Inhibition of Experimental Pulmonary Metastasis of Mouse Colon Adenocarcinoma 26 Sublines by a Sialic Acid:Nucleoside Conjugate Having Sialyltransferase Inhibiting Activity

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ABSTRACT

The total and sialidase-releasable sialic acid contents of mouse colon adenocarcinoma 26 sublines of high (NL-17) and low (NL-44) metastatic potential were found to be positively correlated with their ability to undergo metastasis. Furthermore, sialytransferase activity of intact NL-17 cells was higher than that of NL-44 cells. These findings suggest that sialic acid on the cell surface may play a role in the metastasis of these cells.

Therefore, the effect of a sialyltransferase inhibitor, 5-fluoro-2',3'-isopropylidene-5'-O-(4-N-acetyl-2,4-dideoxy-3,6,7,8-tetra-O-acetyl-1-methoxycarbonyl-D-glycero-α-D-galactoctapopyranosyl)uridine (KI-8110), on the experimental lung metastasis of NL-17 or NL-44 cells was examined. KI-8110 inhibited the transfer of sialic acid to its endogenous acceptor in NL-17 and NL-44 cells. NL-17 or NL-44 cells were injected into the tail veins of mice, and the metastasis-inhibiting activity of KI-8110 was evaluated on the basis of both the lung weight and the number of pulmonary surface nodules about 3 wk after the tumor cell injection and of the survival ratio of mice inoculated with the tumor cells. Pretreatment of tumor cells with KI-8110 together with i.v. injection of KI-8110 caused significant inhibition of pulmonary metastasis of both NL-17 and NL-44 cells. Inhibition of metastasis and prolongation of survival were also observed on i.v. injection of KI-8110 without pretreatment of the tumor cells with KI-8110, but the degree of inhibition was lower than that in the case of the two treatments together.

KI-8110 itself had neither cytostatic nor cytotoxic effects on NL-17 and NL-44 but reduced the retention of tumor cells in the lungs. This antimitastatic effect of KI-8110 may be due to modification of the tumor cell surface resulting from inhibition of sialyltransferase by KI-8110. In addition, a β-linked sialic acid:nucleoside conjugate (KI-8111) and an equimolar mixture of KI-8110 and KI-8111 (KI-414) also inhibited the metastatic ability of NL cells to the same extent as KI-8110 did.

INTRODUCTION

Cancer metastasis is one of the most important problems in cancer research. Metastatic processes are very complicated because they involve various factors, and the overall mechanism is not yet understood.

Recently biochemical studies have provided considerable evidence suggesting differences in the tumor cell surface properties between metastatic and nonmetastatic cells. Some of these findings indicate the close correlation of the sialic acid content of cell membranes with the metastatic potential. Bosmann et al. (1) and Yogeeswaran et al. (2) have found that the amount of neuraminidase-releasable sialic acid on the cell surface of a B16 melanoma subline of high metastatic potential was greater than that of a subline of low metastatic potential. Tao and Burger (3) have shown that a lectin-resistant B16 variant which had lost the experimental metastatic properties also showed a decrease in cell surface sialic acid content. Parallel findings have been observed for RNA tumor virus-transformed sarcoma lines (4) and other systems (5-9). Also reported was evidence which suggests a connection between the sialyltransferase activity of tumor cells and metastatic potential (10).

Thus sialylation of the tumor cell surface seems to be closely related to the metastatic potential of the tumor cells. Consequently it may be possible to modify the metastatic potential of tumor cells by altering the sialic acid metabolism of the cells. As reported in the previous paper, certain sialic acid:nucleoside conjugates show sialyltransferase inhibiting activity in normal murine lymphocytes (11, 12).

In this paper, we describe the effect of these sialic acid:nucleoside conjugates on tumor cell metastasis.

