ALTERATION OF THE NEONATAL PULMONARY PHYSIOLOGY AFTER TOTAL CARDIOPULMONARY BYPASS

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Objectives: The purpose of this study was to analyze the mechanisms associated with lung injury after cardiopulmonary bypass and to propose strategies of prevention. **Methods:** Thirty-two neonatal piglets underwent 90 minutes of hypothermic cardiopulmonary bypass without aortic cross-clamping. Five experimental groups were defined: group I had standard cardiopulmonary bypass (control), group II received continuous low-flow lung perfusion during cardiopulmonary bypass, group III treatment was similar to that of group I with maintenance of ventilation, group IV received pneumoplegia, and group V received nitric oxide ventilation (30 ppm) after cardiopulmonary bypass. Data drawn from hemodynamic and gas exchange values and muscular and pulmonary tissular levels of adenosine triphosphate (in micromoles per gram) and myeloperoxidase (in international units per 100 mg) were used for comparisons before and 30 and 60 minutes after cardiopulmonary bypass. Pulmonary and systemic vascular endothelial functions were assessed in vitro after cardiopulmonary bypass on isolated rings of pulmonary and iliac arteries. **Results:** Pulmonary vascular resistance index, cardiac index, and oxygen tension were better preserved in groups II, IV, and V. All groups disclosed a significant decrease in lung adenosine triphosphate levels and an increase in myeloperoxidase activity whereas these levels stayed within pre-cardiopulmonary bypass ranges in muscular beds. Endothelium-dependent relaxation was preserved in systemic arteries but was strongly affected in pulmonary arteries after cardiopulmonary bypass. None of the methods that aimed to protect the pulmonary vascular bed demonstrated any preservation of pulmonary endothelial function. **Conclusion:** Cardiopulmonary bypass results in ischemia-reperfusion injury of the pulmonary vascular bed. Lung protection by continuous perfusion, pneumoplegia, or nitric oxide ventilation can prevent hemodynamic alterations after cardiopulmonary bypass but failed to prevent any of the biochemical disturbances. (J Thorac Cardiovasc Surg 1997;114:1061-9)

Cardiac operations for repair of acquired or congenital heart defects mandate in the vast majority of cases the use of cardiopulmonary bypass

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(CPB). In the early days of CPB, pulmonary complications caused a significant percentage of the cases of morbidity and mortality.¹ In the past decades, during which time CPB has become commonplace, improvements in technology and better understanding of the physiopathologic processes of lung injury after these procedures have reduced the incidence of pulmonary complications, but they still remain a problem. Acute pulmonary hypertension with decreased gas exchange and low cardiac output are not uncommon, and microscopic alterations of the lung parenchyma have been demonstrated.² Since the initial work of Chenoweth and colleagues,³ who demonstrated the release of complement-derived anaphylatoxins C3a and C5b during and after

CPB, literature on the inflammatory response caused by CPB has been extensive and a number of cytokines have been addressed and demonstrated to take part in the post-CPB inflammatory syndrome.⁴

Recent advances have made neonatal complete repair for many congenital heart defects safer than ever before. However, the neonatal immature lung presents several particularities that make it more vulnerable to CPB, and a higher rate of pulmonary complications have been reported in these neonates than in an adult population.⁵

During total CPB, pulmonary blood flow is completely shut off and the lungs are perfused by bronchial flow alone, putting the lungs at risk for an ischemic insult and reperfusion injury when normal antegrade flow is reestablished through the pulmonary artery after CPB. On the other hand, CPB has been demonstrated to induce inflammatory humoral cascades and cellular activation, which lead to tissue injury.

The primary goal of this study was to investigate the biochemical and hemodynamic variations occurring in the pulmonary vascular bed after total CPB in neonatal piglets. A further objective of this work was to define whether prevention of these variations is feasible.

Material and methods

Thirty-two neonatal piglets (mean age 7 days, mean weight 3.2 kg) were purchased from a local farmer. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). The piglets were fasted for 12 hours before the operation, sedated with intramuscular ketamine (250 mg), and anesthetized with pentobarbital sodium (15 mg/kg intravenously). Subsequently, the animals were intubated and general anesthesia was maintained with N₂O (60%)/O₂ (40%) with intermittent positive ventilation pressure (MMS 107 ventilator, Paris) at 40 breaths/min with a tidal volume of 15 ml/kg.

