Penehyclidine hydrochloride preserves the intestinal barrier function in patients undergoing cardiopulmonary bypass

Ying-jie Sun, PhD, Dan-dan Song, MD, Yu-gang Diao, PhD, Jin Zhou, PhD, and Tie-zheng Zhang, MD

Objective: The study objective was to investigate the protective effect of penehyclidine hydrochloride on intestinal barrier function integrity and its therapeutic potential on endotoxemia and systemic inflammatory response in patients undergoing cardiopulmonary bypass.

Methods: Forty patients undergoing cardiac valve replacement with cardiopulmonary bypass were enrolled in the study. All patients were randomly divided into the penehyclidine hydrochloride or control group (20 patients in each group). Patients in the penehyclidine hydrochloride group received an intravenous injection of 0.05 mg/kg penehyclidine hydrochloride 10 minutes before cardiopulmonary bypass, and those in the control group were given the same volume of saline. Blood samples for blood glucose, lactic acid, intestinal fatty acid binding protein, D-lactate, serum endotoxin (lipopolysaccharide), interleukin-6, and interleukin-10 measurements were collected during the following time points: immediately after anesthesia induction (T₀), 10 minutes after the release of aortic-clamping (T₁), immediately after weaning from cardiopulmonary bypass (T₂), 2 hours postoperatively (T₃), 6 hours postoperatively (T₄), and 18 hours postoperatively (T₅).

Results: Blood glucose, lactic acid, intestinal fatty acid binding protein, D-lactate, lipopolysaccharide, interleukin-6, and interleukin-10 were significantly increased at all postoperative time points. At specific postoperative time points, blood glucose, lactic acid, intestinal fatty acid binding protein, D-lactate, lipopolysaccharide, and interleukin-6 were statistically lower in the penehyclidine hydrochloride group than in the control group. Postoperatively, interleukin-10 did not differ between the penehyclidine hydrochloride and control groups.

Conclusions: Penehyclidine hydrochloride preserves intestinal barrier function integrity, attenuates endotoxemia, and inhibits systemic inflammatory response in patients undergoing cardiopulmonary bypass, possibly by improving intestinal microcirculation and depressing stress response. (J Thorac Cardiovasc Surg 2013;146:179-85)

Cardiopulmonary bypass (CPB) is an indispensable technique that temporarily takes over the function of the heart and lungs for most cardiac operations, maintaining the circulation of blood and the oxygen content of body. The non-pulsatile flow during CPB results in splanchnic ischemia by means of capillary closure and shunting. Intestinal ischemia is paralleled by a disruption of the intestinal mucosal barrier and an increase in intestinal wall permeability, which causes translocation of luminal bacteria and the endotoxins to pass into circulation. In addition, hypothermia associated with CPB has been thought to increase the production of endogenous endotoxins, probably because of intestinal ischemia, decreasing intestinal motility, lower enzyme activity, and impaired function of Kupffer cells. The endotoxemia after CPB adversely affects postoperative recovery in patients with an associated mortality of 12% to 67%. Classic anticholinergics, such as tropane alkaloids, dominantly block acetylcholine receptors and exhibit a wide range of biological activities, including antioxidation and cytoprotective activity. However, these drugs exhibit classic antimuscarinic side effects, including dry mouth and accelerated heart rates. A new anticholinergic drug, penehyclidine hydrochloride (PHC), was developed by the Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, to minimize the side effects harmful to the cardiovascular system. Because PHC selectively blocks M₁, M₃, and N receptors, there are few M₂ receptor–associated side effects, including dry mouth and accelerated heart rates. PHC has both antimuscarinic and antinicotinic activities and retains potent central and peripheral anticholinergic activities. PHC is commonly used in the clinic as an antagonist of organic phosphorus poisoning, asthma, and chronic obstructive pulmonary diseases. More significant, clinical and experiment studies indicate that PHC is beneficial in treating septic shock, acute lung injury, and CPB-associated liver damage.

