Impact of non-di-(2-ethylhexyl)phthalate cardiopulmonary bypass tubes on inflammatory cytokines and coagulation–fibrinolysis systems during cardiopulmonary bypass

Abstract Di-(2-ethylhexyl)phthalate (DEHP), an excellent plasticizer for poly(vinyl chloride) (PVC), is a known endocrine-disrupting chemical. This study was designed to investigate whether a new non-DEHP bilayer tube reduced the release of DEHP, suppressed inflammatory cytokines, and altered coagulation–fibrinolysis systems. Sixteen patients undergoing coronary artery bypass grafting (CABG) were randomly assigned to the non-DEHP bilayer group (group B, \( n = 8 \)), or the noncoated PVC group (group N, \( n = 8 \)). The level of DEHP in the blood was measured before and after cardiopulmonary bypass (CPB). The levels of interleukin-6 (IL-6), D-dimer, and thrombin–antithrombin complex (TAT) were also measured at six points during and after CPB. DEHP was significantly lower in group B (472 ± 141 ng/ml) after CPB compared with group N (2094 ± 1046 ng/ml). The IL-6 level was significantly lower in group B (151 ± 131 pg/ml) than group N (206 ± 224 pg/ml) 180 min after protamine administration. The D-dimer level was significantly lower in group B 60 min after protamine administration (6.2 ± 2.4 μg/ml in group B vs 10.4 ± 4.5 μg/ml in group N) and 180 min after protamine administration (4.4 ± 0.7 μg/ml in group B vs 7.3 ± 2.7 μg/ml in group N). Group B had a tendency toward reduced postoperative bleeding compared with group N at any time. The bilayer tube was superior to the noncoated tube in terms of the inhibition of DEHP release, inflammatory cytokines, and the fibrinolysis system.

Key words Di-(2-ethylhexyl)phthalate (DEHP) · CABG · CPB · Inflammatory response

Introduction

Cardiopulmonary bypass (CPB) is dependent on materials foreign to the patient for its successful application. The continuous interaction of blood with artificial surfaces during CPB leads to substantial damage to cells and plasma proteins, the release of various inflammatory cytokines, and the activation of complement and coagulation–fibrinolysis systems, resulting in the potential dysfunction of several organs. To reduce such adverse effects of blood–tissue interactions, CPB systems need to be adapted to physiologic conditions. These strategies include surface coating of the materials of the circuit designed to suppress the inflammatory response. Heparin-coated circuits were developed to reduce systemic inflammatory reactions by lowering complement activation, reducing neutrophil activation, and reducing plasma levels of inflammatory cytokines such as interleukin (IL)-6 and IL-8. Although heparin-coated circuits are useful for CPB, the industrial procedure for applying heparin coatings to CPB devices adds considerably to the overall cost of CPB. Several research groups have looked at the influence of heparin coatings on di-(2-ethylhexyl)phthalate (DEHP) release.

DEHP is an excellent plasticizer and makes poly(vinyl chloride) (PVC) plastic tubing soft and flexible. Plasticized PVC material has the ability to be welded together, is suitable for steam sterilization (even at 121°C), and has a favorable cost performance. In recent years, animal experiments have shown that DEHP metabolites impair fertility. The most affected cells were shown to be the Sertoli cells of the sperm channel, and, as a result, spermiogenesis. High DEHP levels resulted in various biological effects, including testicular atrophy in rats, proliferation of peroxisomes in rodents, and liver tumors in rats and mice.

Because DEHP may influence the human endocrine system, the search for other costless non-DEHP CPB circuits with high biocompatibility has been a focus of development in bioengineering. Thus, we used a bilayer non-DEHP CPB tube with high biocompatibility in this study. We have previously reported on DEHP release by
non-DEHP tubes. In a previous study, we demonstrated that non-DEHP tubes markedly reduced DEHP levels during CPB in comparison with conventional PVC tubes.\textsuperscript{13} However, we did not demonstrate the influence of non-DEHP tubes on cytokine levels and/or coagulation–fibrinolysis activity. This study aimed to compare a new bilayer tube with a noncoated conventional PVC tube, and focused chiefly on the release of DEHP, inflammatory cytokines, and coagulation–fibrinolysis systems in the blood.

