Turk J Gastroenterol 2009; 20 (3): 180-185

Effects of verapamil, sodium nitroprusside and glutathione addition into perfusion/preservation solutions on preservation-related ICAM-1 molecule expression in rat liver

Organ saklama solüsyonuna, kalsiyum kanal blokürü, nitrik oksit donorü ve glutatyon gibi ajanların eklenmesinin preservasyon hasarına etkileri

Aydın DALGIÇ[1], Ferit TANERİ[1], Mehmet CİNDORUK[2], Aylar POYRAZ[3], Gülen AKYOL[3]

Departments of [1]General Surgery, [2]Gastroenterology, and [3]Pathology, Gazi University, School of Medicine, Ankara, Turkey

Background/aims: *During cold preservation of liver grafts, expression of tissue adhesion molecules has been reported as a factor indicative of preservation injury. Some biochemical agents as well as increased levels of intracellular calcium also play important roles in preservation injury during cold storage. In the current study, we aimed to test if the addition of a calcium channel blocker, sodium nitroprusside, or glutathione into preservation solution would reduce upregulation of adhesion molecules, thus leading to decreased preservation injury in the rat liver.* ***Methods:*** *Fifty Albino Wistar rats, weighing 200±50 g, were divided into 1 control (perfused with Wisconsin solution, without preservation) and 4 study groups of rat livers (10 livers each). Livers in study groups were harvested, perfused, and preserved for 16 hours in 4 different solutions (Wisconsin solution alone, Wisconsin solution+verapamil, Wisconsin solution+sodium nitroprusside and Wisconsin solution+glutathione). At the end of the preservation time, levels of graft tissue adhesion molecule (ICAM-1) expression were analyzed.* ***Results:*** *Preservation for 16 hours with Wisconsin solution alone and Wisconsin solution+verapamil perfusates caused significantly more ICAM-1 expression than did preservation for 16 hours with Wisconsin solution+sodium nitroprusside and Wisconsin solution+glutathione perfusates (p=0.010). No significant difference was found in ICAM-1 expression between the Wisconsin solution+sodium nitroprusside and Wisconsin solution+glutathione groups. In the control group, perfusion with Wisconsin solution alone, without preservation, represented minimal ICAM-1 expression, reflecting minimum preservation injury (p=0.0003).* ***Conclusions:*** *Addition of sodium nitroprusside and glutathione into the Wisconsin solution decreased levels of ICAM-1 molecule expression, which reflects lower levels of preservation injury. In this study, the addition of verapamil to the perfusate/preservation solution for reducing the intracellular calcium accumulation had no effect on tissue ICAM-1 molecule expression.*

Key words: ICAM-1 expression, verapamil, calcium channel blockers, sodium nitroprusside, glutathione, preservation injury

Amaç: *Karaciğer greftlerinin soğuk saklama sürelerinde, adezyon molekül ekspresyonları ve intra selüler kalsiyum birikmesi gibi faktörlerin, preservasyon hasarının bir göstergesi olabileceği önceki çalışmalarda gösterilmiştir. Bu çalışmada, kalsiyum kanal blokörü, sodyum nitroprusside ve glutatyon gibi ajanların saklama solüsyonlarına eklenmesinin, rat karaciğerlerinde, adezyon molekül ekspresyon miktarında bir azalmaya yol açıp açmayacağı araştırıldı.* ***Metot:*** *Ağırlıkları 200±50 g olan elli adet Wistar rat birisi kontrol (sadece perfüzyon) dördü çalışma gurubu olmak üzere, on'ar rat'dan oluşan beş guruba ayrıldı. Greftlerin çıkartılmasını takiben, çalışma grupları, 4 ayrı solüsyonda (yalnızca Wisconsin solüsyonu, Wisconsin solüsyonu+Verapamil, Wisconsin solüsyonu+Sodyum Nitroprusside ve Wisconsin solüsyonu+Glutatyon) 16 saat soğuk preservasyona alındı. Daha sonra karaciğer greftlerinin ICAM-1 molekül ekpresyonları analiz edildi.* ***Sonuçlar:*** *Yalnızca Wisconsin solüsyonu ve Wisconsin solüsyonu+Verapamil ile preservasyon yapılan guruplarda; Wisconsin solüsyonu+Sodyum Nitroprusside ve Wisconsin Solüsyonu+Glutatyon guruplarına göre anlamlı ölçüde fazla ICAM-1 ekpresyonu tespit edildi (p=0.010). Wisconsin solüsyonu+Sodyum Nitroprusside ve Wisconsin Solüsyonu+Glutatyon grupları arasında ICAM-1 ekpresyonu bakımından bir fark gözlenmedi. Yalnızca Wisconsin solüsyonu ile preserve edilip, soğuk iskemiye tabi tutulmayan kontrol gurubunda, minimal preservasyon hasarının bir göstergesi olarak, en az ICAM-1 ekspresyonu tespit edildi (p=0.0003).* ***Tartışma:*** *Wisconsin solüsyonuna, Sodyom Nitroprusside ve Glutatyon gibi ajanların eklenmesi, rat karaciğerlerinde, preservasyon hasarının azaltılmasına yardımcı olabilir. Greft dokusundaki intraselüler kalsiyum akümülasyonunun azaltılması amacı ile preservasyon solüsyonuna eklenen Verapamil, doku ICAM-1 ekpresyonunda bir azalmaya yol açmamıştır.*