On the basis of the potential role of PHC as an antioxidant and a cell membrane stabilizer, we postulated that...
Abbreviations and Acronyms

CPB = cardiopulmonary bypass  
ICU = intensive care unit  
I-FABP = intestinal fatty acid binding protein  
IL = interleukin  
LPS = lipopolysaccharide  
PHC = penehyclidine hydrochloride  
SIRS = systemic inflammatory response syndrome  
TNF-α = tumor necrosis factor-alpha

PHC therapy might be beneficial for the maintenance of intestinal barrier integrity and subsequent attenuation of endotoxemia and systemic inflammatory response syndrome (SIRS). Zhang and colleagues16 demonstrated that PHC post-conditioning reduces small-intestine permeability after limb ischemia–reperfusion by inhibiting oxygen free radicals and inflammatory cytokines for organ damage. More important, our previous research indicated that PCH improves damage resulting from intestinal ischemia, inhibits bacteria translocation, attenuates the release of endotoxins, and further preserves the structure and function of intestinal mucosa in a rat model of CPB.17 The purpose of this study was to investigate whether PHC is effective in preserving the intestinal barrier function and attenuating the endotoxemia and SIRS in patients undergoing CPB. In this study, blood glucose, lactic acid, intestinal fatty acid binding protein (I-FABP), D-lactate, serum endotoxin (lipopolysaccharide [LPS]), interleukin (IL)-6, and IL-10 levels were examined in serum. The dosage of PHC administration, ranging from 0.03 to 0.45 mg/kg, has been proved to be effective and curative in both animal study and clinical investigation.11-17 In the study, the dose of 0.05 mg/kg was administered to minimize the side effects on patients.

PATTERNS AND METHODS

Patients

Forty patients undergoing cardiac valve replacement were followed prospectively from September 2009 to April 2012. This study was approved by the Institutional Committee on Human Research in the General Hospital of Shenyang Military Region, and all patients provided informed consent. Exclusion criteria were as follows: severe anemia, liver and renal insufficiency, any history or signs of endocrine or infectious disease, active or history of nervous or mental disorders, disorders involving the intestines, or disturbances in blood coagulation. All patients had an ejection fraction greater than 50% before cardiac surgery. Eighteen patients underwent mitral replacement, 12 patients underwent aortic replacement, and 10 patients underwent combined mitral and aortic replacement. Bioprostheses and mechanical valve implantation were performed in 14 and 26 subjects, respectively. The patients were randomly divided into 2 groups: those who received the injection of PHC (0.05 mg/kg, Lisite Pharmacology Co, Chengdu, China, No. 080301) through the internal jugular vein 10 minutes before the onset of CPB and those who received the same volume of saline as a control.

Anesthesia

The patients fasted for at least 8 hours and received a glycerin enema before the cardiac procedure. The radial artery was cannulated, and blood pressure, heart rate, oxygen saturation, and electrocardiograph were measured using a Datex-Ohmeda S/5 Anesthesia Monitor (GE Healthcare, Helsinki, Finland). General anesthesia was induced with fentanyl 0.8 μg/kg, etomidate 0.2 mg/kg, pipercuronium 0.1 mg/kg, and midazolam 0.1 mg/kg. Maintenance of anesthesia was performed with infusion of fentanyl 5 to 10 μg/kg/h, boluses of midazolam 0.1 mg/kg and pipercuronium 0.1 mg/kg, and 0.6% to 0.8% end-tidal isoflurane. All patients were ventilated with an oxygen–air mixture equal to an inspired oxygen fraction of 0.7 to maintain an end-tidal carbon dioxide tension of 35 to 45 mm Hg. After oral tracheal intubation, a 3-lumen central venous catheter (Arrow, Reading, Pa) was inserted into the right internal jugular vein.

