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ORIGINAL ARTICLE

Measurement of portal venous flow velocity with an implantable miniature Doppler probe in pig liver transplantation

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Abstract Portal venous flow (PVF) was serially monitored after pig liver transplantation (LTX) with the use of an implantable, miniature Doppler probe developed in our laboratory. Throughout the study period, the mean PVF in pigs that underwent LTX was significantly greater than that in pigs that were sham operated. For three animals with early graft failure secondary to primary nonfunction and for six that survived longer than 7 days, the mean PVF on postoperative day (POD) 1 was 18.7 ± 3.8 cm/s and 41.7 ± 11.2 cm/s, respectively ($P < 0.05$). For animals with acute cellular rejection (ACR), the mean PVF was 61.3 ± 9.9 cm/s on POD 7 and 54.3 ± 6.38 cm/s on POD 14. These values were significantly higher than those for animals without ACR ($P < 0.05$). Moreover, the increase in PVF correlated well with the degree of ACR. The actual PVF volume was measured by ex vivo perfusion, which showed a clear correlation with the PVF velocity obtained with the implanted, miniature Doppler probe. We feel that the liver graft requires increased PVF volume after transplantation to facilitate functional recovery from damage to hepatocytes due to preservation-reperfusion injury, and that ACR is also associated with an increased PVF. We conclude that monitoring the PVF in the early postoperative period after LTX is useful in the evaluation of graft function, particularly for predicting primary nonfunction and severity of ACR.

Key words Portal flow, Doppler, liver transplantation · Doppler, portal flow, liver transplantation · Liver transplantation, pig, portal flow

Introduction

Due, in large part, to recent improvements in clinical management, 5-year patient survival rates after liver transplantation (LTX) have improved over the years [17]. Yet, there is still a significant amount of graft failure in the early postoperative period, with high mortality [19]. Although the causes of early graft failure vary, significant alterations in hepatic hemodynamics are quite common [6]. As portal venous flow (PVF) constitutes a dominant portion of total hepatic flow, monitoring of the PVF to the liver can provide valuable information for physiological evaluation of the graft in the early post-transplant period [7, 11]. To do this, PVF measurement must be performed accurately, with serial monitoring under a steady state condition that would be difficult to achieve with a conventional Doppler flowmeter applied externally to the body.

We describe here an implantable, miniature Doppler probe developed in our laboratory that enables intermittent monitoring of the PVF in animals that have undergone LTX. With this device, we investigated the relationship between PVF and graft outcome, with particular attention to primary nonfunction and acute cellular rejection (ACR). We were able to demonstrate that poor PVF in the early postoperative period was associ-

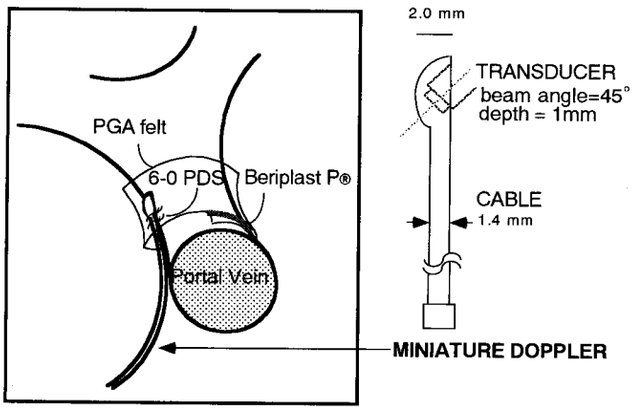

Fig. 1 A schematic representation of how portal venous flow (PVF) can be monitored with an implantable, miniature Doppler probe placed on the portal vein of the animal after liver transplantation

