MOLECULAR PHARMACOLOGY, 28:567–573

Effects of the Inhibitors of IMP Dehydrogenase, Tiazofurin and Mycophenolic Acid, on Glycoprotein Metabolism

JOHN A. SOKOLOSKI AND ALAN C. SARTORELLI

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510

Received July 8, 1985; Accepted September 10, 1985

SUMMARY

The effects of the inhibitors of IMP dehydrogenase, tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) and mycophenolic acid, on the synthesis of cellular glycoproteins were evaluated in Sarcoma 180 cells. Both tiazofurin and mycophenolic acid decreased the rate of incorporation of [2-^{3}H]mannose and [2-^{3}H]fucose into acid-precipitable glycoproteins within 4 hr of exposure; this inhibitory activity was concentration dependent and occurred in the absence of a significant effect on the incorporation of labeled glucosamine and leucine into acid-insoluble material. Interference with the utilization of [^{3}H]mannose for the formation of glycoproteins was paralleled by an inhibition of [^{3}H]mannose incorporation into their lipid-linked oligosaccharide precursors following treatment with cytotoxic concentrations of tiazofurin (100 μM) or mycophenolic acid (10 μM); these actions occurred within 3 hr of exposure to these agents, with maximal reductions being observed at 12 hr. Under these conditions, intracellular GTP levels were reduced by 80%, whereas ATP pools remained unaffected and UTP levels were markedly increased. Guanosine (100 μM) prevented the cytotoxic actions of tiazofurin and mycophenolic acid and reversed the drug-induced decrease in GTP pools and in the incorporation of mannose and other metabolic precursors into acid-insoluble material. Inhibition of fucose and mannose incorporation into lipid-linked oligosaccharides and glycoproteins were preceded by decreases in the labeling of their respective guanosine nucleotide sugars and were followed sequentially by alterations in the plasma membrane as detected by both the binding and the rate of cell agglutination caused by the plant lectin, concanavalin A. The findings that tiazofurin and mycophenolic acid produce alterations in the utilization of [^{3}H]mannose for the formation of glycoproteins and in membrane architecture are indicative of metabolic lesions induced by agents that selectively depress guanine nucleotide synthesis through inhibition of IMP dehydrogenase.

INTRODUCTION

Many of the properties of mammalian cells are expressed through macromolecules associated with the cell surface. Consistent with such function is the demonstration of a variety of changes in the carbohydrate moieties of surface membrane glycoproteins when cells assume a malignant phenotype (1–3). Although the precise role of these carbohydrate groups in surface glycoproteins and the significance of transformation-associated changes are not well understood, it is apparent that modifications in the carbohydrate profile of cell surface glycoproteins contributes to the regulation of several properties altered by the neoplastic process, such as the regulation of cell growth, the immune response and other cellular recognition phenomena (4, 5), metastasis (6), and cell differentiation (7, 8). In addition, other factors which modulate cellular replication, including growth factors and peptide hormones, exert their effects by interacting with cell surface glycoprotein receptors (9, 10). These actions suggest that selective modification of glycoprotein structures might alter the behavior of malignant cells and contribute to their demise. Consistent with such a concept are the membrane alterations produced by the antileukemic agent 6TG[1] demonstrated in previous studies by this laboratory (11, 12). These investigations have shown that treatment of Sarcoma 180 ascites cells with 6TG *in vitro* and *in vivo* produces changes in the plasma membrane which can be detected by the plant lectin concanavalin A as a decrease in the extent of its binding to cell surface receptors and in the resulting rate of cell agglutination produced by this agent. The changes in membrane glycoprotein composition detected by concanavalin A in

This research was supported in part by United States Public Health Service Grant CA-02817 from the National Cancer Institute.

[1] The abbreviations used are: 6TG, 6-thioguanine; PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography; TAD, tiazofurin adenine dinucleotide.

0026-895X/85/060567-07$02.00/0

6TG-treated cells were preceded by pronounced decreases in the rate of fucose and mannose incorporation into cellular glycoproteins and of fucose activation to the nucleotide sugar GDP-fucose (11, 12).

The novel antitumor c-nucleoside tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) has been shown to cause a marked suppression of the formation of guanine nucleotides in a variety of experimental neoplasms as a consequence of pronounced inhibition of IMP dehydrogenase by a nicotinamide adenine dinucleotide metabolite of tiazofurin (13–16). Subsequent decreases in the formation of DNA and RNA, that are believed to be responsible for the cytotoxicity of this antimetabolite, occur in the absence of significant effects on protein synthesis. However, since intracellular GTP levels are also essential for the activation of both fucose and mannose to their respective nucleotide sugars, which in turn provide the precursor pools required for the formation of cellular glycoproteins and their dolichol-linked oligosaccharide precursors (17), it is conceivable that specific lesions affecting glycoprotein assembly may be involved in the cytotoxic actions of tiazofurin. For this reason, we have measured the effects of tiazofurin and the related inhibitor of IMP dehydrogenase, mycophenolic acid (18), on the incorporation of ^{3}H-labeled sugars into the glycoproteins of Sarcoma 180 tumor cells. The results of this study demonstrate that cytotoxic concentrations of inhibitors of IMP dehydrogenase cause alterations in the biosynthesis of cellular glycoproteins in these cells. Furthermore, these changes in glycoprotein metabolism, which represent early events in the expression of the cytotoxicity of these agents, affect the organization of cellular membranes as detected by concanavalin A binding.

