J Food Sci Nutr
Vol 10, p 353~356 (2005)

J Food Science and Nutrition

The Effects of Boron on the Proliferation of Osteoblastic and Neuroblastoma Cells

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Abstract

It has been recently reported that boron affects bone metabolism in humans and animals. In this study we examined whether boron affects the proliferation on various cell types, MG-63, HOS, Raw 264.7 and SK-N-SH. When treated with different concentrations of boron (1, 10, 100 μM) for 24 and 48 hr, the proliferation of MG-63 cells was enhanced at 10 μM ($p<0.05$), for 24 hr. In HOS cells, boron had no effect on cell proliferation at 24 or 48 hr. In addition, treatment of pre-osteoclastic cells (Raw 264.7) with 1, 10, 100 μM boron resulted in no effect on cell proliferation. Proliferation of neuronal cells (SK-N-SH) was enhanced by boron in a concentration dependent manner at low concentrations (0.1, 0.5, 1 μM). Besides proliferation activity, boron has an effect on the enhancement of NO production in SK-N-SH cells in a concentration-dependent manner. These studies showed that boron enhances proliferation of osteoblastic cells (especially MG-63), depending upon the concentration of boron. These results also provide further evidence of the positive effects of boron in neuronal disease.

Key words: boron, proliferation, MG-63, HOS, Raw 264.7, SK-N-SH

INTRODUCTION

Bone fractures resulting from osteoporosis has a great impact on the health of the world's elderly (1), making the development of strategies to prevent this disease and maintain bone health of great importance. Protein and macro-minerals such as calcium, phosphorus and magnesium are essential for normal skeletal structure and function. Furthermore, trace minerals such as iron, zinc, copper and boron are involved in bone metabolism (2-5). Boron is an ultra-trace element and highly concentrated in legumes, vegetables, fruits and seaweeds such as sea tangle and laver (6). Boron is also essential for plant's growth (7). There is circumstantial evidence that boron may affect bone mineral homeostasis in various animal models, including humans (8-11).

Hunt and Nielsen (8) reported that boron deprivation depressed growth and elevated plasma alkaline phosphatase activity in chicks fed inadequate cholecalciferol. Working with boron as a micronutrient, Nielsen et al. (9) also found that adding boron back to boron-deficient diets had salutary effects on indices of bone metabolism and mineral homeostasis in humans. These and other data have contributed to the preliminary conclusion that boron is 'probably essential' in human nutrition (10).

Nielsen et al. (11) found that boron repletion in 12 postmenopausal women, who were previously on a low-boron diet for 119 days, markedly reduced the urinary excretion of calcium.

Although boron seems to affect bone metabolism in animals and humans, there has been little research on the functions of boron at the cellular level. Therefore, more research about the effect of boron on cell proliferation in various cell types is necessary.

Bone that is a dynamic tissue which is continuously resorbed through the action of the osteoclast and reformed through the action of the osteoblast. When resorption exceeds formation, bone loss occurs. Therefore, to investigate the effect of boron on bone formation related cells, we examined osteoblastic and osteoclastic cells. In this study we examined whether boron has an effect on proliferation of ostoblastic (MG-63, HOS) and pre-osteocalstic (Raw 264.7) cells. We also investigated the effect of boron on proliferation and NO production in neuronal cells (SK-N-SH).

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MATERIALS AND METHODS

Chemicals

Unless otherwise indicated, all chemicals including boron were purchased from Sigma (St. Louis, MO). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY).

Cell culture and treatment

Human osteosarcoma cell lines (MG-63, HOS) preosteocalstic (Raw 264.7) and the human neuroblastoma cell line (SK-N-SH) were obtained from ATCC (American type culture collection, USA) and these cells were cultured at 37°C with 5% CO_2 in RPMI 1640 medium containing 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells passaged by means of 0.2 M trypsin/1 mM EDTA (Sigma) in phosphate-buffered saline (PBS).

