

Cyclosporin A가 in vitro

1 . 2 . 2 . 2 . 2 . 2 . 1 . 1

, 1
2

I.

Cyclosporin A(CsA)

9,10).
CsA가

1,2). CsA 가

가

가

11,12) CsA

, , , ,

3,4).

azathioprin

Alkaline phosphatase (ALP)

3)

가 CsA 가 가

12,13).

CsA

CsA

1980

5,6)

, CsA

15 - 45mg/kg, 45 - 95mg/kg

CsA hormone(PTH), prostaglandin E₂ amin D

Parathyroid interleukin - 1, 1,25 dihydroxyvit -

7). CsA

1983

14,15,16). CsA

가

8).

가

Bartold (1989)

CsA

CsA

CsA가

* 1999

:

134

120 - 752

17). CsA
 tumor necrosis factor -
 interferon - 18)

CsA가
 가
 19).
 21 - 24). CsA
 14,17,19)
 25,26)
 Orcegel 12)
 가
 27).
 CsA
 CsA
 CsA
 CsA가
 가
 CsA가
 ALP
 2000 × g

MC3T3
 Alpha Minimum Essential Medium(-
 MEM) Antibiotic antimycotic , Fetal
 Bovine Serum(FBS) GiBCo BRL(USA)
 , ascorbic acid(AA), - glyc-
 erophosphate, CsA, ALP kit(ALP - 10)
 Sigma(USA)
 collagenase
 dispase Wako(Japan)
 Sircol collagen assay kit Biocolor
 Ltd.(USA/Canada)
 (2) CsA
 CsA 가
 CsA 1mg 100μℓ ethanol
 10μℓ Tween 20 가
 - MEM 890μℓ 가 10⁻⁶g/Mℓ
 2.
 (1)
 1 ICR 10
 가
 , 10 Mℓ (0.2% collage-
 nase 0.1% dispase가 - MEM
) 37 10
 CsA가 10 Mℓ
 가 CsA가 37 20
 , ALP 3
 . 2000 × g 5

II.

1.
 (1)
 1 ICR

(2)
 MTT
 96 well
 MC3T3 well 1 × 10⁵
 . 2
 CsA가 0.5, 1, 3μg/Mℓ

2 well 50 μ l . 20 μ l 1 M ALP - 10(Sigma, USA) 30 30 2 p - nitrophenol

MTT (5mg/Ml) 가 37 4

200 μ l dimethyl sulfoxide(DMSO) formazan 405 nm

570nm (5) mRNA Northern blot

(3) CsA Trizol (GIBCO BRL, USA) RNA 20 μ g formaldehyde 1% agarose gel Nylon plus membrane ultraviolet radiation RNA tube

Sircol collagen assay kits 24 well 0.5, 1 가 100 μ l 1M hybridization buffer (0.1mg/Ml salmon sperm DNA가 50% formamide/5x Denhardt's /5xSSC/0.5% SDS) 가 42 가 hybrid minihybridization oven 30 prehybrid ALP hybridization RNA cDNA

3 μ g/Ml CsA가 0.5, 1 가 100 μ l 1M sircol dye 가 30 5000 xg 10 Sircol dye 1M 530nm type I

(4) Alkaline phosphatase 24 well ascorbic acid(50 μ g/Ml) - glycerophosphate(10 mM) CsA가 3 10 well (0.2% collagenase, 0.1% dispase) 100 μ l 가 37 20 , 1.5M tube

PBS 2 100 μ l 0.1% triton X - 100(Sigma, USA) 가 30 . 5000 x g 10 ALP

RNA 20 μ g formaldehyde 1% agarose gel Nylon plus membrane ultraviolet radiation RNA tube hybridization buffer (0.1mg/Ml salmon sperm DNA가 50% formamide/5x Denhardt's /5xSSC/0.5% SDS) 가 42 가 hybrid minihybridization oven 30 prehybrid ALP hybridization RNA cDNA

[³²P]dCTP(3,000 Ci/mmol, Dupont NEN

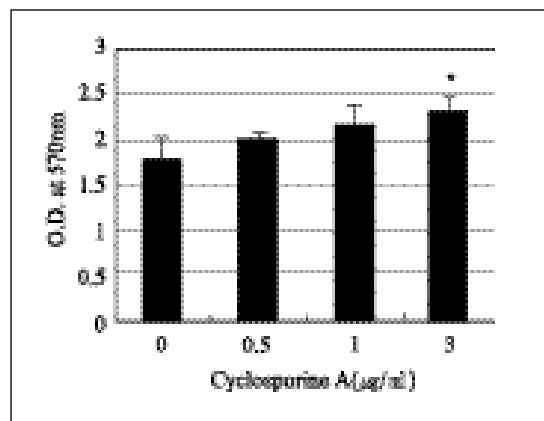


Figure 1. Effect of CsA on proliferation of mouse Calvarial cell. Values represents the mean \pm S.D. from 3 independent experiments. * : Significantly different from the non treated group,

