Genome reprogramming by triggering cardioprotective mechanisms

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Abstract

With increasing life expectancy an ever increasing number of elderly patients will be undergoing cardiovascular and non-cardiovascular surgery. These elderly patients may suffer from cardiovascular diseases or may carry cardiovascular risk factors, which both contribute to an increased risk of perioperative complications including cardiac infarction and cardiac death. The ether-derived halogenated volatile anesthetics (VAs) like isoflurane and sevoflurane can provide cardioprotection as potent as ischemic conditioning when tested on isolated cardiomyocytes in culture or on isolated beating rat hearts. The results indicate that experimentally applied VAs mobilize cardioprotective cellular mechanisms including activation of the mitochondrial ATP-dependent potassium channels and preservation of a functionally intact mitochondrial permeability transition pore. Translation of the experimental findings to the clinical setting with patients undergoing coronary artery bypass graft (CABG) surgery by applying VAs during anesthesia indicates that VAs are able to afford cardioprotection postoperatively over a period of several days. Moreover, patients treated with VAs during operation suffer from fewer adverse cardiac events over a postoperative period of up to one year. Further analysis on a genome-wide basis reveals that treatment of CABG surgery patients in the perioperative phase with VAs reduces the activity of genes involved in noxious pathways and shifts the energy metabolism away from FA oxidation towards the more economical glucose utilization.

Key words: Volatile anesthetics, Cardiac conditioning, Perioperative cardioprotection, K(ATP) channels, Permeability transition pore, Cardioprotective gene program.

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Dedicated in memoriam to Ernest Gutmann

In this overview we like to address the problem of triggering cardioprotective mechanisms and the consequent reprogramming of gene activity profiles. The problem will mainly be illustrated by our own recently published results obtained from in vitro studies on primary cell cultures of adult rat cardiomyocytes and from isolated beating rat heart in the Langendorff setting. The results pertain to the important problem of offering cardioprotection to patients undergoing surgery during the perioperative period. We were able to directly translate the insight into the cardioprotective mechanisms derived from the in vitro experiments to clinical studies on patients subject to coronary artery bypass graft (CABG) surgery.

Several reasons account for an increase in attention and necessity for cardioprotective measures in the perioperative period. With increasing life expectancy and improved surgical technology, an increasing number of elderly patients with cardiovascular diseases or with significant cardiovascular risk factors undergo major surgery. Over 5% of an unselected surgical population undergoing non-cardiac surgery will suffer from severe perioperative cardiovascular complications including cardiac infarction and cardiac death [22, 24]. The incidence of adverse cardiac events may even reach 30% in high-risk patients undergoing cardiovascular surgery. In little more than 100 years, the life span has almost doubled from 40 to about 80 years in industrialized countries. This is mainly due to the introduction of hygiene and improved nutrition in combination with successful defeating of infectious diseases. Despite these beneficial factors, the longer life is laden with ailments including chronic functional degenerations, cancers, cardiovascular diseases and neuropathies. This may be mostly due to the accumulation of mutations for late-onset diseases in the

genome that have not been subjected to evolutionary pressure since natural selection does not select for healthy individuals, but only favors survival of the reproductive phase in order to assure species preservation. Today's medicine is thus confronted with an elderly and disease prone population.

Ischemia-reperfusion injury

In response to stress including severe ischemia, cells undergo necrosis or apoptosis. Mechanical disruption of the cell membrane, the sarcolemma (SL) in the case of the cardiomyocyte, as occurring during reperfusion after an ischemic insult, may be induced by uncontrolled influx of Ca2+ and hypercontraction, which directly leads to necrotic cell death [29, 38]. Cell debris in the necrotic area provokes proliferative inflammation leading in the long-term to scar development. Apoptosis, in contrast, is an energy-consuming process occurring after some delay and represents a transcriptionally regulated response to moderate cell injury. It can be triggered either by extracellular cytokines or by the intracellular mitochondrial pathway both involving proteolytic caspase cascades [for review see Ref. 38]. Apoptosis is characterized by shrinkage of the cell volume, condensation of chromatin, degradation of the genomic DNA, fragmentation into membrane-bound bodies and rapid phagocytosis by neighboring cells without induction of inflammation [21, 42]. The process of necrosis is completed in roughly 24 hours, while apoptosis takes about 4 hours leaving no traces. However, because apoptosis is an energy requiring process, it is conceivable that a portion of the myocytes, which entered the apoptotic pathway and have depleted their energy stores during ischemia, may finally die via the necrotic pathway.