Cardiopulmonary Bypass Technique

After performing a median sternotomy, all patients were given 300 to 400 U/kg heparin. The ascending aorta was cannulated, and venous cannulation was usually accomplished by direct superior vena cava and inferior vena cava cannulation. An extracorporeal circuit was used in all patients, which comprised a Jostra HL-20 heart-lung machine (Jostra USA, Austin, Tex) for nonpulsatile flow and a hollow-fiber membrane oxygenator with an integral heat exchanger (Medtronic Cardiopulmonary, Anaheim, Calif) for gas exchange. The pump priming solution consisted of 1500 mL Ringer’s lactate solution and 100 g of albumin. Activated clotting times were maintained for at least 480 seconds, and hematocrit levels were kept at 25% to 30%. All valvular replacements were performed under moderate hypothermia with a nasopharyngeal temperature of 32°C to 35°C. A cold crystalloid cardioplegic solution was used. During CPB, the pump flow rate was 2.2 to 2.6 L/m²/min and the perfusion pressure was 50 to 80 mm Hg. On discontinuation of the CPB, heparin was neutralized with protamine sulfate. Postoperatively, the patients were admitted to the intensive care unit (ICU). The patients were extubated as soon as clinically indicated.

Clinical Indexes

At the time of hospital admission, demographics and medical diagnoses were recorded. The durations of anesthesia, surgery, aorta crossclamping, and CPB were recorded. At the time of ICU admission, the use of inotropes and vasopressors was recorded. The durations of postoperative ventilation, ICU, and hospital stays were recorded. The transfusion amount, occurrence of severe complications, and deaths were also recorded.

Blood Samples

Blood sampling was performed in all patients at 6 time points: immediately after induction of general anesthesia (T0), 10 minutes after the release of aorta clamping (T1), immediately after weaning from CPB (T2), 2 hours postoperatively (T3), 6 hours postoperatively (T4), and 18 hours postoperatively (T5). All blood samples were obtained from the internal jugular vein in an aseptic fashion. After blood sampling, a complete blood gas analysis was performed using the Bayer Model 865 blood gas analyzer, and blood glucose levels were determined with a glucose analyzer (ABL700, Radiometer Medical, Copenhagen, Denmark). Plasma whole blood samples (5 mL) were centrifuged for 10 minutes at 3500g, and the supernatants were stored at −70°C until measurement. Serum IL-6 and IL-10 were measured by enzyme-linked immunosorbent assay kits (Wuhan Boster Biological Technology, Ltd, Wuhan, China). Serum I-FABP was determined using a enzyme-linked immunosorbent assay kit (Beijing Sunbio Biological Technology, Ltd, China). Serum D-lactate levels were measured using an enzymatic spectrophotometric assay using a centrifugal analyzer at 30°C as described previously.18 Serum endotoxin levels were determined using modified perchloric acid extraction. All data were normalized to remove the influence of hemodilution according to the following formula: the normalized data = the measured data × [Hctpreoperative − Hctpostoperative].
Statistical Analysis
All experimental data were reported as mean ± standard deviation and analyzed using SPSS for Windows 13.0 (SPSS Inc, Chicago, Ill). Student t test was used for comparison among groups. Intergroup comparisons were analyzed using repeated-measures analysis of variance.

RESULTS
Patients
The patients’ clinical data and demographics are summarized in Table 1. Demographic characteristics such as age, gender, and weight did not differ between the groups. There were no differences between groups in the durations of aorta clamping, CPB, and surgery. There were no differences between the groups in the transfusion amount, duration of mechanical ventilation, or length of ICU or hospital stay. All patients recovered uneventfully without having the occurrence of severe complications or death.

Blood Glucose and Lactic Acid
Blood glucose and lactic acid levels from both patient groups at the various time points are presented in Figure 1. Blood glucose and lactate acid levels in both groups were significantly increased after the onset of CPB and peaked at 6 hours postoperatively (P < .05). There were no marked differences in blood glucose or lactic acid levels preoperatively between the 2 groups (P > .05).

Blood glucose levels at all time points after the onset of CPB were lower in the PHC group than in the control group, and these differences reached statistical differences at 10 minutes after the release of aorta clamping (P = .031), and at 2 hours postoperatively (P = .025). Lactic acid levels at all time points after the onset of CPB were lower in the PHC group than in the control group, and this difference was statistically significant immediately after weaning from CPB (P = .019).

