Inhibition of Human Breast Cancer Metastasis in Nude Mice by Synthetic Glycoamines

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Abstract

We have examined the effect of synthetic low molecular weight glycoamine analogues on the metastasis of MDA-MB-435 human breast carcinoma xenografts growing in the mammary fat pads of nude mice. Initial in vitro screening of a panel of synthetic glycoamines was performed using a clonogenic growth assay in 0.9% agarose. Eight of nine compounds manifested a significant dose-dependent inhibition of colony formation by MDA-MB-435 cells in 0.9% agarose. The relative activity rankings of the compounds, based on ID50 independently determined for each synthetic glycoamine analogue, identified N-(1-deoxy-D-lactulos-1-yl)-L-leucine (Lac-L-Leu), N-(1-deoxy-D-fructose-1-yl)-L-leucine (Fru-D-Leu), N-(1-deoxy-D-fructos-1-yl)-L-phenylalanine, and N-(1-deoxy-D-fructos-1-yl)-L-leucine as the most effective inhibitors of colony formation. Two separate experimental treatment protocols were used to examine the effect of selected synthetic glycoamines on human breast cancer growth and metastasis in athymic nude mice. Group A mice were treated intra-peritoneally daily from day 2 after injection of the breast cancer cells until the end of the experiment (17 weeks). In group B, the mice were untreated until the mean tumor diameter was 10 mm, at which time daily i.p. treatment began. After 7 days, the primary tumors were resected, and the mice were treated for an additional 4 weeks (a total of 5 weeks of treatment). The synthetic glycoamines did not have significant antitumor effects, and there was no difference in the tumor incidence or tumor growth rates in mice treated continuously with synthetic glycoamines or PBS. The significant ant metastatic activity of synthetic glycoamines was detected in both experimental treatment protocols. In mice continuously treated with synthetic glycoamines according to protocol A, the incidence of metastasis was decreased 4.6-fold (P = 0.014) and 2.7-fold (P = 0.031) in mice treated with Fru-D-Leu and Lac-L-Leu, respectively. In mice in protocol B, the incidence of pulmonary metastasis was decreased 1.9-fold (P = 0.069) and 2.5-fold (P = 0.042) in mice treated with Fru-D-Leu and Lac-L-Leu, respectively. Correspondingly, the average number of spontaneous pulmonary metastases was reduced from 37 in control mice to 0.2 (P = 0.005) and 0.9 (P < 0.02) in mice treated according to the protocol A with Fru-D-Leu and Lac-L-Leu, respectively. Treatment of mice with N-(1-deoxy-D-fructos-1-yl)-L-leucine did not have significant antitumor effects, and no reduction in metastasis incidence or number was observed in mice treated with this synthetic glycoamine analogue. The treated animals had no apparent toxicity from chronic daily injection (up to 17 weeks of treatment) of synthetic glycoamines, and no obvious pathology was noted in the histological slides of the livers, kidneys, or spleens of the treated mice. Therefore, we have identified two synthetic glycoamines (Fru-D-Leu and Lac-L-Leu) that were the effective inhibitors of spontaneous human breast cancer metastasis in nude mice. Potential mechanisms for antitumor activity of synthetic glycoamines may include the inhibition of β-galectin-mediated homotypic cancer cell aggregation and induction of apoptosis in target cells.

Introduction

Breast cancer is the most common cancer among women in North America and western Europe and is the second leading cause of female cancer deaths in the United States (1, 2). In the United States, age-adjusted breast cancer incidence rates have increased considerably during this century (3). Approximately one in every nine American women will develop breast cancer during her lifetime. Breast cancer incidence rates in the United States have increased about 3% a year since 1980. An estimated 180,000 new breast cancer cases were diagnosed in the United States in 1995, and more than 46,000 deaths from breast cancer were reported (2). Approximately 40% of patients diagnosed with breast cancer have disease that has regional or distant metastases and, at present, there is no efficient curative therapy for breast cancer patients with advanced metastatic disease.

