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Improved liver function following infusion of fructose-1,6-bisphosphate in posthepatectomy patients

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Abstract

The clinical effect of fructose-1,6-bisphosphate (FBP) administered to posthepatectomy patients was examined.　FBP at 0.25 mmol/kg was administered continuously into the hepatic artery for 60 minutes on the 1st postoperative day in 11 cases.　Hepatic arterial infusion of 0.25 mmol/kg glucose was performed in 7 cases.　Furthermore, in 10 cases in which a catheter was not inserted in to the hepatic artery, 0.25 mmol/kg FBP was administered intravenously over a 60-minute period.　Arterial ketone body ratio (AKBR) and serum levels of cyclic adenosine monophosphate, immunoreactive insulin, inorganic phosphorus, glucose, fructose, pyruvate, lactate and pyruvate kinase (PK) in the arterial blood were measured before and after administration.

AKBR hardly changed after hepatic arterial infusion of glucose.　It rose until 3 hours after intravenous or intrahepatic arterial administration of FBP.　Especially, after hepatic arterial infusion of FBP, the AKBR was significantly higher up to 2 hours after administration than that before administration ($P < 0.01$).　With hepatic arterial infusion of FBP, serum pyruvate transiently increased immediately after infusion ($P < 0.01$).　PK activity was significantly elevated after administration of FBP ($P < 0.05$).　Serum lactate levels decreased significantly after hepatic arterial infusion of FBP ($P < 0.05$).　There was no difference in the recovery of protein synthetic ability and the postoperative changes in serum liver function test values among the three groups.

Hepatic arterial infusion of FBP was suggested to promote adenosine triphosphate production by acceleration of the glycolytic pathway and lactate uptake in the hepatic cell.

Introduction

Recently, it has become possible to accurately determine hepatic reserve ability in cases of hepatoma with cirrhosis.　Hepatic surgery has made remarkable progress, enabling extended hepatic re-

section for malignant liver tumor. However, since various complications result in postoperative liver failure in a number of cases, some measure to counter this problem is necessary.

Fructose-1,6-bisphosphate (FBP) acts as a metabolic regulator by stimulating glycolysis and other metabolic processes[1-5]. Experimentally, FBP has an obvious protective effect on hepatic cells and promotes liver regeneration after hepatic resection[6,7]. However, there is no report of clinical administration of FBP after hepatic resection. It has also not been clarified whether FBP has an influence on energy metabolism after hepatic resection. In this study, we examined whether FBP has an influence on postoperative liver function and energy metabolism.

Materials and Methods

Twenty-eight cases of hepatectomy for malignant liver tumors, in which informed consent was obtained in accordance with the Helsinki Declaration, were enrolled in the study. The catheters were retained in 18 cases for the purpose of implementing postoperative anti-cancer chemotherapy. Fructose-1,6-bisphosphate (FBP—Esafosfina®, Biomedica Foscama, Italy) was continuously infused into the hepatic artery at a dose of 0.25 mmol/kg over a 60-minute period on the 1st and 2nd postoperative days in 11 cases as the hepatic arterial infusion group (HAI group). Glucose (0.25 mmol/kg/60 min) was administered into the hepatic artery in 7 cases as the control group. Furthermore, in the intravenous administration group (IV group), 10 cases in which a catheter was not inserted in the hepatic artery were infused 0.25 mmol/kg FBP from a peripheral vein over a 60-minute period on the 1st and 2nd postoperative days.

I. Glucose and energy metabolism dynamics before and after administration of FBP

On the 1st postoperative day, arterial blood was collected immediately before, immediately after, and 1, 2 and 3 hours after administration. The arterial ketone body ratio (AKBR) and serum cyclic adenosine monophosphate (cAMP) were measured as indices of energy metabolism. AKBR was determined as the ratio of acetoacetic acid divided by 3-hydroxybutyric acid determined using the enzyme method. Immunoreactive insulin (IRI), glucose, pyruvate, lactate and pyruvate kinase (PK) in arterial blood were measured as indices of glucose metabolism. IRI and cAMP were determined by radioimmunoassay, the glucose level by the o-toluidine method, pyruvate and lactate by the enzyme method, and PK using a spectrophotometer. Inorganic phosphorus (Pi) and fructose in serum were measured in FBP administration cases by spectrophotometry.

