ORGAN PROTECTIVE EFFECT OF ISOFLURANE ANESTHESIA IN CARDIAC SURGERIES WITH ARTIFICIAL CIRCULATION

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The aim of the investigation is to give full estimation of organ protective effect of isoflurane anesthesia in cardiac surgeries with artificial circulation.

Materials and Methods. There were analyzed the results of clinical, functional, biochemical and morphological examinations of 424 patients operated under the conditions of artificial circulation using two variants of anesthesia: 203 patients were given isoflurane (experimental group); 221 patients — propofol (control group). There were studied clinical progression of rehabilitation period, the change of myocardial contractile function indices, myocardial ultrastructure, and biochemical blood values after the operation.

Conclusion. Isoflurane anesthesia provides an additional protective effect in cardiac surgeries with artificial circulation. This effect is proved by complex clinical studies regarding the heart, liver, and kidneys. Myocardial effect of additional protection is supported by clinical, functional, morphological, and biochemical criteria.

Key words: isoflurane; organ protective effect; surgeries with artificial circulation.

Inhalation anesthetics have protective effect not only on the heart but also on other organs [1]. Experimental studies have shown isoflurane to reduce an inflammatory response of neutrophils preventing them from interacting with endothelial cells after reperfusion [2]. There have been noticed the reduction of pulmonary capillaries permeability and resistance of blood vessels of the lungs, as well as TNF-α level, the main trigger of inflammation cascade [3]. In experiments with isolated liver, inhalation anesthetics reduced oxygen intake and lactate dehydrogenase activity, and for this reason there was made a conclusion of their hepatoprotective effect [4, 5]. Clinical studies of isoflurane anesthesia application in pre-perfusion period stated significant improvement of glomerular filtration indices after the operation. It is regarded as the protection of hepatic parenchyma [6, 7].

However, in spite of a great number of experimental works, only single publications are devoted to the estimation of clinical organ protective effect of isoflurane. So, there can be made no decisive conclusion of protective effect of the anesthetic [1], therefore, full estimation of organ protective effect of isoflurane in operations with artificial circulation (AC) is of considerable interest.

The aim of the investigation is to give a full estimation of organ protective effect of isoflurane anesthesia in cardiac surgeries with artificial circulation.

Materials and Methods. There was carried out retro- and prospective study of 424 patients operated in Specialized Cardiosurgical Clinical Hospital (Nizhny Novgorod, Russia) in the period from January, 2007 to July, 2011, for coronary heart disease (CHD) — 222 patients, and acquired heart valvular disease — 202 patients. Among the patients there were 307 male and 117 female patients aged from 15 to 78 years (mean age — 50.0±0.5 yrs). CHD patients were referred to III (92.7%) and IV (7.2%) functional CCS (Canadian Cardiac Society) class, and the patients with valvular disease — to III (93.5%) and IV (6.4%) class according to NYHA. All the patients were operated under the conditions of AC and pharmaco-hypothermic cardioplegia. Average AC time was 80.2±1.7 min in CHD patients, and

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87.1±2.2 min — in patients with valvular disease. Average time of aortic compression was 54.1±1.2 and 67.6±1.7 min, respectively.

The patients were divided into two groups: the patients of the first one, experimental (n=203) were given isoflurane as the main anesthetic; and in the second, control group (n=221) propofol was used as the main anesthetic.

All the patients received diazepam as premedication (0.15 mg/kg) intramuscularly half an hour before the operation. The patients of both groups were given introductory anesthesia as the combination of diazepam (0.2–0.3 mg/kg) and propofol (2 mg/kg). In the control group, at all stages of the operation, anesthesia was maintained with the help of total intravenous anesthesia (propofol — 2–3 mg/kg/h and phentanyl — 3–5 microgram/kg/h). In pre-perfusion and post-perfusion periods in the main group anesthesia was maintained by means of isoflurane inhalation (1–2% (minimum alveolar concentration — 0.8–1.1)) in all patients. At traumatic stages of the operation there was given phentanyl by bolus injection by 50–100 mcg. Myoplegia was performed in both groups by Arduan, 0.05 mg/kg. AC was performed under normothermic conditions. Crystalloid cardioplegia by Consol was used to protect myocardium in equal number of patients in both groups.

For complex comparative evaluation there were used clinical criteria (character of cardiac resuscitation after cardioplegia, post-ischemic heart rhythm disorders rate, the rate of postoperative congestive heart failure (CHF), hospital lethality in immediate postoperative period); functional criteria (indices of central hemodynamics and myocardial contractile function at the stages of operation); biochemical criteria (activity of total creatine phosphokinase (CPK) and the level of myocardial injury marker of MB-isoenzyme (MB-CPK), the activity of parenchymal organs enzymes); morphological criteria (right atrial myocardial ultrastructure before aortic compression).