The damage inflicted by severe ischemia results from two components [2, 38]. First, the energy production by oxidative phosphorylation ceases because of lack of oxygen supply by the blood. In addition, Na+ increases in the cytoplasm because of the insufficient Na-K-pump activity following the energy shortage. The increase of Na+ is always accompanied by an increase of cytoplasmic Ca2+ due to the reduced extrusion of Ca2+ out of the myocyte by the Na-Ca-exchanger (NCX) because of the lower ion gradients and reduced electrogenic drive. Ca2+ further accumulates in the cytoplasm by leaking out of the sarcoplasmic reticulum (SR) via the ryanodine Ca-release channel (RYR2) and because the SR Ca-pump (SERCA2a) is hypofunctional at low energy supply. Contractility stops altogether.

Second, when the blood supply resumes it might be expected that the myocyte function becomes restored. However far from that, the reappearance of oxygen meets the cell in disarray with high cytoplasmic Ca2+ which immediately triggers various cell activities in an uncoordinated way [2, 27, 38] (Fig. 1). The partially uncoupled mitochondrial metabolism generates massive amounts of reactive oxygen species (ROS) with cytotoxic properties and the newly synthesized ATP may induce supercontraction leading to cell disruption and death. Most of the cytotoxicity attributed to ROS appears to be due to peroxynitrite (POXN) originating from the diffusion-controlled reaction between the superoxide anions and cell produced nitric oxide (NO) [23]. POXN exceeding the antioxidative capacity reacts with and damages membrane lipids, proteins and DNA. The damaging potential of POXN is explained by its peculiar chemistry involving direct oxidation as well as radical-mediated nitration reactions. Experimental data indicate that the damage caused by the reperfusion component is always larger than the one caused by ischemia alone.

Ischemic conditioning

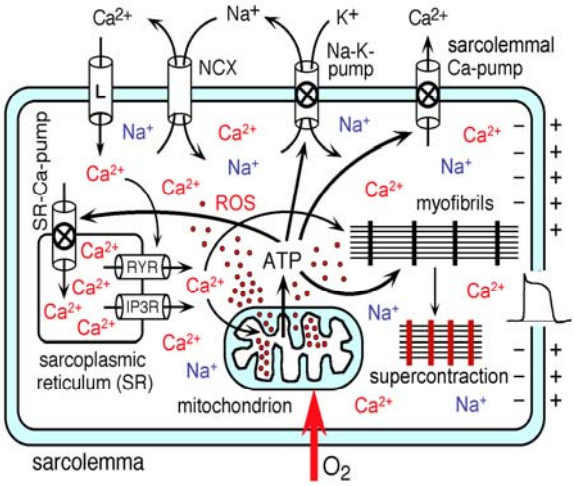

***Fig. 1**: Schematic of cardiomyocyte subject to reperfusion following prior ischemia. Massive production of reactive oxygen species (ROS) in the mitochondria upon reoxygenation (red arrow) when cytoplasmic Na+ and Ca2+ are still increased. The newly formed ATP may lead to supercontraction (for explanations see text). IP3, inositol triphosphate; NCX, Na-Ca-exchager; RYR, sarcoplasmic ryanodine Ca-release channel.*

The phenomenon of ischemic preconditioning (IpreC) was first described in 1986 by Murry and coworkers [20] in the canine myocardium. Several (3-6) brief ischemic episodes interspersed by equally brief reperfusion periods (lasting 2-5 min each) increases the tolerance against subsequent severe ischemia (test ischemia of 30-60 min or more) and significantly reduces the infarct size (extent of necrosis) in the myocardial area at risk. Some years later [19] it was discovered that the immediate cytoprotection subsides after a few hours to be followed by a second phase of protection occurring 12-24 hours later and lasting for 3-4 days. Signs of naturally occurring IpreC in patients with coronary artery disease (CAD) has indeed been inferred from the observation that pre-infarction angina reduces subsequent myocardial infarct size, improves

ventricular function and reduces arrhythmias [reviewed in Ref. 38].

The notion that reoxygenation during reperfusion induces the most severe cell damage led to the idea of modulating this process. Subsequently, a kind of graded reperfusion regimen comprising a series of short bouts of coronary artery occlusions (lasting for 20-60 sec with equally short intervals) proved to offer cardioprotection against the detrimental consequences of a preceding long-lasting ischemic insult comparable to IpreC [41]. This procedure was termed ischemic postconditioning (IpostC). IpostC with four episodes of 1-min balloon inflations starting within one min after reflow was recently performed in patients undergoing percutaneous coronary interventions leading to a significant reduction of myocardial damage as assessed by creatine kinase for surrogate marker [30].