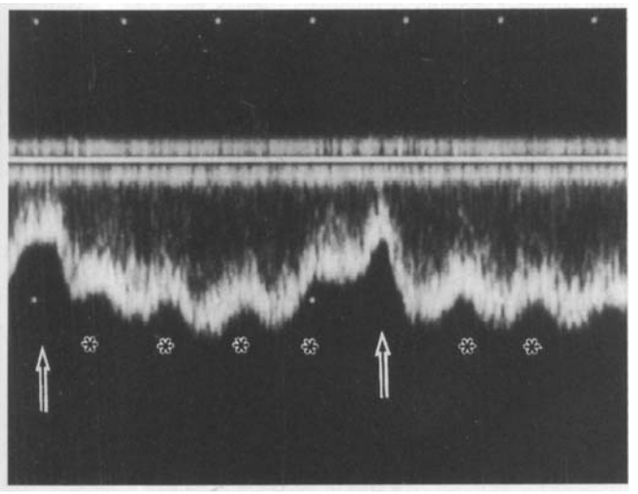

Fig. 2 A typical pattern of portal venous flow (PVF) in an animal 14 days after liver transplantation with an uneventful postoperative course. Flow velocity was recorded as a negative value since the probe was placed in the direction of the blood flow. Inspiratory indentation (*solid arrow*) and arterial systolic indentation (*asterisks*) are indicated. *Dots* at the top indicate (0.5-s interval). The maximum and minimum PVF in this recording are 52 cm/s and 28 cm/s, respectively.

ated with primary nonfunction of the graft, and that there was a positive correlation between the mean PVF and the grade of ACR.

Materials and methods

Young Landrace pigs weighing between 12.5 and 14.5 kg were matched for LTX. The donor liver was harvested and then perfused with 300 ml of University of Wisconsin (UW) solution via the portal vein and 100 ml via the hepatic artery; it was then stored by simple immersion at 4 °C for varying lengths of time between 5 and 8 h. The native liver was removed from the recipient pig while it was on passive veno-venous bypass (main portal vein and common iliac vein to jugular vein).

Engraftment of the liver was performed orthotopically with routine techniques for vascular anastomoses [16]. The portal vein was anastomsed in end-to-end fashion with continuous 6–0 prolene sutures, leaving a generous growth factor. The anastomotic line was usually at the level of the left gastric vein takeoff.

Immunosuppression consisted of a single intravenous bolus injection of methylprednisolone, 200 mg intraoperatively, and cyclosporin, 3 mg/kg body weight, intravenously immediately after the operation. Cyclosporin, 9 mg/kg body weight, was given orally on postoperative days (POD) 1, 2, and 3.

For continuous monitoring of the PVF in the LTX recipients, an implantable, miniature Doppler probe was developed by the authors (U.T. and T.M.). It consists of a piezoelectric ultrasonic transducer, 2×2 mm in size, which holds it at a 45-degree angle to the face of the transducer. It is connected with a coaxial wire that is covered by silicone tubing (0.76 mm ID, 1.4 mm OD, 40 cm in length). This miniature Doppler probe provides a 5-MHz carrier frequency and has a 2-μs duration. The pulse repetition frequency of the Doppler flowmeter is 8 KHz, and the target length ranges from 1 to 10 mm from the face of the probe.

After completing the transplant procedure and before closing the abdominal cavity, the miniature Doppler probe was placed on its longitudinal axis to the anterolateral aspect of the recipient's portal vein, approximately 0.5 cm caudad to the anastomotic suture line. For immobilization, the probe was anchored to a piece of absorbable felt (PGA, Gunze, Kyoto), $10 \times 40 \times 0.3$ mm in size, using absorbable polydioxanone, 6–0 PDS II (Johnson and Johnson Medical, N.J., USA). Both of the free rim ends of the felt were glued with Beriplast P (Behring Berke, Germany). The silicone tube of the probe was directed to the longitudinal axis of the portal vein. It was then guided posterior to the duodenum and placed along with the right posterolateral abdominal wall. Next, it was delivered transcutaneously through the abdominal wall, and a portion of the miniature receptacle was placed in a small, subcutaneous pocket. A schematic representation is shown in Fig. 1.

In order to record the PVF, the subcutaneous pocket was opened under light anesthesia (ketamine sulfate, 0.05 mg/kg, given intramuscularly), and the receptacle of the Doppler probe was connected to a miniature plug with flexible cables from the Doppler flowmeter (Aloka SSD-730, Aloka, Tokyo). With an animal in left decubitus position under very light anesthesia, the Doppler signals were recorded as pulsatile waves of the flow velocity. A typical flow pattern showed a low-frequency inspiratory indentation and high-frequency arterial systolic indentation (Fig. 2). Liver graft biopsies were routinely taken with a Tru-Cut needle on PODs 1, 4 and 7, and either at the time of the animal's death or on POD 14, when they were sacrificed and autopsied. The specimens were submitted for regular histological examinations.

