行政院國家科學委員會補助專題研究計畫成果報告

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行政院國家科學委員會專題研究計畫成果報告

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計畫名稱：肝細胞生長因子與 beta 型轉化生長因子在人類代償性腎生長與腎肥大所伴演的角色
The role of hepatocyte growth factor and transforming growth factor-beta in human compensatory renal growth and renal hypertrophy

中文摘要

我們以超音波檢測嬰兒腎臟體積，共計 29 位膽道閉鎖 17 位新生兒肝炎，10 位猛爆性肝炎，及 32 位健康嬰兒，並檢測其血中 HGF 及 TGF-β1，膽道閉鎖，猛爆性肝炎，及 35% 新生兒肝炎嬰兒均有腎肥大現象，這些病人之腎體積與血中 HGF 呈正相關($r=0.529$, $p<0.001$)但血中 TGF-β1 卻呈負相關($r=-0.505$, $p<0.001$)。

另外對於單一腎臟之代償性腎肥大之病童其血中 HGF 與健康孩童無異(數據未示出)，因此斷定其腎肥大之機轉應與慢性肝傷害因持續性 HGF 刺激引起之腎肥大之機轉並不相同。

關鍵詞：肝細胞生長因子，乙型轉化生長因子。

Abstract

There was significant nephromegaly in infants with biliary atresia as compared with healthy infants ($p < 0.001$ by analysis of covariance). Marked nephromegaly was also noted in all infants with fulminant hepatitis and in 35% of infants with neonatal hepatitis. There was no nephromegaly in infants at 2 months of age with biliary atresia or neonatal hepatitis despite mildly elevated levels of plasma HGF. Irrespective of the duration of HGF exposure and the healthy renal growth by a certain age, there was a positive correlation between plasma HGF and kidney volume ($r = 0.529$, $p < 0.001$), but an inverse correlation between plasma TGF-β1 and nephromegaly ($r = -0.505$, $p < 0.001$) in all diseased infants. These results confirm the presence of large kidneys not only in patients with biliary atresia but also in patients with fulminant hepatitis which suggests the possible pathogenic role of HGF and manifests as elevated HGF/ TGF-β1 ratios in patients with such conditions. Nephromegaly in patients with severe or chronic liver dysfunction may provide a new in vivo model to study the mechanisms of renal growth.

Keywords: hepatocyte growth factor, transforming growth factor β.

Introduction

Hepatocyte growth factor (HGF) is a well-known proliferative growth factor for hepatocytes after liver injury (1). HGF also plays an important role in compensatory renal growth and renal regeneration (2–4). We previously demonstrated elevated levels of plasma HGF and positive correlation between the levels of plasma HGF and nephromegaly in children with biliary atresia (5). Elevated serum HGF levels have also been reported in adults with liver diseases (6).

Transforming growth factor β1(TGF-β1), a prototypical multifunctional cytokine,
is a known inhibitor of cellular proliferation in many types of epithelial cell cultures as well as hepatocyte cultures (7,8). Although the role of TGF-β1 in liver regeneration is not clear, this mito-inhibitor is a logical candidate for terminating the regeneration of liver after partial hepatectomy (8). Interestingly, TGF-β1 is also believed to be a common mediator of renal tubule hypertrophy in different models of epithelial enlargement (7). Considering that TGF-β1 may be a co-regulator of HGF in the model of nephromegaly in biliary atresia, we examined plasma TGF-β1 and HGF concentrations simultaneously in infants with these hepatobiliary diseases.

Materials and Methods

Subjects and specimens

Seventeen infants with neonatal hepatitis, 10 infants with fulminant hepatitis and 29 infants with biliary atresia at National Taiwan University Hospital were enrolled in this study from January 1998 through June 1999. Thirty-two age-matched healthy infants served as controls. All infants with neonatal or fulminant hepatitis were diagnosed using clinical findings followed by liver biopsies. All patients with biliary atresia underwent Kasai's operations before 2 months of age. Renal ultrasound was performed in all diseased and healthy infants to measure renal size. Blood was drawn from each patient for plasma HGF and TGF-β1 determinations.

Kidney measurements

All sonograms were obtained using a commercial unit Toshiba, model SAA 250,
using a 3.5-MHz probe.

HGF and TGF-β1 assays: using ELISA method.

Results

Nephromegaly was significantly more common in infants with biliary atresia compared with healthy infants ($p < 0.001$ by analysis of covariance). Nephromegaly was also noted in all infants with fulminant hepatitis and in 35% of infants with neonatal hepatitis. Table 1 shows levels of plasma HGF and kidney volumes in infants with fulminant hepatitis, biliary atresia and neonatal hepatitis, and healthy children. The highest HGF levels were found in patients with fulminant hepatitis, who also had the most severe nephromegaly. In infants younger than 2 months with biliary atresia and neonatal hepatitis, there was no nephromegaly despite mildly elevated plasma HGF. Table 2 shows some longitudinal data of levels of plasma HGF and kidney volume in 11 patients. As we can see in patients with fulminant hepatitis, either extreme nephromegaly developed in only a few weeks which was associated with highly elevated plasma HGF, or no final nephromegaly was detected when survived from fulminant hepatic failure with ultimately normal levels of plasma HGF.

