Do Antimetabolites Interfere with the Glycosylation of Cellular Glycoconjugates?

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INTRODUCTION

ANTIMETABOLITES are frequently used for the treatment of various neoplastic diseases, including solid tumors and leukemias [1], but the use of these compounds is limited by the occurrence of myeloid and gastrointestinal toxicity. For most of these drugs the mechanism of action has been investigated extensively *in vitro* and *in vivo* [1-5]. These studies, in general, point to interference in purine or pyrimidine biosynthesis (*cf.* Table 1) leading to inhibition of DNA and/or RNA synthesis in dividing cells. Additional mechanisms, however, might be responsible for the cytotoxic effects on non-dividing cells, i.e. the majority of normal cells and tumor cells which are in resting phase.

Several antimetabolites interfere with the manufacture of nucleotides (UTP, GTP and CTP) that are substrates for the manufacture of nucleotide sugars. Since nucleotide sugars are the precursors for the biosynthesis of glycoconjugates (*cf.* Fig. 1), the glycosylation of both secreted and membrane-associated glycoconjugates might be affected in the presence of antimetabolites. The sugar chains extending from the cell surface are important determinants of the phenotype of cells [6] and have been implicated in a variety of cell surface interactions [6-10].

Characteristic changes in cell surface oligosaccharides accompany normal and malignant development and hematopoietic differentiation [11-22]. Thus, for a variety of malignant cells a characteristic increase in molecular weight, caused by changes in branching [11-17] and/or increased amount of sialic acid [11, 12, 16-18], has been observed for cell surface oligosaccharide structures in comparison with those from non-malignant cells. The invasive capacity of cells [17, 19, 20] and the loss of binding of malignant cells to the substratum [12, 21] is related positively to the observed increase in molecular weight. In the last decade the evidence is compelling that the variations in oligosaccharide structures observed coincide with changes in activities of specific glycosyltransferases [e.g. 14, 21, 23-26], indicating the importance of these enzymes in the regulation of the glycosylation process.

The general occurrence of characteristic changes in glycoconjugates in relation to malignancy and differentiation gives sup-

port to the idea that the cell surface oligosaccharide structures are functionally involved in the accompanying change in phenotypic properties. Therefore, antimetabolite-induced alterations in glycosylation (e.g. extent of glycosylation, degree of branching or sialylation) might affect cell function of both normal and cancer cells.

In this article we review the effects of antimetabolites on different aspects of the glycosylation process in normal and tumor cells described thus far.

INVOLVEMENT OF NUCLEOTIDE SUGARS IN THE REGULATION OF THE GLYCOSYLATION PROCESS

A wide variety of oligosaccharide structures are present on cell-membrane glycoproteins and glycolipids. Their biosynthesis requires a non-template post-translational process in the lumen of the endoplasmic reticulum and/or the Golgi complex. A large number of glycosyltransferases are involved in the regulation of this process [27-30]. These enzymes transfer the sugar residues from the nucleotide sugar to the growing carbohydrate chain. Nucleotide sugars are translocated from the cytosol into the Golgi vesicles via specific transporters, in exchange with the corresponding nucleoside monophosphate which are co-products of the transfer reactions (for review see ref. [31]). The glycosyltransferases are not only specific for the nucleotide sugar but also for the acceptor to be glycosylated, the anomeric configuration and the linkage type in which the sugar is attached [28]. Various reports demonstrate direct or indirect evidence that interference in the metabolism of nucleotide sugars or their translocation across the Golgi membrane result in structural changes in the oligosaccharide chains expressed on the cell surface. Thus, incubation of cells with galactosamine (GalNH₂) or glucosamine (GlcNH₂) strongly affected the cellular concentrations of uridine nucleotide sugars, with a concomitant inhibition of aberrant glycosylation of glycoproteins [32-35]. Furthermore, a defective translocation of CMP-sialic acid in the Chinese hamster ovary cell line Lec2 corresponded with a strong decrease in extent of sialylation of galactose or galactosamine residues on cell surface glycoproteins and glycolipids, in comparison to wild-type cells [31, 36]. In another mutant, Lec8, the translocation of UDP-galactose was defective, resulting in a 80-90% reduction in both galactose and sialic acid residues in comparison to wild-type cells [31, 36].