Noninvasive diagnostics of the parameters of central hemodynamics and myocardial contractile function (MCF) was performed using a monitor “Hemosonic 100” (Arrow International, Reading PA, USA); cardiac index (CI), stroke index (SI), aortal blood flow acceleration (ABFA), peak blood velocity (PBV), total peripheral resistance (TPR) were registered at the beginning of the operation (I stage, 1 day), after sternotomy and sterna separation (II stage, 2 day), before AC (III stage, 3 day), after AC (IV stage), and at the end of the operation (V stage).

To estimate the changes of myocardial ultrastructure there were taken right atrial bioptates immediately before AC. For electron-microscopic analysis the material was fixed by 2.5% glutardialdehyde solution with following postfixation by 1% osmic acid solution, dehydration in alcohol with increasing concentration and embedding into the mixture of epoxy resins “Araldit” and “Epon-812”. Ultrathin sections were prepared on ultramicrotome “Ultracut”, Reichert-Jung, Austria. The obtained preparations were examined and photographed using transmission electron microscope “Morgagni 268D” (FEI, USA). Morphometry was performed using AnalySIS program.

The activity degree of CPK and MB-CPK was studied 3, 8, 24, 48 h after the operation. In addition, there were determined the activity of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alpha-amylase, lactate-dehydrogenase (LDG), bilirubin, urea, creatinine on 1, 2, 3 day after the operation. All enzymes were studied using biochemical analyzer “COBAS INTEGRA 400/400” (Roche, Switzerland) and standard reagents.

The obtained data were statistically processed using Statistica 6.0 and parametric criteria (Student t-test) and nonparametric criteria (χ^2, Mann–Whitney, Wilcoxon) for two dependent and independent samples calculating significance test $p<0.05$. The results were presented as average and standard error of mean (M±m).

Results and Discussion. The analysis of the character of cardiac resuscitation after cardioplegia in both groups showed spontaneous resuscitation to be observed statistically significantly more frequently in patients of the experimental group (131 patients, 64.5%) compared to the control group (111 patients, 50.2%; p=0.003), after electric defibrillation cardiac rhythm was recovered in 72 patients (35.5%) of the experimental group, and in 110 patients (49.8%) in the control group (p=0.009).

When comparing clinical course of early postoperative period in the patients of both groups (Table 1) no statistically significant difference was found either in atrioventricular heart block rate, or in hospital lethality, or CHF mortality. Statistically significant differences were revealed only in postoperative CHF rate (p=0.0420).

Thus, clinical criteria of “additional” myocardial protection in isoflurane anesthesia are to be considered the following ones: higher rate of spontaneous cardiac resuscitation and lower rate of postoperative CHF. The study of the values of central hemodynamics and MCF on the stage of operation (Table 2) showed the absence of their statistically significant changes in pre-perfusion period in both groups, though general tendency for changes in control group reflected certain depression of cardiac circulatory dynamics. So, the control group had CI decrease by 13.5% from initial values, SI — by 15.5%, and TPR increase — by 5.0%. By contrast, in the experimental group the change of MCF values demonstrated stability in pre-perfusion period (CI, SI, and TPR values did not statistically differ from initial ones). The course of rehabilitation and post-perfusion periods statistically significantly differed in higher SI values (by 18.5%), ABFA (by 33.5%), and PBV (by 22.6%) against the background of lower TPR (1005.5±90.4 — in the experimental group, and 1303.6±161.4 — in the control one). By the end of the operation there were found

Table 1

Clinical course of post-perfusion and early postoperative periods in groups, absolute number/%

Index	Experimental group (isoflurane)	Control group (propofol)	p
Atrioventricular heart block rate	7/3.40	13/5.80	0.237
CHF rate	9/4.05	21/9.50	0.042
Hospital lethality	3/1.35	9/4.10	0.092
CHF mortality	3/1.35	6/2.71	0.295

Table 2

The changes of myocardial contractile function values at anesthesia stage in patients of both groups (M±m)

