This study presents preventive mechanism of a new polyamine synthesis inhibitor on bone metastasis.

**Introduction**
Polyamine is well-known to reflect cellular proliferation and increase in patients with cancer. We have already reported that a new polyamine synthesis inhibitor, methylglyoxal-bis(cyclopentylamidinohydrazone) (MGBCP) showed a strong inhibitory effect on bone metastasis (44th ORS). Intraarterial administration of MGBCP (20 mg/kg) demonstrated more than 80% inhibition of bone metastasis in an animal model. In this paper, we investigated mechanism of inhibition of bone metastasis by MGBCP.

**Material and Method**

**Antitumor effect of MGBCP on B16 melanoma cells**
B16 melanoma cells were diluted to an initial density of 5x10^5 (fifty thousands) cells/ml for the culture. After 12 hours, the cells were further incubated at 37 °C in the absence or presence of 20,30,40 and 50μM MGBCP for 24,48,72,96 hours. Cell number was measured using a counting chamber and microscope. Viability was determined by the trypan blue staining.

**Assay for DNA fragmentation**
After cultivation in the presence of MGBCP for 48 hours, the cells were pelleted by low speed centrifugation. DNA was isolated from the cells as described by Maniatis et al. Equivalent amounts of DNA were loaded into wells of 2% agarose gel and electrophoresed in 40 mM Tris-acetic acid, pH7.5, containing 2 mM EDTA.

**Inhibition of angiogenesis**
The femur and tibia were removed from female DDY mice, and the bones were transplanted in the dorsal subcutis of other female DDY mice aged 6 weeks. MGBCP was administered topically around transplanted bones at doses of 20 mg/kg/day for 14 days immediately after transplantation. Control mice received saline by topical injections. After 14 days, 0.2 ml of 5%Evans blue solution were injected into the left heart ventricle of the mice under anesthesia. The skin was separated from underlying tissues carefully and evaluated of vascularization around the transplanted bones.

**Results**

**Antitumor effect of MGBCP on B16 melanoma cells**
Decreasing rate of cell proliferation were observed with increasing concentrations of MGBCP in B16 melanoma cells. At 30μM, MGBCP completely inhibited the growth of B16 melanoma cells.

**Assay for DNA fragmentation**
Fragmentation of genomic DNA into oligonucleo-somalized fragments is a characteristic of the occurrence of apoptosis. The amounts of oligonucleosomalized fragments in B16 melanoma cells were increased as the concentration of MGBCP was increased (Fig 1). These data suggested that the apoptotic pathway had been activated by the MGBCP treatment in B16 melanoma cells.

**Inhibition of angiogenesis**
We investigated the effect of MGBCP on the transplanted bone to induce angiogenesis. Twenty mg/kg of MGBCP inhibited angiogenesis significantly.

**Discussion**
MGBCP has been synthesized as a multi-enzyme inhibitor for the polyamine-synthesizing pathway. MGBCP is a potent inhibitor of three polyamine-synthesizing enzymes, S-adenosylmethionine decarboxylase, spermidine synthase and spermine synthase. Our previous experiments indicated that the antitumor effects of MGBCP on human osteosarcoma cell lines (MG-63, G-292 and HOS) resulted from the depletion of polyamine contents. There seems to be two possibilities regarding preventive mechanism of bone metastasis by MGBCP. One is direct inhibitory effect on growth of B16 melanoma cells. Another mechanism is inhibition of angiogenesis around and in the extraskeletal bone. Angiogenesis plays a key role in the development of metastasis. The existence of well developed vascular systems in living bones could be appropriate for analysis of the relationship between angiogenesis and the development of metastasis.

**References**

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**Figure 1. DNA Fragmentation in B16 Melanoma Cells**

- MGBCP 50μM 40μM 30μM 20μM 10μM marker
- Fig 1 DNA fragmentation in B16 melanoma cells

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**Preventive mechanism of bone metastasis by a new polyamine synthesis inhibitor**

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