

G1 Cyclin

Rb - E2F

E

, * , † , †
 * . * . * . * . * . † . †

**The Expression of G1 Cyclins and Rb-E2F, and the Effect of Vitamin E
 on Hepatic Stellate Cells Activated by CCl₄**

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Background/Aims: It is obscure when the activation of hepatic stellate cells and the expression of its related factors occur in acute liver injury. Vitamin E is expected to prevent hepatic fibrosis. The aims of this study were to establish the model of hepatic stellate cell activation in acute liver injury and to confirm the effect of vitamin E for preventing hepatic fibrosis. **Methods:** Male Sprague-Dawley rats were classified into two groups. The one group received a single injection of CCl₄ and the other group received injection of vitamin E daily and a single injection of CCl₄. The serial changes of serum ALT, and [³H]thymidine uptake, -SMA, cyclin D1, CDK4, cyclin E, CDK2, Rb, E2F-1 and NF- B of stellate cells were measured. **Results:** The serial changes of serum ALT levels, [³H]thymidine uptake, and -SMA positive cells showed maximum increase at 32 hours after CCl₄ injection. However, they were significantly decreased with injection of vitamin E. CDK4, cyclin E and CDK2 showed definite band at 16, 32, 48 hours after CCl₄ injection, which diminished or disappeared with injection of vitamin E. Cyclin D1, Rb, E2F-1 and NF- B showed definite band at 32 hours after CCl₄ injection, which also diminished or disappeared with injection of vitamin E. **Conclusions:** We established an *in vivo* model of hepatic stellate cell activation in acute liver injury and confirmed the effect of vitamin E in preventing hepatic fibrosis. (**Korean J Gastroenterol 2001;38:262-269**)

Key Words: Hepatic stellate cell, Hepatic fibrosis, CCl₄, Vitamin E,

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가 가

가

가

E가

NF-kB

가

cyclin D1, CDK (cyclin dependent kinase, E2F-1 NF-kB

CDK) 4, cyclin E, CDK2, Rb,

E 가

1.

100 g Sprague-Dawley

1:3 (vol:vol)

2 mL/kg

, 2 1

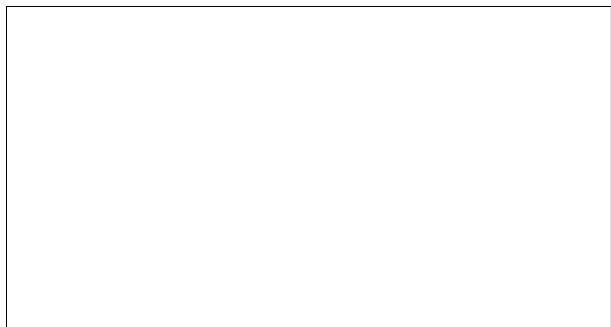


Fig. 1. The classification according to the method. The Sprague-Dawley male rats each received a single intraperitoneal injection of CCl4 in mineral oil (1:3, vol:vol) at a dose of 2 mL/kg only (group 1) or with daily intraperitoneal injection of vitamin E at a dose of 200 mg/kg from 2 days before CCl4 injection (group 2). Five rats were killed before injection of CCl4, at 8 hr, 16 hr, 32 hr, 48 hr, and 60 hr after injection of CCl4, respectively. They received [3H]thymidine at a dose 30 μCi at 4 hr before sacrifice.

200 mg/kg E(-tocopherol, Sigma Chemical Co., St. Louis, MO, U.S.A.)

8 , 16 , 32 , 48

60

5

3,4

4

30 μCi [3H]thymidine

(Amersham Pharmacia Biotech, Buckinghamshire, England)

(Fig. 1).5

2.

1)

(pronase)

(collagenase)(Boehringer Mannheim, Mannheim, Germany)

가 Hank's , 37

30

Schwartz

3-4

Nycodenz (Sigma Chemical Co.,

St. Louis, MO, U.S.A.)

