Effects of vitamin E on osteoblast-like MC3T3-E1 cells

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Introduction

Osteoporosis affects approximately 13 million patients in Japan, and this potentially life-threatening disease has a poor prognosis after fractures in the femoral neck, especially in elderly patients. Bone mass determination is based on the balance between bone formation by osteoblasts and bone resorption by osteoclasts.

It was reported that vitamin E decreases bone mass by stimulating osteoclast fusion[1]. The cytokine interleukin-(IL)-6 and IL-1β can promote hematopoiesis and osteoclastogenesis. However, the effect of vitamin E on osteoblasts remains unclear.

In this study, we examined the effect of vitamin E on osteoblasts to better determine the osteoclast activation mechanism.

1) Fujita K, *et al.*, *Nat. Med.*, **18** (4), 589-595, 2012.

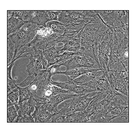
Materials & Methods

Vitamin E

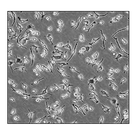
DL-α-tocopherol (α-Toc) MW:430.71
(Wako Pure Chemical Industries, Ltd)

Cells

Mouse calvaria derived osteoblast-like cells (MC3T3-E1)



Mouse pre-osteoclast cells (RAW264.7)



Cell morphology assay

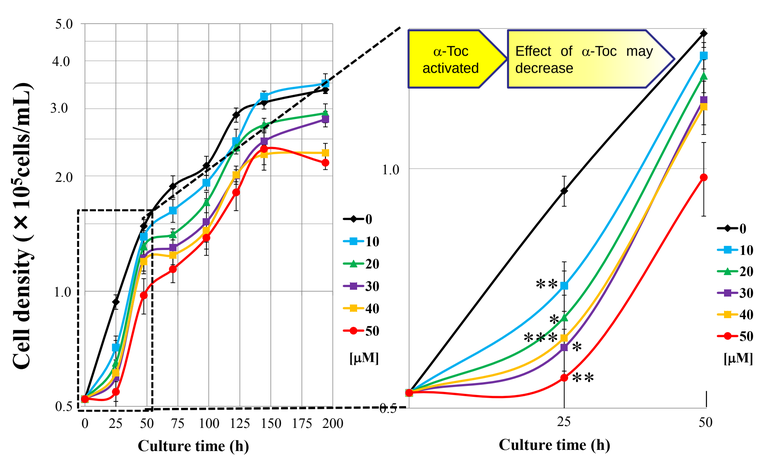
MC3T3-E1 cells were incubated at 310K in a humidified 5% CO_2. The cells were stained by DAPI and phalloidin after culturing for fluorescent observation.

Measurement of released Cytokines

MC3T3-E1 cells were inoculated into a 60mm tissue culture dish at 5×10^4 cells/mL in MEMα containing 10% FBS, and α-Toc at various concentrations, then incubated for 25h at 310K. Supernatants were collected from each well and absolute amounts of IL-6 and IL-1β production from cells were determined using a mouse IL-6 and IL-1β enzyme-linked immunosorbent assay (ELISA) system.

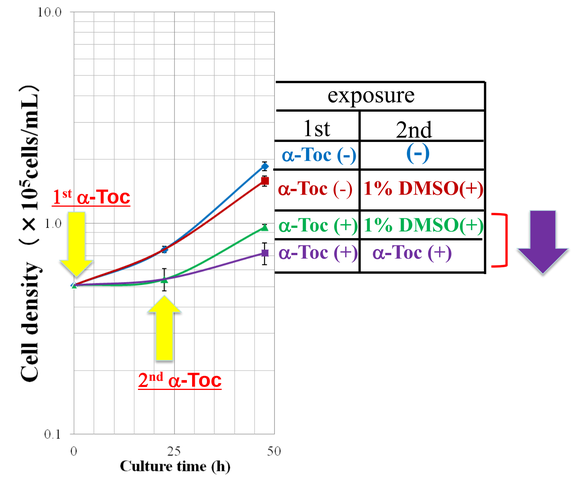
Results

Fig.1 Effect of α-Toc on cell proliferation of osteoblast-like MC3T3-E1 cells



MC3T3-E1 cell proliferation was inhibited by α-Toc in a dose dependent manner.

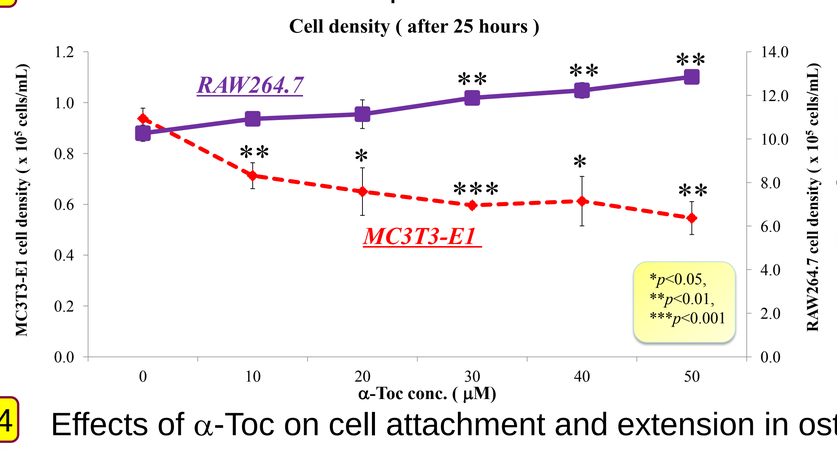
Fig.2 Effects of continuous α-Toc stimuli (50μM) on cell proliferation in MC3T3-E1 cells



