Inhibitory effect of *Artemisia asiatica* on osteoclast formation induced by *Treponema socranskii*

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이 논문을 박사 학위논문으로 제출함

2001년 6월 일

연세대학교 대학원

치의학과

전 승 준

전승준의 박사 학위논문을 인준함

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감사의 글

본 논문이 완성되기까지 시종 세심한 관심을 가져주시고 지도편달을 해 주신 최병재 지도교수님께 진심으로 감사드립니다.

또한 바쁘신 중에도 논문에 많은 조언을 주신 손흥규교수님, 박영철 교수님, 유윤정교수님, 이제호교수님께 감사를 드립니다.

아울러 본 논문 실험을 도와주신 구강생물학교실 옥승호박사님과 교실원 여러분, 소아치과 의국원 여러분께도 감사의 말을 전하고 싶습니다.

또한 지금까지 사랑으로 지켜보시며 면학의 길로 이끌어주신 아버님, 어머님과 장인, 장모님께 감사드리고, 힘든 가운데에서도 항상 힘이 되어주고 내조를 아끼지 않은 사랑하는 아내와 딸 지윤, 지수와 함께 이 결실의 기쁨을 나누고자 합니다.

2001년 6월

저자 씀

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ABSTRACT

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(Directed by Prof. Byung-Jai Choi, D.D.S., M.S., Ph.D.)

Periodontal disease is an inflammatory disorder that leads to the destruction of alveolar bone. It is thought that destruction of alveolar bone is induced by inflammatory reaction to bacteria in periodontal lesion. *Artemisia asiatica* (*A. asiatica*) has been used in traditional oriental medicine as an anti-inflammatory agent, but its effect on bone tissue is not documented. To evaluate the possibility that *A. asiatica* could be used as an inhibitory agent of bone destruction in periodontitis patients, the osteoclast forming ability of *Treponema socranskii*, which is an oral sipochete isolated from periodontal lesion, and the effect of *A. asiatica* on osteoclast differentiation induced by *T. socranskii* was observed. The osteoclast differentiation was estimated by tartrate-resistant acid phosphatase (TRAP; osteoclast differentiation marker)-positive multi-nucleated cell formation in a coculture system of mouse bone marrow and calvarial cells. Prostagalndin E_2 (PGE_2) production in coculture was measured by

enzyme immunoassay system. Effect of *A. asiatica* on cell viability was observed by MTT (3-[4,5-dimethylthiazol-2-yl]-]- 2,5-diphenyl-tetrazolium bromide) assay. *T. socranskii* sonicates (10㎍/ ml) stimulated the formation of TRAP-positive multi-nucleated cells and the production of PGE_2 in coculture. Indomethacin, which is an inhibitor of PGE_2, partailly reduced osteoclast forming activity of sonicates. Ethanol extract of *A. asictica* (100㎍/ ml) inhibited osteoclast formation and PGE_2 synthesis stimulated by *T. socranskii* (10㎍/ ml) in coculture. But eupatilin, which is an ingredient of *A. asiatica* did not show inhibitory effect on osteoclast formation stimulated by *T. socranskii*. *A. asiatica* (100㎍/ ml) did not affect the viability of mouse calvarial and bone marrow cells.

These results suggest that PGE_2 dependent pathway might be involved in the formation of osteoclast induced by *T. socranskii* and that *A. asictica* inhibits osteoclast formation by decreasing of PGE_2 synthesis stimulated by *T. socranskii*.

Key words: *Artemisia asiatica, Treponema socranskii,* Osteoclast

I. INTRODUCTION

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(Directed by Prof. Byung-Jai Choi, D.D.S., M.S., Ph.D.)

Bone remodeling is a process controlled by the action of two major bone cells - the bone matrix forming osteoblast and the bone matrix resorbing osteoclast. The process of bone remodeling requires the balanced activity of these two cells[13]. Osteoclast is tartrate-resistant acid phosphatase (TRAP; osteoclast differentiation marker) positive multi-nucleated cells and the differentiation of these cells consist of multiple steps such as the differentiation of osteoclast precursors into mononuclear prefusion osteoclasts (pOC), the fusion of pOC to form multi-nucleated osteoclasts, and the activation of osteoclast to resorb bone[19]. These steps are controlled by various factors such as parathyroid hormone (PTH)[2], 1α,25-dihydroxy vitamin D_3 ($1\alpha,25(OH)_2D_3$)[21,23], interleukin-11 (IL-11)[24] and prostaglandin E_2 (PGE_2)[1, 6]. These factors induce osteoblast/ stromal cells to express osteoclast differentiation factor (ODF), which induce osteoclastogenesis[12, 22, 25, 29](Fig. 1). Osteoporosis, rheumatic arthritis

and periodontitis are bone disorders which show increased bone destruction and it is thought that bone destruction in these diseases is induced by unbalance of osteoblast and osteoclast differentiation[13].

Periodontitis is a chronic inflammatory disease and the destruction of alveolar bone that supports the teeth is a serious aspect because it leads to the loss of teeth. It is thought that bone destruction in periodontitis is induced by bacteria in periodontal pockets. Various bacteria have been isolated form the subgingival pockets of periodontitis patients. Spirochetae are one of the most common bacteria in subgingival plaque[4,18]. Oral spirochetes fall into the genus Treponema and at least 25 species have been detected. *Treponema soncranskii* is a oral spirochetes which is isolated from periodontal lesion[18]. Although the etiologic role of *T. socranskii* in alveolar bone destruction has been postulated to be an increased number in periodontal lesion, the effect of this bacterium on bone resorption is uncertain.

