

## Zoledronic acid

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|                       |                           |                       |                     |
|-----------------------|---------------------------|-----------------------|---------------------|
|                       | *                         |                       |                     |
| : Bisphosphonate(BPs) | endogenous pyrophosphates | Paget's dis-          |                     |
| ease,                 |                           | ,                     |                     |
| 가                     |                           | . BPs                 |                     |
| (nitrogen-con-        | . 가                       | ,                     |                     |
| taining) BPs          | Zoledronic acid(ZOL)      | nude mouse model      |                     |
| ZOL                   |                           |                       |                     |
| :                     | MG-63 HOS                 | ,                     |                     |
| GFP                   | GFP 가                     | MG-63-GFP, HOS-       |                     |
| 6                     | 10                        | 3×3×3 mm              |                     |
| ,                     | ZOL 120 ug/kg             | 2                     |                     |
| :                     | HOS                       | ,                     |                     |
| mm <sup>3</sup>       | ZOL 131 mm <sup>3</sup>   | 94%                   | 2,520               |
| 72%                   | (P<0.05).                 | , MG-63               |                     |
| :                     | Nude mouse                | 2,866 mm <sup>3</sup> | 209 mm <sup>3</sup> |
| ZOL                   | ZOL                       |                       |                     |
| :                     | Zoledronic acid,          | ,                     |                     |

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Zoledronic acid

가 1. 0.9% HOS

가 18) , MG63 HOS

가 , RPMI medium 1640 10% fetal bovine serum (FBS ,U.S.Bio., USA), 0.2% sodium bicarbonate (Sigma, USA), 1ml 50 µg gentamicin sulfate(Gibco, USA) 가

1,13) Bisphosphonate(BP) MG63 Eagle's minimum essential medium (EMEM) 10% fetal bovine serum (FBS, U.S.Bio., USA), 0.22% sodium bicarbonate (Sigma, USA), 1ml 50 µg gentamicin sulfate(Gibco, USA) 가 5% CO<sub>2</sub>

BPs , Paget ,

BPs ,

3,4,6,8,19,21) BPs Zoledronic acid (Novartis Pharmaceuticals Ltd., Australia)

. BPs 가 2.

1) GFP 20 % MG-63, HOS

BPs pamidronate PT67/pLGFP-METSN pLGFP SN

. Zoledronic acid( ZOL) supernatant 1:1 poly-

BPs , brene 8ug 가 12 .

가 BPs 가 G418 400ug/ml

가 BPs가

2) in vivo

BPs

Charles river 6

HOS, MG-63 BALB/cAnN/CriBgi-nu/nu 20

nude mouse . GFP 가

ZOL MG-63-GFP, HOS-GFP 2×10<sup>7</sup>

tumor mass ZOL

3×3×3 mm , ZOL

120 ug/kg 2

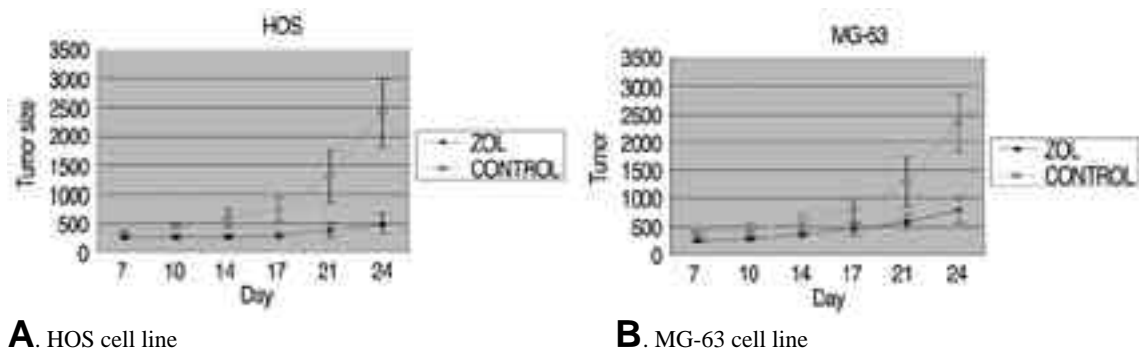
digital caliper 가 T-test P<0.05  
 4/3 r<sup>3</sup> (Fig. 1,2,3).  
 The two-tailed, unequal variance Student's t test(SPSS)