Anahtar kelimeler: ICAM-1 ekspresyonu, verapamil, kalsiyum kanal blokörü, nitrik oksit, glutatyon, preservasyon hasarı

*This study was founded and supported by the Turkish Surgical Society

Address for correspondence: Aydın DALGIÇ
Gazi University Hospital Department of Surgery
Beşevler, 06501 Ankara, Turkey
Phone: + 90 312 202 57 12 • Fax: + 90 312 215 04 94
E-mail: adalgic@yahoo.com

Manuscript received: 23.08.2008 **Accepted:** 18.03.2008

doi: 10.4318/tjg.2009.0004

INTRODUCTION

The goal of organ preservation is to maintain viability of the organ graft prior to transplantation. Physiologic alterations during preservation, such as ischemia and inflammation, or during reperfusion result in interactions between circulating host lymphocytes and graft endothelium. Increased accessory adhesion molecules in graft parenchyma are involved in and mediate these interactions (1). Adhesion molecules also mediate migration of leukocytes into allograft sites and costimulate cell activation and transformation (1, 2). However, if the transplanted organ vascular endothelial cells have been damaged during preservation, or by ischemia, surgical manipulation, reperfusion injury, or some other factor, some proinflammatory cytokines such as interferon (IFN)-gamma, interleukin (IL)-1, tumor necrosis factor (TNF)-alpha, as well as some adhesion molecules like ICAM-1 and P-selectins, will be produced or expressed (3, 4). As a result, inflammatory cells will begin to extravasate and migrate to the allograft site resulting in increased tissue damage. This leads to graft destruction and dysfunction as well as morbidity and rejection (5, 6). Many biochemical agents like calcium and oxygen free radicals also play an important role in this inflammatory process (7).

Anoxic cell swelling is accentuated by cooling, and intracellular calcium accumulation (which causes cellular death) can be prevented by including certain substances in preservation solutions (8, 9). In addition, the deleterious effect of calcium loading and uncontrolled increases in its concentration in intercellular spaces has been shown to have disastrous consequences that can be reduced by manipulating the composition of the perfusate flush solution (10, 11). Calcium channel blockers may inhibit calcium accumulation in hepatocytes and protect against cell damage. Calcium channel blockers also have vasodilating effects on graft capillaries, which cause greater infiltration of the viscous perfusate into the cold graft parenchyma. However, it would appear that blocking the biochemical action of increased intracellular calcium levels by calcium channel blockers also decreases the level of the oxygen free radicals, which leads to cellular damage (12, 13).

On the other hand, oxygen free radicals produced during ischemia, which have hazardous effect on liver cells, have been implicated in many pharmacological interventions involved in liver preservation injury. Glutathione is one of the molecules that decreases oxygen free radicals and prevents hepatocyte injury against ischemia-related oxidative stress during preservation (14). Decreased oxygen free radicals reduce vascular endothelial damage and prevent injury during the preservation process (8, 15, 16). Adding glutathione and verapamil into the perfusate may decrease the hazardous effect of cold ischemia during organ preservation (7).

On the other hand, it has been reported that levels of nitric oxide (NO), which is a natural regulator of microcirculation and a potent vasodilator, were depressed during cold ischemia (17). Sodium (Na) nitroprusside releases NO by a nonenzymatic process (18). Infusion of Na nitroprusside into graft capillaries just before perfusion with Belzer's University of Wisconsin (UW) solution may lead to better infiltration of the viscous perfusate into the graft parenchyma and may decrease the amount of injury that takes place during preservation (12).