Table 1. Effect of CsA on proliferation of mouse Calvarial cell

CsA con. ($\mu\text{g}/\text{Ml}$)	0	0.5	1	3
O.D.	1.8 ± 0.25	2 ± 0.09	2.16 ± 0.21	$2.31 \pm 0.15^*$

Values are mean \pm S.D., n=3

*Significantly different from the non treated group, P<0.05

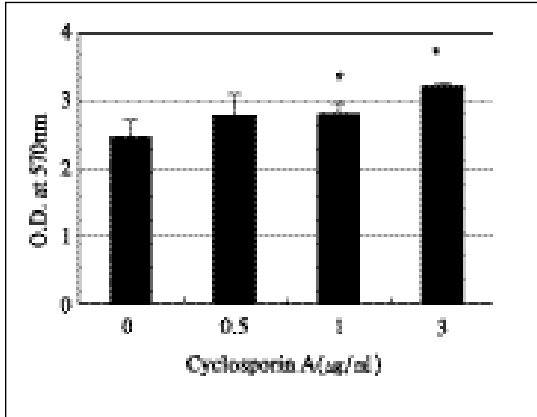


Figure 2. Effect of CsA on proliferation of MC3T3 cell .

Values represents the mean \pm S.D. from 6 independent experiments.

: * Significantly different from the non treated group,

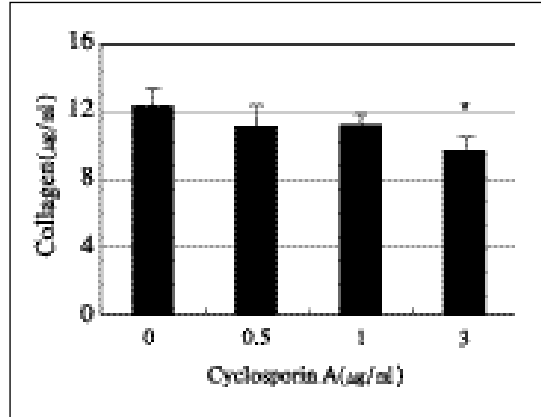


Figure 3. Effect of CsA on Collagen synthesis of mouse Calvarial cell.

Values represents the mean \pm S.D. from 3 independent experiments.

: * Significantly different from the non treated

Table 2. Effect of CsA on proliferation of MC3T3 cell

CsA con. ($\mu\text{g}/\text{Ml}$)	0	0.5	1	3
O.D.	2.45 ± 0.25	2.77 ± 0.30	$2.78 \pm 0.18^*$	$3.20 \pm 0.05^*$

Values are mean \pm S.D., n=6

*Significantly different from the non treated group, P<0.05

Table 3. Effect of CsA on Collagen synthesis of mouse Calvarial cell

CsA con. ($\mu\text{g}/\text{Ml}$)	0	0.5	1	3
Collagen ($\mu\text{g}/\text{Ml}$)	12.70 ± 2.13	11.19 ± 2.03	10.88 ± 0.96	$9.88 \pm 1.18^*$

Values are mean \pm S.D., n=3

*Significantly different from the non treated group, P<0.05

Research Products, Boston, MA, USA)
 random primed DNA labeling
 kit(GIBCO BRL, USA)
 . 2.4kb rat ALP cDNA insert. Nylon

membrane - 70
 Kodak X - OMAT film
 RNA 28S
 rRNA

(6) Nonparametric Test Mann-Whitney Test

III.

1. MC3T3

CsA
 $\mu\text{g}/\text{Ml}$. 2
 MTT 3
 $\mu\text{g}/\text{Ml}$ 가 가
 (Figure 1, Table1).

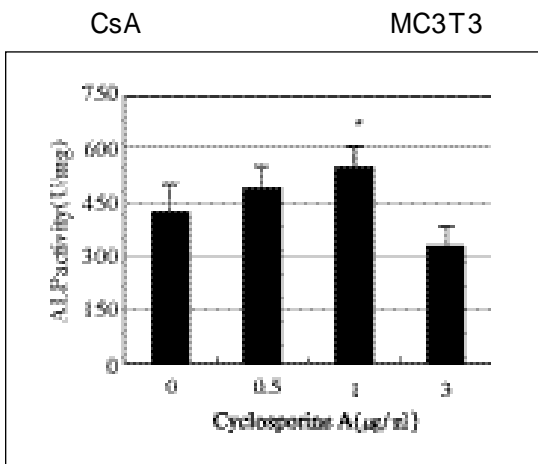


Figure 4. Effect of CsA on ALP activity of mouse Calvarial cell.

Values represents the mean \pm S.D. from 5 independent experiments.

