

C6 세포주 배양에서 All-trans Retinoic Acid와 13-cis Retinoic Acid의 효과*

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= Abstract =

The Effects of All-trans and 13-cis Retinoic Acid on C6 Cell Line Cultures

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Retinoic acid has been used as a trial of chemotherapeutic agent in the field of cancer therapy and resulted some success in leukemia and breast cancer. Recently, it is being tried on the malignant astrocytoma. We evaluated the effect of all-trans and 13-cis-retinoic acid on C6 cell line cultures(14 day incubation) using MTT assay and counting of cell numbers for establishing the basis of clinical trial. The cell number counting showed 51.6% and 43.1% of control in the cell number at $10^{-6}M$ concentration of all-trans and 13-cis-retinoic acid. MTT assay showed 56.4% and 46.1% of control in the optical absorbance at $10^{-6}M$ concentration of all-trans and 13-cis-retinoic acid. These results indicate the possibility of both drug as effective chemotherapeutic agents for glial cell tumors but in-vivo study will be needed for clinical trial.

KEY WORDS : All-trans retinoic acid · 13-cis retinoic acid · Brain tumor · Cell culture · MTT assay · Chemotherapeutic drug · C6 cell line.

서론

Retinoic acid(RA) all-trans, 13-cis and 9-cis re-
tinoic acid isoform()가 N-
(4-hydroxyphenyl)retinamide가
가
가 RA가
가 all-trans RA가
6)7)10)15)17)
Magrassi 10) 1가
, all-trans RA RA
. RA

가 가
3)7)9), all-trans retinoic acid 13-cis
retinoic acid가 12)22)
all-trans 13-cis retinoic acid
가 가
all trans retinoic acid 13-cis retinoic
acid C6
가

연구 범위 및 방법

C6 가

all trans retinoic acid 13 - cis retinoic acid
 $10^{-6}M$ $10^{-12}M$ $10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}M$ 7
 (cell growth inhibition)

MTT

3

1. 세포염색에 의한 생존세포수 측정(Assay for Viability by Dye uptake)

C6 10^5 24well culture flask
 2 10% DMEM(Dolbecco's minimal essential media, Hazleton, USA) . all - trans RA 13 - cis RA $10^{-5}M$ $10^{-11}M$ 7 RA 가 14 가 trypsin solution

$10^5/ml$ 10ul trypan blue 10ul trypan blue hemocytometer

$$\% = \frac{\text{---}}{\text{---}} \times 100$$

2. MTT Assay

MTT tetrazolium

formazan

C6 trypsin (200g, 5) 10cc 5×10^4 /ml 9cm petri dish pipet 200ul 2 11 well 1 37 , 5% CO₂ 1 3 7 , 14 가 MTT(Sigma, USA) 50 μ l(2mg/ml) well 가 , 37 , 5% CO₂ 4 well RA , RA 가 (60lux) . 96 well plate

formazan digital multi - channel pipette(Titertek, Finland) 가 well . 150 μ l dimethyl sulfoxide(DMSO, Sigma, USA) well 가 (plate shaker) formazan 36 10 multi - well ELISA automatic spectrometer recorder(Berhinger ELISA Processor II, Germany) 570nm (absorbance, optical density)

$$\% = \frac{\text{---}}{\text{---}} \times 100$$

결 과

1. Assay for Inhibition of Cell Growth(Fig. 1)

RA 14 C6 가 , RA All - trans RA $51.6 \pm 2.89 \times 10^6/1.9cm^2$ $10^{-10}M$ $49.7 \pm 2.52 \times 10^6/1.9cm^2$, $10^{-9}M$ $42.7 \pm 3.06 \times 10^6/1.9cm^2$, $10^{-8}M$ $37.7 \pm 2.52 \times 10^6/1.9cm^2$, $10^{-7}M$ $31.7 \pm 3.06 \times 10^6/1.9cm^2$, $10^{-6}M$ $26.7 \pm 2.51 \times 10^6/1.9cm^2$, 96.1%, 82.5%, 72.9%, 61.2%, 51.6% . 13 - cis RA $43.3 \pm 3.06 \times 10^6/1.9cm^2$

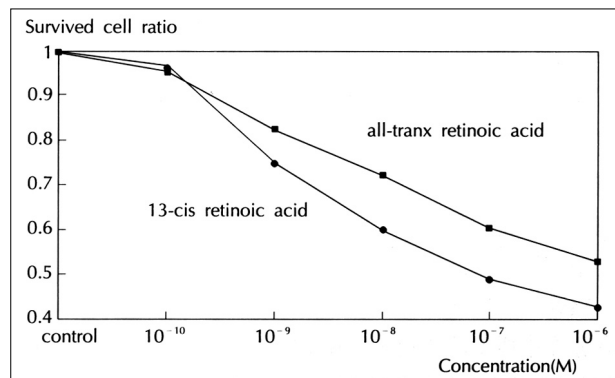


Fig. 1. The cell number counting after 14 days incubation with various concentration of retinoic acid. It showed 51.6% and 43.1% of control in the cell number at $10^{-6}M$ concentration of all-trans and 13 cis-retinoic acid respectively.