The anchorage-independent growth of primary breast cancer cells in a semisolid medium was found to be an independent variable associated with shorter disease-free survival and overall survival after mastectomy (4, 5). The clonogenic growth in agar cultures was also reported to correlate with the progression of breast neoplasms from benign to malignant (4, 5). In several experimental systems using rodent cells, colony-forming efficiency in agarose was shown to be correlated with metastatic potential (6, 7), and a particularly discriminating assay was to test colony formation in higher densities of agar medium (8, 9). It has also been reported that for human tumor cell lines, including breast cancer cells, the ability to grow in dense agarose cultures (>0.6% agarose) corresponds well with metastatic potential tested in nude mice (9–12). Thus, the colony formation assay in agarose cultures of >0.6% density distinguishes cancer cells with high metastatic potential (10–12). Therefore, the clonogenic growth assay could be used for in vitro screening of potential antimetastatic compounds.

The glycoamines are a naturally occurring low molecular weight component of mammalian blood serum that were studied as potential humoral cancer markers (13–15). The prototype compounds were originally isolated from human and rodent blood serum using a combination of conventional chromatography, analytical ultrafiltration, and size-exclusion and reverse-phase high pressure liquid chromatography (13–18). Subsequently, the compounds were structurally characterized as glycoconjugates of amino acids with covalent structures of Schiff base, ester, and Amadori bonding (13, 15–18). The inhibitory activity of high-pressure liquid chromatography-purified natural and synthetic glycoamines on rodent cancer cell aggregation in vitro and experimental metastasis in vivo were reported (13, 14, 19–22). Recently, we described a method of synthesis, purification, and full structural characterization of a series of synthetic glycoamine analogues using nuclear magnetic resonance, mass spectrometry, and X-ray analysis (23–25). The availability of synthetic glycoamines in

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pure form in multigram quantities allowed us to demonstrate a high inhibitory effect of the synthetic glycoamines on clonogenic growth in agarose of metastatic human cancer cells (25). The main objective of this study was to select the most effective synthetic glycoamines acting as inhibitors of clonogenic growth in hard agarose of metastatic breast carcinoma cells and to evaluate the antimetastatic activity of selected glycoamine analogues using the nude mouse model of human breast cancer metastasis (11, 26).

In this paper, we report the significant inhibition (>70%) of the incidence of spontaneous human breast cancer metastasis in the nude mouse model by synthetic glycoamine analogues. We also report that antimetastatic synthetic glycoamines inhibit homotypic aggregation of human MDA-MB-435 breast carcinoma cells and induce apoptosis in metastatic cancer cells.

Materials and Methods

Human Breast Carcinoma Cells

The MDA-MB-435 human breast carcinoma cell line was isolated originally from the pleural effusion of a patient with breast carcinoma and was found to be highly metastatic from the mfp3 tumors (11, 26). The metastatic and tumorigenic properties of human breast carcinoma cell lines in nude mice were found to correspond well with their colony-forming efficiency in dense agarose (11).

Mouse Melanoma Cell Lines

B16 melanoma cell lines exhibiting high (B16-F10 and B16-BL6) and low (B16-F1) metastatic potential (27–30) were kindly provided by Dr. I. Fidler (M. D. Anderson Cancer Center, Houston, TX). The cells were maintained as monolayer cultures as described below.

Cell Culture

The tumor cells were maintained in tissue culture in MEM supplemented with 5 or 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, l-glutamine, and 2-fold vitamin solution (Life Technologies, Grand Island, NY). The cultures were maintained on plastic and incubated in 5% CO2/95% air at 37°C in a humidified incubator. All cultures were free of Mycoplasma and the following murine viruses: Reovirus type 3; pneumonia virus; K virus; Thiel’s encephalitis virus; Sendai virus; minute virus; mouse adenovirus; mouse hepatitis virus; lymphocytic choriomeningitis virus; ectromelia virus; lactate dehydrogenase virus (all assayed by MA Bioproducts, Walkersville, MD).

Tumor cells were harvested from subconfluent cultures (50–70% confluence) by rinsing the monolayer with a 0.25% trypsin/0.02% EDTA solution. After 1 min, the flask was tapped to dislodge the cells, serum-supplemented medium was added, and the suspension pipetted to produce a single-cell suspension. Only single-cell suspensions of >90% viability (determined by trypsin blue dye exclusion) were used for in vivo studies.

