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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執行期間：90年 8月 1日至91年 7月31日

計畫主持人：曹永魁 副教授

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- ☐赴國外出差或研習心得報告一份
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- ☐出席國際學術會議心得報告及發表之論文各一份
- ☐國際合作研究計畫國外研究報告書一份

執行單位：台大醫學院 小兒科

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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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執行機構及單位名稱：台大醫學院 小兒科
計畫名稱：慢性肝傷害型腎肥大之機轉：血中高肝細胞生長因子及低β1型轉化生長因子之角色
Mechanism of nephromegaly in chronic liver injury：the role of elevated plasma HGF/TGF-β1 as a reciprocal balance

一、中文摘要

我們以結紮 25 天大之大白鼠總膽管，做為肝傷害之動物模式檢驗術後一星期，二星期及三星期之腎臟生長及肝細胞生長因子(HGF)及β1 型轉化生長因子(TGF-β1)之變化情形，結果發現實驗組大白鼠於一星期時血中 HGF 與 TGF-β1 有意義降低，於二星期時血中 TGF-β1 仍低於控制組，而腎中 HGF/TGF-β1 比值於二星期時達到有意義的增加，而腎重/體重比值於三星期時實驗組亦有意義高於控制組，且伴隨較高之蛋白質/DNA 比值(此意謂實驗組之腎肥大)。

關鍵詞：肝細胞生長因子，乙型轉化生長因子，慢性肝傷害，腎肥大。

Abstract

Kidney growth was observed each week for 3 weeks after ligation of common bile duct (CBD) in the 25-day-old rats. At one week after surgery, CBD ligation rats had significantly lower plasma HGF and TGF-β1, than sham controls. Compared with sham controls, plasma TGF-β1 was still low but not for plasma HGF at 2nd week. Renal HGF/TGF-β1 start rising since first week and reached a significantly higher level at 2nd week. Kidney weight/body weight ratios were significantly higher, associated with an increased protein/DNA ratio (indicating renal hypertrophy) at 3 weeks after CBD ligation.

Keywords: hepatocyte growth factor, transforming growth factor-β1, chronic liver injury, renal hypertrophy.

Introduction

We have recently reported an entirely new finding not only nephromegaly in children with biliary atresia, but also a correlation between nephromegaly and elevated plasma HGF in these patients (1). However, our animal experiment using low-to-medium dose of HGF injection to rats for a short duration couldn't induce high plasma HGF condition. Our more recent data on infants with subacute or chronic liver injury revealed the extent of nephromegaly correlated positively with plasma HGF but negatively with plasma TGF-β1 (2). To delineate the significance of these correlation results, we should first know whether nephromegaly results from renal hyperplasia or renal hypertrophy. The reciprocal relationship of plasma HGF and TGF-β1 in this situation may reduce antiproliferative effect of TGF-β1 and thus potentiate proliferative effect of HGF resulting in nephromegaly. We create an animal model of chronic liver injury using ligation of common bile duct in rats to observe the nephromegaly (3), to check their renal protein/DNA ratio and thus to determine either hyperplasia or hypertrophy (4). Furthermore, total renal

HGF and total renal TGF-β1 will be measured and correlated with plasma HGF and TGF-β1 in experimental rats to search for a reasonable mechanism.

Methods

Wistar rats, 25-day-old, were performed common bile duct (CBD) ligation and/or division via ventral laparotomy. They were sacrificed at one week, two weeks and three weeks after surgery. Blood was collected and both kidneys were removed after saline perfusion during sacrifice. Body weight and total kidney weight were measured. Kidneys were homogenized with TRI reagent and extracted for tissue lysate. Plasma HGF and TGF-β1, renal tissue protein and DNA, and renal tissue HGF and TGF-β1 were then measured using various specific EIA kits. Renal enlargement was assessed by kidney weight/body weight ratios and renal hyperplasia or hypertrophy by protein/DNA ratio. The relation of nephromegaly to plasma HGF and TGF-β1 and HGF/ TGF-β1 was evaluated. Student t test and Mann-Whitney test were used for statistical analysis.