The correlation between preservation injury and liver tissue ICAM-1 expression has already been demonstrated (11). More specifically, all liver grafts with long preservation times demonstrate at least some degree of ICAM-1 expression. However, the mechanism by which long preservation time leads to the upregulation of ICAM-1 in liver tissue is not known. Expression of ICAM-1 may serve as an early trigger of tissue injury and inflammation (19).

In the current study, we examined the role, if any, of adding a calcium channel blocker, Na nitroprusside, or glutathione, into the perfusate in decreasing upregulation of the ICAM-1 molecule to reduce preservation injury.

MATERIALS AND METHODS

This study was performed after receiving approval from the ethics committee of the Gazi University Medical School and Research Laboratory. All surgical procedures were performed in the experimental surgery laboratories at Gazi University Medical School.

Fifty male Albino Wistar rats, weighing on average 200 g, were divided into five groups (10 livers each). One control (Group 1: perfusion with UW solution and no preservation) and 4 groups of rat livers were harvested, perfused, and preserved for 16 hours (h) with four different solutions (Group 2: perfusion with UW alone, Group 3: perfusion with

UW+calcium channel blocker (verapamil 10 mg/L, Knoll AG, Ludwigshagen, Germany), Group 4: perfusion with UW+Na nitroprusside (60 mg/L, Schwartz Pharma AG, Monheim, Germany) and Group 5: perfusion with UW+glutathione (500 mg/L, Sigma Chemical Co., St. Louis, MO, USA).

Rats were fed preoperatively with standard rat formula. Their oral feeding was stopped approximately 6 h prior to surgery. Subcuticular ketamine (Ketalar, Pfizer Pharmaceuticals, USA, 2 mg/kg subcutaneous) was used as a general anesthetic.

A standard technique was used for the preparation, perfusion, and preservation of the liver grafts (20). Briefly, we opened the abdominal and thoracic cavity with a sternal excised transverse incision (Mercedes incision); cannulated and tied the portal vein (PV); tied the common hepatic artery and bile duct; cut the supra- and infrahepatic inferior vena cava (SHIVC & IHIVC); perfused the liver via the PV (allowing for venting of blood); tied the SHIVC, cannulated the IHIVC; and reperfused the liver through the PV at 25 cm/H2O pressure. As soon as clear effluent began to show in the IHIVC, we excised and preserved the livers in the respective preservation solutions (defined above) at 4°C for 16 h.

Control group (Group 1 livers) were examined without preservation. After 16-h preservation, the samples of liver tissue (Groups 2-5) were taken and stored at -70°C for immunohistochemical analysis of ICAM-1 expression as a marker of preservation injury. ICAM-1 expression was measured semiquantitatively as the degree of staining using a Sigma ICAM-1 kit (Sigma Chemical Co., St. Louis, MO, USA). No staining was read as negative and scored as 0; 1%-30% staining was read as low and scored as 1; 31%-60% staining was read as medium and scored as 2; and staining greater than 61% was read as high and scored as 3 (21, 22). Statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, release 5.0.1, SSPS Inc, Chicago, IL, USA).

Statistical differences for all groups were assessed with the Kruskal-Wallis one-way ANOVA test. Statistical significance between two groups was determined using the nonparametric unpaired two sample (Kolmogorov-Smirnov) test. Values of P less than 0.05 were considered statistically significant.

RESULTS

Samples in the control group showed 0.2±0.1 ICAM-1 expression score (mean±standard deviation). ICAM-1 expression scores in samples after 16-h preservation with UW solution, UW+calcium channel blocker solution, UW+Na nitroprusside solution and UW+glutathione solution were 2.1±0.3, 1.9±0.3, 1.0±0.2 and 0.8±0.3, respectively.

In Kruskal-Wallis one–way ANOVA test for comparing all five groups, samples in the control group (Group 1) demonstrated minimal ICAM-1 expression (p=0.0003). Samples after 16-h preservation with UW solution and UW+Ca channel blocker solution showed significantly more ICAM-1 expression than did samples after 16-h preservation with UW+Na nitroprusside solution and UW+glutathione solution using Kolmogorov-Smirnov unpaired two sample test (p=0.010) (Figure 1). No significant differences were found with regard to ICAM-1 expression between the UW+Na nitroprusside and UW+glutathione groups or between the UW and UW+calcium channel blocker groups with Kolmogorov-Smirnov unpaired two sample test (Figure 2).