With the piglets in a supine position, the femoral vessels were isolated on each side. After femoral arterial cannulation, ventilatory rate and tidal volume were adjusted to establish a normal pH and carbon dioxide tension as determined by arterial blood gas determination (ABL2, Radiometer, Copenhagen). The contralateral superficial femoral artery was isolated and harvested for pre-CPB systemic endothelial function assessment *in vitro*. After completion of a midline sternotomy and pericardiotomy, the ductus was ligated and the great vessels were controlled. A pulmonary artery catheter (Intracath) was introduced into the main pulmonary artery through the right ventricular infundibulum and cardiac output was

recorded with use of a Doppler probe placed around the pulmonary artery trunk (model T106 M, Transonic Systems). The central venous pressure and the left atrial pressure were monitored after placement of indwelling catheters in the right and left atrium. Pressure recording was performed with a P23 ID Statham pressure transducer.

After heparin administration (3 mg/kg), CPB was instituted from the right atrium with the arterial return directed into the ascending aorta. The bypass circuit consisted of a cardiomy reservoir and a membrane oxygenator (Dideco), a heat exchanger, and a roller pump. No arterial filter was used. The circuit was primed (400 ml) with whole blood obtained from a donor pig the day before the operation. Within the first 5 minutes of CPB, a vent was introduced through the left ventricular apex. Mean systemic arterial pressure was maintained at prebypass values with a mean flow rate of 500 ml/min. After a steady state under CPB was obtained, ventilation was stopped and cooling to 28° C was started. The pulmonary artery trunk was crossclamped to prevent any antegrade flow to the lungs while CPB was maintained for 90 minutes without aortic crossclamping. During CPB pulmonary blood flow was measured by collecting the blood that drained from the left ventricle. After this period mechanical ventilation was reinstituted and the piglets were weaned from CPB. After 1 hour of survival, they were killed. The pulmonary arterial vessels were dissected beyond the second-generation branch and mounted in an organ chamber for vascular reactivity evaluation. In addition, systemic vessels from the iliac artery were dissected free and mounted in an organ chamber for vascular physiologic studies.

Physiologic measurements. Hemodynamic measurements were made before institution of CPB and every 30 minutes after cessation of CPB. Cardiac output was determined as pulmonary blood flow (Q_{pa} in liters per minute). Cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated as follows:

$$CI = Q_{pa}/\text{Body weight (kg)}$$

$$\begin{aligned} SVRI (\text{dyne} \cdot \text{sec}^{-1} \cdot \text{cm}^{-5} \cdot \text{kg}) \\ = (MAP - RAP)/CI \cdot 79.9 \end{aligned}$$

$$\begin{aligned} PVRI (\text{dyne} \cdot \text{sec}^{-1} \cdot \text{cm}^{-5} \cdot \text{kg}) \\ = (PAP - LAP)/CI \cdot 79.9 \end{aligned}$$

where MAP is the mean systemic arterial pressure, RAP the mean right atrial pressure, PAP the mean pulmonary arterial pressure, and LAP the mean left atrial pressure.

Throughout the experiment, central, myocardial, and lung temperatures were monitored. Biopsy specimens were obtained before and 30 and 60 minutes after CPB for adenosine triphosphate (ATP), lactate, and myeloperoxidase (MPO) measurements from lung and skeletal muscle tissues.

Evaluation of pulmonary energy metabolism

ATP and lactate measurements. All biopsy specimens of fresh tissues were immediately frozen at a depth of 1 mm

with metal tongs chilled in liquid nitrogen. Once frozen, the tissues were stored at -80°C . ATP and lactate measurements were performed according to the methods described by Date and associates.⁶ The specimens were pulverized in liquid nitrogen and metabolites were extracted in perchloric acid 0.6 N. After centrifugation and neutralization, the supernatants were analyzed for metabolites by the enzymatic methods of Lowry and Passonneau.⁷ Data are given as micromoles per gram of frozen tissue.

Evaluation of pulmonary leukocyte sequestration.