Bisphosphate(BPs) 가  
 endogenous pyrophosphates

1. in vivo model ZOL 30 1 BPs dichloromethylene-1,1-bisphosphonate[CLO] 1-hydroxyethylene-1,1-bisphosphonate[**E제**]  
 1) ZOL R2 side chain 30000  
 BPs 4) 8,9,19) 20,21)

dose(120 ug/kg, 2 /week, s.c. injection) Evdokiou 6)  
 HOS, MG-63 ZOL  
 adriamycin, methotrexate, carboplatin, ifosfamide ZOL

2) HOS 2,520 mm<sup>3</sup> 131 mm<sup>3</sup> HOS 25uM  
 94% , MG-63 , MG-63 100uM  
 2,866 mm<sup>3</sup> 209 ZOL  
 mm<sup>3</sup> 72% (Table 1). MG-63 HOS 가  
 ZOL



**A.** HOS cell line

**B.** MG-63 cell line

**Fig. 1.** Time-enhancement change of tumor size from mice with implanted HOS, MG63 after injection of 120 µg/kg. The Size of the tumor differs significantly during the experimental period.

ZOL

, ZOL

2).

**Table 1.** Time-enhancement change of tumor size.

| <b>A. HOS Group</b> |         |             |
|---------------------|---------|-------------|
| HOS group           | Control | ZOL-treated |
| #1                  | 3315.23 | 130.92      |
| #2                  | 2952.97 | 137.26      |
| #3                  | 2144.66 | 124.79      |
| #4                  | 1767.15 | 113.10      |
| #5                  | 4188.79 | 150.53      |
| MEAN                | 2873.76 | 131.32      |

| <b>B. MG-63 Group</b> |         |             |
|-----------------------|---------|-------------|
| MG-63 group           | Control | ZOL-treated |
| #1                    | 1949.82 | 229.85      |
| #2                    | 2438.64 | 239.04      |
| #3                    | 4315.71 | 268.08      |
| #4                    | 2572.44 | 143.79      |
| #5                    | 3053.63 | 164.64      |
| MEAN                  | 2866.05 | 209.08      |

**T-Test**

**Paired Samples Statistics**

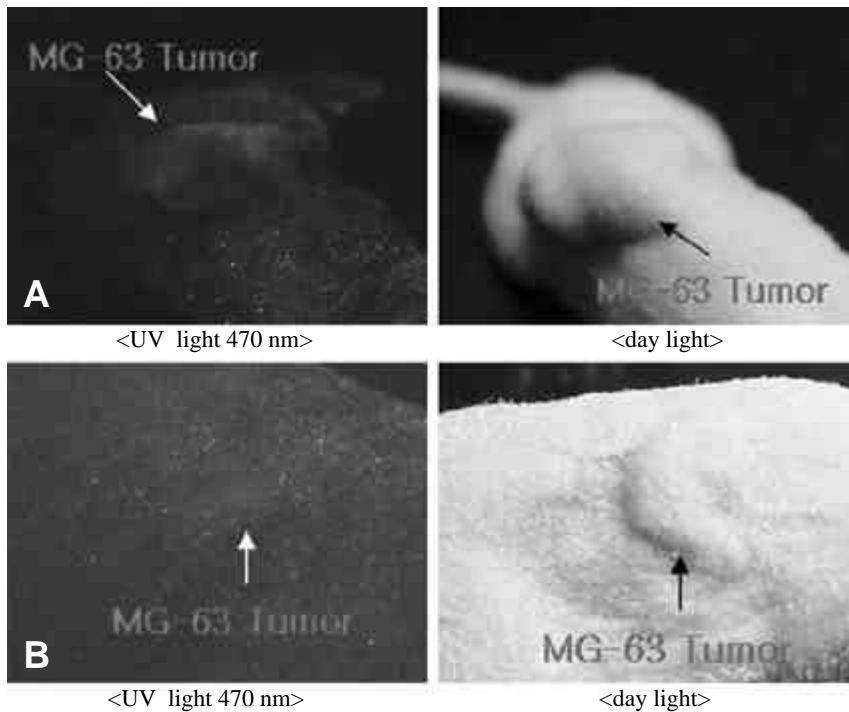
|        |            | Mean      | N | Std. Deviation | Std. Error Mean |
|--------|------------|-----------|---|----------------|-----------------|
| Pair 1 | HOScontrol | 2873,7590 | 5 | 960,03330      | 429,33994       |
|        | HOSzol     | 131,3201  | 5 | 13,95771       | 6,24208         |
| Pair 2 | MGcontrol  | 2866,0484 | 5 | 900,70548      | 402,80774       |
|        | MGzol      | 209,0799  | 5 | 52,55425       | 23,50297        |