The inhibition of colony formation by MDA-MB-435 cells was performed as described previously (25). Briefly, synthetic glycoamines (see below) were suspended in culture medium to achieve a final concentration, after mixing with agarose, of 0.375–6.0 mM and filtered. Single-cell suspensions of MDA-MB-435 cells were mixed in culture medium to achieve a final concentration, after mixing with agarose, of 0.375–6.0 mM and filtered. Single-cell suspensions of MDA-MB-435 cells were counted in at least 10 random fields by comparison with the average colony numbers in triplicate control cultures (cells treated with medium alone).

In Vivo Studies Using MDA-MB-435 Metastasis Model

A model of spontaneous human breast cancer metastasis to the lungs after orthotopic implantation of MDA-MB-435 cells in nude mouse mfps (11, 26) was used for evaluation of antitumor activity of synthetic glycoamines. Mice. Four-to-five-week-old athymic Ncr nu/nu female mice were purchased from the Animal Production Area, National Cancer Institute-Frederick Cancer Research Facility (Frederick, MD). The care and use of the animals were in accordance with institutional guidelines.

Tumor Growth in the mfp. Mice were anesthetized with Metofane (Pitman Moore, Inc., Washington, NJ), and a 5-mm incision was made in the skin over the lateral thorax. The mfp was exposed, and 106 cells in a volume of 0.1 ml of PBS was injected into the fat pad. The incision was closed with wound clips. The growth of the tumors was monitored by weekly examination, and growth rates were determined as the increase in mean tumor diameter, calculated from caliper measurements of two orthogonal diameters.

The Metastasis Therapy Protocol. For each synthetic glycoamine, the mice were divided into two experimental groups. For protocol A, mice were injected daily intraperitoneally, starting two days after tumor inoculation with either 0.2 ml of PBS or 0.2 ml of glycoamines in PBS (a total of 17 weeks of treatment). In group B, the treatment was started only when the mean tumor diameter was 10 mm. After 1 week, the mice were anesthetized, the tumors were resected, and the skin incisions were closed with wound clips. The mice were treated for an additional 4 weeks (a total of 5 weeks of treatment). Mice were killed at the end of treatment schedule, or when moribund, and examined for presence of lung metastases both macroscopically and microscopically. The lungs and tumors were fixed in 10% buffered formalin, and paraffin-embedded sections stained with H&E were examined.

Glycoamine solutions were prepared daily, by suspension of preweighed aliquots in sterile PBS to a final concentration of 10 μM/0.1 ml. The solutions were filtered through a 0.2-μm syringe filter before use.

Statistical analysis of the incidence of lung metastasis was performed using Fisher’s exact test. The significance of differences in the numbers of lung metastasis was evaluated using the Mann-Whitney ranked sum test (31).

Multicell Aggregate Formation Assay

The cell aggregation assay was performed as described previously (25, 32, 33). Briefly, tumor cells were obtained from tumor tissue or harvested from subconfluent cultures using the standard trypsination procedures. Aliquots containing 0.5–1.0 ml of cell suspension (0.5–1.0 × 106 cells/ml in serum-free RPMI 1640 or CMF-PBS) were placed in siliconized glass tubes or plastic cell culture tubes and agitated at 80 rpm for 1 h at 37°C. The aggregation was then terminated by fixing the cells with 1% formaldehyde in CMF-PBS [Ca2+- and Mg2+-free PBS (pH 7.2)]. Three samples from each tube were used for counting the number of single cells using a hemacytometer, and aggregation was calculated according to the equation: (1 − N/Nc) × 100, where N, and Nc represent the number of single cells in the presence of the test compounds and the number of single cells in the control medium (RPMI 1640 or CMF-PBS), respectively. Alternatively, three 20-μl aliquots from each sample were used for preparing total cytological preparations. In each preparation, the numbers of single cells and aggregated cells were counted in at least 10 random high-power microscopic fields, and the average number was calculated for individual samples. All cultures retained >90% viability throughout the duration of the experiments. Each determination was performed in triplicate, and the typical results of at least two separate experiments are presented.