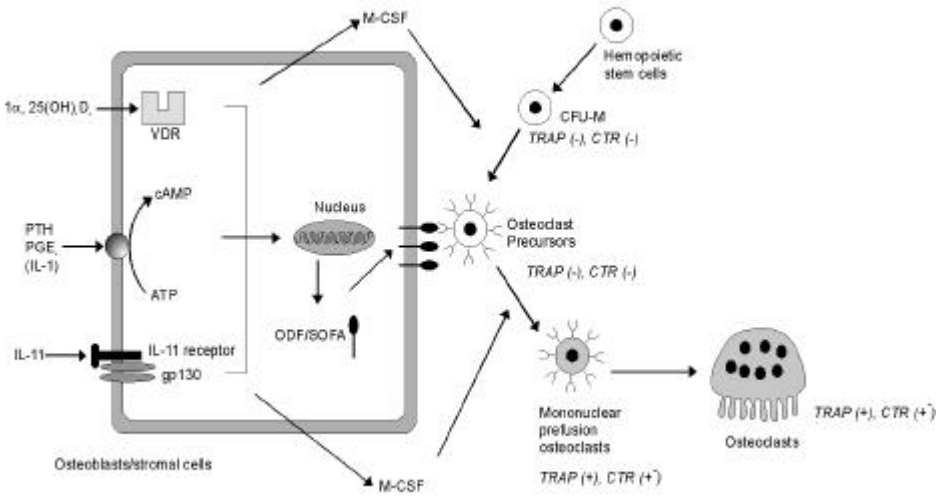

Fig. 1. Schematic diagram of osteoclast differentiation.

Artemisia asiatica, wide spread in nature, has been used in traditional oriental medicine for treatment of inflammation, liver injury, blood diseases caused by disturbance of menses, hematemensis and hemorrhoids[19]. It is thought that alveolar bone destruction in periodontitis is induced by overinduction of osteoclast as a consequence of inflammatory reaction to bactria in subgingival plaque[19]. This suggests the possibility that *A. asiatica* could inhibit osteoclast formation induced by inflammatory reaction to bacteria.

To evaluate the possibility that *A. asiatica* could be used as an anti-resorptive agent to prevent alveolar bone destruction in periodontitis, the osteoclast-forming ability of *T. socranskii* and the effect of *A. asiatica* on osteoclast formation induced by *T. socranskii* were observed. Osteoblast plays important role in osteoclast differentiation. Therefore, osteoclastogenesis-inducing and inhibitory ability of the bacetria and *A. asiatica* were determined in a coculture system of mouse osteoblast and osteoclast precursors.

Ⅱ. MATERIALS AND METHODS

1. Materials and reagents

The ethanol extract of *A. asiatica* (Aasteraceae) and eupatilin were obtained from Dong-A Pham. Co. Ltd. (Seoul, Korea).

Indomethacin, tartrate-resistant acid phosphatase (TRAP) kit and 1α, 25$(OH)_2D_3$ were purchased from Sigma (St. Louis, MO, USA). PGE_2, enzymeimmunoassay (EIA) system was purchased from Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, England). ICR mice were obtained from Bio Korea Co. (Seoul, Korea).

2. Bacterial culture and preparation of whole cell sonicates

T. socranskii subsp. socranskii ATCC 35536 was obtained from American Type Culture Collection, and cultured by the method of Wyss *et al.*[28] It was inoculated into 10ml of OMIZ-Pat broth and incubated at 37℃ for 3days in an atmosphere of 80% N_2, 10% CO_2 and 10% H_2. This culture broth (1.5ml) was then inoculated into 50ml of OMIZ-Pat broth. After 3days culture, cells were harvested by centrifugation at 5,000×g for 10min at 4℃ and washed twice with phosphate buffered saline (PBS). The bacterial cells were then disrupted with an ultrasonic processor at 3Watts for 4minutes in ice. The sonicates were centrifuged at 15,000×g for 5minutes at 4℃ and the supernatant was collected. A protein assay kit (Bio-Rad) was

used to determine protein concentration of sonicates with bovine serum albumin as a standard.

3. Preparation of primary calvarial and bone marrow cells

Calvarial cells were isolated from 1 2-day-old newborn mice by Suda's method with a slight modification[21]. 30 50 calvariae were subjected to digestions using 10ml of enzyme solution containing 0.2% collagenase (Wako, Japan) and 0.1% dispase (GiBCO BRL, U.S.A) for 20minutes at 37℃ in a shaking water bath. The supernatant was discarded and 10ml of enzyme solution was added. After shaking at 37℃ for 20minutes, the supernatant was then collected carefully and transferred into a new tube. This digestion of calvariae with collagenase-dispase was repeated three times. Collected supernatant (30ml) was applied to centrifuge at 1,500×g for 10minutes to collect the osteoblastic cells. Collected cells were resuspended in α-MEM containing 10% fetal calf serum (FCS) and cultured in 10cm culture dishes at a concentration of 10^5cells/ dish to confluence. Cells were then detached from culture dishes by trypsin-EDTA, suspended in α-MEM with 10% FCS and used for coculture as osteoblastic cells. Femorial and tibial bone marrow cells were collected from 4-week-old mice. Tibiae and femora were removed and dissected free of adhering tissues. The bone ends were removed and the marrow cavity was flushed by slowly injecting

media in at one end using a 25-gauge needle. The collected calvarial and bone marrow cells were washed and used for the coculture.

4. In vitro formation of osteoclasts

Mouse calvarial cells (1 x 10^4 cells/ well) were cocultured with bone marrow cells (1 x 10^5 cells/ well) in α-MEM containing 10% fetal calf serum in 48-well plates (Corning Inc., Corning, NY). Culture volume was made up to 400㎕ per well with medium. All cultures were maintained at 37℃ in a humidified atmosphere of 5% CO_2 in air. The sonicates of bacteria, heat-treated sonicates, extract of *Artemisia asiatica*, 1α,25$(OH)_2D_3$, eupatilin or indomethacin was added to the coculture immediately after exchanging the medium on day 3. The cocultures were then cultured for additional 4days. Differentiation of osteoclasts was monitored using TRAP (a marker enzyme of osteoclasts) staining supplied. TRAP-positive multinucleated cells showing more than 3 nuclei were counted as osteoclast cells.

5. Prostaglandin E_2 assay

Total cellular concentration of PGE_2 was measured with PGE_2 enzymeimmunoassay (EIA) system (Amersham Pharmacia Biotech UK Ltd., England). Mouse calvarial cells and bone marrow cells were cocultured in 48 well plate. The sonicates of bacteria, and/ or extract

of *A. asiatica* was added to the coculture immediately after exchanging the medium on day 3. The cocultures were then cultured for additional 24hours. Total cellular concentration of PGE_2 was measured and calculated by the method described in the manual.