Stage	Group	CI, l·min/m²	SI, ml/beats	ABFA, m/s²	PBV, cm/s²	TPR, dyne·s/cm⁵
I	Experimental	2.31±0.43	36.52±2.50	11.01±1.42	55.60±5.90	1673.10±190.20
	Control	2.51±0.18	38.00±2.85	11.58±1.57	57.80±9.41	1953.40±271.51
II	Experimental	2.30±0.31	37.75±1.47	10.45±1.10	56.30±5.11	1620.68±188.90
	Control	2.48±0.14	36.30±2.68	10.60±1.34	52.50±6.29	1753.30±153.62
III	Experimental	2.35±0.24	35.90±2.11	16.02±2.71	62.84±5.23	1404.20±143.10*
	Control	2.17±0.18	32.10±2.84	13.01±1.89	53.90±6.62	2055.60±228.04
IV	Experimental	3.51±0.41	46.02±2.06*	20.16±1.51*	78.85±3.22*	1005.45±90.43
	Control	2.79±0.22	37.50±2.98	13.40±1.63	61.00±3.49	1303.57±161.41
V	Experimental	3.63±0.40	45.88±2.89	20.05±1.90*	82.05±3.14*	970.80±97.34*
	Control	2.93±0.20	39.30±3.42	13.47±2.11	69.40±3.57	1480.50±176.50

* — statistically significant difference of values in the groups, p⩽0,05.

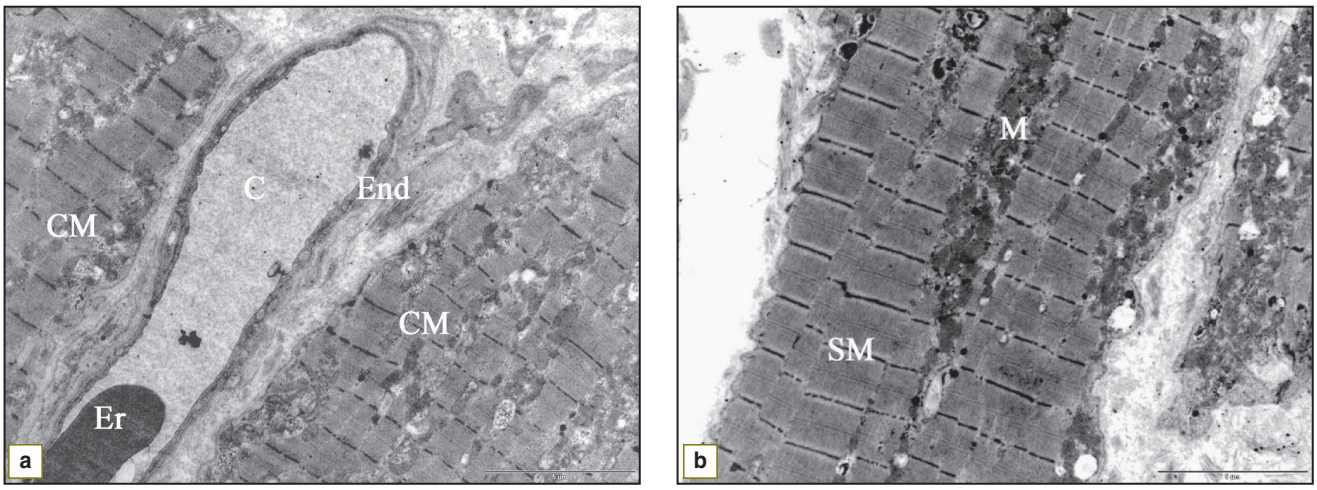

Fig. 1. Isoflurane anesthesia: *a* — myocardial capillary, x4400; *b* — cardiomyocytes, x4400. C — capillary lumen, CM — cardiomyocyte, End — endothelium, Er — erythrocyte, SM — sarcomere, M — mitochondria

statistically significantly higher acceleration rate values (by 32.8%), PBV (by 15.4%), and TPR (by 34.4%).

Thus, the data of functional studies during an operation showed no marked cardiodepressive effect when using both propofol, and isoflurane. On the other hand, statistically significantly higher MCF values in post-perfusion period in the experimental group showed better functional safety of cardiomyocytes. And the revealed phenomenon can be defined as a functional effect of "additional" myocardial protection in isoflurane anesthesia.

The carried out study of ultrastructural myocardial changes enabled to reveal their characteristics depending on anesthetic effect. In isoflurane anesthesia there could be observed the capillaries having wide lumens with fine-grained osmiophilic amorphous material (plasma proteins) and erythrocytes with clear membranes, and capillary endothelium had preserved structure with numerous pinocytic vesicles (Fig. 1). In propofol anesthesia the microcirculation in myocardium changed: along with a wide lumen there were some collapsed capillaries. A half of the observations showed vesicles and membrane structure (microclasmatosis). Capillary endothelium had numerous cytoplasmic outgrowths of luminal membrane, and basal layer (BL) was swelled (Fig. 2). The study of cardiomyocyte (CM) ultrastructure revealed secretory CM to have a great deal of secretory granules. In isoflurane patients CM had slightly reduced sarcomeres, their length being 1.50 micrometer on the average. CM nuclei had euchromatin, and some nuclei had a well-marked nucleolus (Fig. 1, *b*).

