

Bisphosphonate가

가 Paget's disease,
 가 4 - 6),
 4,5,7 - 10),
 etidronate disodium
 가 1000
 alendronate sodium
 . bisphosphonate
 11 - 15), lactic acid
 16,17), proton 14), lysosomal
 enzyme 18), prostaglandin
 19).
 Sharpey's fiber가 Bisphosphonate
 bisphosphonate
 가 1,2),
 13,20 - 22).
 Giuliani 13) bisphosphonate가
 , Garcia -
 Moreno 22)
 bisphosphonate
 가, osteonectin
 가 3).
 bisphosphonate

가
Lekic²³⁾ etidronate
disodium

DMEM

3

4 - 6

(Olympus,
Japan)

alendronate sodium
etidronate disodium 가

2.

가

96 well plate (Nunc, Denmark) well
1 × 10⁴ cell/200 μl

10⁻¹² mol/L 10⁻⁶ mol/L
alendronate sodium (Merk, USA)

etidronate disodium(,)

II.

1.

가

가

3

3

phosphate - buffered saline
(PBS; Sigma, USA) 3
1/3

, 7 trypan blue

MTT (Dimethyl thiazol - 2 - YL - 2,5 -
diphenyl tetrazolium bromide)

2% (100 U/ml ampicillin,
100 μg/ml streptomycin, Gibco, USA)가

PBS 2

PBS 5 . 1 mm²

0.4% trypan blue (Gibco, USA)

35 mm (Nunc, Denmark)

. MTT

가 . 30

1/10 20 μl MTT (Sigma, USA)

가 3 37 , 5%

1% 10% fetal bovine
serum (FBS; Gibco, USA) 가 Dul -
becco's modified Eagle's medium (DMEM;
Gibco, USA) 가 ,

, 3 MTT

3 20 30

DMSO (Dimethyl sulfox -
amide, Sigma, USA) 50 μl 가

(confluency)

fumazone ELISA

PBS 0.05% trypsin - 0.53 mM
EDTA (Gibco, USA) 1 ml 가 37 ,
5% 5 .

immunoplate (Nunc, Denmark)

가 10% FBS가

ELISA reader (Emax precision microplate
reader, Molecular Devices Corp., USA)

DMEM 가

630 nm 570 nm

1% 10% FBS가 가

3.

가

96 well plate well 1 × 10⁴ cell/200 μl
 10⁻¹ mol/L 10⁻¹² mol/L 10⁻⁶ mol/L
 alendronate sodium etidronate disodium 가 가
 2 5 3
 PBS 1% Triton-X 100 (Sigma, USA) 50 μl 가 20 37, 5%
 405 nm 1, 2 40 μl (Sigma, USA) 가
 p - NPP (4 mM p - nitrophenyl phosphate)

ascorbic acid, 10 mM - glycerophosphate (Sigma, USA), 10⁻⁷ M dexamethasone (Sigma, USA) 가 3 50 μg/ml
 28 3% formalin - PBS solution 500 μl 가 10
 . PBS 2
 Ca⁺⁺ 2% alizarin red (pH 4.1 - 4.3, Sigma, USA) 15 , 3

III.

4. 가

96 well plate well 2 × 10⁴ cell/500 μl DMEM
 10⁻¹ mol/L 10⁻¹² mol/L 10⁻⁹ mol/L 10⁻⁶ mol/L
 alendronate sodium etidronate disodium 가 가

1.

1) Alendronate sodium

2

, 7

10⁻⁹ mol/L

. 2

10⁻¹⁰

mol/L

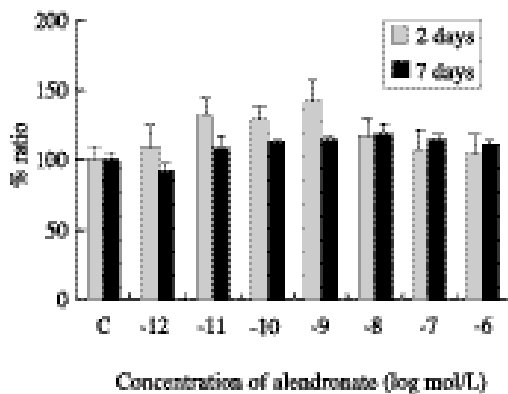


Figure 1. Effect of alendronate on the proliferation of human periodontal ligament cells (C : control).

