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ORIGINAL ARTICLE

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The significance of tissue-type plasminogen activator for pretransplant assessment of liver graft viability: analysis of effluent from the graft in rats

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Abstract We studied the significance of tissue-type plasminogen activator (tPA) on the pretransplant assessment of liver graft viability in rats. The liver grafts were excised from the rats and then divided into two groups. Group 1 consisted of grafts preserved for 4 h in chilled, lactated Ringer's solution (4 °C) and group 2 consisted of grafts preserved for 6 h in the same solution. After preservation, the liver grafts were flushed out through the portal vein using 5 ml of chilled, lactated Ringer's solution (4 °C). The entire effluent from the hepatic veins was then collected and analyzed for tPA, ammonia, lactate, pyruvate, glutamic oxaloacetic transaminase, and lactate dehydrogenase. The tPA concentration of effluent in group 2 was significantly higher than that in group 1 (0.80 ± 0.23 ng/ml vs 0.42 ± 0.08 ng/ml, $P < 0.05$). The lactate, pyruvate, and ammonia levels in group 2 were also higher than those in group 1 (134 ± 13 mg/dl vs 120 ± 2 mg/dl, 0.34 ± 0.40 mg/dl vs 0.09 ± 0.01 mg/dl, and 183 ± 79 μg/dl vs 102 ± 40 μg/dl, respectively). However, the discriminative power of tPA was stronger than that of the other parameters. Histological findings revealed a higher number of trypan blue-stained sinusoidal lining cells that were detached and swollen in group 2. We conclude that the amount of tPA in the effluent flushed from the graft can serve as a sensitive and reliable indicator of cold-preserved liver grafts in rats.

Key words Liver graft viability, rat · Plasminogen activator, rat, liver viability

Introduction

For the pretransplant assessment of liver graft viability, we previously reported the usefulness of the ammonia and lactate levels in the effluent flushed out of the liver graft before revascularization [18, 20]. We also previously reported that a liver graft with 4-h cold ischemia using lactated Ringer's solution is viable, while the liver graft with a 6-h cold ischemia using the same solution is nonviable in rats [8].

In orthotopic liver transplantation (OLTx), tissue-type plasminogen activator (tPA), which is produced by the vascular endothelium and is a key enzyme in activating the initial step of the fibrinolytic system, is known to increase both during the anhepatic phase and immediately after revascularization. tPA has been reported to play an important role in hemorrhagic diathesis after the revascularization of the graft [5, 16, 17]. Furthermore, severe fibrinolysis after revascularization is a well-known feature of either poor function or nonfunction of the liver graft in clinical OLTx [5, 16, 17]. Therefore, it is quite possible that there is a close relationship between liver graft viability and the release of tPA from the graft.

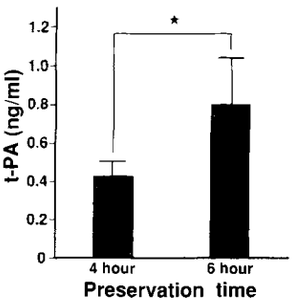
In this study, we evaluated the significance of tPA in the effluent flushed out of the liver graft on the assessment of liver graft viability prior to reperfusion in rats.

Table 1 Comparison of parameters measured in the effluent (*tPA* tissue-type plasminogen activator, *LDH* lactate dehydrogenase, *GPT* glutamic pyruvic transaminase)

Parameter	Group 1	Group 2	*P* value
tPA (ng/ml)	0.42 ± 0.08[a]	0.80 ± 0.23	0.0159
Lactate (mg/dl)	120.3 ± 1.5	134.4 ± 13.1	0.0286
Pyruvate (mg/dl)	0.09 ± 0.01	0.34 ± 0.4	0.0571
Ammonia (μg/dl)	101.9 ± 39.5	182.7 ± 79.2	0.1111
LDH (IU/l)	1431 ± 1064	4304 ± 5137	0.4206
GPT (IU/l)	167 ± 126	291 ± 225	0.7302

[a] Mean ± SD

Fig. 1 The difference in tissue-type plasminogen activator (*tPA*) in the effluent between group 1 and group 2. Data are expressed as the mean ± SD. * $P < 0.05$



Materials and methods

Inbred male Lewis rats, weighing 250–300 g and supplied by Sea Inc. (Fukuoka, Japan), were used for the experiment. A total hepatectomy was performed using a method described elsewhere [8]. Briefly, the rats were anesthetized with an intraperitoneal administration of pentobarbital sodium (50 mg/kg) and were given 500 units of heparin intravenously. The liver was perfused in situ through the distal aorta with 20 ml of chilled, lactated Ringer's solution (4 °C). The liver was then excised and placed in a bath of lactated Ringer's solution (4 °C).

