The influence of anaesthetic and ischaemic preconditioning on generation of reactive oxygen species in the coronary sinus in coronary artery bypass graft patients

Wpływ anestetycznego i niedokrwiennego hartowania serca na generację wolnych rodników tlenowych w zatoce wieńcowej u pacjentów poddanych chirurgicznej rewaskularyzacji mięśnia sercowego

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Abstract

Aim: This study on patients scheduled for elective coronary artery bypass graft surgery was designed to establish whether different preconditioning protocols affect the generation of coronary sinus sampled reactive oxygen species.

Material and Methods: Thirty patients were randomized into three groups: anaesthetic preconditioning (APC) with 20 minutes of sevoflurane 2 vol% inhalation and 10 minutes washout before aortic cross-clamping (n = 10); ischaemic preconditioning (IPC) (n = 10), with 2 cycles of 2-minute ischaemia followed by 3-minute reperfusion before cross-clamping; or control without preconditioning (n = 10). ROS content was measured in coronary sinus blood: before cardiopulmonary bypass (CPB), 10 minutes after APC or IPC protocol or 10 minutes after CPB in the control group, and 10 minutes after aortic declamping in all groups. Electron paramagnetic resonance spectroscopy (EPR) was used to quantitatively measure ROS activation in coronary sinus blood. Lactate, interleukin-6, interleukin-10, and troponin I blood content was measured. Haemodynamic data were collected.

Results: ROS generation in both preconditioning groups after protocol was significantly higher compared to control. It was 108% in the IPC and 96% in the APC group, compared to 64% in the control group. Interleukin-6 arterial blood concentration was significantly higher in all groups after surgery, but was significantly lower in the APC group, 83 (60.88-200.9) pg/ml, compared to the IPC, 177.46 (60.1-293.4) pg/ml, and control group, 200.81 (117.1-325.8) pg/ml, measured six hours after surgery.

Conclusion: The results of the study may suggest that anaesthetic as well as ischaemic preconditioning increased ROS activity

Streszczenie

Cel: Celem pracy była kliniczna ocena wpływu niedokrwiennego (IP) i farmakologicznego hartowania (AP) z zastosowaniem sewofluranu na generację wolnych rodników w zatoce wieńcowej, badaną za pomocą spektroskopii elektronowego rezonansu paramagnetycznego (EPR). Obserwację przeprowadzono u chorych poddawanych planowym zabiegom rewaskularyzacji wieńcowej w warunkach normotermicznego krążenia pozaustrojowego.

Materiał i metody: Prospektywnym i randomizowanym badaniem objęto 30 pacjentów. Chorych podzielono na trzy grupy: w grupie AP włączano wziewnie sewofluran w stężeniu 2 vol%, którego podaż kontynuowano przez 20 min, a następnie odłączano 10 min przed zaklemowaniem aorty. W grupie IP aortę klemowano dwukrotnie na 2 min z 3-minutową reperfuzją po podłączeniu CPB. Aktywność wolnorodnikową mierzono z krwi z zatoki wieńcowej. Badanie przeprowadzono przed podłączeniem CPB, po procedurze hartowania oraz po 10 min reperfuzji.

Wyniki: Aktywność WR uzyskanych w grupie AP i IP była znamiennie wyższa w porównaniu z grupą kontrolną p = 0,02 i p = 0,005, w okresie po stymulacji, bez różnic między grupami AP i IP (p = 0,6). 10-minutowa reperfuzja powodowała istotny spadek aktywności WR we wszystkich badanych grupach do poziomu zbliżonego na początku badania.

Wnioski: Sewofluran podawany wziewnie w stężeniu 2 vol% przez 20 min z 10-minutową przerwą po ekspozycji przed zaklemowaniem aorty stymuluje aktywację wolnorodnikową mierzoną we krwi zatoki wieńcowej za pomocą EPR. Intensywność tej reakcji ma nasilenie podobne do aktywacji wolnorodnikowej uzyskiwanej po procedurze hartowania niedokrwiennego z dwoma 2-minutowymi cyklami niedokrwienia i 3-minutowej

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in coronary sinus blood, measured with EPR quantitative analysis, compared to control.
Key words: volatile anaesthetics, pharmacological preconditioning, ischaemic preconditioning, reactive oxygen species, electron spin spectroscopy.

reperfuzji u chorych poddawanych chirurgicznej rewaskularyzacji wieńcowej z zastosowaniem CPB.
Słowa kluczowe: anestetyki wziewne, hartowanie farmakologiczne, sewofluran, hartowanie przez niedokrwienie, wolne rodniki tlenowe.

