Oxidative Damage in Erythrocytes During Cold Storage With Organ Preservation Solution

Abstract: It is known that erythrocyte aggregation in renal tissue during preservation is cause of microcirculation defects in the reperfusion period. The aim of our study is to investigate oxidative damage in erythrocytes relative to the time of cold ischemia during organ preservation and relationship between lipid peroxidation and development of these damages.

In experiments with a rabbit model, explanted kidneys were exposed to perfusion and 96 hours preservation with Euro-Collins (EC) in the 1st group, and University of Wisconsin (UW) solution in the 2nd group. Electron-microscopic examinations in renal tissues were performed. Lipid peroxidation and glutathione levels in erythrocytes preserved in the same solutions were investigated.

Agglutination of the erythrocytes and adhesion to the endothelium with ≥24 hours preservation were observed in the kidney tissue of the first group, and after ≥48 hours preservation in the second group. During preservation of the erythrocytes in organ protection solutions, a marked increase was seen in lipid peroxidation level in the first group with regard to preservation time, while there was less increase in the second group.

Consequently, we suggest that membrane damage of red blood cells (RBC) due to increase in lipid peroxidation levels may be the cause of the agglutination and adhesion of the erythrocytes to the endothelium. We believe that these degenerative changes may be a major cause of allograft dysfunction in preservation by simple cold storage.

Key Words: Erythrocyte, renal preservation, lipid peroxidation, glutathione, organ preservation solutions

Introduction

In cadaveric organ transplantation, the erythrocyte aggregation that occurs in the capillaries following initial perfusion is considered to be one of the causes of ischemic-reperfusion damage (1). This is called RBC trapping and is thought to be a major factor in the “no reflow” phenomenon by impeding microcirculation during reperfusion (2). It is known that a cold ischemia time of more than 24 hours causes an increase in the incidence of posttransplant renal allograft dysfunction (3).

Recently, it has been proved that the reactive oxygen metabolites play a role in the irreversible cellular damage of allografts during ischemic and post-ischemic periods (4). Oxygen metabolism products such as superoxide radicals, hydroxyl radicals, hydrogen peroxide and lipid peroxides are formed by autooxidation in the course of reperfusion, adenosine monophosphate to transformate uric acid and other catabolic products in hypoxic conditions. Free oxygen radicals cause irreversible cell membrane and organelle damage by increasing lipid peroxidation (5).

Erythrocytes are unique biological structures containing high concentrations of polyunsaturated fatty acids, oxygen and iron ions in ligand state. Thus, they are expected to be sensitive to the potential damage from aerobic conditions such as oxygen radicals (6).

The major components of UW solution, glutathione and allopurinol, which are used frequently in cadaveric renal transplantation, have been found to have influences on the decreasing of free oxygen radical formation and reducing their cytotoxic effects (7).

The aim of our research is to study ultrastructural damage of the endothelium and erythrocytes in the capillaries occurring during renal preservation with EC and UW solutions, and additionally to investigate oxidative damage in erythrocytes preserved in these solutions in the exvivo conditions.
Material and Methods

Experiments were performed in New Zealand rabbits weighing 2.25-2.5 kg. Laparotomy and bilateral nephrectomy were performed under anesthesia with IV pentobarbital (20mg/kg) + ketamine (25mg/kg bolus + 1mg/kg/min infusion). During the procedure, 50ml/kg physiological serum was infused.

In the first group (n:12), IV 250 U/kg heparin was given 3 minutes before nephrectomy. A 20G plastic cannula was placed into the renal arteries, and the kidneys were perfused in ice slush with 4°C EC solution in 100 cm H2O pressure for 20 min (warm-ischemic time was less than 2 min.)

In the second group (n:12), perfusion and preservation were accomplished with UW solution.

The kidneys were examined with EM at 12, 24, 48, 72 and 96 hours at 4°C following perfusion. Samples from cortical and medullary zones of renal tissue were placed in 4% glutaraldehyde solution and, after prefixation with Milloning phosphate buffer, post-fixated in 1% OsO4 and, after dehydration, embedded in araldite medium. During the operation, 10 ml arterial blood samples were taken from both groups by cannulization iliac artery. In the 1st group, blood samples were put into 1ml citrate/ 9ml EC solution, and in the 2nd group, into citrate/ UW solution.

During hypothermic preservation for 1, 24, 48, 72 and 96 hours in 4°C malondialdehyde (MDA), the end product of lipid peroxidation was measured by the method of Torotta et al. (8), and glutathione levels (9) and haemolysis incidence (10) were also measured.

The data obtained were evaluated statistically with Student’s t test.