MATERIALS AND METHODS

Reagents. The sialic acid:nucleoside conjugate (KI-8110) (Fig. 1), the β-anomer of KI-8110 (KI-8111), and an equimolar mixture of the α- and β-anomers of the above sialic acid:nucleoside conjugate (KI-414) were synthesized by the method of Kijima et al. (11). CMP-N-acetyl-[4,5,6,7,8,9-3H]neuraminic acid (247 mCi/mmol) and [6-3H]thymidine (15 Ci/mmol) were purchased from New England Nuclear (Boston, MA); neuraminidase (Arthrobacter ureafaciens), from Nakarai Chemicals, Ltd. (Kyoto, Japan); trypsin (1:250), from DIFCO Laboratories (MI); and heparin sodium from Novo (Denmark).

Mice. Eight-wk-old female BALB/c mice were obtained from Charles River Japan (Kanagawa, Japan) and kept under pathogen-free conditions.

Tumor Cells. Tumor cell lines named NL-17 and NL-44 were established from colon adenocarcinoma 26 by Tsuruo et al. (13) and kindly provided by Dr. T. Yamori (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan). NL-17 is a subline that shows high pulmonary metastatic potential on i.v. injection, but NL-44 shows low metastatic potential under the same conditions. Cells were maintained in vitro RPMI-1640:FCS. The generation time of NL-17 and NL-44 cells is almost the same (NL-17, 21.2 ± 0.2 h; NL-44, 22.6 ± 2.2 h).
was measured as follows. Tumor cells with or without pretreatment with KI-8110 for 24 h were washed twice with Ca2+, Mg2+-free Hank’s balanced salt solution and then centrifuged, the supernatant was collected and assayed for sialic acid content. The extracts were pooled and dried in a scintillation vial, and the sialic acid was released by incubation with neuraminidase. The acid-insoluble materials were collected by centrifugation and then washed twice with 2 ml of phosphotungstic acid and once with 5% trichloroacetic acid. The washed pellet was extracted twice with chloroform:methanol (2:1, v/v) and once with chloroform:methanol (1:2, v/v). The extracts were pooled and dried in a scintillation vial, and the sialic acid content was measured by the thiobarbituric acid method of Aminoff (15), and the pellet was assayed for protein by the method of Lowry et al. (16).

Assay of Sialyltransferase Activity. Tumor cells were harvested by trypsinization (0.05% trypsin:0.02% EDTA solution) for 2 min. The reaction was stopped by the addition of ice-cold RPMI-1640:FCS. The cells were washed twice with the medium and once with 0.01 M sodium phosphate buffer containing 0.12 M NaCl and 1 mM MgCl2 (assay buffer). Cell numbers and cell viability were determined by the trypan blue exclusion test. Ectosialyltransferase activity was measured according to the method of Painter et al. (14). Briefly, an incubation mixture containing 5 x 106 cells and 2 μM CMP:N-acetyl-[14C]neuraminic acid in 100 μl of assay buffer was shown to work with or without the addition of 10–4 M KI-8110 at 37°C in a shaking water bath for 2 h, and the reaction was terminated by the addition of 2 ml of ice-cold 0.5 M HCl containing 1% phosphotungstic acid. The acid-insoluble materials were collected by centrifugation and then washed twice with 2 ml of phosphotungstic acid and once with 5% trichloroacetic acid. The washed pellet was extracted twice with chloroform:methanol (2:1, v/v) and once with chloroform:methanol (1:2, v/v). The extracts were pooled and dried in a scintillation vial, and the pellets were solubilized with Soluene-350 (Packard Instrument Company, Inc.). The radioactivity was determined with a liquid scintillation system.

Determination of Sialic Acid. Neuraminidase-releasable sialic acid was measured as follows. Tumor cells with or without pretreatment with 10–4 M KI-8110 for 24 h were washed twice with Ca2+, Mg2+-free Hank’s balanced salt solution and then detached from the culture dish by treatment with 0.02% EDTA for 5 min. The detached cells were then incubated with 0.02 units of neuraminidase in 1 ml of 0.01 M sodium phosphate buffer (pH 6.5) containing 0.15 M NaCl at 37°C for 2 h. After centrifugation, the supernatant was collected and assayed for sialic acid by the thioarbituric acid method of Aminoff (15), and the pellet was assayed for protein by the method of Lowry et al. (16). Released sialic acid was not detectable in the absence of enzyme. Total sialic acid was determined as follows. Cells harvested as described above were suspended in 0.05 M H2SO4, and the suspension was kept for 1 h at 80°C. After centrifugation, the supernatant was assayed for sialic acid by the method of Roboz et al. (17), and the pellet was assayed for protein by the method of Lowry et al. (16).

