Effect of lipid on proliferation and activation of rat hepatic stellate cells (I)

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**Subject headings** Hepatic stellate cell; triglyceride; very low-density lipoprotein; cell proliferation

**Abstract**

**AIM** To study the effect of lipid (triglyceride and very low-density lipoprotein, VLDL) on proliferation and activation of rat hepatic stellate cells (HSC).

**METHODS** HSC were isolated and cultured from liver of Wistar rats by in situ perfusion with pronase and collagenase and density gradient centrifugation with Nycodenz. HSC proliferation was examined with MTT colorimetric assay.

**RESULT** Triglyceride of 12.5 mg/L had a promoting effect on proliferation of HSC ($P < 0.05$), 25, 50, 100 and 200 mg/L had no effects ($P > 0.05$), but 400 mg/L had an inhibiting effect ($P < 0.01$). VLDL of 6.25 and 12.5 mg/L had no effect on proliferation of HSC ($P > 0.05$), but increased concentration of VLDL could promote the HSC proliferation ($P < 0.05$).

**CONCLUSION** Lipid had an effect on proliferation of HSC. Triglyceride and VLDL may promote HSC proliferation and may be associated with fatty liver and hepatic fibrogenesis.

**INTRODUCTION**

Despite the early controversy over the primary cellular source of extracellular matrix proteins in liver fibrosis, compelling in vitro and in vivo experimental evidence is now available to implicate activated hepatic stellate cells (HSC) in the pathogenetic role$^{1,3}$. Mechanisms by which collagen-producing cells are activated under pathological conditions remain unknown and continue to be a topic of research interest$^{1,2,4,5}$. Although the role of hepatocytes in lipid metabolism and transportation have been discussed intensively, that of HSC was not known. More and more studies showed that HSC took part in lipid metabolism and transportation$^{6}$, their abnormality was related to the pathogenesis of fatty liver and liver fibrosis$^{7}$. In order to seek a possible explanation for the role of lipid in activation of HSC in liver fibrogenesis, we observed the effects of triglyceride and very low-density lipoprotein (VLDL) on proliferation of rat HSC.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats, weighing 400g-450g were fed ad libitum with standard rodent chow.

**Preparation of HSC**

HSCs were prepared by the methods of Friedman et al$^{8}$ and Baroni et al$^{9}$ with slight modifications. The rats were anesthetized with intraperitoneal pentobarbital (30 mg/kg). The liver was perfused in situ through the portal vein with 500 ml of calcium-free Gey’s balanced salt solution for 10 min at a flow rate of 40 ml/min-50 ml/min and then enzymatically digested with perfusate containing 0.05% collagenase (Sigma) and 0.1% pronase E (Merek) for 10 min-15 min. After being removed, the liver was cut into small pieces and incubated in 50 ml fresh GBSS-BSA containing 0.05% collagenase and 0.1% pronase E and stirred at 37°C for 30 min. After passing through gauze, cell suspension was centrifuged at 5000 g for 7 min, the supernatant discarded at the cells washed twice further with Dulbecco’s modified Eagle’s medium (DMEM) (Gibco). The cell suspension was mixed with 18% (W/V) of Nycodenz (Sigma) in GBSS without NaCl. The gradient was centrifuged at 1450
xg for 17min at 4°C. The white, diffuse, flufly band in the lower region of the DMEM layer just above the Nycodenz cushion, which contained highly enriched HSC, was gently aspirated, diluted in about 30ml DMEM and centrifuged at 450×g for 7min at 4°C. The cell pellet was suspended in the incubation medium and seeded in the culture flasks with DMEM containing 20% fetal calf serum. Sterile condition was maintained during the entire isolation and purification procedures. Cell viability was assessed by trypan blue exclusion, and cell counting were conducted in a hemocytometer.

**Culture and determination of HSC**

Purified HSC suspended in DMEM containing HEPES (15mmol), penicillin (100U/ml), streptomycin (100 mg/ml) and fetal calf serum (20% V/V) were seeded at a density of 1×10³ cells/cm² in the culture flasks. The medium was changed 20 to 24 hours after plating, and every 3 to 4 days thereafter. Cells were grown at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. To evaluate the purity of the cultures, HSC at day 2 or 3 and 7 after plating were tested by immunohistochemistry staining for desmin, lysozyme and factor VIII-related antigen, and by ultraviolet excited fluorescence microscopy at the length of 328 nm.