II. Changes in postoperative liver functions and protein synthetic ability

Serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) were measured preoperatively and for 7 days postoperatively. GOT GPT and LDH were determined spectrophotometrically.

On the preoperative and 7th postoperative day, measurement of transferrin (TF), prealbumin (PA) and retinol-binding protein (RBP) as rapid-turnover proteins, and prothrombin time (PT) and hepaplastin test (HPT—total activity of coagulation factor II, VII and X) as indices of protein synthetic ability were performed. TF was determined by nephelometry. PA and RBP were measured by single radial immunodiffusion method[8]. PT and HPT were determined by an autoanalyzer. The recovery rate on the 7th postoperative day was expressed as the percentage of the preoperative value.

Statistical differences between the values of glucose and energy metabolism dynamics before and after administration were analyzed by paired Student's t-test. The significance of differences in results of postoperative liver function tests and protein synthetic ability among the three groups was de-

termined by analysis of variance with multicomparison. All values in the text are expressed as mean $\pm$ SE. Significance was defined as $p < 0.05$.

Results

Table 1 shows the patient background characteristics. There was no difference among the three groups in age, ICG K (disappearance rate: $K = 0.693/T_{1/2}$) value and AKBR. The incidence of liver cirrhosis was high at over 70%, and there was no difference among the three groups.

I. Glucose and energy metabolism dynamics before and after administration of FBP

Table 1 Patient group profiles

	Control (n=7)	HAI (n=11)	IV (n=10)
Age (year)	60±5	67 ±3	57±5
ICG K value	0.136±0.015	0.142 ±0.009	0.137±0.013
AKBR	1.72±0.28	1.62 ±0.16	1.77±0.35
Liver cirrhosis	5 cases	10 cases	7 cases

Values are means ± SE.

ICG K, indocyanine green disappearance rate;

AKBR, arterial ketone body ratio.

Table 2 Changes of the Indices in Energy and Carbohydrate Metabolism

		Before	Immediately	1h	2h	3h
Glucose (mg/dl)	Control	174 ±14	188 ±13 **	177 ±13	175 ±14	173 ±13
	HAI	167 ±17	177 ±17	169 ±16	168 ±17	169 ±17
	IV	170 ±19	176 ±21	173 ±20	179 ±22	174 ±24
IRI (μU/ml)	Control	32 ±8	35 ±9	38 ±9	39 ±7	39 ±9
	HAI	35 ±12	37 ±12	42 ±15	42 ±15	43 ±16
	IV	24 ±4	40 ±9	47 ±13	41 ±11	43 ±16
cAMP (pmol/ml)	Control	19 ±4	18 ±3	15 ±2	15 ±2	16 ±2
	HAI	17 ±2	17 ±2	17 ±2	17 ±2	18 ±2
	IV	14 ±3	13 ±2	14 ±3	14 ±2	14 ±2
Pyruvate (μmol/l)	Control	156 ± 27	172 ±36	159 ±31	153 ±26	150 ±26
	HAI	172 ±24	193 ±26 **	171 ± 26	170 ±28	161 ±21
	IV	117 ±12	135 ±13	115 ±12	127 ±16	120 ±13
PK (U/10^{10} RBC)	Control	5.6 ±0.3	6.2 ±0.5	5.4 ± 0.3	6.1 ±0.4	5.9 ±0.4
	HAI	6.0 ±0.5	5.8 ±0.6	6.1 ±0.5	6.5 ±0.5 *	6.2 ±0.5
	IV	6.5 ±0.3	6.4 ±0.3	6.9 ±0.4	6.6 ±0.3	7.0 ±0.5 *
Lactate (mmol/l)	Control	2.0 ±0.3	2.1 ±0.4	2.0 ±0.3	1.9 ±0.3	1.8 ±0.2
	HAI	2.8 ±0.5	2.8 ±0.5	2.4 ±0.4 *	2.3 ±0.3 *	2.1 ±0.3 *
	IV	1.6 ±0.1	1.8 ±0.1	1.6 ± 0.2	1.5 ±0.1	1.5 ±0.2

Values are means ± SE. * P<0.05 vs. Before; ** P<0.01 vs. Before.

