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Usefulness of ^{31}P-MRS as a method of evaluating the viability of preserved and transplanted rat liver

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Abstract. We used phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) to evaluate the viability of transplanted rat liver. Wistar rats were used as donors and recipients. The donor livers were preserved in saline (group 1), Euro-Collins solution (group 2), or in University of Wisconsin (UW) solution (group 3) for 3 and 6 h in groups 1, 2 and 3 and for 9 h in groups 2 and 3. Thereafter the livers were orthotopically transplanted. ^{31}P-MRS spectra were measured after portal reperfusion. Finally, all the recipients were divided into survivors and non-survivors. Survival rates were better in group 3 than in groups 1 and 2. In the 9-h-preserved livers, the livers in group 3 showed a significantly higher β-ATP/Pi ratio than those in group 2. Comparing survivors and non-survivors in the 6-h-preserved livers in group 2, survivors' livers showed significantly higher β-ATP/Pi ratio than those of non-survivors. We concluded that ^{31}P-MRS is a useful method for assessing viability of rat liver grafts.

Key words: ^{31}P-MRS – β-ATP/Pi ratio – Liver transplantation – Graft viability

Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-MRS) is a noninvasive technique for monitoring the energy metabolism of tissues and organs at the cellular level. With this method, we evaluated the viability of preserved and transplanted rat liver and studied the relationship between ^{31}P-MRS and prognosis.

Materials and methods

A total of 72 male Wistar rats, weighing 250–400 g were used as donors and recipients. All animals were treated according to the ethical rule for experimental animals of Tokyo Women's Medical College, and all operations were performed under ether anaesthesia.

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Prior to excision, the donor livers were perfused with saline via the portal vein. Then the livers were excised and perfused with each preservation solution. The livers were preserved at 4°C in saline (group 1), in Euro-Collins solution (EC) (group 2), or in University of Wisconsin (UW) solution (group 3) for 3 and 6 h in groups 1, 2 and 3 and for 9 h in groups 2 and 3. In group 1, the 6-hour-preserved livers showed such a low survival rate that 9-hour-preservation was not performed. After cold storage, the livers were orthotopically transplanted using the Kamada method. ^{31}P-MRS spectra were measured before the donor hepatectomy (control), and every 10 min from 15 to 105 min after portal reperfusion. Finally, all the recipients were divided into survivors (recipients surviving more than 48 h after transplantation) and non-survivors.

^{31}P-MRS spectra were recorded using a 2.1 Tesla bore superconducting magnet interfaced with a spectrometer (BEM 250/80, Otsuka Electronics, Osaka, Japan) operating at 34.45 MHz for phosphorus and 85.12 MHz for protones. A surface coil was placed on the surface of the liver graft. ^{31}P-MRS spectra were attained using a radiofrequency of 34 μsec wave length applied every 2 s. For each study, data from 300 scans were accumulated and summed to produce each spectrum. β-ATP/Pi ratios were calculated from the peak areas. The data were statistically analyzed with two sample *t*-test.

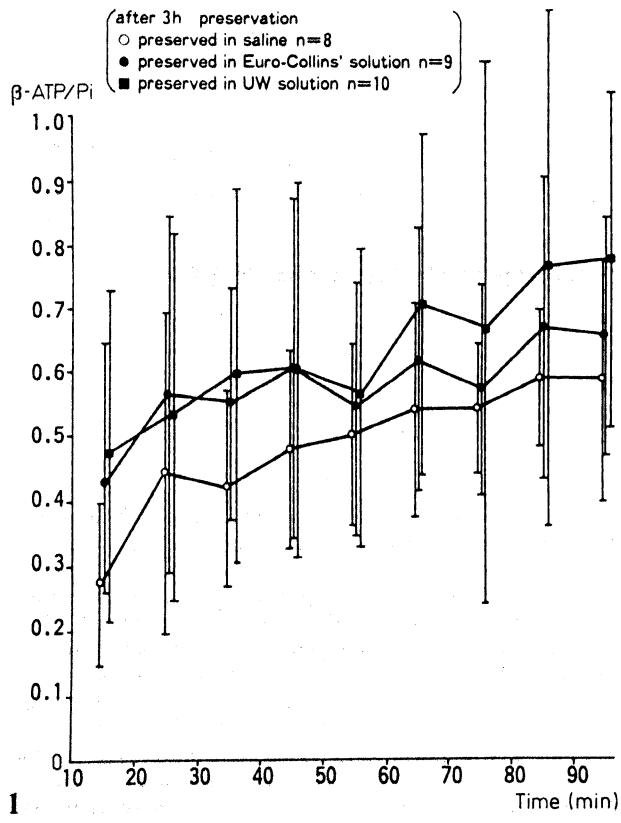
Results

The mean β-ATP/Pi ratio in the controls was 1.18 ± 0.16 (mean ± SD). Table 1 shows the number of survivors and