Competition of Synthetic Glycoamine Analogues for Binding with β-Galactoside-specific PNA Lectin

Three synthetic glycoamines (Lac-β-Leu, Fru-β-Leu, and Fru-Gly) were analyzed for their abilities to compete for binding with β-galactoside-specific PNA lectin to the MDA-MB-435 cells as well as to the reference glycoprotein ASF (Sigma Chemical Co., St. Louis, MO), which displays β-Gal as a terminal sugar residue. The ASF was dissolved in transfer buffer [20 mmol/liter
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Fig. 1. Effect of synthetic glycoamines on MDA-MB-435 colony formation in 0.9% agarose.

Clonogenic growth assay in agarose was performed as described in "Materials and Methods." The presence of synthetic glycoamines significantly inhibited the colony formation ability in hard agarose of MDA-MB-435 cells compared with control PBS-treated cells. The results are presented as the mean of triplicate experiments. Bars, SD.

Tris-base and 150 mmol/liter glycin (pH 7.5), immobilized for 30 min at room temperature in humidified chamber (1 y of glycoprotein per dot) on the nitrocellulose membranes (Gene Screen Hybridization Transfer Membrane; DuPont New England Nuclear), and blocked with 5% BSA-TBS (0.01 mol/liter Tris-HCl and 0.15 mol/liter NaCl (pH 7.4)) for 1 h at room temperature. The membranes were washed twice for 10 min each in TBS and once with buffer 1 and incubated for 1 h at room temperature in buffer 1 [0.05 mol/liter Tris-HCl, 0.15 mol/liter NaCl (pH 7.5), 1 mmol/liter MgCl₂, 1 mmol/liter MnCl₂, and 1 mmol/liter CaCl₂ (pH 7.5)], containing 6.25 y/ml of horseradish peroxidase-PNA (Sigma) alone or with addition of 3 mm (final concentration) synthetic glycoamines. The membranes were subjected to 2 x 10 min washes in TBS [0.05 mol/liter Tris-HCl and 0.15 mol/liter NaCl (pH 7.5)] and developed with 3,3'-diaminobenzidine. Each determination was performed in duplicate, and the typical results of the three separate experiments are presented.

Apoptosis Induction Experiments

Induction of apoptosis in MDA-MB-435 human breast carcinoma cells by synthetic glycoamine analogues was performed as described previously (32). Cell viability in RPMI 1640 containing 0.1% FCS was assayed by acridine orange staining and/or trypan blue exclusion as reported earlier (32). Identification of apoptotic cells using the TUNEL method was performed exactly as described (32). The in situ cell death detection kit POD (Boehringer Mannheim DuPont New England Nuclear), and blocked with 5% BSA-TBS [0.01 mol/liter Tris-HCl and 0.15 mol/liter NaCl (pH 7.5)] and saturation density (32), except that the induction of apoptosis in MDA-MB-435 cells following exposure to a panel of nine synthetic glycoamines was performed as described in "Materials and Methods." The presence of synthetic glycoamines significantly inhibited the colony formation ability in hard agarose of MDA-MB-435 cells compared with control PBS-treated cells. The results are presented as the mean of triplicate experiments. Bars, SD.

Results

Selection of Inhibitors of Colony Formation by MDA-MB-435 Cells in Dense Agarose

We have reported previously the inhibition of colony formation by MDA-MB-435 cells following exposure to a panel of nine synthetic glycoamines (25). Fig. 1 summarizes the results that led to the identification of four compounds that were most effective in the in vitro assay: Lac-L-Leu, Fru-D-Leu, Fru-L-Phe, and Fru-L-Leu. The IC₅₀ doses of these compounds in the agarose assay were 2.3, 2.3, 2.7, and 3.0 mm, respectively. The three Leu analogues were selected for the in vivo study.

Nude Mouse-Human Breast Cancer Xenograft Experiments

Two experimental treatment protocols were used to determine the in vivo activity of the three selected synthetic glycoamines. In protocol A, the mice were treated daily from 2 days after tumor cell injection until the end of the experiment (17 weeks). In group B, daily treatment started when the mean tumor diameter was 10 mm. After 7 days of treatment, the primary tumors were removed, and the mice were treated for an additional 4 weeks (a total of 5 weeks of treatment).