**Proliferation of HSC**

HSC proliferation was studied by colorimetric MTT assay[10]. HSC (1×10⁵cells/100µl) were cultured in multiwell tissue culture plates (96 well/plate) (Corning, New York, NY, USA) for 2 days and 48hrs in the presence or absence of triglyceride and VLDL. Twenty µl of MTT solution (5mg/L) was added to all wells of an assay, and plates were incubated at 37°C for 4hrs. Then 100µl dimethyl sulfoxide was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After 20min-30min at room temperature to ensure that all crystals were dissolved, the plates were read on a E-Liza Mat-300 reader, using a test wavelength of 570nm, a reference wavelength of 630nm. Plates were normally read within 1hr of adding the dimethyl sulfoxide. All samples were analyzed in pentaplicate.

**Statistical analysis**

All data were expressed as mean ±SD. Statistical differences were assessed by the standard t test and P values of <0.05 were judged to be statistically significant.

**RESULTS**

**The yeild, viability and purity of HSC**

The yield of HSC ranged from 5×10⁶ to 1×10⁷ cells per liver. HSC displayed fading green-blue fluorescence with fluorescence microscopy at a wave length of 328nm. After the first washing at 24hr, the HSC purity in culture exceeded by 95% as assessed by phase contrast microscopy and ultraviolet excited fluorescence microscopy. Viability of HSC assessed by trypan blue exclusion exceeded by 95%. HSC positive for desmin by immunohistochemistry in primary culture was above 90%, and in subculture above 95%. Lysozyme and factor VIII-related antigen were negative for HSC.

**Effect of triglyceride on proliferation of rat HSC**

Triglyceride was directly administered to HSC at concentrations of 12.5, 25, 50, 100, 200 and 400mg/L, respectively. The HSC proliferation was measured with colorimetric MTT assay. HSC proliferation in the presence of triglyceride is shown in Table 1. Compared with the contrast (0.1395±0.0276), 12.5 mg/L of triglyceride had a promoting effect on proliferation of HSC (P<0.05), 25, 50, 100 and 200mg/L had no effects (P>0.05), but 400mg/L had an inhibiting effect (P<0.01).

**Table 1 The effects of triglyceride on HSC proliferation**

| Group | Concentration (mg/L) | Value of OD |
|-------|----------------------|-------------|
| Triglyceride | 400 | 0.0990±0.0163<sub>a</sub> |
|         | 200 | 0.1226±0.0138 |
|         | 100 | 0.1212±0.0275 |
|         | 50  | 0.1450±0.0264 |
|         | 25  | 0.1637±0.0243 |
|         | 12.5| 0.1894±0.0316<sub>b</sub> |

<sub>a</sub>P<0.05, <sub>b</sub>P<0.01 vs normal control.

**Effect of VLDL on proliferation of rat HSC**

VLDL was directly administered to HSC at concentrations of 6.25, 12.5, 25, 50 and 100 mg/L of VLDL, respectively. The HSC proliferation was measured with colorimetric MTT assay. HSC proliferation is shown in Table 2. Compared with the contrast (0.1395±0.0276), 6.25 and 12.5mg/L of VLDL had no effect on proliferation of HSC (P>0.05), but 50 and 100mg/L of VLDL could promote HSC proliferation (P<0.05 or P<0.01).

**Table 2 The effects of VLDL on HSC proliferation**

| Group | Concentration (mg/L) | Value of OD |
|-------|----------------------|-------------|
| VLDL  | 6.25 | 0.1395±0.0276 |
|        | 12.5 | 0.1395±0.0276 |
|        | 25   | 0.1450±0.0264 |
|        | 50   | 0.1637±0.0243 |
|        | 100  | 0.1894±0.0316 |

<sub>P</sub><0.05 or <sub>P</sub><0.01 vs normal control.
in steatosis. Vicente et al. studied the lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor-β and promotes hepatic stellate cell activation during oxidant-stress-induced fibrogenesis. FASEB J 1997;11(11):851-857.

Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med 1997;126(2):137-145.

Vicente CP, Guzagnia RM, Borovjek R. Lipid metabolism during in vitro induction of the lipocyte phenotype in hepatic stellate cells. Mol Cell Biochem, 1997;168(1):31-39.

Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med, 1997;126(2):137-145.

Vicente CP, Guzagnia RM, Borovjek R. Lipid metabolism during in vitro induction of the lipocyte phenotype in hepatic stellate cells. Mol Cell Biochem, 1997;168(1):31-39.

Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med, 1997;126(2):137-145.

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