Angiotensin-Converting Enzyme Inhibition by Enalapril: A Novel Approach to Reduce Ischemia/Reperfusion Damage After Experimental Liver Transplantation

MATTHIAS ANTHUBER,[1] STEFAN FARKAS,[2] MARKUS RIHL,[2] MICHAEL D. MENGER,[3] FRIEDRICH WILHELM SCHILDBERG,[4] KARL-WALTER JAUCH,[1] AND KONRAD MESSMER[2]

Angiotensin-converting enzyme (ACE) inhibitors have proven to be effective in the reduction of ischemia/reperfusion damage after myocardial ischemia. Whether this favorable effect can be related to other models of ischemia and reperfusion has not yet been investigated. Therefore, we studied in a model of syngeneic liver transplantation in the rat the effect of recipient enalapril treatment on postischemic liver injury. Untreated animals served as the control group. Treatment with enalapril was started 5 minutes before reperfusion by intravenous infusion of enalapril at a dosage of 5 mg/kg/h. By means of *in vivo* microscopy, the sinusoidal perfusion rate and leukocyte adherence in sinusoids and postsinusoidal venules were analyzed during 45 to 60 minutes of reperfusion. Liver function was monitored by measuring bile output over a period of 60 minutes. Analysis of coagulation factors (prothrombin time, factor V, fibrinogen) and liver enzymes (alanine transaminase [ALT], aspartate transaminase [AST]) served for the evaluation of organ dysfunction and damage secondary to ischemia/reperfusion injury. The sinusoidal perfusion rate was significantly improved by enalapril treatment (94.7% [1.0] vs. 75.3% [3.8]; mean [SEM]; $P = .005$). In addition, leukocyte-sticking in both liver sinusoids and postsinusoidal venules was remarkably reduced in enalapril-treated animals as compared with controls (stickers/lobule: 21.0 [3.3] vs. 59.2 [2.1]; $P = .0004$; stickers/mm^2 venular surface: 20.5 [4.7] vs. 110.3 [18.1]; $P = .0004$). Moreover, bile output was increased (1.13 [0.35] vs. 0.43 [0.18] g bile/60 min · 100 g liver; $P = .06$). Values for PT (22.5% [2.1] vs. 9.7% [1.8]; $P = .005$), factor V 99.4% [9.5] vs. 49.5% [8.5]; $P = .007$), and fibrinogen (64.1% [7.7] vs. 12.8% [3.2]; $P = .001$) were significantly improved, paralleled by a remarkable reduction in serum ALT (1,428 U/L [190] vs. 2,315 [248]; $P = .02$). Our data show for the first time that ACE inhibition in the liver recipient by enalapril attenuates hepatic ischemia/reperfusion damage after experimental liver transplantation. Our results may offer a novel approach to reduce ischemia/reperfusion injury in clinical liver transplantation. (HEPATOLOGY 1997;25:648-651.)

After more than 20 years of clinical experience, liver transplantation is an established treatment modality for end-stage liver disease, acute liver failure, and selected hepatic malignancies.[1,2] The timing of transplantation and selection of suitable donors and recipients are crucial cornerstones for a successful outcome of the costly procedure. In up to 22% of cases, primary graft dysfunction after transplantation is observed, initiating a cascade of severe postoperative complications with considerable impact on morbidity and mortality.[3] Pathophysiologically, graft dysfunction is based on preexisting donor liver damage and insults from cold/warm ischemia and reperfusion.

In recent years, many attempts have been made to improve organ preservation, e.g., to prolong the tolerable ischemic time, and to better protect the graft from both anoxic and oxygen free radical–mediated damage. Therapeutic strategies in recipients were aimed particularly at the reduction of reperfusion injury mediated by oxygen free radicals, which are generated with reoxygenation of the liver. Results in animal studies of myocardial ischemia showed cardioprotective properties of angiotensin-converting enzyme (ACE) inhibitors,[4-6] perhaps related to favorable effects of these drugs on vasomotor tone and bradykinin-prostacyclin metabolism.[7] In the present work, using a model of syngeneic liver transplantation in rats, we tested whether similar beneficial effects of ACE-inhibitor enalapril occur following hepatic ischemia and reperfusion.

MATERIALS AND METHODS

All experiments were performed with the permission of the Government Authorities and in accordance with the German Legislation on Laboratory Animal Experiments.

