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Effect of Calcium Antagonists on the Kidney Graft Injury Induced by Long-Term Cold Ischemia

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Ultrastructural changes in kidney graft induced by long-term cold ischemia and effect of calcium antagonists on these alterations are studied. It is shown that calcium antagonists prevent activation of lipid peroxidation and erythrocyte agglutination and adhesion in capillaries.

Key Words: *ischemia; calcium antagonists; malonic dialdehyde*

Long-term storage of kidney allograft required for choosing a recipient, preoperative procedures or transportation of the organ is a pressing medical problem. Acute tubular necrosis is often caused by cold ischemia longer than 24 h, while 72 h is the maximum time of graft storage [4]. However, long-term cold ischemia is associated with increased occurrence of acute tubular necrosis and graft dysfunction [12].

Calcium antagonists exerting vasodilating, mitochondrion-protective, and antitoxic (with respect to cyclosporine treatment) effects improve functional capacity of kidney grafts [3,8,11].

The present study explores ultrastructural changes in kidney graft during 96-h storage and the effect of calcium antagonists on these changes. To investigate peculiarities of oxidative stress induced by long-term cold ischemia in the kidney we compared electron microscopic picture and data on the intensity of lipid peroxidation in kidney parenchyma.

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MATERIALS AND METHODS

Experiments were carried out on New Zealand rabbits weighing 2.25-2.5 kg. The animals were intravenously narcotized with Phenobarbital (20 mg/kg) and Ketamine (25 mg/kg single injection+1 mg/kg/min infusion) and laparotomy and bilateral nephrectomy was performed. The animals were intravenously injected with 250 U/kg heparin 3 min before surgery and 50 ml/kg 0.9% NaCl was injected intraoperative.

In group 1 animals (n=12), renal arteries were cannuled, the kidneys were placed into physiological saline at the ice melting temperature and perfused with Euro-Collins solution at a perfusion pressure of 100 cm H_2O (heat ischemia did not exceed 2 min).

Group 2 animals (n=12) were intravenously injected with 0.35 mg/kg verapamil 30 min before nephrectomy, other procedures were as in group 1.

The kidneys were stored at 4°C.

In both groups the aorta was cannuled and 160 ml blood was drawn and mixed with citrate (4°C).

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Forty-eight (n=6) or 96 h (n=6) later blood was heated to 37°C, and the kidney was reperfused via the renal artery for 30 min at 100 cm H_2O.

Tissue samples for electron microscopy were obtained from the cortical and medullar zones. Sections were treated with 4% glutaraldehyde, fixed in 1% OsO_4, and after dehydration embedded in Araldite. Contralateral kidneys were perfused with Euro-Collins solution, stored for 12, 24, 48, 72, and 96 h and treated analogously.

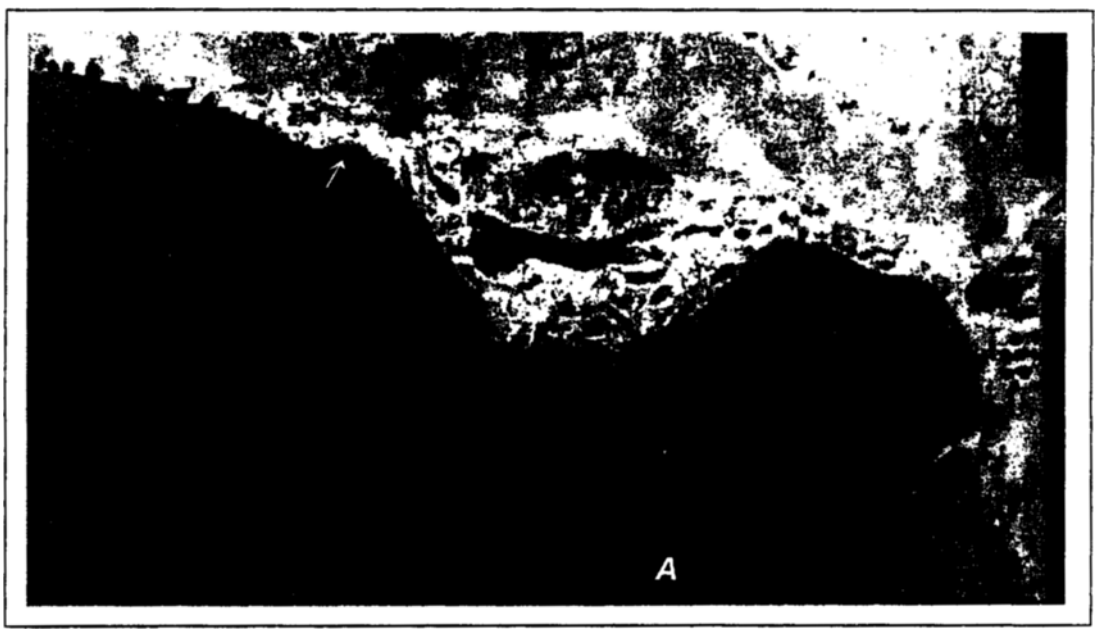

Fig. 1. Erythrocyte agglutination and adhesion (indicated by an arrow) to endothelium of peritubular capillaries in group 1 rabbits after 96-h storage. ×12,800.

