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Amelioration of Disordered Hepatic Protein Synthesis by the Deleted Form of Hepatocyte Growth Factor in Models of Liver Failure in Rats

HIROAKI MASUNAGA, NOBUAKI FUJISE, AKIRA SHIOTA, YASUSHI YAMASHITA, HIROSHI YASUDA AND KANJI HIGASHIO

Life Science Research Institute, Snow Brand Milk Products Co. Ltd, 519 Ishibashi-machi, Shimotsuga-gun, Tochigi 329-05, Japan

Abstract

Because the liver plays an important role in protein synthesis and cholesterol metabolism and reductions in these functions are observed in almost all hepatic disorders, the effects of the deleted form of hepatocyte growth factor (dHGF) on disordered hepatic protein synthesis were studied in various liver-injured rat models using Wistar male rats.

In the 70% hepatectomized rats, plasma clotting time was prolonged and the serum level of total protein and the liver protein content were decreased. The treatment of the animals with dHGF (100–500 $\mu g\ kg^{-1}$, i.v., twice daily) ameliorated these parameters at 48 or 72 h. The administration of carbon tetrachloride or D-galactosamine to hepatectomized rats induced a marked prolongation of plasma clotting time and hypoproteinaemia. In the animals treated with dHGF (500 $\mu g\ kg^{-1}$, i.v., twice daily) these parameters were rapidly reversed compared with those of control groups. In a hepatocellular necrosis model induced by dimethylnitrosamine, the plasma clotting time was extremely prolonged, and liver protein content, serum total protein, albumin, HDL-cholesterol (as an index of lipoprotein) and plasma lecithin-cholesterol acyltransferase activity severely reduced. In this severely injured model, dHGF (5–500 $\mu g\ kg^{-1}$, i.v., twice daily for 28 days) dose-dependently prevented the loss of liver protein content and improved the disordered plasma coagulability and serum protein levels.

These results suggest that dHGF is useful for ameliorating the disorders in hepatic functions such as protein synthesis.

In 70% hepatectomized rats, the remaining liver tissue shows rapid cell proliferation and regains its original size within 10 to 14 days (Higgins & Anderson 1931). The mechanisms that initiate and maintain the regenerative process have been intensively studied, and it has been indicated that many factors such as insulin, glucagon (Bucher & Swaffield 1975), epidermal growth factor (Olsen et al 1988), hepatocyte growth factor (HGF; Nakamura et al 1984) and transforming growth factor (TGF-α, Mead & Fausto 1989; TGF-β, Russell et al 1988) play roles in control of liver regeneration.

HGF is known to be a factor stimulating DNA synthesis in primary mature hepatocytes (Nakamura et al 1984). Ishiki et al (1992) have reported that HGF injections stimulate hepatic DNA synthesis in 30% hepatectomized mice. Fujiwara et al (1993) have also demonstrated that HGF acts as a trigger and promoter of liver growth because of increasing hepatic putrescine content in rats. Roos et al (1992) have reported that HGF is protective in the model of intrahepatic cholestasis induced by α-naphthylisothiocyanate.

The deleted form of hepatocyte growth factor (dHGF) was purified as a tumour cytotoxic factor from the culture broth of human embryonic lung diploid fibroblasts (Higashio et al 1990). dHGF is a basic protein with an approximate molecular mass of 80 kD which lacks a five-amino-acid sequence in the first kringle domain of HGF. dHGF has a higher mitogenic activity for rat hepatocytes than HGF (Shima et al 1991a) and is different from HGF in physicochemical, immunological and biological properties (Shima et al 1994). Because plasma dHGF levels in patients with liver diseases are higher than those of normal subjects, dHGF itself may act as one of the effector molecules responsible for repair of injured liver tissue in patients (Shima et al 1991b).