Surgical Technique. Syngeneic, male Lewis rats (donors: 150-200 g body weight; recipients: 190-250 g) underwent orthotopic liver transplantation according to the cuff technique described by Kamada and Calne.[8] In contrast to Kamada's original technique, grafts were rearterialized and simultaneously reperfused by the portal and arterial route as described by Post et al.[9] Livers were preserved by retrograde aortal flush with University of Wisconsin solution and stored at 4°C for 24 hours. Before reperfusion, the grafts were flushed with 10 mL of Ringer's lactate solution at room temperature via the portal route to remove the preservation solution.

A control group of animals (n = 10) received 0.9% sodium chloride by an intravenous line in the internal jugular vein during reperfusion. Animals of the enalapril study group (n = 8) received enalapril (Xanef, Merck, Sharp, and Dohme, Munich, Germany) at a dosage of 5 mg/kg/h intravenously. Treatment started 5 minutes before reperfusion and was continued until the end of the experiment, approximately 90 to 120 minutes after reperfusion. Mean arterial blood pressure was monitored continuously via a catheter placed in the left carotid artery.

Abbreviations: ACE, angiotensin-converting enzyme; ALT, alanine transaminase; AST, aspartate transaminase.

From the [1]Department of Surgery, University of Regensburg, Regensburg, the [2]Institute for Surgical Research, Ludwig-Maximilians-Universitat Munich, Munich, the [3]Institute for Clinical-Experimental Surgery, University of Saarland, Homburg/Saar, and the [4]Department of Surgery, Klinikum Grosshadern, Ludwig-Maximilians-Universität Munich, Munich, Germany.

Received May 3, 1996; accepted October 15, 1996.

Address reprint requests to: Matthias Anthuber, M.D., Department of Surgery, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany.

0270-9139/97/2503-0026$3.00/0

TABLE 1. Body Weight, Liver Wet Weight, Anhepatic Period, and Cold Ischemic Time for the Liver Transplantation Group Without Treatment (LTx; n = 10) and the Enalapril-Treated Transplant Group (LTx/Ena; n = 8)

	LTx (n = 10)	LTx/Ena (n = 8)
Body weight (g)	225.7 (6.9)	211.6 (3.0)
Liver wet weight (g)	7.2 (0.2)	7.6 (0.2)
Anhepatic period (min)	19.0 (0.3)	18.8 (0.7)
Ischemic time (h)	24.3 (0.1)	24.0 (0.2)

NOTE. Data are means (SEM).

Abbreviations: LTx, liver transplantation without treatment; LTx/Ena, liver transplantation with enalapril treatment.

In Vivo *Microscopy.* For assessment of microvascular liver perfusion and leukocyte-endothelial interaction, *in vivo* microscopy was used according to the technique described by Menger et al.[10] In brief, 30 minutes after reperfusion and at a stable mean arterial pressure above 50 mm Hg, the left liver lobe was exteriorized on a specially designed stage. To avoid major fluid loss and drying, the abdominal cavity was covered with Saran wrap. After 30 to 35 minutes of reperfusion, sodium fluorescein (2.0 μmol/kg) and rhodamin 6G (0.1 μmol/kg) were injected intravenously for fluorescent staining of hepatocytes and leukocytes, respectively. The following *in vivo* microscopy parameters were assessed in 10 randomly selected acinar areas and postsinusoidal venules:

1. Sinusoidal perfusion rate: percentage of perfused sinusoids from all sinusoids visible in a defined acinar area.
2. Permanent leukocyte adherence ("sticker") in sinusoids and postsinusoidal venules: number of leukocytes adhering for at least 20 seconds in sinusoids (number per liver lobule); number of leukocytes attached for at least 20 seconds to the venular surface of postsinusoidal venules (number per square millimeter of venular endothelial surface).
3. Temporary leukocyte adherence in postsinusoidal venules ("rollers"): leukocytes moving along the wall of postsinusoidal venules with a velocity of less than 30% of the central stream velocity (percentage of rollers of all free-moving leukocytes during the observation period of 20 seconds).

Bile Flow. As an indicator of reestablished liver function, bile production was assessed. The amount of bile draining from the bile duct via a polyethylene tube over a period of 60 minutes was collected and weighed, and was related to 100 g of liver wet weight for comparison between individual animals and groups.

Blood Coagulation and Liver Enzymes. At the end of the experiment (90-120 minutes after reperfusion), arterial blood samples were drawn for analysis of coagulation factors (prothrombin time, factor V, and fibrinogen) and liver enzymes (alanine transaminase [ALT], aspartate transaminase [AST]). The samples were processed by standard tests used for human blood analysis.