Ischemic conditioning (pre- or post-) thus induces cytoprotection by subjecting the tissue to short-lasting ischemic stress. The immediate early protection phase relies on signaling events which activate innate cellular protective mechanisms [38]. The second delayed phase of protection occurring only ~20 hours later seems to depend on de novo protein synthesis after the initially triggering signals have subsided.

Despite the clinical observation mentioned before, treatments involving IpreC or IpostC are not advisable to be employed in patients with already compromised cardiovascular status. Pharmacological agents in lieu of ischemic conditioning may provide a more benign approach for eliciting cardioprotection in the clinical setting.

Cardioprotective mechanisms

From the large body of literature, it emerges that a wide variety of noxious stimuli can trigger cardioprotection including ischemia, hypoxia, heat shock, hypothermia, and Ca2+ overload [12, 27, 31, 33, 38]. Such insults produce the liberation of transmitters and hormones (adenosine, purines, angiotensin-II, endothelin, opioids, bradykinin, catecholamines, acetylcholine) which act systemically or in a paracrine fashion after accumulation within the interstitial tissue mainly by stimulating G-protein-coupled receptors (GPCR). Depending on the type of stimuli and their temporal occurrence, several different GPCRs may become activated together or in succession. Accordingly, different downstream signaling pathways may be activated in parallel or in temporal succession leading to cytoprotection. Many of the targeted receptors are coupled to phospholipase-C (PLC) whose activation catalyses the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), generating the secondary messengers inositol 1,3,4-trisphosphate (IP3) and diacylglycerol (DAG), which in turn, stimulate protein kinase-C (PKC) (Fig. 2). PKC is thought to be a central mediator of ischemic conditioning, which requires its translocation from the cytoplasm to anchoring proteins associated with the cytoskeleton and with membranes of cell organelles. PKC may directly phosphorylate the effector proteins or switch the signaling to other kinase cascades [38]. Additional signaling pathways involved comprise the protein kinases–G, and –A, members of the MAPK (mitogen activated protein kinases) family (ERK1/2, p38, JNK and BMK1), the PI3K – PDK1 – PKB(Akt) cascade, and the JAK – STAT pathway. In particular, the PKB(Akt) and the ERK1/2 pathways may play a dominant role in cytoprotective signaling after ischemic conditioning and have thus been called the RISK (reperfusion injury salvage kinase) pathways. The different signaling pathways finally converge and affect the target sites of some major cytoprotective mechanisms associated with the mitochondria including the mitochondrial ATP-dependent potassium channels (mitoKATP channels) and the mitochondrial permeability transition pore (mPTP) (Fig. 2).

The sarcolemmal KATP channels (sarcoKATP channels) seem also to play some role under certain conditions, particularly in small animal models like mice [12, 27, 33, 38]. Activation of the sarcoKATP channels (potassium flows out of the cell) cause membrane hyperpolarization and shorten the duration of the action potential (AP) thus reducing Ca2+ influx into the cell. Activation of the mitoKATP channels at the inner mitochondrial membrane (IMM) causes potassium to flow into the mitochondrial matrix (MM) for regulation of the mitochondrial matrix volume, supports coupling of the respiratory chain and ATP synthesis, attenuates Ca2+ entry from the cytoplasm, and inhibits apoptosis. Activation of the KATP channels during ischemia is thought to be due to phosphorylation by Ser/Thr-kinases PKC and/or PKG (Fig. 2).

The mPTP represents a multiprotein complex comprising the adenine nucleotide transporter (ANT) at the IMM and the voltage-dependent anion channel (VDAC or porin) at the outer mitochondrial membrane (OMM). Additional proteins may be associated with the mPTP at these contact sites between the OMM and IMM including the mitochondrial creatine kinase (mCK), hexokinase (HK), glycerol kinase (GK), the proapoptotic Bax and the antiapoptotic Bcl2 [1, 5, 27, 38]. The mPTP and the mitoKATP channel are functionally coupled in so far as opening of the mPTP counteracts all effects of the activated mitoKATP channel [10, 31]. When the mPTP opens irreversibly solutes exchange between the cytoplasm and the MM, Ca2+ enters the MM, ROS are generated, the proton gradient driving ATP synthesis collapses, and the energy production comes to a halt. Under such conditions the ATP synthase (complex-V) was observed to hydrolyze ATP thus using up what was left as energy source. Activation of the mitoKATP channels favors closure of the mPTP and may support survival while irreversible opening of the mPTP irrevocably leads to death. High Ca2+ in the MM, cyclophilinD (peptidyl-prolyl cis-trans isomerase), and depolarization promote

opening of the mPTP. Fatal opening of the mPTP most likely occurs during the early reperfusion phase [9, 38].