IRI, immunoreactive insulin; cAMP, cyclic adenosine monophosphate; PK, pyruvate kinase.

Control, group receiving hepatic arterial infusion of glucose; HAI, group receiving hepatic arterial infusion of fructose-1,6-bisphosphate; IV, group receiving intravenous administration of fructose-1,6-bisphosphate.

There was no change of AKBR following a glucose load of 0.25 mmol/kg/60 min. AKBR rose until 3 hours after intravenous or hepatic arterial infusion of FBP. Especially, with hepatic arterial infusion of FBP, AKBR was 1.33 ± 0.17, 1.55 ± 0.16, 1.68 ± 0.18, and 1.71 ± 0.26, before, immediately after, one hour after, and 2 hours after administration, respectively. All values were significantly higher after administration than before administration ($P<0.01$). AKBR was 1.65 ± 0.28 at 3 hours after administration, showing a higher value than before administration (Fig. 1).

On the morning after hepatectomy, three cases had an AKBR less than 1.0, and the AKBR rose after hepatic arterial infusion of FBP (Fig. 2). In one case, AKBR was 0.68 before FBP administration, rose to 1.58 after one hour, and was maintained above 1.0 until 3 hours. In another case, AKBR was markedly decreased before FBP administration at 0.47, rose to 0.8 immediately after FBP administration, and remained at about 0.7 after administration.

The blood glucose value transiently rose immediately after glucose administration in the control group ($P<0.01$). It did not show a significant change in the other groups. IRI and cAMP hardly changed after administration in the three groups (Table 2). The serum pyruvate level transiently rose immediately after hepatic arterial infusion of FBP ($P<0.01$). PK activity was significantly increased 2 hours after hepatic arterial infusion and 3 hours after intravenous administration of FBP ($P<0.05$). For the serum lactate level, the value before administration was high in the FBP hepatic arterial infusion group. It was significantly decreased for one to three hours after hepatic arterial administration of FBP ($P<0.05$). However, these three parameters did not change after glucose ad-
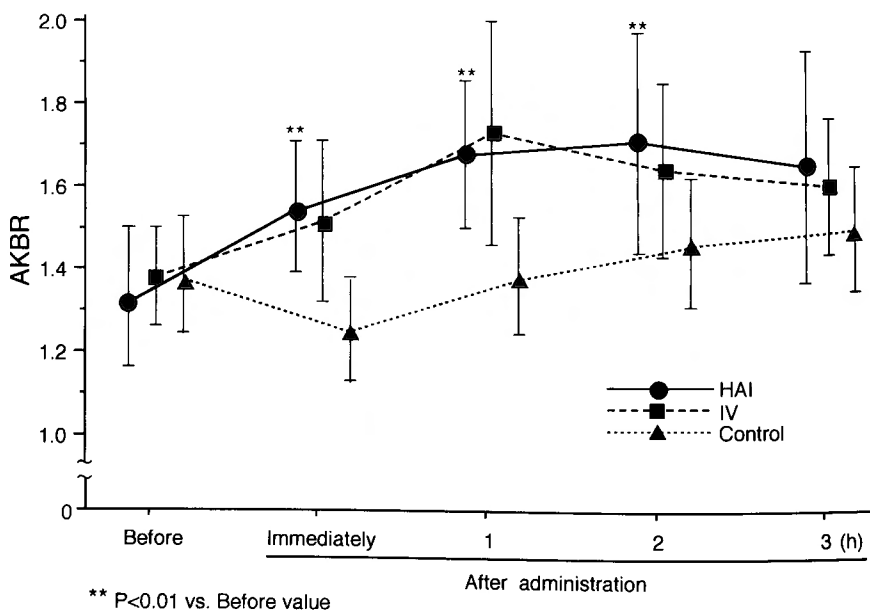