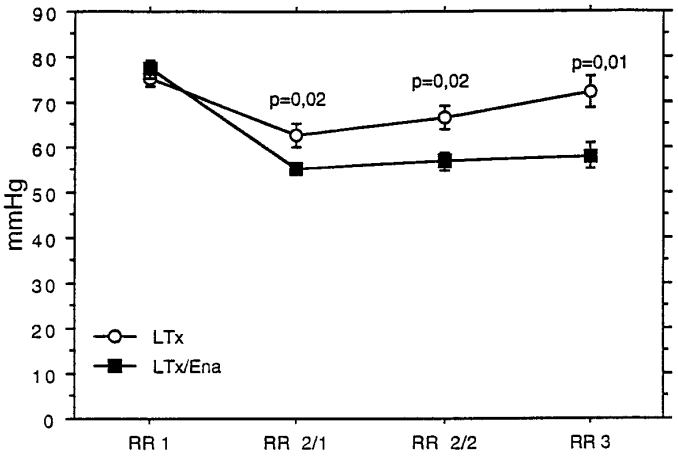

FIG. 1. Mean arterial blood pressures of recipient animals after liver transplantation without treatment (○) and with enalapril treatment during reperfusion (■) (mean [SEM] by the Mann-Whitney *U* test). RR1 = MAP after laparotomy; RR2/1 = MAP before exterioration of the left liver lobe; RR2/2 = MAP after exterioration of the left liver lobe; RR3 = MAP at the end of *in vivo* microscopy.

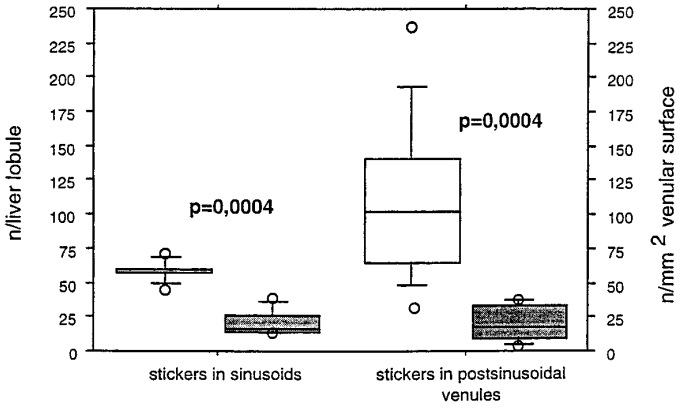

FIG. 2. Permanent leukocyte adherence in liver sinusoids and postsinusoidal venules after liver transplantation (□) and liver transplantation with enalapril treatment (■). *Horizontal lines* of box plots depict 10th, 25th, 50th, 75th, and 90th percentile; *empty circles* display outliers.

Statistics. Statistical differences between groups were calculated using the Student's *t* test for normally distributed data and the Mann-Whitney *U* test for nonparametric data. Differences were considered significant at $P < .05$. All data are presented as means (SEM).

RESULTS

There were no significant differences between the two groups in regard to body weight, liver wet weight, duration of anhepatic period, and total ischemic time (Table 1). However, the mean arterial pressure at time points "before exterioration of left liver lobe," "after exterioration of left liver lobe," and "end of experiment" was significantly depressed in enalapril-treated animals as compared with controls (Fig. 1).

Analysis of microvascular perfusion showed remarkable improvement in enalapril-treated animals as compared with controls. The mean sinusoidal perfusion rate of 75.3% (3.8) in untreated liver recipients increased to 94.7% (1.0) in animals receiving enalapril ($P = .005$), which amounts to an almost normal perfusion rate (98%-100%), usually observed in sham-operated controls. In parallel, permanent leukocyte adherence (leukocyte-sticking) in both sinusoids and postsinusoidal venules was found to be significantly reduced by enalapril treatment (Fig. 2).

Regarding temporary leukocyte adherence in postsinusoidal venules (leukocyte-rolling), enalapril treatment did not result in major changes (control vs. enalapril group: 10.0 [2.1] vs. 7.2 [1.4]).

The results of the functional and biochemical evaluations of the postischemic liver damage are listed in Table 2. Bile

TABLE 2. Bile Flow, Transaminases, and Coagulation Profile

	LTx	LTx/Ena	*P*
Bile flow (g/60min · 100g liver)	0.43 (0.1)	1.13 (0.3)	.06
PT (%)	9.7 (1.8)	22.5 (2.1)	.005
Factor V (%)	49.5 (8.5)	99.4 (9.5)	.007
Fibrinogen (mg%)	12.8 (3.2)	64.1 (7.7)	.001
ALT (U/L)	2,315 (248)	1,428 (190)	.02
AST (U/L)	1,853 (369)	1,418 (274)	.06

NOTE. Data are means (SEM).