DISCUSSION

Many reports have shown that when compared with recipients with a shorter period of graft preservation, those with liver grafts preserved for more than 16 hours develop remarkable preservation injuries, early graft function abnormalities, in-

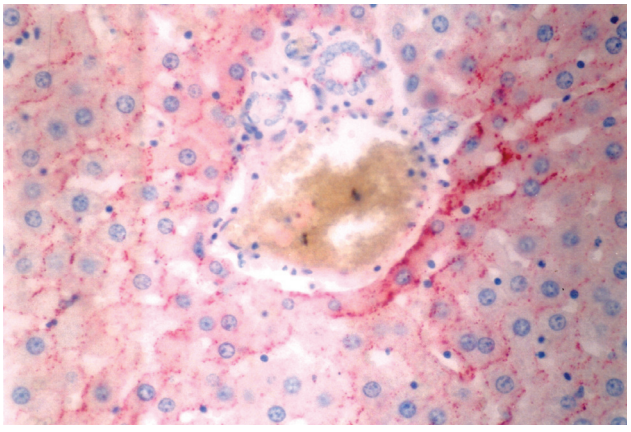

Figure 1. Moderate ICAM-1 expression in liver graft after 16-h preservation with UW solution alone. Moderate ICAM-1 expression was observed in Groups 2 and 3 in which liver grafts were perfused and preserved 16 hours with UW alone and UW+verapamil solution (ICAM-1 expression scores were 2.1 ± 0.3 and 1.9 ± 0.3, respectively).

creased retransplantation rates, inferior long-term outcomes, increased incidences of hepatic arterial thrombosis, and an increased number of nonanastomotic biliary strictures (23). It also has been shown that long-term preservation causes mediators of cold preservation injury, which may trigger vascular endothelial damage to the graft (24).

The mechanisms of vascular endothelial and sinusoidal cell injury during cold preservation remain unknown. It is unclear whether this injury is caused directly by hypothermia or whether cold storage results in the production of ischemic chemical mediators, which affects the hypothermic preservation injury.

Demonstration of ICAM-1 expression in the graft could reflect early inflammation and preservation injury that starts before any histopathologically proven tissue damage (11). More specifically, all liver grafts with long preservation times demonstrate at least some degree of ICAM-1 expression (11). This may represent one aspect of a generalized, nonspecific cytokine release phenomenon triggered by tissue ischemia. The upregulation of adhesion molecules increases cell-to-cell and cell-to-mesenchymal matrix adhesion and allows for transendothelial migration of proinflammatory cells into sites of graft inflammation (25, 26).

Despite the fact that a correlation between preservation injury and liver tissue ICAM-1 expression has already been demonstrated, the mechanism by which long preservation time leads to the upregulation of ICAM-1 in liver tissue is not known. Expression of ICAM-1 may serve as an early trigger of tissue injury and inflammation (11). In this study, we did not correlate the ICAM-1 expression within the groups with histopathologically proven tissue damage. This analysis may prove the idea that ICAM-1 expression in the graft could reflect early inflammation and preservation injury that starts before any histopathologically proven tissue damage.

Vasoconstriction and vasodilatation in the capillary bed are responsible for the vasoregulation of a transplanted graft organ. Ischemia and reperfusion affect this balance, causing vasoconstriction in the capillary bed (7, 27). This may result in leukocytes being trapped within the vessels and plugging the capillaries (28).

The lower ICAM-1 expression demonstrated in Group 4 (in which liver grafts were perfused with

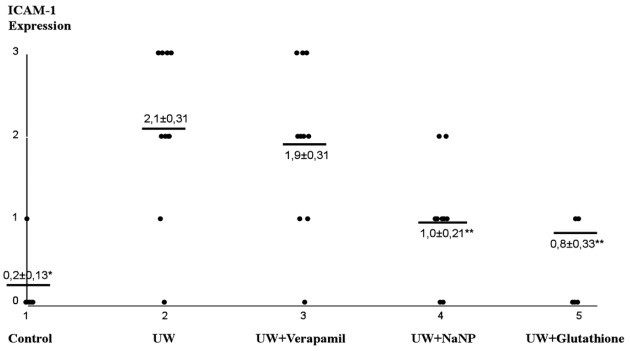

Figure 2. ICAM-1 expression scores according to groups.

* Minimum ICAM-1 expression observed in control (Group 1) group (0.2±0.13; p=0.0003).