6. MTT assay

After mouse calvarial cells (1 x 10^4 cells/ well) had been cocultured with bone marrow cells (1 x 10^5 cells/ well) in α-MEM containing 10% FCS in 96-well plates for 3days, various concentrations of *A. asiatica* extract were added to each well and cultured for an additional 4days. The viability of cells were determined by MTT (3-[4,5-dimethylthiazol-2-yl]-]- 2,5-diphenyl-tetrazolium bromide) assay[16]. After 4days of incubation, 50㎕ of MTT solution (5mg/ ml) was added to each well and continued for 4 hours at 37℃. 200㎕ of DMSO (dimethyl sulfoxide) was added to dissolve the formazan crystals and the optical density of the formazan solution was measured at 570nm.

7. Statistical analysis

Statistical differences were determined by the Mann-Whitney U test.

Ⅲ. RESULTS

1. Induction of osteoclast formation by sonicates of *T. socranskii*

To demonstrate osteoclast-forming ability of *T. socranskii*, sonicated extract was used in coculture. Several concentrations of sonicate (0.1, 1.0, 10, 25μg/ml) were added to the coculture (Fig. 2). When 0.1μg/ml of the sonicate was added to the coculture, it did not induced any formation of osteoclast while 10μg/ml of sonicate induced osteoclast formation with similar extent as that of 1α,25$(OH)_2D_3$(100μM). The number of osteoclasts induced by sonicate of *T. socranskii* was increased in a dose-dependent manner. Osteoclasts formed by sonicates showed intense TRAP activity which is known as a marker enzyme of osteoclasts, and their morphological appearances were similar to those of osteoclasts induced by 1α,25$(OH)_2D_3$. 1α,25$(OH)_2D_3$ was used as a positive control to determine the differentiation of osteoclast cells. Osteoclast cells having more than 3nuclei were detected at 10^{-8}M of 1α,25$(OH)_2D_3$. α-MEM containing 10% fetal calf serum was used as a negative control. No TRAP-positive osteoclast was formed in a negative control.

2. Effect of heat treatment of *T. socranskii* sonicates on osteoclast formation

Several bacterial cellular components such as LPS[26], capsular polysaccharide[17], surface-associated protein or chaperon[9, 14] have been reported that have been involved in bone resorption. Gopalsami *et. al.*[4] reported that a heat-stable, LPS-like material present in the outer membrane of *T. denticola* might be responsible for bone resorption. Therefore, sonicate of *T. socranskii* (10㎍/ ml) was treated with heating at 80℃ for 30minutes to demonstrate whether heat stable, LPS-like material is involved in induction of osteoclast differentiation. As shown at Fig. 3, heating the sonicates reduced the stimulatory effect of *T. socranskii* on osteoclast formation down to 42 TRAP positive cells/ well while intact sonicate produced 550 TRAP positive cells/ well. Therefore, It can be inferred that heat-liable, protein-like components might be involved in the induction of osteoclast formation. However, heating the sonicate did not completely inhibited the formation of osteoclast. This result implies that more than one components might be involved in osteoclast formation induced by *T. socranskii*.

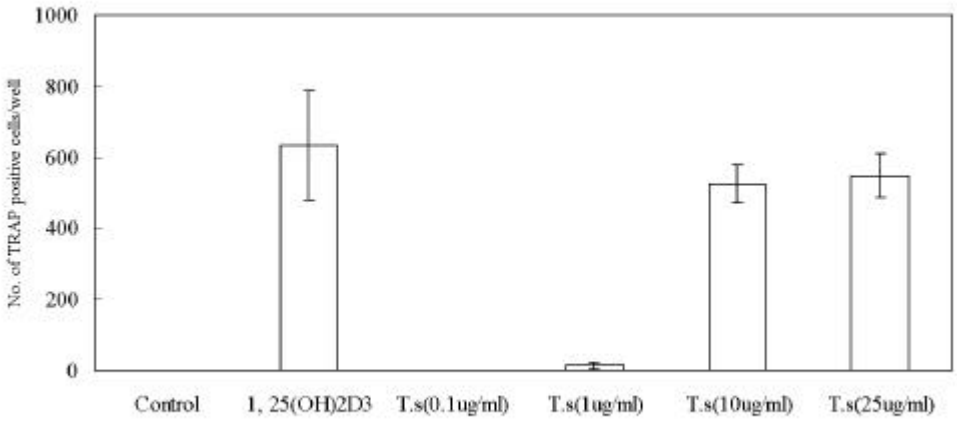

Fig. 2. Effect of *T. socranskii* sonicates on the osteoclast formation. Concentration of $1\alpha,25(OH)_2D_3$: 100 μM

3. Effect of indomethacin on osteoclast formation induced by sonicates of *T. socranskii*

To elucidate the mechanism of osteoclast differentiation induced by *T. socranskii*, indomethacin was added to the coculture system with or without sonicate of *T. socranskii*. Indomethacin has been reported to inhibit PGE_2 synthesis[3]. On adding 0.1mM of indomethacin with sonicate of *T. socranskii*(10μg/ml), 118 TRAP-positive cells/well were formed, whereas 784 TRAP-positive cells/well were formed by sonicate of *T. socranskii* (Fig. 4). This result implies that PGE_2 mediated pathway might be involved in the induction of osteoclast differentiation by *T. socranskii*.

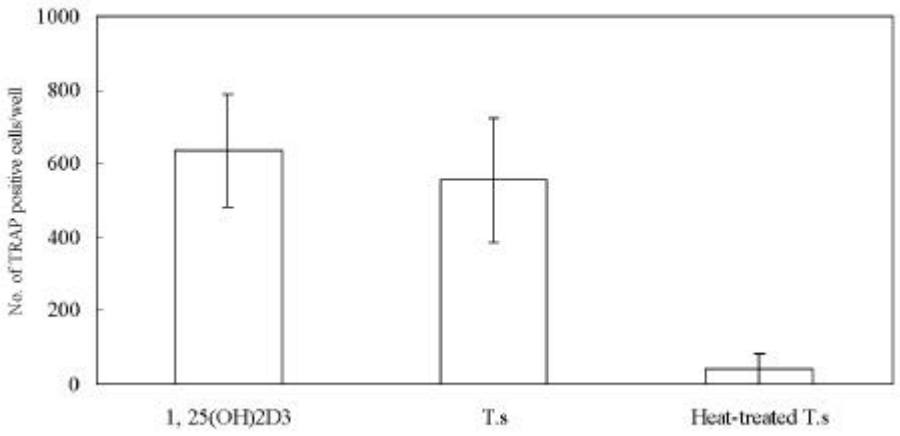

Fig. 3. Effect of heat treatment of *T. socranskii* sonicates on osteoclast formation.

