Anaesthetic preconditioning

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Key points
Preconditioning refers to changes at the biomolecular level that enable specialized tissues to tolerate a major adverse event (e.g., ischaemia) better if those tissues have already been exposed to minor adverse events.

Ischaemic preconditioning is an evolutionary conserved response that can protect the myocardium from hypoperfusion and ischaemia.

Ischaemic preconditioning can be mimicked pharmacologically by a variety of substances including the volatile anaesthetic agents—this is termed anaesthetic preconditioning.

There is evidence of improvements in biochemical markers, echocardiographic findings, and clinical data after anaesthetic preconditioning.

Anaesthetic preconditioning may reduce perioperative myocardial injury during cardiac surgery and possibly non-cardiac surgery.

For survival within different and changing environments, the body has evolved a number of adaptive physiological and biomolecular responses both to changes in the availability of substrate and to tissue injury.

It is well recognized that some of these mechanisms are present within the myocardium, constituting physiological and molecular adaptations referred to as myocardial preconditioning.

Initially described for the physiological and molecular responses to ischaemia and reperfusion, there is increasing evidence suggesting that these mechanisms can be mimicked and manipulated pharmacologically by a variety of substances, including volatile anaesthetic agents.

In an ageing population, the incidence of coronary artery disease is increasing; a NCEPOD report in 1999 identified more than 9000 deaths per annum occurring in the post-surgical period with an associated cardiac cause.

Perioperative myocardial ischaemia occurs in 18–74% of patients undergoing surgery and is associated with significant morbidity and mortality. If the deleterious myocardial effects of the surgical insult can be modulated, then both patients and the resource–limited healthcare system may benefit.

Preconditioning
Change at the biomolecular level that enables specialized tissues to tolerate a major adverse or ischaemic event better if these tissues have already been exposed to several minor adverse or ischaemic events.

There are three occasions when conditioning of the myocardium can occur or could be applied.

Preconditioning of the myocardium can occur before the insult, peri-conditioning can occur during the insult, and post-conditioning can occur after the insult. Regardless of the timing when the preconditioning trigger is applied, there is an early phase where cellular signalling, cascades, and amplification lead to immediate cardioprotection, and a later phase (a second window) where various triggers and mediators lead to genetic re-programming with de novo synthesis of proteins that offer sustained cardioprotection.

History of preconditioning research
In a landmark paper in 1986, Murry and colleagues demonstrated ischaemic preconditioning in the canine heart and by 1993, late preconditioning after myocardial stress had also been reported.

Interest in the possible methods of modulating myocardial stress and injury resulted, in 1997, in Kersten and colleagues describing the preconditioning effect of the volatile agent isoflurane in an animal model, although observational studies as far back as 1976 had identified such an effect.

Since then there has been a growing body of evidence to support volatile anaesthetic agent preconditioning of the myocardium in both animal and human studies.

Definitions
Ischaemic injury
A tissue or cellular injury that occurs as a result of the interruption of a vital substrate, in particular oxygen.

Reperfusion injury
A tissue or cellular injury that occurs after the re-introduction of the vital substrate—in particular oxygen—following an interruption in supply.

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rather than cell death produces the ability to tolerate an exacerbation of those adverse conditions subsequently.

For example, patients who present with acute myocardial infarction have smaller infarcts if they have previously suffered from angina pectoris; patients who exercise until their anginal threshold are then able to continue exercising without angina symptoms; patients undergoing angioplasty who have intermittent rather than continuous occlusion have smaller cardiac enzyme changes; patients undergoing both on-pump and off-pump cardiac surgery have improved biochemical, echocardiographic, and clinical outcomes if the myocardium is subjected to ischaemic preconditioning.

This is an endogenous defence mechanism common to all tissues and has been demonstrated for the brain, liver, kidney, muscle, and myocardium. It is likely that this is a critical ability for cell survival. Jia and Crowder demonstrated preconditioning in invertebrates suggesting a fundamental and evolutionary conserved cellular response.

Preconditioning may play an increasingly important role in our understanding of protecting the myocardium during both cardiac and non-cardiac surgery, but it is quite clear that preconditioning can only be part of a multimodal myocardial protection strategy.

**Mechanisms involved in preconditioning**

There are a number of different mechanisms occurring simultaneously as all natural systems have a degree of redundancy within them and there are both similarities and differences between the mechanisms of ischaemic and anaesthetic preconditioning in both early and late preconditioning (see Fig. 1 for overview).