Effect of Synthetic Glycoamines on Incidence and Growth of Primary Tumors. The growth curves of MDA-MB-435 mfp tumors in mice treated continuously from day 2 after cancer cell injection with Fru-D-Leu or Fru-L-Leu did not differ from the growth curves of the PBS-treated mice (data not shown). The weights of tumors on the control and treated mice at time of autopsy were similar, and the mean weights showed no statistical difference. The incidence of tumors in these mice did not differ, with 82% incidence in the PBS-treated group and 75% incidence in mice treated with either Fru-D-Leu or Fru-L-Leu. In mice that were scored as not having tumor at the end of the experiment, either no tumor was found at the site of injection or a mass of 2-3 mm was found that had shown no progressive growth. Histological evaluation of the small masses showed residual tumor...
cells, surrounded by fibrous connective tissues. The growth curves of MDA-MB-435 mfp tumors in mice treated continuously with Lac-l-Leu showed a trend toward inhibition of the local tumor, although a comparison of the weights of tumors removed from these mice at the time of autopsy (control mean weight = 0.69 + 0.6 g (mean + SD), test mean weight = 0.44 + 0.54 g) showed no statistically significant difference (Student’s t test). A 100% incidence of tumors was recorded in group A mice treated with Lac-l-Leu.

Thus, the synthetic glycoamines did not have significant antitumor effects, and there was no difference in the tumor incidence or tumor growth rates of MDA-MB-435 tumors in mice treated continuously with synthetic glycoamines or with PBS.

**Effect of Synthetic Glycoamines on Tumor Host.** The treated animals had no apparent toxicity from the daily injection (up to 17 weeks of treatment) of synthetic glycoamines. Animal weights, skin texture, and overall behavior were unchanged between the control and the treated groups. No obvious pathology was noted in the histological slides of the livers, kidneys, or spleens of the treated mice. Thus, chronic treatment with synthetic glycoamines did not apparently lead to toxicity.

**Effect of Synthetic Glycoamines on Metastasis.** As shown in Table 1, daily treatment with synthetic glycoamines caused a significant reduction in both the incidence and number of spontaneous pulmonary metastases of MDA-MB-435 human breast carcinoma. The antimetastatic activity of synthetic glycoamines was detected in both experimental treatment protocols. Only 18–22% of control mice (4 of 18 control mice in group A and 3 of 17 control mice in group B) failed to develop pulmonary metastases compared with 83% to 57% of mice treated with Fru-D-Leu (5 of 6 treated mice in group A and 4 of 7 treated mice in group B) or 71% to 67% of mice treated with Lac-l-Leu (5 of 7 treated mice in group A and 4 of 6 treated mice in group B). In mice continuously treated with synthetic glycoamines according to the protocol A, the incidence of metastasis was decreased 4.6-fold ($P = 0.014$) and 2.7-fold ($P = 0.031$) in mice treated with Fru-D-Leu and Lac-l-Leu, respectively (Table 1). The antimetastatic activity of synthetic glycoamines was less profound in mice treated with Fru-D-Leu and Lac-l-Leu (Table 1). A similar trend toward inhibition of the number of pulmonary metastasis in mice treated according to the protocol B was noticed in mice treated with Fru-D-Leu and Lac-l-Leu (Table 1). Treatment of mice with Fru-l-Leu did not have significant antimetastatic effect, and no reduction in metastasis incidence or number was noted in mice treated with this synthetic glycoamine analogue.

Therefore, we have identified two synthetic glycoamines (Fru-D-Leu and Lac-l-Leu) that were the effective inhibitor of spontaneous human breast cancer metastasis in nude mice.