MATERIALS AND METHODS

Cell culture. Sarcoma 180 cells in exponential growth were propagated in Fisher's medium supplemented with 10% horse serum (Grand Island Biological Co., Grand Island, NY) and penicillin/streptomycin (Grand Island Biological Co.) in a 5% CO_2 humidified atmosphere at 37°. Cells used in these experiments were maintained in suspension culture by subdividing stock cultures twice weekly. For cytotoxicity and biochemical studies, Sarcoma 180 cells were incubated in the presence of varying concentrations of tiazofurin (generously donated by Dr. David G. Johns of the National Cancer Institute, Bethesda, MD) or mycophenolic acid. Cell numbers and size were recorded with a Coulter model ZB_1 particle counter equipped with a Channelizer (Coulter Electronics, Inc., Hialeah, FL). Cell stocks were periodically checked for the presence of mycoplasma using the Hoecht staining method (19).

Analysis of ribonucleotide pools. Sarcoma 180 cells were suspended at levels of 0.5 to 1 × 10^6 cells/ml of fresh complete medium containing the appropriate inhibitor. At selected intervals, duplicate portions containing 2 × 10^6 cells were removed, and cells were washed with ice-cold PBS and then extracted with cold perchloric acid (4%, v/v) for 20 min. The acid-soluble material was collected by centrifugation and neutralized with 4 N KOH. The potassium perchlorate precipitate was removed by centrifugation and the sample was stored at −20° before analysis by HPLC. Cell extracts were fractionated using a microparticulate Partisil-10 SAX (Whatman, Inc., Woburn, MA) anion-exchange column and a Rainin gradient HPLC system equipped with a WISP 710A autoinjector (Waters Associates, Milford, MA). Nucleoside triphosphates were sequentially eluted using a 0.4 M $NH_4K_2PO_4$ isocratic buffer system (pH 3.7) maintained at a constant flow rate of 1.5 ml/min. The ultraviolet absorbance of eluants was monitored using an Altex (Berkeley, CA) model 153 detector set at 254 nm, and the peak areas were determined with a Shimadzu (Kyoto, Japan) C-R1A Chromatopac integrator and compared with appropriate standards.

The incorporation of [^{3}H]fucose and [^{3}H]mannose into nucleoside diphosphate sugars was measured with a similar system, using a linear gradient of buffer A (0.05 M $NH_4H_2PO_4$, pH 3.5) and buffer B (0.4 M $NH_4H_2PO_4$, pH 3.3). Buffer A was initially pumped isocratically for 3 min prior to the establishment of a linear gradient consisting of 0 to 100% of buffer B over 45 min; 1.0 ml fractions of the column eluant were collected and the radioactivity therein was measured by liquid scintillation spectrometry.

Incorporation of labeled sugars into glycoprotein and dolichol oligosaccharides. The effects of tiazofurin and mycophenolic acid on total cellular glycoproteins were analyzed as previously described by Lazo *et al.* (11, 12) using either D-[2-^{3}H]mannose (2.25 mCi/ml; 14.5 Ci/mmol), L-[5,6-^{3}H]fucose (1.0 mCi/ml; 56 Ci/mmol), or D-[6-^{3}H]glucosamine hydrochloride (1.0 mCi/ml; 30 Ci/mmol) obtained from New England Nuclear (Boston, MA). Dolichol-containing oligosaccharides were isolated by selective organic extraction (20). Cultured Sarcoma 180 cells (2 × 10^6 cells/ml), pretreated with the appropriate inhibitor for 2 hr, were exposed to 2.0 μCi/ml of either [^{3}H]glucosamine or [^{3}H]mannose for various periods of time. Incorporation was terminated by washing cells three times with ice-cold PBS, followed by extraction with 2.0 ml of chloroform/methanol (2:1, v/v). The insoluble pellet was washed three times with water and dried under nitrogen, and the dolichol-linked oligosaccharides were extracted using chloroform/methanol/water (1:1:0.3, v/v). The remaining pellet, which contained cellular glycoproteins, was hydrolyzed in 1 N NaOH and neutralized, and an aliquot in Aquasol was counted by liquid scintillation spectrometry.

Cell agglutination and lectin binding studies. Cell agglutination by concanavalin A was carried out according to the method of Hwang *et al.* (21). Briefly, washed Sarcoma 180 cells (1 × 10^6 cells/ml) in Ca^{2+}, Mg^{2+}-free PBS were added to 1-ml quartz cuvettes containing 100 to 200 mg of concanavalin A. After gentle mixing, cell agglutination was measured at 546 nm using a Beckman model 25 recording spectrophotometer thermostated at 37°. Cells preincubated with the haptenic inhibitor α-methyl-D-mannoside were used to determine nonspecific agglutination and cell settling.

