**WHAT SHOULD THE ANAESTHETIST KNOW ABOUT ISCHAEMIA-REPERFUSION INJURY?**

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Ischaemia-reperfusion situations routinely occur in a variety of clinical situations like during transplant, heart-, vascular or neurosurgery. The anaesthetist may also encounter ischaemia-reperfusion injury without surgical interventions, like after a transient myocardial ischaemia during a stressful anaesthetic induction. Depending on the severity and duration of ischaemia, the lack of oxygen supply may result in reversible or irreversible damage of the tissues. Early restoration of arterial blood flow and surgical measures to improve the ischaemic tolerance of the tissue (like organ cooling or cardioplegic solutions) are the main therapeutic options currently used.

In the last years, we have not only gained a lot of new insights into the pathomechanisms involved, but we have also learned that anaesthetic drugs can interfere with those mechanisms, providing either protection, being inert or even increasing the damage by blocking protective mechanisms. Thus, by the choice of the anaesthetic drugs, the anaesthetist may interfere substantially with the process of perioperative organ protection - a new concept still widely ignored by both, surgeons and anaesthetists. As most research was about the interaction of anaesthetics with ischaemia-reperfusion damage of the heart, this review will focus on myocardial protection.

If an ischaemia-reperfusion situation occurs, there are three time windows when a substance may interact with the injury process: during ischaemia – before ischaemia – and after ischaemia (i.e., during reperfusion).

**INTERACTION WITH ISCHAEMIC INJURY**

A beneficial role of volatile anaesthetics during myocardial ischaemia was observed as early as 1969 by Spieckermann and colleagues (1), who found a prolonged tolerance to global ischaemia and enhanced preservation of high energy compounds in dog hearts during halothane anaesthesia. Several studies demonstrated that volatile anaesthetics reduced myocardial oxygen demand during ischaemia, thereby reducing ischaemic damage (2-4). In patients with coronary heart disease, isoflurane improved the tolerance to pacing-induced myocardial ischaemia (5). Sevoflurane and desflurane also showed anti-ischaemic properties (6-8). The mechanisms behind this protection might be the negative inotropic and negative chronotropic action of the substances. In addition, volatile anaesthetics maintain myocardial energy stores and might increase collateral blood flow to the ischaemic area, thereby reducing the severity of ischaemia. However, the overall direct anti-ischaemic effect of the anaesthetics is relatively small compared to their pre-ischaemic (=preconditioning) effects or their effects against reperfusion injury. Therefore, the clinical benefit from the direct anti-ischaemic action of anaesthetics appears to be very limited.

**FIGURE 1**

![Figure 1](image)

Modified from Ref. [20]. Patients with preceding angina have a much better outcome after myocardial infarction. This finding underlines the clinical importance of the preconditioning mechanism.
EFFECTS AGAINST REPERFUSION INJURY

DEFINITION

The "reperfusion injury" was defined as "metabolic, functional and structural changes after restoration of coronary perfusion, which can be reduced or prohibited by modification of the reperfusion conditions" (9). The reperfusion injury can be divided into a non-lethal, reversible cellular damage and a lethal, irreversible damage.

MECHANISMS AND CLINICAL MANIFESTATION OF REPERFUSION INJURY

Non-lethal reperfusion injury includes myocardial arrhythmias and the post-ischaemic reduction of myocardial function. The reversible, but delayed recovery of myocardial function after complete restoration of coronary blood flow is called "myocardial stunning" (10). The clinical equivalent for the anaesthetist is often the patient needing short term inotropic support after coming off cardiopulmonary bypass and then makes a full recovery. Lethal reperfusion injury is characterised by irreversible cell death (myocardial necrosis) and can be divided into an early (immediately at the beginning of reperfusion) and a late phase of myocardial damage. The different characteristics of the reperfusion injury are caused by distinct pathomechanisms (Table 1), which can be modified by therapeutic interventions. A recent review describes the pathomechanisms and how anaesthetics can interact in more detail (11).