In principle, similar molecular mechanisms are also operative during the delayed second window of protection. However, the signaling pathways are supported by de novo protein synthesis induced by NF–kB and other transcription factors. Notably, the survival proteins iNOS (inducible nitric oxide synthase), Bcl2 (antiapoptotic protein), COX2 (cyclooxygenase-2), MnSOD (mitochondrial manganese superoxide dismutase), and heat shock proteins (HSP27/70) display an increased expression after ischemic conditioning. NF–kB is activated by dissociation from its inhibitor IkB when the latter has been phosphorylated by PKC [40].

Pharmacological conditioning

According to the different signaling pathways triggered by IpreC and IpostC for induction of cytoprotection, also different classes of pharmaceuticals have been identified to be able to elicit cardioprotection including opioids, adenosine and its analogues, KATP channel openers (KCO) such as cromakalim, pinacidil, diazoxide, nicorandil, and ether-derived halogenated volatile anesthetics (VA) like isoflurane and sevoflurane [31, 33, 39]. While opioids and adenosine have proven their cardioprotective potential in animal experiments, they did not make their way into the clinical armamentarium for this purpose. Neither could the KCOs be clinically used for cardioprotective therapy because their activity is not selective for the mitoKATP channels and their major effects in vivo relate to the sarcoKATP channels of smooth muscle cells causing vasodilatation. An interesting pharmacodynamic profile has been demonstrated for the anti-arrhythmic drug bepridil which exhibits a unique combination of inhibiting the sarcoKATP channels while activating the mitoKATP channels.

On the other hand, the VAs can afford powerful cytoprotection against severe ischemia in experimental animal models as well as in humans with coronary artery disease [11, 25, 34, 37, 38]. Furthermore, they are the best studied pharmaceuticals with regard to their mode of cytoprotective action not only in the heart but also in brain, kidney and liver.

Cardioprotection by volatile anesthetics

In a series of experiments we have delineated the major signaling pathways for VAs in cardioprotection on isolated ventricular adult rat cardiomyocytes (VARC) in short-term culture by life cell imaging microscopy [22, 23] and on isolated beating rat hearts in the Langendorff apparatus [3, 6, 7, 32, 43]. Signaling by VAs was usually compared to that elicited by ischemic conditioning. The experiments on isolated beating rat hearts combine functional contractile parameters with morphological (immunohistochemistry) and biochemical (immunoblotting and enzyme activity) parameters, and were finally complemented by studies on gene activity profiles [4, 16, 17, 28].

The effects of different commonly used anesthetics was tested on the intrinsic fluorescence of mitochondrial flavoproteins as a direct measure of mitoKATP channel activity and on survival of isolated cardiomyocytes exposed to 60 min of ischemia using subsequent hypoosmolar trypan blue staining. Diazoxide-induced mitoKATP channel opening was inhibited by the anesthetics R-ketamine and the barbiturates thiopental and pentobarbital. S-ketamine, propofol, midazolam and etomidate did not affect mitoKATP channel activity, while the VAs positively activated these channels. The significance of these modulatory effects of the different anesthetics on the mitoKATP channels was substantiated in the cellular model of simulated ischemia [35, 36].

Infarcts size and recovery of contractile parameters were similarly improved by VAs applied for pre-conditioning (VApreC) and for postconditioning (VApostC) when compared to IpreC and IpostC. The signaling pathways for conditioning were delineated by using inhibitors and activators specific for individual signaling components. The signaling pathways utilized by VAs for the early protection phase are summarized in Fig.2. Inhibition of beta-adrenergic receptors by propranolol or of alpha1-adrenergic receptors by prazosin had no effect on VA cardioprotection [35].

The two major signaling routes for VApreC are i) AdR1 – activation of PLC – production of IP3 and DAG – phosphorylation of PKC isoforms and subcellular translocation resulting in activation of mitoKATP channels, and ii) probably direct activation of NOS by VAs – production of NO – activation of soluble GCs by binding of NO to the heme group of the protein – generation of the second messenger cAMP – activation of PKG and stimulation of the mitoKATP channels (for details and abbreviations see legends of Fig.2). Inhibition of either of the two signaling routes by any of the involved blockers partially reduces cardioprotection. That both signaling routes converge on activating the mitoKATP channels can be demonstrated by the almost complete inhibition of cardioprotection when these channels are specifically blocked by 5HD. On the other hand, application of the specific channel opener diazoxide induces the cardioprotective effects like the VAs. Together with ROS, NO leads to formation of POXN, which may directly activate PKC by oxidation and/or nitration. This signaling route can be inhibited by the ROS scavenger MPG. In contrast, application of exogenous NO derived from SNAP (nitroso acetyl penicillamine) induces cardioprotection [3, 32].