It is well known that the liver plays an important role in protein synthesis (Majumdar et al 1967) and cholesterol metabolism (Dietschy & Wilson 1970; Strandberg et al 1984), and reductions in these functions are observed in almost all hepatic disorders. We therefore examined the modulating effects of a dHGF in various models of liver dysfunction in rats.

Materials and Methods

Materials

The dHGF used was purified from the culture broth of Namalwa cells transfected with expression vector containing human dHGF cDNA (Shima et al 1991a). The purified dHGF was diluted with sterile phosphate-buffered saline (PBS) containing 0·25% human serum albumin (Kaketsuken, Kumamoto, Japan) and 0·01% Tween 80. dHGF solution was intravenously injected into rats in a volume of 1 $mL\ kg^{-1}$. Control rats received the same volume of the vehicle.

Animals

Male Wistar rats, 160 to 190 g, were obtained from Charles

Correspondence: H. Masunaga, Life Science Research Institute, Snow Brand Milk Products Co. Ltd, 519 Ishibashi-machi, Shimotsuga-gun, Tochigi 329-05, Japan.

River Japan, Inc. (Kanagawa, Japan). The rats were housed under conditions of controlled temperature ($23 \pm 1°C$), humidity ($55 \pm 5\%$) and lighting (0700–1900 h); standard food pellets and water were freely available. The rats were left to become acclimatized to the facility for a week before an experiment. Acute liver injured models were prepared as follows: the 70% partial hepatectomy was performed by excising the medial and left hepatic lobes under ether anaesthesia according to the method of Higgins & Anderson (1931). The hepatectomized rats (n = 6 per group) received dHGF (100 and 500 $\mu g\ kg^{-1}$) every 12 h from just after the 70% hepatectomy.

Other hepatectomized rats were given carbon tetrachloride (CCl_4, Kanto Chemicals, Tokyo, Japan) or D-galactosamine (Wako Pure Chemicals, Tokyo, Japan) to examine the effect of these compounds on regeneration and protein synthesis in injured livers. CCl_4 (0·3 mL kg^{-1}, dissolved in the same volume of olive oil) was administered orally to the rats immediately after the hepatectomy; D-galactosamine (500 mg kg^{-1}) was administered to the rats subcutaneously, immediately after the hepatectomy. These rats received dHGF (500 $\mu g\ kg^{-1}$) every 12 h from just after each induction of liver injury.

A chronic hepato-cellular necrosis was induced by dimethylnitrosamine (Sigma, St Louis, MO, USA), according to the method of Ala-Kokko et al (1989). Rats received dimethylnitrosamine (10 $\mu L\ kg^{-1}$ diluted 100-fold with 0·15 M NaCl) intraperitoneally weekly on the first three consecutive days for 4 weeks. dHGF (5, 50 and 500 $\mu g\ kg^{-1}$) was injected twice daily from the beginning of the experiment up to day 28.

Sampling and analysis

Blood was collected using vacuum tubes (Venoject, Terumo) from the inferior caval vein of rats under ether anaesthesia. For plasma sampling for the thrombotest and for lecithin-cholesterol acyltransferase activity, vacuum tubes containing sodium citrate solution (3·8%) or EDTA, respectively, were used. Plasma-clotting time was assayed using reagents of the Eisai (Tokyo, Japan) thrombotest kit and an Amelung (Lemgo, Germany) KC-40 coagulometer, and was expressed as the time (s) in thrombotest time. The fibrinogen level was assayed using reagents of the Eisai fibrinogen kit and a KC-40 coagulometer. Serum levels of total protein, albumin and high density lipoprotein (HDL)-cholesterol were determined with an Hitachi (Tokyo, Japan) type 7150 automatic analyser, using commercial test reagents (Daiichi Pure Chemicals, Tokyo, Japan). Lecithin-cholesterol acyltransferase activity in plasma (containing EDTA) in dimethylnitrosamine-treated rats was determined by the method of Gromset & Wright (1981). The liver in all rats was excised for weighing and a piece of liver tissue was homogenized in cold saline and stored at − 80°C until assay. The liver protein content was determined using a Bio-Rad protein assay kit.