Results

There was a tendency of, but not significant decrease in body weight and total kidney weight in CBD ligation rats compared with sham operation controls. Plasma HGF was significantly decreased at one week and plasma TGF-β1 was persistently low through one and two weeks after surgery in experimental rats. Renal HGF/ TGF-β1 start rising since first week and reached a significantly higher level than sham controls at 2nd week postoperatively. This was followed by a higher kidney weight/body weight ratio and an elevated protein/DNA ratio (suggesting renal hypertrophy) at 3rd week in CBD ligation rats. These results are summarized in Table 1.

Discussion

To examine if nephromegaly occurred in rats with chronic liver injury, this model provides a rather satisfied but not perfect situation when compared with biliary atresia (after Kasai operation) in human. Complete obstruction of CBD may cause severe liver damage resulting in poor food intake and thus poor weight gain and initial kidney growth. Liver injury may be severe enough to progress to end stage liver disease rapidly and results in death of a half of rats before 3 weeks postperatively (3). Unlike the situation of nephromegaly in biliary atresia with elevated plasma HGF and decreased plasma TGF-β1 and thus elevated plasma HGF/ TGF-β1 ratio (2), plasma HGF and TGF-β1 in CBD ligation model were deceased at one week after surgery and plasma TGF-β1 remained low (this may result in a high HGF/ TGF-β1) at two weeks after surgery. Actually the plasma HGF/ TGF-β1 was similar to control one. However, our data provide evidence to solve the mechanism of nephromegaly in chronic liver injury. Increased renal HGF/ TGF-β1 at two weeks after CBD ligation followed by elevated kidney weight/body weight ratios and renal tissue protein/DNA ratios at 3 weeks may relate renal hypertrophy to these causative growth factors. Unfortunately, why the renal HGF/ TGF-β1 changes in liver injury remains unknown. From human (2) and present rat model, the low TGF-β1 after liver injury may be the most important factor to result in renal hypertrophy in this situation.

計劃成果自評

由於本計劃原為三年計劃，但只通過並減縮為一年，限於經費，只能選擇部分重要者執行。雖然發現選擇之動物模式並非完美，但以上之實驗結果仍能證實我們的假設，得到以前未知的結果，但如能繼續進行實驗，有 renal tissue c-met protein 之結果，則合併寫成論文，將更有價值。

Reference

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Table 1 Changes in total kidney growth after ligation of common bile duct (CBD) in 25-day-old rats.

	Weeks after surgery					
	One week		Two weeks		Three weeks	
	CBD ligation	Sham	CBD ligation	Sham	CBD ligation	Sham
Number of rats	11	8	12	9	4	8
Initial body weight (g)	72.2±3.3	70.3±7.5	74.8±15.8	68.0±14.1	76.2±14.1	72.0±19.0
Final body weight (g)	95.4±14.9	107.6±20.5	134.4±29.5	152.2±15.4	163.1±28.3	197.1±27.7
Total kidney weight (g)	1.158±0.311	1.270±0.266	1.561±0.392	1.799±0.230	1.886±0.304	2.093±0.352
Kidney weight/body weight (g/100g)	1.21±0.22	1.18±0.09	1.17±0.17	1.18±0.11	1 .16±0.05*	1.05±0.07
Protein/DNA (mg/mg)	7.92±3.54	6.52±4.50	10.18±9.29	11.04±5.16	14.36±6.02+	3.84±1.90
Plasma HGF (ng/ml)	1.12±0.70**	2.43±1.05	2.26±2.42	4.24±3.04	1.89±1.20	4.20±2.931
Plasma TGF-β1 (ng/ml)	0.83±0.51++	1.66±0.75	0.86±0.61#	1.68±0.77	0.55±0.21	1.29±0.75
Renal HGF/TGF-β1 (ng/ng)	62.78±95.18	15.92±10.45	54.93±67.11##	10.48±2.80	14.51±10.27	22.27±10.20

* P=0.021 + P=0.001 ** P=0.004 ++ P=0.01 # P=0.013 ## P=0.015