**Materials and methods**

**Patients**

The subjects were 16 patients undergoing first-time elective coronary artery bypass grafting (CABG) scheduled in 2005 or 2006. Subjects were randomly divided into two groups: the first group using a noncoated PVC tube (group N; \( n = 8 \)), and the second group using a bilayer non-DEHP tube (group B; \( n = 8 \)). Exclusion criteria included previous cardiac surgery, renal or liver dysfunction, and preoperative coagulopathy. Anticoagulation or antiplatelet therapy was discontinued 7 days before surgery in all patients. Informed consent for the study was obtained from all patients before surgery.

**Materials used in cardiopulmonary bypass**

In group N, components consisted of a cardiopulmonary bypass circuit, an oxygenator, a hard shell venous reservoir, a cardiotomy reservoir, an arterial filter, and a centrifugal pump; circuits were commercially available noncoated circuits made of PVC including DEHP as a plasticizer. In group B, all components and materials used were similar to those of group N except the cardiopulmonary bypass tube. This cardiopulmonary bypass tube consisted of a bilayer arterial line and venous line. The inner layer was made of a microdomain, which consisted of a soft segment and a hard segment including polyester as a plasticizer and the outer layer was made of PVC with tri-(2-ethylhexyl) trimellitate (TOTM) as a plasticizer. The length of tubing used in both groups was similar.

**Anesthesia and cardiopulmonary bypass**

Anesthesia was induced after premedication with midazolam, and then maintained with fentanyl, midazolam, and vecuronium bromide. The circuits were primed with a mixture of 1300 ml of lactated Ringer’s solution, 250 ml of human serum albumin (250 mg/ml), 200 ml of mannitol (200 mg/ml), and 100 ml of sodium bicarbonate (84 mg/ml). Standard ascending aortic cannulation and right atrial cannulation were performed.

Before aortic cannulation, all patients received a 300 IU/kg dose of bovine heparin. Activated clotting time (ACT) was measured using a Hemochron 801 (International Technidyne, Edison, NJ, USA). The ACT was maintained at 400 s or above by the administration of heparin during CPB, as required. No patients received antifibrinolytic agents or aprotinin during the operation. While the patient was fully heparinized, a cardiotomy suction device was used to return pericardial blood. At all other times during the operation, a cell-saving device (Hemomedics Cell-Saving Device 5 model 2005; Hemoenetics, Braintree, MA, USA) was used.

**Data collection and measurements**

Intraoperative variables including the duration of aortic cross clamping, the duration of CPB, initial and total doses of heparin, and the protamine dose were recorded. Blood samples were obtained at the following six points in both groups: before the induction of anesthesia (pre); 60 min after the initiation of CPB (CPB 60); 10 min after aortic declamping (declamp 10); 5, 60, and 180 min after protamine administration (post 5, post 60, and post 180, respectively). Hematocrit and platelet counts were measured with an automatic cell counter (MAXM-Retic, Beckman Counter, CA, USA). Plasma was separated from blood cells by centrifugation at 3000 \( g \) for 10 min and stored at ~80°C until analysis.

**Di-(2-ethylhexyl)phthalate**

Quantitative analysis of DEHP was performed by selected ion monitoring gas chromatography, as reported earlier.\textsuperscript{8}

**Inflammatory cytokines**

IL-6 levels were measured by enzyme-linked immunosorbent assay. The values obtained during CPB and up to 60 min after protamine administration were corrected for hemodilution and normalized to the hematocrit before the operation.

**Coagulation and fibrinolysis**

The effects of the two tubes on the coagulation and fibrinolytic systems were studied. The coagulation system was investigated by measuring the plasma concentration of the thrombin–antithrombin complex. The fibrinolytic system was investigated by measuring plasma D-dimer concentrations.

**Statistical analysis**

All data were analyzed using standard computer software (Statview 5.0 and Super ANOVA 1.11; Abacus Concepts, Berkeley, CA, USA). All results are reported as mean values ± standard deviation. Analysis of variance was performed to evaluate differences between the two groups. If a significant difference was found, the Wilcoxon rank-sum test was used.