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The new nitric oxide generator for delivery to the extracorporeal circuit: technical and medical characteristics, experimental and clinical studies of its effectiveness and safety

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ABSTRACT

A medical plasma-chemical apparatus for synthesizing nitric oxide (NO) has been developed for the first time. It is designed to deliver it directly into the extracorporeal circuit of heart-lung machine and extracorporeal membrane oxygenation (ECMO) machines. This paper presents the technical characteristics of the apparatus, the results of its experimental use, and clinical studies during CPB surgeries.

In the apparatus's plasma-chemical reactor, the primary synthesis of NO was achieved in the plasma of diffuse low-current (<1 A) microsecond-duration discharges uniformly distributed within the annular discharge gap. This ensured a long service life of the electrode system—up to 6,000 hours—and low levels of unwanted impurities.

Experimental results showed that the oxygenator with a polypropylene hollow fiber membrane retained its mass-transfer properties in the presence of NO at a concentration of 500 ppm for 6 hours, with no signs of chemical interaction between polypropylene and NO. Analysis of the surface condition of hollow fiber membrane samples from oxygenators revealed a significantly lower incidence of fibrin deposits when NO was administered via CPB.

Clinical studies have shown that administering NO to the extracorporeal circuit exerted an antioxidant effect, reduced oxidative stress, maintained high ATP levels in red blood cells, effectively prevented increased red blood cell aggregation, and had a protective effect on endothelial function during prolonged CPB. Perioperative administration of NO was associated with a statistically significant reduction in the incidence of acute kidney injury within 7 days postoperatively and a protective effect on the myocardium.

Keywords: *nitric oxide, administration and dosage, supply and distribution, therapeutic use, cardiopulmonary bypass.*

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Introduction

Since clinical introduction of the AIT-NO-01 inhaled nitric oxide (NO) therapy device (TIANOX; registration certificate No. RZN 2020/10977 dated June 22, 2020), developed by the Russian Federal Nuclear Center — All-Russian Research Institute of Experimental Physics (Sarov), there were many studies devoted to clinical efficacy of NO across various medical fields [1—3]. Researches have covered this device in pulmonology, neonatology and pediatrics, cardiology and cardiac

surgery, intensive care, rehabilitation and other areas. The need for a next-generation NO device capable of delivering the gas into the bloodstream via oxygenator membranes of extracorporeal circulation and circulatory support systems is driven primarily by requirements of modern cardiac surgery. Many reconstructive cardiac procedures are performed under cardiopulmonary bypass (CPB), and its duration depends on complexity of surgery. Prolonged CPB significantly increases the incidence of postoperative complications and mortality [4—6]. There is a clear correlation between CPB time and rate of postoperative complications. Specifically, the incidence of postoperative acute cardiovascular failure (ACVF), acute respiratory failure (ARF) and acute renal failure is higher after CPB >90 min [7]. Postoperative mortality is also associated with CPB time. Studies demonstrated a nearly 4-fold increase ($p<0.05$) in mortality rate when CPB time exceeded 90 min. CPB time over 180 min is followed by almost 6 times higher mortality ($p<0.05$) [8]. Thus, organ and tissue protection during CPB is of particular importance in cardiovascular surgery to reduce the incidence of severe postoperative complications. No prior experimental or clinical studies investigated efficacy and safety of administering exogenous NO generated by a new AT-NO-01 (TIANOX KS) device into oxygenator of extracorporeal circulation circuit. This underscores the relevance of this report.

This comprehensive study presents technical capabilities of a new TIANOX KS device (developed at the Russian Federal Nuclear Center — All-Russian Research Institute of Experimental Physics, Sarov), experimental data on safety and efficacy of NO delivery into extracorporeal circuit (Almazov National Medical Research Centre, St. Petersburg) and clinical data about appropriate on-pump procedures at the Korolev Research Institute — Specialized Cardiac Surgery Hospital (Nizhny Novgorod) and Tomsk National Research Medical Center (Tomsk).

The purpose of the study was to analyze medical and technical characteristics of a new TIANOX KS nitric oxide generator, experimental data regarding safety and efficacy of delivering NO into extracorporeal circuit oxygenator and clinical results of on-pump procedures.

