Anti-metastatic Effect of the Sialyl Lewis-X Analog GSC-150 on the Human Colon Carcinoma Derived Cell Line KM12-HX in the Mouse

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We investigated the inhibitory effect of the sialyl Lewis-X (sLeX) analog, GSC-150, on hepatic metastasis of the human colon carcinoma derived cell line, KM12-HX, which highly expresses sLeX antigen on the cell surface. The number of cancer nodules found in BALB/c nude mouse liver 6 weeks after intrasplenic injection of KM12-HX cells was significantly reduced by co-administration of GSC-150. The amount of [3H]thymidine-labeled KM12-HX cells distributed in liver was also significantly reduced by GSC-150 co-administration in lipopolysaccharide (LPS)-treated mice at 48 h after administration of the tumor cells, while GSC-150 did not reduce the amount of HX cells distributed at 30 min. Considering our previous report that the initial phase of the distribution of KM12-HX cells in liver is governed by their being trapped in the hepatic microvessels because of their large size (Mizuno et al., J. Hepatol., 28, 865–877, 1998), these results suggest that GSC-150 does not inhibit this first-pass trapping by microvessels, but inhibits the subsequent process which is more directly related to final metastasis. GSC-150 inhibited the adhesion of KM12-HX cells to tumor necrosis factor-α (TNF-α)-activated human umbilical vein endothelial cells (HUVECs). These findings imply that the anti-metastatic effect of GSC-150 in vivo could be explained by its inhibition of cell–cell interactions between cancer and host cells.

Key words metastasis; GSC-150; KM12-HX; sialyl Lewis-X; liver

Cancer metastasis occurs through a sequence of steps such as invasion of the circulation from the primary tumor, immigration to distant organs, adhesion to endothelial cells, infiltration into the tissue interstitial space and production of metastatic tumors. Different types of inter-cellular adhesion molecules are involved in each step. Cell adhesion molecules also mediate the adhesion and accumulation of leukocytes at inflammation sites. Many studies have been carried out in an attempt to develop pharmaceutical agents which exhibit an anti-inflammatory effect while avoiding the leukocyte accumulation at the inflammation sites. GSC-150, an analog of sialyl Lewis-X (sLeX) (Fig. 1), was developed as a selectin-blocker and suppressed inflammation caused by Ovalbumin in BALB/c mice. Carbohydrate antigens, including sLeX, are expressed on the cancer cell surface and play an important role in metastasis. Various human cancer cells that express sLeX antigens adhere very tightly to cytokine-activated endothelial cells. Accordingly, anti-inflammatory agents which inhibit leukocyte adhesion may be used to inhibit the interaction between cancer and host cells, thereby arresting metastasis.

KM12-HX cells are derived from human colon carcinoma and highly express sLeX sugar antigen on the cell surface. This cell line exhibits final metastasis in the liver. The frequency of such metastasis depends on the cumulative amount of cancer cells extracted by the liver, much of which can be accounted for by non-specific trapping caused by them being larger than microvessels. On the other hand, cell–cell interactions mediated by the sugar antigen on their surface may also play an important role in metastasis. Thus, the degree of final metastasis is determined by two factors, the first-pass trapping by microvessels and the molecular interaction mediated by sLeX antigen. Accordingly, an sLeX analog may suppress the final metastasis of cancer cells by inhibiting the latter mechanism without having any effect on the former. In the present study, we investigated the anti-metastatic effect of GSC-150 on the hepatic metastasis of KM12-HX cells to demonstrate the importance of these two factors in metastasis.

MATERIALS AND METHODS

Materials

GSC-150 was provided by Kanebo Co., Ltd. (Osaka, Japan). Fetal bovine serum was purchased from BioWhittaker (Walkersville, MD, U.S.A.). [3H]Thymidine was purchased from New England Nuclear (Boston, MA, U.S.A.). Lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α) was purchased from SIGMA (St. Louis, MO, U.S.A.). Human umbilical vein endothelial cells (HUVECs) were purchased from Kyokuto Pharmaceutical Industry (Ibaraki, Japan). Collagen-coated flasks and collagen-coated 24-well plates were purchased from IWaki (Tokyo, Japan).

