

Changes in erythrocyte membrane ATPases and plasma lipid peroxides in upper abdominal surgery under intravenous procaine-balanced anesthesia

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Abstract

AIM To observe the changes in erythrocyte membrane ATPases and plasma lipid peroxides (LPO) patients with in abdominal surgery under intravenous procaine-balanced anesthesia.

METHODS By determining the ATPase activities of erythrocyte membrane, effects of upper abdominal surgery under intravenous procaine-balanced anesthesia on the function of erythrocytes were observed in 15 patients undergoing cholecystectomy and gastrectomy (5 males and 10 females, aged 45.9 ± 10.20 years and weighed $60.60 \text{ kg} \pm 11.93 \text{ kg}$). All patients were free from severe renal, hepatic, pulmonary, cardiac, metabolic and endocrinological diseases and acute infection for at least 2 weeks before surgery. Patients receiving any drug known to affect carbohydrate metabolism prior to anesthesia were excluded from the study.

RESULTS Erythrocyte membrane Na^+ , K^+ -ATPase, Mg^{2+} -ATPase, Ca^{2+} , Mg^{2+} -ATPase activities were not significantly changed 60min-90min after incision as compared with 30min before anesthesia, but were decreased markedly 10min and 24 hours after completion of operation ($P < 0.01$). Plasma lipid peroxides (LPO) were

increased significantly 24 hours after surgery ($P < 0.01$) following an initially marked but transient reduction. Plasma LPO changes were not correlated with erythrocyte membrane ATPase activities, $r = -0.0396$, -0.0097 and 0.4383 , respectively ($P > 0.05$).

CONCLUSION Abdominal surgical trauma under intravenous procaine-balanced anesthesia may be associated with the decreased ATPase activities of erythrocyte membrane and increased LPO in plasma.

INTRODUCTION

Red blood cells are responsible for the transportation of oxygen and carbon dioxide into and out of tissues and organs of the body. Its shape, deformability, exchanges of intra and extra-cellular electrolyte homeostasis and membrane integrity, and the normal erythrocyte functions all depend upon normal activities of the three kinds of ATPases on erythrocyte membranes. It has been demonstrated that anesthesia and surgical trauma can lead to a significant decrease in the activities of these ATPases^[1-3]. The mechanism of decreased activities of ATPases, however, is still unclear. In the study, we observed perioperative alterations in the activities of erythrocyte membrane Na^+ , K^+ -ATPase, Mg^{2+} ATPase and Ca^{2+} , Mg^{2+} ATPase and plasma lipid peroxides (LPO), attempting to probe into the mechanism mentioned above.

MATERIALS AND METHODS

Fifteen patients underwent elective upper abdominal surgery (5 males and 10 females, aged 45.90 ± 10.20 years and weighed $60.60 \text{ kg} \pm 11.93 \text{ kg}$) including cholecystectomy (14) and gastrectomy (1). All patients had been free from significant renal, hepatic, pulmonary, cardiac, metabolic and endocrinological diseases and acute infection for at least two weeks before surgery. Patients receiving any drugs known to affect carbohydrate metabolism prior to anesthesia were excluded from this study.

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After fasting for 12-14 hours and premedicating with phenobarbitone 0.1 g and atropine 0.5 mg intramuscularly 30min before anesthesia, general anesthesia was induced with intravenous diazepam 0.2 mg·kg⁻¹, and thiopentone 4 mg·kg⁻¹ - 6 mg·kg⁻¹ and fentanyl 0.1 mg, 100 mg succinylcholine (SCC) were given to facilitate intubation. Anesthesia was maintained with an intravenous combined solution of 1% procaine and 0.06% SCC at a rate of 0.1ml·kg⁻¹·min⁻¹. Intravenous fentanyl 10 µg·kg⁻¹ and droperidal 0.15mg·kg⁻¹ were administered prior to incision. Additional thiopentone was given if needed. Lactated Ringer's solution was used during the intra and post operative periods. Ventilation was controlled to a PET CO₂ of 4.67kPa-5.33kPa at the same time, electrocardiogram (ECG), heart rate and blood pressure were continuously monitored with Datex Cardiacap.