Although there were no statistical differences in levels of plasma TGF-β1 among various age groups of diseased and healthy infants (data not shown), a tendency toward decreased plasma TGF-β1 was noted in infants with nephromegaly. Figure 2 shows a negative correlation between plasma TGF-β1 and kidney volume in all diseased infants ($r = -0.505$, $p < 0.001$), along with a positive correlation between plasma HGF and nephromegaly ($r = 0.529$, $p < 0.001$). All figures were not shown here.

Discussion

The data in this study again demonstrated nephromegaly in infants with biliary atresia. These infants also had high levels of plasma HGF. Meanwhile in this study, extreme nephromegaly associated with highly elevated levels of plasma HGF was noted in patients with fulminant hepatitis. Nephromegaly was not found in most infants with neonatal hepatitis with non-elevated levels of plasma HGF. These findings provide new evidence for the possible role of HGF in nephromegaly. Moreover, irrespective of the duration for HGF exposure that was hard to estimate in this study and healthy renal growth by a certain age, there was a positive correlation between kidney volume and plasma HGF concentration in all patients with biliary atresia and hepatitis. This further suggests that HGF, rather than the disease (biliary atresia) per se, may involve the development of nephromegaly.

Our data suggest that high circulating HGF may be important in patients with nephromegaly. However, during early infancy of biliary atresia and neonatal hepatitis and in the initial stages of fulminant hepatitis, no nephromegaly was noticed despite elevated or even extremely high levels of plasma HGF. Also, higher levels of plasma HGF seemed to result in larger kidney volume in less time (Table 1 and 2). A reasonable explanation for these findings is that nephromegaly may result from continued stimulation of HGF under the influence of dose effect and exposure duration. Thus, it is expected that most infants with neonatal hepatitis and some patients who survived fulminant hepatic failure had no final nephromegaly.

Theoretically, nephromegaly resulting from hyperplasia and/or hypertrophy on long-term high HGF stimulation in these patients is very likely. Our data regarding correlation between nephromegaly and elevated levels of plasma HGF may provide indirect evidence in this situation. TGF-β1, another candidate mediator of nephromegaly (7), is an inhibitor of renal cell growth because it actually inhibits DNA synthesis and cell proliferation. However TGF-β1 can convert a mitogenic stimulus into hypertrophic signal, promote RNA and protein synthesis, and thus enhance cellular hypertrophy. To our surprise, instead of elevated levels of plasma TGF-β1, which may act as a hypertrophic factor (7), there was an inverse correlation between kidney volume and plasma TGF-β1 concentration in our patients . If TGF-β1 were also involved in the development of nephromegaly, our results provide a reasonable explanation. It is believed that in liver regeneration after partial hepatectomy, HGF is the initial mitogenic stimulus and TGF-β1 stops the proliferation leading to termination of regeneration (8). If renal growth proceeds in a similar way , the extent of nephromegaly from this new model may depend not only on HGF but also on TGF-β1. The low plasma TGF-β1 may implicate the decreased antiproliferative effect on renal cell growth and actually potentiate the mitogenic action of HGF. Thus, nephromegaly inversely correlated with the level of plasma TGF-β1 in this situation.

計畫成果自評：

由於本計畫原為三年計畫，但祇通過並減縮為一年，因此限於經費與病人之收集，祇能選擇部分重要者執行。在結果顯示代償性腎肥大與慢性肝傷害之腎肥大機轉可能不同後，我們直接檢測慢性肝傷害引起腎肥大模型之 TGF-β1 相關角色。出乎我們意料之外。腎臟之大小與血中 TGF-β1 呈負相關。此結果雖可解釋得通。但與我們原先的考量與動物實驗的設計背道而馳。由於計畫被刪大部分(僅有一年)動物實驗無法進行。不過如果將來有機會進行實驗。則必須修改計畫。(即應以 TGF-β1 antibody 代替 TGF-β1 給予老鼠)。不過本計畫合併上一計畫之結果已寫成論文。並試投 SCI 期刊。

Reference:

1 Aldana PR, Goerke ME, Carr SC, Tracy TF: The expression of regenerative growth factors in chronic liver injury and repair. J Surg Res 57: 711-717, 1994.