** P<0.01 vs. Before value

Fig. 1　Arterial ketone body ratio (AKBR) on the 1st postoperative day after hepatic arterial infusion of fructose-1,6-bisphosphate (HAI—solid dots), intravenous administration of fructose-1,6-bisphosphate (IV—closed squares) and hepatic arterial administration of glucose (Control—closed triangles). Values are expressed as mean±SE. There is little change of AKBR after glucose load of 0.25 mmol/kg. AKBR rose until 3 hours after administration of fructose-1,6-bisphosphate. Especially, after hepatic arterial infusion of fructose-1,6-bisphosphate, AKBR was significantly increased up to 2 hours after administration compared to that before administration ($P<0.01$).
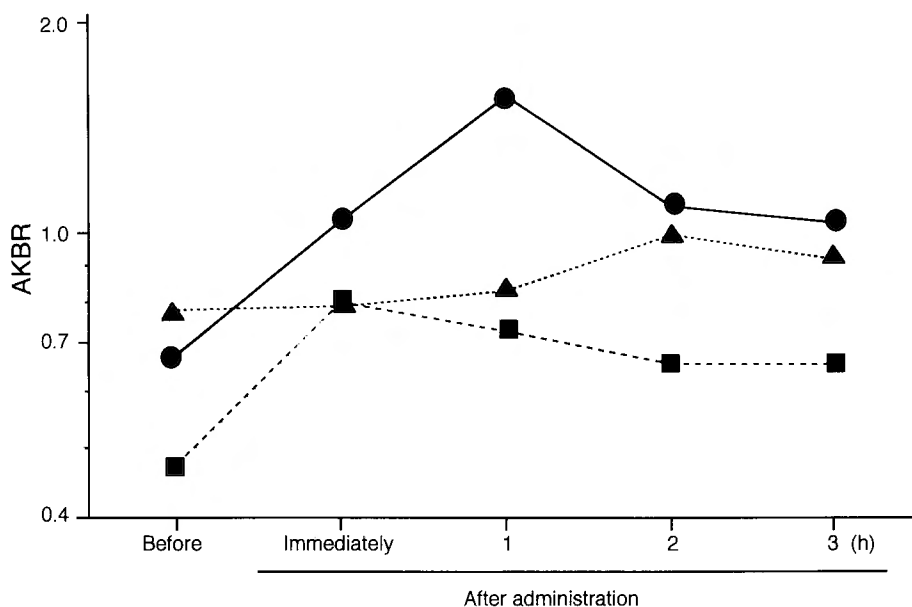
Fig. 2   Three cases in hepatic arterial infusion group with an arterial ketone body ratio (AKBR) of less than 1.0.   In all cases, AKBR rose after hepatic arterial infusion of fructose-1,6-bisphosphate.
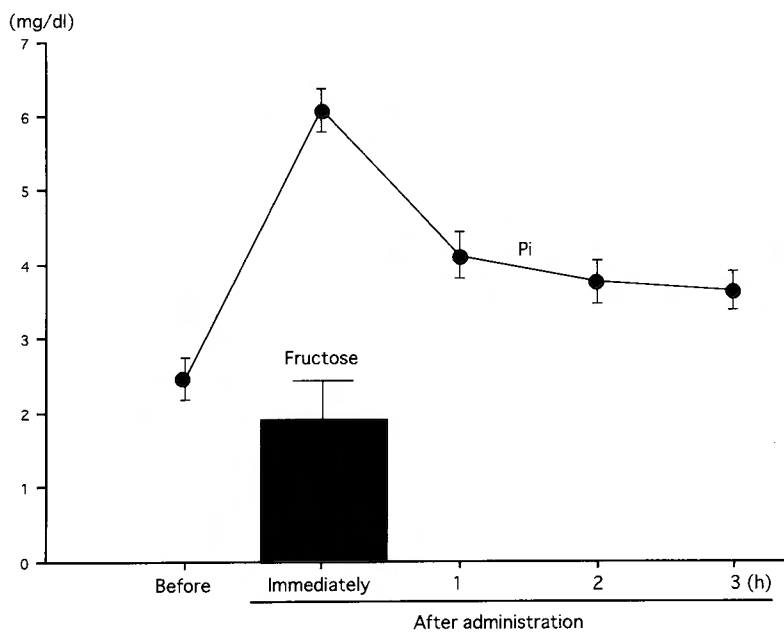

Fig. 3   Inorganic phosphorus (Pi) and fructose after FBP hepatic arterial infusion.   Values are expressed as mean $\pm$ SE.   Pi showed a peak immediately after administration, and returned to within normal limits after one hour.   It was also significantly higher 3 hours after administration than before administration (P $<$ 0.01).   Fructose was detected only immediately after FBP administration, and was already below the detection limit after one hour.

ministration (Table 2).