TABLE 1: MECHANISMS OF REPERFUSION INJURY

<table>
<thead>
<tr>
<th>myocardial stunning (sublethal reperfusion injury)</th>
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</thead>
<tbody>
<tr>
<td>- oxygen radicals</td>
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<tr>
<td>- changes of cellular Ca2+-homeostasis</td>
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<tr>
<td>- reduced Ca2+-sensitivity of the myofilaments</td>
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<tr>
<td>- endogenous mediators (adenosine, KATP-channels)</td>
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<tr>
<th>early lethal reperfusion injury</th>
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<tr>
<td>- Ca2+-paradox</td>
</tr>
<tr>
<td>- oxygen-paradox</td>
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<tr>
<td>- changes of intracellular pH and osmolarity</td>
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<tr>
<td>- increased sarcolemmal fragility</td>
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<tr>
<th>delayed lethal reperfusion injury</th>
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<tr>
<td>- adhesion and activation of polymorphonuclear neutrophils</td>
</tr>
<tr>
<td>- release of free radicals from activated neutrophils</td>
</tr>
<tr>
<td>- adhesion and activation of platelets</td>
</tr>
<tr>
<td>- activation of the complement system</td>
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<td>- apoptosis</td>
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INTERACTION OF ANAESTHETICS WITH REPERFUSION INJURY

In 1996, a specific protection against myocardial reperfusion injury by halothane was described (12). While all previous studies could not discriminate between anti-ischaemic effects and effects against reperfusion injury, this study demonstrated for the first time that modification of the reperfusion conditions by administration of a common volatile anaesthetic specifically reduced reperfusion damage. A similar cardioprotective effect was confirmed for enflurane, isoflurane, sevoflurane and desflurane and the noble gas xenon under a variety of experimental conditions in vitro and in vivo; cardioprotection against reperfusion damage was also maintained when the heart was already protected against ischaemic damage by cardioplegic solutions [for review see (11)]. The amount of cardioprotection in all these studies was substantial, leading to an infarct size reduction of about 50%. In addition, several specific mechanisms could be identified: a direct action at the myocardial cell against immediate damage by an interaction with the ryanodine receptor of the sarcoplasmic reticulum (13) and an action against the neutrophil mediated secondary damage (14). While there comes a large body of evidence of a specific cardioprotective effect of the inhalational anaesthetics against reperfusion injury, only one study confirmed this findings in patients: in patients undergoing coronary bypass grafting, 1.7 Vol.% of isoflurane given for the first 15 min after the release of the aortic cross clamp led to a substantial reduction in the need for inotropic support and to a reduction of myocardial damage assessed by post operative troponin release (15).
At variance with potent inhalation anaesthetics, intravenous anaesthetics have shown little evidence of cardioprotection during ischaemia-reperfusion situations. Propofol, for example, is known as a free oxygen radical scavenger and inhibits calcium influx across plasma membranes, but does not improve post-ischaemic myocardial function (16). Given only during the reperfusion period, propofol provided no protective effect against cellular damage in isolated rat hearts (17).

**FIGURE 2**

Modified from Ref. [24]. Five min inhalation of 1 MAC sevoflurane have a similar infarct size reducing effect as the „endogenous“ cardioprotective mechanisms „early“ and „late preconditioning“.

**EFFECTS OF ANAESTHETICS BEFORE ISCHAEMIA: PRECONDITIONING**

**THE CONCEPT OF PRECONDITIONING**

It seems that most of our cells have an endogenous protection system that – if activated before ischaemia – partially protects the cells against the consequences of ischaemia-reperfusion. The protection mechanism is called “preconditioning”, and the activating stimulus can be a short ischaemia, oxidative stress, a short change in temperature or some drugs.

Preconditioning was first discovered in the myocardium by Murry and co-workers (18) when they tried to increase infarct size in dogs by multiple periods of short ischaemia preceding a longer ischaemia. Surprisingly
at that time, infarct size was not increased but reduced by more than 50% if the infarct inducing long ischaemia was preceded by short ischaemic periods. An exploding number of studies has confirmed these findings for all species tested so far and also for different tissues. The first described early phase of protection (classic or early preconditioning, EPC) begins shortly after the preconditioning stimulus and disappears after 2-3 h. Twelve to twenty four hours after the initial preconditioning stimulus, cardioprotection from preconditioning reappears lasting for 2-3 days. This phenomenon has been termed "late preconditioning" (LPC) or "second window of protection"(19). An actual review of the mechanisms of ischaemic preconditioning can be found at (20).