Concentration of $1\alpha,25(OH)_2D_3$: 100 μM

Concentration of *T. socranskii* : 10μg/ml

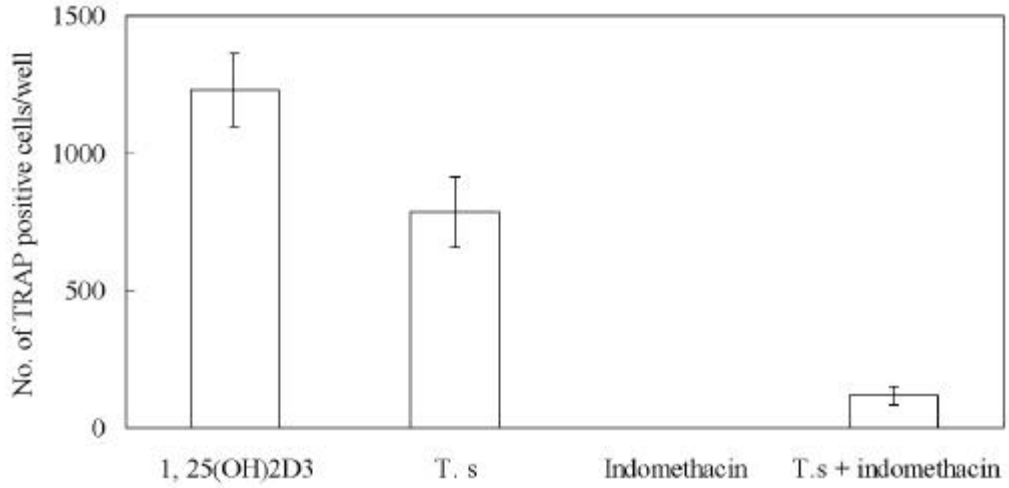

Fig. 4. Effect of indomethacin on osteoclast formation induced by *T. socranskii* sonicates.

Concentration of $1\alpha,25(OH)_2D_3$: 100 μM

Concentration of *T. socranskii*: 10μg/ml

Concentration of indomethacin: 100 μM

4. Inhibition of osteoclast formation by *Artemisia asiatica*

To evaluate the inhibitory effect of *A. asiatica* on osteoclast formation induced by *T. socranskii*, we applied sonicate of *T. socranskii* as an inducer of osteoclast differentiation in coculture. Ethanol extract of *Artemisia asiatica* was simultaneously added to the coculture with or without *T. socranskii* sonicate (Fig. 5). When 100㎍ / ml of *A. asiatica* extract and 10㎍/ ml of *T. socranskii* sonicate were added to the coculture simultaneously, no TRAP-positive cell was detected, while 491 TRAP-positive cells were formed with sonicate of *T. socranskii* only. When several concentrations (1.0, 10, 100㎍/ ml) of *A. asiatica* extract and 10㎍/ ml of *T. socranskii* sonicate were added to the coculture simultaneously, the number of TRAP-positive multinucleated cells formed was decreased in a dose-dependent manner (Fig. 6). Morphological appearances of TRAP- positive osteoclast cells induced by sonicate of *T. socranskii* and its inhibition by extract of *A. asiatica* were shown at Fig. 7.

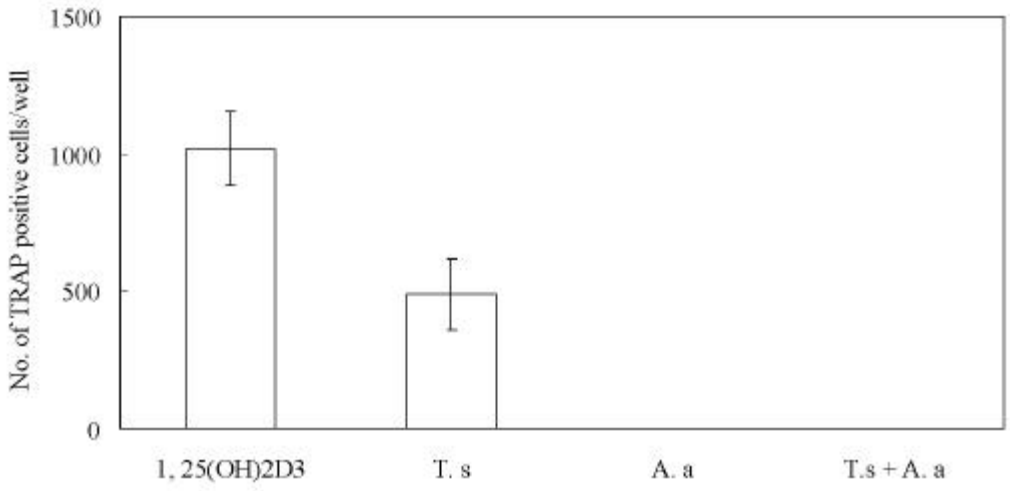

Fig. 5. Effect of *A. asiatica* extract on osteoclast formation induced by *T. socranskii* sonicates.