Statistical analysis

The results are expressed as the means ± s.e. The data were analysed by analysis of variance. Differences between groups were analysed using Fisher's least significance difference test. Significance was established at $P < 0·05$ or $P < 0·01$.

Results

In the 70% hepatectomized rats the thrombotest time was prolonged and serum total protein and HDL-cholesterol were reduced after the hepatectomy (Table 1).

The remaining liver weight and liver protein content did not return to normal 72 h after hepatectomy (Table 2). Treatment of the animals with 500 $\mu g\ kg^{-1}$ dHGF improved the disordered thrombotest time, total protein and HDL-cholesterol to almost their normal levels 72 h after hepatectomy. The remaining liver weights and liver protein contents were also rapidly attained. Administration of CCl_4 or D-galactosamine more markedly induced prolongation of thrombotest time and reductions in total protein and HDL-cholesterol in 70% hepatectomized rats (Table 1) and apparently delayed the recovery of liver weight and liver protein content after hepatectomy (Table 2). In the animals treated with 500 $\mu g\ kg^{-1}$ dHGF, thrombotest time, total protein and HDL-cholesterol rapidly attained almost the same level as those in the control rats 72 h after the normal liver resection.

In a chronic hepatocellular necrosis model induced by dimethylnitrosamine, the liver weights and liver protein contents were dramatically reduced (Table 3). In these rats thrombotest time was severely prolonged and the levels of total protein, albumin and fibrinogen were markedly reduced. Further, lecithin-cholesterol acyltransferase activity and HDL-cholesterol were also reduced. Treatment of the animals with dHGF (50 and 500 $\mu g\ kg^{-1}$) restored the liver weight and liver protein content. It also dose-dependently prevented the prolongation of thrombotest time and the reductions in total protein, albumin, fibrinogen, lecithin-cholesterol acyltransferase activity and HDL-cholesterol.

Discussion

Plasma clotting time, serum protein and HDL-cholesterol levels are known to be closely related indices of hepatic function, because the liver plays an important role in the synthesis of albumin, blood coagulating factors, high density lipoprotein, cholesterol and lecithin cholesterol acyltransferase, etc. (Radding et al 1958; Majumdar et al 1967; Calandra et al 1971; Brown & Goldstein 1986).

In this study we have indicated that dHGF ameliorates protein synthesis disorders in various hepatic failure models. In 70% hepatectomized rats given dHGF a dose-dependent elevation in liver weight was observed. Bucher et al (1978) have reported that epidermal growth factor alone has little effect on hepatic DNA synthesis but that epidermal growth factor combined with glucagon has an appreciable effect. In this study dHGF alone strongly stimulated liver regeneration in partially hepatectomized rats. Our findings indicate that dHGF has potent hepatotrophic activity. Indices of liver function such as thrombotest time, total protein and HDL-cholesterol were also improved; this was followed by recovery of liver size. These results suggest that dHGF appears to act as a physiological inducer of liver regeneration.

The mechanism of hepatocellular necrosis by CCl_4 is considered to be enzymatic activation of CCl_4 to a CCl_3 free radical in the membrane of the endoplasmic reticulum (Stachura et al 1981); D-galactosamine, on the other hand, causes disruption of liver glucose metabolism, leading to a decrease in

Table 1. Effects of dHGF on thrombotest time, total protein and HDL-cholesterol levels in acute liver failure models after 70% hepatectomy in rats.