Introduction

Heart ischaemic preconditioning (IPC) was first described by Murry and was rapidly recognized as one of the most powerful protective mechanisms against ischaemia [1]. The clinical application of IPC protocols is still controversial [2, 3.]. Volatile anaesthetics contribute to myocardial protection, in a similar fashion to IPC. The acute cardioprotective effect lasts beyond their elimination and the effect is known as anaesthetic preconditioning (APC) [4]. The distinct advantage of APC compared to IPC is that it does not require ischaemia to produce this protective effect. Formation of reactive oxygen species (ROS) is one of the key mechanisms involved in myocardial preconditioning and postconditioning [5, 6].

Cardiopulmonary bypass (CPB) enhances and stimulates endogenous production of inflammatory mediators and cytokines in several organs. An influx of those substances to the systemic circulation during reperfusion is responsible for a systemic inflammatory response [7]. Whether preconditioning can modulate the inflammatory response to CPB is not clearly elucidated yet.

Reperfusion of ischaemic hearts results in an explosive release of ROS, which leads to contractile heart dysfunction. *In vivo* investigation of these reactions is complicated. Electron paramagnetic resonance spectroscopy (EPR) is one method used for ROS detection.

ROS are present only for a limited time in high concentrations in blood and tissue due to their short half-life.

To be able to investigate ROS levels, a small reactive molecule (spin trap like) can be used. Spin traps can react with ROS to generate more stabile radicals, indirectly making it possible to obtain information about the primary ROS [8]. ROS activation can thus be studied quantitatively and used to evaluate different preconditioning protocols.

The aim of this study was to evaluate whether IPC and APC with sevoflurane affect ROS activation in coronary sinus blood, measured by EPR analysis, in patients scheduled for elective coronary artery bypass graft surgery (CABG). Additionally the purpose of the study was to define whether preconditioning can modulate the inflammatory response to CPB measured with interleukin-6 (IL-6) and interleukin-10 (IL-10) plasma.

Material and Methods

Study subjects

After institutional approval of the study design, informed consent was obtained from all patients. Thirty patients scheduled for first-time elective CABG under CPB were included in the study. Patients with poor myocardial function (ejection fraction < 40%), unstable angina, diabetes, renal or liver failure were excluded.

Preconditioning protocol

The patients were computer randomized before surgery, in a prospective blind fashion for the patient, to one of three groups. In the ischaemic preconditioning (IPC) group (n = 10) CPB were established and the pump was running to empty the heart. Then the ascending aorta was occluded by cross-clamping for 2 minutes, followed by 3 minutes of reperfusion. The clamping procedure was repeated once.

In the anaesthetic preconditioning (APC) group (n = 10) sevoflurane 2 vol% was added from the vaporizer on the anaesthesia machine or, when on bypass, from a vaporizer connected to the heart-lung machine for 20 minutes with 10 minutes washout time prior to aortic cross-clamping to mimic the ischaemic preconditioning protocol. Propofol was disconnected during sevoflurane administration.

In the control (C) group (n = 10) the pump had been operating for 10 minutes before the operation commenced.

Anaesthesia

All patients were premedicated one hour before the operation with morphine 0.1 mg/kg intramuscularly and midazolam 7.5 mg orally. Anaesthesia was induced with propofol TCI 2-4 µg/ml and fentanyl 5-7.5 µg/kg. Pancuronium 0.1 mg/kg was given to facilitate intubation. Anaesthesia was maintained with propofol TCI 2-3 µg/ml and fentanyl 5-10 µg/kg/h as a continuous intravenous infusion. The depth of anaesthesia was evaluated using Entropy Sensor (Datex-Ohmeda). Ventilation was controlled artificially with a mixture of air and oxygen (FiO_2 0.6), while maintaining the end tidal carbon dioxide concentration of 4.7-6 kPa. In all patients, a pulmonary artery catheter (Criticath® – BD USA) was advanced into the pulmonary artery. Surgical techniques were the same in all patients and were performed by 1 of 2 cardiac surgeons using a uniform myocardial protective technique. Cardiopulmonary bypass with non-pulsatile perfusion flow (2.2-2.4 l/min/m^2) was conducted using Dideco Compacflo (Sorin Group) membrane oxygenators, which are permeable to anaesthetic agents. Normothermia (37°C) was maintained during the entire procedure. Aortic root and two-stage single venous cannula were used for CPB. A retrograde self-inflating cardioplegia cannula was guided into the coronary sinus. A nine-gauge cannula was placed in the aortic root for anterograde cardioplegia and for venting.