Assay of Pulmonary Metastasis. NL-17 and NL-44 cells were cultured with (or without) 10–4 M KI-8110 for 24 h and harvested by trypsinization as described above. The final cell suspension contained 2.5 x 106 cells in 1 ml of 0.9% NaCl supplemented with 1% BALB/c mouse serum. The cell suspension (0.2 ml) was injected into the tail veins of mice, followed immediately by the injection of 0.2 ml of KI-8110 solution (2.5 mg/ml) or 0.9% NaCl solution. Then the mice were given 0.2 ml of KI-8110 solution (2.5 mg/ml) or 0.9% NaCl solution i.v. every 3 days. About 3 wk after the tumor cell implantation, the mice were subjected to autopsy, lung weights were determined, and, after fixation of the lungs in 10% formaldehyde:picric acid solution, pulmonary metastases were grossly estimated by counting the number of metastatic nodules on the pulmonary surface. It has been suggested that the "pulmonary i.v. colonization assay" does not truly reflect the state of spontaneous metastatic potential. For the sake of brevity, "pulmonary metastasis" in our experimental metastasis assay will mean i.v. implantation—survival and growth of NL cells.

Measurements of Cell Growth or Viability. Cells were incubated with or without KI-8110 in RPMI-1640:FCS at 37°C. At various culture times, the cell number and the cell viability were determined by trypan blue dye exclusion in a hemacytometer.

In Vivo Tumor Cell Retention. Labeled tumor cells were injected into the tail veins of mice, and the retention of radioactivity in the lungs was studied. NL-17 cells were incubated with [3H]thymidine for 24 h at a concentration of 25 μCi/ml. The labeled tumor cells were injected into the tail veins of mice, and then at various times after the injection, mice were sacrificed and their lungs were excised. The excised lungs were put into scintillation vials, solubilized with NCS (Amersham, Arlington, IL), and then assayed for radioactivity with a liquid scintillation system.

RESULTS

Effects of KI-8110 on Sialyltransferase Activity in NL-17 and NL-44 Cells. When tumor cells were incubated with N-acetyl-[14C]neuraminic acid in the presence of KI-8110, the incorporation of N-acetyl-[14C]neuraminic acid into glycoproteins and glycolipids of the tumor cells was significantly inhibited (Table 1). In this assay, cell viability did not decrease during the incubation. Table 1 also shows that the incorporation of N-acetyl-[14C]neuraminic acid was about 2-fold higher in NL-17 cells than in NL-44 cells. Since NL-17 is experimentally a highly metastatic cell line and NL-44 a cell line of low metastatic potential, sialyltransferase activity may be positively correlated to the metastatic potential of tumor cells.

Change in Sialic Acid Content of NL-17 and NL-44 Cells on Treatment with KI-8110. As shown in Table 2, the neuraminidase-releasable sialic acid and total sialic acid contents of the tumor cells decreased on treatment with 10–4 M KI-8110 for 24 h. For both NL-17 and NL-44, the effect of the drug on the neuraminidase-releasable sialic acid content of the cells was found to be more significant than that on the total sialic acid content. It is also shown in Table 2 that both the neuraminidase-releasable and total sialic acid contents are about 2-fold higher in NL-17 cells than in NL-44 cells. These results suggest that the sialic acid content is positively correlated with the metastatic potential in these cell lines like the sialytransferase activity discussed above.