The direct binding of [^{3}H]concanavalin A (specific activity 42.4 Ci/mmol) to intact cells was assayed as previously described (21). Cells were washed and resuspended in Ca^{2+}, Mg^{2+}-free PBS (pH 7.4) containing 0.5% bovine serum albumin. Concanavalin A (100 to 200 μg/ml) was added and the reaction mixtures were gently shaken for 30 min at ambient temperature. Concanavalin A binding was terminated by the addition of 2.0 μl of ice-cold PBS containing 0.5% bovine serum albumin followed by the immediate centrifugation of the glass reaction tube. Washed cells were lysed with 1 N NaOH and the radioactivity present in an aliquot was determined in Hydrofluor. Specific binding was calculated as the difference between total and nonspecific binding, with nonspecific binding being determined in parallel samples containing 50 mM α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, MO). The nonspecific binding of [^{3}H]concanavalin A to Sarcoma 180 cells was less than 10% of the total observed binding.

RESULTS

To determine the growth-inhibitory potency of tiazofurin and the related IMP dehydrogenase inhibitor, mycophenolic acid, on Sarcoma 180, cultured cells were exposed to concentrations of tiazofurin ranging from 10^{-7} to 10^{-6} M or to mycophenolic acid from 10^{-7} to 10^{-5} M continuously for 24 hr. Under these conditions, a concentration-dependent inhibition of cellular replication occurred, with mycophenolic acid (ID_{50} = 0.4 μM) showing considerably greater cytotoxicity than tiazofurin (ID_{50} = 7.0 μM) (Fig. 1). Previous studies have linked the

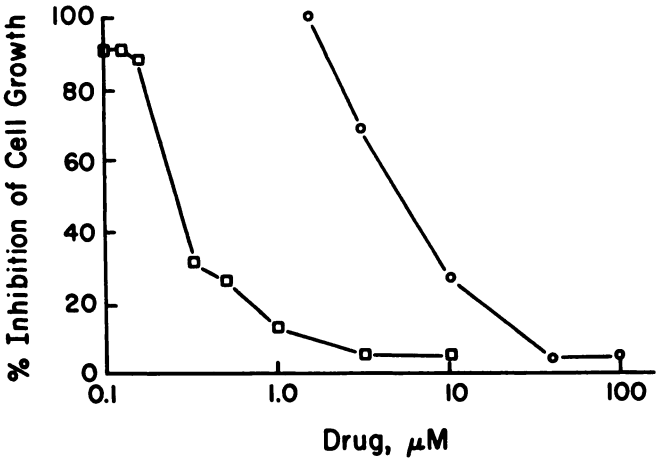

FIG. 1. *Inhibition of the proliferation of Sarcoma 180 cells by tiazofurin and mycophenolic acid*

Sarcoma 180 cells were incubated with various concentrations of tiazofurin (○) or mycophenolic acid (□) for 24 hr. Results are given as the per cent inhibition of cell growth from a typical experiment run in duplicate.

TABLE 1

Effects of tiazofurin and mycophenolic acid on nucleoside triphosphate levels of Sarcoma 180 cells

Sarcoma 180 cells (8 to 10 × 10^5 cells/ml) were exposed to various concentrations of tiazofurin or mycophenolic acid for 2 hr. Duplicate aliquots were removed, washed with ice-cold PBS, and analyzed as described in "Materials and Methods." Each value represents the mean of results from a typical experiment analyzed in duplicate. Average control nucleotide concentrations in nanomoles/10^6 cells were: CTP, 0.20; UTP, 0.63; ATP, 3.08; GTP, 1.86.

Inhibitor	Concentration	Percentage of control			
		CTP	UTP	ATP	GTP
	μM				
Tiazofurin	0.1	100	112	86	78
	1.0	96	114	85	66
	10.0	96	120	87	65
	100	134	160	90	44
Mycophenolic acid	0.1	108	118	92	55
	1.0	200	183	91	37
	10.0	221	208	95	25

cytotoxicity of tiazofurin and mycophenolic acid to the selective depletion of intracellular guanine nucleotides resulting from inhibition of IMP dehydrogenase (13–16, 18). To examine these effects in Sarcoma 180 cells, changes in the concentrations of acid-soluble nucleotides were examined. After exposure to tiazofurin and mycophenolic acid for 2 hr, a concentration-related reduction in GTP levels occurred (Table 1). A greater reduction in intracellular GTP was observed in cells treated with mycophenolic acid, which presumably is a reflection of the higher potency of this metabolic inhibitor. The decrease in GTP levels produced by these agents occurred with minimal or no reduction in intracellular concentrations of ATP, supporting the reported selectivity of these inhibitors on the purine nucleotide biosynthesis pathways. In contrast, the content of pyrimidine nucleoside triphosphates increased concomitantly in response to these inhibitors, with mycophenolic acid causing more pronounced increases in CTP and UTP levels than those caused by tiazofurin. A similar increase in pyrimidine nucleoside triphosphate levels has been noted in other cell lines in response to tiazofurin and may reflect an increased availability of 5-phosphoribosyl-1-pyrophosphate (22). The time course of the depletion of intracellular GTP relative to other nucleoside triphosphate pools of Sarcoma 180 cells was examined over an 8-hr period of exposure to either 10 μM mycophenolic acid or 100 μM tiazofurin. As seen in Fig. 2A, exposure to tiazofurin produced a rapid reduction in GTP levels. This decrease in intracellular GTP content was maximal at 1 hr and was maintained for the duration of the experiment with both inhibitors of IMP dehydrogenase. Under these conditions, drug treatments caused a marked increase in UTP levels and minor perturbations in the concentration of ATP.