1. Medical and technical characteristics of the TIANOX KS device

1.1. High-durability plasma-chemical reactor (PCR). NO synthesis was carried out in air-based thermally nonequilibrium plasma ($T_e/T>>1$) of a diffuse discharge involving vibrationally excited nitrogen molecules (N_2^*) [9]; T_e — electron temperature, T — gas temperature in the channel. Current and voltage oscillography in discharge channel indicates that pulsed energy with a specific density of at least 1.5 J/cm^3 is deposited into the gas during diffuse discharge phase. Under these conditions, a chain mechanism for NO synthesis involves the following pair of reactions:

$$N_2^* + O \rightarrow NO + N (k_1 \approx 10^{-9}\ cm^3 \cdot s^{-1}\ at\ T_e = 1-2\ eV), \quad (1)$$

$$N + O_2^* \rightarrow NO^* + O (k_2 \geqslant k^0\ eV T_e = 1-2\ eV), \quad (2)$$

as well as chain-branching reaction

$$NO^* + O_2 \rightarrow NO_2 + O (E_a \approx 1\ eV), \quad (3)$$

Here, $k^0 \approx 3 \cdot 10^{-13}$ cm$^3 \cdot$s^{-1} is reaction rate threshold (2) ensuring self-sustaining nature of reaction pair (1), (2) capable of compensating for the loss of nitrogen and oxygen atoms in concurrent termolecular oxidation reactions; k1 and k2 are rate constants for reactions (1) and (2), respectively, Ea — activation energy for reaction (3). Oxidation reactions result in small quantities of toxic by-products: O_3, NO_2, and NO_3. However, almost no ozone (O_3) was detected in reactor's output flow (significantly less than maximum permissible concentration 0.1 mg/m^3), as O_3 rapidly decomposes within the discharge channel via dissociation reaction $O_3 + M \rightarrow O_2 + O + M$, where M — molecule or atom. As for the higher nitrogen oxides (NO_2 and NO_3), they are selectively and completely chemically adsorbed by passing the gas stream through soda lime (approximately 400 g). NO concentration is determined by volumetric flow rate (v_g) and frequency f.

A diffuse discharge lasting up to t≈6 μs was periodically excited at a repetition frequency (f) 300—10000 Hz by a current source featuring a series resonant circuit that included plasma-chemical reactor. Resonant circuit impedance limited current density in discharge channel to j≈2.9 × 10^2 A/cm^2. This reduced stainless-steel electrode erosion rate and concentration of metal vapors (iron, nickel, chromium) below OSHA and NIOSH limits (1 mg/m^3).

At specific tangential air flow rates (v_g) through the wide annular discharge gap of plasma-chemical reactor (diameter≈56 mm), the discharge channel forms in a new location each time. This location is shifted in gas flow direction by a fixed distance (Δ) exceeding channel diameter (d_{ch}) [9]. Uniform sequential channel formation with a step (Δ) 0.5—1.5 mm (annular gap perimeter≈170 mm) combined with short-term (≈ 6 μs) attachment of channel to electrode surfaces dramatically extends plasma-chemical reactor service life (by up to three orders of magnitude). Peak NO synthesis efficiency (ω≈2 × 10^{16} molecules/J) and peak NO concentration (≈ 1,200 ppm) at v_g=1 L/min remained within 15% measurement error over a period of ≈ 6,000 hours (including≈2,000 hours of maximum discharge power).

Extending continuous operating time to several thousand hours eliminates the need for urgent electrode replacement during NO therapy in intensive care and reduces the risk of potential adverse outcomes.

1.2. Device for plasma-chemical synthesis of nitric oxide. We present front **(Fig. 1a)** and rear **(Fig. 1b)** views of device [10] designed to synthesize NO from ambient air within an advanced discharge chamber [11], deliver NO to extracorporeal circuit and maintain NO concentration at membrane oxygenator inlet.

The apparatus is configured as a vertical assembly mounted on a mobile base (1) comprising several interconnected units: gas mixture generation and preparation unit (2), gas mixture monitoring unit (3), data acquisition and processing unit (4) featuring a graphic touchscreen display **(Fig. 1a)**. In on-pump cardiac surgery or extracorporeal membrane oxygenation (ECMO), the apparatus is connected to CPB or ECMO circuit prior to the procedure following the gas flow scheme [12]. The "NO outlet" port **(Fig. 1b)** is connected to the inlet of gas-air mixture supply line leading to oxygenator, while the "Control" port is connected to the outlet of line linked to oxygenator. Power is supplied via the "220 V" switch.