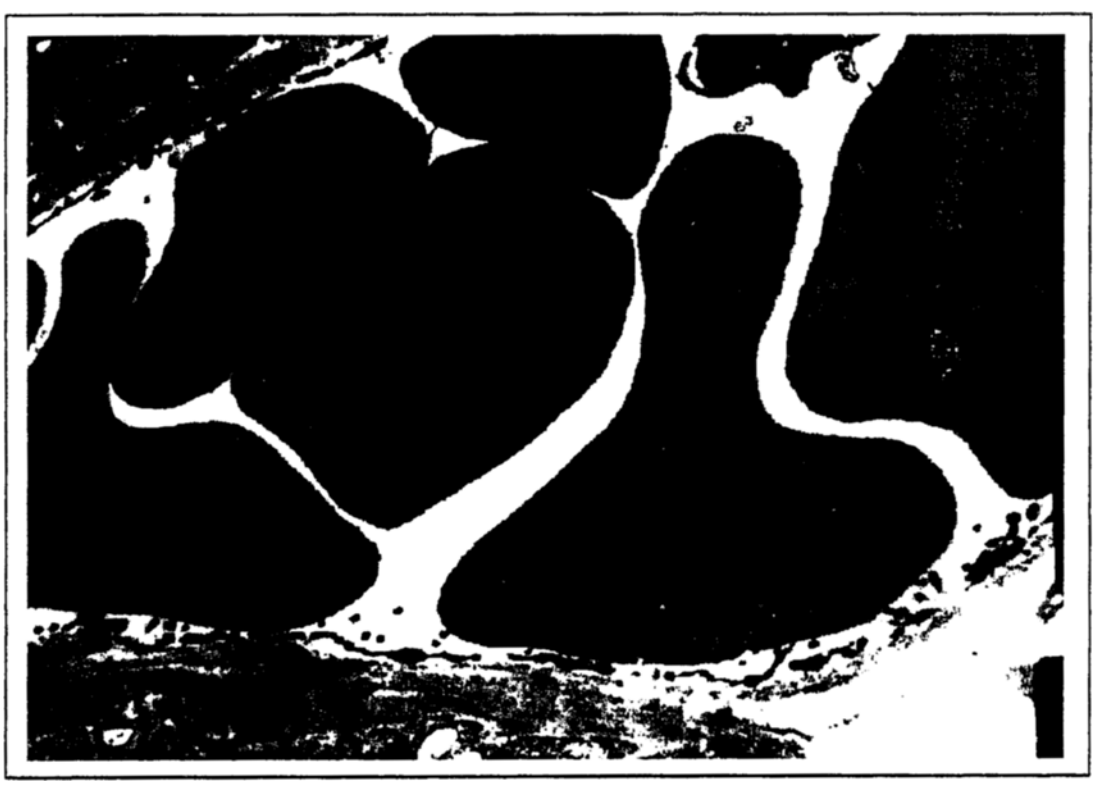

Fig. 2. Absence of erythrocyte agglutination and adhesion to endothelium of peritubular capillaries in group 2 rabbits after 96-h storage. ×10,600.