To ascertain the effects of depletion of GTP pools on glycoprotein biosynthesis, Sarcoma 180 cells were exposed to a variety of concentrations of tiazofurin or mycophenolic acid and the incorporation of several monosaccharide precursors of glycoprotein was measured. Such treatment produced concentration-dependent reductions in the rate of utilization of ^{3}H-labeled fucose and mannose (Table 2). Thus, at a level of 100 μM tiazofurin and 10 μM mycophenolic acid, the highest concentrations of each of these inhibitors employed, a 40 to 50% reduction in incorporation was observed; in con-

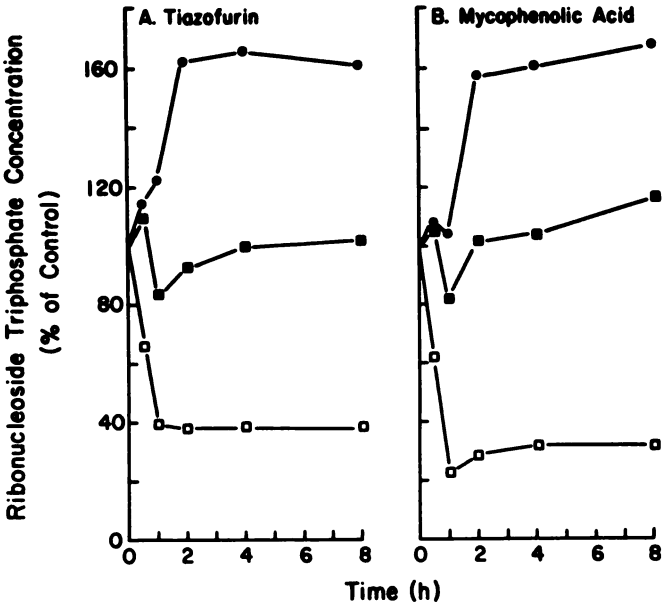

FIG. 2. *Changes in acid-soluble nucleoside triphosphate concentrations in Sarcoma 180 cells treated with tiazofurin and mycophenolic acid*

Sarcoma 180 cells (8 × 10^5 cells/ml) were exposed in culture to 100 μM tiazofurin or 10 μM mycophenolic acid. Duplicate aliquots were removed at the indicated times, washed with ice-cold PBS, and extracted with 0.2 ml of 4% perchloric acid. The supernatant fractions were neutralized with 2 N KOH and analyzed by HPLC as described in "Materials and Methods." Each value represents the mean of typical results obtained from duplicate samples from two separate experiments: ●, UTP; ■, ATP; □, GTP. Average control nucleotide concentrations in nanomoles/10^6 cells were: UTP, 0.63; ATP, 3.08; GTP, 1.86.

TABLE 2

Effects of tiazofurin and mycophenolic acid on the incorporation of ^{3}H-labeled precursors into glycoproteins

Sarcoma 180 cells were preexposed to various concentrations of tiazofurin or mycophenolic acid for 2 hr; ^{3}H-radiolabeled precursor (2.0 μCi/ml) was added and incubations were continued for an additional 2 hr. Samples were extracted and analyzed as described in "Materials and Methods." Results for each labeled precursor are the means of the percentage of control ± standard error from at least three independent determinations. Average control radioactivities (cpm/2 × 10^6 cells) were: mannose, 2.5 × 10^3; fucose, 1.6 × 10^3; glucosamine, 6.7 × 10^3; leucine, 4.1 × 10^3.

^{3}H-labeled precursor	Tiazofurin (μM)				Mycophenolic acid (μM)		
	0.1	1.0	10.0	100	0.1	1.0	10.0
Mannose	93.5 ± 5.7	85.7 ± 1.9	70.4 ± 11.7	55.9 ± 7.3	72.6 ± 2.5	62.0 ± 4.1	49.7 ± 3.1
Fucose	90.6 ± 15.1	79.2 ± 5.6	66.6 ± 7.6	60.5 ± 9.5	80.8 ± 3.6	72.0 ± 11.1	50.2 ± 3.7
Glucosamine	113.7 ± 10.2	111.3 ± 11.4	110.1 ± 6.4	92.5 ± 8.1	109.0 ± 15.0	87.9 ± 2.0	80.5 ± 4.5
Leucine	103.4 ± 0.4	99.2 ± 1.0	100.2 ± 0.7	87.6 ± 0.4	126.4 ± 0.7	99.4 ± 7.2	86.7 ± 14.7

trast, incorporation of glucosamine, a sugar precursor not activated by GTP, was decreased at most only minimally. Furthermore, under these conditions, the basal rate of protein synthesis, as measured by the incorporation of [^{3}H]leucine into acid-insoluble material, was relatively unaffected, despite the marked perturbations in the formation of cellular glycoproteins produced by these agents.