Student *t* test
**p*<0.05,
***p*<0.01,
****p*<0.001

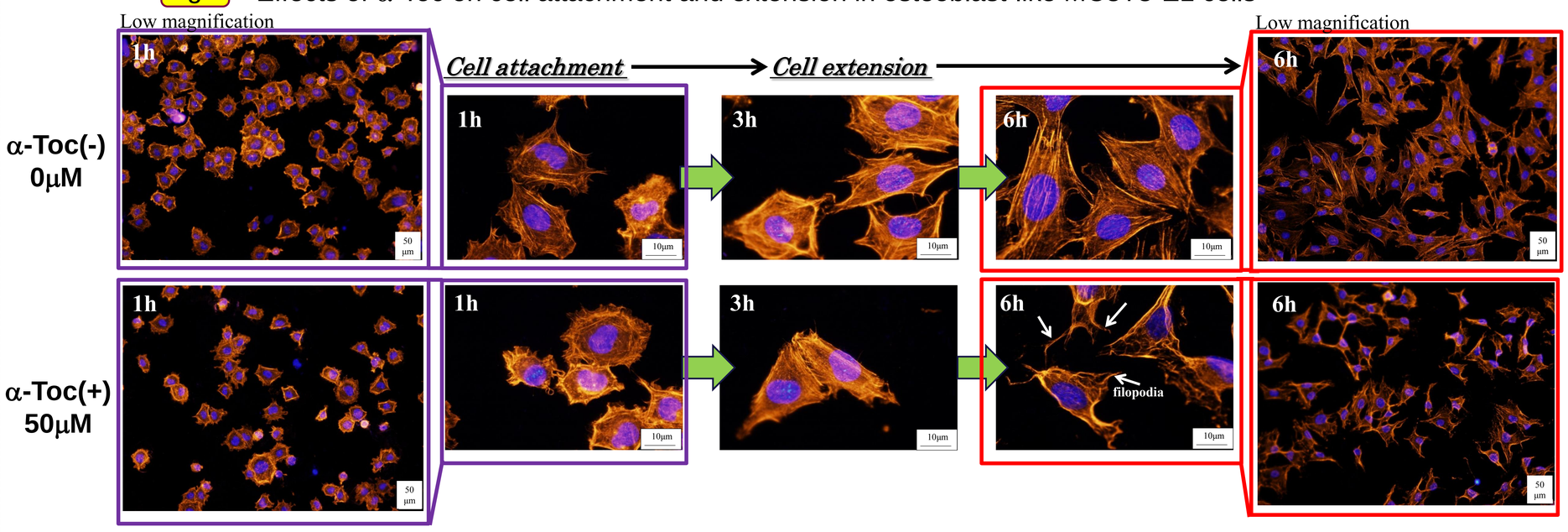
The inhibitiory effect on cell proliferation by α-Toc was maintained following continuous α-Toc stimulation.

Fig.3 Effects of α-Toc on cell proliferation of RAW-264.7 and MC3T3-E1 cells



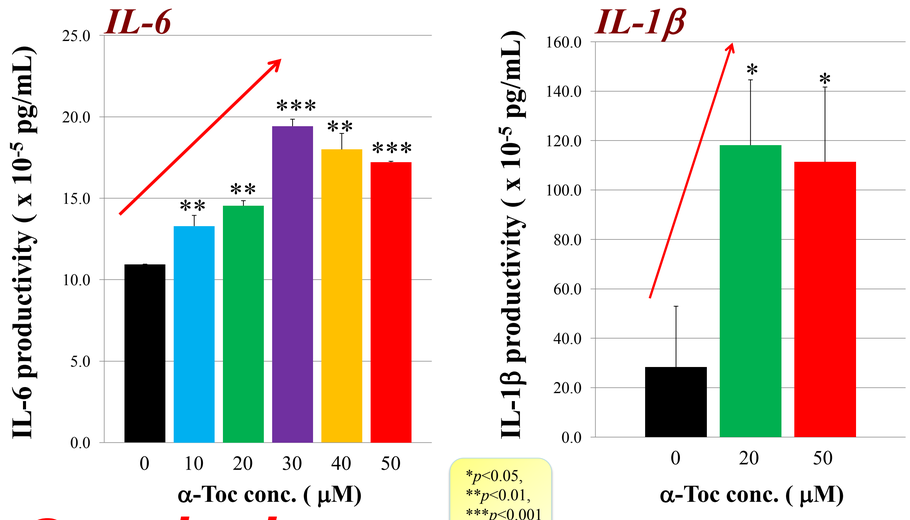
RAW264.7 cell proliferation was stimulated by α-Toc in a dose dependent manner. The effect of α-Toc on RAW264.7 cells was different compared with MC3T3-E1 cells.

Fig.4 Effects of α-Toc on cell attachment and extension in osteoblast-like MC3T3-E1 cells



MC3T3-E1 cell attachment and extension were inhibited in the presence of 50μM α-Toc stimulation.

Fig.5 Effects of α-Toc on cytokine productivity from MC3T3-E1 cells after 25 hours (IL-6, IL-1β)



Levels of IL-6 in the supernatant of MC3T3-E1 cells were high following 30μM α-Toc and were secreted in a dose-dependent manner (0-30μM) . Levels of IL-1β were similar to IL-6 levels.

Conclusion

Vitamin E (α-toc) likely induces activation of osteoclasts by inhibiting osteoblast growth and proliferation as well as promoting cytokine productivity of IL-6 and IL-1β in favor of osteoclast activation.