Blood samples were drawn from the pulmonary artery catheter and left atrial line for total and differential (neutrophils) white blood cell counts (Argos 3, ABX France, Montpellier) before the start of CPB, at the time of pulmonary reperfusion, and 30 and 60 minutes after the discontinuation of CPB. The percentage of pulmonary leukosequestration was expressed as the difference between the pulmonary artery and left atrial white blood cell count divided by the pulmonary artery white blood cell count all multiplied by 100.

MPO activity. The method described by Mullane, Kraemer, and Smith⁸ was used to measure MPO activity in the lungs. Before and 30 and 60 minutes after CPB, biopsy samples were frozen in liquid nitrogen and stored at -80°C . They were then pulverized and homogenized in 10% wt/vol hexadecyltrimethyl ammonium bromide buffer (0.5% hexadecyltrimethyl ammonium bromide in 50 mmol/L phosphate buffer at pH 6.0) with a Polytron homogenizer. The homogenate was sonicated on ice for 15 seconds, frozen at -70°C , and thawed three times, then centrifuged at 40,000g for 15 minutes. Supernatant was assayed for MPO activity spectrophotometrically. Twenty microliters of supernatant was combined with 12 μL of 25 mM H_2O_2 , 30 μL of 40 mmol/L O-dianisidine hydrochloride, and 1.938 ml of 50 mmol/L phosphate buffer (pH 6.0). The change in absorbance was measured at 460 nm on a Beckman spectrometer (model 25). One unit of MPO activity was defined as the activity degrading 1 μmol of peroxide per minute at 25°C .

Isolated pulmonary arterial ring studies. At the end of each experiment, left and right intrapulmonary arterial segments were dissected out and placed in warm Krebs-Henseleit buffer composed of (in micromoles per liter) 118.3 NaCl, 4.7 KCl, 2.5 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.2 KH_2PO_4 , 1.2 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 25 NaHCO_3 , 0.03 ethylenediaminetetraacetic acid, and 11.1 glucose. Isolated pulmonary arteries were cleaned and cut into rings 3 to 4 mm in length (1 to 2 mm outer diameter). Three to four rings were obtained from each animal. The rings were then mounted on stainless steel hooks, suspended in 10 ml tissue baths, and connected to force displacement transducers (LB-5, Showa-sokki) to record changes in force by the use of a chart recorder (LR 4210, Yokogawa). The baths were filled with 10 ml of Krebs-Henseleit buffer and aerated at 37°C with a gas mixture of 95% O_2 and 5% CO_2 . Pulmonary arterial rings were initially stretched to produce a preload of 1g of force and equilibrated for 60 to 90 minutes. A preload of 1g has been proved to be the optimal resting tension on the basis of length tension analyses performed in pilot studies. This value is close to the optimal resting tension ($1.060 \pm 0.040\text{g}$) found by Liu

and associates⁹ in piglet neonatal pulmonary arterial rings. During this period, the Krebs-Henseleit buffer in the tissue baths was replaced every 10 minutes. After incubation with indomethacin (10^{-5} mol/L) for 60 minutes, a concentration-response curve to phenylephrine was obtained. The rings were then washed, and the developed force was allowed to return to the baseline level. The rings were then precontracted with phenylephrine to generate about 1g of developed force. Once a stable contraction was obtained, cumulative doses of acetylcholine (10^{-9} to 10^{-3} mol/L) were added to the bath to assess changes in endothelium-dependent relaxation. These rings were washed again and allowed to equilibrate to baseline levels. The procedure was repeated with a single dose (10^{-5} mol/L) of sodium nitroprusside, an endothelium-independent vasodilator.

In addition to the change in force, responses were assessed by determination of the concentration that produced 50% of the maximal response (EC_{50}) extrapolated from a plot of log concentration versus percentage of maximal response. The contractile responses to phenylephrine were expressed in absolute values (milligrams) and the maximum relaxation to acetylcholine and sodium nitroprusside was expressed as the percentage of the phenylephrine-induced precontraction: 0% indicates no relaxation and 100% a relaxation that equals the magnitude of the precontraction.

Pulmonary arterial rings obtained after CPB were compared with control pulmonary artery rings obtained from matched piglets that did not undergo CPB.