Once CPB (or ECMO) is commenced with delivery of oxygen-air mixture to oxygenator supply line at a volumetric flow rate of 2—4 L/min, the data acquisition and processing unit is activated. This unit automatically brings the other constituent units into operational state. We establish NO concentration at the oxygenator inlet using the display screen, for example, 100 ppm and initiate NO synthesis in the generation and gas mixture preparation unit. Gas mixture pu-



Fig. 1. **TIANOX KS device.**
a — front view, 1 — base, 2 — gas mixture generation and preparation unit, 3 — gas mixture monitoring unit, 4 — data acquisition and processing unit; b — rear view, where 1 — "Control" port, 2 — "NO output" port, 3 — "220 V" power switch, 4 — "Vent" port.

rified of NO_2 exits via the "NO outlet" port and enters initial part of ECMO circuit. Here, gas mixture mixes with gas-air flow as it moves towards oxygenator. This flow is predominantly directed into oxygenator for blood processing and oxygenation, while a part is diverted back to the gas analysis module. After passing through this unit, gas mixture is purified of NO_2<0.5 ppm and vented to the atmosphere via the "Vent" port.

Specialists control concentrations of free hemoglobin and methemoglobin, blood gases and other parameters approximately every 30 minutes throughout CPB. Analysis may necessitate adjustments to composition or airflow rate within the circuit. This can cause NO concentration in oxygenator to deviate from pre-set value. Processor of the data acquisition and processing unit calculates deviation and, following a pre-programmed algorithm, adjusts resonant current power and NO synthesis rate in reactor automatically restoring NO concentration to baseline level. Before terminating ECC and CPB, NO synthesis is stopped via the display screen, and the data acquisition and processing unit is powered down.

1.3. Technical specifications of device. 1. The device is operated via a touchscreen. 2. The device is designed to operate when connected to extracorporeal circuit with a flow rate of 2—4 L/min and overpressure 200—900 mm Hg. 3. Volumetric flow rate of NO-containing gas mixture at the device outlet is 0.7±0.2 L/min. 4. Volumetric flow rate of gas mixture in sample harvested for monitoring is 0.7±0.2 L/min. 5. Settable NO concentration range at the inlet to membrane oxygenator is 0—200 ppm. Permissible deviation from NO concentration is ±20%. 6. The device monitors NO and NO_2 concentrations in gas mixture. 7. Power consumption is no more than 250 W. 8. Mass of device is no more than 50 kg. 9. Overall dimensions of device (maximum): height 1300 mm, width 750 mm, depth 550 mm.

2. Experimental research of nitric oxide delivery into extracorporeal circuit (CPB, ECMO).

Further development of NO-based blood treatment in CPB oxygenators required an in-depth study of chemical interaction between NO and polypropylene hollow fibers of membranes. This work was carried out at the Almazov National Medical Research Centre (St. Petersburg) with participation of scientists from the world-class laboratory "SMART Polymer Materials and Technologies" (headed by I.V. Vorotyntsev).

At the first stage, scientists analyzed stability of polypropylene membranes upon contact with high concentrations of NO significantly exceeding those used in clinical practice (500 ppm). A novel experimental test rig was developed to study stability of polypropylene hollow fibers of the Inspire 8M oxygenator (Sorin Group S.p.A., Italy) when supplied with NO-containing gas mixture. The rig incorporated a system of gas flow controllers connected to a mixing chamber, as well as thermostated container with distilled water and 0.9% sodium chloride (NaCl) solution. Analytical system included a Pfeiffer Prisma-Pro QMG 250 M2 (1—200 u) mass spectrometer (Pfeiffer Vacuum GmbH, Germany). We prepared gas mixture for delivery to oxygenator using a dynamic flow-mixing method. Contact between two phases occurred in hollow polypropylene fibers of oxygenator upon simultaneous introduction of gas mixture and saline solution.

At baseline, we simulated basic operating mode by supplying N_2/O_2 gas mixture (50:50 vol%) and saline solution for 6 hours. Throughout the experiment, we analyzed composition of gas mixture exiting the oxygenator over time using a mass spectrometry system. This enabled determination of system separation factor for baseline operating mode. Subsequently, we delivered N_2/O_2/NO gas mixture (49.975:49.975:0.05 vol%, corresponding to NO content 500 ppm) and saline solution into the system after previous work with N_2/O_2 mixture. Over a 6-hour period, we analyzed composition of gas mixture exiting the oxygenator over time using a mass spectrometry system. Gas-transport characteristics of membrane (separation factor) were assessed considering nitrogen and oxygen concentrations normalized to 100%. Separation factor characterized mass transfer in oxygenators under various gas mixture compositions and allowed for conclusions regarding stability of polypropylene membrane when exposed to NO concentration of 500 ppm [13].