			48 h	72 h
Thrombotest (s)	Normal		23·3 ± 0·4	23·4 ± 0·4
	Hepatectomy	Vehicle	28·7 ± 1.9	25·2 ± 0·6
		dHGF 100 μg kg^{-1}	25·4 ± 0·9	23·7 ± 0·4
		500 μg kg^{-1}	25·0 ± 0·1*	23·2 ± 0·2**
	Hepatectomy	Vehicle	40·1 ± 1·4	27·0 ± 0·7
	+ Galactosamine	dHGF 500 μg kg^{-1}	34·5 ± 1·1**	25·1 ± 0·6
	Hepatectomy	Vehicle	49·8 ± 4·3	29·5 ± 3·0
	+ CCl_4	dHGF 500 μg kg^{-1}	37·7 ± 3·3*	23·6 ± 0·4*
Total protein (g dL^{-1})	Normal		5·2 ± 0·1	5·3 ± 0·1
	Hepatectomy	Vehicle	4·5 ± 0·1	5·0 ± 0·1
		dHGF 100 μg kg^{-1}	4·7 ± 0·1	5·1 ± 0·1
		500 μg kg^{-1}	4·7 ± 0·1	5·3 ± 0·1*
	Hepatectomy	Vehicle	4·2 ± 0·1	4·4 ± 0·1
	+ Galactosamine	dHGF 500 μg kg^{-1}	4·3 ± 0·1	4·6 ± 0·1*
	Hepatectomy	Vehicle	3·9 ± 0·1	4·3 ± 0·2
	+ CCl_4	dHGF 500 μg kg^{-1}	4·3 ± 0·1*	4·8 ± 0·1**
HDL-cholesterol (mg dL^{-1})	Normal		36·8 ± 3·0	43·2 ± 1·2
	Hepatectomy	Vehicle	28·9 ± 3·1	33·5 ± 3·0
		dHGF 100 μg kg^{-1}	31·9 ± 2·0	41·0 ± 2·6*
		500 μg kg^{-1}	33·5 ± 2·6	54·7 ± 2·9**
	Hepatectomy	Vehicle	9·7 ± 1·9	25·6 ± 2·1
	+ Galactosamine	dHGF 500 μg kg^{-1}	12·8 ± 1·4	31·5 ± 2·8
	Hepatectomy	Vehicle	10·1 ± 1·9	29·4 ± 3·8
	+ CCl_4	dHGF 500 μg kg^{-1}	15·3 ± 2·0	35·3 ± 2·0

dHGF was given intravenously every 12 h for 48 h or for 72 h from just after 70% hepatectomy. Data represent means ± s.e. for 6–10 rats. Significant difference against each control group receiving vehicle indicated by $*P < 0.05$ and $**P < 0.01$.

Table 2. Effects of dHGF on liver protein content and liver weight in acute liver failure models after 70% hepatectomy in rats

			48 h	72 h
Liver protein (g/100 g)	Normal		0·522 ± 0·026	0·535 ± 0·016
	Hepatectomy	Vehicle	0·305 ± 0·009	0·378 ± 0·007
		dHGF 100 μg kg^{-1}	0·326 ± 0·008	0·405 ± 0·009
		500 μg kg^{-1}	0·346 ± 0·008*	0·436 ± 0·009**
	Hepatectomy	Vehicle	0·281 ± 0·008	0·381 ± 0·011
	+ Galactosamine	dHGF 500 μg kg^{-1}	0·295 ± 0·074	0·427 ± 0·004**
	Hepatectomy	Vehicle	0·242 ± 0·009	0·322 ± 0·018
	+ CCl_4	dHGF 500 μg kg^{-1}	0·312 ± 0·019*	0·396 ± 0·015**
Liver weight (g/100 g)	Normal		4·758 ± 0·236	4·938 ± 0·081
	Hepatectomy	Vehicle	2·999 ± 0·086	3·648 ± 0·118
		dHGF 100 μg kg^{-1}	3·061 ± 0·143	4·072 ± 0·071*
		500 μg kg^{-1}	3·265 ± 0·050	4·195 ± 0·073**
	Hepatectomy	Vehicle	2·636 ± 0·087	3·642 ± 0·099
	+ Galactosamine	dHGF 500 μg kg^{-1}	2·763 ± 0·087	3·939 ± 0·049**
	Hepatectomy	Vehicle	2·637 ± 0·083	3·458 ± 0·129
	+ CCl_4	dHGF 500 μg kg^{-1}	3·190 ± 0·116**	4·131 ± 0·106**

dHGF was intravenously given every 12 h for 48 h or for 72 h from just after 70% hepatectomy. Data represent the means ± s.e. for 6–10 rats. Significant difference against each control group receiving vehicle indicated by