The liver grafts were divided into two groups. Group 1 consisted of grafts with 4 h of cold ischemia in chilled, lactated Ringer's solution (4 °C), while grafts in group 2 had 6 h of cold ischemia in the same solution.

After preservation, the liver graft was flushed out through an indwelling catheter in the portal vein with 5 ml of chilled, lactated Ringer's solution (4 °C) in the same way that had been used to flush the graft before implantation in the rat OLTx model. Then, the entire effluent from the hepatic veins was carefully collected in a sterile glass cup through the suprahepatic vena cava to analyze the levels of tPA, ammonia, lactate, pyruvate, glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH).

tPA was determined using an IMULYSE tPA test kit (Biopool, Nmea, Sweden). The other parameters were measured using the same method described elsewhere [20].

Histological assessment

The liver grafts were perfused for 2 h with an isolated perfusion pump with a warm Krebs-Henseleit buffer solution (37 °C) and then with a Krebs-Henseleit solution (37 °C) containing 200 μM of trypan blue, followed by a 5-min infusion of 2 % glutaraldehyde in the perfusate for fixation. Subsequently, the tissue was stained with eosin in order to identify the nuclei of nonviable cells stained with trypan blue.

Statistical analysis

Data are expressed as the mean ± SD. Wilcoxon's rank sum test was applied for comparisons between the two groups.

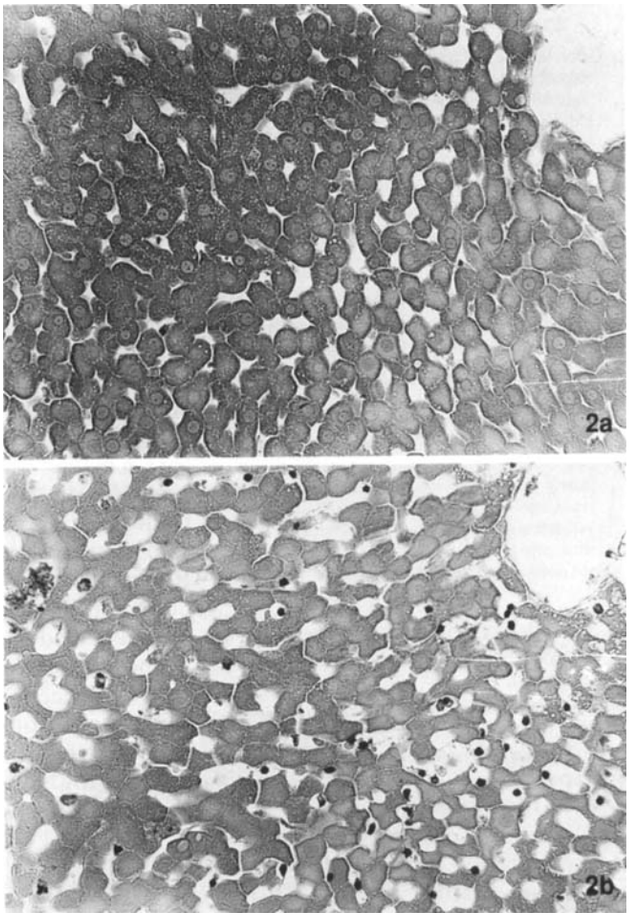


Fig. 2a, b Photomicroscopic views of liver grafts cold preserved for: **a** 4 h and **b** 6 h (eosin × 220). In the former, there are few trypan blue-stained cells in the sinusoids, whereas in the latter many trypan blue-stained cells are seen in the sinusoids, which are swollen and detached from the hepatocytes

Results

Table 1 shows a comparison of the parameters measured in the effluent. The tPA concentration in group 2 was significantly higher than that in group 1 ($P = 0.0159$), as was the lactate value ($P = 0.0286$; Fig. 1). The pyruvate and ammonia levels in group 2 were also higher than those in group 1.