Concentration of $1\alpha,25(OH)_2D_3$: 100 μM

Concentration of *T. socranskii*: 10μg/ml

Concentration of *A. asiatica*: 100μg/ml

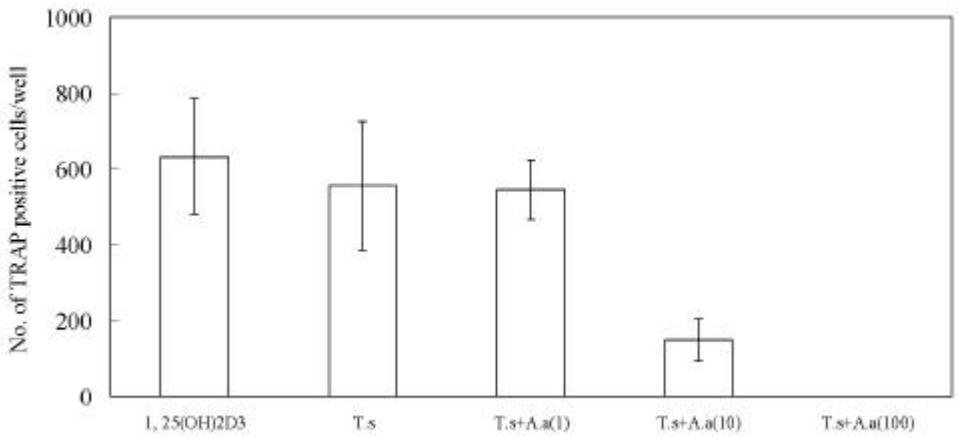

Fig. 6. Effect of *A. asiatica* on the *T. socranskii*-induced osteoclast formation at various concentrations.

Concentration of $1\alpha,25(OH)_2D_3$: 100 μM

Concentration of *T. socranskii*: 10μg/ml

Concentration of *A. asiatica*: 1, 10, 100μg/ml

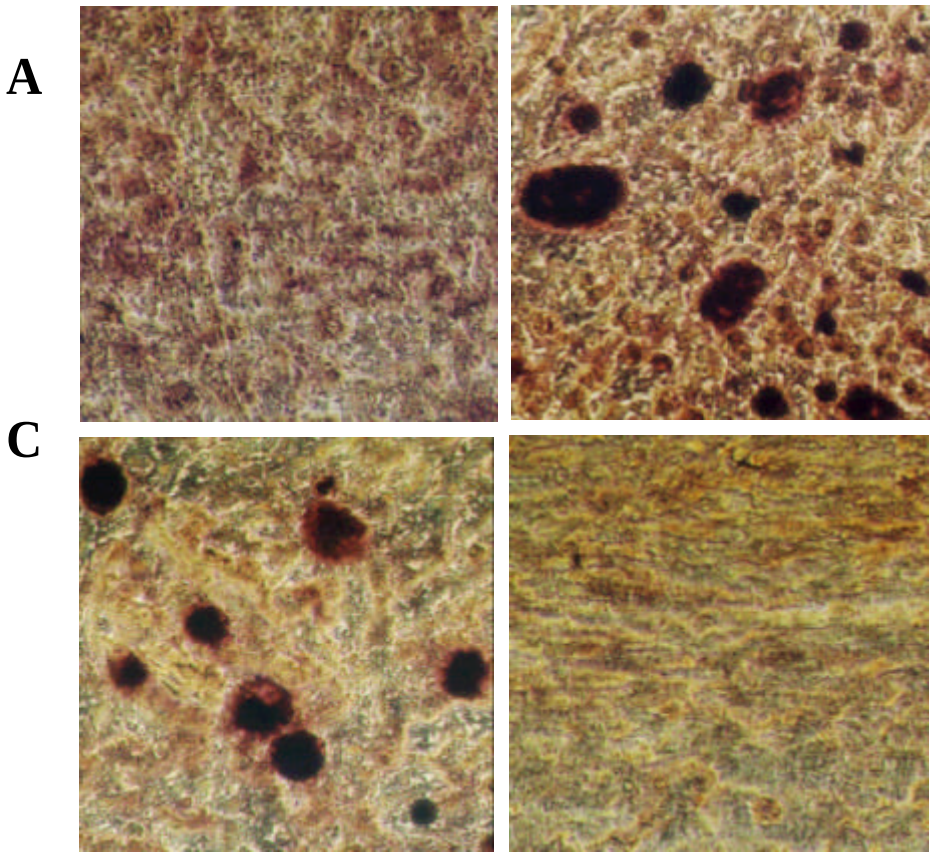

Fig. 7. Inhibition of *T. socranskii*-induced osteoclast formation by *A. asiatica*.

A, non-treated; B, 100 μM of 1α,25$(OH)_2D_3$; C, 10μg/ml of *T. socranskii*; D, *T. socranskii* with 100μg/ml of *A. asiatica*

5. Effect of *A. asiatica* on PGE_2 synthesis stimulated by sonicates of *T. socranskii*

It is known that the level of PGE_2 was increased in osteoclast differentiation caused by several periodontal pathogens[26, 30]. At Fig. 4, indomethacin that has an inhibitory effect on PGE_2 synthesis showed partial inhibitory effect on osteoclast formation induced by *T. socranskii*. Therefore, quantitative analysis of total cellular concentration of PGE_2 has been carried out to confirm the involvement of PGE_2 in the induction of osteoclast by *T. socranskii* and the osteoclast formation inhibitory effect of *A. asiatica*. Total cellular concentration of PGE_2 was increased when the coculture was treated with sonicate of *T. socranskii*. PGE_2 was increased to 134pg/ml whereas control was 83pg/ml. It was decreased down to 42 pg/ml when treated with *T. socranskii* and *A. asiatica* simultaneously (Fig. 8).

6. Effect of *A. asiatica* on the proliferation of osteoblast/bone marrow cell coculture

The effect of *A. asiatica* on the viability of the coculture was determined by MTT assay. Extract of *A. asiatica* showed no effect on the viability of coculture up to a concentration of 100μg/ml, as compared with non-treated cells (Fig 9), but at higher concentrations of *A. asiatica* extracts (>100μg/ml), it inhibited coculture growth.