ANTIMETABOLITES AND GLYCOSYLATION

Antimetabolites can interfere in three ways with nucleotide sugar metabolism. Firstly, the antimetabolite itself can be converted into a nucleotide sugar analog; secondly, the antimetabolite can deplete the levels of normal nucleotides leading to a depletion of nucleotide sugars; and thirdly, the concentration

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Abbreviations: AraC, arabinofuranosylcytosine; 5FU, 5-fluorouracil; FUR, 5-fluorouridine; FUDR, 5-fluoro-2'-deoxyuridine; MTX, methotrexate; PALA, N-(phosphonacetyl)-L-aspartate.

Table 1. Summary of known mechanisms of several antimetabolites

Antimetabolite	Active intermediate	Target enzyme	Postulated effect leading to cell kill
MTX	MTX + polyglutamates	DHFR	depletion of TTP inhibition purine <i>de novo</i>
6MP; 6MMPR	6-thio-IMP 6-thio-GMP		inhibition purine <i>de novo</i> incorporation nucleic acids
6TG	6-thio-GMP		incorporation nucleic acids
Tiazofurin Mycophenolic acid	TAD	IMP-DH IMP-DH	depletion GTP depletion GTP
AraC	Ara-CTP	DNA polymerase	incorporation DNA depletion dCTP
PALA		ATC	depletion UTP + CTP
Pyrazofurin 6-Azauridine	monophosphate aza-UMP	OPRT OPRT	depletion UTP + CTP depletion UTP + CTP
Acivicin		CPS-II CTP synthetase 6MP synthetase purine <i>de novo</i>	depletion purine + pyrimidine nucleotides
5FU	FdUMP	thymidylate synthase	depletion TTP
	FUTP		incorporation into RNA
Hydroxyurea		ribonucleotide reductase	depletion deoxyribonucleotides

The data are taken from refs. [1–5]. DHFR, dihydrofolate reductase; IMP-DH, IMP dehydrogenase; ATC, aspartate transcarbamylase; OPRT, orotate phosphoribosyltransferase; CPS II, carbamylphosphate synthetase II. TAD is the diphosphate of tiazofurin. 6MP, 6-mercaptopurine; 6MMPR, 6-methyl-mercaptopurine riboside; 6TG, 6-thioguanine; FdUMP, 5-fluoro-deoxyUMP; FUTP, 5-fluoro-UTP.

of nucleotide sugars can be enhanced directly or indirectly. All mechanisms may lead to an interaction with glycosylation of proteins, because the kinetics of glycosyltransferase reactions are dependent on the presence and local concentration of the corresponding nucleotide sugar [29]. The latter compounds can be divided into two classes on the basis of the type of nucleotide: (i) pyrimidine-derived nucleotide sugars, UDP-hexoses, UDP-*N*-acetylhexosamines and CMP-sialic acid, and (ii) purine-derived nucleotide sugars, GDP-mannose and GDP-fucose. Therefore, in this article the effects of pyrimidine and purine antimetabolites on nucleotide sugar formation will be treated separately. The reviewed effects of antimetabolites on glycosylation are summarized in Tables 2 and 3.

A. Nucleotide sugar formation

(i) *Effects of pyrimidine antimetabolites.* 5FU has been reported to interfere with the incorporation of sugars into glycoproteins in murine L1210 leukemia cells [37] and to disturb cellular wall mucocomplex synthesis in bacteria [38]. In the last decade the possible pathway of interference of 5FU in glycoconjugate metabolism has been studied in various cell systems such as L1210 [39], colon cancers [40], sarcoma 180 [41], melanoma cells [42], S49 cells [43–45], TA3 mammary tumor cells [46], hepatoma [47, 48] and isolated hepatocytes [48]. It appeared that substantial amounts of 5-fluoro-derivatives of UDP-hexoses and/or UDP-*N*-acetylhexosamines could be formed during incubation of the cells in the presence of FUR

[48, 49]. Also with the deoxynucleoside substrate, FdUR, the synthesis of a deoxynucleotide sugar, FdUDP-*N*-acetylglucosamine, could be demonstrated [50].