In some of the animals in which the miniature Doppler probe was placed, blood flow volume was correlated with the flow velocity. After induction of general anesthesia, the animals were fully heparinized. The blood was exsanguinated and collected in the reservoir. The animals were then sacrificed and the splenic vein cannulated with a 20 Fr polyethylene catheter, which connects it to the blood reservoir. The superior mesenteric vein and other portal tributaries were ligated. The inferior vena cava above the diaphragm was also cannulated as an exit conduit for the effluent blood from the liver. By infusing the blood through the portal vein from different heights above the body level, which corresponded to the differing flow volume, the PVF velocity could be

recorded with the aid of the previously placed miniature Doppler probe, thereby calibrating the flow volume.

The principles and regulations of the Animal Research Laboratory of Nagoya University School of Medicine were followed in all of the animal experiments.

The data were expressed as mean ± the standard deviation of the mean. An analysis of variance and a paired Student's *t*-test were used to determine significance. A P value below 0.05 was considered significant.

Results

Three of 12 animals included in the study died by POD 3. The cause of death in these animals was ascribed to primary graft nonfunction as histological examinations showed diffuse, patchy, parenchymal necroses without any other demonstrable etiologies. Nine animals survived longer than 7 days. One animal died on POD 10 due to a bleeding gastric ulcer. The rest of the animals survived for 14 days, at which time they were sacrificed and autopsied.

Three animals were sham operated so as to provide a control group; their mean PVF was 31.2 ± 4.5 cm/s at the time of operation. Postoperatively, their mean PVF was 32.4 ± 4.3 cm/s, 30.7 ± 4.0 cm/s, and 32.2 ± 3.3 cm/s on PODs 1, 4, and 14, respectively. For the animals that underwent LTX, the mean PVF was 41.7 ± 11.2 cm/s, 62.2 ± 8.2 cm/s, 54.3 ± 6.3 cm/s, 52.9 ± 10.8 cm/s, and 40.4 ± 16.7 cm/s on PODs 0, 1, 4, 7, and 14, respectively. For the three animals that died of primary nonfunction, the mean PVF was 22.3 ± 8.3 cm/s and 18.3 ± 4.3 cm/s on PODs 0 and 1, respectively, which was significantly lower than that in animals that survived longer.

Acute cellular rejection (ACR) was graded from the histological findings as: "none to minimal", "mild", or "moderate to severe", based on criteria in clinical liver transplantation [8]. On POD 4, none to minimal ACR was noted in six animals and mild ACR in three animals. On POD 7, none to minimal ACR was noted in four pigs, mild ACR in one, and moderate to severe ACR in three. On POD 14, none to minimal ACR was noted in four pigs, mild ACR in two, and moderate to severe ACR in two. The mean PVF in these three grades of ACR was 43.4 ± 9.2 cm/s, 52.3 ± 5.3 cm/s, and 62.1 ± 7.3 cm/s, respectively, with a significant difference between the grades none to minimal and moderate to severe ACR ($P < 0.05$, Fig. 3). One animal that showed histological evidence of severe rejection on POD 7 had the highest PVF of all: 64 cm/s. Another pig with severe rejection on POD 14 had the second highest PVF, 60 cm/s.

The animals were subdivided into groups with primary nonfunction and grades of ACR. On POD 7, the mean PVF in animals with mild or moderate to severe ACR was 61.3 ± 10.1 cm/s, which was significantly greater than the 46.3 ± 7.2 cm/s in animals with none to minimal ACR ($P < 0.05$). Similarly, on POD 14, the mean PVF in animals with mild or moderate to severe ACR was 54.3 ± 6.1 cm/s, significantly greater than the 37.9 ± 2.8 cm/s in animals with none to minimal ACR ($P < 0.05$). The changes in mean PVF in relation to time after LTX in these subdivided groups of animals are shown in Fig. 4.

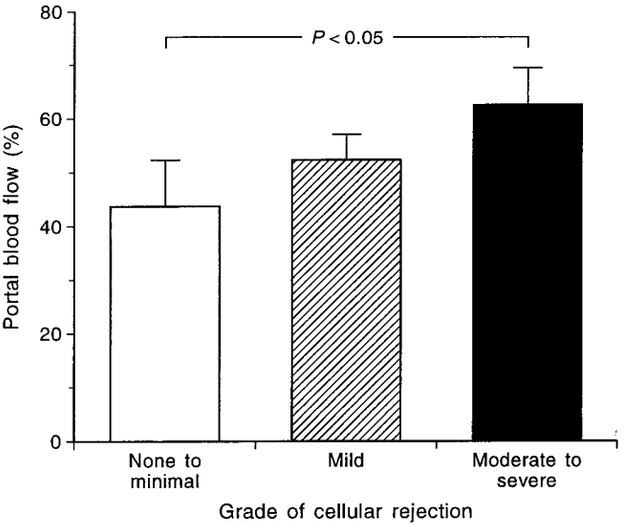

Fig. 3 Comparison of the mean PVF in the three groups of animals with episodes of "none to minimal", "mild", and "moderate to severe" acute cellular rejection (ACR). There was a significant difference between the "none to minimal" and "moderate to severe" ACR groups ($P < 0.05$)