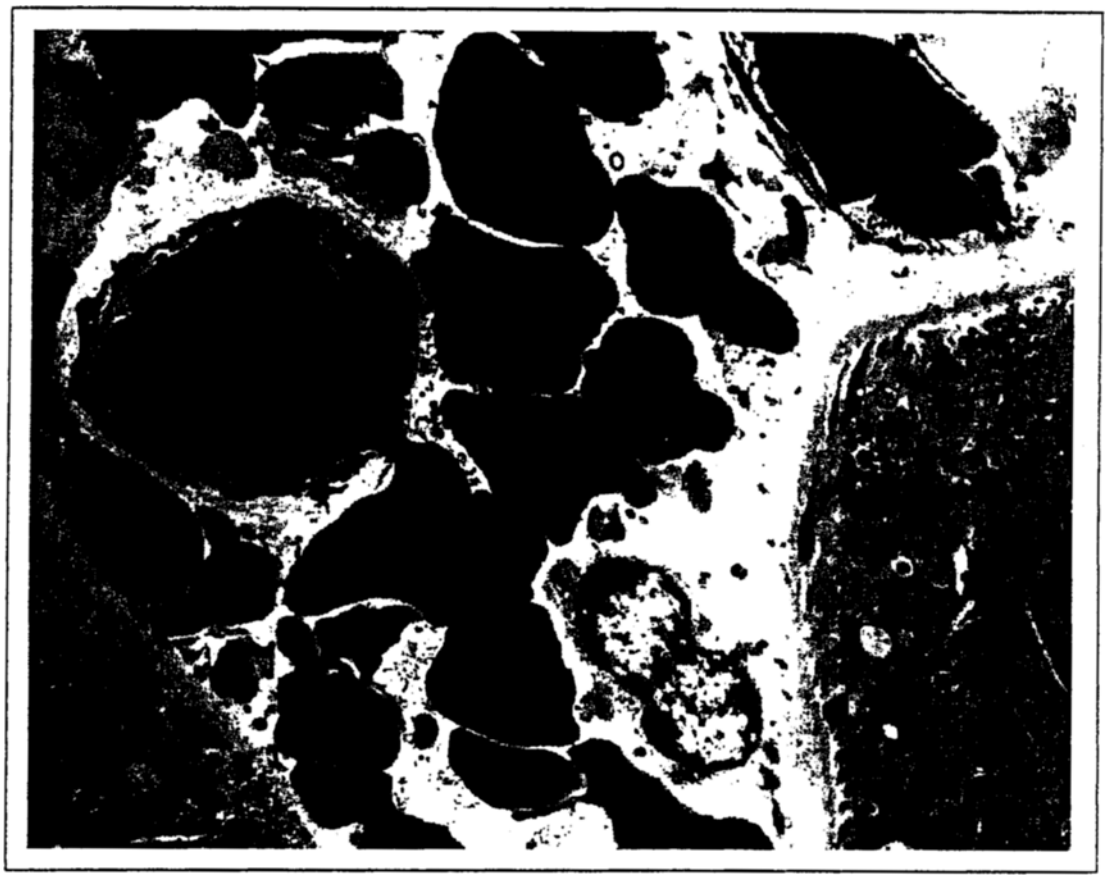

Fig. 3. Endothelial defects and erythrocyte extravasation in group 1 rabbits after 96-h storage and reperfusion with autologous blood. ×3600.

The content of malonic dialdehyde (MDA) after perfusion and reperfusion was measured as described elsewhere [13].

Significance of differences was evaluated using the Student *t* test.

RESULTS

In contralateral kidneys of both groups (without reperfusion) we observed erythrocyte aggregation in the peritubular and glomerular capillaries. In control kidneys stored for 24 and 48 h, erythrocyte agglutination and adhesion to capillary endothelium were seen (Fig. 1). These changes became more pronounced after 72- and 96-h storage and were accompanied by degenerative alterations in the endothelium.

Erythrocyte aggregation was also noted in verapamil-treated animals; however, in kidneys stored for 96 h erythrocyte aggregation and adhesion to vascular wall were absent (Fig. 2), the endothelium was better protected.

Erythrocyte aggregation in kidney capillaries is sometimes considered as the cause of ischemia-reperfusion damage [5,9]. However, it not always leads to obstruction of the vessel and is not restricted by scintigraphically nonperfused zone of the kidney [2,5].

We found no publications on the role of erythrocyte agglutination and adhesion to the endothelium in the development of reperfusion damage. We believe that these alterations induced by cold ischemia lasting more than 24 h play a key role in the genesis of no-reflow phenomenon, when microcirculation cannot be restored during the reperfusion period.

In kidneys of group 1 rabbits reperfused with autologous blood after 48-h storage we found pro-

TABLE 1. Lipid Peroxidation in Liver Parenchyma (*M±m*)

Group		Concentration of MDA, μg/g
Group 1	After perfusion	7.04±0.72
	After 48 h	16.51±2.63**
	After 96 h	29.4±3.53**
Group 2	After perfusion	6.39±0.61
	After 48 h	8.32±1.23*
	After 96 h	11.34±1.38**

Note. *$p<0.05$, **$p<0.01$ within the group, *$p<0.01$ compared with group 1.

nounced degenerative alterations such as hydropic vacuolization and reduction and loss of mitochondrial cristae in the proximal tubules. These changes became more pronounced after 96-h storage and were accompanied by degeneration of the endothelium, appearance of microdefects, and erythrocyte extravasation.

In animals treated with calcium antagonist degenerative changes in the proximal tubules were less pronounced, endothelial defects and erythrocyte extravasation were absent.

The first few minutes of postischemic reperfusion are characterized by massive generation of oxygen radicals [1]. These metabolites induce phospholipid peroxidation and damage to cell membranes [6].

The concentration of MDA in liver parenchyma rose proportionally to the duration of cold ischemia (Table 1).

However, the content of MDA in verapamil-treated animals was significantly lower than in the control group ($p<0.01$), suggesting that membrane-protective effect of calcium antagonists is mediated through inhibition of lipid peroxidation.

Our experiments demonstrated a new pathogenetic mechanism in the effect of calcium antagonists: prevention of excessive induction of lipid peroxidation during long-term cold ischemia of kidney allograft. Calcium antagonists apart from other beneficial effects possess membrane-protective properties and prevent erythrocyte agglutination and adhesion to vascular wall, thus improving microcirculation in the graft during reperfusion.

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