The kinetics of radiolabeled precursor incorporation into cellular glycoproteins and dolichol-linked oligosaccharides of Sarcoma 180 cells, preexposed to 100 μM tiazofurin or 10 μM mycophenolic acid for 2 hr prior to addition of radiolabeled precursors, was ascertained (Fig. 3). After a total period of 3 hr of exposure to 100 μM tiazofurin or 10 μM mycophenolic acid, an approximately 40% decrease in the rate of incorporation of [^{3}H]mannose into glycoproteins occurred. Further reductions in the degree of incorporation of mannose into glycoproteins took place with time and these leveled off at a maximum of 60% inhibition by 6 hr. In contrast, [^{3}H]glucosamine incorporation into glycoproteins was only slightly affected at the earliest time measured; by 6 hr, however, the utilization of [^{3}H]glucosamine for the formation of glycoprotein diminished in a manner analogous to that seen with [^{3}H]mannose. Incorporation of [^{3}H]leucine into protein was also inhibited at the latest time points measured, which presumably is a reflection of the metabolic toxicity of the inhibitor in treated cells. A corresponding decrease in the utilization of ^{3}H-labeled sugars for the formation of dolichol-linked intermediates of glycoprotein biosynthesis also occurred.

Since the antiproliferative effects of tiazofurin and mycophenolic acid are believed to result from the selective depletion of guanine nucleotides consequent to the inhibition of IMP dehydrogenase, the simultaneous exposure of cells to inhibitor and guanosine, employed as a source of preformed guanine to circumvent the blockage of IMP dehydrogenase, was measured to determine whether the physiological purine nucleoside was capable of preventing cytotoxicity. The continuous exposure of cultured Sarcoma 180 cells to 20 μM tiazofurin for 24 hr resulted in greater than a 40% inhibition of cell growth (Fig. 4). Increasing concentrations of guanosine progressively decreased the cytotoxicity of tiazofurin, with complete reversal being attained at 100 μM guanosine. Guanosine at this concentration by itself had little effect on cellular proliferation; however, 200 μM guanosine was inhibitory to Sarcoma 180 cells and for this reason did not rescue cells from the cytotoxic actions of tiazofurin. Under identical conditions, exogenous guanosine pro-

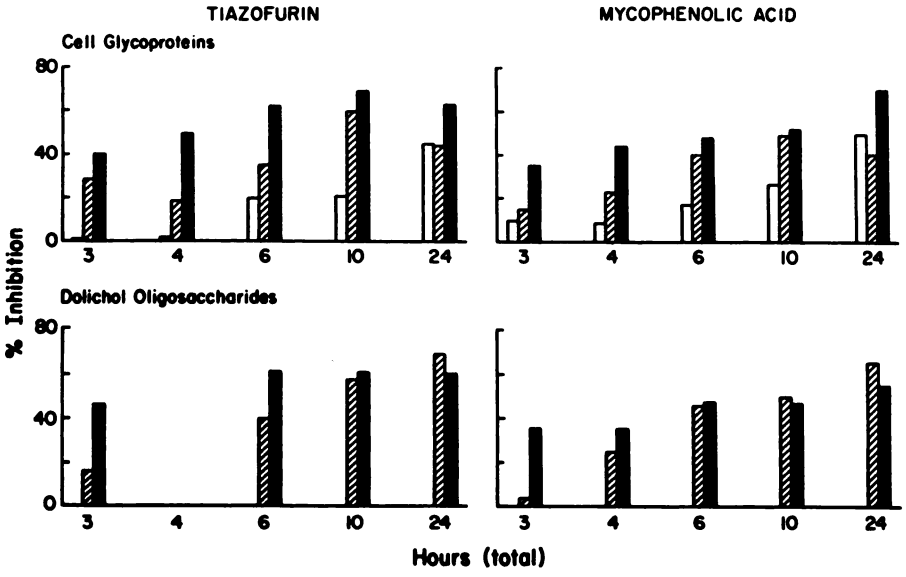

FIG. 3. *Inhibition of the synthesis of glycoproteins and lipid-linked intermediates by tiazofurin and mycophenolic acid*

Sarcoma 180 cells (1 × 10^6 cells/ml) were treated with 100 μM tiazofurin or 10 μM mycophenolic acid for 2 hr. ^{3}H-radiolabeled precursor (final concentration 1.0 μCi/ml) was added and incubations were continued for various periods of time. Triplicate samples were removed at selected intervals and dolichol-linked oligosaccharides and cellular glycoproteins were extracted as described in "Materials and Methods." Data represent the means of two or more experiments in each treatment group: □, leucine; ▨, glucosamine; ■, mannose.

FIG. 4. *Prevention of tiazofurin toxicity to Sarcoma 180 cells by exogenous guanosine*

Sarcoma 180 cells were incubated with various concentrations of guanosine (10 to 200 μM) in the presence (○) or absence (△) of 20 μM tiazofurin for 24 hr, and cell numbers were determined at 24 hr. Results are the average of three independent experiments, each run in duplicate.

