PREVENTION OF BONE METASTASIS IN HUMAN BREAST CANCER BY THE THROMBIN INHIBITOR

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Introduction

Blood-bone metastasis begins with the formation of a new blood supply (angiogenesis) to the primary tumor. This is followed by invasion of tumor cells into the blood, arrest and lodgment in the microvasculature of the target organ, extravasations into the tissue and secondary tumor growth. The association between blood coagulation and cancer growth and metastatic dissemination is not yet completely understood. We have already reported the effect of thrombin on cell motility (47th and 49th ORS). Thrombin stimulated chemotaxis and chemokinesis of highly metastatic osteosarcoma LM8 cells and B16 melanoma cells. And hirudin or argatroban, the thrombin inhibitor, inhibited the stimulatory effect by thrombin. Argatroban inhibited bone metastasis on B16 melanoma cells in animal model. In this study, we investigated that argatroban, the thrombin inhibitor, inhibited bone metastasis in human cancer cells.

Material and Method

Motility assays

Chemotaxis was assayed by phagokinetic tracks. Colloidal gold-coated coverglasses were placed in a 6-well plate, and MDA-231, human breast cancer cell, were seeded at 3x10^5/well with thrombin (0.02-1µM). After 20h incubation with RPMI-1640 and 10% FCS, the phagokinetic tracks were visualized using dark-field illumination in a confocal laser microscope.

MDA-231 cells activated by thrombin, most effective concentration on cell migration of this assay, were seeded at 3x10^5/well with argatroban (0.1-10µM). After 20h incubation, the phagokinetic tracks were measured. Images were processed and measured using NIH Image software. The significance of the differences between argatroban-treated and untreated groups was estimated by ANOVA using the STAT VIEW program.

Effect of argatroban on bone metastasis

MDA-231 cells (1x10^6) suspended in 0.1ml of PBS were injected into the left heart ventricle of the mice with the use of a 29-gauge needle under the anesthesia with pentobarbital. Argatroban was administered intraperitoneally at doses of 9mg/kg/day for 28 days immediately after the inoculation of MDA-231 cells. Untreated mice received saline by intraperitoneal injection. After day 28, incidence of bone metastasis was evaluated in hind limbs by radiography. The significance of the differences between argatroban-treated and untreated groups was estimated by student t-test using the STAT VIEW program.

Results

Motility assays

Thrombin concentration of 0.08µM was most effective in cell migration for MDA-231 cells with 10% FCS for 20 hours incubation. Argatroban dose-dependently inhibited cell migration. The stimulatory activity of thrombin on MDA-231 cells was inhibited significantly by above concentrations of 0.01µM argatroban (Fig 1).

Inhibition of bone metastasis by argatroban

In the untreated mice, bone metastasis developed in 77.5% (31/40 bone) of hind limbs. In the hind limbs, bone metastasis was found in 47.5% (19/40) of the treatment mice (Fig 2). Thus, this drug inhibited bone metastases significantly in this animal model (p < 0.05). No adverse effects on other organs were observed macroscopically or microscopically.

Discussion

Cancer metastasis is a multi-step process in which tumor cell interactions with endothelium and subendothelial matrix play an essential role in determining the organ specificity of metastasis and fate of metastasizing cells. The coagulation system plays a role in tumor invasion and metastasis. The coagulation-derived products, thrombin is known as a stimulator of tumor cell motility. Active motility of tumor cells is crucial in the establishment of metastasis. The important role that activation of the coagulation system plays in the process of tumor invasion and metastasis has been well documented. It has been reported that anticoagulant therapy suppresses the invasion of cancer cells. Argatroban, a synthetic small molecule direct thrombin inhibitor, selectively inhibits the catalytic site of thrombin in a reversible manner. In this study, we showed that argatroban inhibited bone metastases in human cancer cell line, MDA231. Argatroban is thought to exert an inhibitory effect on bone metastasis by mechanisms inhibiting tumor cell motility. It is advantageous that argatroban is available for clinical use to inhibit bone metastasis since it has already been used clinically against thrombosis. Anticoagulant therapy, such as argatroban therapy, may be a new preventable agent for bone metastases.

Fig. 1 Motility assays. Argatroban dose-dependently inhibited cell migration.

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<thead>
<tr>
<th>Hindlimbs</th>
<th>Untreated</th>
<th>Argatroban</th>
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<td>31 / 40 bone (77.5 %)</td>
<td>19 / 40 bone (47.5%)</td>
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Fig. 2 Incidence of bone metastasis treated argatroban by intraperitoneal injection (n=10). Argatroban inhibited bone metastases significantly in animal model (p < 0.05).