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Effects of Fuzhenghuayu decoction on collagen synthesis of cultured hepatic stellate cells, hepatocytes and fibroblasts in rats *

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Abstract

AIM To study the mechanism of Fuzhenghuayu (FZHY) decoction on anti-liver fibrosis.

METHODS FZHY 10% decoction sera was incubated with rat normal subcultured hepatic stellate cells (HSC) and fibrotic primarily cultured HSC, normal and fibrotic hepatocytes and subcultured skin fibroblasts seperately. Cell intracellular and extracellular collagen synthesis rates were measured by the method of [^{3}H] Proline impulse and collagenase digestion.

RESULTS For primarily cultured HSC and hepatocytes, both of intracellular and extracellular collagen synthesis rates decreased in the drug sera group. For the normal subcultured HSC and primarily cultured hepatocytes, the extracellular collagen secretion was decreased obviously by the drug sera, and intracellular collagen synthesis rates were inhibited to some extents. For fibroblasts, both intracellular and extracellular collagen synthesis rates were inhibited somewhat, but no significant differences were found.

CONCLUSION The mechanism of FZHY decoction on anti-liver fibrosis may be associated with inhibition of liver collagen production.

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INTRODUCTION

Liver fibrosis is the common pathological feature of chronic liver diseases, and is closely associated with changes of liver cell functions. In order to investigate the mechanisms of Fuzhenghuayu decoction action on liver fibrosis, the drug serum was collected and incubated with cultured rat hepatic stellate cells (HSC), hepatocytes and fibroblasts, and then cellular functions were observed.

MATERIALS AND METHODS

Animals

Wistar male rats were purchased from Shanghai Animal Center, Chinese Academy of Sciences, among them, rats weighing 180g-200g rats were used for isolations of hepatocytes, 350g-450g rats were used for isolation of HSC. SD rats, pregnant for 12-14 days were the gifts of Shanghai Institute of Family Planning and used for skin fibroblast isolation and culture. All rats were maintained with food and water available *ad labium*.

Reagents

Minimum essential medium Eagle (MEM), 199 Medium (M199) and Dubocal modified Eagle's Medium (DMEM) were purchased from GIBCO, USA. Pronase E, type IV collangenase, Metrizimide, 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) from Sigma Co., USA. And L-[5-^{3}H] proline ([^{3}H]Pro) from Amersham Co., England.

Drug

Fuzhenghuayu (FZHY) decoction consists of *Cerdeceps, Semen Persiciae, Radix Salviae* Miltiorrhizae, etc. Shanghai Zhonghua Pharmaceutical Factory made the decoction into a kind of fluid extract. Each gram of the fluid extract contained 2703g of the above raw herbs.

Cell isolation and culture HSC isolation and culture were performed according to the modified Freidman method[1], and hepatocyte isolation according to the modified method[2]. Fibroblast followed E Zheng's method[3].

Model establishment[4] Male Wistar rats received 0.5% DMN dissolved in 0.15mol/L NaCl, at a dose of 10μl of DMN/100kg i.p., for 3 consecutive days each week for 3 weeks. The pair fed controls

received an equivalent amount of saline.

Drug sera were prepared Using our own method[5].

Grouping and drug sear incubation The cultured cells were divided into control and drug serum groups. The control was incubated with 199 medium containing 10% normal rat serum, and the drug group was incubated with 199 medium containing 10% drug serum, for 72h in HSC and fibroblast cells, for 48h in hepatocytes and 24h for fibrotic HSC.

Assay of collagen synthesis rates Greets method was used[7].

Statistics Two tails student t test was used for statistical analysis.

RESULTS

Effects on HSC collagen synthesis

The drug sera markedly inhibited the intracellular collagen synthesis in normal subcultured HSC, and both intracellular and extracellular collagen productions in fibrotic HSC.

Table 1 Effects of drug sera on HSC collagen synthesis rate (n = 4, % $\bar{x}$±s)

Group	Normal subcultured HSC		Fibrotic HSC	
	Intracellular	Extracellular	Intracellular	Extracellular
Drug sera	0.31±0.21	2.70±0.10[a]	0.12±0.09[a]	0.80±0.34[b]
Control	0.57±0.37	4.15±0.95	0.33±0.10	1.72±0.53

[a]P<0.05,[b]P<0.01,*vs* control.

Effects on hepatocyte collagen synthesis

The drug sera could inhibit extracellular collagen synthesis more obviously in fibrotic hepatocytes, than in the normal cells. The drug sera could also inhibit intracellular collagen synthesis of fibrotic hepatocytes (Table 2).

Table 2 Effects of drug sera on hepatocyte collagen synthesis rate (n = 4, % $\bar{x}$±s)

Group	Normal subcultured HSC		Fibrotic HSC	
	Intracellular	Extracellular	Intracellular	Extracellular
Drug sera	0.34±0.05	0.23±0.04[a]	0.84±0.16[a]	0.43±0.12[b]
Control	0.31±0.09	0.30±0.02	1.24±0.50	0.60±0.14

[a]P<0.05, [b]P<0.01, *vs* Control.

Effects on NIH/3T3 fibroblast collagen synthesis

The drug sera could inhibit both intracellular and extracellular collagen synthesis to some extent, but no significant difference was found (Table 3).

Table 3 Effects of drug sera on NIH/3T3 fibroblast collagen synthesis rate (n = 4, % $\bar{x}$±s)

Group	Intracellular	Extracellular
Drug sera	1.53±0.50 (6)	8.89±3.66 (6)
Control	2.03±0.75 (5)	12.62±1.03 (4)

In bracket was the case numbers.

DISCUSSION

Collagens are the main components of extracellular matrix, which play an important role in keeping liver structure and functions. If collagen production increased or decomposition decreased, its metabolism would break and lead to liver fibrosis. It was found that HSC could transform to myofibroblasts under stimulation of cytokines induced by hepatocyte injury, and was the key cell for synthesis and secretion of collagen in liver. In the paper, both normal subcultured HSC and fibrotic HSC showed significance in collagen production. Although hepatocyte has the function of collagen production, which is low in normal hepatocytes, and increased obviously in fibrotic hepatocytes. This was also observed in our study. Fibroblasts had many subtypes, all of which could produce extracellular matrix, and were used for cell models in investigating liver fibrosis instead of HSC[8,9]. In this study, rat skin fibroblast showed ability of collagen production.

Besides anti-etiology therapy, regulation of collagen metabolism, including inhibition of collagen synthesis and increase of collagen decomposition, could protect or delay the formation of liver fibrosis, while inhibition of collagen production in liver is one of key steps for anti-liver fibrosis. In our previous clinical and animal studies, Fuzhenghuayu decoction showed good effects on liver fibrosis[10-13]. In the present study, serum was collected from rats fed on Fuzhenghuayu decoction by seropharmacological method and incubated with 3 kinds of cells. The results showed that the drug sera could decrease normal and intracellular collagen synthesis of fibrotic hepatocyte, decrease extracellular collagen production in subcultured HSC and normal hepatocyte, inhabit fibroblast collagen production and intracellular collagen production in HSC to some extent, but no action on intracellular collagen synthesis in normal hepatocytes. It is suggested that one of important mechanisms of Fuzhenghuayu decoction action on liver fibrosis may be the inhibition of liver collagen production.

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