(density

gradient)

(1,000 g, 4 , 20

),

3,4,6

328 nm

oil red

가 desmin

3

95%

-70

2)

0.5% NP-40, 10 mM

(NaF) 10 mM

(Na pyrophosphate)

5% (citric acid)

, 30%

(sucrose) 1%

(4,000 g, 4 , 30)

-70

5,7

3) ALT

1 mL

-70

ALT

(Hitachi)

ALT

4) (block), 1
 -SMA (Vector Laboratories, Burlingame, CA, U.S.A.)
 (biotin) 2 (DAKO, Glostrup, Denmark)
 (alkaline phosphatase)가

NBT-BCIP
 2 가 400
 -SMA 10

5) [³H]thymidine
 (Trizol) (GIBCO BRL Life Technologies, Grand Island, NY, U.S.A.)
 (12,000 g, 4, 15)
 100% 가 DNA
 DNA (DNA pellet) 0.1 M (sodium citrate) 75% 8 mM (NaOH) OD 260 nm 10 μg (beta counter)

6) Western blot
 RIPA
 (10,000 g, 4, 10)
 OD 595 nm 10 μg (CDK2, CDK4, Rb) 20 μg (cyclin D1, cyclin E), 10% (cyclin E, Rb) 16% (cyclin D1, CDK2, CDK4)
 (nitro-cellulose membrane) 2 4
 5% (nonfat milk)
 1 cyclin D1, CDK4, cyclin E, CDK2, Rb (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) HRP 가 2 (DAKO)
 (chemiluminescence, NEN™ Life Science, Boston, MA, U.S.A.)

7) Electrophoretic mobility shift assay (EMSA)
 4 15
 (12,000 g, 4, 5)
 OD 260 nm 3 μg ³²P
 E2F-1 oligonucleotide (5'T TCG CGC 3')

NF-kB oligonucleotide (5'GGG GAC TTT CCC 3'), (band shift) polydIdC 6%
 10% (acetic acid), 20%
 1
 -70
³²P
 oligonucleotide 4,5,7,8

1. ALT

ALT (IU/L) 1
 8, 16, 32, 48, 60 74 ± 20.7, 170 ± 54.3, 258 ± 83.5, 1178 ± 381.3, 274 ± 174.0, 92 ± 74.0, 2
 64 ± 21.9, 152 ± 58.9, 156 ± 62.3, 576 ± 141.5, 70 ± 35.4, 62 ± 55.4 32
 ALT가 가 가 (p<0.05),
 1 2 ALT 가 (p<0.05)(Fig. 2).

2. [³H]thymidine

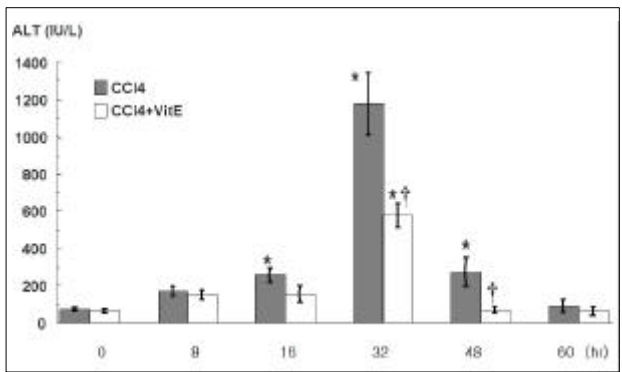


Fig. 2. The change of serum ALT level. Serum ALT level before injection of CCl₄, at 8 hr, 16 hr, 32 hr, 48 hr, and 60 hr after injection of CCl₄ showed 74 ± 20.7, 170 ± 54.3, 258 ± 83.5, 1178 ± 381.3, 274 ± 174.0, 92 ± 74.0 (IU/L) in group 1, and 64 ± 21.9, 152 ± 58.9, 156 ± 62.3, 576 ± 141.5, 70 ± 35.4, 62 ± 55.4 (IU/L) in group 2. Serum ALT level was significantly increased at 32 hr in group 1, but there was significant difference between group 1 and group 2.
 *p<0.05 compared to time 0 (control) of each group.
 † p<0.05 compared at the same time between group I & II.

³H] thymidine 1 76.4
 ± 14.7, 76.6 ± 19.7, 78.8 ± 23.8, 529.2 ± 284.8, 299.0 ± 161.6,
 179.6 ± 63.9 cpm , 71.6 ± 19.9, 90.4 ±
 9.6, 85.0 ± 24.0, 223.0 ± 86.3, 171.2 ± 47.8, 127.8 ± 19.3 cpm
 가 (p<0.05), 1 2
³H]thymidine 가
 (p<0.05)(Fig. 3).

