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Development of New Therapy in Fulminant Hepatitis from Glucagon-Insulin Therapy to Bone Marrow Cell Transplantation

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Abstract Application of cell therapy using stem cell into intractable diseases has been noted as the medicine in the next generation taking the place of organ transplantation. Bone marrow cells have been focused as a candidate of the source of this therapy. Liver failure is a terminal state of all advanced liver diseases and thousands of patients are dying of decompensated liver cirrhosis in every year in Japan. Liver transplantation must be the strongest weapon to relieve those patients. However, particularly in Japan, liver transplantation has not been popularized. In this sense, we thought of autologous bone marrow cells transplantation for decompensated liver cirrhosis. Basic studies using animal model revealed that transplanted bone marrow cells into syngenic mouse suffering from liver cirrhosis migrated into the damaged liver and transdifferentiated to the hepatocytes. Based upon the animal studies, clinical trial of this new cell therapy supported by the Ministry of Health, Labor and Welfare is ongoing. It is early to estimate this therapy. However, free from liver failure has been observed in some cases.

Key words: fulminant hepatitis, fulminant hepatic failure, glucagon-insulin therapy bone marrow cell transplantation, stem cell

Introduction

Fulminant hepatic failure (FHF) and decompensated liver cirrhosis are recognized to be very intractable liver diseases. According to the survey by the study group of intractable liver disease appointed by the Ministry of Health, Labor and Welfare, approximately 10,000 people are diagnosed of FHF and a half of the patients who did not have liver transplantation are died. On the other hand, in Japan we have approximate 150 to 200 million people carrying chronic liver disease, mainly caused by infection of HBV and HCV and among them approximate 3,000 people are died of decompensated liver cirrhosis and

40,000 people are died of hepatocellular carcinoma.

Liver transplantation is the best way for relief such patients and the survival rate among the patients transplanted is pretty high. The issue of liver transplantation is donor shortage. Especially, in Japan liver transplantation is performed by using of the liver from living donor and the number of the patients transplanted is so limited in comparison with the Europe and USA where transplantation has been performed using brain death donors.

Our strategy to improve the survival rate among the patients is to develop more effective regenerative medicine. From the development of glucagons-insulin (G-I) therapy to bone marrow cell transplantation (BMT), the track of the battle against intractable liver disease in our institution will be introduced.

Glicagon-Insulin therapy

In 1975, Bucher and Swaffield observed that synergic action of glucagons and insulin promoted cultured rat primary hepatocytes.[1)2)] Subsequently, her group reported simultaneous injection of glucagons and insulin improved the survival rates among the mice with fulminant hepatic failure by inoculation of murine hepatitis virus (MHV).[3)] We followed their experiments and confirmed synergic action of glucagons and insulin which improved liver damage and subsequently relieved the rats with fulminant hepatic failure due to injection of high dose of d-galactosamin.[4)] In 1978, we administered 1mg of glucagons and 10 units of insulin simultaneously intravenously to the patients with fulminant hepatic failure in a condition of coma grade 2. She was relieved with remarkable improvement of liver dysfunction. Since our first publication on G-I therapy in fulminant hepatic failure in 1979,[5)] several investigators have followed this G-I therapy with some modification as shown in Table 1 .[6-9)] Fèhér and co-workers ascertained the improved survival rates among the patients of acute alcoholic hepatitis accompanied with FHF who treated with G-I therapy by means of single blind control study.[9)] Whereas, the other study groups could not improve the survival rate in spite of improvement of cholestasis. We estimated the usefulness of

Table 1 Trials of glucagon-insulin therapy reported

Investigator	Year reported	Administration
1. Okita K, et al.[5]	1979	continuous infusion for 24 hours, 500ml of 5% glucose containing, Glucagons 2.0mg Insulin 20unit
2. Baker AL, et al.[6]	1980	continuous infusion for 12 hours, 200ml of 5% Dextrose containing, Glucagon 2.4mg Insulin 24unit
3. Redecker AG, et al.[7]	1982	continuous infusion for 24 hours, 960ml of D10W solution containing, Glucagon 4.8mg Insulin 48unit
4. Jaspan JB, et al.[8]	1984	continuous infusion for 24 hours, 500ml of 20% Glucose containing, Glucagon 4.8mg Insulin 96unit Albumin 5ml
5. Fèhér J, et al.[9]	1987	drop infusion, 2 or 3 times daily, 500ml of 5% Glucose containing, Glucagon 1.0mg Insulin 10unit

