Trilinolein improves erythrocyte deformability during cardiopulmonary bypass

S. K. TSAI, P. CHAN, T. Y. LEE, J. M. YUNG & C. Y. HONG

Departments of Anesthesiology and Medicine, Veterans General Hospital; Institutes of Traditional Medicine and Clinical Medicine, National Yang-Ming Medical College; Taipei, Taiwan, ROC

The *in vitro* effect of trilinolein, a triglyceride with linoleic acid as the major fatty acid residue in the esterified positions of glycerol, on erythrocyte deformability was studied in blood samples collected from 12 patients before and after cardiopulmonary bypass (CPB). Erythrocyte deformability was measured with a filtration method and expressed as red cell filtration rate (RFR). RFR was reduced after CPB and the reduction was time dependent. Trilinolein at a concentration of 10^{-7} M significantly reversed the CPB-induced reduction of RFR when it was mixed with blood samples collected 30, 60 and 90 min from the start of CPB. This study confirmed the effect of CPB on erythrocyte deformability and showed that this damage could be significantly improved by mixing blood with trilinolein.

Keywords erythrocyte deformability trilinolein cardiopulmonary bypass

Introduction

In patients undergoing cardiopulmonary bypass (CPB), erythrocyte (red blood cell, RBC) damage is well documented. During extra corporeal circulation, the shape of RBCs is altered, their membrane becomes less pliable, and calcium ion is accumulated in RBCs [1, 2]. These changes may cause a reduction of RBC deformability which disturbs normal microcirculation, leads to organ dysfunction and increases morbidity and mortality of patients [3–7].

Recently, we purified from *Panax pseudoginseng*, a medicinal herb widely used for the treatment of cardiovascular diseases in China, an active component that improved the deformability of calcium-loaded RBCs. Since the active component was identified as trilinolein [8], we undertook a study to investigate the effect of trilinolein on CPB-induced decrease of RBC deformability in 12 patients undergoing elective open-heart surgery. RBC deformability was monitored by a microfiltration method [9].

Methods

Patients

Twelve male adult patients undergoing elective open-heart surgery for coronary artery bypass grafting were studied. Their mean age was 60.1 ± 2.3 (mean $\pm$ s.d.) years. None received blood transfusion before CPB.

All patients were given a fentanyl- O_2 -pancuronium anaesthesia. Standard CPB was employed in all cases. A heart-lung machine using a Sarns roller pump and a membrane oxygenator (Maxima, Medtronic, USA) was primed with 2000 ml Ringer solution and 3000 units heparin. Patients received systemic heparinisation and the activated clotting time was maintained between 400 and 500 s. The CPB was performed at moderate hypothermia (28° C).

RBC preparation

Blood samples (20 ml) were collected for the measurement of RBC deformability before, and 30, 60 and 90 min after the start of CPB. The heparinised venous blood was mixed with an equal volume of trilinolein (Sigma, USA) and incubated at 37° C in a water bath for 2 h. Trilinolein had been dissolved in Joklik's minimum essential medium (J-MEM, Gibco Lab-oratories, USA) containing 1% DMSO and 0.5% bovine albumin. RBC incubated in a drug-free J-MEM containing DMSO and albumin was used as a control. Then blood was centrifuged at 2000 g for 5 min (Sorvall RT6000B, Du Pont, USA). The buffy coat and the upper 5 mm of RBCs was discarded. Packed RBCs were washed twice with 0.9% NaCl solution.

RBC deformability

RBC deformability was tested by a Reid's microfiltration technique [9]. Five minutes prior to the test,

washed packed RBCs were diluted 10-fold with a J-MEM. 0.5 ml of diluted RBC-drug mixture in a disposable syringe was made to flow through the 5 micron pores of a 13 mm diameter polycarbonate membrane filter (Nuclepore Corporation, Pleasanton, California, USA) by gravity to a recipient bottle. The filtration time was recorded and the mean of three readings was calculated for each sample. Each filter was initially tested with saline solution and only filters with a mean flow time of 2.0 ± 0.2 s were used. Deformability was expressed as red blood cell filtration rate (RFR) in $\mu\text{l s}^{-1}$ and the following formula was used for calculation of RFR [5].

$$\text{RFR } (\mu\text{l s}^{-1}) = \frac{0.5 \text{ ml} \times \text{flow time of saline in seconds}}{2.0 \text{ s} \times \text{flow rate of RBC suspension in s}} \times 1000$$

Statistics

All data are expressed as mean $\pm$ s.e. mean. ANOVA test was used for comparison of drug effects. Statistically significance was assumed for $P < 0.05$.