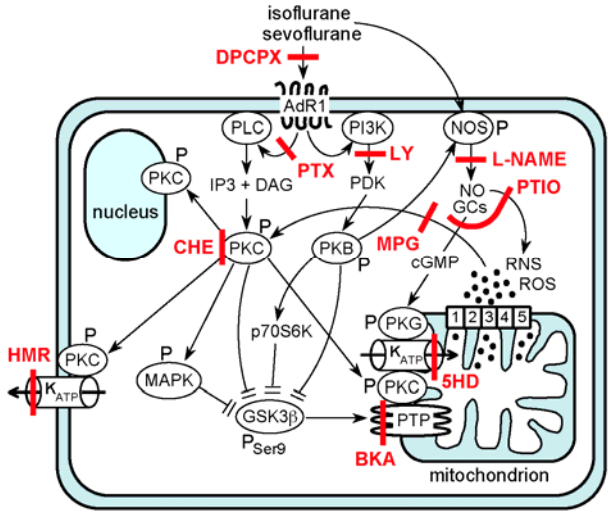

***Fig. 2**: Major signaling pathways for cardioprotective mechanisms by volatile anesthetics (VA, isoflurane and sevoflurane) in rat cardiomyocyte as assessed by using inhibitors specific for certain signaling components. The red bars and abbreviations denote inhibition of the corresponding signaling route. DPCPX (cyclopentyl dipropylxanthine) specifically inhibits the adenosine receptor1. PTX (pertussis toxin) inhibits the protein Gi signaling. CHE (chelerythrine) inhibits phosphorylation of protein kinase-C isoforms and their translocation to specific subcellular sites. DPCPX, PTX or CHE each partially inhibit cardioprotection by VAs. L-NAME (nitro L-arginine methyl ester) is a nonspecific inhibitor of the different nitric oxide (NO) synthase (NOS) isoforms (eNOS, iNOS and nNOS). Signaling by NO can also be inhibited by the NO scavenger PTIO (carboxyphenyl tetramethylimidazoline-1-oxyl-3-oxide). NO forms reactive nitrogen species (RNS) which react with the reactive oxygen species (ROS) originating from the partially uncoupled mitochondrial metabolism to form peroxynitrite which may directly activate PKC. This side-route can be inhibited by the free radical scavenger MPG (N-2-mercaptopropionyl glycine). Inhibition of the mitoKATP channel with the specific inhibitor 5HD (5-hydroxydecanoate) completely abrogates VA and ischemic preconditioning. The inhibitor HMR (HMR1098, cardiac selective sulphonurea derivative) specific for the sarcoKATP channels has no effect in either VA or ischemic preconditioning. Signaling from AdR1 via the PI3K (phosphoinositide-3 kinase) – PKB (protein kinase-B) path inhibits GSK3beta (glycogen synthase kinase-3 beta) which, in turn, keeps the mitochondrial permeability transition pore (PTP) closed is more prominent in postconditioning. This path can be inhibited by LY (LY294002, morpholinyl-phenyl-benzopyran) abrogating cardioprotection (PTP opens during reperfusion), while BKA (bongkrekic acid) mimicking conditioning inhibits opening of the PTP. In addition, MAPK (mitogen activated protein kinase) pathways may be involved in conditioning to various degrees. PDK = phosphoinositide-dependent kinase-1; p70S6K = ribosomal protein S6 kinase.*

VA signaling from the AdR1 probably via the G-beta-gamma component can also activate the axis PI3K – PDK – PKB resulting in inhibiting GSK3beta by phosphorylation at Ser9 (Fig. 2). Inactivation of GSK3beta is associated with keeping the mPTP closed during reperfusion by an as yet unknown mechanism. Pore opening and mitochondrial integrity can be assessed in two ways, i) biochemically by measuring the tissue content of NAD+ which is released from inactive dysfunctional mitochondria upon pore opening, and ii) morphologically by visualizing the active uptake of the red-fluorescing MitoTracker Red 580 by viable mitochondria, while dysfunctional mitochondria do not accumulate the stain [7]. The involvement of mPTP opening during reperfusion and cell damage can be shown by application of the pore opener atractyloside (a dipertenoid glycoside) or the inhibitor bongkrekic acid (BKA). As the mPTP typically opens during reperfusion, it seems natural that the signaling for mPTP closing plays a more prominent role in VApostC and in IpostC than in preconditioning procedures [6, 7, 43]. In addition to the PI3K – PKB pathway, cardioprotection may also be supported by inhibition of GSK3beta by MAPKs including ERK1/2 and p38. PKC seems to function as upstream kinase for the MAPKs pathways. This illustrates the intimate and complex cross-connections in cardioptrotective signaling (Fig. 2). The lack of effect of the specific HMR-1098 inhibitor indicates that the sarcoKATP channels are not significantly involved in cardioprotection induced by VA or ischemic conditioning in the rat heart.