G-I therapy in FHF experienced in our institution (Table 2).[10] In conclusion, G-I therapy alone or combination with G-I therapy and plasma pheresis improved the survival rate among the patients with FHF, particularly in acute form. However, G-I therapy could not contribute to the improvement of mortality rate among the patients in subacute form. In 1985, Takahashi, who was a chairman of the study group of FHF appointed by the Ministry of Health and Welfare at that moment, mentioned superiority of G-I therapy in combination with plasmapheresis from the results of nationwide survey of FHF (Table 3) .[11] Since our report on G-I therapy , this therapy has been estimated to be standard therapy in FHF. Concerning ineffectiveness of G-I therapy in subacute form of FHF, Masuhara disclosed the difference of growth factors between acute- and subacute form of FHF.[12] That is, he measured the levels of HGFmRNA, TGF α mRNA, TGF β_1 mRNA and DNA synthesis using biopsy samples from the liver of acute hepatitis, subacute form of FHF, chronic hepatitis and liver cirrhosis. He has concluded that FHF is likely a state of impaired liver regeneration, because expressions of HGFmRNA and TGF α mRNA were the lowest, whereas TGF β_1 mRNA was the highest. This observation indicates that FHF is suppressed condition in regeneration, and consequently induction of capacity to regenerate the impaired liver must be difficult.

Liver transplantation which replaces the damaged liver to the healthy liver has been focused since the establishment of the technique by Starzl in Pittsburgh.[13] Mortality of FHF in Europe and USA has been improved by means of liver transplantation. However, in Japan liver translatation has been performed mainly using the liver from living donor. Accordingly, liver transplantation has not been applied to all patients who need.

Table 2 Estimation of Glucagon-Insulin Therapy in Fulminant Hepatitis[10]

Form	Therapeutic Regimen	Survival(%)
Acute	PD	0/2 (0)
	PD + BE	1/4 (25)
	G-I	4/8 (50)
	G-I + BE	1/3 (33%)
	G-I + PP	4/6 (67)
Subacute	PD	0/8 (0)
	PD + BE	0/1 (0)
	G-I	2/6 (33)
	G-I + BE	0/2 (0)
	G-I + PP	2/13(15)

PD: prednisolon G-I: glucagons and insulin therapy
BE: blood exchange PP: plasmapheresis

Table 3 Survival Rates among the Patients with Fulminant Hepatitis and Therapy[11]

Therapy	(+)/(-)	1982	1983	1984	total
G-I	(+)	15/59(25.4)	20/64(31.3)	30/95(31.6)	65/218(29.8)
	(-)	4/22(18.2)	4/21(19.0)	3/22(13.6)	11/65(16.9)
BE	(+)	2/5(40.0)	3/6(50.0)	2/6(33.3)	7/17(41.1)
	(-)	17/76(22.4)	21/79(26.5)	31/111(27.9)	69/266(25.9)
PP	(+)	15/45(33.3)	18/50(36.0)	25/84(29.8)	58/179(32.4)
	(-)	4/36(11.1)	6/35(17.1)	8/33(24.2)	18/104(17.3)
SD	(+)	13/58(22.4)	13/57(22.8)	19/71(26.8)	45/186(24.1)
	(-)	6/23(26.1)	6/22(27.2)	14/46(30.4)	26/91(28.5)

G-I: Glucagon-Insulin Therapy BE: Blood Exchange
PP: Plasmapheresis SD: Steroid Hormone

Difficulty of liver transplantation using the liver from brain death donor indicates the development of new therapy for FHF.

Bone marrow cell transplantation

Ever since the transdifferentiation of BMC into hepatocytes was documented following a bone marrow transplant from a man donor to a woman recipient diagnosed of leukemia,[14] BMC has been an attractive cell source in regenerative medicine. We collected BMC from GFP-*Tg* mice and transplanted into non GFP-*Tg* mouse in which liver cirrhosis was prepared by multi-injection of CCl_4. After transplantation of green fluorescence positive BMC, recipient mouse were sacrificed weekly up to 4 weeks. The livers taken were perfused, incubated with 4% paraformaldehyde, subsequently soaked in 30% sucrose for a few more 3 days, and frozen in dry ice. Eighteen μm slices were applied to immnohistochemistry using anti GFP antibody. The number of cells stained with anti GFP antibody increased at the zone 3 in every week up to the 4th week.[15] Transdifferentiation of BMCs to hepatocytes was confirmed by either localization of albumin in the GFP positive cells or increase of serum albumin level in the mice transplanted bone marrow cells as compared with the control mice[15] (Fig. 1). BMCs are divided into two fractions such as hematopoietic cells and non-hematopoietic cells. We succeeded the separation of these 2 fractions by AutoMACS using anti Liv8 antiserum which recognized hematopoietic cells. After separating Liv8-positive