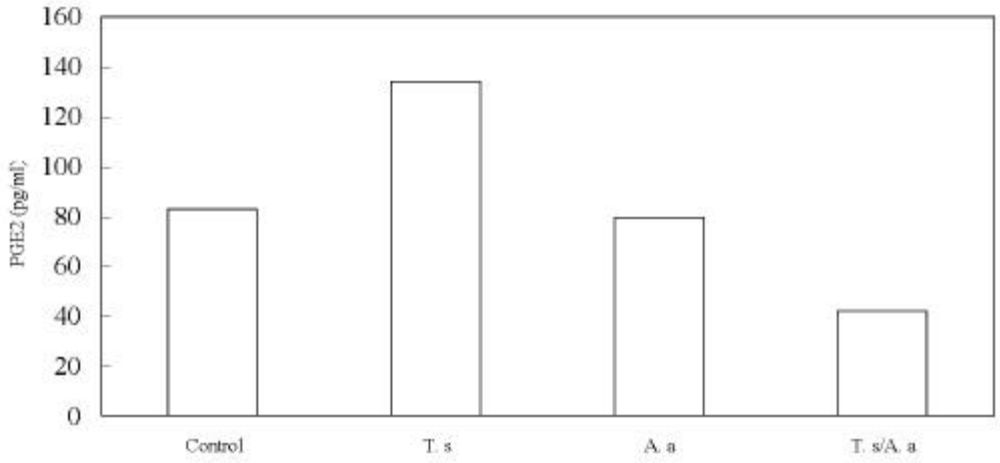

Fig. 8. Effect of *A. asiatica* on PGE_2 synthesis stimulated by *T. socranskii* sonicates.

Concentration of *T. socranskii*: 10㎍/ ml

Concentration of *A. asiatica*: 100㎍/ ml

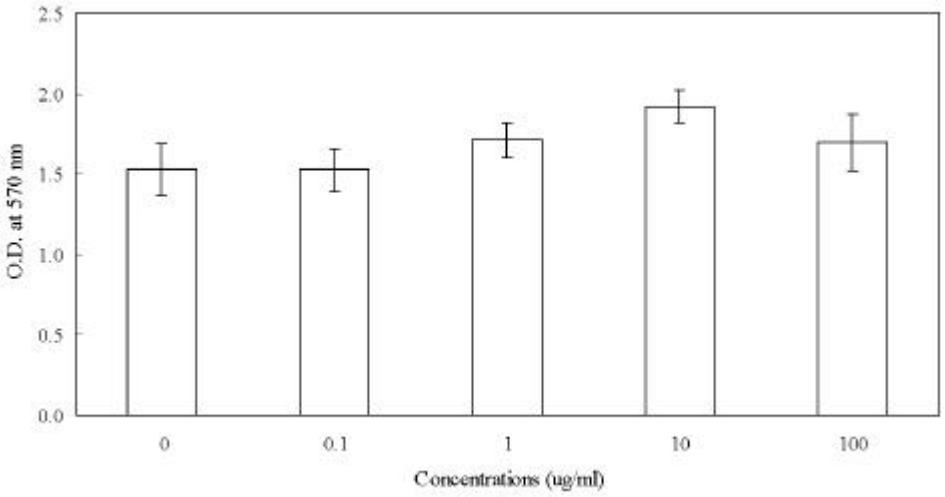

Fig. 9. Effect of *A. asiatica* on the proliferation of osteoblast/ bone marrow cell coculture.

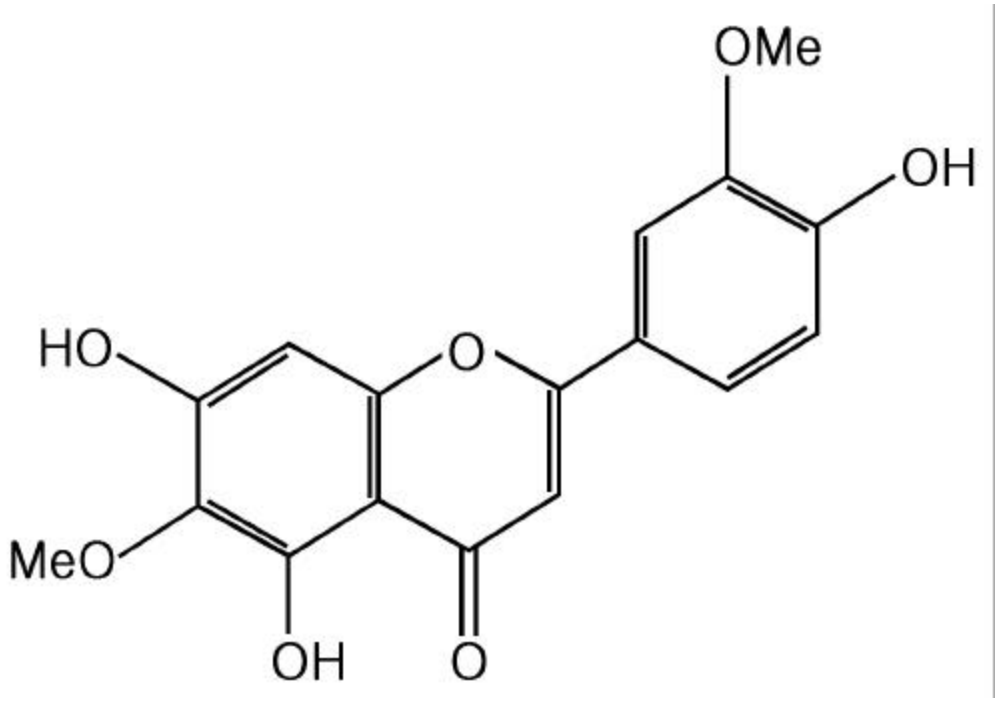

Fig.10. Structure of eupatilin (5,7-dihydroxy 3',' 4',' 6'-trimethoxy-flavone).

7. Effect of eupatilin on the proliferation of osteoblast/bone marrow cell coculture

The effect of eupatilin on the viability of the coculture was determined by MTT assay. Eupatilin showed no effect on the viability of coculture up to a concentration of 10mM, as compared with non-treated cells (Fig. 11).

8. Effect of eupatilin on osteoclast formation stimulated by sonicates of *T. socranskii*

Eupatilin has been reported to be an active compound of *A. asiatica*[11]. Therefore, the effect of eupatilin on osteoclast formation was observed in coculture with or without sonicate of *T. socranskii*. However, eupatilin did not show any significant inhibitory effect on osteoclast formation induced by *T. socranskii* (10㎍/ml) when 100㎛M of eupatilin was added to the coculture (Fig. 12).