Table 1. The number of survivors and non-survivors in each group

	Preservation time	Survivors	Non-survivors	Total	Survival rate
Group 1					
Saline	3 h	7	1	8	0.88
Saline	6 h	4	5	9	0.44
Group 2					
EC	3 h	8	1	9	0.89
EC	6 h	7	3	10	0.70
EC	9 h	6	3	9	0.67
Group 3					
UW	3 h	8	2	10	0.80
UW	6 h	6	2	8	0.75
UW	9 h	8	1	9	0.89

Change in β-ATP/Pi ratio after portal reperfusion
(mean±SD)



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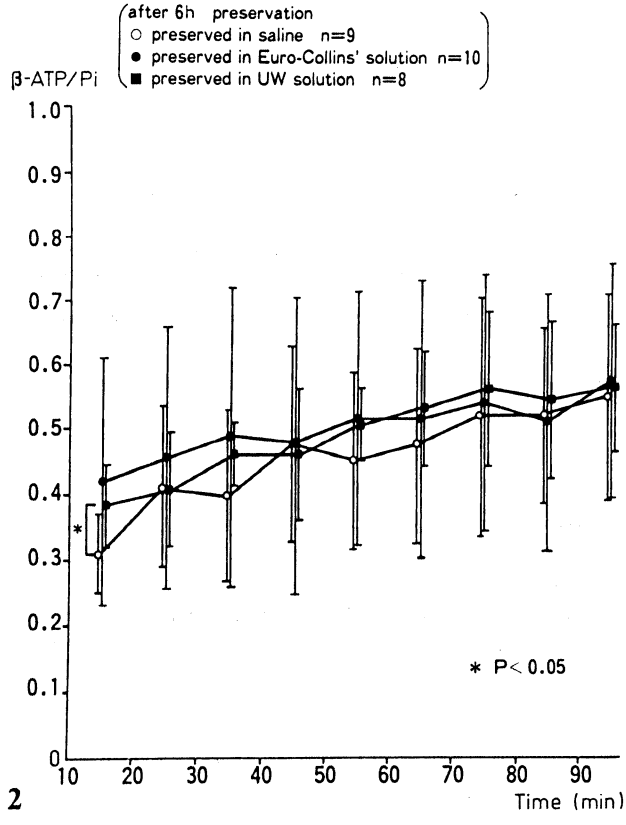
Change in β-ATP/Pi ratio after portal reperfusion
(mean±SD)



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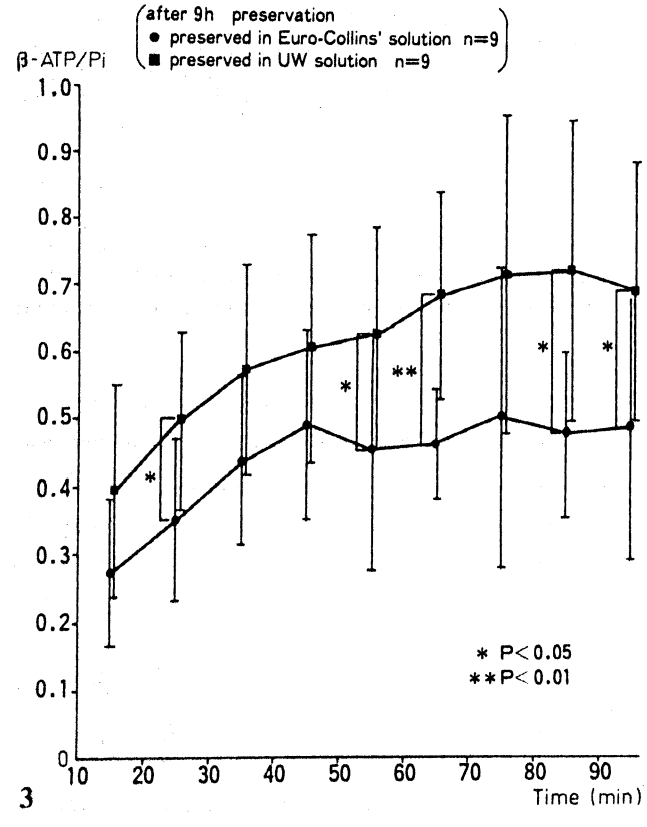
Change in β-ATP/Pi ratio after portal reperfusion
(mean±SD)