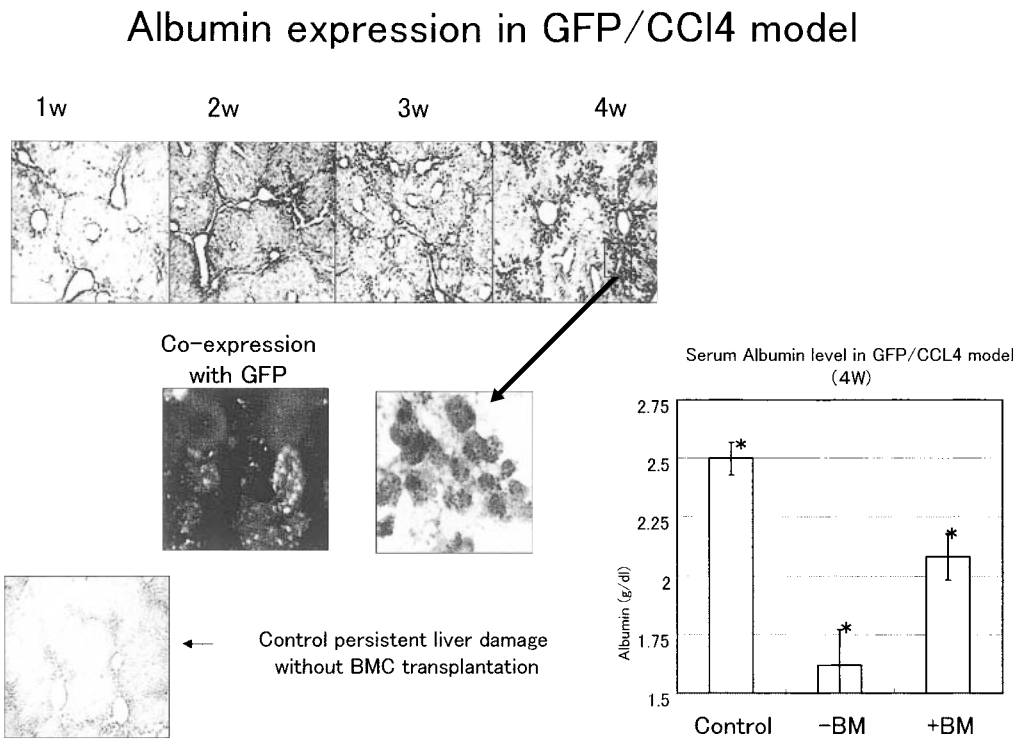

Fig. 1 Albumin expression in GFP/CCl_4 model

After transplantation of bone marrow cells from GFP transgenic mouse, number of GFP positive cells stained with anti GFP antisrum increased within a observation period until the 4th week. These GFP positive cells indicated by the arrow were also stained immunohistochemically with anti albumin antiserum (left side of the magnified view of GFP positive cells). On the othe hand, in the cirrhotic liver without transplantation of bone marrow cells, any GFT positive cells were not observed. As shown in the graph, serum albumin level was recovered significantly in the mice transplanted (+BM), as compared with the mice without transplantation (-BM).

cells and Liv8-negative cells, each cells were transplanted to recipient mice with CCl_4-induced liver cirrhosis. In the both groups, the number of GFP-positive cells in the liver tended to increase, although the positive cells was larger significantly in the group transplanted Liv8-negative cells. At four weeks after transplantation, the number of GFP-positive cells was significantly lower in the Liv8-positive cell group. These observations suggest non-hematopoietic BMCs are involved in transdifferentiation to the hepatocytes.[16] Transdifferentiation of the transplanted BMCs to the hepatocytes was also supported by the gene expression concerning organogenesis of the liver and drug metabolizing enzyme.[17] On the other hand, Lagasse demonstrated that hematopoietic stem cells have been shown to differentiate into hepatocytes under selective pressure like fumarylcetylacetate-deficient mice simply fused with resident hepatocyte.[18] This fusion event has been demonstrated to occur between resident hepatocytes and myelomonocytes[19][20] and also in normal mice using the Cre-cox system.[21] However, using the same approaches Harris et al. recently demonstrated that epithelial cells can develop from BMC without cell fusion.[22] Also, many recent publications have suggested that bone marrow derived hepatocytes could have originated from the mesenchymal compartment rather than the hematopoietic compartment.[14–16][23] Those results support our concept that transplanted BMCs can transdifferentiate to hepatocytes under specific circumstances (Fig. 2).