Experimental Hepatic Metastasis of KM12-HX Cells

Male BALB/c nude mice (6 weeks, 20—23 g, Nihon Ika-gaku, Tokyo, Japan) were injected intraperitoneally with 0.5 ml saline containing 0.08 mg LPS 3 h before administration of the tumor cells. KM12-HX cells were harvested by treatment with 0.05% trypsin/0.02% EDTA and washed three times with PBS. Viable tumor cells were suspended in PBS or PBS containing GSC-150 (final GSC-150 concentration was 10 mg/ml) and injected into the spleen of lightly anesthetized nude mice at a dose of 6×10^5 or 15×10^5 cells/mouse in 0.1 ml. 1mg GSC-150 alone was then injected

Fig. 1. Chemical Structure of GSC-150
into GSC-150-treated mice via the tail vein 2 h after injection of the cells. The number of hepatic tumor lesions was determined 6 weeks later. This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**Effect of GSC-150 on the Distribution of KM12-HX Cells to Liver** KM12-HX cells were labeled with [3H]thymidine (50 μCi) for 24 h as described previously, and then injected into the spleen at a dose of 3×10⁵ cells/mouse in 0.1 ml PBS. These cell suspensions contain various concentrations of GSC-150 (0, 0.3, 1, 3, 10 mg/ml). At a specified time, the liver was removed and washed for 72 h in three changes of 70% ethanol to remove soluble [3H]thymidine. The 70%-ethanol precipitable fraction thus obtained was solubilized and the radioactivity was measured.

**Adhesion of KM12-HX Cells to TNF-α-Activated HUVECs** HUVECs were maintained in a collagen-coated flask with E300 medium in a humidified atmosphere of 5% CO₂ at 37 °C. HUVECs at passage 2—7 were plated at a density of 2×10⁴ cells/well 24 h prior to the assay and stimulated with 200 units/ml TNF-α for 4 h before the assay. The medium was replaced with GSC-150 dissolved in 1% BSA/Hanks solution and incubated for a further 30 min. After preincubation, 25 μl of [3H]thymidine-labeled KM12-HX cell suspension (2×10⁴ cells/ml, 0.5 μCi/ml) was added and incubated for 30 min at 37 °C. After washing three times with PBS, the attached cells were solubilized in 0.1 N NaOH and the radioactivity was measured.

### RESULTS

**Effect of GSC-150 on Experimental Metastasis** When KM12-HX cells at a dose of 15×10⁵ cells/mouse were administered to nude mice, the number of cancer nodules originating from the metastasis was significantly reduced by GSC-150 (Table 1). At a dose of 6×10⁵ cells/mouse, production of cancer colonies was increased by LPS treatment, which activates vascular endothelial cells, compared with the control (Table 1). The number of colonies were significantly reduced by GSC-150 co-administration to LPS-treated mice (Table 1).

**Distribution of [3H]Thymidine-Labeled KM12-HX Cells to Liver** KM12-HX cells labeled with [3H]thymidine were administered to the spleen and the ethanol-precipitable radioactivity was measured in the liver (Fig. 2). In the early phase after injection of the cells (30 min and 5 h), the

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### Table 1. Production of Experimental Hepatic Metastasis by KM12-HX Cells

<table>
<thead>
<tr>
<th>Injection cell number (cell/mouse)</th>
<th>Groups</th>
<th>Incidence (%)</th>
<th>Mean b)</th>
<th>Each No. of nodules c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15×10⁵</td>
<td>Control</td>
<td>6/6 (100)</td>
<td>14.17</td>
<td>7, 10, 10, 14, 18, 26</td>
</tr>
<tr>
<td></td>
<td>GSC-150</td>
<td>6/6 (100)</td>
<td>5.33*</td>
<td>2, 3, 5, 6, 6, 10</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5/6 (83.3)</td>
<td>1.67</td>
<td>0, 1, 1, 2, 2, 4</td>
</tr>
<tr>
<td></td>
<td>GSC-150</td>
<td>4/6 (66.7)</td>
<td>1.33</td>
<td>0, 0, 2, 2, 2, 2</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>6/6 (100)</td>
<td>37.3</td>
<td>3, 10, 39, 55, 58, 61</td>
</tr>
<tr>
<td></td>
<td>LPS+GSC-150</td>
<td>5/6 (83.3)</td>
<td>5.83**</td>
<td>0, 1, 1, 9, 10, 14</td>
</tr>
</tbody>
</table>

- a) The dose of GSC-150 was 1 mg/mouse twice.
- b) Mean value of nodules.
- c) The number of cancer nodules on liver surface being counted. *p<0.005 (compared with control). **p<0.05 (compared with control).