Abbreviations: LTx, liver transplantation without treatment; LTx/Ena, liver transplantation with enalapril treatment.

flow as an indicator of restored energy-dependent liver function was increased in the enalapril study group, although the difference to controls did not reach statistical significance. Blood coagulation as assessed by the prothrombin time, factor V activity, and fibrinogen concentration was significantly better preserved in enalapril-treated recipients. With regard to hepatocellular integrity, untreated recipients released considerably higher amounts of transaminases, in particular ALT, indicating a higher degree of hepatocellular damage.

DISCUSSION

During cadaveric liver transplantation, there are inevitable periods of hypothermic anoxia, partial normothermic hypoxia, lack of hepatotrophic substances, and toxic insults from oxygen free radicals, which result in organ damage, generally referred to as ischemia/reperfusion injury. In clinical transplantation, this damage presents as graft dysfunction or nonfunction in 16% and 6% of cases, respectively.[11] Introduction of the University of Wisconsin solution by Belzer marked a considerable progress in liver preservation[12]; however, despite safe prolongation of the tolerable ischemic time, rates of early graft dysfunction remained a major concern. Primary graft viability is not fully preserved by simply flushing and storing the liver in ice-cold University of Wisconsin or Brettschneider's solution (HTK solution). Although studies on modified flush solutions and pretreatment of recipients with oxygen free radical scavengers or calcium channel blockers have already proven to have favorable effects on early graft function,[13-16] none of these has been introduced into routine clinical practice.

Ischemia/reperfusion injury was effectively reduced by different types of ACE inhibitors in animal models on temporary coronary blood flow occlusion. In the case of thiol-containing ACE inhibitors (captopril, zofenopril), the cardioprotective effects were in part attributed to scavenging of oxygen free radicals.[17,18] However, what may be more important is the common property of all ACE inhibitors to induce vasodilation by different pathways. By inactivating kinase II, ACE inhibitors block both the conversion of angiotensin I to vasoconstrictory angiotensin II and additionally break down vasodilating bradykinin to its inactive metabolites. Increased concentrations of bradykinin stimulate endothelial prostacyclin synthesis. Prostacyclin by itself acts vasodilative and has potent antiaggregatory, and yet unexplained, cytoprotective properties. Stabilization of cellular and subcellular membranes, as well as protection against lipid peroxidation by oxygen free radicals, may play a role in cytoprotection. By investigations on cardioprotective actions of ACE inhibitors, Li and Chen proved that the beneficial effects of captopril were almost completely abolished by indomethacin, and that the protective effect of captopril on coronary flow after reperfusion was similar to that of exogenous prostacyclin.[19,20] These findings led them to their conclusion that the cardioprotective action of ACE inhibitors may be mediated by local release of prostacyclin.

In the present study, we were able to prove by means of *in vivo* microscopy, that continuous enalapril treatment during reperfusion almost normalizes nutritive liver perfusion after experimental liver transplantation. Moreover, leukocyte–endothelial cell interaction was significantly reduced within the microvascular liver network, indicating attenuation of ischemia/reperfusion–induced inflammatory response. Oxygen free radical scavenging by the enalapril cannot explain the effects observed, because enalapril does not possess the sulphydryl-moiety. Favorable modulation of the angiotensin, bradykinin, and prostacyclin metabolism may be the prevailing mechanism of action, which results in improved liver microcirculation. Cytoprotective, platelet-, and granulocyte-inhibiting effects of locally increased prostacyclin concentrations may have to some extent contributed to the restoration of hepatic perfusion and reduced leukocyte-endothelial interaction after grafting.[21-23]

Disturbances of the liver microcirculation were in accordance with the results of the hepatic ischemia/reperfusion injury as assessed by the functional and biochemical parameters. Bile flow is a reliable indicator of energy-dependent liver function[24,25]; thus, increase after enalapril treatment reflects accelerated restoration of hepatic energy charge. Why the difference to untreated controls did not reach the level of significance may be related to both an inadequate collection period and a considerable variance in a group size of eight individuals.