A study by Kalenka and colleagues on the proteome of the left ventricle (LV) using 2D gel electrophoresis and mass spectrometry demonstrated that volatile agents alter cardiac gene and protein expression. They showed a two-fold change in 106 different proteins predominantly associated with glycolysis, mitochondrial respiration, and stress. This distinct change in myocardial protein expression was the same for desflurane, isoflurane, and sevoflurane, suggesting a class action of these agents lasting for 72 h. Furthermore, the proteome changes were similar to those found after ischaemic preconditioning which suggests a common functional mechanism for volatile agent and ischaemic preconditioning.

Sergeev and colleagues used microarray technology to look at gene expression in 8799 genes after exposure to ischaemic or anaesthetic preconditioning and found both increased up- and down-regulation of gene expression compared with the control groups. This confirmed an overlap in the changes in gene expression between ischaemic and anaesthetic preconditioning—specifically in genes known to be involved with cellular stress, cell defence, and apoptosis, but also suggested a distinct cardioprotective phenotype for each method of preconditioning. Further analysis of the changes in the gene expression suggests that anaesthetic preconditioning, and ischaemic preconditioning, might have a late phase of cardioprotection.

These studies also demonstrate the complexity of the mechanisms underlying preconditioning of tissues, and a number of mechanisms have been postulated for preconditioning and these are outlined below.

**Receptor activation**

Different triggers can lead to receptor activation, effecting changes within the cellular environment and leading to a final pathway which then results in preconditioning.

These triggers include—among others—halogenated volatile anaesthetic agents, opiates, adenosine, β-blockers, and lidocaine; all these substances have been shown to reduce infarct size in animal studies.

**Intracellular signalling cascades**

The second messenger systems include the phosphatidylinositol 3 kinase (PI3K) and the protein kinase C (PKC) systems, both systems can be triggered by volatile agents and the inert gas xenon (nitrous oxide has no effect), leading to significant reductions in infarct size and apoptosis as demonstrated in a number of animal studies.

A variety of studies have also suggested that reactive oxygen species (ROS), formed as a result of the exposure to volatile agents and complex III in the electron transport chain, may be part of the activated signalling system, possibly by changing the activation thresholds of those intracellular systems.

Nitric oxide synthases (inducible and endothelial NOS) are also part of the intracellular signalling cascade. A study in rabbits showed that the benefits of preconditioning with desflurane in terms of infarct size reduction could be blocked by NOS inhibitors and it is known that chronic ethanol consumption, which modulates NOS, enhances the preconditioning effect of volatile agents. Whether NOS acts as a trigger, as part of the second messenger cascades, or as an effector in its own right is not yet fully established, but we know that second messenger systems and cascades facilitate preconditioning.

**Intracellular mitochondrial structural changes**

After initiation of a second messenger signalling cascade by receptor activation, an intracellular component mediates an immediate intracellular conditioning response.

Evidence points to the $K_{ATP}$ channels and the mitochondrial permeability transition pore (MPTP) as the important intracellular components. $K_{ATP}$ channels exist both on the surface of the sarcoplasm and on the mitochondria and the MPTP is an integral component of the mitochondria. Their activation or modulation gives rise to preconditioning.

Drugs that open $K_{ATP}$ channels such as nicorandil, pinacidil, and diazoxide can precondition the myocardium and have been
found to enhance preconditioning in animal studies and these studies suggest lasting sensitization, and early preconditioning, from $K_{\text{ATP}}$ channel opening.

Modulation of the MPTP via $K_{\text{ATP}}$ channels has been associated with reduced infarct size and biochemical damage after exposure to desflurane. It is also known that cyclosporin—a modulator of the MPTP—can pharmacologically mimic ischaemic preconditioning via this pathway—the benefits of the use of cyclosporin during acute myocardial infarction have been recently demonstrated.

**Modulation of calcium homeostasis**

The final pathway seems to lie within the mitochondrial cellular structure and within the electron transfer chain.

The opening of $K_{\text{ATP}}$ channels and the prevention of the opening of the MPTP ultimately can protect the cell by decreasing its cytosolic and mitochondrial $Ca^{2+}$ load. Studies showing the attenuation of diastolic and systolic $Ca^{2+}$ flux by sevoflurane and isoflurane could demonstrate the effect of preconditioning on the calcium homeostasis.

