Selective Protection of Nonmalignant Cells by a Novel Cell Surface Glycopeptide

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ABSTRACT

A novel glycopeptide inhibitor of cell division, isolated from bovine cerebral cortex cell surfaces, was shown to selectively protect nonmalignant cells from the cytotoxic action of 5-bromo-2-deoxyuridine (5-BrdUrd). When mouse LM-22 cells (nonmalignant and devoid of gangliosides) were preincubated with G_{48} ganglioside (3.0 µg/ml), the cell surface glycopeptide inhibitor effectively arrested cell division. In contrast to LM-22 cells, transformed mouse fibrosarcoma (No. 1316) cells were insensitive to the glycopeptide inhibitor whether or not they were preincubated with G_{48} ganglioside. Mixed cultures of LM-22 cells preincubated with G_{48} ganglioside and 1316 fibrosarcoma cells at an approximate ratio of 1:1 were established. Since LM-22 cells are resistant and 1316 fibrosarcoma cells are sensitive to 3.0 mM ouabain, the identity of surviving cells following BrdUrd treatment could easily be determined. Three hr after the establishment of the mixed cell population, 250 ng protein per ml of the purified bovine glycopeptide inhibitor was added to selectively arrest the mitosis of the LM-22 cells. After an additional 3 hr of incubation, 5-BrdUrd was added to a final concentration of 5.0 mM. Twelve hr later, cells were serially diluted and seeded into duplicate plates with and without 3.0 µM ouabain. LM-22 cells were effectively protected from the cytotoxic action of 5-BrdUrd (92 to 94% survival) while the majority of the 1316 fibrosarcoma cells were killed (21 to 30% survival). The selective protection of LM-22 cells was shown to be independent of differences in plating efficiency, cytotoxicity of 5-BrdUrd in the absence of the glycopeptide inhibitor, and the generation time of the two cell lines.

INTRODUCTION

We have recently described the isolation and partial purification of a growth inhibitory glycopeptide isolated from bovine cerebral cortex cell surfaces that can inhibit protein synthesis and cell growth in baby hamster kidney (BHK-21) cells but not in their polyoma virus transformed analogues (py-BHK) (3). The inhibitor isolated from bovine brain appears to have physical, chemical, and biological properties similar to a growth-inhibitory glycopeptide that we have previously isolated from the mouse cerebral cortex (3, 4). Both mouse and bovine glycopeptides inhibit cell division in a completely reversible and nonlethal manner. In contrast to BHK-21 cells and other nonmalignant cell lines, the glycopeptide growth inhibitors are relatively or completely ineffective against transformed or tumor-derived cell lines (3–5). We have reported 2 ways in which cells escape inhibition; py-BHK cells can bind the glycopeptide in a saturable fashion but inactivate the inhibitor by enzymatic degradation (3). The mouse fibrosarcoma cell line 1316 avoids inhibition due to a reduced level of the ganglioside G_{48}, in its cell surface membrane (6). The 1316 cells preincubated with G_{48}, ganglioside, under conditions in which the ganglioside degradation does not occur (0–4°), were sensitive to the inhibitor although incubation at 37° resulted in the degradation of the ganglioside and a reversion of the cells to a state of insensitivity to the glycopeptide (6).

Since the cell surface glycopeptide primarily inhibited division of nontransformed cells, we reasoned that the molecule may be useful in protecting "normal" cell populations of cytotoxic agents that influence proliferating cells. Such a strategy may find eventual application in multiple-drug cancer chemotherapy. To this end, this initial study examines the ability of the bovine glycopeptide inhibitor to selectively protect nonmalignant cells, in mixed culture with transformed cells, to the cytotoxic action of 5-BrdUrd.

MATERIALS AND METHODS

Isolation and Purification of Glycopeptide Cell Growth Inhibitor. The glycopeptide cell growth inhibitor was isolated from bovine cerebral cortex cell surfaces by mild Pronase treatment as described previously (4). The macromolecules, released by mild proteolysis, were purified by ethanol precipitation, chloroform:methanol extraction, Bio-Gel P-100 filtration, and affinity chromatography on an UEA-1 column (4). The final preparations were dialyzed against 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid:120 mM KCl:5 mM MgCl₂, pH 7.1, buffer filter sterilized, and stored at −85°.