**Induction of Apoptosis in MDA-MB-435 Human Breast Carcinoma Cells by Synthetic Glycoamines**

It had been demonstrated previously that synthetic glycoamines could inhibit homotypic aggregation of murine B16 melanoma cells by interfering with $\beta$-galactoside-specific lectin-mediated cell-cell interactions (25). Blocking of cell surface galectin-mediated cell-cell interactions resulted in induction of apoptosis in highly metastatic B16-F10 murine melanoma cell variants (32). We investigated whether these mechanisms are relevant to the bioactivity of synthetic glycoamines on MDA-MB-435 human breast carcinoma cells as well. Similar to the murine B16 melanoma model, synthetic glycoamines significantly inhibited homotypic aggregation of MDA-MB-435 cells and blocked binding of $\beta$-galactoside-specific PNA lectin to the MDA-MB-435 cells (Fig. 2). More than 50% reduction in homotypic aggregation of human breast carcinoma cells treated with $5 \mu$m of Fru-D-Leu and Lac-l-Leu was observed. Treatment of MDA-MB-435 cells with synthetic glycoamines caused a 4–6-fold increase in the apoptosis level as determined by the TUNEL assay (Fig. 3A). Induction of apoptosis in glycoamine-treated highly metastatic MDA-MB-435 human breast carcinoma cells was confirmed using DNA fragmentation analysis, which clearly demonstrated an accumulation of low molecular weight apoptotic DNA in MDA-MB-435 cells at 48 h after treatment with antimetastatic synthetic glycoamines (Fig. 3B).

**Discussion**

We have identified two synthetic glycoamines that were effective inhibitors of spontaneous human breast cancer metastasis in nude mice. Continuous i.p. injection of Lac-l-Leu or Fru-D-Leu, beginning

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**Table 1. Spontaneous lung metastasis of MDA-MB-435 human breast carcinoma in nude mice treated with synthetic glycoamines**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lung metastasis number$^a$</th>
<th>Incidence of lung metastasis$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protocol A</td>
<td>Protocol B</td>
</tr>
<tr>
<td>Control (PBS)</td>
<td>37 (0–150)</td>
<td>32 (0–150)</td>
</tr>
<tr>
<td>Fru-D-Leu (FDL)</td>
<td>0.2 (0–1)</td>
<td>22 (0–100)</td>
</tr>
<tr>
<td>Lac-l-Leu (LL)</td>
<td>0.9 (0–5)</td>
<td>8 (0–40)</td>
</tr>
<tr>
<td>Fru-l-Leu (FLL)</td>
<td>39 (0–150)</td>
<td>37 (0–150)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Protocol A</th>
<th>Protocol B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (PBS)</td>
<td>78% (14/18)</td>
<td>82% (14/17)</td>
</tr>
<tr>
<td>Fru-D-Leu (FDL)</td>
<td>17% (16)</td>
<td>43% (37)</td>
</tr>
<tr>
<td>Lac-l-Leu (LL)</td>
<td>29% (27)</td>
<td>33% (26)</td>
</tr>
<tr>
<td>Fru-l-Leu (FLL)</td>
<td>67% (46)</td>
<td>78% (79)</td>
</tr>
</tbody>
</table>

$^a$ Average number (and range) of lung metastases. Metastasis scored both macroscopically and microscopically. The mice that did not develop a primary tumor have been eliminated from the count. $P$ were calculated using the Mann-Whitney ranked sum test.

$^b$ Mice with lung metastases/mice with mfp tumors. Metastasis scored both macroscopically and microscopically. The mice that did not develop a primary tumor have been eliminated from the count. $P$ were calculated using Fisher’s exact test.

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The synthetic glycoamines did not have a significant effect on tumor growth, and there was no difference in the primary tumor incidence of MDA-MB-435 mfp tumors in treated or control group mice. This observation is not surprising because synthetic glycoamines were described as inhibitors of cancer cell aggregation and adhesion mediated by cell surface β-galactoside-specific lectins (19, 25). Carbohydrate-mediated homotypic and heterotypic cancer cell aggregation and adhesion is thought to be one of the key events in the metastatic process (19—22, 23), which apparently does not play a significant role in growth of primary neoplasms. A similar selective antimetastatic effect without noticeable antitumor activity has been reported recently for another carbohydrate-based metastasis inhibitor. A modified citrus pectin was reported to act by interfering with β-galectin-mediated adhesion of metastatic cancer cells.