If one assumes that for the heart, angina is the physiological equivalent of preconditioning, the findings of Kloner and co-worker (21) underline the immense clinical importance of this phenomenon: patients in whom a myocardial infarction is preceded by angina have a much better outcome in terms of survival (fig. 1). Similarly in the brain, preceding transient ischaemic attacks seem to improve outcome after an ischaemic insult (22). During cardiac surgery, ischaemic preconditioning of the heart can be elicited as an effective adjunct to myocardial protection, but may not get widespread use because for the on-pump procedures, it requires repeated aortic cross clamping (for review of the partly conflicting results see (23)). Pharmacological preconditioning may be more interesting in this setting; and as anaesthesiologists we may become involved as some of our anaesthetic agents can stimulate preconditioning while others may block it.

3.2 ANAESTHETIC PRECONDITIONING

According to new data, not only ischaemia, but also pharmacological stimulation by volatile anaesthetics can elicit this profound cardioprotective effect. Figure 2 gives an example of the extent of infarct size reduction seen after only short administration of a volatile anaesthetic (24). Interestingly, similar to the volatile anaesthetics, the inert gas xenon also induces a profound preconditioning effect despite being haemodynamically "inert" (25). Anaesthetic-induced preconditioning did not only reduce infarct size, but also post-ischaemic myocardial contractile dysfunction (“myocardial stunning”) and endothelial dysfunction in various experimental models. To induce these changes that persist long after the volatile anaesthetic has been discontinued, the anaesthetic must have lasting effects on intracellular protein and signal transduction. A detailed discussion of the molecular mechanisms is beyond the scope of this refresher course and the interested reader is referred to two excellent recent reviews (26;27). Figure 3 gives an overview of a possible concept of the intracellular signal transduction of anaesthetic preconditioning. Opioids can also stimulate preconditioning in vitro and in vivo. Unfortunately, opioid induced preconditioning depends on activation of the δ₁-receptor, which is not activated by most of the clinically used opioids. As differences in signal transduction pathways may exist amongst species, it is reassuring that some of the key mechanisms of anaesthetic preconditioning (i.e., K<sub>ATP</sub>-channel opening, adenosine receptor involvement, free radicals, mitochondrial activated protein (MAP)-kinases) have been confirmed in isolated human trabecula or myocardial cells (for review see: (27)).

While some evidence is accumulating that ischaemic preconditioning not only works under experimental conditions, but also may have clinical implications in transplant or plastic surgery, there are only a very few recent studies addressing anaesthetic preconditioning of organs other than the heart: probably, anaesthetics can precondition the kidney (28), and anaesthetic preconditioning had neuroprotective effects in various experimental settings (29-31). The clinical significance of these findings is still unknown.

**POTENTIAL HARMFUL MECHANISMS: BLOCKADE OF CARDIOPROTECTION BY ANAESTHETICS AND ORAL ANTI-DIABETICS**

Opening of the (mitochondrial) K<sub>ATP</sub>-channel is a central mechanism in the signal transduction of preconditioning (fig. 3). Barbiturates and ketamine can block K<sub>ATP</sub>-channels in isolated cells. While thiopental appeared to be safe and did not block experimental preconditioning at clinical doses (32), several studies found that ketamine completely blocked the cardioprotection of ischaemic preconditioning both in vitro and in vivo (for example: (33)); the effect was stereospecific for the R(-)-enantiomer. In experimental models, the substances propofol, etomidate, midazolam, dexmedetomidine and mivazerol had no effects on K<sub>ATP</sub>-channel activity (for review see: (34)). While the clinical importance of this findings is still unknown, it is probably safer to avoid racemic ketamine in clinical settings where ischaemia-reperfusion is likely to occur. Sulfonylurea oral anti-diabetics like glibenclamide can block the K<sub>ATP</sub>-channel and prevent cardioprotection by preconditioning. Recent evidence suggest that a patient with type II diabetes and coronary artery disease may profit from changing the treatment to insulin (by having less ischaemia induced myocardial dysfunction) (35).
Treatment with volatile anaesthetics leads to an activation of the mKA TP (i.e. opening) – PKC (i.e. phosphorylation) complex which play a central role in ischaemic as well as in anaesthetic induced preconditioning. This complex is closely linked to activation of TK and release of ROS. Blockade of KA TP channel opening, PKC activation, and intracellular release of ROS blocks the protection by anaesthetic induced preconditioning. Also the blockade of NO-synthesis, the activation of adenosine receptors, and G-proteins inhibit the protection. The definite order of activation in the signal transduction cascade is still a matter of debate. Downstream of KA TP channel opening and PKC activation, phosphorylation of the mitogen activated protein kinase p38 and its downstream target HSP27 play an important role. The involvement of MKKs and MKKKs appear likely as there are needed to activate the mitogen activated protein kinase p38.