Effects of KI-8110 on Pulmonary Metastasis Resulting from i.v. Injection of NL-17 and NL-44 Cells. As shown in Table 3 and Fig. 2, pulmonary metastasis of NL-17 and NL-44 cells was significantly inhibited by pretreatment and i.v. injection of KI-8110. Increases in lung weight due to the growth of metastasized colonies were also inhibited by KI-8110. Administration of KI-8110 i.v. without pretreatment inhibited pulmonary metastasis, and the effect of the combination of pretreatment with KI-8110 with i.v. administration of the drug was additive, as shown in Table 3. The survival of mice inoculated with NL-17 cells was prolonged significantly by the administration of KI-8110 (Fig. 3). KI-8111, β-anomer of KI-8110, and KI-414, an equimolar mixture of KI-8110 and KI-8111, also inhibited the metastatic ability of NL cells to the same extent as KI-8110 did (Table 4). Furthermore, KI-8111 inhibited sialic acid transfer to the endogenous acceptors of NL-17 cells (Table 1). This suggests that the β-linked sialic acid:nucleoside conjugate as well as the α-linked sialic acid:nucleoside conjugate (KI-8110) has an inhibitory activity toward the metastasis of NL cells. KI-8110 (0.1 mM) had no effect on the growth and viability of NL-17 and NL-44 cells until 5 days after addition of KI-8110 (data not shown).
INHIBITION OF EXPERIMENTAL PULMONARY METASTASIS

Table 1
Effect of KI-8110 and KI-8111 on sialic acid transfer to the endogenous acceptors of NL-17 and NL-44 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Protein</th>
<th>Lipid</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N-acetyl-[^14C]neuraminic acid incorporated (dpm/10^7 cells)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL-17</td>
<td>None</td>
<td>3005.3 ± 115.5 a</td>
<td>3157.6 ± 202.2</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>2007.4 ± 473.2 b</td>
<td>1047.3 ± 246.4</td>
</tr>
<tr>
<td></td>
<td>KI-8111 (10^-4 M)</td>
<td>2689.2 ± 67.2 c</td>
<td>629.1 ± 35.4</td>
</tr>
<tr>
<td>NL-44</td>
<td>None</td>
<td>1398.9 ± 8.5</td>
<td>1816.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>892.7 ± 142.9 d</td>
<td>448.3 ± 24.0</td>
</tr>
<tr>
<td></td>
<td>KI-8111 (10^-4 M)</td>
<td>1457.8 ± 32.7 e</td>
<td>272.4 ± 14.8</td>
</tr>
</tbody>
</table>

a Mean ± SD for three experiments.
b Treatment group versus nontreated group, Student's t test, P < 0.05.
c Treatment group versus nontreated group, Student's t test, P < 0.01.
d Treatment group versus nontreated group, Student's t test, P < 0.001.

table 2
Cell surface (neuraminidase-releasable) and total sialic acid contents of KI-8110-treated or nontreated NL-17 and NL-44 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Neuraminidase releasable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL-17</td>
<td>None</td>
<td>3.3 ± 0.22 a</td>
<td>13.5 ± 1.69</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>2.75 ± 0.15 b</td>
<td>9.9 ± 1.72 c</td>
</tr>
<tr>
<td>NL-44</td>
<td>None</td>
<td>1.53 ± 0.04 d</td>
<td>7.78 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>1.22 ± 0.04 e</td>
<td>6.50 ± 0.25 f</td>
</tr>
</tbody>
</table>

a Mean ± SD for three experiments.
b Non-treated group versus treated group, Student's t test, P < 0.05.
c Non-treated group versus treated group, Student's t test, P < 0.01.
d Non-treated group versus treated group, Student's t test, P < 0.001.