MTT assay for proliferation

Cell proliferation was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay adapted from Mosmann (12). Briefly, the cells were seeded in 96-well plates at suitable densities of cells per well with various concentrations of Boron (13). After an incubation period of 24 or 48 hr, respectively, the enzyme activities of viable cells were measured by addition MTT to each well. After 4 hr of additional incubation, the amount of formazan was determined by absorbance at 540 nm using a microplate reader (Menlo Park, CA).

Nitrite determination

The cells were treated with the control media for 24 or 48 hr in the presence or absence of various doses of the test compounds and the accumulation of nitrite in culture supernatant was measured using the assay system described by Ding et al. (14). 100 μL aliquots of culture supernatants were mixed with an equal volume of Griess reagent (mixture at 1:1 of naphthylethylenediamine dihydrochloride and 1% sulphanilamide in 5% H_3PO_4) and incubated at room temperature for 10 min. The absorbance at 540 nm was measured using a microplate reader. Nitrite concentration was calculated from a $NaNO_2$ standard curve.

Statistical analysis

The data were expressed as mean±SE. The statistical differences between the groups were determined using Student's *t*-test.

RESULTS AND DISCUSSION

Effect of boron on osteoblastic cells proliferation

Osteoblastic cells (MG-63, HOS) were treated with various concentrations of boron (1, 10, 100 μM) for 24 and 48 hr, respectively. The proliferation of MG-63 cells was enhanced at 10 μM ($p<0.05$), for 24 hr (Fig. 1). These data indicated that the effect of boron on osteoblastic cells proliferation is variable depending upon the concentration of boron. In HOS cells, cell proliferation was slightly increased in a concentration dependent manner at 24 hr, although there was no statistical significance, and it had no effect at the same concentration in same cells at 48 hr (Fig. 2).

There is little information on the effect of boron on osteoblastic cells. However, in a recent animal study using rats, Choi et al. (15) reported that serum osteocalcin was significantly higher in high-calcium, boron supplemented, and ovariectomized (OVX) groups than those

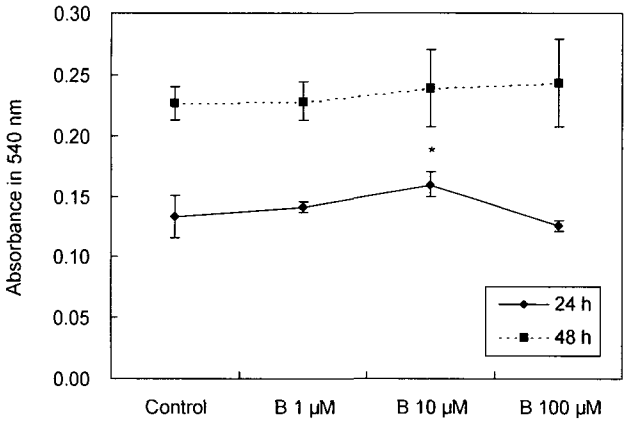

Fig. 1. Effect of boron on proliferation of MG-63 cells (1×10^4 cells/well) treated with boron at various concentrations for 24 and 48 hr. The proliferation of cells was determined by MTT assay. Cell density was measured at 540 nm. The data represents the mean±SE of quadruplicate experiments. *$p<0.05$; significantly different from the control (no treatment).

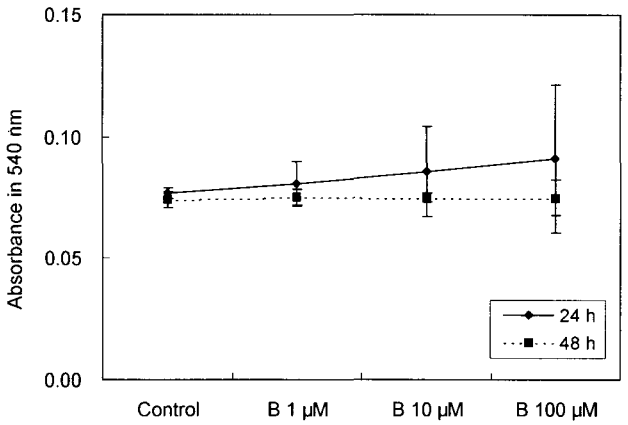

Fig. 2. Effect of boron on proliferation or HOS cells (1×10^4 cells/well) treated with boron at various concentrations for 24 and 48 hr. The proliferation of cells was determined by MTT assay. Cell density was measured at 540 nm. The data represents the mean±SE of quadruplicate experiments.