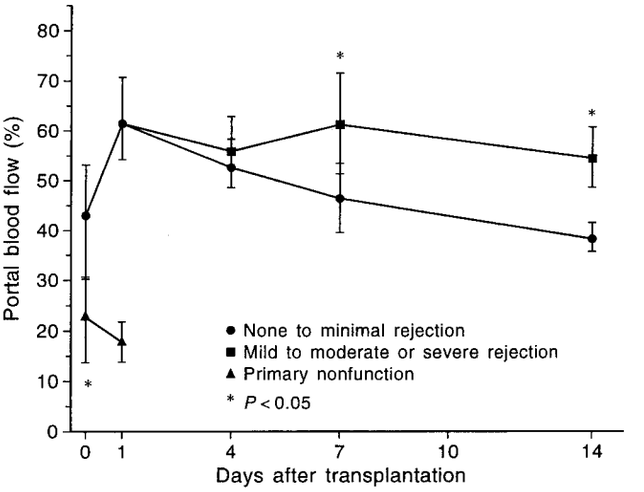

Fig. 4 Changes in the mean PVF in relation to time after liver transplantation in animals with primary nonfunction (▲) and with (●) or without (■) acute cellular rejection (ACR)

The above data on PVF prompted us to investigate whether the arterial flow to the liver would be significantly altered. We attempted to use the miniature Dop-

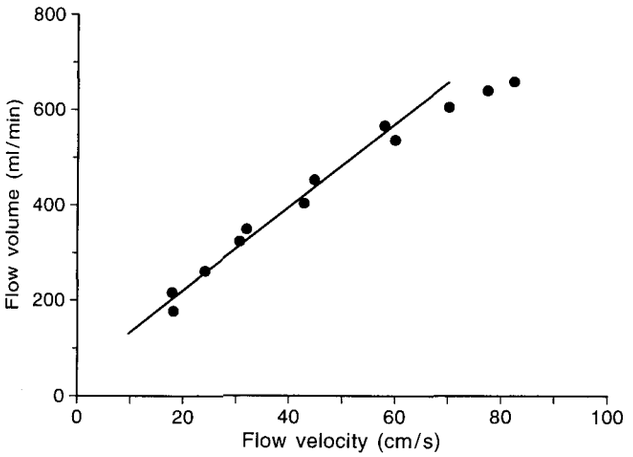

Fig. 5 Actual portal blood flow volume (*vertical axis*) measured by ex vivo perfusion plotted against PVF (*horizontal axis*). There was a linear relationship between them in the range of 10–65 cm/s, but there seemed to be a decreased rate of proportionality in volume as compared with flow velocity greater than 65 cm/s

pler probe for hepatic artery flow monitoring, but this mostly failed due to the technical difficulty of maintaining the probe, which is larger than the artery, in an appropriate position. Nevertheless, we did manage to complete the study in one animal that survived throughout the study period without any significant episode of either preservation injury or rejection. In two other animals, arterial flow could only be measured only on PODs 1 and 4 because the probe became dislodged thereafter. However, the flow was measured on POD 14 by replacing the probe under direct vision at laparotomy before the autopsy. Although one of these two animals showed mild rejection, the arterial flow did not differ remarkably from that in the other two animals that had minimal ischemic injury or rejection. The mean arterial flow in these three animals was 28.0 cm/s, 46.3 cm/s, and 32.7 cm/s on PODs 1, 4, and 14, respectively.

A postmortem flow-volume correlation study of the portal vein showed that there was a linear relationship between them in the range 10–65 cm/s, although there seemed to be a decreased rate of proportionality in volume as compared with flow velocity greater than 65 cm/s (Fig. 5).

After completion of the study, the miniature Doppler probe was manually removed from the recipient pig's body without much difficulty.

Discussion

The implantable, miniature Doppler probe described in this report allows for intermittent recordings of hemodynamic changes in the postoperative period after pig LTX, as needed. This technique enables one to determine serial changes in the PVF with great accuracy and reproducibility. This miniature Doppler probe can be removed without the need for re-exploration after.