After aortic cross-clamping, warm blood cardioplegia was delivered with perfusate temperature according to the Calafiore protocol [9]. Next the cardioplegic solution was delivered retrograde to the coronary sinus, after each distal peripheral anastomosis was completed or every 15 minutes.

Postoperatively, the patients were transferred to ICU with ongoing mechanical ventilation. Sedation with propofol 0.5-1 mg/kg/h was continued until the patient was eligible for weaning from the ventilator and the tracheal tube was removed. The time from the end of surgery to tracheal tube removal was noted. Postoperative course was monitored to detect myocardial ischaemia, need for inotropic pharmacological support, and heart rhythm disturbances.

Haemodynamic data

Haemodynamic data were collected at four separate time points: after induction of anaesthesia, 30 minutes after emerging from CPB, 6 hours after surgery and 24 hours after surgery. Haemodynamic monitoring included heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and central venous pressure (CVP). Cardiac output was measured with the bolus thermodilution method. Cardiac indexes (CI) and systemic and pulmonary vascular resistance index (SVRI, PVRI) were calculated.

Laboratory measurements

ROS and lactate were measured in all patients by drawing 10 ml of blood from the coronary sinus at three different time points. Blood was drawn before CPB, at the end of IPC or APC protocol or 10 minutes after CPB in the control group, and 10 minutes after cross-clamping release in all groups.

Blood for IL-6 and IL-10 measurements were collected from the coronary sinus before CPB, 10 minutes after cross-clamping release and 6 hours after declamping from arterial blood. Blood for troponin I (Tn I) measurement was collected from arterial blood before CPB, and 6 and 24 hours after surgery.

Laboratory methodology

Coronary sinus blood samples for ROS were collected in syringes containing 1 ml 120 mmol/l solution of the spin trap α-phenyl-tert-butylnitrone (PBN) (Sigma–Aldrich UK). Blood was allowed to clot at freezing temperature in the dark, to protect from trap photolytic degradation, for 10 minutes before being centrifuged for the next 10 minutes at 3500 rpm. A volume of 0.5 ml HPLC-grade toluene was added to the serum/adduct and vortex mixed for 30 seconds. The PBN adduct was extracted and placed in an ESR-sample tube. The sample was then vacuum de-gassed using a turbo pump and stored in liquid nitrogen for analysis the same day.

EPR analyses were performed on ESR spectroscope model 300E (Bruker-Karlsruhe, Germany) in series, X-band spectrometer with 100 kHz frequency modulation using the microwave frequency (ca. 9 GHz). For quantitative assessment ROS generation was expressed in arbitrary units.

The accuracy of the assays is all very technique dependent and for that reason quality control was provided before the study, to confirm that the methodology was robust.

Plasma samples for interleukin evaluation were stored at –70°C. Commercially available kits measured by ELISA for the analyses of IL-6, IL-10 (Milenia, Germany) and troponin I (Boehringer, Mannheim, Germany) were used.

Blood lactate concentration was measured by a blood gas analyzer (Ciba Corning 860; Chiron diagnostics GmbH, Fernwald, Germany).

Statistics

Statistical analyses were performed using, depending on the distribution of data (distribution analysis – Shapiro-Wilk test), parametric (univariate and multivariate ANOVA) and non-parametric (ANOVA rank Kruskal-Wallis, Mann-Whitney test, Wilcoxon test) tests as appropriate. Descriptive statistics are presented as medians (min-max values), since most values did not follow a normal distribution. MS Statistica 7.0 was used for statistical analysis. A probability value of $p < 0.05$ was considered statistically significant.