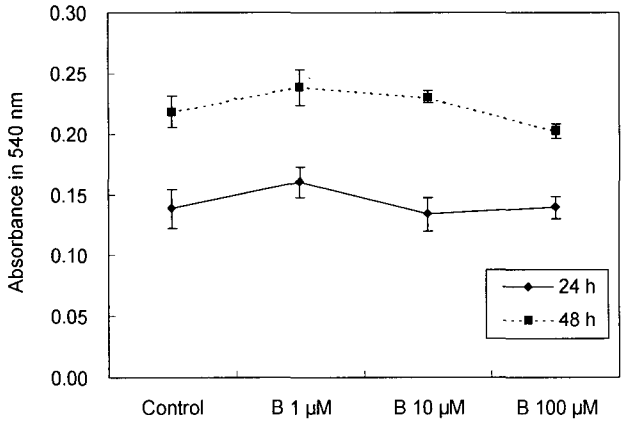

Fig. 3. Effect of boron on proliferation of Raw 264.7 cells (1×10^4 cells/well) treated with boron at various concentrations for 24 and 48 hr. The proliferation of cells was determined by MTT assay. Cell density was measured at 540 nm. The data represents the mean±SE of quadruplicate experiments.

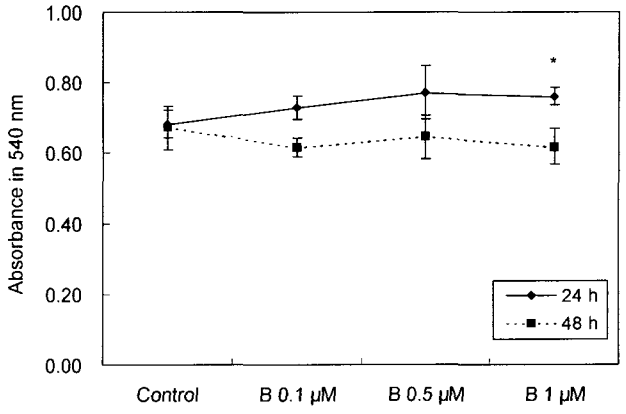

Fig. 4. Effect of boron on proliferation of SK-N-SH cells (5×10^4 cells/well) treated with boron at various concentrations for 24 and 48 hr. The proliferation of cells was determined by MTT assay. Cell density was measured at 540 nm. The data represents the mean±SE of quadruplicate experiments. *p <0.05; significantly different from the control (no treatment).

in other groups. Osteocalcin is the most abundant noncollagenous protein in bone, and serum concentrations of osteocalcin can provide a measure of osteoblast activity or bone remodeling (16).

Effect of boron on pre-osteoclatic cells proliferation

Pre-osteoclastic cells (Raw 264.7) were treated with various concentrations of boron (1, 10, 100 μM) for 24 and 48 hr, respectively. There were no proliferative effects for the concentration of boron used and times of the treatment (Fig. 3).

It is known that in vivo system, bone resorption is indicated by biochemical markers such as urinary calcium, hydroxyproline, pyridinoline, and deoxypyridinoline (DPD). Recently, Choi et al. (15) reported that boron supplementation had no effect on urinary DPD excretion, but boron treatment diminished urinary calcium excretion. Nielsen et al. (11) also reported that boron supplementation of 3 mg B/d decreased urinary Ca and P excretion in human, but did not affect urinary Ca excretion in growing barrow (5).