According to experimental data, oxygenator with polypropylene hollow-fiber membrane maintained mass-transfer characteristics under NO 500 ppm for 6 hours. Importantly, there were no signs of chemical interaction between polypropylene and NO [13, 14].

To evaluate potential chemical reactions between NO and polypropylene hollow-fiber membranes of oxygenator, we performed Fourier-transform infrared (FTIR) spectroscopy of membrane material [13]. Polypropylene underwent no changes upon exposure to 500 ppm NO, as evidenced by no band shifts in polypropylene IR spectrum typical for NO chemisorption. There were no signs of physical NO sorption onto polypropylene. We revealed no differences between control polypropylene membrane samples and those exposed to NO [13].

The second experimental stage involving large animals was devoted to effects of NO administered to oxygenator on functional status and morphology of internal organs. Ten pigs undergoing on-pump cardiac surgery were included. Five animals in the experimental group received NO 100 ppm via oxygenator (CPB-NO group), while five pigs

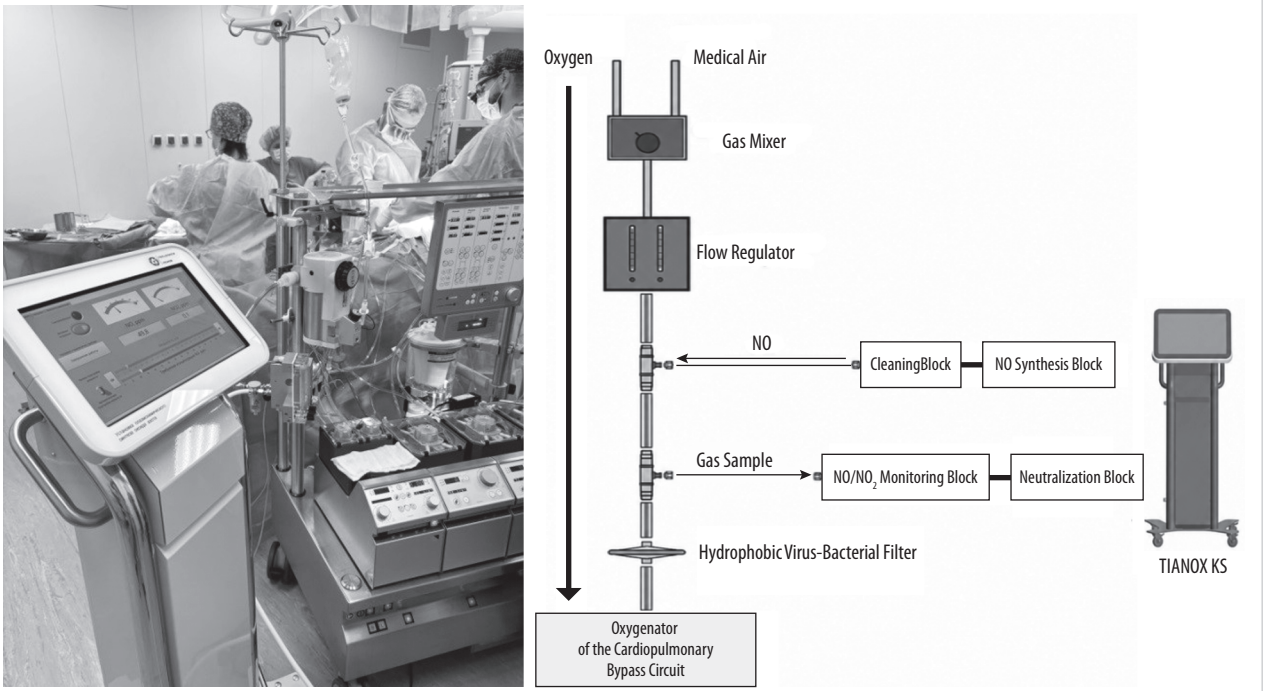

Fig. 2. **Connection of the TIANOX KS device to gas-air mixture delivery line of extracorporeal circulation system.**