Gene activity profiles in cardioprotection of rat hearts

VApreC resembles IpreC in so far as the major signaling pathways (PKC and NO routes) are also activated in IpreC [3, 6, 7, 32, 35, 36, 43]. On the other hand, the MAPKs (ERK1/2 and p38) are activated during the conditioning phase of IpreC but not of VApreC. However, the postischemic cellular and functional recovery proved to depend also in VApreC on activation of ERK1/2. These findings indicate that different gene activity profiles may be generated by the two different conditioning procedures (VApreC versus IpreC), despite a similar cardioprotective potential in both cases. Moreover, the profiles may also differ after the 40 min test ischemia was applied [4, 28]. Transcriptome profiles induced by VA or ischemic conditioning triggers and those obtained after ischemia-reperfusion injury are summarized in Fig. 3.

Without further analysis, it is clear from inspection of the gene expression matrix that the VA and the ischemic conditioning triggers regulate a number of genes in parallel, while a large portion of genes are differentially regulated as indicated by non-corresponding colored rows (first two column groups in Fig. 3). On the other hand, a large portion of genes display a similar color pattern after test ischemia alone

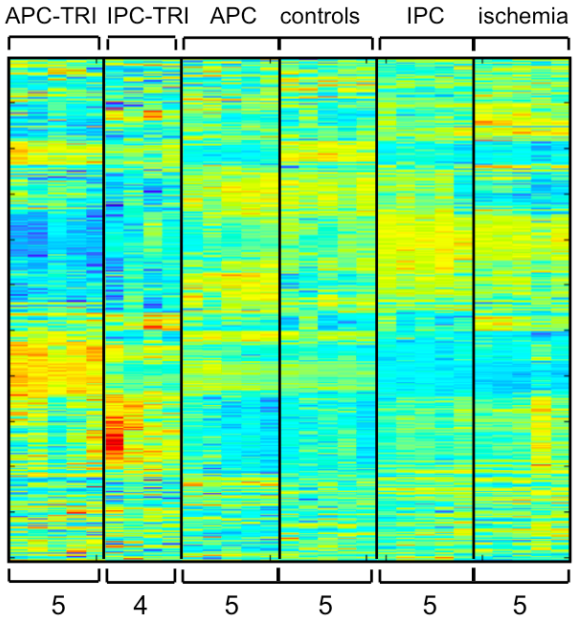

Fig. 3*: Transcriptome profiles of isolated beating rat hearts after conditioning trigger alone and after ischemia-reperfusion injury. Global gene expression matrix (heat map) of 2'212 ANOVA-filtered genes that are either upregulated (red) or downregulated (blue) derived from coupled two-way clustering with six experimental groups. Horizontal rows correspond to genes, and columns correspond to individual hearts (number of hearts per group is given underneath on the abscissa). APC-TRI = trigger by volatile anesthetic isoflurane (15 min followed by 10 min washout); IPC = ischemic conditioning trigger (3 cycles of 5 min ischemia interspersed by 5 min reperfusion); APC = APC-TRI followed by 40 min test ischemia; controls = time-matched perfusion without treatment; IPC = IPC-TRI followed by 40 min test ischemia; ischemia = 40 min test ischemia alone. Samples for gene chip analysis were taken in all cases at the time point corresponding to 180 min after completion of the 40 min test ischemia. Figure modified from Ref. 33.*

and after IpreC followed by test ischemia (last two column groups in Fig. 3). Finally, a similar correspondence of commonly regulated genes appears in time-matched perfusion controls when compared with VApreC followed by test ischemia (middle two column groups in Fig. 3).

The difference of activity profiles between the two conditioning triggers and the substantial overlapping of the profiles, first, between VA protection and untreated controls, and second, between ischemic protection and unprotected ischemic hearts, can be visualized by the method of principal component analysis, a technique used to reduce multidimensional data sets to lower dimensions [4].