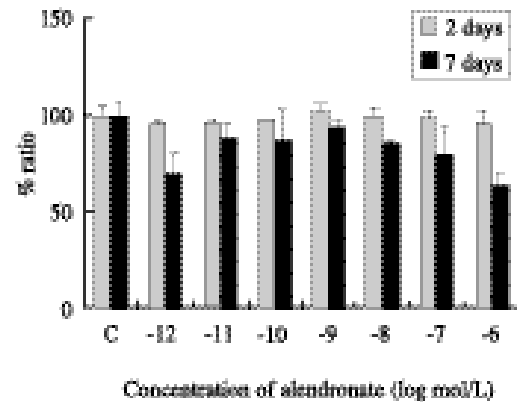


Figure 2. Effect of alendronate on the cellular activity of human periodontal ligament cells (C : control).

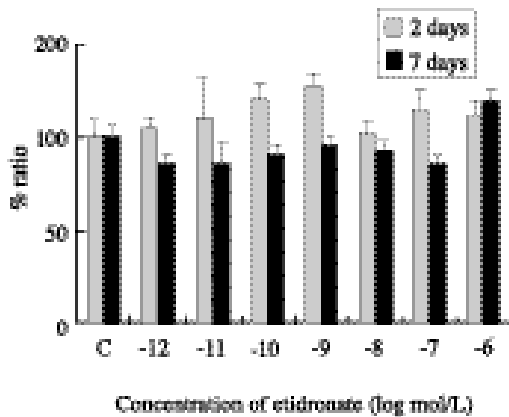


Figure 3. Effect of etidronate on the proliferation of human periodontal ligament cells (C : control).

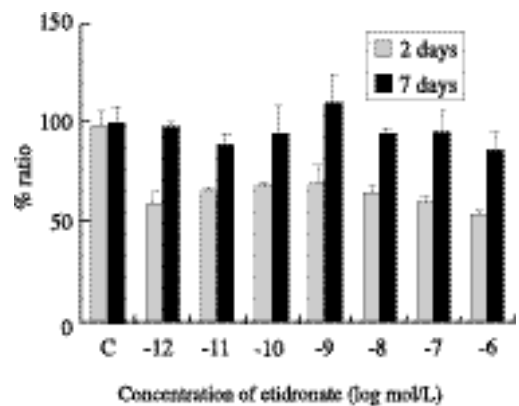


Figure 4. Effect of etidronate on the cellular activity of human periodontal ligament cells (C : control).

(Photo 1, 2).

2.

2) Etidronate disodium

1) Alendronate sodium

2

2

, 7

가

10^{-8} 10^{-6} mol/L

10^{-9} mol/L

가

7

(Photo 3).

10^{-12} mol/L

가

10^{-8} mol/L

가

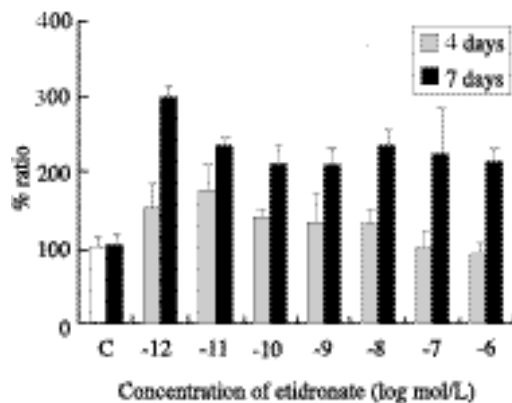


Figure 5. Effect of alendronate on the alkaline phosphatase activity of human periodontal ligament cells (C : control).