Although numerous authors have reported on the value of Doppler ultrasound monitoring in healthy or diseased conditions of the liver [9, 10, 22], there have been only a few reports on the evaluation of hemodynamic changes after clinical renal [5] or liver transplantation [7, 11] with the use of external duplex ultrasound or an implanted Doppler probe. Moreover, these studies have been limited to periodic measurements of blood flow, thus providing little information about hemodynamic changes during the early post-transplant period. There has been only one clinical report of serial monitoring after coronary artery bypass surgery [18]. Therefore, ours is the first experimental study on serial monitoring with an implantable, miniature Doppler probe after LTX.

The main early postoperative insult to the graft that occurs immediately after LTX is preservation-reperfusion injury [3]. The pathophysiological mechanism underlying this type of injury primarily involves endothelial cell damage to the blood vessels [3]. An activated coagulation mechanism and the liberation of vasoactive substances including reactive oxygen are factors that contribute to severely impaired microcirculation, an increased vascular resistance and resulting decrease in PVF [21].

In the present study, three animals died of primary nonfunction. The incidence may be considered high, given the fact that the maximum preservation time was 8 h. One was that mitochondrial respiratory function and adenine nucleotides during preservation decreased significantly after 6 h [14]. Another is that the pig appears to be less tolerant than other species [1], perhaps due to an interspecies difference in susceptibility to preservation damage to sinusoidal endothelial cells [15]. As all of the grafts with primary nonfunction showed a mean PVF of less than 30 cm/s on POD 1, one may conclude that adequate PVF is necessary for hepatocyte recovery from preservation-reperfusion injury. After the immediate postoperative period, when preservation-reperfusion injury is no longer a threat to the graft, ACR episodes seem to become a dominant factor contributing to the increase in PVF. The precise mechanism underlying the increased mean PVF is not well understood but may be due to a hemodynamically enhanced state resulting from a physiological response to tissue damage secondary to rejection. However, the actual cause may be obscured in the earlier postoperative period when preservation injury predominates, and this may, in turn, explain the lack of a significant difference in the mean PVF on POD 4 in this study.

Further experience with this method will expand our knowledge of portal hemodynamics during the post-LTX period, as well as in the normal physiological con-

dition. Indeed, we have seen enormous variation in the portal flow velocity pattern, even after changing the position of the animal. We have also confirmed well-known characteristic findings of portal flow as influenced by respiration [12] and other physiological conditions [13], as well as circadian change [4].

The measurement of hepatic arterial flow is certainly beneficial, not only in the detection of arterial complications such as stenosis or thrombosis but also for the understanding of hitherto unknown changes in hemodynamics after LTX. Unfortunately, we were unable to overcome the technical difficulty, and so the data were available in only a limited number of animals. Still, it is worth noting that a concomitant increase in hepatic arterial flow was also observed in the early post-transplant period. As the diameter of the artery was small (1.45–2.10 mm outer diameter), the mean arterial flow volume (approximately 30–100 ml/min) comprised a much smaller portion of the total blood flow to the liver than the portal flow volume (approximately 200–700 ml/min). Therefore, the concomitant increase in arterial blood flow may not have made a significant contribution to the entire hepatic hemodynamics. In any case, the arterial blood flow did not decrease, as might well be expected based on the finding that the portal and arterial flows did not correlate well with each other [20].

In conclusion, serial monitoring of PVF with the implantable Doppler probe enables accurate detection of flow changes and provides a reliable parameter for predicting early graft function after LTX. Further investigation should provide a better understanding of the diverse changes in hemodynamics after LTX. We consider the method herein described to be applicable in clinical LTX.