If the activation of HSP27 is causally linked to the protection or only co-activated is not yet known. Another presumably parallel pathway seems to be the activation of ERK1-2, but only preliminary data about this pathway are yet available. The further steps to the still unknown end-effector mediating the protection by ischaemic and anaesthetic induced preconditioning are still under investigation.

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\begin{align*}
\text{AKT} (\text{PKB}) & = \text{protein kinase B} \\
\text{eNOS} & = \text{endothelial nitric oxide synthase} \\
\text{ERK1-2} & = \text{extracellular signalling regulated kinase 1 and 2} \\
\text{mKA TP} & = \text{mitochondrial ATP-sensitive potassium channel} \\
\text{HSP27} & = \text{heat shock protein 27} \\
\text{HSP90} & = \text{heat shock protein 90} \\
\text{JNK} & = \text{c-june NH2-terminal kinase} \\
\text{MKKs} & = \text{mitogene activated protein kinase kinases} \\
\text{MKKKs} & = \text{mitogene activated protein kinase kinase kinases} \\
\text{NO} & = \text{nitric oxide} \\
\text{p38} & = \text{mitogen activated protein kinase p38} \\
\text{PDK} & = \text{phosphatidylinositoltrisphosphat dependent kinase} \\
\text{PKC} & = \text{protein kinase C} \\
\text{PLC} & = \text{protein lipase C} \\
\text{ROS} & = \text{free oxygen radicals} \\
\text{TK} & = \text{tyrosine kinase} \\
\text{Calphostin C and Staurosporin} & = \text{blocker of protein kinase C} \\
\text{L-NAME} & = \text{blocker of nitric oxide synthesis} \\
\text{MnTBAP and MPG} & = \text{intracellular oxygen radical scavenger} \\
\text{PD98059} & = \text{blocker of ERK1-2} \\
\text{5HD} & = \text{blocker of mitochondrial ATP-sensitive potassium channels}
\end{align*}
IS ANAESTHETIC INDUCED CARDIOPROTECTION CLINICALLY RELEVANT?

With regard to anaesthetic preconditioning, i.e. administration of the volatile anaesthetic before aortic cross clamping, several studies have shown a preconditioning effect for isoflurane (36-38), enflurane (39) and sevoflurane (40). All studies used relatively small groups of patients ranging from 20 to 72 and consequently, had to focus on surrogate outcome markers like post-ischaemic dysfunction and release of markers of cellular damage like troponins. Most studies found a better myocardial function (or less dysfunction) (39-41), and / or a decrease in myocardial injury markers (36) (only a tendency: (38)). Two of the studies also demonstrated an increase of biochemical markers indicating crucial signal transduction steps of preconditioning in biopsies of human myocardium (38;40). One study also reported less renal injury after sevoflurane preconditioning before cardiopulmonary bypass (40). With regard to a protection against reperfusion injury, a better post-bypass ventricular function and a decrease in troponin C release (15) was observed after short term isoflurane treatment starting with the release of the aortic cross clamp.

A more “clinical” approach that probably combines the different protective mechanisms was used by De Hert and co-workers: they simply gave the volatile anaesthetic sevoflurane throughout the procedure (coronary bypass surgery) and compared the volatile anaesthetic based anaesthesia with total intravenous anaesthesia by propofol (42). Although only 20 patients with good preoperative left ventricular function were enrolled, the study could show a clear difference: a better ventricular function after coming off bypass in the sevoflurane group and less myocardial damage measured by a markedly reduced troponin release in the following 26 h. These findings could be confirmed in elderly patients with poor ventricular function (43) and the intermediate results from a multicentre study by the same authors also confirmed less troponin release with sevoflurane or desflurane anaesthesia compared with propofol or midazolam (44).

In conclusion, there are strong organ protective mechanisms that can be influenced by anaesthetic agents. Anaesthetics may induce organ protection like the volatile anaesthetics (by preconditioning and by an action against reperfusion injury) or they may also block protection mechanisms (like the blockade of preconditioning by racemic ketamine). From recent studies comes increasing evidence that cardioprotection by anaesthetic agents can be elicited in the clinical setting and may add to other organ protection strategies. Thus, it is conceivable that the choice of the anaesthetic drug may have an impact on patient outcome in ischaemia-reperfusion situations. However, this still has to be confirmed by large studies looking for definite outcome parameters.

REFERENCES
44. Stefan de Hert, personal communication.