Antimetastatic Activity of a Synthetic Serine Protease Inhibitor, FOY-305 (Foypan®)

MOTOHIRO OHKOSHI1 and YUTARO SASAKI2

1Department of Health Care, Showagakuin University, Arakino 3-29-20, Abiko-shi, 270-1114; 2ONO Pharm. Ltd., Japan

Abstract. Metastasis is one of the major causes of mortality in cancer. It is well known that the activities of cell surface serine proteases are especially enhanced in malignant tumors. Proteolytic degradation of the extracellular matrix and basal membrane is a crucial event for tumor cell invasion and metastasis formation. FOY-305 (Foypan®), a remedy for tumor pancreatitis, is a broad spectrum synthetic serine protease inhibitor which inhibits enzymatic activities including trypsin, thrombin, kallikrein and plasmin. Using Lewis lung carcinoma cell, we found that FOY-305 inhibited both spontaneous and experimental pulmonary metastasis. Furthermore, the combined treatment of FOY-305 and a traditional anti-cancer drug, 5-FU or bleomycin, resulted in marked enhancement of anti-pulmonary metastatic activity.

FOY-305 (Foypan®) is a broad spectrum synthetic serine protease inhibitor which inhibits enzymatic activities including trypsin, thrombin, kallikrein and plasmin (1). Previous studies have shown that FOY-305 suppressed not only 3-methylcholanthrene-induced carcinogenesis in mice, but also the growth of spontaneous solid tumors in mice (2-6). A phase analysis by flow cytometry showed that FOY-305 suppressed the cell cycle progression at G1-or S-phase (7).

During the sequential steps of metastasis, the interaction of the tumor cell with various host cells (platelets, lymphocytes, endothelial cells) and extracellular matrix (EM) and basement membrane (BM) is a crucial event for tumor cell invasion and metastasis formation.

It is well known that tumor cells secrete various proteolytic enzymes. In particular, uPA-activated plasmin, as well as matrix metalloproteinase (MMPs), plays an important role in tumor cell invasion (8).

We have already reported that FOY-305 potently inhibited the invasion of HT-1080 cells into the reconstituted BM matrigel, as well as u-PA activity (9).

In the present study, we investigated whether FOY-305 would inhibit the metastasis of Lewis lung carcinoma and possibly enhance the anti-metastatic activities of 5-FU and bleomycin (BLM).

Materials and Methods

Materials. FOY-305 (Foypan®) was obtained from Ono Pharmaceutical Co., Ltd. (Osaka, Japan), 5-FU was obtained from Sankyo Co., Ltd., Tokyo, Japan, bleomycin (BLM) from Nippon Kayaku Co., Ltd., Tokyo, Japan. Leupeptin (10) was obtained from Nippon Kayaku Co., Ltd., Tokyo, Japan.

Animals. Female C57/B1 black mice weighing between 17g and 19g, obtained from the Shizuoka Laboratory Animal Center, Shizuoka, Japan, were used. The mice were fed normal chow purchased from Oriental Fermention Co., Ltd., Tokyo, Japan.

Metastasis experiment with mouse Lewis lung carcinoma. i) Spontaneous metastasis: Lewis lung carcinoma was subcutaneously grown in female C57/B1 mice and continuously maintained by transplantation from mouse to mouse. A tumor was surgically removed under sterile conditions and excised with scissors. Sliced tissues were then trypsinized in PBS for 30 min at 37°C with stirring and the obtained cell suspension was adjusted to 3.3 x 10^6 cells/ml. Ten cells were inoculated into a foot pad of female C57/B1 black mice weighing between 17g and 19g. The mice were fed on a diet containing 0.1% FOY-305 or 0.1% leupeptin, starting Day 1 after tumor inoculation, while control mice were fed on the basal diet. The primary tumor was surgically removed on Day 11. Sacrifice and lung examination for metastasis were performed on Day 20. The number of colonies on the lung surfaces was counted. ii) Experimental metastasis: A Lewis lung carcinoma cell suspension was prepared as described in the method for the spontaneous metastasis experiment. In addition, the combined treatment of FOY-305 with 5-FU or bleomycin was performed. 1.5x10^6 viable Lewis lung carcinoma cells were inoculated into the tail vein of female C57/B1 black mice weighing between 17g and 19g. The mice were fed on a diet containing 0.1% ...

Correspondence to: Motohiro Ohkoshi, Department of Health Care, Showagakuin University, Arakino 3-29-20, Abiko-shi, 270-1114, Japan. Tel/Fax: +81-471-88-5089.

Key Words: Lewis lung carcinoma, metastasis, protease inhibitor, Foypan.
FOY-305, Days 1–15, while control mice were fed on the basal diet. During the experiment, 5mg/kg of 5-FU or 2.5mg/kg of BLM dissolved in physiological saline were intraperitoneally injected into the mice once every two days. Sacrifice and assay of pulmonary metastasis were performed on Day 15. All removed tumors and lungs in the experimental or control mice were fixed in 10% formalin, embedded in paraffin and cut into sections. Paraffin sections were stained with hematoxylin and eosin for microscopic examination. Statistic analysis was performed by Student’s t-test.

**Results**

Table I shows data on the effect of FOY-305 or leupeptin on the spontaneous metastasis of Lewis lung carcinoma in mice. 0.1% FOY-305 in the diet significantly reduced the number of metastases of the pulmonary surface, the dry weight of pulmonary metastasis and the tumor diameter, respectively (p<0.001). FOY-305 inhibited more potently the pulmonary metastasis of Lewis lung carcinoma than leupeptin.

Table II shows data on the comparative effect of FOY-305 and the combined treatment with 5-FU on the experimental pulmonary metastasis in mice. 0.1% FOY-305 in the diet also significantly inhibited the experimental pulmonary metastasis...
Single application of 5mg/kg 5-FU did not significantly inhibit the pulmonary metastasis in this study, but the combined treatment of FOY-305 with 5-FU resulted in marked enhancement of the anti-metastatic activity (p<0.001).

Figure 1 shows the pathological findings of the experimental pulmonary metastasis in the mice.

Table III shows data on the comparative effect of FOY-305 in combined treatment with 2.5mg/kg of BLM on the experimental pulmonary metastasis. Single application of 2.5mg/kg of BLM did not significantly inhibit the pulmonary metastasis in this study, but the combined treatment of FOY-305 with BLM resulted in marked enhancement of the anti-metastatic activity (p<0.005).

Discussion

Serine proteases like trypsin or plasmin degrade the components of the extracellular matrix (ECM). Through this function, malignant tumor cells can migrate and invade distant organs (11). Tumor invasion into the ECM and basement membrane (BM) is a crucial step of tumor metastasis. In order to investigate the possible therapeutic intervention against tumor invasion, we had investigated the anti-invasive activities of several synthetic serine protease inhibitors. FOY-305, a serine protease inhibitor, at a non-cytotoxic concentration, was effective at inhibiting HT-1080 fibrosarcoma invasion in vitro (9). It is
well known that leupeptin has an inhibitory effect on tumor metastasis (12).

In the present study, spontaneous pulmonary metastasis of Lewis lung carcinoma was more significantly inhibited by FOY-305 ($p<0.001$) than leupeptin ($p<0.05$). Furthermore, the combined treatment of FOY-305 and 5-FU or bleomycin resulted in marked enhancement of the experimental anti-metastatic activity ($p<0.005$).

In conclusion, the present study suggests the possible use of the invasion-associated protease inhibitor FOY-305 in controlling tumor metastasis.

References


Received August 18, 2004
Revised December 6, 2004
Accepted December 16, 2004