RNA and protein synthesis and to cell necrosis (Yoshitake et al 1991). Administration of CCl_4 or D-galactosamine to 70% hepatectomized rats apparently delayed the recovery of their liver weight and induced severe hepatic failure. Under these disordered hepatic conditions, dHGF also accelerates the synthesis of hepatic proteins such as clotting protein, serum protein, lipoprotein and liver structural protein.

Dimethylnitrosamine is activated by microsomal enzymes such as dimethylnitrosamine demethylase (Mostafa et al 1981) and then appears to block liver protein synthesis and to act primarily as a liver poison, producing severe liver necrosis. It markedly prolonged plasma clotting time and reduced the levels of plasma protein such as fibrinogen, albumin and total protein.

In this study, dHGF ameliorated all these conditions and liver protein content dose-dependently, indicating acceleration of hepatic protein synthesis. Such beneficial effect may act by protecting against liver injury and by stimulation of recovery from injury by proliferation of the remaining hepatocytes or restoration of the function of injured hepatocytes. dHGF also drastically restored abnormal lecithin-cholesterol acyltransferase activity and cholesterol level. These results indicate

Table 3. Effects of dHGF on plasma proteins, liver protein content and liver weight in chronic hepatocellular necrosis models induced by dimethylnitrosamine in rats. Rats received 10 μL kg^{-1} dimethylnitrosamine intraperitoneally on the first three consecutive days weekly for four weeks. dHGF was given intravenously to rats every 12 h for 28 days.

Assay	Normal	Dimethylnitrosamine-induced model			
		Vehicle	dHGF (μg kg^{-1})		
			5	50	500
Thrombotest (s)	22.3 ± 0.2	162·2 ± 37·2	93·4 ± 23·3*	48·6 ± 20·7**	24·5 ± 0·5**
Fibrinogen (mg dL^{-1})	227 ± 5	110 ± 6	123 ± 6	150 ± 13*	201 ± 8**
LCAT activity (dpm min^{-1} mL^{-1})	1453 ± 84	606 ± 79	711 ± 74	954 ± 81**	1264 ± 73**
Total protein (g dL^{-1})	6·2 ± 0·1	4·0 ± 0·1	4·3 ± 0·2	5·5 ± 0·2**	6·7 ± 0·1**
Albumin (g dL^{-1})	2·8 ± 0·0	1·9 ± 0·1	2·1 ± 0·1	2·6 ± 0·1**	3·2 ± 0·0**
HDL-cholesterol (mg dL^{-1})	46·1 ± 2·6	15·8 ± 1·7	22·1 ± 2·2	33·6 ± 3·4**	60·3 ± 3·0**
Liver protein (g/100 g body weight)	0·731 ± 0·022	0·250 ± 0·029	0·323 ± 0·041	0·545 ± 0·043**	0·751 ± 0·016**
Liver weight (g/100 g body weight)	4·423 ± 0·0051	2·102 ± 0·183	2·509 ± 0·274	4·085 ± 0·254**	5·092 ± 0·123**

Each value represents the mean ± s.e. of 8–10 rats. *$P < 0.05$, **$P < 0.01$ significantly different from control group.

that dHGF not only accelerates protein synthesis but improves cholesterol metabolism under such severe hepatic failure condition.

In conclusion, these findings suggest that dHGF has strong hepatotrophic activity and accelerates the amelioration of disordered hepatic functions such as protein synthesis and cholesterol metabolism.

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