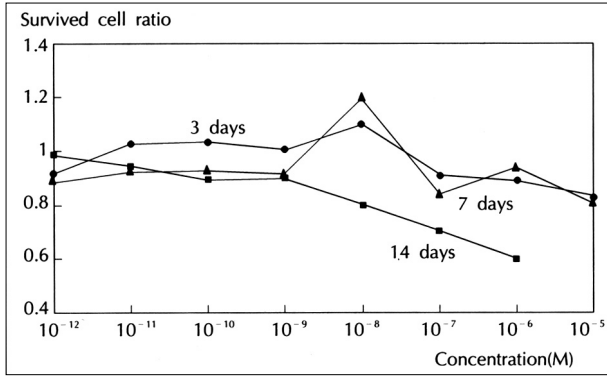


Fig. 2. The results of MTT assay after 3 day, 7day and 14day culture of C6 cell line with all-trans retinoic acid. Only the group of 14day culture with all-trans retinoic acid shows good correlation between the cell survival ratio and concentration of retinoic acid.

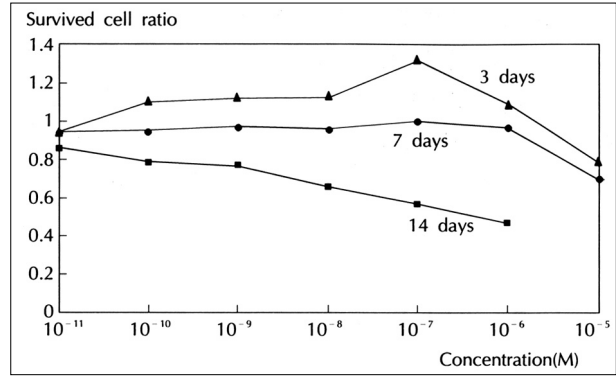


Fig. 3. The results of MTT assay after 3 day, 7day and 14day culture of C6 cell line with 13-cis retinoic acid. Only the group of 14day culture with 13-cis retinoic acid shows good correlation between the cell survival ratio and concentration of retinoic acid.

$10^{-10}M$ $42.0 \pm 2.65 \times 10^6 / 1.9cm^2$, $10^{-9}M$
 $32.7 \pm 3.06 \times 10^6 / 1.9cm^2$, $10^{-8}M$
 $26.3 \pm 2.52 \times 10^6 / 1.9cm^2$, $10^{-7}M$ 20.7 ± 2.08
 $\times 10^6 / 1.9cm^2$, $10^{-6}M$ $18.7 \pm 2.08 \times$
 $10^6 / 1.9cm^2$
 , 96.9%,
 75.3%, 60.7%, 47.6%, 43.1% .

2. MTT Assay

RA	C6	MTT
assay		, 3 7
RA		14
RA		. All - trans RA
3		$10^{-11}M$ $10^{-5}M$
		93.9 ± 24.8 , 103.4 ± 21.3 , 103.7 ± 17.4 , 97.4
		± 19.8 , 109.7 ± 9.8 , 92.2 ± 25.9 , 90.1 ± 18.4 , $82.0 \pm 13.6\%$
		, 7 91.2 ± 24.5 , 94.6 ± 20.6 ,
		93.2 ± 13.0 , 90.4 ± 22.1 , 118.7 ± 17.6 , 84.1 ± 32.8 , 94.7
		± 18.8 , $81.1 \pm 25.5\%$ (Fig. 2). 13 - cis RA 3
		$10^{-11}M$ $10^{-5}M$
		94.8 ± 14.2 , 109.5 ± 45.5 , 113.3 ± 42.5 , $113.0 \pm$
		39.1 , 133.4 ± 49.7 , 108.1 ± 40.3 , $75.8 \pm 22.0\%$,
		7 94.5 ± 5.6 , 94.1 ± 10.6 , 96.7 ± 8.0 ,
		96.3 ± 15.6 , 100.4 ± 7.9 , 95.0 ± 24.8 , $67.9 \pm 5.5\%$
		(Fig. 3).
14	All - trans RA	
	$10^{-11}M$	$95.5 \pm 12.7\%$,
$10^{-10}M$	$90.5 \pm 18.6\%$, $10^{-9}M$	89.4
± 22.4 , $10^{-8}M$	79.2 ± 12.1 , $10^{-7}M$	
69.9 ± 18.6 ,	$10^{-6}M$	56.4 ± 18.2

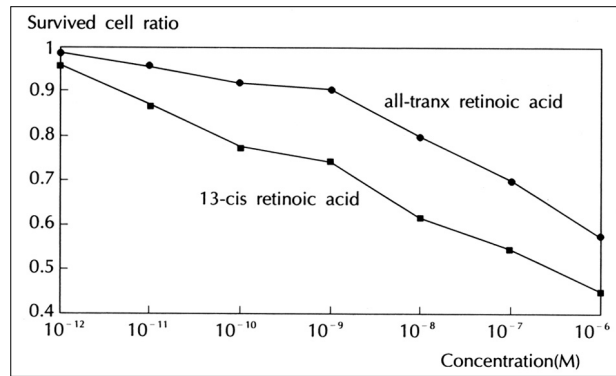


Fig. 4. MTT assay showed 56.4% and 46.1% of control in the optical absorbance at $10^{-6}M$ concentration of all-trans and 13 cis-retinoic acid respectively after 14 days incubation with various concentration of retinoic acid.

