine, bradykinin, opioids

and other extracellular signalling molecules. All these triggers have been shown to bind to G-protein coupled receptors, but surprisingly they then activate different pathways that converge upon PKC. Additionally, the mediator phase of conditioning, including enzymes from the so 'called' reperfusion injury salvage kinase (RISK) pathway, is discussed. All signalling finally seems to lead to inhibition of the mitochondrial permeability transition pore (mPTP), which is currently suggested to be the end-effector of ischaemic preconditioning.

Several years after the discovery of *pre*-conditioning it became clear that the obviously strong endogenous protective intervention had to occur *before* an ischaemic insult, making the clinical application very difficult, as ischemia often already has taken place, for example, in patients presenting with an acute myocardial infarction. This is when the idea of protective pharamacological interventions *during* reperfusion, possibly achieved by an intervention as ischaemic *post*-conditioning, was born.

However, as Cohen and Downey (2015) point out, irrespective of the logical implications of the successful interventions seen in animal experiments, several clincial trials using ischaemic mimicking agents like, adenosine, atrial natriuretic peptide, or cyclosporine A, have yet remained little successful.

In the last part of their review, Cohen and Downey focus on the role of platelets, platelet activating factor (PAF) and platelet $P2Y_2$ inhibitors in cardioprotection (Cohen & Downey, 2015). It is nowadays quite accepted that in patients presenting with acute myocardial infarction (AMI), complications occurring during and after stenting can in fact be significantly improved by the use of antiplatelet drugs like aspirin, thienopyridines (clopidogrel and prasugrel) or triazolopyrimidins (tricagrelor, Cohen & Downey, 2015). So far, the anti-aggregation properties are suggested to be responsible for the protective effect against re-occlusion of a stent, but Cohen and Downey shed light upon a complete different aspect. They suggest that especially the $P2Y_2$ ADP receptor inhibitors (thienopyridines and triazolopyrimidines), in fact (already) activate the *post*-conditioning pathway: This means that the attempt to add another 'conditioning' stimulus by e.g. ischaemic or pharmacological conditioning might fail (Cohen & Downey, 2015).

Cohen and Downey (2015) lead the reader to a very critical but also future-oriented view on 'conditioning' of the heart. They support further laboratory research for elucidating alternative pathways that may extend the spectrum of the anti-ischaemic armamentarium beyond the platelet inhibitors used in standard care.

Baxter and Bice take a closer look into the phenomenon of '*post*-conditioning' of the heart (Bice & Baxter, 2015). This more recently described form of 'conditioning' has been in the focus of research over the last decade. Bice and Baxter (2015) distinctively introduce the mechanisms that have been implicated in ischaemic post-conditioning and which are quite similar to those known from pre-conditioning. Again, inhibition of the mPTP resembles the potential end-effector in post-conditioning and pathways like the RISK, SAFE, GSK-3 beta and cGMP/PKG are involved (Bice & Baxter, 2015). Bice and Baxter (2015) come to the conclusion, that even though promising results from initial clinical studies using both, ischaemic and pharmacological post-conditioning exist, here the final translation to the clinical situation fails. They suggest that study design, timing, drug administration, technical limitations with respect to the end-point measurements and – most importantly – existing co-morbidities in our patients might limit the translation of protection by conditioning to the clinic (Bice & Baxter, 2015). However, as Cohen and Downey, they end with a very promising view on the dead end some of the 'conditioning' researchers might feel to be stranded in. In their view the variability within the group of AMI patient makes it probably impossible to find a one-size-fits-all cure for each patient, but taking into account the enormous amount of patients with cardiovascular diseases, any standard drug in a single dose might make a huge difference at least for some of the respective patients (Bice & Baxter, 2015).

Another more clinically applicable form of 'conditioning' the heart, *remote* ischaemic conditioning (RIC), is described in the review of Schmidt *et al*. (2015). RIC is defined as short-lasting periods of ischaemia applied to a distant organ form the heart, which eventually lead to the protection of the heart itself against ischaemia reperfusion injury. Schmidt *et al*. draw a picture of the most recent and promising mediators and targets involved in remote ischaemic conditioning. Among these, especially the recently extensively investigated dialysate from plasma, containing most probably not one but several potent cardioprotective factors (factor X), is discussed. Also MicroRNA (especially miR-144) and exosome release during RIC have recently entered the focus of RIC research. Schmidt *et al*. suggest that these might be very promising strategies to develop future therapies mimicking RIC (Schmidt *et al*., 2015). In addition, Pzyklenk points out that among all forms of 'conditioning' without doubt 'post-conditioning' and RIC are the most promising strategies to be translated to the clinic. In her view, especially the extremely complex and time sensitive signalling network involving all conditioning forms limits translation from promising preclinical trials to larger clinical trials. She also critically questions the study design (patient population) of recent clinical trials examining conditioning the heart as well as the choice of the experimental protocol (Przyklenk, 2015). In her review, the reader will be confronted with the hypothesis that despite the assumption that translatability of preclinical data to the clinic would implicate a study design as close as possible to the meanwhile well-established pathways of cardioprotection, there is an enormous heterogeneity among and within the clinical studies that have been performed so far (Przyklenk, 2015).