In the changes of Pi and fructose levels after FBP hepatic arterial infusion, Pi showed a peak immediately after administration (Fig. 3). It returned to within the normal range $(4.1 \pm 0.3 \mathrm{mg/dl})$ after one hour. It was also significantly higher 3 hours after administration than before administration $(P < 0.01)$. Fructose was detected $(1.9 \pm 0.5 \mathrm{mg/dl})$ only immediately after FBP administration, and fell to below the detection limit one hour after administration (Fig. 3).
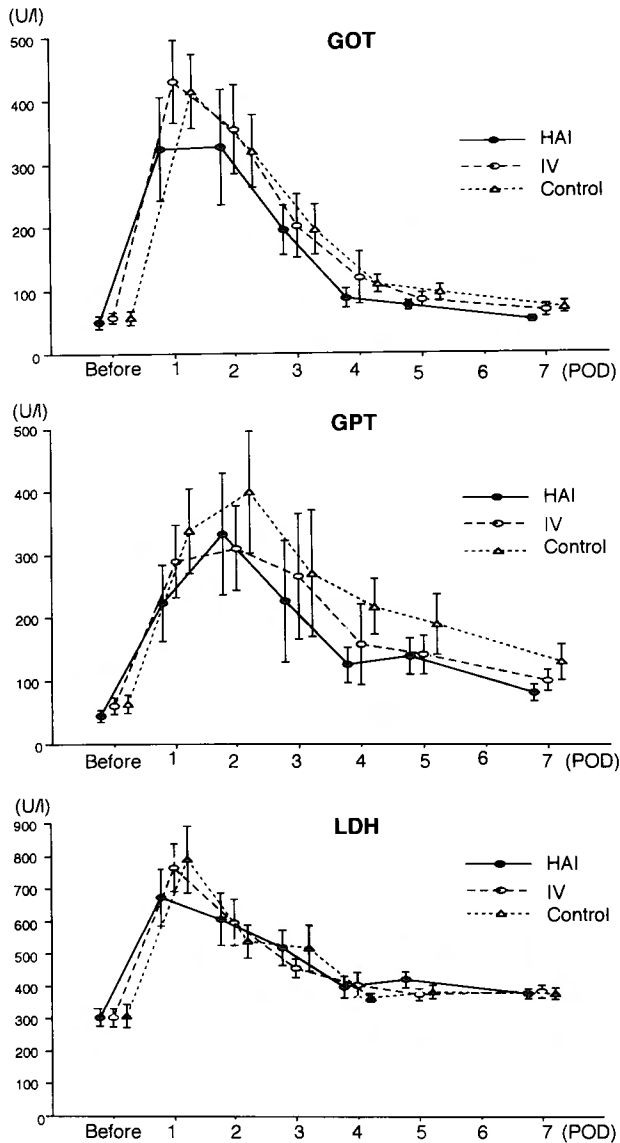
Fig. 4 Levels of serum biochemical indices reflecting liver function in hepatic arterial infusion of FBP (HAI—solid dots), intravenous administration of FBP (IV—open circles) and hepatic arterial administration of glucose (Control—open triangles). Postoperative serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) levels were not different among the three groups.
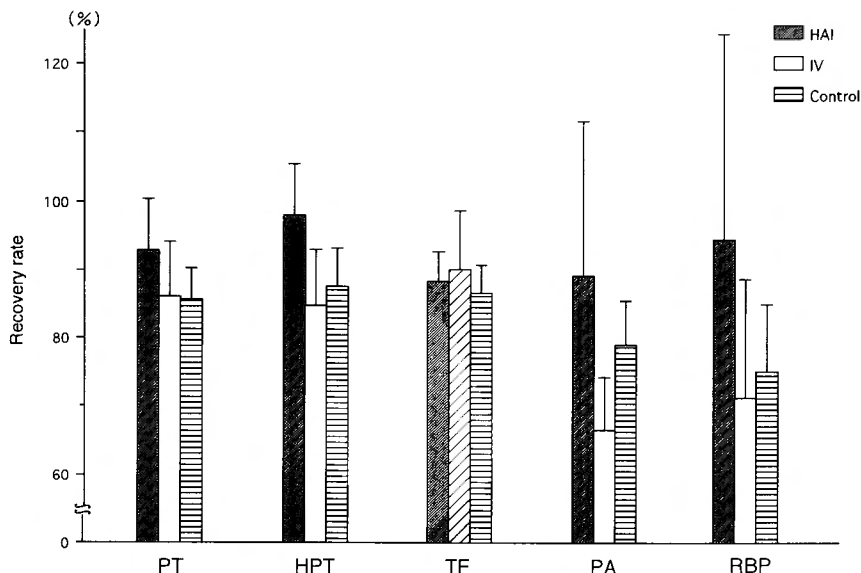
Fig. 5 Recovery rate of protein synthetic ability on the 7th postoperative day (percent of preoperative value). Values are expressed as mean ± SE. Values in FBP hepatic arterial infusion group were greater than those in the control group for all indices. PT, prothrombin time; HPT, hepaplastin test; TF, transferrin; PA, prealbumin; RBP, retinol-binding protein.

