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ORIGINAL ARTICLE

The effect of hemodilution by cardiopulmonary bypass on protein binding of olprinone

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Abstract
Purpose Olprinone is a phosphodiesterase type III inhibitor that is often used to increase cardiac output after cardiopulmonary bypass (CPB). Hemodilution by CPB is likely to decrease total olprinone concentration, but it may also increase the free (unbound) concentration of olprinone due to reduced protein binding. The aim of this study was to investigate the effect of hemodilution on the protein binding of olprinone.
Methods Eleven patients scheduled for elective cardiac surgery with CPB were enrolled in our study. Olprinone was continuously infused at a rate of 0.2 μg/kg/min from the time of the first surgical incision until the patient arrived at the recovery unit. Protein binding was evaluated twice, just before the start of CPB and at the beginning of withdrawal from CPB. Olprinone concentration and protein binding were determined with high-performance liquid chromatography and ultrafiltration methods, respectively. Olprinone protein binding was also evaluated in vitro.
Results Olprinone protein binding to albumin was 63 % in vitro, but it did not bind to alpha-1 acid glycoprotein. Olprinone protein binding in patients before CPB was 81.5 ± 4.3 %, whereas protein binding at withdrawal from CPB was 63.3 ± 14.3 %.
Conclusions Unbound olprinone concentration increased by 20 % during CPB, which suggests that the pharmacological effects of olprinone might be enhanced during and after CPB. Close hemodynamic monitoring is necessary to control the effects of olprinone after CPB, because CPB alters olprinone's pharmacokinetics.

Keywords Olprinone · Cardiac surgery · Cardiopulmonary bypass · Protein binding · Albumin

Introduction

Olprinone (Coretec®; Eisai, Tokyo, Japan) is a phosphodiesterase type III inhibitor (PDEI) with both strong inotropic and vasodilatory effects [1] that is often used in cardiac surgery for weaning from cardiopulmonary bypass (CPB) [2, 3]. However, due to its low clearance and small volume of distribution, overdosing has been known on occasion to cause severe hypotension [4, 5]. Overdosing does not simply reflect high plasma concentrations, since the drug is highly bound to plasma protein, and the free (unbound) drug concentration may increase without a change in total drug concentration. This may enhance the drug's pharmacological actions, especially in cardiac surgery where CPB requires blood to be diluted to reduce viscosity. Although dilution decreases the total concentration of the drug in the blood, it may increase the free drug concentration that is responsible for the drug's pharmacological actions. In this study, we investigated the effect of CPB on the protein binding of olprinone.

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Materials and methods

After receiving approval by the ethics committee of our institute, we recruited 11 patients scheduled for elective cardiac surgery with CPB from January 2006 to January 2007. All patients gave written informed consent. Patients with anemia, hypoproteinemia, renal dysfunction or hepatic dysfunction based on preoperative evaluations were excluded.

All oral intake was prohibited for at least 6 h before surgery, and patients were transferred into the operating theater without premedication. After the placement of monitors for direct arterial pressure measurement via the radial artery, electrocardiogram, pulseoximetery and bispectral index, anesthesia was induced with 600–800 μg fentanyl, 3–5 mg midazolam and 3 % sevoflurane. The trachea was intubated after administration of 0.1 mg/kg of vecuronium. Patients were artificially ventilated in a pressure-controlled mode with 40 % oxygen to maintain the end-tidal carbon dioxide between 35 and 40 mmHg. Anesthesia was maintained with 1–2 % sevoflurane and additional fentanyl. During CPB, anesthesia was changed from sevoflurane to a target-controlled infusion of propofol (target concentration 2.0 μg/mL). The CPB priming solution consisted of 500 mL Salinhes® (Fresenius Kabi Japan, Tokyo, Japan), 500 mL Bicarbon® (Ajinimoto Pharmaceuticals, Tokyo, Japan) and 300 mL Mannitol® (Yoshindou, Toyama, Japan). The target temperature was set at 34 °C during CPB.

Olprinone was started at an infusion rate of 0.2 μg/kg/min at the time of the first incision, and this rate was kept constant until the patient was transferred to the recovery unit. For weaning from CPB, 5 μg/kg/min dopamine was also administered. When it was difficult to keep the systolic pressure above 80 mmHg, 0.1 mg phenylephrine was administered as a bolus or noradrenaline was infused. After completion of surgery, patients were transferred to the recovery unit while they were still unconscious.