The reviews of Pagliaro and Penna (2015) and Inserte and Garcia-Dorado (2015) deal with the complex mechanism underlying *reperfusion* injury. In this phase of I/R injury, redox signalling (Pagliaro & Penna, 2015) and cGMP/PKG signalling (Inserte & Garcia-Dorado, 2015) are critically involved in the development of cardiac damage. Pagliaro and Penna (2015) point out to the fact that undifferentiated diminishment of redox signalling [reactive oxygen species (ROS) and reactive nitrogen species (RNS)] by antioxidants cannot be the future therapy in I/R damage, as in fact redox signalling is vital to several physiological processes (Pagliaro & Penna, 2015). The authors suggest a more site- and time-specific inhibition of ROS/RNS without affecting survival pathways relying on ROS/RNS, however, clinical data are again sparse regarding this topic (Pagliaro & Penna, 2015).

Next to ROS signalling, a pivotal role for cGMP/PKG signalling during reperfusion injury and cardioprotection by 'conditioning' has been described (Inserte & Garcia-Dorado, 2015). According to Inserte and Garcia-Dorado (2015) extensive amounts of pre-clinical data definitively support the role of cGMP/PKG in cardioprotection. Moreover, the authors are convinced that targeting these key players of the signal transduction cascade in the early phase of reperfusion is a valuable and very promising therapeutic option toward diminished cardiac damage (Inserte & Garcia-Dorado, 2015). Unfortunately, the narrow dose-response curve that has to be followed when cGMP levels are increased might dampen the positive view, as too high cGMP levels have recently been shown to be rather harmful than protective (Inserte & Garcia-Dorado, 2015).

The role of the extracellular signalling molecules (autacoids): adenosine, bradykinin and opioids, is extensively described in the review by Kleinbongard and Heusch (2015) and for opioids by Headrick *et al*. (2015). These endogenous signalling molecules are un-doubtfully all involved in the different forms of 'conditioning' however, once again translations to clinical trials were disappointing. Although e.g. adenosine has been tested in several trials of AMI, elective PCI or CABG patients, and some studies are positive and promising, there is still no consensus on whether adenosine reduces infarct size in the clinical scenario (Kleinbongard & Heusch, 2015). Headrick *et al*. point out that although small clinical trials showed a benefit of morphine and remifentanil in CABG surgery patients, targeting the opioid receptors (OPR) more specifically (e.g. δ-OPR agonists) in order to avoid cardiorespiratory effects of unspecific OPR agonists would be a more promising approach (Headrick *et al*., 2015). Especially for opioids one has to take into account that maintaining anaesthesia during surgical procedures might in itself already be cardioprotective and thus stocking up on cardioprotective interventions might be difficult in this setting (Kleinbongard & Heusch, 2015).

Both expert groups agree upon the fact that ageing, co-morbidities and – most importantly – relevant drugs during the surgical procedure are the challenge that has to be overcome before autacoid mimicking drugs can find their way into the clinic (Kleinbongard & Heusch, 2015) (Headrick *et al*., 2015). In this context, sustained ligand-activated protection (SLP) might have a future role in clinical applications as it has been shown to be effective also in diseased animal models (Headrick *et al*., 2015). Kleinbongard and Heusch (2015) end with a conclusion that might frustrate those of us working on 'pharmacological' induced conditioning. The authors argue that probably the search for more 'drugs' to induce cardioprotection should stop soon, and the development of more reliable RIC models in the clinic could be the future (Kleinbongard & Heusch, 2015).

The two reviews dealing with the probable most *easily* translatable conditioning strategies using anaesthetics or noble gases extensively describe the mechanisms underlying such cardioprotection (Kikuchi *et al*., 2015, Smit *et al*., 2015). Protection induced by volatile anaesthetics (Kikuchi *et al*., 2015) and later on noble gases, like xenon and helium (Smit *et al*., 2015), has been recognized for the last two decades. Unfortunately, these two reviews come to the disappointing conclusion that the application of these substances, although already clinically used, is still limited and advances in this field are minimal (Kikuchi *et al*., 2015, Smit *et al*., 2015). Ageing, diabetes, hyperglycaemia and drugs frequently used in AMI patients (beta blockers, glibenclamide) have been shown to diminish this form of conditioning in animals as well as in humans (Kikuchi *et al*., 2015). Thus once again, more laboratory research using adapted animal models and models that properly mimic the clinical anaesthesia models are needed (Kikuchi *et al*., 2015, Smit *et al*., 2015).