Interestingly, we demonstrated remarkable fibrolysis in the mice with liver cirrhosis after transplantation of the BMCs resulting in an improved survival rate.[24] Double fluorescent immunohistochemistry showed the expression of MMP-9 on the GFP positive cell surface. Film in situ zymographic analysis revealed strong gelatinolytic activity in the periportal area coinciding with location of MMP-9 positive BMC. This finding introduces not only regenerative medicine for intractable liver diseases, but also a new concept for the therapy of liver cirrhosis.

After getting approval from the ethical

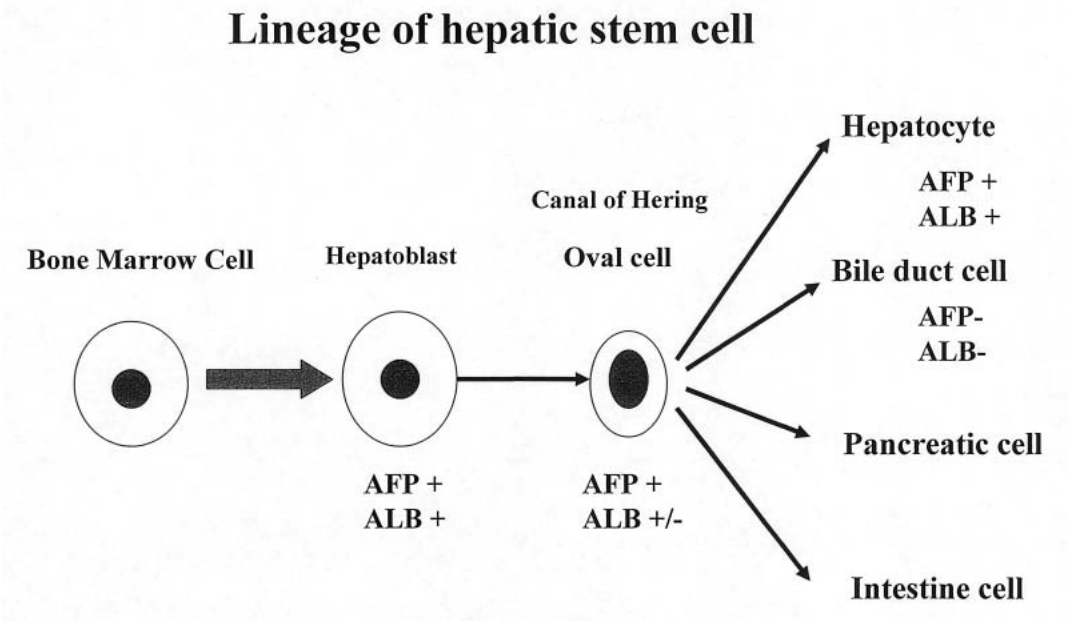

Fig. 2 Lineage of hepatic stem cell
Based upon the data taken, hepatic stem cells in the bone marrow differentiate to the hepatoblast, and subsequently to the mature hepatocytes. Consequently, the presence of bone marrow cells in the liver does not result from fusion between hepatocytes and bone marrow cells, but from the transdifferentiation of hepatic stem cells locating in the bone marrow fraction to the mature hepatocytes.

committee of Yamaguchi University School of Medicine, we initiated a phase 1 clinical study named "autologous BMC transplantation for liver cirrhosis patients" from November 2004. Up to August 2005, we transplanted autologous BMC to the 8 cirrhotic patients. Characteristically, in all patients transplanted BMC, serum albumin levels and platelet counts increased during 8 months' observation period, and PⅢP level reflecting grade of liver fibrosis decreased. So far, all patients transplanted are surviving. We will proceed to this translational research to develop a new cell therapy to cure liver cirrhosis patients. We are not sure this new cell therapy is also available to relieve the patients with FHF. At first, we will estimate the role of BMC mediating between G-I therapy in combination with plasmapheresis and liver transplantation.

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