2.Nagaike M, Hirao S, Tajima H, Noji S, Taniguchi S, Matsumoto K, Nakamura T: Renotropic functions of hepatocyte growth factor in renal regeneration after unilateral nephrectomy. J Biol Chem 266: 22781-22784, 1991.

3.Ishibashi K, Sasaki S, Sakamoto H, HoshinoY, Nakamura T, Marumo F: Expressions of receptor gene for hepatocyte growth factor in kidney after unilateral nephrectomy and renal injury. Biochem Biophys Res Commun 187: 1454-1459, 1992.

4.Joannidis M, Spokes K, Nakamura T, Faletto D, Cantley LG: Regional expression of hepatocyte growth factor/ *c-met* in experimental renal hypertrophy and hyperplasia. Am J Physiol 267: F231-F236, 1994.

5. Tsau YK, Chen CH, Chang MH, Teng RJ, Lu MY, Lee PI: Nephromegaly and elevated hepatocyte growth factor in children with biliary atresia. Am J Kidney Dis 29: 188-192, 1997.

6.Shiota G, Okano JI, Kawasaki H, Kawamoto T, Nakamura T: Serum hepatocyte growth factor levels in liver diseases: clinical implications. Hepatology 21: 106-112, 1995.

7. Sharma K, Ziyadeh FN: The emerging role of transforming growth factor-β in kidney diseases. Am J Physiol 266: F829-F842, 1994.

8. Michalopoulos GK, DeFrances MC: Liver regeneration. Science 276: 60-66, 1997.

Table 1. The plasma HGF levels and renal volume in patients and healthy children

Group	Age(months)	No. of children	HGF(pg/mL)	Renal volume(cm^3)
Fulminant hepatitis	5.18 ± 1.51	6	8026 ± 5499^{++}	65.00 ± 16.18^{+}
	13.58 ± 3.96	4	8552 ± 5409**	82.62 ± 20.88*
Biliary atresia	2.05 ± 1.00	8	1511 ± 879 $^{\#}$	26.22 ± 7.39
	6.27 ± 1.06	10	4265 ± 2774^{++}	49.89 ± 12.95^{+}
	12.38 ± 3.07	11	3575 ± 1883**	68.28 ± 16.53*
Neonatal hepatitis	2.17 ± 0.93	7	1728 ± 767 $^{\#}$	24.54 ± 6.44
	6.35 ± 1.05	5	3510 ± 2477^{++}	36.97 ± 14.72
	13.85 ± 5.16	5	1643 ± 1795	38.07 ± 13.26
Healthy control	2.07 ± 1.01	11	485 ± 221 $^{\#}$	19.47 ± 5.22
	6.07 ± 1.01	9	563 ± 291^{++}	26.30 ± 3.96^{+}
	13.54 ± 2.83	12	551 ± 279**	31.82 ± 4.66*

*, + $p < 0.001$; **, ++, # $p < 0.005$ (patients versus healthy controls)

Table 2. Longitudinal changes in plasma HGF, plasma TGF-β1 and kidney volume in eleven patients

Patient no.	Age at check-up (months)	Plasma HGF (pg/mL)	Plasma TGF-β1 (ng/mL)	HGF/TGF-β1 (pg/ng)	Kidney volume (cm^3)
Fulminant hepatitis					
1.	1.8	15570	13.45	1157	31.02
	3	15570	10.71	1454	72.05
2.	6	14469	14.05	1030	46.51
	6.8	9440	5.51	1714	92.26
3.	2.8	2200	11.33	194	50.89
	3.25	886	8.89	100	85.44
	4.5	2053	7.89	260	65.69
4.	6	3708	23.12	160	39.54
	7	1365	22.27	61	52.46
	10	570	17.76	32	27.68
Severe neonatal hepatitis					
5.	2.5	3312	27.19	122	21.34
	6.5	7197	12.60	571	58.47
6.	7.5	4783	44.58	107	32.96
	9.25	4783	8.25	580	49.63
Biliary atresia					
7.	1.8	1718	46.19	37	20.45
	5	2710	46.72	58	49.03
8.	10.2	3835	30.34	126	45.29
	18	4935	32.70	151	73.82
9.	2	1243	21.66	57	36.99
	9	4870	8.44	577	83.28
10.	5	3943	46.01	86	30.00
	11.5	6473	13.18	491	64.40
11.	1.5	466	9.54	49	19.56
	4.5	2735	11.34	241	38.56
	6.7	7067	11.18	632	53.49

Reference ranges for data: plasma HGF 531 ± 258 pg/mL (n = 32), plasma TGF-β1 26.20 ± 19.29 ng/mL (n = 20), plasma HGF/ TGF-β1 33.1 ± 25.0 pg/ng (n = 20), kidney volume at 2 months 19.47 ± 5.22 cm3 (n = 11), kidney volume at 13 months 31.82 ± 4.66 cm3 (n = 12).