**Paired Samples Correlations**

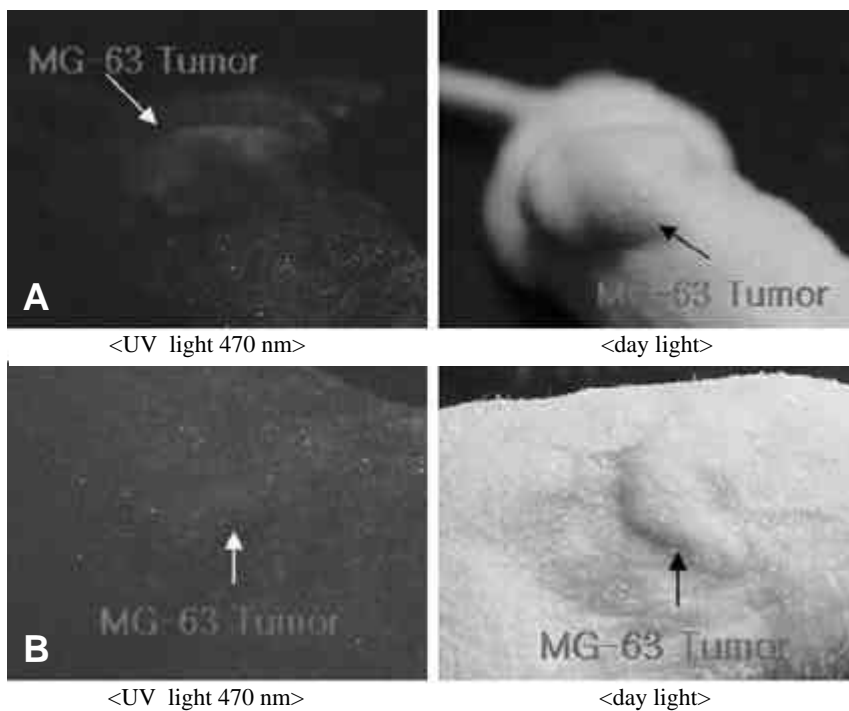
|        |                     | N | Correlation | Sig. |
|--------|---------------------|---|-------------|------|
| Pair 1 | HOScontrol & HOSzol | 5 | .942        | .017 |
| Pair 2 | MGcontrol & MGzol   | 5 | .341        | .575 |

**Paired Samples Test**

|        |                     | Paired Differences |                |                 |   | t         | df    | Sig. (2-tailed) |       |
|--------|---------------------|--------------------|----------------|-----------------|---|-----------|-------|-----------------|-------|
|        |                     | Mean               | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference |           |       |                 |       |
|        |                     |                    |                |                 | Lower                                     |           |       |                 | Upper |
| Pair 1 | HOScontrol - HOSzol | 242,43889          | 946,09750      | 423,49226       | 1006,71067                                | 910,10715 | 8,476 | 4               | .003  |
| Pair 2 | MGcontrol - MGzol   | 656,96848          | 864,17517      | 326,41516       | 1259,12000                                | 254,81605 | 8,719 | 4               | .003  |



**Fig. 2.** The photograph of GFP-expressing HOS tumor 4 weeks after subcutaneous injection in nude mice. (A) control-HOS, (B)ZOL-HOS



**Fig. 3.** The photograph of GFP-expressing MG-63 tumor 4 weeks after subcutaneous injection in nude mice. (A) control-MG-63, (B) ZOL-MG-63

— : —

BPs 가 , ZOL caspase-3 가 .