Intestinal Fatty Acid Binding Protein, D-Lactate, and Lipopolysaccharide
Serum I-FABP, D-lactate, and LPS levels from both patient groups at the various time points are presented in Figure 2. Serum I-FABP, D-lactate, and LPS levels in both groups were significantly increased after the onset of CPB (P < .05). I-FABP peaked at 6 hours postoperatively, D-lactate peaked at 2 hours postoperatively, and LPS peaked immediately after weaning from CPB. There were no marked differences in serum I-FABP, D-lactate, and LPS levels preoperatively between the 2 groups (P > .05).

Serum I-FABP, D-lactate, and LPS levels at all time points after the onset of CPB were lower in the PHC group than in the control group. These differences in serum I-FABP were statistically significant 10 minutes after the release of aorta clamping (P = .015), at 2 hours postoperatively (P = .033), and at 6 hours postoperatively (P = .046). These differences in D-lactate were statistically significant immediately after weaning from CPB (P = .039) and at 2 hours postoperatively (P = .028). These differences in LPS were statistically significant immediately after weaning from CPB (P = .036), 2 hours postoperatively (P = .029), and 6 hours postoperatively (P = .034).

Interleukin-6 and 10
Serum IL-6 and IL-10 levels from both patient groups at the various time points are presented in Figure 3. Serum IL-6 and IL-10 levels in both groups were significantly increased after the onset of CPB and peaked at 6 hours postoperatively (P < .05). There were no marked differences in IL-6 and IL-10 levels preoperatively between the 2 groups (P > .05).

Serum IL-6 levels at all time points after the onset of CPB were lower in the PHC group than in the control group, and these differences were statistically significant at 2 hours postoperatively (P = .024) and 6 hours postoperatively (P = .043). There were no differences in serum IL-10 levels at all time points after the onset of CPB between the 2 groups (P > .05).

DISCUSSION
The intestine has been an organ of interest in the initiation and perpetuation of the inflammatory response after surgery. The intestines also act as a barrier to prevent microorganisms and toxins contained within the lumen from spreading to distant tissues and organ. Impairment of the intestinal barrier often occurs in CPB because of inherent nonpulsatile flow and hypoxia, resulting in the increased intestinal permeability and subsequent translocation of bacteria and endotoxins from gut. Bacteria translocation and endotoxemia play a key role in the development of SIRS...
and multiple organ failure. Thus, the protection of intestinal barrier integrity is extremely critical for the attenuation of endotoxemia and SIRS after CPB. Our previous study demonstrated that PHC protected the structure and function of the intestinal mucosa in a rat model of CPB. The recent study focused on intestinal barrier injury, endotoxemia, SIRS, and the therapeutic potential of PHC in patients undergoing CPB.

CPB is a nonphysiologic intervention that leads to a major surge in counter-regulatory hormones, including adrenal cortex hormone, glucagon, and catecholamines, which are accompanied by an insulin-resistant state with subsequent stimulation of glucose and hyperglycemia. The accumulation of lactic acid is the direct result of oxygen deprivation and anaerobic metabolism. The nonpulsatile perfusion during CPB causes splanchnic ischemia by inducing capillary closure and shunting. Conversely, a continuous-flow left ventricular assist device is able to diminish vascular impedance, increase vascular compliance, and improve splanchnic perfusion. During periods of the ischemia and hypovolemia, the intestine vasoconstricts and shunts blood toward more vital organs, such as the heart and brain. The intestine is one of the most susceptible organs to hypoperfusion during conditions of stress. Thus, blood glucose and lactic acid concentrations are the useful markers to assess stress response and intestinal ischemia after CPB. In this study, the levels of blood glucose and lactic acids were significantly increased after the onset of CPB.

I-FABP is a 14-kDa cytosolic protein uniquely located in mature small-intestinal enterocytes. It is involved in the uptake and transport of fatty acids from the small-bowel lumen. I-FABP normally is almost undetectable in serum. During ischemic events, the mucosa is injured and intestinal permeability is increased, leading to the translocation of gut bacteria. Serum D-lactate in mammals normally is low.

