Anti-Invasive and Metastatic Activities of Evodiamine

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We have recently reported that evodiamine can suppress in vitro invasion and lung metastasis by colon 26-L5 carcinoma cells. To extend our study, we examined the anti-invasive and metastatic effects of evodiamine on Lewis lung carcinoma (LLC) and B16-F10 melanoma in addition to colon 26-L5 carcinoma. Critical structures of evodiamine for the activities were also evaluated by comparison with compounds possessing structures similar to that of evodiamine. Evodiamine concentration-dependently inhibited the invasion of B16-F10, LLC and colon 26-L5 cells with IC50 values of 2.4 μM, 4.8 μM and 3.7 μM, respectively. Pre-treatment of colon 26-L5 cells with evodiamine before inoculation into mice caused significant suppression of the liver metastasis as well as the lung metastasis. Lung metastasis by LLC is also inhibited significantly by pre-exposure to evodiamine. When the anti-migratory activity of evodiamine was compared with that of evodiamine-like compounds, rutacearpine lacking a methyl group at N-14 and a hydrogen at C-13b exhibited much less effect than evodiamine. In addition, reserpine, having β-configurated hydrogen at C-13b, inhibited tumor cell migration more potently than yohimbine, having α-configurated hydrogen at the same position. These results suggest that evodiamine may be useful as a leading compound for agents in tumor metastasis therapy. Also, the presence of a methyl group at N-14 and the configuration of hydrogen at C-13b may be responsible for the inhibitory activities of evodiamine.

Key words evodiamine; tumor cell; invasion; migration; metastasis

Tumor metastasis is a major cause of death in cancer patients, and its blockade has been considered to enable cancer patients to survive.1 Thus, it is important to find promising agents with anti-metastatic activity.

The cascade of cancer metastasis comprises a complex multistep process, and tumor invasion into the extracellular matrix plays an important role in tumor metastasis.2–5 Various products have been shown to possess anti-invasive activities, and some of them are demonstrated to be able to prevent tumor metastasis.6–9 We have recently reported that evodiamine can markedly inhibit invasion and lung metastasis by colon 26-L5 cells.10

Evodiamine is one of the main constituents of Evodiae Fructus11 and has been shown to possess anti-tumor growth,12 anti-aldosterone releasing,13 anti-obesity,14 bronchoconstrictive,15 anti-nociceptive,16 vasorelaxant17 catecholamine-secretory18 and anti-nitric oxide producing19 properties. On the other hand, the anti-invasive and metastatic effects of evodiamine have not been fully elucidated.

To extend our study, we investigated here the anti-invasive and metastatic activities of evodiamine on lung carcinoma and melanoma cells, and in addition to colon carcinoma cells, and estimated critical structures of evodiamine for the inhibition of tumor cell invasion, migration and metastasis using compounds having structures similar to that of evodiamine.

MATERIALS AND METHODS

Materials Evodiamine, 14-methyl-8,13,13b,14-tetrahydro-7H-indolo-[2′,3′:3,4] pyrido[2,1-b] quinazolin-5 one, was purchased from Matsuura Yakujiyo Co., Ltd. Rutacearpine, yohimbine, reserpine and indole were purchased from Wako Pure Chem. Ind., Ltd. 2-Mercapto-4(3H)-quinazolinone was purchased from Tokyo Kasei Kogyo Co., Ltd. These compounds were dissolved in dimethylsulfoxide and used in this study.

Cells and Cell Culture Murine colon 26-L5 adenocarcinoma was kindly provided by Prof. I. Saiki (Toyama Med. Pharm. Univ., Inst. of Natural Medicine, Toyama, Japan). Murine B16-F10 melanoma was kindly provided by Dr. S. Wakuzawa (Hokuriku Univ., Kanazawa, Japan). Lewis lung carcinoma (LLC) was purchased from RIKEN Cell Bank. Colon 26-L5 cells and B16-F10 cells were maintained in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 2-mercaptoethanol, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. LLC was maintained in DMEM with 10% FCS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin.

Animals Inbred 6 week-old female Balb/c mice were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. All mice were housed in a controlled environment with a 12 h light/dark cycle, a temperature of 24±2°C and humidity at 55±10%, and they were given commercial food and tap water ad libitum. After an acclimatization period of 1 week, these mice were used in the present study.

Cell Invasion and Migration Assays Tumor cell invasion through a reconstituted basement membrane (Matrigel) were assayed according to the methods reported previously.20 Briefly, in transwell cell culture chambers, filters of 8 μm pore size were coated with Matrigel on the upper surface and 0.5 μg of fibronectin on the lower surface. Volumes of Matrigel were 50 μg for colon 26-L5 cells, and 30 μg for LLC and B16-F10 cells. Tumor cells (2×105) suspended in medium containing 0.1% bovine serum albumin were pretreated with various concentrations of evodiamine for 30 min on ice, then added to the upper compartment. Evodiamine was also added to the lower compartment. The control was the vehicle given in the same way. After a 6 h incubation at 37°C, cells were fixed and stained with crystal violet. In a cell migration assay, colon 26-L5 cells were pretreated with test compounds, as described above, in the chambers with filters coated with fibronectin on the lower surface, which were then incubated for 3 h. Cells that had invaded or migrated to
the lower surfaces of filters were extracted, and absorbance of the cell lysate was measured at 590 nm. IC50 values in the invasion assay were determined using the computer software, DeltaGraph Pro 3.5 (Nippon Polaroid KK, Tokyo, Japan).