Venous blood were collected in heparin (20 U/ml) 30min before induction of anesthesia (T₀), 60min-90min after incision (T₁), 10min (T₂) and 24 hours (T₃) after completion of operation. Plasma samples were collected for the detection of LPO (TBA method). According to Beutler's method^[4], the packed red blood cells were filtered through a special cotton column to remove white blood cells and platelets, and subsequently washed and centrifuged three times with 50mmol/L tris-EDTA-NaCl buffer (EDTA -1mmol/L and NaCl 150mmol/L, pH 7.4), and finally the packed red blood cells were made into 50%-60% red blood cell suspension (all procedures were performed below 4°C) and preserved separately in liquid nitrogen for assay.

Samples taken out of liquid nitrogen were put into ice-water to melt for assay. Red blood (0.5ml) cell suspension was drawn, diluted precisely with more than 20-fold red cell membrane washing solution (10mmol/L tris-EDTA buffer, EDTA

1mmol/L, pH 7.4, 0°C-4°C) and centrifuged (10000r/min for 20min, at 0°C-4°C) for 4 times. Opalescent red cell membranes were obtained and made into 2ml red blood cell membrane suspension with a 50mmol/L tris-sucrose-buffer (sucrose 75mmol/L, pH 7.4, 0°C-4°C). According to the modified Lowry's method^[5], the contents of membrane protein were determined and adjusted to 1.5g/L approximately. The activities of red blood cell membrane Na⁺, K⁺ ATPase, Mg²⁺ ATPase and Ca²⁺, Mg²⁺-ATPase were determined by Charalambous' method^[6] and expressed by nmolPi·mgPro⁻¹·min⁻¹.

RESULTS

Changes in activities of red blood cell membrane ATPase

There were no significant changes of the activities of three kinds of ATPases on red blood cell membranes since the initial anesthesia to 60min-90min after incision. Up to 10min after the completion of surgery, however, Na⁺, K⁺-ATPase, Mg²⁺-ATPase and Ca²⁺, Mg²⁺ ATPase activities decreased significantly to 2.962 ± 1.245 , 4.125 ± 1.006 and 16.642 ± 11.346 from 3.745 ± 1.233 , 5.050 ± 1.115 and 32.705 ± 9.585 nmolPi·mgPro⁻¹·min⁻¹ ($P < 0.001$, respectively) and were still at a lower level 24 hours after completion of surgery

Changes in plasma LPO

Plasma LPO increased significantly following an initially marked but transient reduction. 60min-90min after incision, plasma LPO decreased to 8.831 ± 1.180 from $9.815 \text{ nmolMDA/ml} \pm 1.100$ nmolMDA/ml prior to anesthesia ($P < 0.05$), and then gradually increased and reached to the peak ($11.782 \text{ nmolMDA/ml} \pm 1.218$ nmolMDA/ml) 24 hours after surgery.

Table 1 Results of red blood cell membrane ATPases and plasma LPO

	T ₀	T ₁	T ₂	T ₃
Na ⁺ , K ⁺ -ATPase (nmolPi·mgPro ⁻¹ ·min ⁻¹)	3.745±1.233	3.546±1.466	2.962±1.165 ^{b,c}	2.900±1.055 ^{a,b}
Mg ²⁺ -ATPase (nmolPi·mgPro ⁻¹ ·min)	5.050±1.150	4.905±1.192	4.125±1.008 ^{b,c}	3.978±1.183 ^{b,c}
Ca ²⁺ , Mg ²⁺ -ATPase (nmolPi·mgPro ⁻¹ ·min ⁻¹)	32.71±5.85	32.93±7.61	16.64±11.35 ^{b,c}	10.22±6.46 ^{b,c,d}
LPO (nmolMDA·ml ⁻¹)	9.815±1.100	8.831±1.180 ^a	9.135±1.174	11.78±1.22 ^{b,c,e}

Compared with T₀, ^a $P < 0.05$; ^b $P < 0.01$; compared with T₁, ^c $P < 0.01$; compared with T₂, ^d $P < 0.05$; ^e $P < 0.01$.