Fig. 2. Inhibition of ASF-induced B16-F1 murine melanoma and spontaneous MDA-MB-435 human breast carcinoma cell aggregation (A) and binding of β-galactoside-specific PNA lectin to the MDA-MB-435 cells (B and C) is accompanied by induction of apoptosis in target cells (D and E). Cell aggregation (A), lectin binding (B and C), and apoptosis induction (D and E) experiments were performed as described in “Materials and Methods.” The evaluation of both cell aggregation and PNA lectin-binding activities was performed after 1 h of incubation of MDA-MB-435 cells. The apoptosis level was determined using the TUNEL assay after 2 days of cultivation of MDA-MB-435 cells in RPMI 1640 containing 0.1% FCS without (Control) and with addition of 2 ms (final concentration) synthetic glycoamines. Representative results of one of two similar experiments are presented. Bars, SD.

2 days after the inoculation of MDA-MB-435 human breast carcinoma cells in mfps of athymic nude mice, decreased the incidence of pulmonary metastasis by 2.7-fold and 4.6-fold, respectively. The average number of spontaneous pulmonary metastases was reduced from 37 in PBS-treated mice to 0.9 in Lac-α-Leu-treated mice and 0.2 in Fru-α-Leu-treated mice. The relative number of metastasis-free mice (without either macroscopic and microscopic metastases) was increased 3.8-fold in Fru-α-Leu-treated mice and 3.2-fold in Lac-α-Leu-treated mice. Chronic treatment (daily i.p. injection during 5 or 17 weeks of treatment) with synthetic glycoamines did not lead to any apparent toxicity.

In vitro screening of potential antimetastatic compounds using colony formation assay in dense agarose could be useful for selecting promising compounds for subsequent in vivo studies. According to our results, the most promising compounds were those that produced more than 70—80% inhibition of the clonogenic growth of the MDA-MB-435 cells in dense agarose. The effective in vivo antimetastatic compounds Fru-α-Leu and Lac-α-Leu caused 74 and 82% inhibition, respectively, of the colony formation in 0.9% agarose. Fru-α-Leu showed a 51% inhibition of colony formation in 0.9% agarose by MDA-MB-435 cells, yet did not produce any significant antimetastatic effect in vivo.

In Fig. 3, it was observed that the induction of apoptosis in MDA-MB-435 human breast carcinoma cells by antimetastatic synthetic glycoamines. MDA-MB-435 cells were incubated in RPMI 1640 containing 0.1% FCS without (Control) and with addition of 2 ms (final concentration) synthetic glycoamines. After 2 days of cultivation, the apoptosis level was determined using the TUNEL assay (A) and DNA fragmentation analysis by electrophoresis in 1.2% agarose gel and ethidium bromide staining (B) as described in “Materials and Methods.” The counts of apoptotic cells were made on a minimum of 200 cells per culture. Representative results from one of two similar experiments are presented as a mean of triplicate determinations. Bars, SD.
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Two apparently unrelated chemical modifications of Fru-L-Leu molecule (substitution of L-Leu for D-Leu in Fru-L-Leu and addition of β-Gal residue in Lac-L-Leu) resulted in a dramatic increase in biological activities, as determined in both the clonogenic growth of α-Gal residue in Lac-L-Leu) resulted in a dramatic increase in pounds. The observation that synthetic glycoamines induce apoptosis in vitro in human metastatic breast cancer cells certainly may add a new dimension to our understanding of the molecular mechanisms of the biological activity of this class of molecules. However, it is not yet clear how the in vitro apoptosis induction activity of synthetic glycoamines is related to their in vivo antitumor activity and, obviously, further experiments are needed in this area.

This study used a single metastatic human tumor; experiments using additional cancer cell lines are necessary to fully establish the effectiveness of the synthetic glycoamines as antitumor agents. However, our data add further support to the concept of anticell adhesion therapy for cancer, particularly for treatment of a cancer metastasis of breast, ovarian, prostate, and colon tumors, and malignant melanoma (19–22, 25, 33, 34). Interference with the carbohydrate-mediated cell-cell recognition and adhesion may lead to reduced metastatic dissemination and may be useful as an adjuvant treatment along with surgery, radiation, and chemotherapy.

References

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