table 3
Effect of KI-8110 on the experimental metastasis of NL-17 and NL-44 cells in mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Lung wt (mg)</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL-17</td>
<td>None</td>
<td>140 ± 26 a</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>520 ± 183 b</td>
<td>81 ± 26 c</td>
<td>72 ± 77 d</td>
<td>47-130 8/8</td>
</tr>
<tr>
<td>NL-44</td>
<td>None</td>
<td>201 ± 03 e</td>
<td>23 ± 19 a</td>
<td>21 ± 53 b</td>
<td>0-53 7/8</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>165 ± 55 f</td>
<td>8 ± 12 g</td>
<td>2 ± 34 h</td>
<td>0-34 5/8</td>
</tr>
<tr>
<td></td>
<td>KI-8111 (10^-4 M)</td>
<td>201 ± 47 i</td>
<td>37 ± 7.1 j</td>
<td>35 ± 52 k</td>
<td>29-52 6/8</td>
</tr>
<tr>
<td></td>
<td>KI-8110 (10^-4 M)</td>
<td>161 ± 54 m</td>
<td>18 ± 12 n</td>
<td>18 ± 34 o</td>
<td>1-34 6/8</td>
</tr>
<tr>
<td></td>
<td>KI-8111 (10^-4 M)</td>
<td>138 ± 31 p</td>
<td>10 ± 5 q</td>
<td>10 ± 27 r</td>
<td>2-17 8/8</td>
</tr>
</tbody>
</table>

a Number of mice showing metastasis per number of mice used.
b Mean ± SD.
c Non-treated NL-17 group versus nontreated NL-44 group, Student's t test, P < 0.001.
d Non-treated group versus treated group, Student's t test, P < 0.001.
e Cells were pretreated with 10^-4 M KI-8110 for 24 h before inoculation.
f The size of colonies was much smaller than that in the case of NL-17.
g Non-treated group versus treated group, Student's t test, P < 0.001.

Effect of KI-8110 on In Vivo Tumor Cell Retention. Thirty min after tumor cell injection, approximately 80% of nontreated NL-17 cells were retained in the lungs, but less than 50% of KI-8110-treated NL-17 cells injected with KI-8110 were retained in the lungs at this time. After 30 min, the number of cells retained in the lungs decreased with time. The retention time in the lungs was shorter for KI-8110-treated NL-17 cells than for nontreated cells (Fig. 4).

DISCUSSION

Metastasis occurs via a complex cascade of events or a series of sequential steps whereby tumor cells invade neighboring tissue and penetrate into the lymphatic and/or circulatory systems, become detached from the primary tumor mass, and spread to near and distant sites where they become arrested, invade, and finally proliferate to form new metastatic colonies (16). In this cascade, tumor cells interact with themselves; with a number of other cells, vascular, endothelial, and circulating host cells; and with soluble blood components. Such interactions may affect the metastatic nature of the tumor cells. Therefore, the tumor cell surface is one of the most important targets for research on tumor metastasis. In fact, Hagmar and Norby (19) and Fidler (20) used trypsin to alter tumor cell surface properties and found that the metastasis potential was reduced by this treatment. Irimura et al. (21) used a cell glycosylation inhibitor, tunicamycin, to modify the biosynthesis of surface glycoproteins of B16 melanoma cells, and they found that the tunicamycin-modified B16 cells failed to form experimental pulmonary tumor colonies after i.v. injection.

Sialic acids are terminal sugars of cell surface glycoconjugates. Recently it has been suggested that the sialic acid content or sialyltransferase activity of the tumor cell surface is positively related to the metastatic potential of the tumor cells (1-10). However, some authors have failed to observe this correlation (22, 23). This inconsistency may be due to variations in cell lines. In the present study, it was observed that NL-17, a highly metastatic cell line when implanted i.v., did in fact show higher sialyltransferase activity and a higher sialic acid content than the cell line of low metastatic potential, NL-44. As we reported previously (12), the sialic acid:nucleoside conjugate, KI-8110, has a sialyltransferase inhibiting activity that specifically depends on the acceptor. Sialic acid transfer to O-glycosidically linked sugar chains of glycoproteins was specifically inhibited, and this inhibition was found to be long lasting when compared with the case of CDP, a known sialyltransferase inhibitor. KI-8110 inhibited the sialyltransferase activity of both NL-17 and NL-44 cells and decreased their sialic acid contents.