Inhibitors of pyrimidine *de novo* synthesis, such as PALA [45, 47] and 6-azauridine [45–47] can cause a depletion of the UTP pool with a concomitant sharp decrease of the total uridine nucleotide sugar pool. Such conditions favor the synthesis of

Fig. 1. Nucleotide-sugar-dependent reactions. The brackets embrace the group of nucleotide sugars that can participate in a certain metabolic route. Route a, asparagine-linked oligosaccharide structures; route b, serine- or threonine-linked oligosaccharide structures. Abbreviations used: Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Man, mannose; SA, sialic acid.

Table 2. Summary of literature on effects of fluoropyrimidines on glycosylation

Fluoro-pyrimidine	Cell type	Nucleotide pools*	Metabolic incorporation	Miscellaneous	Combination	Ref.
5 FU	L1210 leukemia		[³ H]Fuc ↓ , [³ H]GlcN ↑	cell surface hydrophobicity ↑		[37]
	L1210 leukemia		[³ H]Fuc ↑ , [³ H]GlcN ↑ , [³ H]Gal ↑ , [³ H]Man ↑			[78]
	<i>E. coli</i>			accumulation precursors cell wall mucocomplex		[38]
	L1210 leukemia	FUDP-sugars (25%)				[39]
	sarcoma 180	FUDP-sugars (16%)			guanosine doubles effect	[41]
	colon tumor	FUDP-Glc+				[40]
	colon	FUDP-Glc-				
	spleen	FUDP-Glc+				[43]
	S49 lymphoma	FUDP-sugars			uridine doubles effect	
	melanoma	FUDP-sugars (6–30%)			PALA no significant effect	[42]
	ovarian tumor			galactosyltransferase ↓		[70]
	TA3 mamma carcinoma	FUDP-HexNAc+			percentage dependent on addition of GlcN and/or 6-azauridine	[46]
FUR	rat hepatocytes	FUDP-Hex+ , FUDP- HexNAc+			thymine increases effect 2–6 times	[48]
	H35 hepatoma	FUDP-Hex+ , FUDP- HexNAc+				[48]
	S49 lymphoma	FUDP-GlcNAc+ , FUDP-GalNAc+				[44]
	Morris hepatoma	UDP-Glc ↓ , FUDP- Hex+ , FUDP- HexNAc+			in presence of GalN and 6-azauridine or PALA	[47]
	AS-30D hepatoma	FUDP-Hex (38%) , FUDP-HexNAc (16%)			in presence of GalN and 6-azauridine or PALA	[45]
FUDR	AS-30D hepatoma	FUDP-HexNAc+ more than FUDP-Hex+		FUDP sugars substrates for enzymes of UDP sugar metabolism		[49]
	TA3 mamma carcinoma	FUDP-HexNAc (6–58%)			percentage dependent on addition of GlcN and/or 6-azauridine	[46]
	lymphoblast	dUTP+ , dUDP- GlcNAc+ , FdUDP- GlcNAc+				[50]
FUDP-sugars	chick embryo microsomes			Glc-P-Dol formation same K_m and V_m for FUDP-Glc and UDP- Glc (GlcNAc) ₂ -PP-Dol formation $2K_m$ and $\frac{1}{3}V_m$ for FUDP-GlcNAc compared to UDP- GlcNAc		[69]

*Figures in parentheses are percentages of total fluoro-nucleotides formed. FUDP, 5-fluoro-UDP; Dol, dolichol; GlcN, glucosamine; GalN, galactosamine; Hex, hexoses; HexNAc, N-acetyl-hexoseamines. See for other abbreviations Table 1 and Fig. 1.

↑ increased; ↓ decreased; = not changed; + formed; – not detectable.

5FU-nucleotide sugars. The cytotoxic effect of the combination PALA and 5FU against rat hepatoma could be increased by D-galactosamine [47], which is also able to decrease UTP pools and to inhibit glycosylation. Another inhibitor of glycosylation, D-glucosamine [32–34], enhanced the cytotoxicity of 6-azauridine and 5FU against TA3 mammary tumor cells [46]. The addition of amino sugars induced a further depletion of the UTP pool, respectively, by formation and trapping of normally not occurring (F)UDP-galactosamine, and by a very large increase in the concentration of (F)UDP-N-acetylglucosamine and (F)UDP-N-acetylgalactosamine. The pools of other uridine nucleotide sugars were strongly decreased. In both studies it was postulated that the marked increase in 5-fluoro derivatives of the UDP-N-acetylhexosamines was related to the cytotoxic effects of 5FU. Co-incubation with other sugar analogs, D-galactosone [51] or D-glucosone [52], also resulted in trapping of uridine nucleotide sugar pools and increased incorporation of 5FU.