DISCUSSION

The result showed that the activity of red blood cell membrane Na^+ , K^+ -ATPase decreased significantly 10min and 24 hours after completion of the operation. It was in accordance with other reports. Changes in activities of Mg^{2+} -ATPase and Ca^{2+} , Mg^{2+} -ATPase were similar to Na^+ , K^+ ATPase. The mechanisms why the activities of red blood cell membrane ATPases are affected postoperatively, however, are still unclear. There is no doubt that the inhibition of ATPases, to some extent, reflects the fact that the noxious factors were produced in the blood during and after anesthesia and/or surgical trauma, which may directly and/or indirectly destruct or inhibit red cell membrane functions or ATPase activities. It was demonstrated that many lipid-soluble anesthetics and tranquilizers reversibly bind themselves to red blood cell membranes, and this is accompanied by alterations in membrane lipid-protein interaction and conformation, and alterations of coagulo-soluble state between the cytoplasmic side of the red blood cell membrane and cytoplasm, leading to decrease of membrane fluidity and activities of various membrane bound enzymes. Hudgins and Bond^[7] reported that highly or lipid-soluble low anesthetics could reduce the activities of various ion pumps by competitive or non-competitive mechanism, such as dibucaine and procaine. However, their inhibitive mechanisms differ from each other according to the various ion pumps involved. For instance, the highly lipid soluble agents (e.g. dibucaine) inhibit various enzymes by nonspecific binding to red blood cell membranes. On the other hand, low lipid-soluble agents, such as procaine, might interact more specifically with monovalent cation binding sites or with one of the two conformations. The size of inhibited enzyme activities is also greatly correlated with the effect and toxicity of the anesthetics employed. Red blood cell membranes might be directly or indirectly destructed by disturbances in hormone homeostasis, and the release of various body fluid factors such as complements, oxygen radicals and LPO, and the increased activities of phospholipidase A_2 ^[8] after anesthesia and/or surgical trauma. The injured membranes might be associated with disturbances in renewal of normal red cell membrane lipid and alterations in membrane lipid-protein interactions and conformations, together with changes in red blood cell morphology and a decrease in deformability, which finally leads to a decrease in membrane fluidity and enzymatic activities.

It was demonstrated that significantly inhibited activities of red cell membrane ATPases and

significant increases in plasma LPO after surgery were found in surgical patients under intravenous balanced anesthesia^[2]. Palmer^[9] suggested that decreased activity of Na^+ , K^+ ATPase might be correlated with direct destructive effects of some fluid factors such as complements, oxygen radicals and/or LPO on enzymatic molecular conformations and functions. Our data, however, showed that decreased activities of Na^+ , K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} , Mg^{2+} ATPase were not related to perioperative alterations in plasma LPO ($r = -0.0396$, -0.0097 and -0.4383 , $P > 0.05$, respectively). Plasma LPO increased significantly following an initially marked but transient reduction. It was likely that significantly decreased LPO during intraoperative period was related to blood dilutions caused by intraoperative massive infusion, and/or to inhibited LPO oxidation induced by protective effects of anesthetics such as procaine hydrochloride on red blood cell membranes. The significantly increased LPO after surgery demonstrated that noxious fluid factors such as LPO, complements and oxygen radicals were produced during and after anesthesia and/or surgical trauma due to the absorption of necrotizing tissues and broken cells from the sites of surgical trauma, inflammatory and non-inflammatory responses after surgical trauma, potential injuries resulting from highly fractional oxygen, and direct or indirect effects of some anesthetics such as thiopentone. These factors may directly or indirectly result in the activation of the complement system in the body and the loss of neutrophil granulocyte functions, and then induce a series of oxidative reactions "respiratory bursts" by activated complements, lysosome enzymes, hydrogen peroxidase and myeloperoxidase^[10]. As a result, plasma LPO increased significantly after surgery. The increased LPO may further lead to decreased activities in red blood cell membrane ATPases and other enzymes.

The intra- and post-operative inhalation of high fractional oxygen (more than 80%) may be directly associated with potential injuries to red blood cell membranes ATPases due to increases in superoxide anion (O_2^-) and other toxic oxygen intermediates^[11-13].

The energy of ATPases directly depends upon intracellular ATP, which is only synthesized in the glycolytic pathway, and this energy accounts for about 30% - 50% of the total energy consumption of red blood cells. Consequently, decreased synthesis of intracellular ATP may be directly accompanied by decreased activities in red blood cell membrane ATPases and a reduction in red blood cell

deformability^[14-16].

The decreased activities of membrane ATPases may result in disturbances in intra and extra-cellular electrolyte homeostasis and in increased permeability of red blood cell membranes by various ions and small molecular substances, and then induce massive potassium exflux and sodium influx^[1,16], thereby leading to a reduction of red cell membrane fluidity and deformability and an increase in membrane fragility. Such alterations may directly affect gas exchanges between the blood and tissues, and microcirculatory functions, resulting in failures in microcirculation and in multiple organ system.

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