in the control group (CPB-control) received no nitric oxide. CPB time was 4 hours, postoperative follow-up — 12 hours. This CPB time was selected to maximize adverse effects on the bodies. To assess liver and kidney damage, we analyzed serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and creatinine at baseline, upon weaning from CPB, 6 and 12 hours later. In addition, morphological examination of liver and kidneys followed withdrawal of animals from experiment [15, 16]. Prolonged CPB consistently caused liver damage. Baseline ALT and AST were similar in both groups. In the CPB-control group, serum ALT increased from baseline 43 (34; 44) to 82 (53; 99) U/L after 12 hours (p<0.05). Serum AST in this group increased from baseline 25 (17; 26) to 269 (164; 376) U/L after 12 hours (p<0.05). There was no significant increase of ALT or AST in the CPB-NO group. In the CPB-control group, creatinine increased from baseline 131 (129; 133) to 273 (241; 306) μmol/L after 12 hours (p<0.05). We did not find significant increase of creatinine in the CPB-NO group. Serum creatinine was lower in the CPB-NO group after 12 hours: 183 (168; 196) versus 273 (241; 306) μmol/L (p=0.008). Morphological examination of liver and kidneys revealed tissue alterations, circulatory disturbances and inflammatory infiltration. Collectively, these findings reflected CPB-induced tissue damage. In the CPB-NO group, scores of these changes were lower, although differences were not significant [15, 16].

We studied surface of hollow-fiber oxygenator membranes after 4-hour CPB using scanning electron microscopy (JEOL 1610LV microscope, JEOL Limited, Japan). Three oxygenator membrane samples were examined: 2 from the CPB-NO group and 1 from the control group [13, 14, 17]. Comparison of polypropylene hollow-fiber membrane surfaces revealed a significantly higher number of fibrin deposits on the control samples (by 2—7 times) after 4-hour CPB [13].

3. Clinical data on effect of delivering gaseous nitric oxide into CPB circuit on markers of oxidative stress, endothelial dysfunction and erythrocyte status

Scientists evaluated the impact of gaseous NO delivery on markers of oxidative stress, endothelial dysfunction and erythrocyte status, as well as clinical outcomes (postoperative complication rates and mortality) after CPB >90 min. The local ethics committee of the Korolev Specialized Cardiac Surgery Hospital approved the study protocol (protocol No. 6 dated July 8, 2024). There were 86 patients. We calculated sample size a priori considering expectable incidence of postoperative complications (acute heart failure, acute respiratory failure and multiple organ failure) within 7 days after surgery with CPB >90 minutes. According to the literature, the complication rate was approximately 35—40%. We hypothesized that NO would reduce this value to 15—20% (20% absolute risk reduction). With α=0.05 and 80% power, comparison of binary outcomes in 2 independent groups required a minimum of 37 patients per a group. Accounting for potential 15% dropout rate, target sample size was set at 86 patients (43 per a group). No interim analysis was planned. There was intention-to-treat analysis. All patients underwent heart valve surgery and combined procedures under CPB. Patients were randomized into two groups: the first group (control) — standard protocol for anesthesia and CPB; the second group (experimental) — NO (40 ppm) administered into gas delivery line throughout the entire perfusion period. There were no significant between-group differences in sex, age or baseline status (p>0.05). All patients underwent heart valve surgery alone or in combination with CABG under normothermic cardiopulmonary bypass. Crystalloid cardioplegia was employed for myocardial protection (Custodiol, Dr.F. Köhler Chemie GmbH, Germany). The Newman–Keuls test (ANOVA) for surgery type found no significant between-group differences.

CPB time was 99.3±6.5 and 111.3±7.9 min, aortic cross-clamping time — 75.1±4.9 and 84.1±5.1 min, respectively. There were no significant between-group differences.

In the control group, CPB was performed according to standard protocol. In the experimental group, gaseous NO (40 ppm) was administered into the gas delivery line of heart-lung machine (TIANOX KS device). NO dosing was controlled via built-in monitor. NO flow rate was 250—300 ml/min with a concentration of 44.5±0.72 ppm in fresh oxygen flow. Mean NO_2 concentration was 0.2—1.1 (0.80±0.06) ppm. NO delivery into CPB circuit began once the target volumetric perfusion rate was reached and continued until the end of CPB **(Fig. 2)**.

We analyzed biochemical parameters at the following stages: 1 — baseline (before induction of anesthesia); 2 — after 5-minute CPB; 3 — after 60-minute CPB; 4 — after 90-minute CPB; 5 — at the end of CPB; 6 — at the end of surgery; 7 — on the first postoperative day. We assessed free-radical lipid oxidation in plasma by measuring intermediate molecular products of lipid peroxidation (diene and triene conjugates) and Schiff bases as end products. Free-radical lipid oxidation in erythrocytes was estimated by measuring malondialdehyde (MDA). Analysis of antioxidant system implied erythrocyte catalase activity. We also measured erythrocyte electrophoretic mobility, erythrocyte aggregation, concentrations of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes, circulating endothelial cells [18, 19].