Cell Culture. Mouse LM-22 cells resistant to 3.0 mM ouabain and devoid of detectable surface gangliosides and mouse fibrosarcoma 1316 cells, induced by UV irradiation of C3H mice (10), were routinely grown as monolayer cultures. All cultures were maintained at 37° in a 5% CO₂:95% air atmosphere in humidified incubators (3). Fetal calf serum was purchased from Dutchland Laboratories, Inc. (Denver, Pa.), and all other media components were purchased from Grand Island Biological Company (Grand Island, N. Y.).

Cell Growth Assays. LM-22 or mouse fibrosarcoma 1316 cells were harvested by trypsinization and seeded into 24-well tissue culture plates (Costar, Cambridge, Mass.) at an initial cell density of 1 x 10⁴ cells/well. After a 24-hr period to allow the cells to attach to the culture vessel surface, ganglioside G_{48} (Supelco, Inc., Bellefonte, Pa.) was added to a final concentration of 3.0 µg/ml. The cell monolayers were incubated for 3 hr with ganglioside G_{48}, washed 3 times with complete medium, and then fed with either complete medium supplemented with 250 ng protein per ml of the UEA-1 purified bovine glycopeptide inhibitor in 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid:120 mM KCl:5 mM MgCl₂, pH 7.1, buffer, or complete medium alone (controls). Triplicate cell samples were taken every 6 hr by trypsinization, diluted in Difluor 3 (Baker...
Mixed Cell Culture. LM-22 cells, pretreated with 3.0 µg/ml of ganglioside GM1 for 3 hr to confer sensitivity to the bovine growth-inhibitory glycopeptide, were mixed with 1316 fibrosarcoma cells and seeded into 24-well tissue culture plates at a total cell density of 2 x 10⁴ cells/well. After 3 hr, cells were fed with fresh medium (controls) or fresh medium containing 250 ng protein per ml of the UEA-1 purified bovine glycopeptide. After an additional 3 hr of incubation, 5-BrdUrd (Sigma Chemical Co., St. Louis, Mo.) was added to all cultures to a final concentration of 5.0 mM. The mixed cell cultures were then incubated for 12 hr when cells were removed by trypsinization, serially diluted, and seeded into duplicate 35-mm dishes with and without 3.0 mM ouabain (200 or 400 cells/dish). After incubation for 6 to 7 days, the media were removed, the cells were fixed and stained with crystal violet in 10% ethanol, and the colonies were counted. The colony count on the plates without ouabain revealed total surviving cells, and those on plates with 3.0 mM ouabain indicated the proportion of the total that were LM-22 cells.

RESULTS

No inhibition of cell growth was measured when LM-22 or mouse fibrosarcoma 1316 cells were incubated with 250 ng protein per ml of the UEA-1 affinity purified bovine glycopeptide (Chart 1). This is consistent with our previous report that cells devoid of GM1 ganglioside are refractory to the protein synthesis and cell growth-inhibitory activity of these glycopeptides (6). When LM-22 cells, however, were preincubated with GM1 ganglioside (3.0 µg/ml) for 3 hr prior to the addition of the glycopeptide, cell division was markedly inhibited by the bovine glycopeptide for approximately 12 hr (Chart 2, left). After the 12-hr arrest period, the cells escaped from the inhibitory action of the glycopeptide, and the cell number in the treated population quickly approached that of the control culture. Important to our subsequent experiments were the observations that the inhibition of the LM-22 cells was completely reversible and that the kinetics of cell division within 6 hr of escape showed that virtually all cells escaped from the growth arrest (Chart 2, left). Unlike the LM-22 cells, pretreatment of 1316 fibrosarcoma cells with the ganglioside GM1 did not lead to cell cycle arrest (Chart 2, right). This observation was consistent with our previous report that showed that 1316 fibrosarcoma cells rapidly metabolize the GM1 ganglioside at 37° (6). As a result, little if any GM1 would be found in fibrosarcoma 1316 cell membranes 3 hr after the ganglioside was added.