These findings are partly unexpected in so far as both VA and ischemic conditioning provide cardioprotection by signaling for activation of the mitoKATP channels and for prevention of mPTP opening by activation of the reperfusion injury salvage kinase pathways, yet the two conditioning procedures induce two different gene activity profiles. Ischemic conditioning elicits cardio-protection by activation of endogenous reaction mechanisms against the afflicted sublethal cell damages. One may speculate that a number of these cellular reactions are similar or identical to those induced by severe tissue ischemia in unprotected hearts, thus contributing to the similar transcriptome profiles. On the other hand, the similar profiles after test ischemia with prior VA conditioning and that of untreated controls clearly favors this conditioning procedure for clinical application rather than ischemic conditioning. Moreover, VAs represents an accepted and widely used class of pharmaceuticals.

Clinical application of volatile anesthetics for cardioprotection

The general experimental findings on VA conditioning could be directly translated to clinical randomized trials [8, 13, 18]. In one set of 72 patients (40-80 years old) undergoing coronary artery bypass graft (CAGB) surgery (2-6 grafts) on cardiopulmonary bypass circuit (PBC) with cardioplegic arrest, preconditioning was applied by administering sevoflurane (4 vol%) or an oxygen-air gas mixture (placebo) for 10 min after initiating the anesthesia with either propofol or etomidate and immediately before cross clamping the aorta [13]. In a second set of 20 patients (50-80 years old) undergoing off-pump CABG surgery (2-5 grafts), anesthesia was maintained with either sevoflurane gas or with intravenous propofol from the moment of taking the first atrial biopsy (B1) before vessel surgery started until 180±40 min later when the second atrial biopsy (B2) was collected after termination shortly before chest closing [18]. The atrial biopsies were used for microarray analysis using Affymetrix GeneChip Human Genome U_133 Plus 2.0 arrays. Among several biomarkers measured in blood samples taken at 0, 24, 48 and 72 hours in both studies, plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) were significantly lower from 24 up to 72 hours post-surgery in patients treated with sevoflurane (Fig. 4A). High plasma levels of NT-preBNP are indicative of cardiac dysfunction due to myocyte damage. In addition, this finding indicates that in both clinical studies the sevoflurane treatment provided cardioprotection covering the delayed protection phase over several days [13, 18].

In a one-year follow-up of the first study with 72 patients, six patients (17%) of the placebo group developed adverse cardiac events such as coronary artery reocclusions and congestive heart failure (CHF), while only one patient (3%) of the sevoflurane group was newly affected by CHF [8].

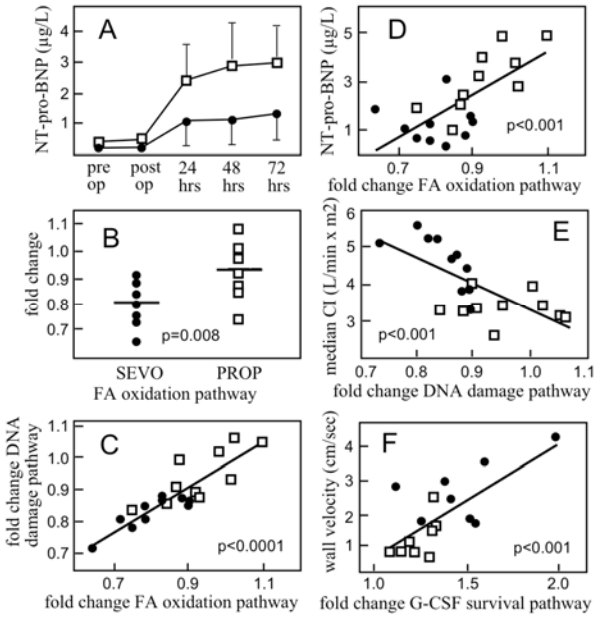

***Fig. 4**: Perioperative plasma levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and correlations between anesthetic-induced transcriptional phenotypes and post-surgery cardiac function of patients with coronary artery bypass graft (CABG) surgery (data taken from Ref. 40). (A) Plasma levels of NT-proBNP are significantly higher in the propofol than in the sevoflurane group from 24 up to 72 hours after the operation (time effect p <0.001; treatment effect p =0.002. (B) Differential regulation of the FA oxidation pathway. (C) Relation between metabolic changes in fatty acid (FA) oxidation and DNA-damage signaling. (D) NT-proBNP levels best correlate with FA oxidation path. (E) Cardiac index best correlates with DNA-damage signaling. (F) Early diastolic left ventricular wall movement best correlates with the granulocyte colony-stimulating factor (G-CSF) survival pathway. Black dots = sevoflurane; open squares = propofol; SEVO = sevoflurane; PROP = propofol.*

The perioperative peak concentration of the myocardial injury marker NT-preBNP was significantly higher in all patients subject to late cardiac events.