Mitochondria have less $Ca^{2+}$ uptake and have better preserved respiration and ATP synthesis after preconditioning treatment with volatile anaesthetic agents and this leads to improved indices of myocardial $O_2$ utilization (a marker of oxidative stress), contractile and relaxation indices, and can result in increased coronary blood flow.

**Concurrent hypotheses**

As discussed above, these mechanisms are complex and other hypotheses have been put forward. Hormonal, cytokine, immunological and metabolic modulators of cellular activity, and intercardiac myocyte cellular signalling are also implicated in preconditioning.

For example, COX-2 inhibitors abolish any cardioprotection by anaesthetic preconditioning with isoflurane; this might explain the higher rate of myocardial infarctions if COX-2 inhibitors have been given. 12-Lipoxygenase (12 LO) expression is increased with
isoflurane and provides delayed and early cardioprotection, resulting in a reduction in infarct size—an effect abolished by selective 12 LO inhibitors.

Immunologically, there is less pulmonary neutrophil accumulation after preconditioning treatment with volatile agents—these have been implicated in acute lung injury, and volatiles can improve LV dysfunction associated with platelet-activating factor-stimulated neutrophils via adenosine receptor activation.

Metabolically, preconditioning may enhance ATP preservation by a reduction in energy consumption and metabolic down-regulation. Apoptosis may also be slowed down, and by a reduction in metabolic toxic by-products, cell fragility and swelling may be reduced.

The gap junctions within the cardiac syncytium play a role too—alterations in the opening and closing of the tight junctions may prevent necrosis from spreading or it may support necrotic cells with nutrients from adjacent less damaged cells.

**Gene transcription and expression**

As shown by Kalenka and Sergeev, late phase or second window preconditioning is likely to occur as a result of gene transcription and expression facilitated through receptors activation. The activation of these receptors, intracellular signalling pathways, KATP channels, the MPTP, and the molecular components of the calcium homeostasis all form part of late phase preconditioning.

**Summary**

Preconditioning is a very complex and evolutionary response with a number of postulated mechanisms (Table 1) leading to more effective glycolysis and mitochondrial respiration and also reduced stress and apoptosis.

Early preconditioning is likely to be mediated by receptor activation and transduction of the cellular signal through the internal milieu of the cellular environment to the effector site within the mitochondria, resulting in changes to calcium homeostasis.

Late preconditioning is likely to be mediated through receptor activation and subsequent changes in gene transcription and expression.

**Clinical evidence**

In addition to the animal studies discussed above, there are now an increasing number of in vivo studies in humans, looking at ischaemic and anaesthetic preconditioning; studies have looked at off-pump and on-pump CABG, other cardiac surgery, non-cardiac surgery, and paediatric cardiac surgery (Table 2).

**In vivo data**

Although the literature is populated by underpowered studies, in cardiac surgery at least, the effects of anaesthetic preconditioning have demonstrated reduced myocardial lactate, oxygen utilization and free radical production, and lower troponin I and BNP levels; studies using echocardiography could show less anterior wall hypokinesis and improved LV function. Some studies even could demonstrate improved cardiac indices, reduced inotrope requirements, and shorter intensive care and hospital lengths of stay.

Studies have also looked at the dose dependence in early and late preconditioning as it is of interest as to whether the dose and interval strategies of agent administration affect preconditioning or not. An in vivo study by De Hert and colleagues looking at dosing and intervals suggested that the administration of a volatile agent throughout the case reduced troponin release, improved cardiac function on echocardiography, and shortened intensive care stay compared with interval dosing or either pre- or post-insult dosing regimes.

**Table 1** Possible mechanisms leading to preconditioning

<table>
<thead>
<tr>
<th>Receptor activation</th>
<th>Volatile agents</th>
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<tbody>
<tr>
<td></td>
<td>Pacing</td>
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<td></td>
<td>Opiates</td>
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<td>Bradykinin</td>
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<td></td>
<td>Adenosine</td>
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<td>Lidocaine</td>
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<td>Intracellular second messengers</td>
<td>( K_{ATP} ) channels</td>
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<tr>
<td>PKC</td>
<td>Mitochondrial permeability transition pore</td>
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<tr>
<td>PFK</td>
<td>ATP preservation</td>
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<tr>
<td>Tyrrosine kinase</td>
<td>Maintenance of mitochondrial integrity</td>
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<td></td>
<td>and structure</td>
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<td>Concurrent hypotheses</td>
<td>Cytokines</td>
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<td>Immunological and neutrophil attenuation</td>
<td>Metabolic changes</td>
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<td>Reduced apoptosis</td>
<td>Gap junction control</td>
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<td>Late phase</td>
<td>Gene transcription and expression</td>
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</table>