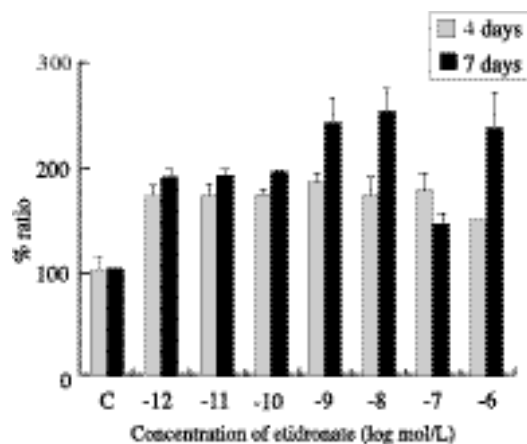


Figure 6. Effect of etidronate on the alkaline phosphatase activity of human periodontal ligament cells (C : control).

2 . MTT
7 가 (Figure 1, 2).

2) Etidronate disodium
2 가
10⁻⁹ mol/L 가
7 10⁻⁶ mol/L
가

. MTT
2
, 7
(Figure 3, 4).

3.

1) Alendronate sodium
4 10⁻⁷, 10⁻⁶
mol/L 가 가

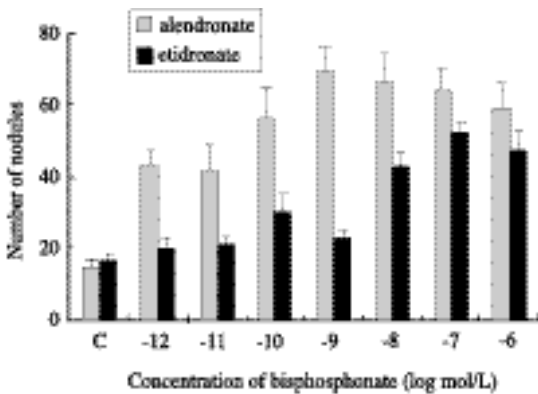


Figure 7. Effect of bisphosphonate on formation of mineralized nodules by human periodontal ligament cells (C: con-

10⁻¹¹ mol/L 가
7 가 10⁻¹² mol/L 가
(2)(Figure 5).

2) Etidronate disodium
4 가 가 . 7
가
10⁻⁸ mol/L 가
(1.6)(Figure 6).

4.

가
alizarin red
bisphosphonate
(Photo 4).

1) Alendronate sodium
가
alizarin red
가
(Photo 5).

가 10⁻⁹ mol/L 가
(3.8)(Figure 7).

2) Etidronate disodium
Alendronate sodium
가
(Photo 6).

가 10⁻⁷ mol/L 가
(2.3)(Figure 7).

IV.

. Meraw³³⁾ Meraw

Reeve³⁴⁾ bisphosphonate 가

4) 가 , bisphosphonate가

prostaglandin, IL - 1

가

24 - 27).

1)

, 2)

PDGF - BB, IGF - 1, TGF -

가 , 3)

28,29).

4)

가

35)

Lin³⁰⁾ 가

Garcia - Moreno²²⁾ 10⁻¹²~10⁻¹ mol/L alendronate sodium 가

, Giuliani¹³⁾

10⁻¹⁴ 10⁻⁵ mol/L

bisphosphonate 가 가

alendronate sodium 10⁻¹³ 10⁻⁴ mol/L etidronate disodium 가

11 - 15).

가

. Lekic²³⁾ etidronate disodium 5 × 10⁻⁵ mol/L, 2 × 10⁻⁴ mol/L 가 bisphosphonate가

bisphosphonate가

4,5,7 - 10).

Igarashi³¹⁾

bisphosphonate

가

alendronate sodium

가

가

, Teronen³²⁾

etidronate disodium 10⁻¹² 10⁻⁶ mol/L

bisphosphonate가

가

(neu -

trophil collagenase MMP - 8)

bisphosphonate

가 가
가

, alendronate sodium

1)

2

가

, 2)

가

가

, 7

가

(7), 3) 가

2 , 7

(14) 4)

. Etidronate disodium

가 (19)

40). Cho 41)

2

50 µg/Ml

가

, 7

ascorbic acid, 10 mM - glycerophosphate, 5 µ M dexamethasone 가

2 , 7

Mukai 42)

가

Giuliani 13)

가

alendronate sodium

etidronate

가

disodium

가 가

가

가

가

가

가 , alen -
dronate sodium etidronate disodium

,

36,37).