References

1. Boudjema K, Lindell SL, Southard JH, Belzer FO (1990) The effects of fasting on the quality of liver preservation by simple cold storage. Transplantation 50: 943–948
2. Cardoso JE, Gautreau C, Jeyaraj PR, Patrzalek D, Cherruau B, Vaubourdolle M, Legendre C, Wroblewski T, Houssin D (1994) Augmentation of portal blood flow improves function of human cirrhotic liver. Hepatology 19: 375–380
3. Clavien PA, Harvey RC, Strasberg SM (1992) Preservation and reperfusion injuries in liver allograft. Transplantation 53: 957–978
4. Garcia-Pagan JC, Feu F, Castells A, Luca A, Hermida RC, Rivera F, Bosch J, Rodes J (1994) Circadian variations of portal pressure and variceal hemorrhage in patients with cirrhosis. Hepatology 19: 595–601
5. Gilabert R, Campistol JM, Bru C, Vilardell J, Bianchi L, Andreu J (1991) Utility of pulsed Doppler in the diagnosis and follow-up of acute vascular graft rejection treated with OKT3 monoclonal antibody or antilymphocyte serum. Transplantation 51: 614–618
6. Grenvik A, Gordon R (1987) Postoperative care and problems in liver transplantation. Transplant Proc 19 [Suppl 3]:26–33
7. Houssin D, Fratacci M, Dupuy P, Vigouroux C, Gatecel C, Payen D, Chapuis Y (1989) One week of monitoring of portal and hepatic arterial blood flow after liver transplantation using implantable pulsed Doppler microprobes. Transplant Proc 21: 2277–2278
8. International Working Party (1995) Terminology for hepatic allograft rejection. Hepatology 22: 648–651
9. Moreno AH, Burchell AR (1982) Respiratory regulation of splanchnic and systemic venous return in normal subjects and in patients with hepatic cirrhosis. Surg Gynecol Obstet 154: 257–267
10. Moriyasu F, Ban N, Nishida O, Nakamura T, Miyake T, Uchino H, Kanematsu Y, Koizumi S (1986) Clinical application of a ultrasonic duplex system in the quantitative measurement of portal blood flow. J Clin Ultrasound 14: 579–588
11. Paulsen AW, Klintmalm GBG (1992) Direct measurement of hepatic blood flow in native and transplanted organs, with accompanying systemic hemodynamics. Hepatology 16: 100–111
12. Rabinovici N, Navot N (1980) The relationship between respiration, pressure and flow distribution in the vena cava and portal and hepatic veins. Surg Gynecol Obstet 151: 753–763
13. Sabba C, Ferraioli G, Genecin P, Colombato L, Buonamico P, Lerner E, Taylor KJW, Groszmann RJ (1991) Evaluation of postprandial hyperemia in superior mesenteric artery and portal vein in healthy and cirrhotic humans: an operator-blind echo-Doppler study. Hepatology 13: 714–718
14. Sakurada M, Ohkohchi N, Koizumi M, Fujimori K, Satomi S, Sasaki T, Taguchi Y, Mori S (1989) Mitochondrial respiratory function, adenine nucleotides and antioxygenic enzymes in pig liver transplantation. Transplant Proc 21: 1321–1322
15. Shimada M, Yanaga K, Kishikawa K, Kakizoe S, Itasaka H, Ikeda T, Suehiro T, Sugimachi K (1993) Prediction of hepatic graft viability before reperfusion: an analysis of effluent from porcine allografts. Transpl Int 6: 4–7
16. Starzl TE, Iwatsuki S, Esquivel CO, Todo S, Kam I, Lynch S, Gordon RD, Shaw BS Jr (1985) Refinements in surgical technique of liver transplantation. Semin Liver Dis 5: 349–356
17. Starzl TE, Demetris AJ, Van Thiel D (1989) Medical progress: liver transplantation. I. N Engl J Med 321: 1014–1022
18. Takayama T, Suma H, Wanibuchi Y, Tohda E, Matsunaka T, Yamashita S (1992) Physiological and pharmacological responses of arterial graft flow after coronary artery bypass grafting measured with an implantable ultrasonic Doppler miniprobe. Circulation 86 [Suppl 2]:217–223
19. Todo S, Nery J, Yanaga K, Podesta L, Gordon RD, Starzl TE (1989) Extended preservation of human liver grafts with UW solution. JAMA 262: 711–714
20. Yanaga K, Shimada M, Makowka L, Esquivel CO, Tzakis AG, Starzl TE (1989) Significance of blood flow measurement in clinical liver transplantation. Transplant Proc 21: 2330–2331
21. Yokoyama I, Kobayashi T, Negita M, Hayashi S, Yasutomi M, Katayama A, Uchida K, Takagi H (1996) Liberation of vasoactive substances and its prevention with thromboxane A_2 synthase inhibitor in pig liver transplantation.Transpl Int 9: 76–81
22. Zoli M, Marchesini G, Cordiani MR, Pisi P, Brunori A, Trono A, Pisi E (1986) Echo-Doppler measurement of splanchnic blood flow in control and cirrhotic subjects. J Clin Ultrasound 14: 429–435