Results

Baseline and preoperative characteristics

There was no difference between the APC, the IPC and the control groups with respect to age, weight, or number of distal anastomoses, use of left internal mammary artery, or use of venous grafts. Similar operative course was noted including total CPB time and aortic cross-clamp time. Recovery of spontaneous electrical heart activity was noted in all patients during reperfusion after aortic cross-clamp release (Table I). The course after operation was similar in all groups with respect to the time of artificial ventilation and tracheal tube removal. Inotropic support in the early

Tab. I. Patients' demographics and surgical data – data are presented as medians (min-max). Anaesthetic preconditioning (APC) and ischaemic preconditioning (IPC)

	APC (n = 10)	IPC (n = 10)	Control (n = 10)
Gender f/m	2/8	2/8	3/7
Age (years)	63.5 (48-73)	60 (55-74)	62 (55-72)
Body weight (kg)	71 (62-86)	71 (60-88)	68 (58-91)
Height (cm)	168 (154-180)	170 (150-179)	164 (159-178)
Ejection fraction (%)	67.5 (45-86)	65 (55-80)	69 (45-80)
Number of grafts	3.1 (2-4)	3.0 (2-4)	2.9 (2-4)
CPB time (min)	81 (49-116)	76 (38-126)	81.5 (32-120)
Aortic cross-clamp time (min)	41 (27-70)	37 (16-66)	38 (21-60)
Extubation time (h)	6.9 (3.8-9.15)	6.95 (3.6-9.1)	7.22 (4.0-9.2)

** $p < 0.05$ between the study groups.*

Tab. II. Haemodynamic parameters collected at certain measurement times. Data are presented as medians (min.-max.)

Parameter		T1	T2	T3	T4
HR (1/min)	APC	73.0 (54-84)	91 (66-117)	96 (78-114)	82 (74-102)
	IPC	63.0 (52-98)	80. (71-89)	89 (79-115)	87 (80-105)
	C	66.5 (53-92)	81 (66-97)	90 (77-124)	82 (70-108)
MAP (mm Hg)	APC	80 (71-106)	85 (69-109)	87 (68-96)	75.5 (60-95)
	IPC	92 (81-99)	81 (74-88)	87 (79-100)	76 (54-99)
	C	85 (56-118)	74 (62-84)*	78.5 (62-86)*	77.5 (68-90)
MPAP (mm Hg)	APC	14 (5-22)	19 (14-38)	24.5 (18-32)	18 (10-27)
	IPC	19 (7-24)	21 (12-25)	24 (17-28)	20 (15-24)
	C	22 (12-25)*	20.5 (14-24)	24 (20-27)	21 (19-24)
CVP (mm Hg)	APC	9 (0-10)	8.5 (1-15)	10 (6-14)	8 (5-14)
	IPC	8 (1-13)	10 (4-14)	12 (9-14)	9. (5-14)
	C	8.5 (5-11)	9.5 (6-12)	11 (8-16)	10 (9-12)
PCWP (mm Hg)	APC	8.5. (1-13)	12 (7-21)	13.5 (10-18)	8 (5-16)
	IPC	10 (3-19)	13 (6-20)	13 (9-16)	12 (10-15)
	C	11.5 (8-14)	11.5 (7-13)	13.5 (11-16)	12. (9-16)
CI (l/min/m^2)	APC	2.2 (1.9-3.3)	2.8 (1.4-4.9)	3.2 (2.4-3.9)	2.7 (1.9-3.4)
	IPC	2.3 (1.6-2.9)	2.5 (1.9-3.3)	2.7 (1.7-4.2)	3.1 (2.5-4.3)
	C	2.3 (2.1-2.5)	2.1 (1.9-3.1)	2.4 (1.9-4.0)*	2.4 (2.0-3.6)
PVRI (dyne*cm^5/m^2)	APC	229 (38-428)	187 (114-442)	241 (110-377)	253 (160-365)
	IPC	239 (136-593)	227 (131-326)	361 (87-465)	191 (55-332)
	C	307 (137-443)	343 (77-547)	378 (156-457)	333.5 (110-378)
SVRI (dyne*cm^5/m^2)	APC	2668 (1529-4592)	2168 (1224-3472)	1800 (1200-2573)	1936 (1270-3407)
	IPC	2852 (2282-4254)	2377 (1757-3201)	1967 (898-3545)	1529 (909-2194)
	C	2782 (1727-3870)	2460 (1116-2694)	2217 (1120-2863)	2066 (1318-2729)

** $p < 0.05$ between the study groups.*

post-CPB period was necessary in 3 patients in the APC group, two in the IPC group and 3 in the control group. Atrial fibrillation was noted on the first day after surgery in one patient in the IPC group, one patient in the APC group and 2 in the control group. Administration of vasopressors was similar in all groups.