Groups. Five groups were studied. Group I ($n = 7$) was a control group in which no intervention was made to protect the lungs during CPB. In group II ($n = 7$) the pulmonary vascular bed was perfused (mean flow 35 ml/min, mean perfusion pressure 14 mm Hg) with venous blood during CPB with an independent pump. In group III ($n = 6$) the ventilation was maintained during CPB. In group IV ($n = 6$) Cambridge pneumoplegic solution was administered during CPB at crossclamping of the pulmonary artery, and in group V ($n = 6$) the piglet lungs were ventilated with nitric oxide (NO; 30 ppm) at the time of pulmonary reperfusion.

Drugs. The following drugs were used (Sigma Chemical): indomethacin, phenylephrine, acetylcholine, and sodium nitroprusside. All drugs were freshly prepared on the day of the experiment.

Statistical analysis. All results are expressed as mean plus or minus the standard error of the mean. Intergroup and intragroup mean values were compared by two-way analysis of variance to analyze the combined effect of time and group. The Newman-Keuls multiple-sample comparison test was used to evaluate any differences in the results.

Results

Hemodynamic results (Fig. 1). Pulmonary venous return drained to the reservoir was reduced to a mean of 0.5% of bypass flow. This was only increased to a mean of 15% in group II. The systemic vascular resistance index increased in all groups

Fig. 1. A, Variations in the systemic vascular resistance index. B, Variations in the pulmonary vascular resistance index. C, Variations in the cardiac index. D, Variations in the oxygen tension values. *t0*, Before CPB; *t1*, 30 minutes after weaning from CPB; *t2*, 60 minutes after weaning from CPB. The groups are those described in the text. **p* = 0.001 versus *t0*.

after 90 minutes of hypothermic CPB without cross-clamping of the aorta but this reached statistical significance only in groups I and III.

Pulmonary vascular resistance values and pulmonary vascular resistance index were significantly increased after CPB in groups I and III. Lung protection by continuous lung perfusion, by pneumoplegia, or by NO ventilation at the time of pulmonary reperfusion seems to prevent the increase in pulmonary vascular resistance and pulmonary vascular resistance index.

Cardiac output and cardiac index were better preserved in groups IV and V at 30 and 60 minutes after the cessation of bypass. Although both cardiac output and cardiac index were initially (at 30 min-

utes) well preserved in group II, they demonstrated a significant trend to decrease 1 hour after CPB. On the other hand, groups I and III showed a severe reduction of cardiac output and cardiac index after bypass and inotropic support was necessary in four of the piglets to maintain survival.

Oxygen delivery was also significantly affected in groups I and III.

Pulmonary energy metabolism (Fig. 2). All groups showed a significant decrease in pulmonary parenchymal ATP stores. However, groups I and III had the lowest levels at 30 minutes after the cessation of bypass, which then plateaued until the animals were killed. On the other hand, groups II, IV, and V demonstrated better preservation of ATP

Fig. 2. Variations in the lung ATP content before (*T0*) CPB and 30 minutes (*T1*) and 60 minutes (*T2*) after weaning from CPB. The groups are those described in the text. **p* = 0.005 versus *T0*; †*p* = 0.002 versus *T1*.

Fig. 3. Variations in the lung MPO activity before (*T0*) CPB and 30 minutes (*T1*) and 60 minutes (*T2*) after weaning from CPB. The groups are those described in the text. **p* = 0.001 versus *T0*.

stores at 30 minutes after bypass, which continued to decrease at 60 minutes. In this model, muscular ATP stores were preserved to pre-CPB levels throughout the experimental procedure (1.33 ± 0.13 $\mu\text{mol/gm}$ fresh tissue before bypass vs 1.32 ± 0.12 $\mu\text{mol/gm}$ fresh tissue 60 minutes after weaning from bypass).

The inverse profile was observed with lactate accumulation in lung parenchyma (1.76 ± 0.28 $\mu\text{mol/gm}$ fresh tissue before bypass vs 5 ± 0.8 $\mu\text{mol/gm}$ fresh tissue 60 minutes after cessation of bypass).