** In study groups, 16-h preservation with UW+Na nitroprusside and UW+glutathione showed lower ICAM-1 molecule expression in liver tissue compared with the UW and UW+verapamil groups (p=0.010).

UW+Na nitroprusside solution) may be a reflection of less preservation injury than that in liver grafts perfused with UW alone. The capillary vasodilatory effect of Na nitroprusside as a NO donor, just before the cold perfusion, could prevent local and systemic vasoconstriction in the graft and may allow for more satisfactory infiltration of the perfusate into the graft parenchyma, leading to better perfusion of cold and viscous UW solution into the capillary area.

During cold ischemia, inactivation of the calcium transport enzyme system causes increases in cytolytic calcium and activates the phospholipase A2 enzyme, which lyses cellular membranes (29, 30). Increased intracellular calcium has harmful effects on preserved organs. There are many reports that show that adding calcium channel blockers (diltiazem, verapamil, and others) into an existing preservation solution has protective effects against ischemic injury in transplanted allografts (31, 32). However, in this study, liver grafts stored in verapamil-added perfusate did not show less ICAM-1 expression than that in liver grafts perfused with UW alone (Group 2). This may be related to the cytoprotective effect seen in calcium channel blockage in preservation solution that did not affect liver graft adhesion molecule expression in Group 3.

Oxygen free radicals in cold storage inactivate many specific cellular enzymes and are involved in the inflammatory process of reperfusion injury (7). Lipid peroxidation in the cell membrane is another major damaging effect of oxygen free radicals that takes place during cold storage and reperfusion. Reactive oxygen species, as well as peroxides,

cell membrane phospholipids, and polyunsaturated fatty acids, can cause structural and functional cell damage of graft organs during cold storage (33). The cellular defense of graft organs in cold storage against reactive oxygen species is provided by many antioxidants such as glutathione and specific enzymes like superoxide dismutase, catalase, and glutathione peroxidase. These compounds, located in a variety of cellular compartments, are regulated by the level of ischemia.

In Group 5, addition of glutathione into the perfusate also reduced the level of ICAM-1 expression in liver tissue. This may be related to the decreased level of cytotoxic reactive oxygen species seen during cold preservation.

In summary, in this study, addition of verapamil to the perfusate/preservation solution for inhibition of intracellular calcium accumulation had no effect on tissue ICAM-1 molecule expression. Addition of Na nitroprusside (for capillary vasodilatation) and glutathione (to reduce oxygen free radicals and produce an antioxidant effect) to the perfusion/preservation solution led to decreased levels of ICAM-1 molecule expression, reflecting less preservation injury to the graft.

Therefore, during long-term cold preservation, this might prove beneficial in preventing graft complications; however, further studies of this effect on larger groups of human donor livers are warranted.