II. Changes in results of postoperative liver function tests and protein synthetic ability

The postoperative changes in serum GOT, GPT and LDH levels showed no difference among the three groups (Fig. 4).

The recovery rate of protein synthetic ability on the 7th postoperative day with respect to PT, HPT, TF, PA and RBP was greater in the FBP hepatic arterial infusion group (93%, 98%, 88%, 89%, 95%) than in the control group (86%, 87%, 86%, 79%, 75%); however, the differences were not significant (Fig. 5).

Discussion

As one of the complications after hepatectomy, postoperative hepatic failure can occur due to liver cirrhosis which almost always coexists with hepatocellular carcinoma, massive intraoperative hemorrhage, vascular procedures used to control bleeding during hepatectomy, combined removal of the portal vein and decreased hepatic blood flow due to liver mobilization. Therefore, it is important to reduce liver injury during operation and to maintain hepatic cellular viability in order to prevent postoperative hepatic failure.

FBP is a substrate of the glycolytic pathway, whose effects include activation of glycolysis[1,4], inhibition of glyconeogenesis[4], stimulation of phospholipid synthesis[4], activation of the reticuloendothelial system[1], and inhibition of the production of free radicals[1].

In experimental study, the significant effect of FBP was observed by virtue of administration of 0.25 mmol/kg or more[6]. The inhibition of superoxide production in incubation of human nuetrophils with FBP was dose-dependent over the range 0.2 mM–5 Mm[9]. It is well established that perfusate containing 1 mM–10 mM FBP allows the maintenance of hepatic cellular viability

dose-dependently[10]. The recommended doses for single intravenous infusion of FBP are 0.15 to 0.4 mmol/kg[1]. Therefore, we applied the administration of 0.25 mmol/kg FBP as minimal effective dose in this clinical study.

The half-life of FBP in serum is short at 10-15 minutes[11]. This, however, does not indicate degradation of FBP in the circulating blood, but represents the elimination rate from the blood, that is, transfer from blood to tissues[1]. Urinary excretion is low at 3-4%[1]. Thus, minimal FBP is degraded in the blood; rather, it is concentrated in individual organs[11], where it is degraded gradually by the action of phosphatase on the cell membrane[11]. Therefore, bolus administration of FBP has a relatively longer action and is expected to exert a greater effect[12]. In this study, only about 4% of fructose, the skeleton of the degraded FBP, was detected in the blood immediately after administration of FBP. Inorganic phosphorus showed a peak immediately after administration of FBP with an increased value until 3 hours after administration compared to before administration, indicating that FBP was metabolized slowly.