When mice were given i.v. injections of NL-17 or NL-44 cells pretreated with KI-8110 and then given KI-8110 i.v., pulmonary metastasis was significantly suppressed by these treatments in both NL-17 and NL-44 cells, and the survival of the mice was prolonged. Administration of KI-8110 alone i.v. also inhibited pulmonary metastasis, but the degree of inhibition was lower than that with the above combined treatment. Actually, the effects of the two treatments are additive.

KI-8110 itself had neither cytostatic nor cytotoxic effects on NL-17 and NL-44 cells, yet it reduced the retention of tumor cells in the lungs. Therefore, KI-8110 may have an effect at least on
INHIBITION OF EXPERIMENTAL PULMONARY METASTASIS

Fig. 2. Gross appearance of lungs from tumor cell-inoculated mice. In a are control mice; in b, mice were inoculated with NL-17 cells at 5 x 10⁴ cells/mouse; in c, mice were inoculated with NL-17 cells at 5 x 10⁴ cells/mouse and treated with KI-8110 at 0.5 mg/mouse/3 days; in d, mice were inoculated with NL-17 cells, which had been pretreated with 10⁻⁴ M KI-8110 for 24 h at 5 x 10⁴ cells/mouse, and treated with KI-8110 at 0.5 mg/mouse/3 days; in e, mice were inoculated with NL-44 cells at 5 x 10⁴ cells/mouse; in f, mice were inoculated with NL-44 cells at 5 x 10⁴ cells/mouse and treated with KI-8110 at 0.5 mg/mouse/3 days; in g, mice were inoculated with NL-44 cells, which had been pretreated with 10⁻⁴ M KI-8110 for 24 h at 5 x 10⁴ cells/mouse, and treated with KI-8110 at 0.5 mg/mouse/3 days. The lungs were excised from the mice 21 days after the i.v. injection of tumor cells and then washed and fixed with Bouin’s solution.

Fig. 3. Effect of KI-8110 on the survival ratio of mice inoculated i.v. with NL-17 cells. Mice were inoculated with NL-17 cells at 5 x 10⁴ cells/mouse ( ); NL-17 cells which had been pretreated with 10⁻⁴ M KI-8110 for 24 h, at 5 x 10⁴ cells/mouse, and KI-8110 at 0.5 mg/mouse/3 days ( ); or NL-17 cells at 5 x 10⁴ cells/mouse and KI-8110 at 1.0 mg/mouse/day ( ), into the tail vein.

Since KI-8110 inhibits sialyltransferase, it is possible that this antimetastatic effect is due to modification of tumor cell surface properties affecting the sialic acid content of the cells. We are now studying the effect of KI-8110 on other metastatic cells, Lewis lung carcinoma, and B16 melanoma cells. Sinha and Goldbery (24) noted that neuraminidase treatment tends to change the distribution of metastatic colonization. Furthermore it was reported that the natural killer susceptibility of tumor cells decreased as the tumor cell surface sialic acid content increased (25), and it has been proposed that sialic acid is closely related to cell surface antigenicity and sometimes has a masking effect on tumor antigens (26, 27). These findings suggest that the change in sialic acid content on the tumor cell surface may affect the susceptibility of tumor cells to the host immune system.

Furthermore some metastatic tumor cells are known to contain a platelet-aggregating material, which is a trypsin-sensitive glycoprotein and sensitive to neuraminidase treatment (28, 29). Platelet-aggregating material activity decreased on treatment of NL-17 cells with KI-8110, and this might have resulted from the inhibition of tumor cell sialyltransferase by KI-8110. Since this tumor cell-induced platelet aggregation plays a crucial role in tumor cell arrest (30, 31), loss of platelet-aggregating material activity may decrease the metastatic potential of tumor cells.

Thus sialic acid can be said to be a key substance in tumor metastasis, and the antimetastatic effect of KI-8110 observed in the present study may possibly be related to the change in sialic acid metabolism on the tumor cell surface. It is possible that ³¹Kijima-Suda, Y. Miyamoto, S. Toyoshima, M. Itoh, and T. Osawa, unpublished data.