Clinical endpoints included postoperative ventilation time, length of ICU- and hospital-stay, incidence of postoperative complications (acute heart failure, acute respiratory failure, multiple organ failure) and in-hospital mortality.

Statistical analysis was performed using Microsoft Excel 2003 and STATISTICA 6 software. Analysis of contingency tables was conducted online via the medstatistic.ru portal. We analyzed distribution of quantitative variables using the Kolmogorov-Smirnov and Shapiro-Wilk tests. To evaluate differences in quantitative variables with parametric distribution between two independent groups, we used analysis of variance (ANOVA and Repeated Measures ANOVA) and Dunnett's test for multiple pairwise post-hoc comparisons. Differences were significant at $p<0.05$.

Results

Analysis of the above-mentioned technologies in on-pump surgeries with CPB time >90 min yielded the following findings. Analysis of lipid peroxidation products in both groups revealed significantly lower levels of triene conjugates after 60-minute CPB and at the end of surgery, as well as lower levels of Schiff bases after 60-minute CPB, at the end of CPB and surgery. Assessment of erythrocyte catalase activity showed significant decrease at all stages of surgery and CPB in the control group, whereas the experimental group demonstrated significant increase in erythrocyte catalase activity after initiation of CPB and NO delivery into extracorporeal circuit. There was a significant between-group difference in this parameter after onset of CPB until the end of surgery. Serum MDA as a stable secondary lipid peroxidation product and marker of oxidative stress in erythrocytes significantly increased in both groups. In the control group, serum MDA maximally increased by 501% compared to baseline value by the 90th minute of CPB. In the experimental group, this increment was only 90% at onset of CPB with subsequent significant decline. Furthermore, serum MDA was significantly lower in the experimental group throughout CPB. Analysis of erythrocyte ATP content revealed no significant changes at any stage in the control group. In the experimental group, erythrocyte ATP significantly increased upon initiation of CPB and NO delivery to extracorporeal circuit. This elevation persisted until the first postoperative day. Analysis of 2,3-DPG revealed its decrease with minimum value by the 90th minute of CPB in both groups and no statistical significance. There were no significant differences in content of 2,3-DPG at all stages in both groups. Electrophoretic mobility of erythrocytes was similar in both groups. Erythrocyte aggregation significantly increased in the control group after onset of CPB and reached maximum values on the 1st postoperative day. There were no significant changes of erythrocyte aggregation in the experimental group and its decrease on the 1st day after surgery. Analysis of circulating endothelial cells revealed significant decrease of their number in both groups. There was significantly less number of circulating endothelial cells in the experimental group after 60-min CPB and at all subsequent stages. Finally, ventilation time and length of hospital-stay were similar in both groups. Experimental group was characterized by significantly shorter ICU-stay and less incidence of acute heart failure, acute respiratory failure and multiple organ failure.

Thus, NO in on-pump surgeries causes antioxidant effect with significant increase in catalase activity and lower oxidative stress as evidenced by significant decrease of lipid peroxidation products in plasma and erythrocytes. The effects of NO on erythrocytes are significantly higher ATP in erythrocytes compared to baseline values and effective prevention of excessive erythrocyte aggregation. NO has a protective effect on endothelial function during long-term cardiopulmonary bypass and promotes favorable postoperative outcomes. There was no in-hospital mortality in both groups.

4. Clinical effect of NO delivery into extracorporeal circuit on intraoperative kidney protection

We analyzed clinical effect of NO delivery into extracorporeal circuit on intraoperative kidney protection at the Tomsk National Research Medical Center. Previously, the same authors carried out several experimental studies and demonstrated clear organ protection and safety of NO derived through plasma-chemical synthesis in cardiac surgery and extracorporeal circulation [20—22]. Considering appropriate fundamental data, the authors carried out a randomized controlled trial DEFENDER devoted to effectiveness and safety of NO derived through plasma-chemical synthesis in high-risk cardiac surgery [23]. The TIANOKS KS device was used as a NO generator. The purpose of the study was to evaluate the effectiveness and safety of perioperative NO conditioning in patients with chronic kidney disease undergoing elective on-pump cardiac surgery for prevention of postoperative acute kidney injury. Study design was approved by the local ethics committee (protocol No. 237 of December 20, 2022). According to baseline hypothesis, replenishment of perioperative NO deficiency in these patients would reduce the incidence of cardiac surgery-associated acute kidney injury and slow further progression of renal dysfunction without increasing the incidence of adverse events.