ROS activation

The data obtained for ROS measurement allowed us to evaluate data from 25 patients (Fig. 1). In 5 cases spin trap samples were not eligible for further analysis or the profiles were incomplete. Some ROS activity was observed before CPB was started, with no difference between groups. ROS generation activity was significantly increased at the end of the preconditioning protocols and 10 minutes after CPB in the control group, compared to the initial values. ROS generation measured in blood from the coronary sinus in the IPC group and APC group was significantly higher than the ROS generation in the control group, $p < 0.05$. No difference was seen between the IPC and APC groups at that time. Ten-minute reperfusion after aortic cross-clamping release yielded a significant decrease of ROS activity in all groups comparable to baseline values with no significant difference between groups.

Haemodynamics, inflammation and other laboratory findings

There were no differences between groups in measured or calculated haemodynamic variables during the time period, except for lower cardiac index in the control group, six hours after surgery, and lower MAP in this group at six and 24 hours after surgery, $p < 0.05$.

A significant increase in cardiac index was noted over time in all groups (Table II).

IL-6 and IL-10 concentrations were similar in all groups at the beginning of the observation (Table III). IL-6 significantly increased in all groups during reperfusion. The highest concentration of IL-6 was noted 6 hours after surgery, $p < 0.05$. Intensity of increase was smaller in the APC group compared to the IPC and control group six hours after surgery, $p < 0.05$. This sampling however for obvious reasons was drawn from arterial blood and can be considered rather as an indicatory value. IL-10 concentration increased significantly ($p < 0.05$) in all groups during reperfusion. The decrease at six hours after surgery for the same reason as IL-6 measurement can be treated as an indicatory value.

A significant ($p < 0.05$) increase in lactate generation was noted in the coronary sinus in all groups over time with no difference between groups.

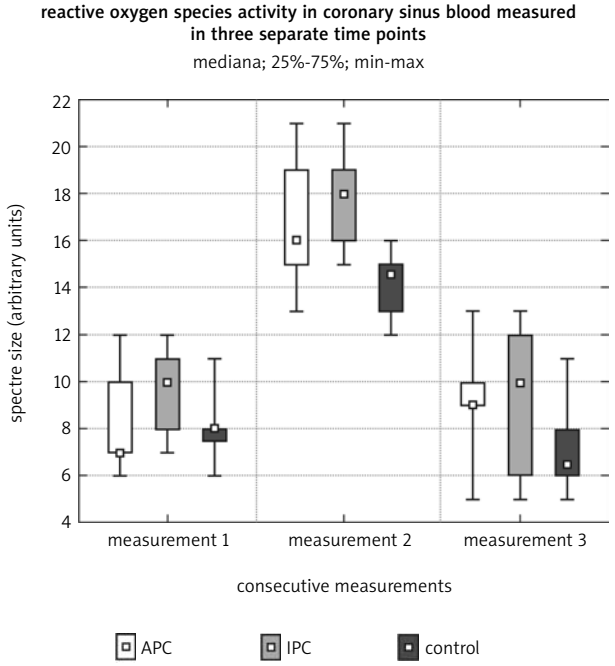

Fig. 1. Reactive oxygen species activity on coronary sinus blood measured in three separate time points. Consecutive measurements were made: before CPB (measurement 1), 10 minutes after preconditioning protocol or 10 minutes after CPB in control group (measurement 2), 10 minutes after aortic cross-clamping release (measurement 3). Reactive oxygen species activity is expressed using arbitrary units. Data are presented as medians and min-max. Anaesthetic preconditioning (APC) n = 9 and ischaemic preconditioning (IPC) n = 8, control = 8

Tab. III. Interleukin 6 (IL-6), pg/ml and interleukin 10 (IL-10), pg/ml in plasma. Sampling times were: before CPB from coronary sinus (1), 10 minutes after aortic cross-clamping release from coronary sinus (2), and 6 hours after operation from arterial blood (3). Data are presented as medians (min-max). Anaesthetic preconditioning (APC) and ischaemic preconditioning (IPC)

		APC	IPC	Control
Il-6	sample 1	36.8 (4.2-64,1)	39.5 (3.8-62.1)	40.9 (3.7-61.3)
	sample 2	50.85 (9.7-173.4)	47.1 (10.9-149.6)	13.45 (5.8-234.7)
	sample 3	83 (60.88-200.9)*	177.46 60.1-293.4	200.81 (117.1-325.8)
Il-10	sample 1	3.34 (1.2-6.8)	5.01 (1.4-9.8)	4.89 (0.9-11.5)
	sample 2	341 (25-1988)	268.75 (87.5-825)	115 (12.5-512)
	sample 3	122.7 (62.5-187.5)	156.25 (12.5-662)	111.25 (50-337.5)

** p < 0.05 between the study groups.*

Troponin I concentration increased 6 and 24 hours after surgery in all groups, with no differences between groups (data not shown).