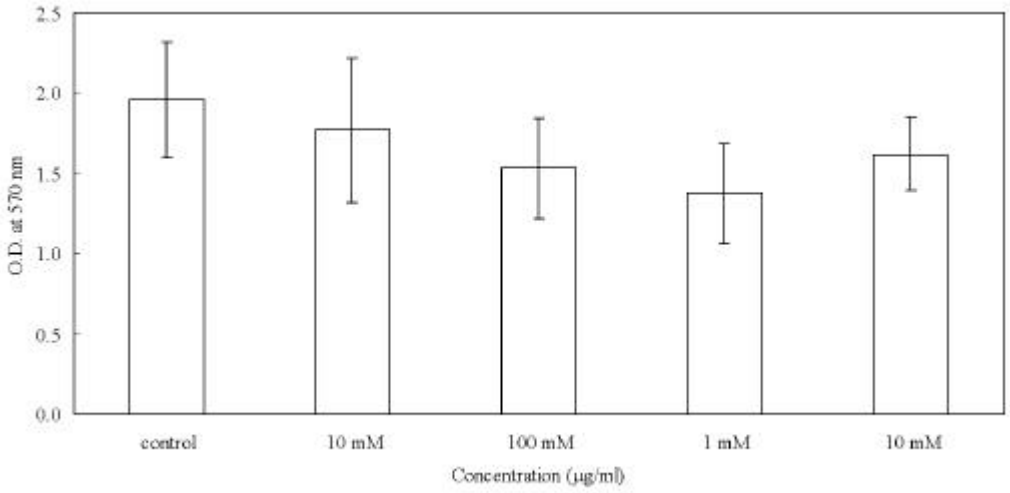

Fig.11. Effect of eupatilin on the proliferation of osteoblast/ bone marrow cell coculture.

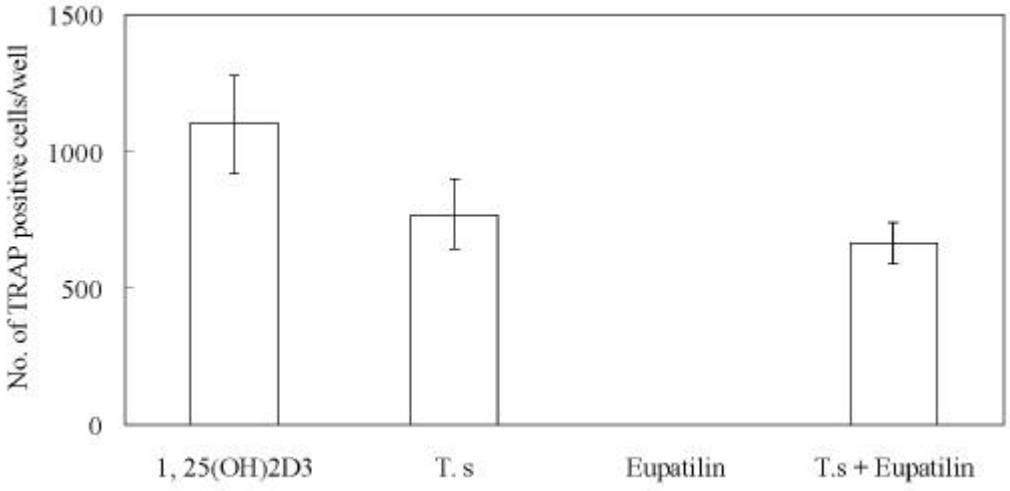

Fig.12. Effect of eupatilin on osteoclast formation induced by *T. socranskii* sonicates.

Concentration of *T. socranskii*: 10㎍/ ml

Concentration of eupatilin: 100㎛M

Ⅳ. DISCUSSION

In this study, we demonstrated inhibitory effect of *A. asiatica* on *T. socranskii*-induced osteoclastogenesis. *T. socranskii*, which is frequently isolated from periodontal lesion, showed stimulatory effect on osteoclast differentiation. This was confirmed with TRAP staining of the coculture treated with or without *T. socranskii* extract. Several bacterial factors involved in bone resorption have been reported. In *Actinobacillus actinomycetemcomitans*, cellular components such as LPS[26], capsular polysaccharide[17], surface-associated protein or chaperon[9, 14] showed bone-resorbing activity. In the case of *Porphyromonas gingivalis*, LPS[15], surface-associated protein[27] and fimbriae[7] are reported as stimulators of bone resorption. Gopalsami *et. al.*[4] showed that the outer membrane of *T. denticola* induced bone resorption and heating the outer membrane preparation did not abolish the effect. They concluded that a heat-stable, LPS-like material present in the outer membrane might be responsible for bone resorption. These reports imply that several cellular components of *T. socranskii* might be involved in osteoclast formation induced by *T. socranskii*. To identify the components of *T. socranskii* which induces osteoclast differentiation, we treated the extract of *T. socranskii* with heat. Heating the extract at 80℃ for 30 minutes reduced the stimulatory effect down to 7.6%. This suggests that heat-labile substances of *T.*

socranskii might be involved in the induction of osteoclast formation.

Zubery *et al*.[30] reported that induction of osteoclast by *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Campylobacter rectus* was partially inhibited by indomethacin, an inhibitor of PGE_2 synthesis, and suggested that there might be PGE_2 dependent pathway in osteoclast formation induced by these bacteria. Meghji *et. al.* also reported that LPS of *A. actinomycetemcomitans* induced osteoclast formation through PGE_2 dependent pathway[13]. These studies imply that PGE_2 might be important mediator in the process of bacteria induced osteoclastogenesis. In this study, the addition of *T. socranskii* sonicates increased cellular production of PGE_2 and indomethacin reduced *T. socranskii*-induced osteoclast formation. These data indicate that induction of osteoclast differentiation by *T. socranskii* might be mediated by PGE_2 dependent pathway.