**Table 2** Clinical evidence for the benefits of anaesthetic preconditioning

<table>
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<tr>
<th>Surgical groups studied</th>
<th>Off-pump CABG</th>
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<tr>
<td></td>
<td>On-pump CABG</td>
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<tr>
<td>Non-coronary graft cardiac surgery</td>
<td>Paediatric cardiac surgery</td>
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<tr>
<td>Myocardial lactate reduced</td>
<td>Troponin I reduced</td>
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<tr>
<td>CK MB reduced</td>
<td>BNP reduced</td>
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<tr>
<td>Free radical production reduced</td>
<td>TOE/TTE showed reduced wall akinesis</td>
</tr>
<tr>
<td>TDI show better LV function</td>
<td>Oxygen utilization improved</td>
</tr>
<tr>
<td>Cardiac indices improved</td>
<td>Inotropes requirement reduced</td>
</tr>
<tr>
<td>Ventilated time reduced</td>
<td>Incidence of MI reduced</td>
</tr>
<tr>
<td>Hospital and ICM stay reduced</td>
<td>No mortality data yet though</td>
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</tbody>
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Observational data
Further clinical evidence for preconditioning and its mechanisms can be gathered from an assessment of the observed effect of co-morbidities or concurrent medications on anaesthetic preconditioning.

For example, both diabetes mellitus and hyperglycaemia may abolish preconditioning, although this can be reversed by blocking the mitochondrial permeability transition pore. Glibenclamide, which interferes with the $K_{ATP}$ channels, has been shown in some studies to abolish the preconditioning benefit of volatile agents.

Senescence can abolish the benefits of preconditioning. In the ageing isolated rat heart, there may be an inability for ATP replenishment or for effective $Na^+$ or $Ca^{2+}$ extrusion from the cells, hence preventing the possible benefits of preconditioning.

Further examination of the interplay between other co-morbidities, medications, and preconditioning may throw further light on the mechanisms underlying this phenomenon, but the very existence of such interplay supports the proposed effects of preconditioning.

Meta-analyses and registry data
Two meta-analyses and one review of data from the Danish cardiac surgery register have shed further light on the clinical evidence and give anaesthetic preconditioning greater credibility.

Two recent meta-analyses in the British and the Canadian Journals of Anaesthesia looked at 27 trials with 2797 patients and 32 trials with 2841 patients respectively, and suggested that there was a significant difference in the patients who received volatile agents. They had a lower troponin I increase, improved cardiac indices, less inotrope use, and less mechanical ventilation, although there was no difference in overt myocardial ischaemia, myocardial infarction rates, intensive care stay, hospital stay, or mortality.

A review of the Danish registry data demonstrated that sevoflurane had beneficial effects in patients who were not already preconditioned by pathological ischaemia/angina in terms of improved biochemical markers, echocardiographic findings, and clinical outcomes. No effect, though, was seen in patients who had suffered episodes of ischaemia before the exposure of sevoflurane. In other words, anaesthetic preconditioning could not be superimposed on a myocardium that was already preconditioned by ischaemia/angina pectoris.

These larger studies support the benefits of anaesthetic preconditioning.

Implications in cardiac and non-cardiac anaesthesia
What then are the implications for anaesthesia?

There is probably sufficient evidence to support the use of volatile agents during cardiac surgery, and perhaps specifically where no ischaemic preconditioning has previously occurred.

Animal studies and small clinical trials also suggest that the use of volatile agents to precondition the myocardium against ischaemic episodes in the non-cardiac surgery perioperative period may be beneficial in patient groups in whom, and in surgical procedures where, there is likely to be an increased risk. The data suggest that there are improved biochemical markers, improved LV function, reduced myocardial ischaemic damage and infarct size, fewer arrhythmias, and in terms of clinical outcomes, there may be a reduction in the use of inotropes, ventilated and intensive care days, and a reduced hospital length of stay.

As yet though, there is no evidence of a reduction in mortality. In the interim, pending further evidence-based studies, volatile agents should contribute to, and be used as part of, a multimodal strategy to protect the myocardium.

References

Please see multiple choice questions 4–6