In addition to depletion, the concentration of uridine nucleotides can also be increased experimentally. In cancer chemotherapy, this is used to reverse cytotoxic effects of antimetabolites such as PALA [53] and 5FU [54–57], both in experimental systems [54, 55, 57] and patients [56]. Uridine is able to increase the UDP-glucose concentration in various tissues, including brain [58], and in cell culture [59]. It was shown that both in tumors and normal tissues uridine increased the pyrimidine nucleotide levels greatly [60]. For isolated rat hepatocytes, we could show that uridine also increased the levels of nucleotide sugars (Pels Rijcken WR, Peters GJ, Ferwerda W, unpublished results). On the other hand, in HT-29 cells uridine at 0.1 mM did not reverse the cytotoxic effects of D-glucosamine [59] (which involves depletion of the UTP pool via formation of UDP-N-acetylhexosamines with a concomitant inhibition or aberrant glycosylation of glycoproteins [32–34]). In rat C6 glioma cells the cytotoxic effect of 20 mM glucosamine was even enhanced by 2 mM uridine [61]. This supports the idea that changes in nucleotide sugar pools in itself can be responsible for the cytotoxic effects.

One study has been directed to the effects of antimetabolites which deplete CTP pools [62] and therefore might influence the formation of CMP-sialic acid. Indeed, in HL-60 cells the depletion of CTP by acivicin could be correlated with inhibition of the sialylation of cell surface glycoconjugates. Inhibition of sialylation was also exhibited by AraC and hydroxyurea. The effects of the antimetabolites on sialylation could be (partially) restored by the addition of cytidine to the culture media.

MTX can induce the formation of dUDP-N-acetylglucosamine [50]. Another deoxynucleotide sugar, dTDP-N-acetylglucosamine, could be synthesized in a cell-free system, but not in experiments *in vivo* [63]. No studies have appeared describing deoxynucleotide sugars as substrates in the glycosylation reactions.

(ii) *Effects of purine antimetabolites.* Purine antimetabolites mainly interfere with nucleotide sugar metabolism by depletion of guanine nucleotides. Inhibition of IMP-dehydrogenase by e.g. tiazofurin and mycophenolic acid leads to a reduction of GTP [64]. In sarcoma 180 cells these antimetabolites decreased the cellular concentration of both GDP-mannose and GDP-fucose, but exhibited no effect on e.g. UDP-N-acetylglucosamine. In the same cell system a decrease in the GDP-fucose pool was induced by 6-thioguanine *in vitro* but also *in vivo* after injection of the antimetabolite into the host animal [65].

Changes in cellular GDP-sugar concentrations have also been reported upon treatment of HL-60 cells with 6-thioguanine, tiazofurin and mycophenolic acid [66–68]. However, using a variant HL-60 cell line, deficient in hypoxanthine-guanine phosphoribosyl transferase, Sartorelli and co-workers showed that the depletion of guanine nucleotides induced by purine antimetabolites resulted in differentiation rather than direct cell death [66]. Moreover, they could show that the concomitant decrease in GDP-sugar concentration was related also to the differentiation process.

B. Effects of antimetabolites on glycosylation

(i) *Effects on glycosyltransferases.* Only a few studies describe a direct effect of FUDP-sugars on metabolic routes involved in the glycosylation process. FUDP-glucose, FUDP-galactose and FUDP-N-acetylglucosamine have been tested *in vitro* as substrates for glycosyltransferases in the glycosylation of glycoconjugates and for enzymes of the nucleotide sugar metabolism. Decreased product formation, in terms of V_{max} , was reported for several FUDP-sugars [49, 69]. Thus, FUDP-N-acetylglucosamine and FUDP-glucose were less efficient substrates than the natural UDP-sugars in the formation of lipid-linked precursors for the oligosaccharide chains of glycoproteins in *in vitro* studies with crude microsomal fractions of primary chick embryo cells [69]. A 50% reduction in product formation was observed with FUDP-N-acetylglucosamine as substrate instead of the natural UDP-sugar. The K_m for the enzyme was decreased, indicating that the FUDP-sugar exhibited a higher affinity than UDP-N-acetylglucosamine for the enzyme.