FBP has been reported to completely protect the liver against galactosamine hepatotoxicity[13] and carbon tetrachloride-induced liver damage[14], and also to prevent hepatic ATP and creatine phosphate depletion following colloidal carbon injection in rats[15]. In addition, the perfusate containing FBP has also been shown to be capable of maintaining hepatic cellular viability in cold liver perfusion [10][16]. It is well established experimentally that FBP has a protective effect on the hepatic cell and promotes liver regeneration for 24 hours after hepatectomy[6,7]. However, the influences that FBP exerts on energy metabolism in patients with hepatectomy have not been examined clinically.

AKBR reflects the function of mitochondria in the liver, and is an important index of hepatic energy metabolism[17-19]. AKBR was increased by hepatic arterial infusion of FBP, but not by hepatic arterial infusion of glucose. Especially, in cases of poor AKBR, hepatic arterial infusion of FBP had a significant effect. This means that FBP promotes adenosine triphosphate production in the condition of energy crisis in the liver soon after hepatectomy. This action was observed immediately after administration, and was maintained until 3 hours after administration. However, it is expected that the effect of FBP continues more than 3 hours following administration as stated above.

The mechanism, involved in this phenomenon was considered to be activation of the glycolytic pathway, because of the sharp decrease following the transient rise in serum pyruvate, and the rise of PK activity. The decrease of serum lactate suggested that lactate uptake by the liver increased. Consequently, FBP is presumed to maintain cell viability due to ATP production in hepatic cell. After hepatic arterial infusion of FBP, blood glucose levels did not show significant fluctuation. Therefore, it is unlikely that fructose, the skeleton of the degraded FBP, was transformed to glucose and was simply supplied as an energy source.

FBP is generally believed to exert its effects through interaction with the cell membrane, modifying the ion permeability[3,12,20], and resulting in the maintenance of cell viability. Glycophosphate ester generally is not considered to pass through the cell membrane[21]. In the past several years, there have been reports that radiolabeled FBP crosses the cell membrane as such and can be utilized not only as a metabolic regulator but also as a substrate[22]. In addition as the membrane permeability of the liver cell changes in posthepatectomy patients and liver injury, FBP may enter the liver cell, resulting in activation of the glycolytic pathway.

Nevertheless, beneficial effects on postoperative liver function and protein synthetic ability were not observed after administration of FBP 0.25 mmol/kg/day on the 1st and 2nd postoperative days. Further studies using higher doses and longer asminiration periods, are expected to show a more

pronounced therapeutic effect of FBP in the future.

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和文抄録

肝切除術後における fructose-1,6-bisphosphate 投与による
肝機能改善効果

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　肝切除例に対して，術後に fructose-1,6-bisphos-phate (FBP) を投与し，その臨床効果を検討した．肝動脈内にカテーテルを留置した肝癌切除18例のうち，11例に対して FBP 0.25 mmol/kg を60分間持続肝動脈内投与し，glucose 0.25 mmol/kg を肝動脈内に投与した7例を対照とした．なお，肝動脈カテーテル非留置10例に対して，FBP 0.25 mmol/kg を60分間持続全身投与した．投与前後における AKBR, c-AMP, IRI, iP, glucose, fructose, pyruvate, lactate, pyruvate kinase (PK) の血中変化を測定し，術後の肝機能検査値の推移を検討した．

　Glucose の肝動注では AKBR はほとんど変化しなかったが，FBP は肝動注でも全身投与でも，投与3時間後まで上昇した．とくに FBP 肝動注では投与前値に比べ，2時間後まで統計学的に有意に上昇した (P<0.01)．FBP の肝動注では，血清 pyruvate は FBP 投与直後に一過性に上昇し (P<0.01), PK 活性は FBP 投与2時間後に有意に上昇していた (P<0.05)．また，血清 Lactate は FBP の肝動脈内投与後有意に低下した (P<0.05)．術後の肝逸脱酵素の推移や蛋白合成能の回復は3群で有意な差はなかった．

　以上より，FBP の肝動脈内投与は肝細胞における解糖系亢進や乳酸摂取の亢進により ATP 産生を促進する可能性が示唆された．