Results

In both groups, there were no findings of damage on erythrocytes and endothelium with EM studies following perfusion after 12 hours simple cold storage. After 24 hours preservation, the initial phase of erythrocyte's agglutination and adhesion to the endothelium was determined in the first group (Fig. 1). In the 2nd group, there were no signs of adhesion after the same period (Fig. 2). After 48 hours of preservation, erythrocyte agglutination and adhesion to the endothelium were seen in both groups. After hypothermic preservation of 72 and 96 hours, there were increased signs of agglutination, and adhesion and endothelial damage were more marked in the 1st group. In the same group, the damage of erythrocyte membrane completeness was observed after 96 hours of preservation (Fig. 3).

In comparing the groups’ levels of erythrocyte lipid peroxidation (MDA concentration), we found no statistically significant difference following perfusion (p> 0.05). A marked increase was seen in the levels of lipid peroxidation in the erythrocytes of the 1st group after cold storage for 24, 48, 72 and 96 hours (p< 0.05); in the 2nd group, there was no significant increase in the levels of lipid peroxidation at 24 hours (p> 0.05). MDA
concentration at 48 hours preservation and more was found relatively low, when compared to the marked increase in the 1st group (p> 0.01; Fig. 4).

In the following perfusion, we also found no statistically significant changes in the glutathione levels of erythrocytes in these groups (p>0.05). After preservation times of 24 hours and more, glutathione levels of erythrocytes had decreased (p< 0.01). Although in the UW group a decrease was observed, the glutathione level was clearly higher than in the EC group throughout the preservation period (p< 0.01; Fig 5).

While the incidence of haemolysis in the erythrocytes in the both groups was low in initial phase, it increased according to time of preservation (p> 0.01, Fig 6).

Discussion

It has been emphasized that RBC trapping plays a role in the development of ischemic-reperfusion damage in renal preservation by simple cold storage (1, 2). Erythrocytes in the renal capillaries during cold storage become rigid as a result of calcium binding to the membrane proteins due to the depletion of adenosine 3 phosphate (ATP) at the ischemic period or inactivation of Ca²⁺-ATP’ase activity by hypothermia. Elasticity loss of
erythrocytes causes capillary obstruction during reperfusion (11). In our study, marked RBC trapping was determined in arteriole and capillaries in the both groups.

But also Booster et al. determined that erythrocyte aggregation in the ischemically damaged renal allografts was not limited to scintigraphically non-perfused areas (12). Glomerular RBC trapping occurring during perfusion with Collins solution does not always cause mechanic obstruction, as stated by Anaise et al. (1). The conclusions of these studies lead to the logical necessity to study damage in the endothelium and the erythrocytes occurring in the vascular set of the renal allograft during hypothermic preservation.

In the EM examinations in the present study, the initial phase of erythrocyte agglutination and adhesion to the endothelium were seen in the 1st group 24 hours of hypothermia. After 48 hours and more preservation time, these signs increase and endothelial degeneration develops. We believe that this may be one of the most important reasons of acute tubular necrosis when time of cold storage is ≥24 hours.

Our findings suggest that UW solution prevents erythrocyte agglutination and adhesion during 24-hour preservation. At the same time we observed a decrease in endothelial damage at 72 hours preservation time, parallel to data in the literature (13).
In erythrocytes, oxidative stress causes an increase in lipid peroxidation levels and, consequently, damage to membrane proteins (14). UW solution inhibits the effects of free oxygen metabolites and lipid peroxides because of the glutathione it contains (15). The results of our study also show that in erythrocytes preserved in a hypothermic environment with EC solution, there are increases in lipid peroxidation and decreases in glutathione levels proportional to the preservation time. The changes in both parameters in the UW group are relatively low in comparison to the EC group. This situation can be shown in the EM examination as a cause of prevention of erythrocyte agglutination and adhesion with UW solution at 24 hours preservation of erythrocyte agglutination and adhesion.

Vicens et al. found that a cold ischemic period of over 18 hours enhances RBC trapping in the renal medulla, and that superoxide dismutase, as a factor inhibiting lipid peroxidation, reduces aggregation (16).

Hemoglobin and hemoglobin/haptoglobin complexes, crossing to the vessel lumen during degeneration of erythrocyte membrane integrity or haemolysis, cause vasoconstriction and inhibit endothelial relaxing factor (17). Our intent was to study the haemolysis incidence of erythrocytes in the various organ preservation solutions. As seen in Table 3, the longer the perfusion time, the greater the increase in the haemolysis incidence of erythrocytes, but no statistical difference between groups was found (p>0.05).

We observed agglutination of erythrocytes and adhesion to the endothelium as a consequence of a decrease in glutathione levels and an increase in lipid peroxidation at preservation times of 24 hours and more with EC solution. We believe that this effect plays an important role in the pathogenesis of ischemic-reperfusion damage to the renal allograft. It is thought that UW solution may reduce damages caused by ischemia due to its protective effect on erythrocyte agglutination and endothelial adhesion occurring during hypothermia of longer than 48 hours’ duration by decreasing lipid peroxidation.

References