A. asiatica have been frequently described as an anti-inflammatory agent in traditional oriental medicine. Because osteoclast formation induced by *T. socranskii* was mediated by PGE_2, and PGE_2 was reported as a mediator of inflammatory reaction, the effects of *A. asiatica* on osteoclast differentiation and PGE_2 synthesis stimulated by *T. socranskii* were identified at the concentration which *A. asiatica* did not affect cell viability. Osteoclast differentiation by *T. socranskii* was inhibited by the extract of *A. asiatica*. Furthermore, total cellular concentration of PGE_2 was decreased to about half of

control when treated with *T. socranskii* and *A. asiatica* simultaneously. These data show that *A. asiatica* inhibit osteoclast formation through inhibition of *T. socranskii* induced PGE_2 synthesis. Eupatilin, as an active ingredient of *A. asiatica*, was also applied to osteoblast/ bone marrow cell coculture, but eupatilin did not inhibited the *T. socranskii*-induced osteoclast formation. Therefore, subsequent investigations regarding active components of *A. asiatica* will be required.

In this report, we showed inhibitory effect of *A. asiatica* on *T. socranskii*-induced osteoclast formation. Because other periodontal pathogens are also involved in alveolar bone destruction[19], inhibitory effects of *A. asiatica* on osteoclastogenesis induced by these pathogens should be presented to confirm the possibility of *A. asiatica* as a candidate for the inhibitory agent of bacteria induced-bone resorption.

Ⅴ. CONCLUSION

To evaluate the possibility that *A. asiatica* could be used as an inhibitory agent of bone destruction in periodontitis, the osteoclast forming ability of *Treponema socranskii*, which is an oral sipochete isolated from periodontal lesion, and the effect of *A. asiatica* on osteoclast differentiation induced by *T. socranskii* was observed. The osteoclast differentiation was estimated by TRAP (osteoclast differentiation marker)-positive multi -nucleated cell formation in a coculture system of mouse bone marrow and calvarial cells. PGE_2 production in coculture was measured by enzyme immunoassay system. Effect of *A. asiatica* on cell viability was observed by MTT assay.

T. socranskii sonicates (10㎍/ ml) stimulated the formation of TRAP-positive multi-nucleated cells and the production of PGE_2 in coculture. Indomethacin, which is an inhibitor of PGE_2, partailly reduced osteoclast formation activity of sonicates. Ethanol extract of *A. asictica* (100㎍/ ml) inhibited osteoclast formation and PGE_2 synthesis stimulated by *T. socranskii* sonicates (10㎍/ ml) in coculture. But eupatilin, which is an ingredient of *A. asiatica* did not show inhibitory effect on osteoclast formation stimulated by *T. socranskii*. *A. asiatica* (100㎍/ ml) did not affect the viability of mouse calvaria cell and bone marrow cells.

These results suggest that PGE_2 dependent pathway might be involved in the formation of osteoclast induced by *T. socranskii* and that *A. asictica* inhibited osteoclast formation by decreasing of PGE_2 synthesis stimulated by *T. socranskii*.

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국문요약

*Treponema socranskii*에 의해 유도된 파골세포 형성에 대한 쑥 추출물의 억제효과

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치주염은 치아 지지조직인 치조골이 파괴되는 염증성질환으로서 치아상실의 주된 원인이다. 치조골 파괴는 치태세균에 대한 염증반응에 의하여 야기되는 것으로 생각되고 있다. 오래 전부터 민간요법에서 많이 사용 되어온 쑥은 항염작용이 있는 것으로 기록되어 있으나 뼈에 대한 작용은 밝혀져 있지 않다. 쑥 추출물이 치주염 예방 및 치료의 보조법으로서의 활용가능성을 제시하고자 먼저 치주염환자의 치태 내 존재하는 것으로 보고된 *Treponema socranskii*의 파골세포분화 유도능을 분석한 후, 쑥 추출물이 본 세균에 의한 파골세포형성을 억제할 수 있는지 평가하였다. 조골세포는 파골세포분화 유도인자를 생성하여 파골세포의 형성에 관여한다. 따라서 세균에 의한 파골세포 형성능 및 쑥의 파골세포분화 억제능은 조골세포와 파골세포 전구세포를 같이 배양하는 혼합배양법을 이용하여 측정하였으며 형성된 파골세포는 파골세포분화 표식인자인 tartrate-resistant acid phosphatase (TRAP) 염색을 통하여 확인하였다. 세균 및 쑥에 의한 PGE_2 생성 유도능 및 억제능은 PGE_2에 대한 enzyme immunoassay system을 이용하여 측정하였으며 쑥에 의한

세포독성능은 MTT방법으로 평가하였다.

초음파 분쇄기를 통하여 분리된 *T. socranskii* 분쇄액을 혼합배양액에 첨가한 결과 균 분쇄액 (10μg/ ml)이 첨가된 실험군에서 파골세포의 형성이 유도되었으며 PGE_2 생성도 증가하였다. PGE_2의 합성을 억제하는 indomethacin을 균 분쇄액과 함께 첨가한 경우, 균에 의한 파골세포 형성이 억제됨을 확인할 수 있었다. *T. socranskii* 분쇄액 (10μg/ ml)과 쑥 추출물 (100μg/ ml)을 동시에 첨가한 경우 균에 의하여 유도된 파골세포의 형성 및 PGE_2 생성이 억제되었다. 쑥 추출물은 파골세포의 형성을 억제하는 농도인 100μg/ ml에서 조골세포 및 파골세포 전구세포에 대한 세포독성을 나타내지 않았다. 쑥의 주요 활성성분으로 알려진 eupatilin은 파골세포 형성을 억제하지 않았다.

이와 같은 연구결과들은 구강내에 존재하는 병원성 세균인 *T. socranskii*가 PGE_2 관련 경로를 통하여 파골세포의 형성을 유도하며, 쑥 추출물은 본 세균에 의한 PGE_2 생성을 억제하여 파골세포형성을 억제할 가능성을 시사한다. 그러나 쑥의 주요 활성성분인 eupatilin은 파골세포의 형성에 아무런 영향을 미치지 못하였으므로 eupatilin 이외의 다른 물질이 파골세포 형성의 억제에 관여하는 것으로 추정되어진다.

핵심되는 말 : *Treponema socranskii*, 파골세포, 쑥