Discussion

All protocols stimulated ROS activity in coronary sinus blood measured with EPR analysis. This may indicate the usefulness of the method for quantitative analysis of ROS activation in coronary sinus blood in different clinical conditions. Statistical comparison between the study groups may suggest higher ROS activation in both preconditioning groups when compared to the control. ROS reactions are for many reasons difficult to follow. Spin trapped radicals retain their activity; therefore the ROS concentration measured in the present study may be lower than the amount actually generated after the preconditioning protocol and during the reperfusion period. Some radicals undergo degradation before being PBN trapped; others can be lost during preparation, storage and transport. Detected ROS signals during measurement were however distinct enough to allow comparative analysis.

Direct ROS generation in response to IPC in CABG patients was evaluated by Wu et al. [2]. According to our knowledge there has not been reported any clinical study on the possible impact of sevoflurane on ROS activation with EPR analysis.

The role of ROS in IPC was suggested by Murry, who demonstrated that radical scavenging systems had the ability to abolish preconditioning effects during reperfusion [10]. Experimental data indicted that ischaemic and anaesthetic preconditioning share many common intracellular signalling pathways, with the mitochondrial ATP-dependent potassium channels playing a pivotal role in both types of cardioprotection. Although the exact signalling pathway is not yet fully understood, all of these observations suggest that volatile anaesthetics precondition the myocardium by mechanisms similar to IPC but they have the distinct advantage of not requiring ischaemia to produce this effect [11]. Volatile anaesthetics are lipophilic and can easily diffuse through cellular and subcellular membranes. They do not require ionic or covalent binding to specific receptors but can interact with lipophilic amino acids to cause conformational changes in membranes, channels, and enzymes. In this way, they can alter mitochondrial electron transport, and can cause enhanced electron leakage and ROS generation, which may act as a trigger for preconditioning [12]. Such a mechanism was postulated in reference to isoflurane and partially to sevoflurane. Direct ROS generation was demonstrated during and after anaesthetic exposure in animal heart models [13, 14]. APC can be abolished by ROS scavengers [14-16].

ROS appear to play a dual, and apparently paradoxical role in APC, as formation of a small quantity of ROS is required to trigger APC, while decreased ROS formation during subsequent ischaemia and reperfusion may underlie, at least in part, the functional and structural preservation [13]. Some studies strongly implicate the electron transport chain of mitochondria as the most likely source of anaesthetic-induced ROS generation, but the exact mechanism of this activity is unclear [13, 17].

Some separate investigations using EPR have demonstrated that most ROS activation in the coronary sinus hap-

pens at the beginning of the reperfusion [2]. This is in some contrast with our findings. Differences in protocol such as the type, temperature and the way of cardioplegia solution delivery during CPB, which all directly influence heart preconditioning, may explain the different results. An important factor is antioxidant and scavenging properties of the blood itself. Cardioplegic protocol with the use of warm blood cardioplegia during aortic cross-clamping and perfusion of the heart in a definite time interval causes periodically recurrent ROS scavenging during the intermittent perfusion of the heart. This could explain the lack of intensified ROS activity at the beginning of reperfusion in our study and confirmed efficacy of such a protection method during CPB. Volatile anaesthetic APC application has been given with short periods of exposure and washout of the drug [18]. However, now it seems that the best results are gained when volatile anaesthetics are administered during all the time of operation before and during CPB, as well as during reperfusion [19, 20]. Recently it has been reported that sevoflurane use during CABG surgery prolonged and improved the prognosis in one-year observation [21]. Our intention was to use an APC protocol similar to the IPC protocol, since we found it the most correct methodologically. However, we were not able to follow the exact time sequences of events. One of the problems was duration of sevoflurane delivery: shorter time of sevoflurane delivery with low flow of fresh gas delivery, both on anaesthesia and CPB machine, could cause the problem to reach target sevoflurane concentration, and higher sevoflurane concentration could cause circulatory collapse.