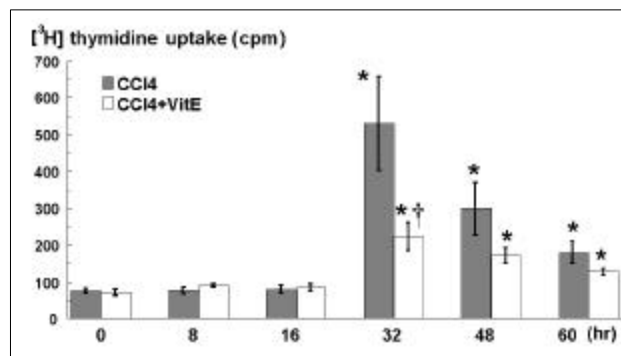


Fig. 3. The change of [³H]thymidine level. [³H]thymidine level before injection of CCl₄, at 8 hr, 16 hr, 32 hr, 48 hr, and 60 hr after injection of CCl₄ showed 76.4 ± 14.7, 76.6 ± 19.7, 78.8 ± 23.8, 529.2 ± 284.8, 299.0 ± 161.6, 179.6 ± 63.9 (cpm) in group 1, and 71.6 ± 19.9, 90.4 ± 9.6, 85.0 ± 24.0, 223.0 ± 86.3, 171.2 ± 47.8, 127.8 ± 19.3 (cpm) in group 2. [³H]thymidine level was significantly increased at 32 hr in group 1, but there was significant difference between group 1 and group 2.

*p<0.05 compared to time 0 (control) of each group.
 †p<0.05 compared at the same time between group I & II.

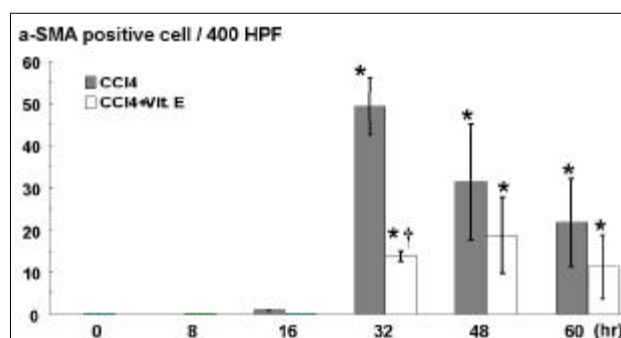


Fig. 4. The change of α-SMA expression. α-SMA in liver tissue before injection of CCl₄, at 8 hr, 16 hr, 32 hr, 48 hr, and 60 hr after injection of CCl₄ showed 0, 0, 2.0 ± 0.8, 49.2 ± 6.8, 31.2 ± 13.6, 21.8 ± 10.1 (α-SMA positive cell/400x high power field) in group 1, and 0, 0, 0, 14.8 ± 1.4, 18.0 ± 9.1, 12.2 ± 6.2 (α-SMA positive cell/400x high power field) in group 2. α-SMA activities was significantly increased at 32 hr in group 1, but there was significant difference between group 1 and group 2.

*p<0.05 compared to time 0 (control) of each group.
 †p<0.05 compared at the same time between group I & II.

3. -SMA

-SMA⁹
 1 0, 0, 2.0 ± 0.8, 49.2
 ± 6.8, 31.2 ± 13.6, 21.8 ± 10.1 (-SMA /400
) , 2 0, 0, 0, 14.8 ± 1.4, 18.0 ±
 9.1, 12.2 ± 6.2 1 16
 32 가
 (p<0.05), 1 2 -SMA
 (p<0.05)(Fig. 4).

4. G1 cyclin

G1 CDK CDK G1
 S G1 cyclin cyclin D1
 1 32 8, 16, 32, 48, 60
 , 2
 (Fig. 5). G1 cyclin cyclin E 1
 16 , 32 , 48
 2 8, 16, 32, 48, 60

(Fig. 6).

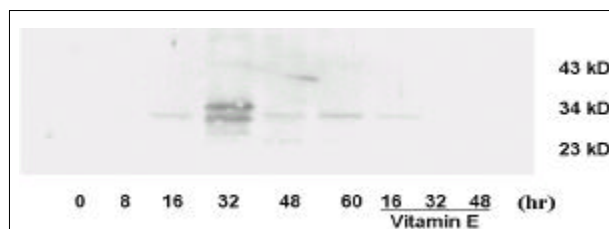


Fig. 5. The change of cyclin D1 expression. Cyclin D1 activities measured by Western blot were increased at 32 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2.