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![Fig. 2. Time-Profiles of the Distributed Amount of [3H]Thymidine-Labeled KM12-HX Cells to Liver after Intrasplenic Injection into Mice](image)

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**DISCUSSION**

Attempts to inhibit each step in hematogenous metastasis have been performed with the aim of arresting metastasis. We have previously suggested that two important mechanisms are involved in the hepatic metastasis of KM12-HX cells. The first mechanism is a physical trapping caused by the difference in size between cancer cells and the microvessels in liver. Therefore, this mechanism may not be a suitable target for the suppression of metastasis. Actually, the distribution of KM12-HX cells to liver was not inhibited by GSC-150 treatment during the initial (ca. 5 h) phase (Fig. 2). LPS treatment also had no effect on such distribution (Fig. 2). Thus, the GSC-150 dose not affect such physical trapping of KM12-HX cells in the liver microvessels. The second mechanism involves an interaction involving the ad-
hesis of molecules and seems to be a more appropriate target. The distribution of cancer cells in LPS-treated mice at 48 h was significantly increased, compared with the control group (Fig. 2), and this distribution was significantly inhibited by GSC-150 (Figs. 2, 3A). GSC-150 also inhibited the specific attachment of KM12-HX cells to HUVECs (Fig. 3B). These results suggest that GSC-150 preferentially inhibits the metastatic process which is closer to the final metastasis, probably interaction of the cancer cells with the host endothelial cells, resulting in a reduction in metastasis as shown in Table 1.

According to our previous kinetic analysis, the amount of final cancer metastasis can be represented as \( AUC_{\text{liver}} \times CL_{\text{int}} \) where \( AUC_{\text{liver}} \) is the area under the concentration curve of the distribution of cancer cells to the rapid-association compartment, originating from physical trapping by microvessels, while \( CL_{\text{int}} \) represents the intrinsic clearance of transfer from the rapid-association compartment to the final metastasis.\(^9\) The distribution of KM12-HX cells to the rapid-association compartment accounted for most of the early phase distribution of cancer cells.\(^9\) This \( CL_{\text{int}} \) should include various processes up to metastasis, such as adhesion and extravasation. The present finding suggests that GSC-150 does not affect the \( AUC_{\text{liver}} \), but reduces the \( CL_{\text{int}} \). This result supports the validity of our physiological model\(^9\) which describes the hepatic distribution and amount of metastasis after injection of cancer cells.

The inhibition by GSC-150 of experimental inflammation caused by ovalbumin was found to occur at a dose of 3—30 mg/kg in the mouse.\(^3\) In the present study, the inhibitory effect of GSC-150 on cancer cell distribution was observed at a lower dose (0.03 mg/mouse, Fig. 3A). Considering such a difference in dose-dependence, the inhibitory effect of GSC-150 both on cancer cells and inflammation cannot simply be governed by the same mechanism. The IC\(50\) for the inhibition of HX cell adhesion to HUVECs was 0.3—1 mM (Fig. 3B), which was comparable with that for the inhibition by GSC-150 (0.28 mM) of specific binding of sLeX to E-selectin.\(^4\) Considering that few KM12-HX cells were attached to non-activated HUVECs (data not shown), the inhibitory effect of GSC-150 shown in Fig. 3B seems to be directed against the cell adhesion molecules expressed on TNF-\(\alpha\)-activated HUVECs. Since TNF-\(\alpha\) activation induces the expression of E-selectin on HUVECs,\(^1,2\) one possible target of GSC-150 is E-selectin. However, we should also note a previous report that the adhesion of HX cells to activated HUVECs was inhibited to 60% of the control value by E-selectin antibodies.\(^3\) From this result, adhesion of HX cells to HUVECs cannot be simply explained by the action of E-selectin. LPS is also known to induce the expression of many adhesion molecules.\(^11,12\) GSC-150 has an affinity for not only E-selectin but also L-selectin and P-selectin,\(^9\) and, therefore, GSC-150 may possibly inhibit cell adhesion molecules other than E-selectin. The molecular mechanism of the antimetastatic effect of GSC-150 needs to be clarified by further investigations.

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REFERENCES
10) Mizuno N., Kato Y., Shirota K., Izumi Y., Irimura T., Harashima H.,
    Kiwada H., Motoji N., Shigematsu A., Sugiyama Y., J. Hepatol., 28,
13) Morodomi T., Ogata Y., Sasaguri Y., Morimatsu M., Nagase H.,
14) Okada Y., Morodomi T., Enghild J. J., Suzuki K., Yasui A., Nakanishi
15) Saiki I., Iida J., Murata J., Ogawa R., Nishi N., Sugimura K., Tokura
16) Mulligan M. S., Vaporciyan A. A., Miyasaka M., Tamatani T., Ward P.