TABLE 3

Effects of tiazofurin and mycophenolic acid on the incorporation of ^{3}H-labeled carbohydrates into nucleotide sugar precursor pools of Sarcoma 180 cells

Sarcoma 180 cells were treated with 100 μM tiazofurin or 10 μM mycophenolic acid for 2 hr. ^{3}H-radiolabeled precursor (2.0 μCi/ml) was added and the incubation was continued for an additional 2 hr. Cells were pelleted and analyzed as described in the text. Data represent the average percentage of control incorporation from two separate experiments. Average control activities (cpm/2 × 10^6 cells) were: GDP-mannose, 1.3 × 10^3; GDP-fucose, 4.3 × 10^3; UDP-*N*-acetylglucosamine, 2.0 × 10^3.

Treatment	Percentage of control		
	GDP-mannose	GDP-fucose	UDP-*N*-acetylglucosamine
Tiazofurin	43.5	53.6	89.2
Mycophenolic acid	50.8	67.2	103.2

vided only a partial rescue of cells from the toxicity produced by 100 μM tiazofurin (data not shown). Reversal of tiazofurin-induced cytotoxicity by guanosine was paralleled by a complete reversal of the depletion of GTP levels and the consequent inhibition of glycoprotein, DNA, and RNA synthesis by this agent (data not shown).

To further establish the relationship between the observed decrease in glycoprotein synthesis and the reduction in intracellular GTP concentration, the effects of tiazofurin and mycophenolic acid on the incorporation of ^{3}H-labeled sugars into nucleotide sugar precursor pools was measured. Tiazofurin and mycophenolic acid caused a selective reduction in the formation of both GDP-fucose and GDP-mannose, whereas the incorporation of *N*-acetylglucosamine into UDP-*N*-acetylglucosamine was not depressed by these treatments (Table 3).

Evidence for the presence of altered glycoprotein structures on the external surface of Sarcoma 180 cells was obtained through binding studies with the plant lectin concanavalin A. The incubation of cells with 100 μM tiazofurin or 10 μM mycophenolic acid for 4 hr produced a reduction in the specific binding of concanavalin A by 36% and 53%, respectively (Table 4). This decrease in

TABLE 4

Effects of tiazofurin and mycophenolic acid on the specific binding of concanavalin A and on the resulting agglutination of Sarcoma 180 cells

Binding of [^{3}H]concanavalin A to Sarcoma 180 cells was measured 4 hr after treatment with 100 μM tiazofurin or 10 μM mycophenolic acid as described in "Materials and Methods." Each value represents the average of at least three experiments with three determinations of concanavalin A binding in each experiment. Cell agglutinations are representative A_{546} values from one of four experiments measured from 2 to 10 min as described in the text.

Treatment	Specific binding (μg Con A/10^6 cells)	Percentage of control	Cell agglutination (ΔA_{546})
None	1.35		0.085
Tiazofurin	0.87	64.5	0.020
Mycophenolic acid	0.64	47.2	0.015

specific binding was paralleled by a decrease in the rate of agglutination by the plant lectin. In contrast, the specific binding of wheat germ agglutinin, a lectin specific for *N*-acetylglucosamine, remained unaffected under these conditions (data not shown).

DISCUSSION

The activity of IMP dehydrogenase has been shown to be directly correlated with tumor cell proliferation, leading to the suggestion that this enzyme is an important target for cancer chemotherapy (23–25). IMP dehydrogenase, which catalyzes the conversion of inosinic acid to xanthylic acid, is the first enzyme uniquely involved in the biosynthesis of guanine nucleotides; therefore, interference with the activity of this enzyme leads to a marked reduction in the level of guanine nucleotides. Since guanine nucleotides serve as substrates and activators for a number of cellular metabolic processes, including essential roles in DNA, RNA, and protein synthesis, the inhibition of IMP dehydrogenase by agents such as tiazofurin and mycophenolic acid would be expected to produce major alterations in the metabolic behavior of malignant cells. Consistent with these expectations, this laboratory demonstrated that the purine-thiol inhibitor of IMP dehydrogenase, 6TG, interfered with the biosynthesis of cellular glycoproteins, presumably as a consequence of drug-induced depletion of intracellular GTP (11, 12). Since this interpretation is complicated by the fact that 6TG causes a variety of other metabolic lesions, including its incorporation into cellular DNA (26), we have measured the effects of the potent IMP dehydrogenase inhibitors tiazofurin and mycophenolic acid on glycoprotein biosynthesis in cultured Sarcoma 180 cells. Consistent with previous reports (13, 18), inhibition of cell growth by tiazofurin and mycophenolic acid was accompanied by a marked suppression of guanine nucleotide biosynthesis that preceded a substantial inhibition of DNA and RNA biosynthesis. Furthermore, exposure of Sarcoma 180 cells to tiazofurin or mycophenolic acid produced a concentration- and time-dependent decrease in the rate of incorporation of ^{3}H-labeled mannose and fucose into cellular glycoproteins and their dolichol-linked intermediates. These effects occurred before the detection of drug-induced changes in the incor-

poration of glucosamine or leucine into glycoproteins and proteins, respectively, supporting the concept that the early effects on glycoprotein biosynthesis are due to depletion of guanine nucleotides in drug-treated cells. Such drug-induced depletion of guanine nucleotide pools results in a decrease in the activation of both mannose and fucose to their respective guanosine diphosphate sugars. The observed reduction in labeled fucose and mannose incorporation into GDP-fucose and GDP-mannose, respectively, in tiazofurin- and mycophenolic acid-treated cells is consistent with this expectation. In support of the importance of these lesions to drug action is the finding that exogenous guanosine completely protected Sarcoma 180 cells from the antiproliferative effects of relatively low levels of these inhibitors, and both restored GTP levels to normal and reversed blockade of glycoprotein biosynthesis.