<sup>5,14)</sup> , BPs

. Stearns SCID mice <sup>3)</sup> . chlodronate etidronate  
 , taxol alendronate BPs가 , ATP

가

<sup>22)</sup> . Magnetto ibandronate tax- <sup>7)</sup> . pamidronate, iban-  
 oid dronate, alendronate, zoledronate

<sup>16)</sup> . BPs Ras Rho small GTP-bind-  
 ing protein(GTPase) isoprenylation  
 mevalonate pathway enzyme <sup>15)</sup> .

adriamycin, methotrexate, carbaplatin, farnesyl-pyrophosphates geranyl-  
 ifosfamide pyrophosphates isoprenoid lipid  
 , Ras, Rho,  
 GTPase transla-  
 tion prenylation .

ZOL <sup>6)</sup> , Rac Rab GTPase  
 in vitro ZOL tation prenylation .  
 ZOL GTPase ,

Jadjev ZOL taxol <sup>12)</sup> . <sup>23)</sup> . 가 BPs가 protein  
 in vivo ZOL 가 BPs가 ,  
 . 가 ,  
 meval-

Evdokiou <sup>6)</sup> ZOL onate <sup>6)</sup> .  
 48 ~ 72 nude mouse ZOL

BPs , ZOL , BPs 가 , 가  
 prenylation . 가  
 prenylated pro- 가

tein <sup>17)</sup> .

HOS, MG-63 ZOL 가 ZOL  
 S-phase . ZOL 가

ZOL S-phase

<sup>4,9,20)</sup>

BPs 가 caspase cascade ZOL in vivo  
 가 , ,  
 ZOL caspase-3 cascade , ZOL

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## Abstract

### Tumor Suppressive Effect of Zoledronic Acid on Human Osteosarcoma Cells in Vivo

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**Purpose:** Bisphosphonates (BPs) are the analogues of endogenous pyrophosphates: they have been used in the treatment of skeletal diseases such as Paget 's disease, osteoporosis, and tumor-inducing osteolysis, and are used in treatment of osteolytic metastasis of breast cancer recently. They are also used as one of the therapeutic agents for metastasis of prostatic cancer of which metastasis makes the mixed nature of osteolysis and osteogenesis. Although the action mechanism of BPs are well known for diseases with excessive osteoclastic bone resorption, the direct effect of BPs has not been known yet. This study was intended to see the tumor suppression capability of Zoledronic acid(ZOL) using nude mouse with osteosarcoma.

**Materials and Methods:** MG-63 and HOS osteosarcoma cell lines were used and the transfected MG-63-GFP and HOS-GFP cells, which were made for detection under fluorescent light, were subcutaneously injected to make osteosarcoma. The five 6-week male mice were used for the experiment at each group. After the injection, mice were cultivated until tumor pieces grow up to  $3 \times 3 \times 3$  mm<sup>3</sup> and ZOL of 120 ug/kg was subcutaneously injected twice a week. Sizes of tumor were measured twice a week and photographed under fluorescent light.

**Results:** In in vivo test with HOS osteosarcoma cell lines, mean size of tumors was 2,520 mm<sup>3</sup> in control group and was 131 mm<sup>3</sup> in ZOL group, which showed 94% of reduction comparing with the control ; with MG-63 osteosarcoma cell lines, mean size of tumors was 2,866 mm<sup>3</sup> in control group and was 209 mm<sup>3</sup> in test group with 72% of reduction (p<0.05).

**Conclusion:** In in vivo tests with nude mice, we suggest that ZOL has direct effect on osteosarcoma cells and it would be used as one of the therapeutic agents for osteosarcoma, especially to ZOL-sensitive osteosarcoma cells.

**Key Words:** Zoledronic acid, Osteosarcoma, Nude mouse.

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