Direct inhibition of glycosyltransferases by 5FU does not seem likely to occur, since for galactosyltransferase inhibition was observed only in the millimolar concentrations range of 5FU [70]. In patients, peak levels in plasma are usually not higher than 0.5 mM [71], and decrease within a few hours to below the micromolar level. Furthermore, in colon tumors and healthy mucosa from 5FU treated patients the level of 5FU is not higher than 10 μ M [72]. Direct effects on glycosyltransferases by other antimetabolites, however, can occur since a competitive inhibition of serum sialyltransferase has been reported for AraC [73, 74].

(ii) *Effects on cell membrane glycoconjugates.* In some studies regarding the antimetabolite-induced changes in nucleotide sugar concentrations, accompanying alterations in the binding of lectins to cell-surface glycoproteins have been observed [62, 64, 75–77]. Thus decreased binding of Concanavalin A, specific for mannose-containing oligosaccharide structures, was observed in HL-60 and sarcoma 180 cells after treatment with guanine antimetabolites. Furthermore, different binding characteristics were observed for peanut and soybean agglutinins, lectins specific for exposed galactose and/or N-acetylgalactosamine residues, after incubation of desialylated HL-60 cells with AraC, 3-deazauridine, acivicin and hydroxyurea [62].

Both pyrimidine and purine antimetabolites have been reported to induce changes in the metabolic incorporation of radioactive sugars into cellular glycoproteins. In sarcoma 180 cells tiazofurin and mycophenolic acid inhibited the incorporation of both radioactive mannose and fucose into lipid-linked oligosaccharide precursors of cellular glycoproteins, which was accompanied by a decrease in binding of Concanavalin A to the cell surface [64]. Comparable alterations in membrane glycoprotein composition were observed when sarcoma 180 cells

Table 3. Summary of literature on effects of other antimetabolites on glycosylation

Antimetabolite	Cell type	Nucleotide pools	Metabolic incorporation	Lectin binding	Miscellaneous	Combination	Ref.
6 MMPR	sarcoma 180	GTP ↓, UTP ↑	[³ H]Man ↓, [³ H]GlcN=	Con A ↓		adenine counteracts guanosine enhances effects	[76]
6MP	HL-60 leukemia	GTP ↓	[³ H]Man ↓		differentiation	adenine counteracts	[81]
	L1210 leukemia		[³ H]Fuc ↑, [³ H]GlcN=, [³ H]Man=, [³ H]Gal=				[78]
6TG	sarcoma 180	GDP-Fuc ↓	[³ H]Fuc ↓, [³ H]GlcN=				[65]
	6TG resistant sarcoma 180		[³ H]Fuc=	Con A ↓, WGA=, RCA=			[75]
	L1210 leukemia		[³ H]Fuc=, [³ H]GlcN=, [³ H]Gal=, [³ H]Man=				[78]
MTX	lymphoblast	UTP=, UDP- GlcNAc=, dUTP+, dUDP-GlcNAc+					[50]
AraI	WI38 fibroblast		[¹⁴ C]SA ↓		sialyltransferase ↓, CMP-SA synthetase ↓ CMP-SA, hydrolase ↓		[74]
	HL-60 leukemia	CTP ↑	<i>de novo</i> synthesis SA ↓ and resialylation ↓	SBA ↑, PNA ↑			[62]
Ara-CTP	serum				sialyltransferase ↓		[73]
	WI38 fibroblast		[¹⁴ C]SA ↓		same effects as AraC		[74]
3-Deazauridine	HL-60 leukemia	CTP	<i>de novo</i>	PNA ↑			[62]
	L1210 leukemia	UDP-Hex ↑	synthesis SA= and resialylation ↓				[90]
Pyrazofurin	L1210 leukemia	UDP-Hex ↓					[90]
	colon tumor colon, spleen	UTP ↓, CTP ↓ UTP=, CTP=					[40]
Tiazofurin/ mycophenolic acid	sarcoma 180	GDP-Man ↓, GDP- Fuc ↓, UDP- GlcNAc=	[³ H]Man ↓, [³ H]Fuc ↓, [³ H]GlcN=	Con A ↓			[64]
	HL-60 leukemia		[³ H]Man ↓, [³ H]GlcN ↓	SBA ↑, PNA ↑	differentiation		[68]
Acivicin	HL-60 leukemia	CTP ↓	<i>de novo</i> synthesis SA ↓ and resialylation ↓	SBA ↑, PNA ↑			[62]
Hydroxyurea	HL-60 leukemia	CTP ↑	<i>de novo</i> synthesis SA ↓ and resialylation	SBA ↑, PNA ↑			[62]