Anti-inflammatory properties of volatile anaesthetics were suggested by some recently conducted studies [22]. Experimental studies have shown that pro-inflammatory cytokines increase measured in coronary sinus blood takes place in the postoperative period as well. IL-6 concentrations rise constantly and are independent from CPB time. In this study we were not able to measure IL-6 in coronary sinus blood in the postoperative period for obvious reasons. The smaller IL-6 increase in arterial blood found in our study in the early postoperative period may suggest anti-inflammatory properties of sevoflurane already after twenty minutes of inhalation. Experimental studies have shown that pro-inflammatory cytokines increase in coronary sinus blood in the postoperative period as well. IL-6 concentrations have been shown to rise constantly and independently from CPB time. Since ROS stimulation in our study was similar after both preconditioning protocols it seems that sevoflurane anti-inflammatory properties are not necessarily related to ROS activation routes of protection. Sevoflurane in 2 vol% concentrations when given as an adjunct to cardioplegia solution attenuates the inflammatory response after CPB monitored not only with IL-6 but also CD11b/CD18 and TNF-α [23]. IL-10 concentration increased to compensate for the production of IL-6, and sevoflurane did not suppress the increase of this anti-inflammatory cytokine.

In conclusion, our study may suggest that sevoflurane given for twenty minutes in 2 vol% concentration, with 10 minutes washout time before aortic cross-clamping, activates ROS generation in coronary sinus blood. The intensity of this activation is of similar magnitude to two cycles of two-minute ischaemia followed by 3-minute reperfusion IPC protocol before cross-clamping, in patients undergoing CABG surgery with CPB. The role of intermittent warm blood cardioplegia in possible inhibition of ROS activation in the early reperfusion period during CPB should be stressed. EPR is a valuable method for quantitative analysis of ROS activation in coronary sinus blood and is useful to evaluate different preconditioning protocols.

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Komentarz

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Myocardial preconditioning is a well known phenomenon of heart muscle protection against ischaemic injury in experimental models of ischaemia and reperfusion. The principal effect of this protection is a huge reduction of infarct area (in some models 70% reduction of the infarct area) in the preconditioned heart. This is the most powerful endogenous mechanism of protection known in nature. Mechanisms of myocardial protection by preconditioning have been studied for more than thirty years and a lot of new data are accumulating simultaneously with the progress in molecular biology almost every year. Research areas are mainly focusing on two aspects of myocardial preconditioning: **trigger mechanisms which may induce protection** and **mechanisms of this protection**. The clinical relevance of this approach is obvious. It has been shown in the meantime that preconditioning is not only valid for myocardium, but has been observed in other tissues as well. Surprisingly, not only ischaemic insult but other stress factors may induce preconditioning and protection. Beside that there are many types of myocardial protection such as: early (lasting a few hours), late (a few days), remote (trigger not in the heart but for example in the limb) and postconditioning (protection during the reperfusion phase after ischaemia). For many years numerous clinical data, mainly in cardiology and cardiac surgery, have shown the presence of the mechanism in a clinical setting. For more than ten years myocardial "preconditioning" has been a field of interest in cardiac anaesthesia.

Volatile anaesthetic as a trigger of myocardial protection has been recognized and this protection is called "anaesthetic preconditioning" [1]. Modern inhalation agents used for general anaesthesia mimic the protective effects observed in experimental protocols of ischaemic preconditioning. This protective effect was observed in numerous patients undergoing cardiac surgery. The improvement in the outcome of coronary surgery patients receiving sevoflurane was published a few years ago [2]. Protective measures against ischaemic events can be taken before ischaemia (as presented in the paper of Goździk W et al. in this issue of Kardiochirurgia i Torakochirurgia Polska), during ischaemia or after ischaemic insult. The authors of the cited paper did not find a profound cardioprotective effect with sevoflurane or ischaemic preconditioning in the limited number of cases studied, but the increased production of reactive oxygen species (ROS) in coronary sinus blood after anaesthetic as well as after ischaemic preconditioning protocols has been documented. Of note is the presence of ROS activity in coronary sinus blood in the control group of patients after 10 min of cardiopulmonary bypass. This indicates the universal mechanism of ROS production in the human body.

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