In propofol patients cardiomyocytes were slightly reduced (up to 1.37 micrometer on the average), there were the areas of non-uniform contraction, overcontraction and dilatation, with myofibril lysis in some observations. In most cases, cardiomyocyte nuclei had euchromatin, and there were small karyolemma invaginations. In some observations the nuclei were on cell peripheries (Fig. 1, *b*).

Thus, the study of myocardial ultrastructure revealed the damage of myocardial microcirculation and subcellular structure in patients with propofol anesthesia. Morphological criteria of "additional" protection of myocardium in isoflurane anesthesia are adequate microcirculation (wide lumens of capillaries, the preservation of blood state in vessels and endothelium), and the preserved CM structure (slight uniform contraction of sarcomeres, active nuclei).

Total activity of CPK in postoperative period (Table 3) in the experimental group was statistically significantly lower than in the control group by 23.8% three hours after the operation, and by 14.7% — 8 h after the operation. The

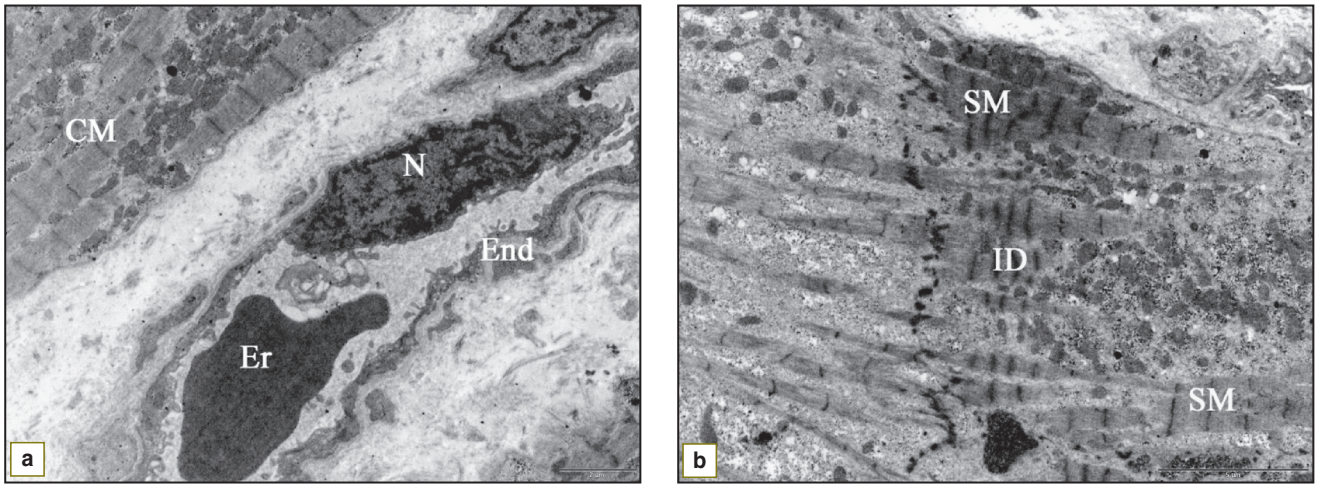

Fig. 2. Isoflurane anesthesia: a — myocardial capillary, x5600; *b* — cardiomyocytes, x4400. Conventional symbols – See in Fig. 1; N — nucleus, ID — intercalated disc

Table 3

Activity indices of total CPK and MB-CPK damage marker in the groups after the operation, units/l

Stage	Group	CPK	MB-CPK
I	Experimental	466.60±35.84*	41.20±2.76**
	Control	612.30±35.44	53.01±2.77
II	Experimental	496.50±35.38	35.27±1.74**
	Control	581.80±40.63	47.36±1.76
III	Experimental	756.10±79.27	33.01±1.53**
	Control	714.25±73.95	26.71±1.92
IV	Experimental	635.40±69.75	39.78±1.62
	Control	624.13±75.63	32.75±2.00

* — statistically significant difference of values in the groups at similar stage, p<0.05; ** — p<0.01.

level of myocardial damage marker MB-CPK was also significantly lower (by 22.3, 25.5, 17.0% — 3, 8, 24 h later, respectively) in the experimental group. Lower MB-CPK level gives the evidence of the lower degree of ischemic and reperfusion damage of myocardium that confirms an "additional" cardioprotective effect in isoflurane anesthesia, and is a biochemical criterion of the protection.