: * Significantly different from the non treated

Table 4. Effect of CsA on ALP activity of mouse Calvarial cell

CsA con. ($\mu\text{g}/\text{Ml}$)	0	0.5	1	3
ALP activity (U/mg)	417.10 \pm 80.74	486.10 \pm 61.14	547.29 \pm 61.46*	327.46 \pm 56.38

Values are mean \pm S.D., n=5

*Significantly different from the non treated group, P<0.05

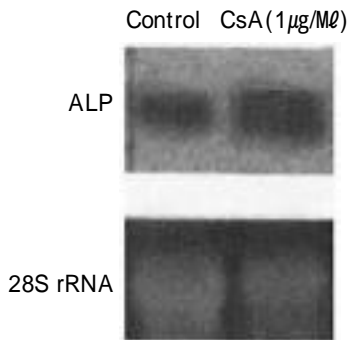


Figure 5. Expression of ALP mRNA of CsA treated mouse Calvarial cell.

Expression of ALP mRNA was analyzed by Northern blot analysis as described in material and methods.

(Figure 2, Table 2).

CsA
 CsA 1, 3 $\mu\text{g}/\text{Ml}$ 가 가
 2.
 CsA가
 (Figure 3, Table 3). CsA
 , CsA 0.5, 1, 3 μg
 /Ml
 CsA 3 $\mu\text{g}/\text{Ml}$

3. ALP

CsA

CsA가 10 ALP (Figure 4, Table 4). CsA 1 $\mu\text{g}/\text{M}\ell$ ALP 가 .

4. ALP mRNA CsA CsA가 1 $\mu\text{g}/\text{M}\ell$ RNA ALP (Figure 5). ALP가 CsA 1 $\mu\text{g}/\text{M}\ell$ ALP mRNA 가 .

IV. CsA CsA (1,4) ROS cell type I collagen mRNA가 CsA가 28). , Schincaglia GP(1992) CsA가 -procollagen 3 가 31) CsA CsA ALP ROS CsA ROS CSA가 1 - 10 $\mu\text{g}/\text{M}\ell$ ALP 1 - 2 28,29) Boran (1996) CsA

CsA가 CsA가 9,10) MC3T3 CsA 가 CsA , CsA 3 $\mu\text{g}/\text{M}\ell$ 가 가 . , CsA가 Laurie (1992)²⁸⁾ Osteosarcoma ROS 17/2.8 cell(Rat Osteoblast - like Osteosarcoma Cell) CsA 가 Laurie 5 $\mu\text{g}/\text{M}\ell$, Takaaki 1 10 $\mu\text{g}/\text{M}\ell$ CsA가 CsA Takaaki(1998)²⁹⁾ CsA 가 .

ALP 가 가 12).
 CsA V.
 10 ALP
 CsA 1 $\mu\text{g}/\text{Ml}$ ALP CsA가 가
 가 3 $\mu\text{g}/\text{Ml}$ CsA가 ,
 ALP 가 ALP MTT , Sircol
 , CsA ALP ALP
 CsA RNA ALP Northern blot
 mRNA , ALP가 1. CsA 3 $\mu\text{g}/\text{Ml}$
 , CsA (1 $\mu\text{g}/\text{Ml}$) ALP 가
 mRNA 가 2. CsA 1 3 $\mu\text{g}/\text{Ml}$
 24 - 48 CsA가 MC3T3 가
 ALP 3. CsA 3 $\mu\text{g}/\text{Ml}$
 10 ALP
 . ALP
 CsA가 ALP 가 ALP mRNA 가
 가
 CsA CsA가
 ALP 가 , CsA
 CsA
 CsA

VI.

Osteosarcoma
 CsA

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4. , , , ,
 , , : Cyclosporin A

ALP
 가 CsA ,

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- Abstract -

The Effect of Cyclosporin A on Osteoblast in vitro

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Cyclosporin A (CsA) is an immunosuppressive agent widely used for preventing graft rejecting response in organ transplantation. The basic properties of CsA to osteoblast has not been well known yet. A better understanding of the mechanisms of CsA function on bone could provide valuable information regarding basic properties of bone remodeling, pharmacotherapeutic intervention in metabolic bone disease, and the consequences of immunosuppression in bone physiology.

The purpose of this study was to investigate the effect of CsA on osteoblast by evaluating parameters of proliferation, collagen synthetic activity, alkaline phosphatase activity, and ALP mRNA expression in mouse calvarial cell.

1. CsA (3 μ g/Ml) treated mouse calvarial cell showed statistically significant increase in cell proliferation. (P<0.05)
2. CsA (1, 3 μ g/Ml) treated MC3T3 cell line showed statistically significant increase in cell proliferation.
3. The amount of collagen of CsA (3 μ g/Ml) treated mouse calvarial cell was decreased statistically significantly.
4. Alkaline phosphatase activity was

increased statistically significantly in CsA treated group (1 μ g/Ml).

5. mRNA expression of ALP was increased in CsA treated group

These results suggest that CsA could affect bone remodeling by modulating proliferation & differentiation of osteoblast.

Key word; Cyclosporin A (CsA), Mouse Calvarial Cell, Osteoblast, Collagen Synthesis, Alkaline Phosphatase, Proliferation