In the second study with CABG off-pump surgery patients, cardiac output and myocardial velocity mapping were determined by pulmonary artery catheter and echocardiography just before (B1) and after (B2) excision of the atrial biopsies [18]. Two atrial samples were collected per patient and used for hybridization (no sample pooling). Sample B1 constitutes the background expression profile before exposure to either sevoflurane or propofol, whereas sample B2 reflects the gene response to surgery in the two anesthetic groups. The existence and direction of gene expression changes over time (from B1 to B2) was reliably detected by the microarray technology, as confirmed by real-time quantitative polymerase chain reaction. The global gene matrix containing the expression values of the 54'675 probe sets was used as input for the Gene Set Enrichment Analysis (GSEA). GSEA considers pre-defined gene sets based on previous biologic knowledge such as signaling or metabolic pathways, or groups of genes pertaining to particular subcellular structural or functional entities, and determines whether the members of these sets are over- or under-represented in a list of genes that correlate with a specific phenotype or class distinction. The output is a normalized enrichment score that represents a measure of the degree of enrichment (positive or negative or no significant correlation) of the gene set in question.

The results revealed over 500 pathways differing over time from B1 to B2 (start and end of bypass surgery) in sevoflurane and propofol patients, the bulk (494) of which was commonly regulated by both anesthetic regimens (Table 1). Among the pathways differently regulated by the two anesthetics, sevoflurane compared to propofol, reduced the transcriptional activity of genes in the fatty acid (FA) oxidation pathway and in the DNA-damage signaling cascade, while the genes in the granulocyte colony-stimulating factor (G-CSF) survival pathway were upregulated (Fig. 4). The tight correlation between these regulated pathways over both anesthetic groups implies a possible link between the metabolic phenotype and cardioprotection.

Conclusions and outlook

The results show that inhalation of sevoflurane for only 10 min immediately before cardiovascular surgery, or alternatively, when used to maintain anesthesia during surgery for around three hours, can provide effective cardioprotection up to several days post-surgery. On a genome-wide basis we have shown that different anesthetics differently modulate the transcriptional activity in response to cardiac surgery in human hearts. The genomic reprogramming induced by the volatile ether sevoflurane, as compared to the intravenous anesthetic propofol, seems to exert cardioprotection over a period of at least one year.

The reduction of FA oxidation leads to a relative increase in glucose utilization via the so-called glucose-fatty acid cycle [26]. Even small but coordinate changes in a majority of genes encoding members of a particular pathway (gene set) may dramatically alter the flux through that pathway. In the postischemic myocardium FA oxidation provides nearly 100% of the cellular energy requirement [14]. The relative shift away from FA oxidation was shown to improve contractile recovery after ischemia and affect long-term outcome [15]. In fact, favoring the energetically more economical glucose (3.17 ATP per oxygen molecule) over FA oxidation (2.83 ATP per oxygen molecule) may be beneficial in the mechanically stressed heart with only limited oxygen supply.

Table 1. Number of pathways (enriched gene sets) up- or down-regulated in atrial biopsies (B2) taken 180 ± 40 min after anesthesia with either sevoflurane gas or intravenous propofol in comparison to biopsies (B1) taken at the start (baseline) of the two anesthetic regimens in patients with off-pump CABG surgery (see text for details).

Regulation of gene pathways	Specific for sevoflurane	Commonly regulated with both anesthetics	Specific for propofol
Up-regulated	14	266	39
Down-regulated	39	228	14

In conclusion, our results indicate that experimentally applied VAs mobilize cardioprotective cellular mechanisms including activation of the mitoKATP channels and preservation of a functionally intact mPTP.

Furthermore, analysis on a genome-wide basis revealed that treatment of CABG surgery patients in the perioperative phase with VAs reduces the activity of genes involved in noxious pathways and shifts the energy metabolism away from FA oxidation towards the more economical glucose utilization. Most importantly, the beneficial effects of the VAs are long-lasting and patients may suffer from fewer adverse cardiac events over a period up to one year or more. Therefore, clinical surveys are required to establish whether the application of VAs in cardiovascular and non-cardiovascular surgery will be beneficial for the patients with regard to peri- and post-operative outcome including cardiovascular complications, hospitalization time and mortality.

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