The analysis of enzyme activity change and a number of biochemical blood values characterizing the functioning of parenchymal organs (liver, kidneys) in postoperative period (Table 4) in the experimental group showed lower activity of some enzymes, in particular ASAT (by 8.4, 12.4, 13% on 1, 2, and 3 day, respectively), alpha-amilase activity (by 20.7, 22.6, 8.8%), LDG activity (by 10.6, 6.3, 16.4% respectively, with statistically significant difference — on the 3rd day). In addition, there was revealed the decrease of total bilirubin in postoperative period (6.4, 11.3, 20.6% on 1, 2, and 3 day, respectively) that indirectly indicates smaller damage and liver function retention.

There was no stated significant change of creatinite level in blood in postoperative period. Urea level increased less significantly in the patients of the experimental group (by 7.0 and 22.9% on the 2 and 3 days, respectively) compared to the control one (by 20.4 and 61.5% on the 2 and 3 days, respectively). And in the experimental group the growth was not statistically significant, while in the control group $p \leqslant 0.05$. The character of these changes indicates an "additional" protective effect of isoflurane on renal tissue.

Thus, the character of activity change of enzymes and a number of biochemical indices in postoperative period prove an "additional" protective effect of isoflurane on parenchymal organs, particularly liver and kidneys.

Complex studies carried out enabled not only to reveal and confirm an "additional" protective effect of isoflurane anesthesia in operations with AC, but also establish specific criteria of this protection. So, clinical criteria of "additional" myocardial protection are to be considered

Table 4

The activity of enzymes and biochemical blood values after the operation with AC

Laboratory findings	Group	I stage	II stage	III stage
ASAT, units/l	Experimental	66.75±7.18	54.79±5.20	48.02±4.89
	Control	75.62±7.72	62.55±5.87	55.29±5.01
ALAT, units/l	Experimental	27.27±2.54	24.39±2.29	29.47±1.92
	Control	25.25±2.85	23.83±2.33	31.82±1.75
Bilirubin (total), mg/dL	Experimental	0.88±0.09	0.63±0.06	0.54±0.05
	Control	0.94±0.05	0.71±0.07	0.68±0.05
Urea (mg/dL)	Experimental	48.40±3.72	51.80±3.88	59.47±5.84
	Control	43.52±3.38	52.40±4.39	70.27±6.98
Creatinine, units/l	Experimental	1.27±0.11	1.08±0.06	1.07±0.07
	Control	1.14±0.07	1.07±0.05	1.04±0.06
Amylase, units/l	Experimental	89.34±11.94	78.68±19.03	80.78±12.79
	Control	112.64±12.86	101.78±19.01	88.57±12.11
LDG, units/l	Experimental	283.20±12.52	285.90±10.14	253.5±11.6**
	Control	316.90±11.87	305.10±11.82	303.30±12.06

* — statistically significant difference of values in the groups at similar stage, p<0.01.

the higher rate of spontaneous cardiac resuscitation and lower CHF rate.

Statistically higher MCF indices in post-perfusion period in the experimental group of patients reflect functional effect of "additional" myocardial protection. Morphological criteria of "additional" myocardial protection are adequate microcirculation and the retention of CM ultrastructure. Lower level of myocardial damage marker MB-CPK gives the evidence of the lower degree of ischemic and reperfusion damage of cardiac muscle that is biochemical criterion of the protection. In addition, isoflurane has an "additional" protective effect on some organs and systems, in particular, the character of enzymes activity change and a number of biochemical indices in postoperative period that proves its protective effect on parenchymal organs functioning.

Conclusion.

Isoflurane anesthesia provides an "additional" protective effect in cardiac surgeries with artificial circulation, and this effect is proved by complex clinical studies. Myocardial effect of additional protection is supported by clinical, functional, morphological, and biochemical criteria. Clinical criteria are to be considered the following ones: the higher rate of spontaneous cardiac resuscitation and the lower rate of congestive heart failure.

Statistically higher indices of myocardial contractile function in post-perfusion period in the examined patients reflect functional effect of "additional" myocardial protection. Morphological criteria are adequate microcirculation, and preserved ultrastructure of cardiomyocytes, biological criteria being the lower level of myocardial damage marker MB-CPK indicating the lower degree of ischemic and reperfusion damage of myocardium. Isoflurane has an "additional" protective effect on both: the parenchymal organs functioning, in particular, enzyme activity, and some biochemical values in postoperative period that suggests its protective effect on liver and kidneys.

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