↑ increased; ↓ decreased; = not changed; + formed; – not detectable. Con A, Concanavalin A; WGA, wheat germ agglutinin; RCA, Ricinus communis agglutinin; SBA, soy bean agglutinin; PNA, peanut agglutinin. See for other abbreviations Tables 1 and 2 and Fig. 1.

were incubated with other purine analogs, 6-thioguanine [65, 75] and 6-methylmercaptapurine riboside [76].

We have studied the effect of various pyrimidine and purine antimetabolites on the incorporation of four radioactive sugar precursors into the cellular glycoconjugates of leukemic mouse L1210 cells, using growth-limiting but non-lethal concentrations of the antimetabolites [78] (cf. Tables 2 and 3). 5FU induced an increase in the incorporation of [^3H]glucosamine, [^3H]galactose, [^3H]fucose, and [^3H]mannose. Mercaptopurine, had no effect on the [^3H]glucosamine [^3H]mannose and [^3H]galactose incorporation, but increased the incorporation of [^3H]fucose. Another purine analog, 6-thioguanine, did not have a significant effect at all. The effects of 5FU and mercaptopurine on the relative incorporation of the radioactive sugars most likely reflects changes in oligosaccharide structures. This conclusion was based on our finding that the changes in incorporation of the various radioactive sugars in the oligosaccharides was not a result of changes in the specific radioactivity of the corresponding nucleotide-sugar pools in the L1210 cells [78]. In an earlier study Kessel [37] also reported that 5FU induced an increase in the incorporation of [^3H]glucosamine into L1210 cells, but a decrease in the [^3H]fucose incorporation. This discrepancy compared with our result most probably can be explained by the limiting amount of glucose in the culture medium used in his study, which is known to affect the incorporation of sugars too [79, 80].

C. Relationship between antimetabolite-induced changes in glycosylation and differentiation or recruitment of cells

Krug *et al.* [59] have postulated that changes in nucleotide sugar metabolism have a causal relation with the onset of differentiation or growth inhibition of HT-29 cells. They reported that the induction of differentiation of these cells by glucosamine could not be prevented by restoring the glucosamine-depleted UTP level of the cells to normal values by adding uridine or cytidine, and, most importantly, that the glucosamine-induced change in the UDP-*N*-acetylglucosamine pool was not reduced. Furthermore, Sartorelli and co-workers have shown that, in HL-60 cells, purine antimetabolites not only deplete guanine nucleotides but are also able to induce differentiation [66, 68, 81]. Preceding differentiation, changes in nucleotide sugar levels and a marked reduction of incorporation of [^3H]mannose into glycoproteins and lipid-linked oligosaccharide precursors of glycoproteins occur. Changes in glycosylation of cellular and secreted glycoproteins have been reported to coincide with differentiation [23, 24, 26, 82–86]. It must be considered, therefore, that some of the antimetabolite-induced changes in glycosylation discussed in this article are a reflection of the onset of maturation of cells.

AraC and MTX have been reported to induce recruitment of quiescent tumor cells to the proliferative state [87–89]. Since it is assumed that the glycosylation changes during the cell cycle [91], it might be possible that the changes in glycosylation of the cells after exposure to these antimetabolites reflect a change in the distribution of the cells over the various phases of the cell cycle. Since AraC has been reported to be a competitive inhibitor for sialyltransferase [73, 74] the causal relationship between the AraC-induced change in glycosylation and recruitment and *visa versa* has to be further investigated.

CONCLUDING REMARKS

From the above-mentioned data it is clear that a wide range of antimetabolites—at low doses—are able to induce changes in

the glycosylation of glycoproteins. It is generally assumed that antimetabolites exert their cytotoxicity *via* their interference with RNA and/or DNA synthesis. Changes in glycosylation induced by antimetabolites might be an additional mechanism to cytotoxicity. It is of interest, therefore, to study the effects of antimetabolites on glycosylation not only of tumor cells but also of normal tissues, especially mucosal cells from the intestinal tract and bone marrow cells.

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