Since the bovine glycopeptide blocked LM-22 cell cycling in a reversible and nontoxic manner, it seemed conceivable that the glycopeptide inhibitor could provide selective protection to "normal" cell populations, in the presence of transformed cells, against a compound that was primarily cytotoxic to proliferating cells. To test this possibility, LM-22 cells, pretreated with GM1 ganglioside (3.0 µg/ml), were mixed with 1316 fibrosarcoma cells at an approximate ratio of 1:1 and plated as a mixed population as described in "Materials and Methods." Three hr later, 250 ng protein per ml of UEA-1-purified bovine glycopeptide were added to the mixed culture to selectively inhibit the growth of LM-22 cells. After 3 hr of incubation, 5-BrdUrd was added to a final concentration of 5.0 mM, and the cells were incubated for an additional 12 hr. The cells were then removed from the culture vessel, serially diluted, and transferred to duplicate plates, with and without 3.0 mM ouabain, at a final concentration of either 200 or 400 cells/well. The surviving colony-forming units of LM-22 and 1316 fibrosarcoma cells were scored after 6 to 7 days of incubation. The result clearly showed that the LM-22 cells were efficiently protected from the cytotoxic action of 5-BrdUrd (92 to 94% survival) while the majority of the 1316 fibrosarcoma cells (21 to 30% survival) were killed (Table 1). The 6 to 8% loss of LM-22 cells might be expected since the bovine glycopeptide efficiently, but not completely, inhibited cell division (Chart 2, left). In fact, calculations of the cell susceptibility based on the growth kinetics of inhibitor-treated LM-22 cells suggested that approximately 9% of the cells would be killed by 5-BrdUrd.

The selective protection of the nonmalignant cells by the glycopeptide inhibitor was independent of differences in plating efficiency between the LM-22 and 1316 fibrosarcoma cells (40% efficiency in both cases), the cytotoxicity of 5-BrdUrd in the absence of the glycopeptide inhibitor (75% in both cases), and their generation time in culture (Chart 1).
DISCUSSION

We have previously shown that glycopeptides isolated from the cell surface of mouse and bovine cerebral cortex can inhibit protein synthesis and cell growth in a completely reversible and nontoxic manner (3, 4). The ability of the inhibitor to inhibit cell growth of GM1-treated LM-22 cells (Chart 2, left) allowed us to protect these cells from the S-phase-specific drug 5-BrdUrd (2, 7), while the insensitive fibrosarcoma 1316 cells (Chart 2, right) were not protected (Table 1).

Various strategies have been approached to provide multiple drug therapeutic regimens that selectively kill proliferating cell populations. As an example, Vadlamudi and Goldin (10) used vinblastine (M-phase inhibitor) and 1-β-D-arabinofuranosylcytosine (S-phase inhibitor) in combination with a mouse L1210 leukemia cell model. Vinblastine was used to synchronize cells and, 16 hr later, a point when most cells were traversing the S phase, 1-β-D-arabinofuranosylcytosine was introduced. Although effective in destroying the synchronized L1210 leukemia cell population, such a regimen does not avoid synchronization and selective killing of normal cell populations in the bone marrow. A similar strategy, with similar limitations, also has been approached with a combination therapy with daunorubicin and 1-β-D-arabinofuranosylcytosine (1). Other approaches of multiple-drug therapy have used techniques that rescue cells from the cytotoxic action of cancer chemotherapeutic agents. As an example, the duration of methotrexate exposure can be controlled by the addition of leucovorin, an antidote that bypasses the methotrexate block (8, 9). The introduction of leucovorin can enhance the antitumor action of methotrexate and affords an amplification of its selective activity.

The strategy described in this study is aimed at protecting normal cells from the cytotoxic action of drugs that primarily influence proliferating cells. In contrast to leucovorin utilization, our aim was to protect nontoxic cells initially and to avoid the necessity of a rescue. We chose 5-BrdUrd because it is well established as a drug that only influences proliferating cell populations (2, 7). Although clinical application, if at all possible, of this novel growth-inhibitory glycopeptide to selectively protect normal cells is in the distant future, we are encouraged with the efficiency with which the substance protected LM-22 from unusually high concentrations of 5-BrdUrd. A variation on this theme may find application in the development and screening of new antitumor drugs. For instance, it may be possible to develop bioassays with and without the inhibitory glycopeptide to discriminate between anticancer drugs that primarily are cytotoxic to only rapidly dividing cells and those compounds that indiscriminantly destroy dividing and mitotic-arrested cells.

REFERENCES

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