Blood sampling and olprinone measurement

Protein binding was measured just before the start of CPB and at the start of weaning from CPB. Arterial blood (5 mL) was collected after systemic heparinization in a tube, and the plasma was separated by centrifugation at 3000 rpm for 10 min and then pipetted into another plastic tube for storage at −70 °C until analysis. Protein binding was evaluated by an ultrafiltration membrane method (described below). Olprinone concentrations were determined using high-performance liquid chromatography at the Niigata University of Pharmacy and Applied Life Science [4]. Hemoglobin, total protein and albumin concentrations were measured three times: 1 or 2 days before surgery, just before the start of weaning from CPB and the day after surgery.

Measurement of protein binding in vitro

Olprinone binding to human serum protein and to human albumin and alpha-1 acid glycoprotein were evaluated in vitro. Human serum protein was obtained from a volunteer. Human serum albumin (albumin from human serum; Wako, Osaka, Japan) and alpha-1 acid glycoprotein (alpha-1 acid glycoprotein from human plasma; Wako) solutions were prepared at room temperature and adjusted to a pH of 7.4 with phosphate buffer. Olprinone was added to the serum to achieve a concentration of 20, 50, 100, 250, 500 or 1000 ng/mL, and the mixture was placed in a shaking apparatus at room temperature for 30 min. Consecutive 0.3-mL aliquots of each mixture were transferred to a centrifugal filter unit and ultrafiltered by centrifugation at 1400 *g* for 30 min at room temperature. Non-specific binding of olprinone to the filter was assessed in Ringer's solution. Protein binding was calculated using the following formula:

$$\text{Protein binding } (\%) = 100 - 100 \times C_{\text{corUF}}/C_{\text{preUF}},$$

where C_{corUF} is the drug concentration in the ultrafiltrate corrected for non-specific binding, and C_{preUF} is the drug concentration before ultrafiltration. Each ultrafiltration experiment was performed in duplicate.

Protein binding to human serum albumin and alpha-1 acid glycoprotein was then measured. Human serum albumin was dissolved in phosphate buffer to achieve a concentration of 1, 2, 2.5, 5, 7.5, 10.0 g/dL, while alpha-1 acid glycoprotein was dissolved in phosphate buffer to achieved a concentration of 25, 50, 100, 200, 250 mg/mL. Olprinone was added to the solutions to achieve a concentration of 20 ng/mL. Protein binding of these solutions was measured as presented above.

Statistics

Data are presented as the mean ± standard deviation (SD). The preoperative CPB and postoperative data were compared with a one-way analysis of variance followed by a Bonferroni post hoc test. A two-sided P value of <0.05 was considered to be significant.

Results

In vitro study

Olprinone binding to human serum protein was 80.1 ± 4.6 % in a concentration range of 20–1000 ng/mL of olprinone. Olprinone did not bind to alpha-1 acid glycoprotein, whereas olprinone binding to human serum albumin was 58.3 ± 2.1 % at albumin concentrations of >3 g/dL.

In vivo study

Eleven patients (7 males, 4 females) were enrolled in this study. All patients had normal preoperative laboratory data except for slight anemia. The demographic data are shown in Table 1. The surgical and anesthesia data are shown in Table 2. All patients were infused with a 5 % albumin solution during or after CPB. Nine patients received a transfusion of red blood cells.

The olprinone concentration was 15.5 ± 8.5 and 49.1 ± 34.9 ng/mL just before the start of and at the beginning of withdrawal from CPB, respectively. Protein binding was successfully measured in nine of the 11 patients: before CPB, it was 81.5 ± 4.3 %, but at the start of CPB weaning it was only 63.3 ± 14.3 % (range 33.0–88.5 %). Figure 1 shows that there was no significant relationship between total protein concentration and protein binding of olprinone. However, serum albumin concentration and protein binding of olprinone were positively correlated (Fig. 2; $R^2 = 0.42$, $P < 0.05$).

Table 3 shows the perioperative changes in hemoglobin, total protein and serum albumin concentrations. Hemoglobin, total protein and serum albumin concentrations decreased to 81, 60 and 70 % of their preoperative values, respectively, due to blood dilution by CPB. The hemoglobin concentration did not increase the day after surgery; however, total protein and serum albumin increased slightly but still remained below their preoperative values.

