

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 . RA tryptic solution

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

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

2. MTT Assay

MTT tetrazolium

formazan

C6 tryptic solution (200g, 5) 10cc 5×10^4 /ml pipet 9cm petri dish well 200ul 2 11 well . 37 , 5% CO₂ 1 well RA 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 $\times 10^6/1.9cm^2$

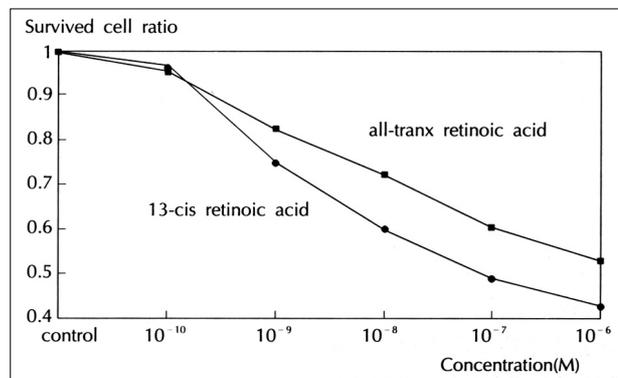


Fig. 1. The cell number counting after 14 days incubation with various concentration of retinoic acid. It showed 51.6% and 43.1% of control in the cell number at $10^{-6}M$ concentration of all-trans and 13 cis-retinoic acid respectively.

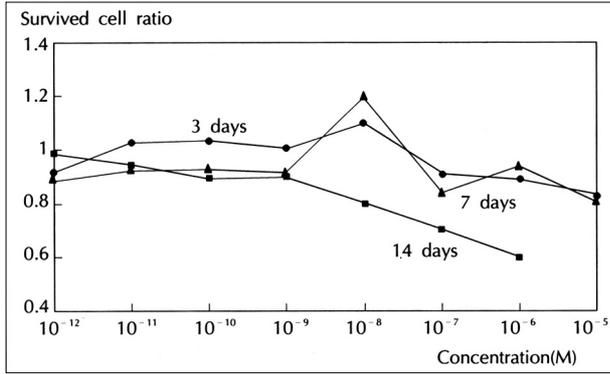


Fig. 2. The results of MTT assay after 3 day, 7day and 14day culture of C6 cell line with all-trans retinoic acid. Only the group of 14day culture with all-trans retinoic acid shows good correlation between the cell survival ratio and concentration of retinoic acid.

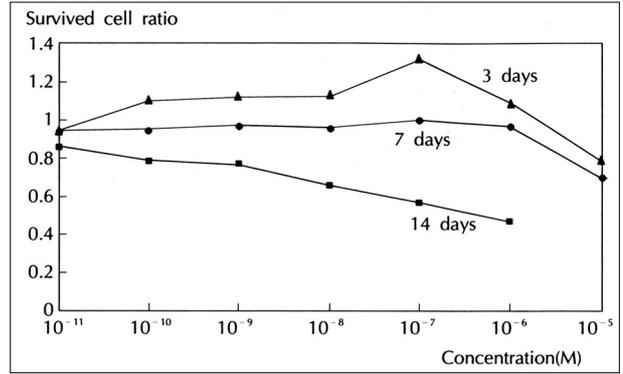


Fig. 3. The results of MTT assay after 3 day, 7day and 14day culture of C6 cell line with 13-cis retinoic acid. Only the group of 14day culture with 13-cis retinoic acid shows good correlation between the cell survival ratio and concentration of retinoic acid.

$10^{-10}M$ $42.0 \pm 2.65 \times 10^6 / 1.9cm^2$, $10^{-9}M$
 $32.7 \pm 3.06 \times 10^6 / 1.9cm^2$, $10^{-8}M$
 $26.3 \pm 2.52 \times 10^6 / 1.9cm^2$, $10^{-7}M$ 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

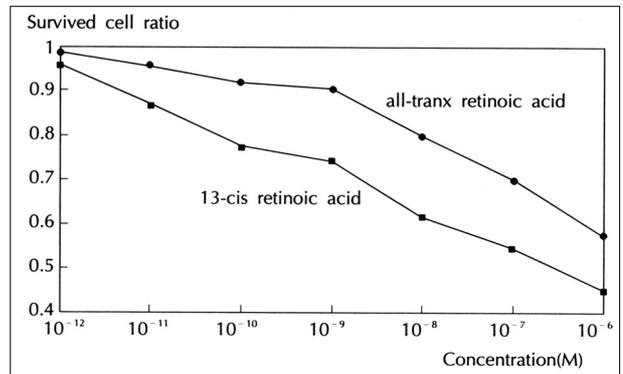


Fig. 4. MTT assay showed 56.4% and 46.1% of control in the optical absorbance at $10^{-6}M$ concentration of all-trans and 13 cis-retinoic acid respectively after 14 days incubation with various concentration of retinoic acid.