In the last four reviews some of the key targets involved in orchestrating different conditioning forms are excellently reviewed (Ong *et al*., 2015, Halestrap *et al*., 2015, Martin *et al*., 2015, Schilling *et al*., 2015). For the sake of brevity I will only be able to highlight some snap shots from these reviews, hereby emphasizing that under no circumstances do I wish to undermine the importance of these contributions to the completion of this themed issue.

Regarding the aforementioned crucial involvement of mPTP in cardioprotection by different conditioning forms, the reviews by Ong *et al*. (2015) and by Halestrap *et al*. (2015) give an excellent detailed overview on the mechanisms by which the mPTP is regulated and in which way hexokinase 2 (HK2) might be of importance for maintenance of the opening of this pore. The opening of the mPTP at the onset of reperfusion leads to cell death. Trials over the past years using cyclosporine A, an inhibitor of mPTP opening, have proven that when administered right at the beginning of reperfusion, it in fact reduces MI (Ong *et al*., 2015). Larger multicentre trials as the CYCLE and CIRUS study are currently running and will reveal very important results regarding the clinical use of cyclosporine A (Ong *et al*., 2015).

The glycolytic enzyme HK2 has been found to bind to the outer membrane of the mitochondria, thereby stabilizing the contact sites of outer and inner mitochondrial membranes. Dissociation of HK2 from the membrane during the ischaemic phase leads to an increased loss of cytochrome C from the mitochondria, which in turn results in opening of the mPTP during reperfusion (Halestrap *et al*., 2015). Ischaemic preconditioning has been shown to involve increased HK2 binding to the mitochondrial membrane, thereby inducing cardioprotection. Halstrap *et al*. critically evaluate the potential development of drugs that might increase binding of HK2 to the mitochondria, as these are yet unavailable (Halestrap *et al*., 2015).

Also caveolins, reviewed by Schilling *et al*. (2015), have more recently been associated with the mitochondria and it has been convincingly shown that they play a pivotal role in different forms of conditioning. Caveolins are structural proteins that are essential for the formation of so called 'caveolae', small plasma membrane invaginations enriched with cholesterol and sphingolipids (Schilling *et al*., 2015). These proteins are thought to regulate protective signalling within a multiprotein (signalosome) complex. Interestingly, many of the above-discussed key players (e.g. opioids, adenosine, PKC) of ischaemic and pharmacological conditioning have been shown to be associated with caveolae/caveolins in a dynamic process. Schilling *et al*. distinctively focus on the fact that caveolins might be key players in overcoming the pathophysiological limits for conditioning (ageing, diabetes), hereby leaving us with the hope that future studies might identify drugs that can specifically increase caveolin expres-

sion, thereby protecting the heart against ischaemia/reperfusion damage (Schilling *et al*., 2015).

Last but not least, Martin *et al*. provide us with an excellent review over the role of a member of the 'stress-activated' kinases family, the p38 MAPK in cardiovascular disease. The activation of p38 during ischaemic preconditioning cycle has been shown to attenuate the detrimental activation of the same enzyme during the lethal ischaemic period. Thus, p38 MAPK activation also might trigger protective effects in the heart, thereby making the inhibition of p38 MAPK quite unpredictable in clinical trials (Martin *et al*., 2015). However, very recent clinical trials with the selective, potent, and orally active p38 MAPK inhibitor Losmapimod (GlaxoSmithKline, Brentford, London, UK) show promising results in the settings of myocardial infarction, and a much larger trial implementing Losmapimod is on its way (Martin *et al*., 2015).

Concluding remarks

This themed issue summarizes a very important portion of all the research ongoing in the area of cardioprotection by several 'conditioning' forms. Of course, it cannot be complete as there is still so much to learn. The main goal of this themed issue was not only to review the current state of the art in this field, but also to provide a *critical overview* upon the opportunities that lie ahead for strong endogenous mechanisms of conditioning to gain broader translatability into the clinical situation.

With regard to this, we hope that studying these review articles will convince the readership that there is indeed a future for 'conditioning the heart' against ischaemic damage. By combining the enormous amount of knowledge we have gained from animal and preclinical studies, and applying this knowledge to the existing co-morbidities and peculiarities occurring during the perioperative period, we will hopefully succeed in our venture/conquest to identify novel candidate drugs for conditioning the heart and for testing in clinical trials.

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