RA	$10^{-11}M$	93.0
$\pm 33.2\%$, $10^{-10}M$	$83.5 \pm 14.5\%$, $10^{-9}M$	
$81.3 \pm 16.5\%$, $10^{-8}M$	$72.6 \pm 13.6\%$,	
$10^{-7}M$	$57.0 \pm 4.0\%$, $10^{-6}M$	
$52.9 \pm 7.2\%$		
(Fig. 4).		

고찰

RA 가 all - trans
 13 - cis isomer
 , all - trans RA 13 - cis RA가
 , all - trans RA
 (10mg/kg) 2×10^{-6}

가 45¹⁸⁾, $10^{-6}M$ RA
 13 - cis RA 가 1.3 가 가 .
 17.4 가 , 30% 가 all - trans -
 RA 4 - oxo - 13 - cis - RA 13 - cis all - trans RA 가
⁵⁾¹⁸⁾
 all - trans - RA
¹²⁾ , all - trans RA 가 C6 Ch -
 , 13 - cis RA all - trans RA apman³⁾ RA $10^{-5}M$ $2.5 \times 10^{-4}M$
¹²⁾²²⁾ 13 - cis RA 가 10 70% 가 , Higashida
 가 가 ⁷⁾ C6BU - 1 $10^{-6}M$ 가
 all - trans RA , $10^{-5}M$ 15%, $5 \times 10^{-4}M$ 50%,
 가 ¹³⁾¹⁶⁾ $10^{-4}M$ 95% 가
 RA , ¹⁾ $10^{-7}M$ 10%, $10^{-6}M$
 RA receptor(RAR)¹⁴⁾ retinoid X receptor 27%, $10^{-5}M$ 27%가
 (RXR)²⁴⁾가 . RA re - $10^{-5}M$ 가
 ceptor(RAR) all - trans RA , RAR
 ligand - dependent transcriptional regulator 가 가 60mg/m² RA
¹¹⁾¹⁴⁾ 가 $0.5 - 1 \times 10^{-5}M$ ¹²⁾¹⁸⁾
 RAR retinoid X receptor(RXR) heterodimer $10^{-6}M$
 , RXR ligand - in -
 dependent auxillary factor ¹⁹⁾²⁴⁾ 가
 RA가
 13 - cis RA RAR RXR ²⁾⁴⁾ , Fischer ⁶⁾ C6
 all - trans - RA 13 - cis - RA가 RA $10^{-8}M$ 가 $10^{-7}M$
⁹⁾ , 20%, $10^{-6}M$ 55%, $10^{-5}M$ 58%
 RAR RXR , Yung ²³⁾
 . RA rat C6
 calbindin mRNA가 가 calbindin RA $10^{-7} - 10^{-6}$
 RA ²¹⁾ , Rutka
 가 RA ¹⁵⁾ U343MGA 13 - cis RA 2
²⁰⁾가 가 RA $10^{-8}M$ 20%
 , $10^{-6}M$ 60%
 murine C - 1300 RA $10^{-9}M$ 가
 $10^{-5}M$ $2.5 \times 10^{-4}M$ 10 70% $10^{-6}M$ all - trans RA 51.1%, 13 -
 가 ³⁾ N18TG - 2 cis RA 43.6%
 $10^{-6}M$ 가 , $10^{-5}M$ 15%, 가
 $5 \times 10^{-4}M$ 50%, $10^{-4}M$ 95%
⁷⁾
 RA가 ,
 Sidell¹⁷⁾ 가 $10^{-5}M$
 , $10^{-5}M$
 all - trans RA $10^{-9}M$ 25 45%,
 $10^{-8}M$ 50 65%, $10^{-7}M$ 75 80%, $10^{-6}M$ 가 14 가
 80 85%가 , Lovat ⁹⁾ RA
 $10^{-9}M$ 가

RA 가 1 2
 13)
 RA
 2
 all -
 trans RA가 13 - cis RA 가
 9) all - trans RA 13 - cis RA
 가 (p<0.01)
 가 ,
 All - trans RA
 13 - cis RA 가
 가 ,
 Kaba 8) cis - RA
 all - trans RA 13 - cis RA가
 가
 C6 가 retinoic acid, all -
 trans RA 13 - cis RA
 10⁻¹⁰M
 , RA 10⁻⁶M
 , all - trans RA
 13 - cis RA
 • : 1998 2 3
 • : 1998 2 13
 • :
 442 - 380 5
 : 0331) 219 - 5664, : 0331) 219 - 6658

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