Table 1 Demographic data

Parameters	Value
Age (years)	66 ± 4.5
Gender (male/female)	7/4
Height (cm)	158 ± 6.1
Weight (kg)	57.5 ± 7.5
Body mass index (kg/m^2)	22.8 ± 1.8

Values are presented as the mean ± standard deviation

Table 2 Surgery and anesthesia data

	Values
Surgical procedure (AVR/MVP/myxoma)	6/4/1
Anesthesia time (min)	365 ± 69
Surgical time (min)	275 ± 55
CPB time (min)	113 ± 21.5
Bleeding (mL)	690 ± 434
Urine (mL)	932 ± 380
Infusion (mL)	3222 ± 650
5 % albumin (mL)	454 ± 148
RBC transfusion (mL)	420 ± 342
FFP transfusion (mL)	232 ± 224

Values are expressed as the mean ± SD

AVR Aortic valve replacement, *MVP* mitral valve plasty, *CPB* cardiopulmonary bypass, *RBC* red blood cell, *FFP* fresh frozen plasma

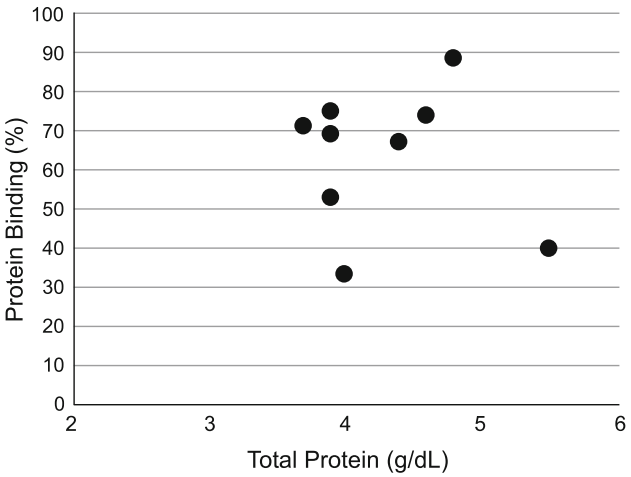

Fig. 1 Relationship between total protein and plasma protein binding. Total protein concentration and protein binding were measured at the start of weaning from cardiopulmonary bypass (CPB). There was no statistically significant relationship

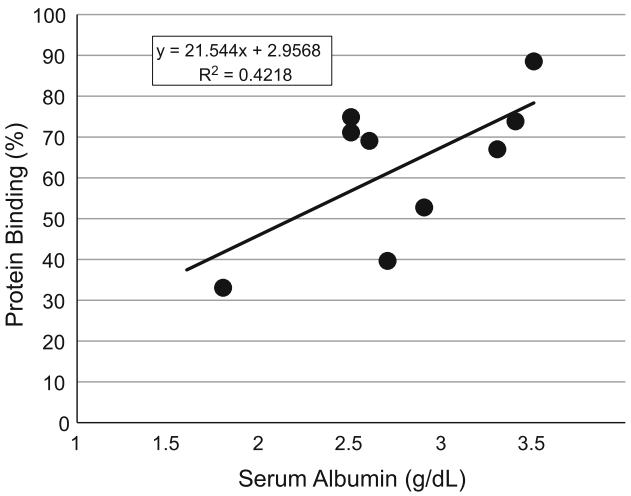

Fig. 2 Relationship between albumin concentration and protein binding. This relationship was positive ($P < 0.05$, simple linear regression analysis). Albumin concentration and protein binding were measured at the start of weaning from CPB

Discussion

Phosphodiesterase type III inhibitors, including olprinone, are water-soluble drugs that have relatively small distribution volumes [4, 5]. Therefore, dilution by CPB has a large effect on the circulating concentrations or protein binding of these drugs. The protein binding of olprinone in

Table 3 Hemoglobin, total protein and serum albumin

	Preoperative data	During CPB	Postoperative data
Hemoglobin (g/dL)	12.0 ± 1.35	9.78 ± 0.57*	9.52 ± 1.25*
Total protein (g/dL)	6.97 ± 0.23	4.20 ± 0.51*	5.13 ± 0.33*,#
Serum albumin (g/dL)	3.87 ± 0.41	2.72 ± 0.48*	3.48 ± 0.38*,#

* $P < 0.05$ vs. preoperative data by one-way analysis of variance (ANOVA) with Bonferroni post hoc test. # $P < 0.05$ vs. during CPB data byone-way ANOVA with Bonnferroni post-hoc test

Preoperative data were obtained 1 or 2 days before surgery. During CPB, data were obtained at the beginning of withdrawal from CPB. Postoperative data were obtained the day after surgery

Values are expressed as the mean ± SD

healthy normal volunteers has been determined to be 81 % [5], and the data obtained from the patients in our study were comparable. In our study, the protein binding of olprinone decreased from 81.5 to 63.3 % during CPB, indicating that the unbound drug fraction increased from 18.5 to 36.7 %. This should have increased the pharmacological effects by almost twofold, assuming the total drug concentration did not change and the unbound concentration remained on the linear portion of the dose–response curve.