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 $10^{-7} - 10^{-6}$
²¹⁾ , Rutka
 RA 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

References

1) . . : Dexamethasone, 6 -methylprednisolone retinoic acid가 C6
 25 : 269-277, 1996
 2) Allenby G, Bocquel M-T, Saunders M, et al : Retinoic acid receptors and retinoid X receptors : Interactions with endogenous retinoic acids. *Proc Natl Acad Sci USA* 90 : 30-34, 1993
 3) Chapman SK : Antitumor effects of vitamin A and inhibitors of ornithine decarboxylase in cultured neuroblastoma and glioma cells. *Life Sci* 26 : 1359-1366, 1980

4) Crettaz M, Baron A, Siegenthaler G, et al : Ligand specificities of recombinant retinoic acid receptors RAR and RAR. *Biochem J* 272 : 391-397, 1990
 5) Finklestein JZ, Krailo MD, Lenarsky C, et al : 13-cis retinoic acid (NSC122758) in the treatment of children with metastatic neuroblastoma unresponsive to conventional chemotherapy : Report from the Children's Cancer Study Group. *Med Ped Oncol* 20 : 307-311, 1992
 6) Fischer I, Nolan CE, Shea TB : Effects of retinoic acid on expression of the transformed phenotype in C6 glioma cells. *Life Sci* 41 : 463-470, 1987
 7) Higashida H, Miki N, Ito M, et al : Cytotoxic action of retinoid butenolides on mouse neuroblastoma and rat glioma cells. *Int J Cancer* 33 : 677-681, 1984
 8) Kaba SE, Langford LA, Yung WKA, et al : Resolution of recurrent malignant ganglioglioma after treatment with cis-retinoic acid. *J Neuro-Onco* 30 : 55-60, 1996
 9) Lovat PE, Irving H, Malcolm AJ, et al : 9-cis retinoic acid-better retinoid for the modulation of differentiation, proliferation and gene expression in human neuroblastoma. *J Neuro Onco* 31 : 85-91, 1997
 10) Magrassi L, Butti G, Pezzotta S, et al : Effects of vitamin D and retinoic acid on human glioblastoma cell lines. *Acta Neurochir (Wien)* 133 : 184-190, 1995
 11) Mangelsdorf DJ, Evans RM : Vitamin A receptors : New insights on retinoid control of transcription. In : Morriss-Kay, G (ed) *Retinoids in Normal Development and Teratogenesis*. Oxford University Press, Oxford, 1992, pp27-50
 12) Meyskens FL Jr, Goodman GE, Alberts DS : 13-cis retinoic acid : Pharmacology, toxicology, and clinical applications for the prevention and treatment of human cancer. *Crit Rev Oncol Hematol* 3 : 75-101, 1985
 13) Phuphanich S, Scott C, Fischbach AJ, et al : All-trans-retinoic acid : A phase II Radiation Therapy Oncology Group study (RTOG 91 -13) in patients with recurrent malignant astrocytoma. *J Neurooncol* 34 : 193-200, 1997
 14) Rudfern CPF : Retinoic acid receptors. *Pathobiology* 60 : 254-263, 1992
 15) Rutka JT, Armond SJ, Giblin J, et al : Effect of retinoids on the proliferation, morphology and expression of glial fibrillary acidic protein of an anaplastic astrocytoma cell line. *Int J Cancer* 42 : 419-427, 1988
 16) Shepherd FA : Alternatives to chemotherapy and radiotherapy as adjuvant treatment for lung cancer. *Lung Cancer* 17 Suppl 1 : S121-S136, 1997
 17) Sidell N : Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells in vitro. *J Natl Cancer Inst* 68 : 589-593, 1982
 18) Smith MA, Adamson PC, Balis FM, et al : Phase I and pharmacokinetic evaluation of all-trans retinoic acid in pediatric patients with cancer. *J Clin Oncol* 10 : 1666-1673, 1992

- 19) Stunnenberg HG : *Mechanisms of transactivation by retinoic acid receptors. BioEssays 15 : 309-315, 1993*
- 20) Takatsuka J, Takahashi N, Luca LM : *Retinoic acid metabolism and inhibition of cell proliferation : An unexpected liaison. Cancer Res 56 : 675-678, 1996*
- 21) Wang YZ, Christakos S : *Retinoic acid regulates the expression of the calcium binding protein, calbindin-D28K. Mol-Endocrinol 9 : 1510-1521, 1995*
- 22) Windhorst DB, Nigra T : *General clinical toxicology of oral retinoids. J Am Acad Dermatol 6 : 675, 1982*
- 23) Yung WKA, Lotan R, Lee P, et al : *Modulation of growth and epidermal growth factor receptor activity by retinoic acid in human glioma cells. Cancer Res 49 : 1014-1019, 1989*
- 24) Zhang X-K, Hoffmann B, Tran PBV, et al : *Retioid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. Nature 355 : 441-445, 1992*