The regulation of glycoprotein biosynthesis is poorly understood at present, but qualitative and quanitative alterations in the incorporation of labeled sugars into glycoproteins may well involve the bioavailability of the appropriate nucleotide-sugar precursors (27). Control of the formation of glycoproteins by modulation of UDP-*N*-acetylglucosamine metabolism has recently been reported (28); however, studies utilizing this nucleotide sugar are complicated by its distribution into cell surface glycolipids and proteoglycans as well as into glycoproteins (29). Conversely, there is more specificity in the utilization of mannose, since this sugar is found predominantly on carbohydrate chains that are N-linked to asparagine residues (30). The unique formation of asparagine-linked glycopeptides by a pathway involving the sequential addition of nucleotide sugar precursors to a dolichol-linked oligosaccharide carrier which is transferred *en bloc* to the growing protein chain suggests that potential regulation of this process may be obtained by manipulating the availability of the endogenous nucleotide sugar precursor pool (17).

Tiazofurin and the related nucleoside analog ribavirin constitute an important group of antimetabolites, with potential application in both antiviral and anticancer chemotherapy. Tiazofurin is initially activated to the monophosphate level, which is then adducted to NAD by the action of NAD pyrophosphorylase to form the active inhibitor of IMP dehydrogenase, TAD (16, 31). In view of its capacity to interfere with a specific enzymatic reaction in the purine nucleotide biosynthetic scheme, tiazofurin, ribavirin, and the related IMP dehydrogenase inhibitor mycophenolic acid are valuable tools to employ in investigations of the metabolic events leading to cell death following guanine nucleotide deprivation. The findings described in this report suggest that tiazofurin and mycophenolic acid produce structural and compositional changes in the membranes of treated cells as a consequence of the perturbation of glycoprotein synthesis. The extent to which modifications in cell surface properties, which can be detected prior to the time of drug exposure required to produce cell death, contribute to the cytotoxicity of these agents is not known. However, if the alterations in membrane glycoprotein composition that are produced by tiazofurin and mycophenolic acid cause disruption of the synthesis of cell surface receptors, it is reasonable to speculate that such changes might produce a variety of metabolic alterations in the malignant cell, including modified immunogenicity, adhesiveness, and membrane transport of nutrients, all of which may play a role in the cytotoxic actions of these agents.