Figure 2 shows the histological findings from the liver grafts in groups 1 and 2. More sinusoidal lining cells were stained by trypan blue in group 2 than in group 1. Moreover, the sinusoidal lining cells in group 2 were swollen and detached from Disse's spaces while those in group 1 were almost normal. The parenchymal

cells, on the other hand, exhibited only minimal changes, such as microvesicular steatosis, in both groups.

Discussion

Although the experimental model used in this study may have some disadvantages that call into question its clinical relevance (e.g., the cold ischemia time, type of preservation solution), the pathogenesis of the preservation and reperfusion injuries of the liver graft has previously been confirmed in this experimental model [8, 18, 20]. We have reported that there is a threshold of cold ischemia time in rat liver preservation for which endothelial cell damage seems to be most important [8]. Furthermore, others have reported that sinusoidal endothelial cell injury is a key mechanism for cold ischemia and the loss of viability of the liver graft [2, 9, 10, 12]. McKeown et al. [12] stated that prolonged cold ischemia caused endothelial swelling and detachment from the hepatocytes, which had no remarkable changes, and that damage to the sinusoidal lining cells resulted in a nonviable graft. Caldwell-Kenkel et al. [2] reported that after prolonged cold ischemia, all parenchymal cells were viable, while approximately 40 % of the nonparenchymal cells were judged to be nonviable by the trypan blue dye exclusion technique.

In clinical OLTx, fibrinolysis following revascularization of the liver graft is not uncommon, and an association between the degree of fibrinolysis and graft viability has been advocated [5, 15, 16]. Moreover, severe fibrinolysis after revascularization of the graft is also known to be the first sign of primary nonfunction of the graft.

Porte et al. [17] reported a gradual increase in tPA activity during the anhepatic period, which was followed by an explosive increase in tPA in association with a reduction in plasminogen activator inhibitor-1 immediately after revascularization of the liver graft. Such hyperfibrinolysis is considered to be a primary fibrinolysis and can be reversed by the administration of aprotinin, which is not an anticoagulant but a potent antifibrinolytic agent [6, 7, 13]. Furthermore, such an explosive increase in tPA immediately after revascularization is considered to be caused by the increased release to tPA from the liver graft produced during preservation. Therefore, it is theoretically possible that severe fibrinolysis in OLTx is caused by tPA that is produced during cold preservation of liver grafts of poor quality and that it is then suddenly released into the systemic circulation upon revascularization.

tPA is a glycoprotein with a molecular weight of approximately 70000 that has a half-life of 3–5 min in the blood [1, 14]. This enzyme is synthesized by the vascular endothelium [11] and is released in response to a number of stimuli including stress, exercise, venous occlusion, and the administration of various vasoactive agents [3, 4, 21, 22].

The present study suggests that the amount of tPA in the effluent is an indicator of liver graft viability and that it may be more sensitive than other parameters we have demonstrated in the past, such as ammonia, lactate, and pyruvate [18, 20]. Since tPA is principally released by the vascular endothelium and increases under critical conditions, the tPA concentration of the graft effluent could reflect the degree of endothelial cell damage of the liver. Therefore, in our effluent analysis, we believe that the ammonia, lactate, and pyruvate levels reflect parenchymal cell damage, while the tPA concentration in the effluent could represent endothelial cell damage, which has previously been shown to correlate with liver graft viability [2, 8, 9, 11, 12]. This assumption also seems to be reinforced by the histological findings in our study that show that trypan blue-positive sinusoidal lining cells were more prominent in group 2 than in group 1. However, in order to confirm our hypothesis, further studies, including clinical trials of tPA, which has been shown to be an indicator of graft viability, would be required.

With regard to the pretransplant assessment of the liver graft, the time of sampling in this experiment might be too late to evaluate the graft before transplantation. However, assessment of the liver graft prior to revascularization is, in practice, so difficult that only a few parameters such as the level of lecithin:cholesterol acyltransferase [19] and the monoethyl glycinexylidine formation after an intravenous injection of 1 mg/kg lidocaine [15] in the donors have been reported as possible predictors of liver graft viability. Moreover, the concept of the present study would be applied to the assessment during preservation if the effluent was sampled from the hepatic veins when the graft was rinsed during preservation.

In conclusion, tPA may indicate damage to endothelial cells, which are known to be the most sensitive to preservation injury and which play a critical role in reperfusion after preservation, while most other well-known parameters reflect parenchymal cell damage. Therefore, the amount of tPA in the effluent flushed out of the cold-preserved liver graft seems to be a sensitive and reliable indicator of liver graft viability.

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