Comparison of coagulation parameters between the study groups revealed significant improvements for animals treated with enalapril. Because blood samples were drawn 90 to 120 minutes after reperfusion, changes may be interpreted as reduced activation of intravascular disseminated coagulation during reperfusion rather than early restored *de novo* synthesis of coagulation factors by the graft. Disseminated intravascular coagulation, hyperfibrinolysis, metabolic derangements, pulmonary hypertension, and systemic hypotension are frequent disorders observed after revascularization of the graft, and are comprised by the term "reperfusion syndrome."[26,27]

A major concern in the conduction of the study was that enalapril may contribute considerably to the postreperfusion hemodynamic instability of recipients. In fact, mean arterial pressure in the enalapril group was significantly reduced compared with untreated controls; however, prolonged periods of severe shock were never observed. Enalapril may have successfully reduced the reperfusion syndrome; otherwise, enalapril-induced hypotension would not have been tolerated by the animals. In a different set of experiments, increasing dosages of enalapril in sham-operated animals were studied to determine the maximum tolerable dosage in terms of hypotension. The dose of 5 mg/kg/h represented the limit at which animals started to react with mild hypotension. In animals receiving a transplant, we actually expected induction of intolerable hypotension by the use of 5 mg/kg/h enalapril before and during reperfusion. Although mild hypotension was observed as compared with controls, systemic perfusion pressure seemed to be sufficient. Animals rapidly awoke after reperfusion, indicating metabolic function of the graft and, even though not measured, urine production as observed by the filling volume of the urine bladder, which seemed to be comparable with untreated animals. These findings suggest life-sustaining cardiocirculatory conditions.

Serum concentrations of ALT and AST after clinical liver transplantation indicate the extent of hepatocellular damage from ischemia/reperfusion injury.[28] Hepatocellular injury, as reflected by the release of liver enzymes, was attenuated in the enalapril-treated recipients. This could be related to both the increase in nutritive perfusion and to cytoprotective actions of prostacyclin. The discrepant levels for ALT and AST may be explained by their different localization in the hepatocyte. While ALT is exclusively a cytoplasmic enzyme, AST is represented to 70% in mitochondria. This finding may indicate either that enalapril during reperfusion is particularly protective at the cytoplasmic level and not in the subcellular compartment, or that there is some unknown, specific effect of enalapril.

Analysis of graft perfusion, liver function tests, and bile flow are established means in clinical and experimental liver transplantation to evaluate the extent of ischemia/reperfusion injury. Whether graft function is sufficiently preserved to sustain life, however, is best proven by data on graft or animal survival, which should be a subject of future studies.

Whether the improvements can be attributed to the modulation of the angiotensin, bradykinin, and prostacyclin metabolism remain to be determined in future studies. More-

over, investigations are needed to clarify whether enalapril and possible other ACE inhibitors have some as-yet-unknown, specific, protective properties. On the basis of our data, clinical application of enalapril should be considered as a novel approach to improve early graft and to decrease the rate of early graft liver failure after liver transplantation.