Reasons for using a crystalloid priming solution are as follows: (1) to decrease blood viscosity and improve peripheral blood flow; (2) to improve oxygenation through the artificial lung; (3) to protect blood cells; (4) to maintain renal function [6]. In our institute, the CPB priming volume for normal-sized patients is 1300 mL of crystalloid, and this volume was used in all cases in our study. The mean body weight of our patients was 57.5 kg, and blood volume was calculated to be about 4000 mL (assuming blood volume is 7 % of body weight). Consequently, CPB initiation expanded blood volume from 4000 to 5300 mL and should have decreased the total olprinone concentration by 25 % of the value measured before CPB, if the olprinone concentration was at steady state.

Despite an increase in blood volume from the CPB priming circuit, the olprinone concentration increased from 15.5 ± 8.5 to 49.1 ± 34.9 ng/mL during CPB. Prior to this study, we had expected that CPB would reduce the total olprinone concentration by dilution. The main reason for the threefold increase was continuous infusion without a bolus or loading dose. In our institute, the duration of the time interval between the first surgical incision and the start of CPB is about 30 min, and that from the start of CPB to the completion of withdrawal from CPB is about 120 min. When olprinone is continuously infused without a bolus or loading dose, the calculated concentration at 150 min is about threefold higher than the value at 30 min (assuming a half-life of 100 min [5]). Thus, it is unlikely that the olprinone concentration had reached steady state at the start of CPB, and therefore we could not evaluate the effect of protein binding changes on pharmacological action during CPB. However, the in vitro study results verified that the protein binding values were accurate over a wide olprinone concentration range; thus, even if the olprinone concentration did not reach steady state, the protein binding values obtained in this study should be correct.

Proteins were also diluted by CPB. In this study, hemoglobin, total protein and serum albumin decreased to 81, 60 and 70 %, respectively, of the values measured before CPB. Before the start of CPB, 81.5 % of olprinone was bound to serum protein and 18.5 % was unbound. During CPD, however, 63.3 and 36.7 % of the olprinone was bound and unbound to serum protein, respectively. The olprinone concentration increased by threefold during CPB, and the synergistic effect between an increase in the unbound fraction and total olprinone concentration may have caused an exaggerated response. The pharmacological actions of olprinone may also be influenced by additional factors, such as dopamine, temperature, pH and water balance. Thus, it was difficult to isolate the effects of changes in protein binding from the effects of other variables that may change hemodynamic variables during weaning from CPB. There was no case in our study where the olprinone infusion had to be stopped. Therefore, further studies are necessary to clarify the effect of protein binding on the pharmacological actions of olprinone.

In our study, the protein binding of olprinone showed a positive relationship with serum albumin concentration but not with total protein concentration. These results indicate that the maintenance of albumin levels is important to obtain stable pharmacological effects. More recently, blood salvaging systems have been used to reduce the need for blood transfusions after CPB. With these systems, red blood cells are recovered, while serum proteins are discarded. When the CPB time is prolonged or when there is an excessive amount of bleeding, the administration of albumin should be considered to obtain the expected effects of olprinone. In our study, protein and albumin levels increased the day after surgery, but they were still lower than those measured prior to CPB. This results suggests that the pharmacological effects of not only olprinone, but also of other highly bound drugs,, may be enhanced the day after surgery.

Albumin binding is not specific to olprinone, and the administration of other drugs may compete with olprinone for albumin binding. This may result in significant drug interactions during CPB. In particular, drugs with high protein binding may influence the unbound concentration and pharmacological action of olprinone. Heparin, a drug used in cardiac surgery, binds extensively to plasma proteins, but its clinical concentration is not high enough to

cause competitive interaction at the albumin binding site. We found no in vitro olprinone binding to alpha-1 acid glycoprotein, which is a protein that binds to basic drugs, such as opioids and local anesthetics [7]. This suggests that olprinone does not competitively interact with other basic drugs used during cardiac surgery.

In conclusion, olprinone protein binding was reduced from 81.5 to 63.3 % during CPB, which suggests that the pharmacological effects of olprinone are enhanced during and after CPB. Since CPB alters olprinone's pharmacokinetics, close hemodynamic monitoring is necessary to control olprinone effects after CPB.

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