가 가

Giuliani

DeBernard³⁸⁾

가

13)

가

alendronate sodium etidronate disodium

가

가 가

Bisphosphonate

11 - 15)

가

Giuliani 13)

가 ,

4,5,7 - 10),

31)

39).

32 - 34),

Lekic 23)

, alendronate sodium

etidronate disodium

phosphonate가

phosphonate가

alendronate sodium 10⁻¹²~10⁻⁸ mol/L, etidronate disodium 10⁻⁹~10⁻⁶ mol/L

가

가

bisphosphonate
가가

V.

Bisphosphonate가

1. Alendronate sodium

2, 7

가

2

, 7

4, 7

가

가

.

2. Etidronate disodium

2

가

, 7

2, 7

4, 7

가

가

bisphosphonate

가

VI.

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(1)



Photo 1

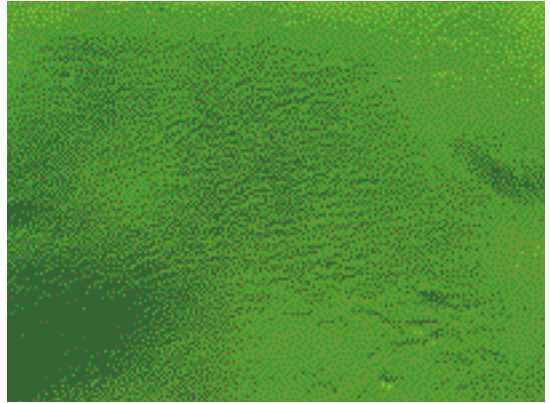


Photo 2

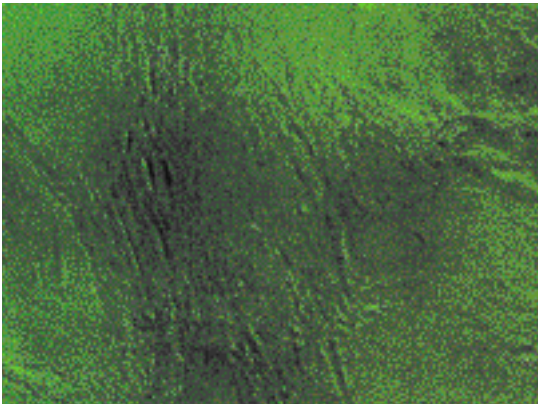


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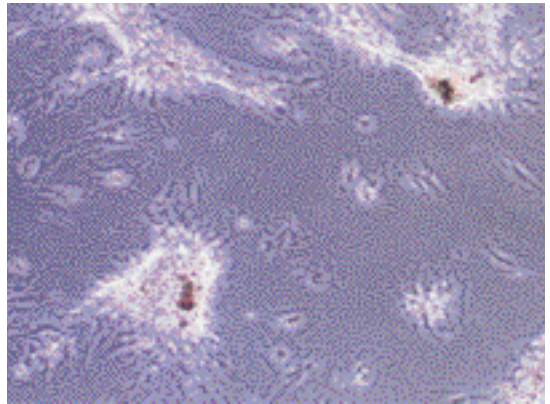


