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The effect of retinoic acid on Ito cell proliferation and content of DNA and RNA

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Subject headings liver fibrosis; retinoic acid; Ito cell; cell culture; microspectrophotometer; DNA; RNA

INTRODUCTION

The development of liver fibrosis is due to an imbalance between synthesis and degradation of extracellular matrix. Recent studies have shown that Ito cells which are located along the sinuses of liver, can store and metabolize Vit A, and are the main cells that produce collagen, among which type I, III and IV and laminin, account for 80%-95% of the total hepatic collagen[1]. Ito cells in the course of proliferation and synthesis of collagen showed a reduction of Vit A contents and retinoic acid receptors[2]. This study was designed to investigate the effect of retinoic acid on Ito cell proliferation and the contents of DNA/RNA and to analyse further its mechanism or pathways.

MATERIALS AND METHODS

Ragents

DMEM medium (Gibco Compony); all trans-retinoic acid (RA), retinol palmitate (RP) (Sigma Company); ^{3}H-TdR (Shanghai Nuclear Energy Research Institute); Solution A: 0.1% Triton X-100, 0.08N HCl, 0.15N NaCl; solution B: 120μM acridine orange (AO), 1mM EDTA, 0.15N NaCl; vidas microspectr ophotometer analysis system.

^{3}H-TdR incorporation test

The isolation and culture of Ito cells were done by the method as published before[3]. Rat Ito cells of 5-day primary culture were suspended on DMEM me dium containing 20% bovine serum. The number of the cells was adjusted to 1×10^5/mL. One mL of the cell suspension was put into each well of the 24 -well culture plate, which contained serial concentrations of RA/RP. The control wells contained no RA/RP. After incubating for 24 h, 8 μci-^{3}H-TdR was added to each well and incubated for another 48h. The cells were collected on the F49 filter paper, fixed with 4mL 10% trichloro-acetic acid and dehydrated with 4mL alcohol. The dried filter papers were put on the bottom of the scinti bottle which contained 5 mL scintillating solution for counting the pulse per minute (CPM). The degree of DNA replication was indicated by CPM/well. We set three wells for each sample.

DNA/RNA content analysis

Ito cell culture and treatment were handled with the same procedure as above and the difference was as follows: drug treatment with RA/RP at 10^{-4}mol/L, small cover glass was put into each well of the plate to bear the growing cells. After the culture medium was discarded, the Ito cells on the small cover g lass were fixed for 30min with 70% alcohol and washed with PBS solution. Solution A (0.4mL) was added into each well, the preparation was set onice bath for reacting 15 seconds, 1.2mL solution B was then used for 8min staining. The small cover glass was put on a glass slide, fluorescent microscopic examinati on was made immediately with the inciting light wave of 488 nm. DNA was examined with a screened filter at a wave-length of 530 nm, with green in stain, whereas RNA examined with a filter at 610 nm showed a red fluorescence. Fifty cells were examined in each group with fluorescent microspectrophotometer-30 (FMSP-30); the fluorescence intensity was converted into grey scale value, corresponding to the relative aver age DNA/RNA contents of Ito cells.

RESULTS

^{3}H-TdR incorporation test

Lower densities of RA/RP (10^{-6} mol/L) had no effect on the ^{3}H-TdR incorporation of Ito cell. With higher densities ^{3}H-TdR incorporation of Ito cell was inhibited as compared with the control (P<0.05)(Table 1).

DNA/RNA content analysis

The results showed that RA/RP at 10^{-4}mol/L

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could reduce DNA/RN A- contents of Ito cells as compared control (P<0.01) (Table 2).

Table 1 ^{3}H-TdR incorporation test

Group	Dosage (mol/L)	(CPM±s)/well
Control		2866.4±253.2
Test groups		
RA	10^{-6}	2657.6±104.8
	10^{-5}	1279.4±236.4[a]
	10^{-4}	182.2±67.6[a]
RP	10^{-6}	2598.4±76.4
	10^{-5}	1182.6±154.4[a]
	10^{-4}	897.2±82.6[a]

[a]P<0.05 *vs* control group (Student's *t* test).

Table 2 DNA, RNA content analysis

Group	Dosage (mol/L)	DNA	RNA
Control		101.98±21.58	89.38±22.03
RA	10^{-4}	61.79±18.31[a]	56.31±14.72[a]
RP	10^{-4}	46.85±11.52[a]	49.20±10.12[a]

[a]P<0.05 *vs* control group (Student's *t* test).

DISCUSSION

Ito cells which have the characteristics of fibroblasts cell and myofibroblasts are the main collagen-producing cells in the liver. Bamard H[4] and Seifert WF[5] reported that RA could reduce the deposition of types I and III collagen in the CCl_4-induced liver fibrosis of the rat through its inhibitory effect on the transformation of Ito cell to myofibroblasts.

Our study showed that RA/RP could inhibit ^{3}H-TdR incorporation of rat Ito cells and reduced the DNA/RNA contents of rat Ito cells. Our previous study indicated that RA could restore retinoic acid receptor content and increase cAMP content in the primary culture of rat Ito cells[6]. The form of biologic effect of RA was similar to that of thyroxin[7], i.e. they both act on nuclear receptor resulting in a change of the second messenger and regulating the gene expression of RAR and collagen in the Ito cells. RA may be expected to be an effective antihepatofibrotic agent.

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