The primary endpoint was AKI rate within 7 days after surgery according to KDIGO 2012 criteria. The main objectives included comparison of severity and duration of AKI, dynamics of glomerular filtration rate in short- and mid-term period, postoperative complications and safety of NO therapy. The study included 136 patients randomized 1:1 to receive perioperative ni-

tric oxide conditioning or standard perioperative management without NO delivery. Analysis of sample size was based on previous studies showing a 66% incidence of cardiac surgery-associated acute kidney injury in high-risk patients, including those with preoperative CKD [24]. In all patients, perioperative care incorporated the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. A conservative estimate in perioperative KDIGO bundle suggested a 16% reduction in the incidence of cardiac surgery-associated acute kidney injury [25]. In earlier studies, perioperative NO therapy—even at high concentrations—demonstrated favorable safety and resulted in a 50% reduction in the incidence of cardiac surgery-associated acute kidney injury [26—28]. Considering the pooled data regarding combination of perioperative NO and KDIGO bundle, the incidence of cardiac surgery-associated acute kidney injury in the NO group was predicted as 25%. According to Lehr's formula, the authors needed for 61 patients in each group to achieve 80% statistical power at α=0.05. Accounting for expectable 10% dropout rate, final sample size was increased to 68 patients per a group (136 participants).

Perioperative management of patients in both groups implied standard nephroprotective strategies, including goal-directed perfusion (maintaining oxygen delivery >280 mL/min/m^2 during cardiopulmonary bypass) and adherence to KDIGO bundle components intraoperatively and for 48 postoperative hours.

Inclusion criteria were CKD with eGFR<60 mL/min/1.73 m^2 for at least 3 months prior to surgery, elective on-pump cardiac surgery. Exclusion criteria were emergency surgery, administration of potentially nephrotoxic drugs within 24 hours prior to surgery, critical preoperative status requiring mechanical ventilation, inotropic/vasopressor support or mechanical circulatory support, pregnancy, participation in another RCT, active endocarditis and/or sepsis, severe pulmonary hypertension, age <18 years previous kidney transplantation.

In the experimental group, NO was administered continuously at a concentration of 80 ppm. We delivered gas early after tracheal intubation through the ventilator (TIANOX device), during CPB through the oxygenator (TIANOX KS device) and finally for 6 postoperative hours through ventilator (TIANOX device), unless early weaning from ventilator necessitated discontinuation of therapy. The authors designated this strategy as "NO conditioning". This strategy of perioperative pharmacological intervention initiates a cascade of physiological and biochemical changes to enhance resistance of biological systems (cells, tissues, organs, systems and organism as a whole) to perioperative injurious factors, to minimize negative effects of stressors during their immediate impact and/or to bolster adaptive capabilities after lesion. This strategy integrates all potential mechanisms of NO-induced organ protection and reflects a three-phase approach comprising the following components. Preconditioning implies pre-stressor stimulation of NO-dependent cytoprotective pathways and intraoperative replacement therapy to correct endogenous NO deficit arising during CPB. Postconditioning includes post-stress minimization of injury via NO-mediated modulation of oxidative stress and inflammation.

Mean duration of NO therapy was 678±30 min. The control group received gas-air mixture without nitric oxide at all perioperative stages.

Perioperative NO administration was associated with significantly less incidence of acute kidney injury within 7 postoperative days (23.5% in the NO group versus 39.7% in the control group, RR 0.59; 95% CI 0.35—0.99; p=0.043). However, there were no between-group differences in severity or duration of acute kidney injury. Six months after surgery, the estimated GFR was higher in the NO group (50 [45; 54] vs. 45 [41; 51] mL/min/1.73 m^2, respectively, p=0.038). CKD progression was less common after NO therapy, although this difference was not significant (10.3% vs. 22.1%, p=0.062) [22].

Additionally, the incidence of postoperative pneumonia was significantly lower in the NO group (14.7% vs. 29.4%, RR 0.50; 95% CI 0.25—0.99; p=0.039). There were no significant between-group differences in other in-hospital outcomes, including mortality, ventilation time, length of ICU- and hospital-stay. Furthermore, patients in the NO group had lower serum troponin I after 6 postoperative hours (2.616 ng/mL [1.639; 3.901] vs. 6.605 ng/mL [3.881; 8.030]; p<0.001) and 24 hours (1.844 ng/mL [1.409; 2.847] vs. 5.346 ng/mL [2.384; 11.161]; p<0.001). This suggests potential concomitant cardioprotective effect of therapy.