Effect of boron on proliferation and NO production in neuronal cells

Boron enhanced proliferation of neuronal cells in a concentration-dependent manner at low concentrations (0.1, 0.5, 1 μM) (Fig. 4). The enhancement of boron on the proliferation of neuronal cells may provide further evidence for beneficial effects of boron on neuronal diseases like Alzheimer's disease.

In addition to proliferation activity, boron has an effect on the enhancement of NO production in SK-N-SH cells in a concentration-dependent manner (Fig. 5). It has been known that nitric oxide, one of the smallest biological

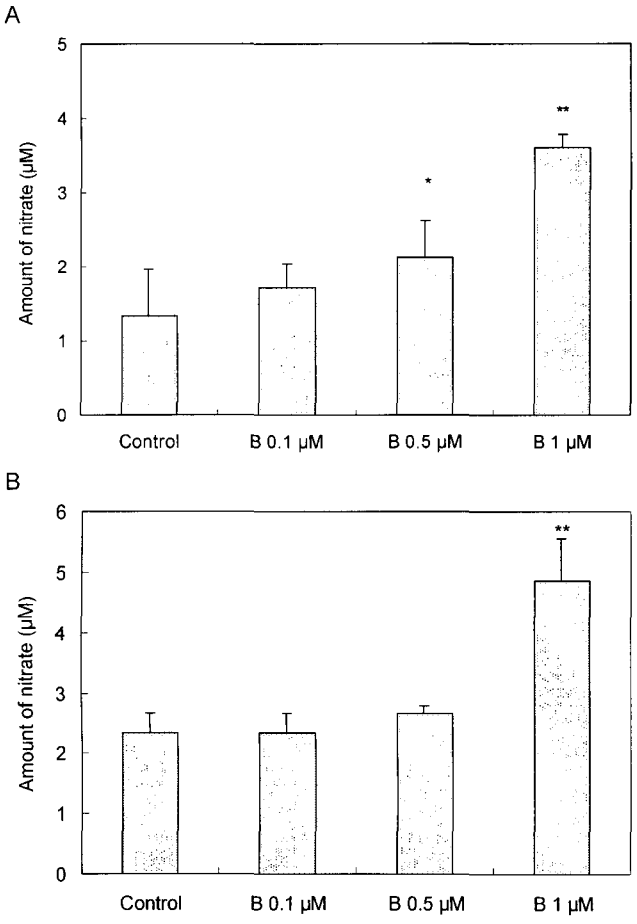

Fig. 5. Effect of boron on nitritie production by SK-N-SH cells. SK-N-SH (5×10^4 cells/well) were treated with boron of various concentration for 24 (A) and 48 hr (B). Culture supernatants were collected and the levels of nitrite were measured as described in materials and method. The data represents the mean±SE of quadruplicate experiments. *p<0.05, **p<0.01; significantly different from the control (no treatment).

molecular mediators, plays important roles in many different aspects of mammalian biology, such as vascular relaxation, neurotransmission, platelet aggregation and immune regulation (17-19).

In recent studies, estrogen has been reported to have many effects on the nervous system, including regulation of neuroendocrine activities, modulation of autonomic functions, neuroprotective effects, and improvement on cognitive tasks (20,21). Many of these effects are mediated or influenced by nitric oxide (NO). Our data indicated that boron increased the production of nitrite in SK-N-SH neuroblastoma cells. Since this effect of boron is similar to that of estrogen, boron might have a positive effect on neuronal disease.

In conclusion, these studies showed that boron increases proliferation of osteoblastic cells (especially MG-63), depending upon the concentration of boron. Therefore, to apply boron to clinical application of treatments for osteoporosis, a suitable concentration of boron must be defined to have the desired effects only on target cells. Also, these results may also provide evidence supporting the positive effect of boron on neuronal disease.

ACKNOWLEDGEMENTS

This work was supported by grant No. RTI05-01-02 from the Regional Technology Innovation Program of the Ministry of Commerce, Industry and Energy (MOCIE).

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(Received October 26, 2005; Accepted December 5, 2005)