MPO activity and pulmonary leukocyte sequestration (Figs. 3 and 4). MPO activity was significantly increased in all groups at 60 minutes after

bypass; however, this increase was delayed in groups I, II, and V. Neutrophil sequestration in the lung parenchyma after CPB was significantly increased at 30 and 60 minutes after bypass only in group V. Throughout the experimental procedure and in all groups, MPO activity in skeletal muscle could not be detected either before CPB or 60 minutes after cessation of bypass.

Isolated pulmonary and femoral arterial ring study (Figs. 5 and 6). In pulmonary vessels, similar maximal contraction to phenylephrine was observed in all groups. The EC_{50} to phenylephrine in each group was not different from that in control animals not subjected to CPB.

Fig. 4. Variations in the percentage of lung neutrophil sequestration before (T_0) CPB and 30 minutes (T_1) and 60 minutes (T_2) after weaning from CPB. The groups are those described in the text. $*p = 0.005$ versus T_0 .

Fig. 5. Endothelium-dependent relaxation in pulmonary vessels. The vessels were precontracted with phenylephrine and cumulative doses of acetylcholine were added to the bath. The groups are those described in the text. $*p = 0.01$ versus Control No CPB.

The maximal relaxant response to acetylcholine obtained in all groups was reduced to 50% of control values. The EC_{50} for acetylcholine was nearly unchanged in all groups and ranged from $8.5 \cdot 10^{-8}$ to $1.8 \cdot 10^{-7}$ mol/L. These results indicate that after CPB endothelium-dependent relaxation of pulmonary artery was impaired. Conversely, the response to sodium nitroprusside was not altered after CPB and ranged from 10% to 120%.

In contrast to the results obtained with pulmonary arterial rings, the endothelium-dependent relax-

ation measured in systemic vessels was not altered after total CPB.

Discussion

This study confirms the observation of previous works that during total CPB the pulmonary bed is subjected to an ischemia-reperfusion injury that leads to a decrease in the energy stores of the lung parenchyma.¹⁰⁻¹⁴ Interesting enough was the failure of general hypothermia to 28°C to prevent the loss of ATP and the accumulation of lactates in lungs

Fig. 6. Endothelium-dependent relaxation in systemic vessels. The vessels were precontracted with phenylephrine and cumulative doses of acetylcholine were added to the bath. The groups are those described in the text.

tissue, whereas these stayed within control values in territories not subjected to ischemic injury.

Neutrophil involvement in the pathophysiologic processes of the pulmonary vascular injury after CPB is a well-described phenomenon.¹⁵⁻²⁰ However, in the present study, the lungs only showed dysfunction whereas the systemic vascular beds, which did not undergo ischemia-reperfusion injury, did not present hemodynamic impairment, leukocyte sequestration, or endothelial dysfunction. These results tend to demonstrate that vascular tissue injury occurs when both endothelial injury and neutrophil activation are present. Activated neutrophils alone or associated with plasmatic inflammatory cytokines, which are known to be released during CPB, failed in the present study to cause peripheral vascular tissue injury because MPO activity and endothelial control of vascular tone were preserved in those territories.

Another consequence of CPB was the loss of vascular tone control by pulmonary artery endothelial cells. This has been already reported in experimental models^{14, 21-23} and in patients.²⁴ Several hypotheses have been proposed to explain this phenomenon. Some reports imply the role of a systemic inflammation reaction including free radicals from activated neutrophils¹⁷ and thromboxane A₂.^{11, 25, 26} More recently, Morita and coworkers²³ have shown

a protective effect of the pulmonary endothelium after CPB by the use of antioxidant agents arguing that hydrogen peroxide and the subsequent production of hydroxyl radical is a strong effector of endothelial damage. Finally, another hypothesis advanced to explain the mechanism of pulmonary endothelial dysfunction after CPB is the existence of an ischemic insult to the endothelial cells. Previous studies on cultured endothelial cells submitted to various durations of hypoxia showed a significant deficiency of intracellular high-energy phosphates^{27, 28} and the cells lost their capacity to contract.²⁹ In addition, reoxygenation is likely to produce a lipid peroxidation with membrane damage and production of reactive O₂ species.³⁰ Additionally, previous work from our laboratory has shown in an isolated perfused lung model that ischemia alone is responsible for a loss of endothelial pulmonary vascular tone control, which was even amplified by reperfusion.³¹

We can therefore speculate that when endothelial cells are subjected to an ischemic insult there is a significant loss of intracellular high-energy phosphates with a parallel activation of glycolysis as evidenced by increased lactate production. Endothelial cells then express surface antigens that are targets for activated neutrophils. The latter adhere at reperfusion on the endothelium and produce a

toxic amount of cytotoxic enzymes that induce tissue destruction.