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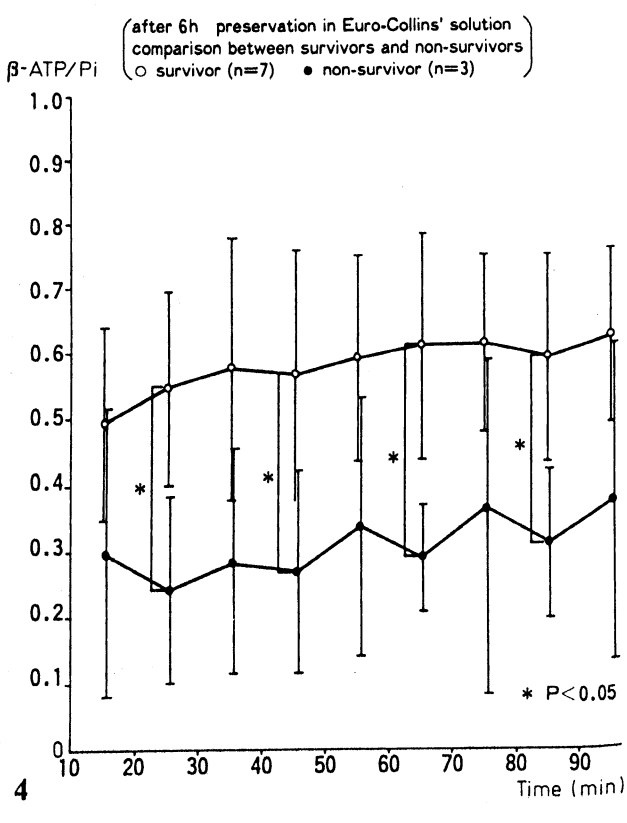
Change in β-ATP/Pi ratio after portal reperfusion
(mean±SD)



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non-survivors in each group. In the saline-preserved group (group 1), the survival rate decreased to 44% in the 6-h-preserved group. In the EC-preserved group (group 2), the survival rate decreased to 67% in the 9-h-preserved group. However, in the UW-preserved group (group 3), recipients showed high survival rates until 9 h of preservation.

After 3 h preservation, no significant difference in β-ATP/Pi ratio was observed between the three groups (Fig. 1), while after 6 h preservation, a significant difference was observed only at 15 to 25 min after portal reperfusion (Fig. 2). After 9 h preservation, liver grafts in group 3 showed significantly ($P < 0.05$) higher β-ATP/Pi ratios than those in group 2 at 25, 55, 65, 85, and 95 min after portal reperfusion (Fig. 3).

Comparing survivors and non-survivors, after 6 h preservation in EC (group 2), a significant difference was observed in the β-ATP/Pi ratio (Fig. 4). In this case, survivors' liver grafts showed significantly higher β-ATP/Pi ratios at 25, 45, 65, and 85 min after the portal reperfusion.

Discussion

Liver transplantations are increasing in number and have been established as the treatment for end-stage liver disease. Although many studies evaluating graft viability have been reported, the results remain controversial. Even now, primary nonfunction occurs in about 5–10% of liver transplants, reflecting a poor prognosis and the need for retransplantation. It has been suggested that graft viability is dependent upon its capacity to regenerate ATP, the energy source indispensable for protein synthesis, restoration of ion gradients, bile production, other factors in metabolism [1, 2].

The measurement of hepatic phosphorus metabolites and inorganic phosphate by ^{31}P-MRS has been performed widely using perfusion models, and it has been proven to be an excellent, objective, noninvasive method for assessing the energy status of liver grafts. In this study, we used ^{31}P-MRS to evaluate the viability of preserved and transplanted rat liver using the β-ATP/Pi ratio which is regarded as reflecting energy availability. The survival rate in group 2 was higher than that in group 1, and it was highest in group 3. Although no significant difference existed in the grafts transplanted after 3 h preservation, the β-ATP/Pi ratios after transplantation in group 2 and 3 were higher than those in group 1 but not significantly so. After 9 h preservation, β-ATP/Pi ratios after transplantation in group 3 were higher than those in group 2 after portal reperfusion. Thus, after 9 h preservation, UW-preserved livers had more satisfactory results compared with those of EC-preserved livers. In the 6-hour-EC-preserved group, survivors' livers showed significantly higher β-ATP/Pi ratios after portal reperfusion compared with those of non-survivors. Therefore, we concluded that the capacity of ATP regeneration at an early stage, namely, 15–105 minutes after portal reperfusion is important in predicting the subsequent recovery of graft function.

We concluded that ^{31}P-MRS could be used in evaluating graft viability and in predicting graft nonfunction at an early stage.

Fig. 1. Change in β-ATP/Pi ratio after portal reperfusion after 3 h preservation

Fig. 2. Change in β-ATP/Pi ratio after portal reperfusion after 6 h preservation

Fig. 3. Change in β-ATP/Pi ratio after portal reperfusion after 9 h preservation

Fig. 4. Change in β-ATP/Pi ratio after portal reperfusion after 6 h preservation in EC-solution

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