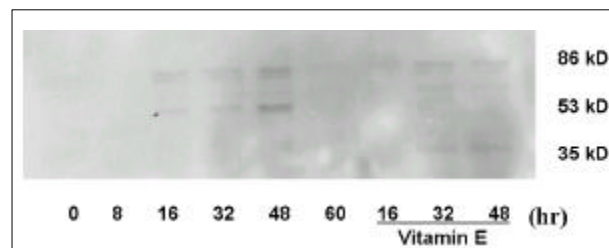
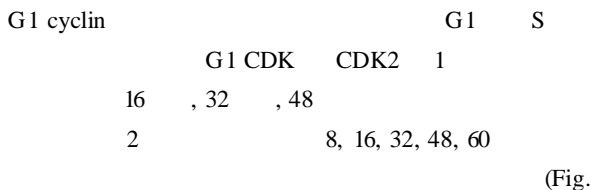


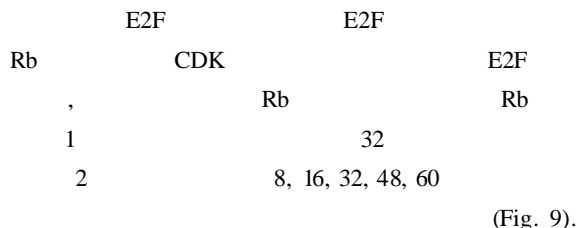
Fig. 6. The change of cyclin E expression. Cyclin E activities measured by Western blot were increased at 16 hr, 32 hr and 48 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2.

5. G1 CDK



7). G1 CDK CDK4 1
16, 32, 48
2 8, 16, 32, 48, 60
(Fig. 8).

6. Rb



7. E2F-1

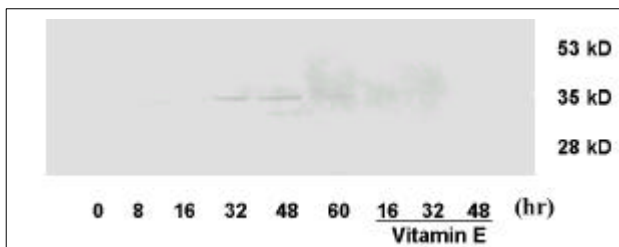
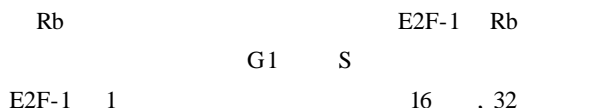


Fig. 7. The change of CDK2 expression. CDK2 activities measured by Western blot were increased at 16 hr, 32 hr and 48 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2.

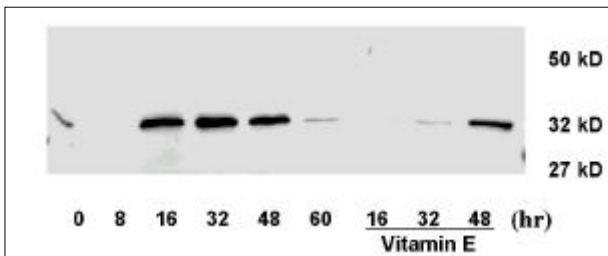


Fig. 8. The change of CDK4 expression. CDK4 activities measured by Western blot were increased at 16 hr, 32 hr and 48 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2.

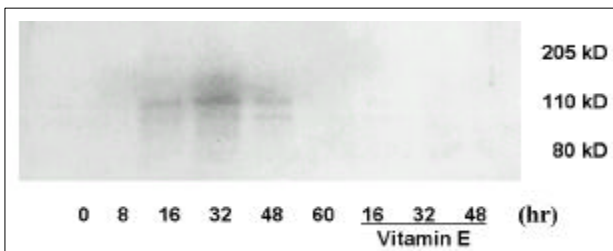


Fig. 9. The change of Rb expression. Rb activities measured by Western blot were increased at 32 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2.