FIGURE 1. Blood glucose and lactic acid levels in both groups were significantly increased after the onset of CPB and peaked at 6 hours postoperatively ($P < .05$). Blood glucose levels were statistically lower in the PHC group than in the control group at 10 minutes after the release of aorta clamping ($P = .031$) and at 2 hours postoperatively ($P = .025$). Lactic acid levels were statistically less in the PHC group than in the control group immediately after weaning from CPB ($P = .019$). PHC, Penehyclidine hydrochloride.
bacteria and its metabolic products, including D-lactate, into circulation. As early as 2 hours postoperatively, elevated plasma levels of D-lactate were measured in subjects with histologically proven intestinal ischemia. The translocation of gut bacteria further results in the elevated concentration of serum endotoxin. Thus, plasma D-lactate and serum endotoxin levels have been shown to be a useful marker used to monitor an increase in intestinal permeability, bacterial translocation, and endotoxia after CPB in early stages. In this study, serum I-FABP, D-lactate, and endotoxin were substantially increased after the onset of CPB. This demonstrated that CPB impairs intestinal barrier function integrity and increased intestinal permeability with resultant bacterial translocation and endotoxia. More significant, our study indicated that PHC therapy inhibits the elevation of I-FABP, D-lactate, and serum endotoxin, illustrating that PHC is beneficial in preserving the integrity of intestinal barrier function and depressing the translocation of gut bacteria and endotoxin. It is possible that PHC protects intestinal barrier function by stabilizing the cell membrane and enhancing the tolerance of enterocytes to hypoxia.

Inflammatory cytokine responses after CPB are physiologic and associated with a host systemic inflammatory response to exogenous stimulation. IL-6 functions as a proinflammatory cytokine and is one of the most important mediators of fever and the acute phase systemic response. The inflammatory response can lead to a compensatory anti-inflammatory reaction. IL-10 is an anti-inflammatory cytokine and is capable of inhibiting synthesis of proinflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha (TNF-α). The severity of the systemic inflammatory response is prominently influenced by the balance between proinflammatory and anti-inflammatory cytokines. In this study, we found that serum levels of IL-6 and IL-10 were significantly increased after...
the onset of CPB. This indicated that CPB initiated a proinflammatory and compensatory anti-inflammatory response. More significant, we also found that PHC therapy inhibits the elevation of IL-6 and did not change serum IL-10, illustrating that PHC attenuates the release of proinflammatory mediators and maintained the anti-inflammatory response.

PHC is a novel therapeutic agent that exhibits protective effects on intestinal barrier function in animal models of CPB and limb ischemia–reperfusion. In addition, previous investigations have reported that PHC therapy has curative effects after liver injury, acute lung injury, and septic shock. Zhan and colleagues demonstrated that PHC was effective in treating lung and liver damage during sepsis, and its mechanism of action was probably that PHC attenuates the release of proinflammatory mediators and maintained the anti-inflammatory response.

PHC mediates these responses by improving intestinal microcirculation and depressing stress response. PHC may preserve intestinal barrier function integrity, attenuate endotoxemia, and inhibit the systemic inflammatory response in patients undergoing CPB. It is likely that PHC mediates these responses by improving intestinal microcirculation and depressing stress response.

**Study Limitations**

The study has 2 major limitations. First, serum indicators were selected to determine the effect of PHC on intestinal barrier function and systemic inflammatory response. Because of the limited availability of tissue specimen, no tissue examination was performed to further confirm the conclusion. Second, although PCH has been proved to be effective on protecting intestinal barrier function, it does not selectively target the intestine and might exert side effects on several organs. Further investigations are required to understand its effects on a single organ.

**CONCLUSIONS**

PCH may preserve intestinal barrier function integrity, attenuate endotoxemia, and inhibit the systemic inflammatory response in patients undergoing CPB. It is likely that PCH mediates these responses by improving intestinal microcirculation and depressing stress response.

**References**