Results

Mean CPB time for these 12 patients was 110 ± 10.1 min. RFR for blood collected before CPB was $58 \pm 4 \mu\text{l s}^{-1}$. Figure 1 showed that RFR was reduced after CPB, and trilinolein reversed the CPB-induced reduction of RFR 30, 60 and 90 min after the start of CPB. The effect of trilinolein was concentration-dependent. Only 10^{-7} M significantly improved RFR while the improvement induced by 10^{-9} and 10^{-8} M were not statistically significant.

Figure 1 Influence of trilinolein on cardiopulmonary bypass (CPB)-induced reduction in RBC deformability as expressed as a reduction in filtration rate. Time 0 indicates RBC filtration rate for blood collected before CPB. Blood samples collected 30, 60 and 90 min after the start of CPB were mixed with either trilinolein-free medium (○), 10^{-9} M (▲), 10^{-8} M (■) or 10^{-7} M (●) trilinolein before filtration rate was measured. *indicates a significant difference between trilinolein-treated and non-treated blood samples (mean $\pm$ s.e. mean for 12 patients; ANOVA, $P < 0.05$).

Discussion

The normal shape and deformability of RBCs are maintained by intracellular ATP, low content of calcium ion and cell membrane fluidity [10–14]. Depletion of intracellular ATP, accumulation of calcium ion or changes in membrane fluidity is associated with a decrease in RBC deformability. In RBCs undergoing prolonged CPB, the cell membrane may lose its elasticity and reduce its flexibility. An increased membrane permeability may result in an increased rate of calcium flux. These alterations during CPB are known to reduce RBC deformability during open-heart surgery. Old patients and long perfusion time heighten RBC susceptibility to the changes during CPB.

Our results showed that RBC deformability was reduced by CPB. Trilinolein added to the blood reversed the CPB-induced reduction in RBC deformability. The effect of trilinolein was dose-dependent, only 10^{-7} M showed a significant improvement while 10^{-8} and 10^{-9} M did not.

We recently found that both linoleate rich triacylglycerol purified from *Panax pseudoginseng* and authentic trilinolein improved the deformability of calcium loaded RBC. We also showed that trilinolein is not a specific antagonist of calcium ion. The mechanism by which trilinolein improves RBC deformability was considered to be related to membrane fluidity [8]. Introduction of specific lipid into the cell membrane may alter the shape of a RBC because its membrane is composed of a lipid bilayer. During our previous study, trilinolein was mixed with washed RBCs. In the present study, it was mixed with blood collected from the patients and the deformability of washed RBCs was measured afterwards. Adding trilinolein to blood samples is more similar to the clinical situation when whole blood rather than RBCs is infused back to patients after CPB. It should be admitted that Reid's filtration method only measured RBC deformability in a low shear test; its relevance to high shear changes of RBCs in circulation need further studies. It should also be pointed out that 10^{-8} M trilinolein was most effective in reversing the calcium-induced reduction of deformability in washed RBCs [8], while 10^{-7} M needed to be mixed with whole blood to show a significant improvement of RBC deformability in CPB.

Currently, there are several drugs, such as pentoxifylline, vinpocetin [15], piracetam [16] and verapamil [17] available for improving RBC deformability. Since they all have pharmacological effects on either myocardial contractility or platelet aggregation, they may not be suitable for cardiac patients undergoing CPB. Trilinolein, on the other hand, is a type of triglyceride normally present in the human body. Its potential application as a drug to mix with blood for the improvement of RBC deformability during CPB could be better than existing drugs. However, its benefit for patients undergoing open heart surgery can be established only after blood samples mixed with trilinolein are infused back to the patients and a reduction in morbidity and mortality is demonstrated.

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