Safety profile of perioperative NO administration was favorable. Nitrogen dioxide concentration in inhaled gas mixture did not exceed 2 ppm and remained below the preset threshold of 3 ppm. Methemoglobin after 6 postoperative hours was higher in the NO group. However, these values did not exceed the clinically significant threshold of 5% in any patient. There were no significant between-group differences regarding the rate of blood transfusions, postoperative blood loss, or platelet count at the first postoperative day. There were no adverse events, organ dysfunction or need to restart NO therapy related to the intervention.

5. Discussion

The authors present a novel plasma-chemical device designed to deliver NO into extracorporeal circuit of membrane oxygenator for cardiopulmonary bypass or ECMO. This device as the first of its kind globally was developed and manufactured in Russia. Technical solution involved NO synthesis in plasma of diffuse low-current (<1 A) microsecond-duration discharges distributed uniformly throughout the volume of annular discharge gap. This ensured a long service life for electrode system (up to 6,000 hours) and low content of unwanted impurities.

Despite nearly 30-year experimental and clinical researches in delivering NO to oxygenators of CPB and ECMO systems, until 2023 there was no conclusive data regarding safety of contact between NO as a highly reactive chemical compound and hollow-fiber polymers of gas-exchange membranes [13, 14]. In 1999, Body S.C. et al. [17] presented the only study devoted to ex vivo interaction of high-concentration NO (224±10 ppm) with CPB oxygenator membrane polymers. Considering blood gas composition, gas mixture pressure at various points in CPB circuit and electron microscopy of hollow-fiber membranes, the authors concluded safety of NO delivery to polypropylene-membrane oxygenators. Importantly, there was no analysis of chemical composition of gas mixture exiting the oxygenator or fluid filling the CPB circuit. According to our experimental data, polypropylene hollow-fiber membrane oxygenator maintains mass-transfer characteristics in the presence of 500 ppm NO throughout 6 hours. An important finding was no signs of chemical interaction between polypropylene and NO [13]. Additionally, Fourier-transform infrared (FTIR) spectroscopy of membrane material was performed [13]. This study indicated that polypropylene underwent no changes upon exposure to NO at a concentration of 500 ppm. This was confirmed by the absence of characteristic band shifts in polypropylene IR spectrum that would reflect

NO chemisorption. There were no signs of physical NO sorption on polypropylene.

The second experimental stage implied analysis of effects of NO delivery into oxygenator on functional status and morphological characteristics of internal organs. There were stable biochemical (AST, ALT, creatinine) and morphological (liver, kidneys, brain, and myocardium) parameters in animals receiving NO during prolonged CPB [15, 16].

Clinical analysis of efficacy and safety of the TIANOX KS device was performed after positive experimental results and approval by local ethics committees of institutions. Studies on effects of extracorporeal NO administration on markers of oxidative stress, endothelial dysfunction, and erythrocyte status were also conducted for the first time in the Russian Federation [21]. NO delivery into extracorporeal circuit provided antioxidant effect, reduced oxidative stress, maintained high ATP in erythrocytes, effectively prevented excessive erythrocyte aggregation and exerted protective effect on endothelial function during prolonged CPB [18, 19, 29].

Impact of perioperative NO administration (generated via plasma-chemical synthesis using the TIANOX KS device) on the incidence of acute kidney injury was analyzed for the first time in the Russian Federation. Extracorporeal NO administration during CPB was associated with significantly less incidence of acute kidney injury within 7 postoperative days, as well as cardioprotective effect (evidenced by lower troponin I at 6 and 24 postoperative hours).

There are important data regarding safety of extracorporeal NO administration. Specifically, methemoglobin exceed the clinically significant threshold of 5% in none patient neither during CPB nor after 6 postoperative hours. There were no significant between-group differences regarding the incidence of blood transfusions, postoperative blood loss or platelet count on the first postoperative day. No adverse events, organ dysfunction, or need to re-initiate NO therapy related to therapy were observed.

Conclusion

Medical plasma-chemical device for nitric oxide synthesis (TIANOX KS) has been developed for the first time to deliver NO into extracorporeal circuit. Safety and efficacy of this procedure were verified in experiments and randomized clinical trials.

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