REFERENCES

1. Heemann UW, Tullius SG, Azuma H, et al. Adhesion molecules and transplantation. Ann Surg 1994; 219: 4-12.
2. Adams DH, Burra P, Hubscher SG, et al. Endothelial activation and circulating vascular adhesion molecules in alcoholic liver disease. Hepatology 1994; 19: 588-94.
3. Toledo-Pereyra LH, Suzuki S. Cellular and biomolecular mechanisms of liver ischemia and reperfusion injury. Transplant Proc 1994; 26: 325-7.
4. Seekamp A, Till GO, Mulligan MS, et al. Role of selectins in local and remote tissue injury following ischemia and reperfusion. Am J Pathol 1994; 144: 592-8.
5. Moolenaar W, Bruijn JA, Schrama E, et al. T-cell receptors and ICAM-1 expression in renal allografts during rejection. Transpl Int 1991; 4: 140-5.
6. Adams DH, Mainolfi E, Elias E, et al. Detection of circulating intercellular adhesion molecule-1 after liver transplantation--evidence of local release within the liver during graft rejection. Transplantation 1993; 55: 83-7.
7. Koo DDH, Fuggle SV. Impact of ischemia/reperfusion injury and early inflammatory responses in kidney transplantation. Transplantation Reviews 2000; 14: 210-24.
8. Pegg O. Principles of tissue preservation. Progress in Transplantation 1985; 2: 69-103.
9. Ar'Rajab A, Ahren B, Bengmark S. Improved liver preservation for transplantation due to calcium channel blockade. Transplantation 1991; 51: 965-7.
10. Quintana AB, Rodriquez JV, Scandizzi AL, et al. The benefit of adding sodium nitroprusside (NPNa) or S-nitrosoglutathione (GSNO) to the University of Wisconsin solution (UW) to prevent morphological alterations during cold preservation/reperfusion of rat livers. Ann Hepatol 2003; 2: 84-91.
11. El-Wahsh M, Fuller B, Davidson B, et al. Hepatic cold hypoxia and oxidative stress: implications for ICAM-1 expression and modulation by glutathione during experimental isolated liver preservation. Cryobiology 2003; 47: 165-73.
12. Rodriguez JV, Guibert EE, Quintana A, et al. Role of sodium nitroprusside in the improvement of rat liver preservation in University of Wisconsin solution: a study in the isolated perfused liver model. J Surg Res 1999; 87: 201-8.
13. Wagner K, Albrecht S, Neumayer HH. Prevention of posttransplant acute tubular necrosis by the calcium antagonist diltiazem: a prospective randomized study. Am J Nephrol 1987; 7: 287-9.
14. Jamieson NV, Lindell S, Sundberg R, et al. Preservation of the canine liver for 24-48 hours using simple cold storage with UW solution. Transplantation 1988; 46: 517-22.
15. Chazouilleres O, Calmus Y, Vaubourdolle M, Ballet F. Preservation-induced liver injury. Clinical aspects, mechanisms and therapeutic approaches. J Hepatol 1993; 8:123-34.
16. Jacobsson J, Sundberg R, Valdiva LA, Starzl TE. Liver preservation with lidoflazine and the UW: a dose - finding study. Transplantation 1993; 56: 472-5.
17. Pinsky DJ, Oz MC, Koga S. Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing the nitric oxide pathway. J Clin Invest 1994; 93: 2291-7.
18. Feelisch M, Noack E. Correlation between nitric oxide formation during degeneration of organic nitrate and activation of guanylate cyclase. Eur J Pharmacol 1987; 139: 19-30.
19. Fuller B, Dijk S, Butler P, et al. Pro-inflammatory agents accumulate during donor liver cold preservation: a study on increased adhesion molecule expression and abrogation by curcumin in cultured endothelial cells. Cryobiology 2003; 46: 284-8.
20. D'Silva M, Pirenne J, Bonnet P, et al. Flexible technique for multiple organ procurement in rats: a training and research tool in transplantation. Microsurgery 1993; 14: 342-9.
21. Abebe W, Cavallari N, Agrawal DK, et al. Functional and morphological assessment of rat aorta stored in University of Wisconsin and Euro Collins solutions. Transplantation 1993; 56: 808-16.
22. Pienaar BH, Lindell SL, Van Gulik T, et al. Seventy-two-hour preservation of the canine liver by machine perfusion. Transplantation 1990; 49: 258-60.
23. Adam R, Bismuth H, Diamond T, et al. Effect of extended cold ischemia with UW solution on graft function after liver transplantation. Lancet 1992; 340: 1373-6.
24. Clavien PA, Sanabria JR, Upadhaya A, et al. Evidence of the existence of a soluble mediator of cold preservation injury. Transplantation 1993; 56: 44-53.
25. McAnulty JF, Ploeg RJ, Southard JH, Belzer FO. Successful five-day perfusion preservation of the canine kidney. Transplantation 1989; 47: 37-41.
26. Kalayoglu M, Sollinger HW, Stratta RJ, et al. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1: 617-9.

27. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332: 411-5.
28. Leaf A. Cell swelling. A factor in ischemic tissue injury. Circulation 1973; 8: 455-8.
29. Pfeiffer DR, Schmid PC, Beatrice MC, et al. Intramitochondrial phospholipase activity and the effects of Ca 2+ plus N-ethylmaleimide on mitochondrial function. J Biol Chem 1979; 254: 11485-94.
30. Beatrice MC, Palmer JW, Pfeiffer DR. The relationship between mitochondrial membrane permeability, membrane potential, and the retention of Ca 2+ by mitochondria. J Biol Chem 1980; 255: 8663-71.
31. Sobh MA, Shehab el-Din AB, Moustafa FE, et al. A prospective randomised study of the protective effect ofverapamil on ischemic renal injury in renal allotransplants. Transplant Proc 1989; 21: 1230-2.
32. Dawidson I, Rooth P, Lu C, et al. Verapamil improves the outcome after cadaver renal transplantation. J Am Soc Nephrol 1991; 2: 983-90.
33. Kellogg EW 3rd, Fridovich I. Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol 1975; 250: 8812-7.