The initial events of the metastatic cascade, i.e., tumor arrest. Since KI-8110 inhibits sialyltransferase, it is possible that this antimetastatic effect is due to modification of tumor cell surface properties affecting the sialic acid content of the cells. We are now studying the effect of KI-8110 on other metastatic cells, Lewis lung carcinoma, and B16 melanoma cells. Sinha and Goldbery (24) noted that neuraminidase treatment tends to change the distribution of metastatic colonization. Furthermore it was reported that the natural killer susceptibility of tumor cells decreased as the tumor cell surface sialic acid content increased (25), and it has been proposed that sialic acid is closely related to cell surface antigenicity and sometimes has a masking effect on tumor antigens (26, 27). These findings suggest that the change in sialic acid content on the tumor cell surface may affect the susceptibility of tumor cells to the host immune system.

Furthermore some metastatic tumor cells are known to contain

Table 4: Effect of KI-8110 and KI-8111 on the experimental metastasis of NL-17 cells in mice

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment</th>
<th>Lung wt (mg)</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Incidencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>127 ± 15b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5/5</td>
</tr>
<tr>
<td>NL-17</td>
<td>None</td>
<td>508 ± 285</td>
<td>53 ± 30</td>
<td>47</td>
<td>20–93</td>
<td>5/5</td>
</tr>
<tr>
<td>NL-17</td>
<td>KI-8111²</td>
<td>165 ± 74²</td>
<td>11 ± 11a</td>
<td>9</td>
<td>0–30</td>
<td>4/5</td>
</tr>
<tr>
<td>NL-17</td>
<td>KI-8110²</td>
<td>130 ± 6²</td>
<td>1 ± 1</td>
<td>0</td>
<td>0–2</td>
<td>2/5</td>
</tr>
<tr>
<td>NL-17</td>
<td>KI-8111²</td>
<td>221 ± 103</td>
<td>25 ± 20</td>
<td>23</td>
<td>8–57</td>
<td>5/5</td>
</tr>
<tr>
<td>NL-17</td>
<td>KI-8111²</td>
<td>125 ± 10²</td>
<td>2 ± 3</td>
<td>1</td>
<td>1–7</td>
<td>5/5</td>
</tr>
</tbody>
</table>

a Number of mice showing metastasis per number of mice used.
b Mean ± SD.
c Mice were given KI-8110 (0.5 mg/body) i.v. every 3 days after tumor cell inoculation.
d Nontreated group versus treated group. Student’s t test, P < 0.05.
e Cells were pretreated with 10⁻⁴ M KI-8110 for 24 h before inoculation.
f Nontreated group versus treated group. Student’s t test, P < 0.01.
g Mice were given KI-8111 (0.5 mg/body) i.v. every 3 days after tumor cell inoculation.
h Cells were pretreated with 10⁻⁴ M KI-8111 for 24 h before inoculation.

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861

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INHIBITION OF EXPERIMENTAL PULMONARY METASTASIS

![Graph showing inhibition of experimental pulmonary metastasis](image)

Fig. 4. Effect of KI-8110 on retention of NL-17 cells in the lung. Mice were given injections of either [3H]thymidine-labeled NL-17 cells at 5 x 10⁶ cells/mouse (C) or [3H]thymidine-labeled NL-17 cells which had been pretreated with 10⁻⁴ M KI-8110 for 24 h, at 5 x 10⁶ cells/mouse, and KI-8110 at 0.5 mg/mouse (•), into the tail vein. Mice were subjected to autopsy at the indicated times, the lung were removed, and the radioactivity in them was determined. Points, mean of two measurements; bars, SE.


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Inhibition of Experimental Pulmonary Metastasis of Mouse Colon Adenocarcinoma 26 Sublines by a Sialic Acid:Nucleoside Conjugate Having Sialyltransferase Inhibiting Activity

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