REFERENCES

1. Bismuth H, Azoulay D, Dennison A. Recent developments in liver transplantation. Transplant Proc 1993;25:2191-2194.
2. Neuhaus P, Blumhardt G, Bechstein WO, Keck H. Progress in the field of liver transplantation in the last 10 years. Langenbecks Arch Chir 1992; 317(suppl):209-216.
3. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, et al. Risk factors for primary dysfunction after liver transplantation—a multivariate analysis. Transplantation 1993;55:807-813.
4. de Graeff PA, de Langen CDJ, van Gilst WH, Bel K, Scholtens E, Kingma JH, Wesseling H. Protective effects of captopril against ischemia/reperfusion-induced ventricular arrhythmias in vitro and in vivo. Am J Med 1988; 84:67-74.
5. Przyklenk K, Kloner RA. "Cardioprotection" by ACE-inhibitors in acute myocardial ischemia and infarction? Basic Res Cardiol 1993;88(suppl 1): 139-154.
6. Scholkens BA, Linz W. Cardioprotective effects of ACE inhibitors: experimental proof and clinical perspectives. Clin Physiol Biochem 1990;8:33-43.
7. Schroer K. Role of prostaglandins in the cardiovascular effects of bradykinin and angiotensin-converting enzyme inhibitors. J Cardiovasc Pharmacol 1992;20(suppl 9):S68-S73.
8. Kamada N, Calne RY. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 1979;28:47-56.
9. Post S, Menger MD, Rentsch M, Gonzalez AP, Herfarth C, Messmer K. The impact of arterialization on hepatic microcirculation and leukocyte accumulation after liver transplantation in the rat. Transplantation 1992; 54:789-794.
10. Menger MD, Marzi I, Messmer K. In vivo fluorescence microscopy for quantitative analysis of the hepatic microcirculation in hamsters and rats. Eur Surg Res 1991;23:158-169.
11. Ploeg RJ, D'Alessandro AM, Hoffmann RM, Eckhoff D, Isaacs R, Knechtle SJ, Pirsch JD, et al. Impact of donor factors and preservation on function and survival after liver transplantation. Transplant Proc 1993;25:3031-3033.
12. Belzer FO, D'Alessandro AM, Hoffmann RM, Knechtle SJ, Reed A, Pirsch JD, Kalayoglu M, et al. The use of UW solution in clinical transplantation. A 4-year experience. Ann Surg 1992;215:579-583.
13. Gao WS, Takei Y, Marzi I, Lindert KA, Caldwell-Kenkel JC, Currin RT, Tanaka Y, et al. Carolina rinse solution—a new strategy to increase survival time after orthotopic liver transplantation in the rat. Transplantation 1991;52:417-424.
14. Lemmens HP, Schoen MR, Blumhardt G, Filler D, Brandau O, Meissler M, Baer P, et al. Influence of SOD, catalase, and epoprostenol on 24-hour liver preservation in pigs. Transplant Proc 1993;25:2549-2553.
15. Liang D, Thurman RG. Protective effects of the calcium antagonists diltiazem and TA3090 against hepatic injury due to hypoxia. Biochem Pharmacol 1992;44:2207-2211.
16. Post S, Rentsch M, Palma P, Gonzalez AP, Otto G, Menger MD. Effect of Carolina rinse solution on Kupffer cell activation after reperfusion in rat liver transplantation. Transplant Proc 1992;24:2703.
17. Chopra M, Scott N, McMurray J, McLay J, Bridges A, Smith WE, Belch JJ. Captopril: a free radical scavenger. Br J Clin Pharmacol 1989;27:396-399.
18. Chopra M, McMurray J, Stewart J, Dargie HJ, Smith WE. Free radical scavenging: a potentially beneficial action of thiol-containing angiotensin converting enzyme inhibitors. Biochem Soc Trans 1990;18:1184-1185.
19. Li K, Chen X. Protective effects of captopril and enalapril on myocardial ischemia/reperfusion damage of the rat. J Mol Cell Cardiol 1987;19:909-915.
20. Chen X, Pi X, Li D, Li Y, Deng H. Prostacyclin-mediated cardioprotection of captopril and ramiprilate against lipidperoxidation in the rat. Prog Clin Biol Res 1989;301:167-173.
21. Alvarez-Lopez A, de Hemptinne B, Hoebeke Y, Lambotte L. Prostaglandin E2 increases the tolerance of the rat liver to warm ischemia in absence of splanchnic congestion. Transplant Proc 1987;19:4105-4109.
22. Araki H, Lefer AM. Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. Am J Physiol 1980;238:176-181.
23. Fantone JC, Kinnes DA. Prostaglandin E1 and prostaglandin I2 modulation of superoxide production by human neutrophils. Biochem Biophys Res Commun 1983;113:506-512.
24. Bowers BA, Branum GD, Rotolo FS, Watters CR, Meyers WC. Bile flow—an index of ischemic injury. J Surg Res 1987;42:565-569.
25. Sumimoto K, Inagaki K, Yamada K, Kawasaki T, Dohi K. Reliable indices for the determination of viability of grafted liver immediately after orthotopic transplantation. Bile flow rate and cellular adenosine triphosphate level. Transplantation 1988;46:506-509.
26. Himmelreich G, Kierzek B, Neuhaus P, Slamer KJ, Jochum M, Riess H. Coagulation changes and the influence of the early perfusate in the course of orthotopic liver transplantation (OLT) when aprotinin is used intraoperatively. Blood Coagul Fibrinolysis 1991;2:51-58.
27. Himmelreich G, Jochum M, Bechstein WO, Machleidt W, Neuhaus P, Slama KJ, Riess H. Mediators of leukocyte activation play a role in disseminated intravascular coagulation during orthotopic liver transplantation. Transplantation 1994;57:354-358.
28. Greig PD, Woolf GM, Sinclair SB, Abecassis M, Strasberg SM, Taylor BR, Blendis LM, et al. Treatment of primary liver graft nonfunction with prostaglandin E1. Transplantation 1989;48:447-453.