Photo 4

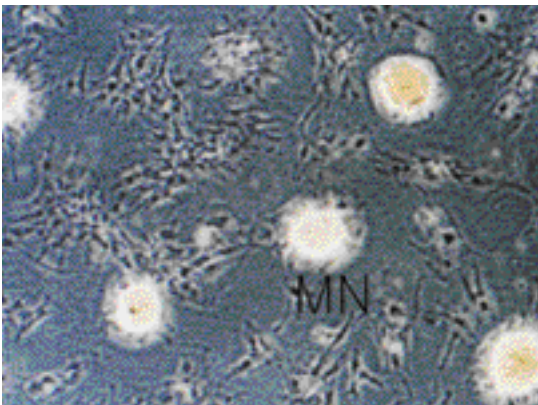


Photo 5

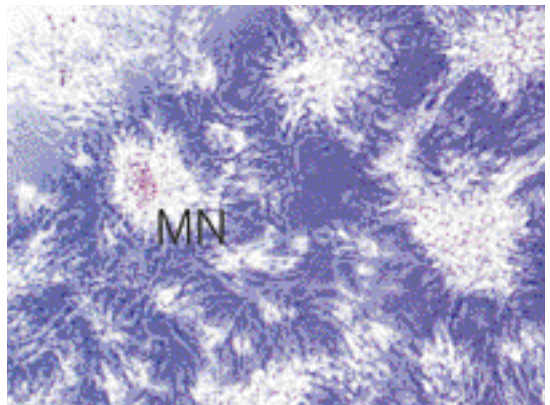


Photo 6

lular matrix proteins during development of mineralized nodules by rat periodontal ligament cells in vitro. *Calcif Tissue Int* 57:52 - 59, 1995

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Photo 1. Control group after 7 days of culture. Cells were confluent(X40).

Photo 2. Alendronate sodium group at the concentration of 10^{-9} mol/L after 7 days of culture. Cells were aggregated, not confluent(X40).

Photo 3. Etidronate disodium group at the concentration of 10^{-7} mol/L after 7 days of culture. Cells were aggregated, not confluent(X40).

Photo 4. Control group after 28 days of culture.

The fewer mineralized nodules stained with red orange color were seen compared to bisphosphonate group.

Photo 5. Alendronate sodium group at the concentration of 10^{-9} mol/L after 28 days of culture. The mineralized nodules stained with red orange color were surrounded by cells. The more round shaped nodules with clear margin than the nodules in etidronate group were seen(X40).

Photo 6. Etidronate disodium group at the concentration 10^{-7} mol/L after 28 days of culture. The larger, irregular shaped mineralized nodules stained with red orange color were seen compared to alendronate group(X40).

Effect of Bisphosphonate on Osteoblastic Activity of the Human Periodontal Ligament Cells in Vitro

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Previous studies have demonstrated an increase in bone mass and density with use of bisphosphonate in osteoporosis. This agent acts as an inhibitor of osteoclastic activity and results in increase of net osteoblastic activity.

The purpose of the present study was to examine the effect of the bisphosphonate on osteoblastic activity of the human periodontal ligament cells in vitro. Periodontal ligament cells were primarily obtained from extracted healthy third molars. Cells of 4th to 6th passage were cultured in Dulbecco's modified Eagle's medium containing alendronate sodium or etidronate disodium at the concentration of 10^{-12} - 10^{-6} mol/L in 5% CO₂ incubator at 37 °C. Cell count and MTT assay for cellular activity were done at 2 to 7 days of culture. Alkaline phosphatase activity at 4 to 7 days of culture and formation of mineralized nodules at 28 days of culture with addition of 50 µg/ml

ascorbic acid, 10 mM - glycerophosphate ,
10⁻⁷ M dexamethasone were evaluated.

1. Alendronate sodium

Compared to the control, the proliferation of periodontal ligament cells was generally increased and the cellular activity was maintained at 2 days of culture and generally decreased at 7 days of culture. Alkaline phosphatase activity of periodontal ligament cells was increased and the formation of mineralized nodules by periodontal ligament cells was enhanced compared to the control.

2. Etidronate disodium

The proliferation of periodontal ligament cells was increased at 2 days of culture and decreased or maintained at 7 days of culture. Compared to the control, the cellular activity of periodontal ligament cells was generally decreased. Alkaline phosphatase activity of periodontal ligament cells was increased and the formation of mineralized nodules by periodontal ligament cells was enhanced compared to the control.

These results suggest that alendronate sodium and etidronate disodium may have a potential effect on osteoblastic lineage of periodontal ligament cells, distinct from their inhibitory action on osteoclasts and could contribute to enhance periodontal regeneration and alveolar bone regeneration.