REFERENCES

1. Warren, L., and C. A. Buck. The membrane glycoproteins of the malignant cell. *Clin. Biochem.* **13**:191–197 (1980).
2. Warren, L., S. R. Baker, D. L. Blithe, and C. A. Buck. The role of carbohydrates bound to protein, in *Biomembranes*, vol. 11 (A. Nowotny, ed.). Plenum Press, New York, 53–78 (1983).
3. Nicolson, G. L. Transmembrane control of the receptors of normal and tumor cells. II. Surface changes associated with transformation and malignancy. *Biochim. Biophys. Acta* **458**:1–72 (1976).
4. Berger, E. G., E. Buddecke, J. P. Kamerling, A. Kobata, J. C. Paulson, and J. F. G. Vliegenthart. Structure, biosynthesis and functions of glycoprotein glycans. *Experientia* **38**:1129–1158 (1982).
5. Yogeeswaran, C. Cell surface glycolipids and glycoproteins in malignant transformation. *Adv. Cancer Res.* **38**:289–350 (1983).
6. Irimura, T., R. Gonzalez, and G. L. Nicolson. Effects of tunicamycin on B16 metastatic melanoma cell surface glycoproteins and blood-borne arrest and survival properties. *Cancer Res.* **41**:3411–3418 (1981).
7. Zieske, J. D., and I. A. Bernstein. Modification of cell surface glycoprotein: addition of fucosyl residues during epidermal differentiation. *J. Cell Biol.* **95**:626–631 (1982).
8. Mizoguchi, A., S. Takasaki, S. Maeda, and A. Kobata. Changes in asparaginase-linked sugar chains of human promyelocytic leukemic cells (HL-60) during monocytoid differentiation and myeloid differentiation: appearance of high mannose-type oligosaccharides in neutral fraction. *J. Biol. Chem.* **259**:11943–11948 (1984).
9. Hedo, J. A., M. Kasuga, E. Van Obberghen, J. Roth, and C. R. Kahn. Direct demonstration of glycosylation of insulin receptor subunits by biosynthetic and external labeling: evidence for heterogeneity. *Proc. Natl. Acad. Sci. USA* **78**:4791–4795 (1981).
10. Soderquist, A. M., and G. Carpenter. Glycosylation of the epidermal growth factor receptor in A-431 cells: the contribution of carbohydrate to receptor function. *J. Biol. Chem.* **259**:12586–12594 (1984).
11. Lazo, J. S., K. M. Hwang, and A. C. Sartorelli. Inhibition of L-fucose incorporation into glycoprotein of Sarcoma 180 ascites cells by 6-thioguanine. *Cancer Res.* **37**:4250–4255 (1977).
12. Lazo, J. S., C. W. Shansky, and A. C. Sartorelli. Reduction in cell surface concanavalin A binding and mannose incorporation into glycoproteins of Sarcoma 180 by 6-thioguanine. *Biochem. Pharmacol.* **28**:583–588 (1979).
13. Jayaram, H. N., R. L. Dion, R. I. Glazer, D. G. Johns, R. K. Robins, P. C. Srivastava, and D. A. Cooney. Initial studies on the mechanism of action of a new oncolytic thiazole nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 286193). *Biochem. Pharmacol.* **31**:2371–2380 (1982).
14. Robins, R. K., P. C. Srivastava, V. L. Narayanan, J. Plowman, and K. D. Paull. 2-β-D-Ribofuranosylthiazole-4-carboxamide, a novel potential antitumor agent for lung tumors and metastases. *J. Med. Chem.* **25**:107–108 (1982).
15. Robins, R. K. Nucleoside and nucleotide inhibitors of inosine monophosphate (IMP) dehydrogenase as potential antitumor inhibitors. *Nucleosides Nucleotides* **1**:35–44 (1982).
16. Gebeyehu, G., V. E. Marquez, J. A. Kelley, D. A. Cooney, H. N. Jayaram, and D. G. Johns. Synthesis of thiazole-4-carboxamide adenine dinucleotide. A powerful inhibitor of IMP dehydrogenase. *J. Med. Chem.* **26**:922–925 (1983).
17. Hubbard, S. C., and R. J. Ivatt. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **50**:555–583 (1981).
18. Lowe, J. K., L. Brox, and J. F. Henderson. Consequences of inhibition of guanine nucleotide synthesis by mycophenolic acid and virazole. *Cancer Res.* **37**:736–743 (1977).
19. Chen, T. R. *In situ* detection of mycoplasma contamination in cell culture by fluorescence Hoechst 33258 stain. *Exp. Cell Res.* **104**:255–262 (1977).
20. Pan, Y.-T., and A. D. Elbien. The formation of lipid-linked oligosaccharides in Madin-Darby canine kidney cells: changes in oligosaccharide profiles induced by glucosamine. *J. Biol. Chem.* **257**:2795–2801 (1982).
21. Hwang, K. H., S. A. Murphree, and A. C. Sartorelli. A quantitative spectrophotometric method to measure plant lectin-induced cell agglutination. *Cancer Res.* **34**:3346–3402 (1974).
22. Lui, M. S., M. A. Faderan, J. J. Liepnicks, Y. Natsumeda, E. Olah, H. N. Jayaram, and G. Weber. Modulation of IMP dehydrogenase activity and guanylate metabolism by tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide). *J. Biol. Chem.* **259**:5078–5082 (1984).
23. Jackson, R. C., G. Weber, and H. Pronnis. IMP dehydrogenase, an enzyme linked with proliferation and malignancy. *Nature (Lond.)* **256**:331–333 (1975).

24. Weber, G. Biochemical strategy of cancer cells and the design of chemotherapy. G. H. A. Clowes Memorial Lecture. *Cancer Res.* **43**:3466–3492 (1983).
25. Jackson, R. C., H. P. Morris, and G. Weber. Partial purification, properties and regulation of inosine 5′-phosphate dehydrogenase in normal and malignant rat tissues. *Biochem. J.* **166**:1–10 (1977).
26. Paterson, A. R. P., and D. M. Tidd. 6-Thiopurines, in *Antineoplastic and Immunosuppressive Agents*, part 2 (A. C. Sartorelli and D. G. Johns, eds.). Springer-Verlag, Berlin, 384–403 (1975).
27. Yurchenco, P. D., C. Ceccarini, and P. H. Atkinson. Labeling complex carbohydrates of animal cells with monosaccharides. *Methods Enzymol.* **50**:175–204 (1978).
28. Hindenburg, A. A., R. N. Taub, S. Grant, G. Chang, and M. A. Baker. Effects of pyrimidine antagonists on sialic acid regeneration in HL-60 cells. *Cancer Res.* **45**:3048–3052 (1985).
29. Struck, D. K., and W. J. Lennarz. The function of saccharide-lipids in synthesis of glycoproteins, in *The Biochemistry of Glycoproteins and Proteoglycans* (W. J. Lennarz, ed.). Plenum Press, New York, 35–83 (1980).
30. Czichi, U., and W. J. Lennarz. Localization of the enzyme system for glycosylation of proteins via the lipid-linked pathway in rough endoplasmic reticulum. *J. Biol. Chem.* **252**:7901–7904 (1977).
31. Cooney, D. A., H. N. Jayaram, G. Gebeyehu, C. R. Betts, J. A. Kelley, and D. G. Johns. The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. *Biochem. Pharmacol.* **31**:2133–2136 (1982).

Send reprint requests to: Dr. Alan C. Sartorelli, Director, Yale Comprehensive Cancer Center, P. O. Box 3333, New Haven, CT 06510.