Experimental Lung Metastasis Assay Colon 26-L5 cells or LLC were harvested and treated with 30 μM evodiamine at a density of 1×105 or 2×105 cells/200 μl on ice for 30 min, respectively. Then, the treated cells were injected with the compound into 6 mice per group. The control was the vehicle given in the same way.

Statistical Analysis All data are expressed as mean±S.D. Statistical differences were evaluated by Student’s t-test, and p<0.05 was considered significant.

RESULTS AND DISCUSSION

We have demonstrated that evodiamine has suppressive activity on the in vitro invasion and lung metastasis of colon 26-L5 cells.10) To extend our observations, anti-invasive and metastatic effects of evodiamine on other tumor cell lines, Lewis lung carcinoma (LLC) and B16-F10 melanoma, were examined here. Evodiamine was applied at a concentration of less than 30 μM, because the concentration range exhibited a marginal effect on tumor cell viability.21) As shown in Fig. 1, evodiamine inhibited the invasion of B16-F10 cells, as well as colon 26-L5 cells, in a concentration-dependent manner and achieved 70—80% suppression at 30 μM in both cell lines. Evodiamine also suppressed the invasive activity of LLC cells, although the effect reached a plateau over 3 μM. IC50 values were determined to be 3.7 μM for colon 26-L5 cells, 2.4 μM for B16-F10 cells and 4.8 μM for LLC cells.

These results suggest that the inhibitory activity of evodiamine on tumor cell invasion is independent of kinds of tumor cells.

The invasive activity of tumor cells is one of the critical factors in the establishment of tumor metastasis.2) In fact, several products possessing anti-invasive effects have been shown to inhibit tumor metastasis.3—9) We have also shown that the treatment of colon 26-L5 cells with evodiamine before inoculation into mice caused a marked reduction in lung metastasis.10) On the other hand, it has been demonstrated that tumor metastasis is affected by the microenvironment of the target organs22) and that the metastatic properties of tumor cells vary according to different kinds of tumor cells.23) Therefore, the anti-metastatic effect of evodiamine was further examined in terms of the liver metastasis of colon 26-L5 cells and the lung metastasis of LLC cells. As shown in Table 1, the exposure of colon 26-L5 cells to 30 μM evodiamine before inoculation caused significant suppression (p<0.01) of liver metastasis, which was almost the same inhibition rate as that in lung metastasis. Lung metastasis by LLC is also reduced significantly (p<0.01) by pre-treatment with 30 μM evodiamine. These results suggest that evodiamine may exert anti-metastatic activities independent of the microenvironment of target organs or type of tumor cell.

The cell invasion by the transwell chambers with Matrigel and fibronectin used in this study involves three sequential steps which are cell adhesion to Matrigel, enzymatic degradation of the gel by tumor cell-derived enzymes, and cell migration to fibronectin through the degraded gel area.24) We have previously demonstrated that evodiamine inhibited the migration of colon 26-L5 cells with an IC50 value of 4.1 μM.21) And in the present study, almost the same IC50 value was determined for the tumor cell invasion, as described in Fig. 1.19) These observations indicate that the anti-invasive activity of evodiamine may be mainly based on the inhibition of tumor cell migration. Then, to estimate structures contributing to the anti-invasive and migratory activities of evodiamine, the effects of compounds having structures similar to that of evodiamine on tumor cell migration were examined. As shown in Fig. 2, rutaecarpine, lacking a methyl group at position 14 and a hydrogen at position 13b, had a more potent effect on tumor cell migration. The stereochemical importance of hydrogen at position 13b could be supported by the observation that reserpine, having a β-configurated hydrogen at position 13b, had a more potent effect on tumor cell migration. These observations suggest that the presence of a methyl group at position 14 and the configuration of hydrogen at position 13b may be responsible for the inhibitory effect of evodiamine. The stereochemical importance of hydrogen at position 13b could be supported by the observation that reserpine, having a β-configurated hydrogen at position 13b, had a more potent effect on tumor cell migration.

Table 1. Metastasis by Tumor Cells Treated with Evodiamine before Inoculation

<table>
<thead>
<tr>
<th>Tumor cells</th>
<th>Metastasis</th>
<th>Treated with</th>
<th>No. of nodules</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon 26-L5</td>
<td>Lung</td>
<td>DMSO</td>
<td>83±45</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Evodiamine</td>
<td>25±17</td>
<td></td>
</tr>
<tr>
<td>LLC</td>
<td>Lung</td>
<td>DMSO</td>
<td>115±61</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Evodiamine</td>
<td>23±17</td>
<td></td>
</tr>
</tbody>
</table>

Tumor cells were treated with 30 μM evodiamine or DMSO for 30 min, and then inoculated into 6 mice per group. Tumor colonies on the surface of lungs or livers were counted under a dissection microscope on day 14 (colon 26-L5) or 21 (LLC). Tumor colonies are expressed as mean±S.D.
inhibitory effect than yohimbine with an α-configurated hydrogen at the same position. The anti-migratory activities of evodiamine with α- and β-configurated hydrogen at position 13b should be tested to clarify this point. Additionally, partial structures of evodiamine, indole and 2-mercapto-4(3H)-quinazolinone were found to have much less activity compared to evodiamine, indicating that both indole and quinazolinone moieties may be necessary to exert the complete inhibitory effect of evodiamine.

In this study, we demonstrated that evodiamine is effective in inhibiting invasion and metastasis by LLC and colon 26-L5 cells, and invasion by B16-F10 melanoma cells. In addition, functional groups at position 14 and the configuration of hydrogen at position 13b of evodiamine may affect its inhibitory effects on tumor cell invasion, migration and metastasis. These results suggest that evodiamine may be useful as a leading compound for agents in tumor metastasis therapy.

REFERENCES