On the assumption that lung ischemia-reperfusion syndrome was the main cause of the endothelial dysfunction observed in pulmonary arteries and that activated neutrophils mandate an endothelial injury to further exert detrimental effects on vascular tissues, we attempted to avoid lung ischemia-reperfusion injury by several methods. Reduction of energy demand was achieved by general hypothermia, which alone failed to protect the pulmonary vascular bed (group I). Provision of energy substrates to the lungs during CPB was achieved either by continuous lung perfusion (group II) or by ventilation (group III). Pneumoplegia (group IV) was performed because of the similitude that may exist with lung transplantation, and, finally, post-CPB ventilation with NO (group V) was used because NO has been demonstrated to avoid neutrophil lung sequestration and to reduce reperfusion injury after lung ischemia and reperfusion in neonates.³²

Although the post-CPB hemodynamic status was better preserved when the lungs were protected by continuous perfusion, pneumoplegia, or inhalation of NO at reperfusion, there were no significant differences among the five groups in the values reflecting the lung energy metabolism, neutrophil lung sequestration, or capacity of the pulmonary arterial rings to relax to an endothelium-dependent agonist. However, groups that received lung protection by continuous perfusion, pneumoplegia, or NO at reperfusion demonstrated a better initial conservation of high-energy phosphates, which secondarily decreased with time. The absence of a correlation between post-CPB hemodynamic status and biologic results might be explained by several hypotheses. Continuous lung perfusion in the present model was performed under suboptimal and nonphysiologic conditions, with a low, nonpulsatile flow, and probably resulted in nonperfused areas. In this group, to maintain a determined rate of flow, we adapted the flow to the pulmonary arterial pressure kept at about 15 mm Hg. This pressure level was achieved with a suboptimal flow rate of 35 ml/kg per minute. Any increase in flow rate was associated with an extraphysiologic increase in pulmonary arterial pressure. The reasons for such reaction, although unclear, could be explained by the facts that during operations with an open thorax lung compliance is modified and that hypothermia per se may lead to pulmonary vasoconstriction. In addition, pneumoplegia was not repeated during CPB and the

solution was quickly washed out by the collateral flow from the bronchial arteries; therefore its beneficial effects, if any, were only transient.

The most surprising results were obtained with NO inhalation at lung reperfusion and after cessation of CPB. In a model of isolated neonatal lung ischemia and reperfusion, NO provided at lung reperfusion was able to prevent reperfusion injury with conservation of pulmonary endothelial function and an absence of neutrophil sequestration.³² In the present model, NO inhalation at the end of CPB was associated with the highest rate of lung neutrophil sequestration and failed to provide protection of the pulmonary endothelium. Several methodologic factors between isolated lung perfusion and CPB might explain these different results: (1) in CPB, the bronchial circulation although minimal is still patent and can provide to the ischemic endothelial cells circulating activated neutrophils and cytokines and (2) innervation is maintained intact during CPB and might have physiologic implications for the pulmonary microcirculation with vasoconstriction and neutrophil entrapment. This paradoxical effect of NO has been previously documented³³ and was supported by the evidence of interaction between NO and superoxide, which can combine to form peroxynitrite anion, which can generate hydroxyl anion, both of which are potent oxidizing components.

In conclusion, neonatal CPB leads to severe ischemic damage of the pulmonary vascular endothelium, which then interacts with activated neutrophils at reperfusion. More refined cellular and subcellular experimentation seems necessary to determine the different sequences of cellular damage and then to propose preventive therapy. Among the different methods that aim to protect the lungs during CPB, continuous lung perfusion, pneumoplegia, and NO ventilation at lung reperfusion prevent more severe hemodynamic deterioration.

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