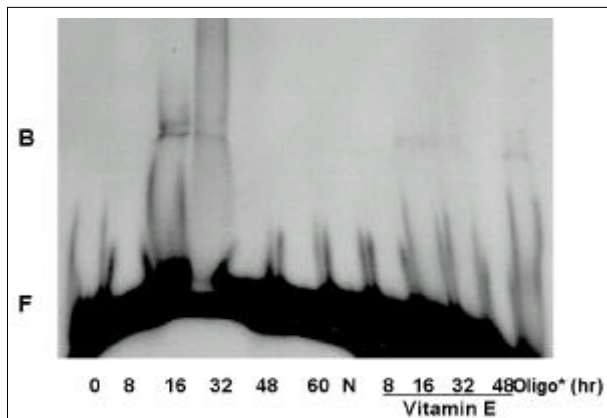


Fig. 10. The change of E2F-1 expression. E2F-1 measured by electrophoretic mobility shift assay were increased at 16 hr and 32 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2. The activities were decreased with unlabelled oligonucleotide (Oligo*).

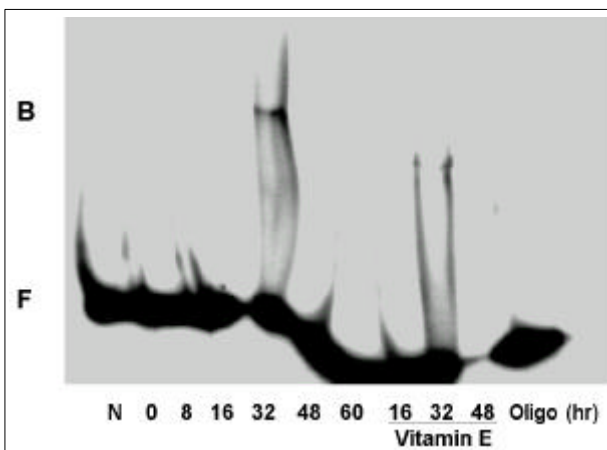


Fig. 11. The change of NF-kB expression. NF-kB measured by electrophoretic mobility shift assay were increased at 32 hr in group 1, but decreased or absent at 8 hr, 16 hr, 32 hr, 48 hr and 60 hr in group 2. The activities were decreased with unlabelled oligonucleotide (Oligo*).

2 8, 16, , 가
 32, 48, 60

oligonucleotide

(Fig. 10).

8. NF- κ B

NF- κ B

가

NF- κ B 1

32

2

8, 16, 32, 48, 60

oligonucleotide

NF- κ B

NF- κ B

가

(leukotriene),

(Fig. 11).

¹³

^{14,15}
 chrome p-450

microsomal cyto-
 (trichloromethyl radical)

가

가

(cytokine)

methyl peroxy radical)

(trichloro-

(oxygen free radical)

가

(microtubule)

가

가

가

가

(glycoprotein)

(pro-

가

teoglycan)

가가

¹⁰

가

가

³

가

⁴

TGF-beta 1

가 가

¹⁶

가

E가

가

가

E

NF- κ B

가

가

가 ^{11,12}

(myofibroblast)

¹³

E

(cytoskeletal filament)

B

C

가

가

^{17,18}

E가 ALT 가 C B

가 가

가 G1, S, G2, M 가

cyclin G1 G2 cell division cycle

(CDC) (p53, Rb E

gene) (E1A, E2F1)

8 cyclin, cyclin A, B1, B2, C, D1, D2, D3, E가 G1 G2 S M :

cyclin, G2 cyclin cyclin G1

S M , cyclin D1, CDK4, cyclin E, CDK2, Rb, E2F-1 NF-kB

G1 cyclin A-type cyclin, D-type E-type cyclin CDK2 CDK4 가 E

G1 S . G1 cyclin : Sprague-Dawley 1

D-type cyclin CDK4 1 , 2

가 2

cyclin D E

cyclin D CDK4 CDK6 8 , 16 , 32 , 48 60

CDK Rb 5

¹⁹⁻²¹ Cyclin E-CDK2 cyclin D RB ALT , , [³H] thymidine

가 RB 가

E2F, Elf-1, c-Abl 가

G1 S DNA

²²⁻²⁴ 가

G1 S G1 G1 CDK ALT, [³H]thymidine ,

cyclin D, cyclin E, CDK2, CDK4, Rb, E2F-1 32 가 가 . Cyclin E, CDK2, CDK4 16, 32, 48 , cyclin D1, Rb, E2F-1, NF-kB 32

E

16, 32, 48

가 가 E

16, 32, 48 가 .

-
- E
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