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Changes in Glycosylation of L1210 Cells After Exposure to Various Antimetabolites

Truus W. De Graaf, Sabrina S. Slot, Godefridus J. Peters and Willem Van Dijk

This study establishes that antimetabolites do have the potency to change cellular glycosylation, as was suggested in our previous review (*Eur J Cancer* 1990, 26, 516–523). Murine leukaemia L1210 cells were exposed to various antimetabolites under non-lethal conditions. The antimetabolites 5-fluorouracil (5FU), arabinofuranosylcytosine (AraC), methotrexate (MTX) and 6-mercaptopurine (6MP), but not 6-thioguanine, induced considerable changes in the metabolic incorporation of radioactively labelled monosaccharides. Each antimetabolite exhibited a different effect. Significant differences were found between the radioactivity incorporated from the monosaccharides glucosamine, fucose, mannose and galactose, relative to control values. Polyacrylamide gel electrophoresis indicated that changes were induced in the glycosylation of individual glycoproteins. 5FU, AraC, MTX and 6MP all influenced both pyrimidine- and purine-mediated sugar incorporation. This excludes, therefore, direct effects of the antimetabolites on their analogue nucleotide-sugars. The antimetabolite-induced changes in glycosylation did not directly correlate with the observed cell-cycle effects of the antimetabolites.
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INTRODUCTION

ANTIMETABOLITES ARE widely used in the treatment of neoplastic diseases. They are able to interfere with nucleotide metabolism, thus inhibiting RNA/DNA synthesis, which can lead to cell death. Various additional effects, possibly interrelated, have been reported: (i) induction of differentiation [1–3], (ii) blockade in a particular phase of the cell cycle [4–6], (iii) augmentation of the antigenic activity of glycoproteins [7], (iv) change of cellular nucleotide-sugar metabolism and/or glycoconjugate biosynthesis [8].

Changes in the glycosylation of cells occur during normal maturation and differentiation, but also during malignant development [9–11]. The evidence is compelling that cell–cell interactions are affected by changes in cell–surface glycosylation, e.g. during the processes of adhesion, invasion and metastasis [12–15].

Some examples of observed antimetabolite-induced changes in glycosylation [8] are: (i) altered glycosyltransferase activity [16], (ii) changed lectin binding [17], and (iii) altered incorporation of ^{3}H-labelled sugars [2, 3, 18, 19]. In the latter studies it was not excluded, however, that antimetabolite-induced changes in specific radioactivities of the incorporated label might have caused the alterations.

The mechanism of action by which antimetabolites can affect glycosylation is not completely understood [8]. Pyrimidine and purine antimetabolites could interfere with, respectively, the cellular pyrimidine and purine nucleotide-sugar pools (e.g. UDP-glucosamine and GDP-fucose), which are precursors for glycans. Furthermore, antimetabolite-induced changes in the cell-cycle distribution could be responsible for changes in cellular glycosylation [20, 21].

This study has been performed in order to investigate: (i)

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whether antimetabolites can indeed influence glycoconjugate synthesis, and if so, (ii) whether the mechanism(s) of action mentioned above are actually taking place. L1210 murine leukaemia cells were used as a model system, and were exposed to various antimetabolites under non-lethal conditions.

MATERIALS AND METHODS

Chemicals

D-[6-^{3}H]Glucosamine hydrochloride (GlcN, 1110 GBq/mmol), L-[6-^{3}H]fucose (Fuc, 740 GBq/mmol), D-[2-^{3}H]mannose (Man, 1110 GBq/mmol), D-[6-^{3}H]galactose (Gal, 2220 GBq/mmol), and *N*-acetyl-D-[6-^{3}H]mannosamine (ManNAc, 1110 GBq/mmol) were purchased from NEN (Boston, Massachusetts, U.S.A.), diluted with Hank's balanced salt solution (HBSS, GIBCO, Grand Island, New York, U.S.A.) to the desired concentrations, and stored at 4°C. D-[1-^{14}C]Glucosamine hydrochloride (GlcN, 2 GBq/mmol) was obtained from Amersham (Buckinghamshire, U.K.), and stored at −20°C. 5-Fluorouracil (5FU), 6-mercaptopurine monohydrate (6MP), and 6-thioguanine (6TG) were obtained from Sigma (St. Louis, Missouri, U.S.A.), methotrexate (MTX) from Lederle (Etten-Leur, The Netherlands), and 1-β-D-arabinofuranosylcytosine hydrochloride (AraC), from Mack (Illertissen, Germany). The antimetabolites were dissolved in HBSS or 0.04 mol/l NaOH [6-mercaptopurine (6MP) and 6-thioguanine (6TG)], diluted with HBSS to the desired concentrations, set at pH 7, and stored at −20°C until use. All other chemicals were of analytical quality and obtained from commercial sources.

Cell culture

The murine leukaemic cell line L1210 was maintained in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum, 20 mol/l Hepes (GIBCO), 2 mmol/l L-glutamine (Flow, Irvine, U.K.), and 60 mol/l mercaptoethanol (Fluka, Buchs, Switzerland). Cells were kept in an incubator at 37°C in a saturated humidified 5% CO_2 atmosphere and were passaged every 2–3 days. Routine testing for mycoplasma revealed no infections. Flat bottom 96-microwell plates (Greiner, Alphen a/d Rijn, The Netherlands) were used for the determination of effects of antimetabolites on cell growth and incorporation of radioactivity from radioactively labelled sugar precursors and leucine. In each experiment one plate was used for the determination of viability and growth inhibition of the cells, and another similarly composed plate was used for the metabolic labelling of the cells. Per well, 1–6 × 10^4 cells were seeded in a volume of 200 μl and antimetabolites and/or radioactively labelled precursors in HBSS were added in a volume of 10 μl (control cells received HBSS instead of antimetabolite solution). Each condition was assayed on both plates in quadruplicate and controls in 8- to 12-fold. Plates were covered with plate sealer (Flow) and were stored in the incubator until harvesting.

Growth inhibition

At the start and end of the incubation period, cell concentrations were determined with a Coulter counter model DN from Coulter Electronics (Bedfordshire, U.K.). The cell viability was determined by trypan blue exclusion. Percentage growth inhibition (GI) was calculated as follows:

$$GI(\%) = 100 - 100 \times [X_t(\text{exposed}) - X_0]/[X_t(\text{control}) - X_0].$$

X_t = cells/ml at end of incubation, X_0 = cells/ml at start of incubation.

Radioactivity incorporated into acid precipitates

Cellular glycoconjugates were labelled metabolically for 24 h with 3.7–37 kBq/well of ^{3}H-labelled monosaccharides or 37–74 kBq/well of [^{14}C]glucosamine. The incubation was arrested by addition of 50 μl ice-cold (4°C) 25% trichloroacetic acid (TCA) per well and keeping the plate at 4°C for 20 min. The acid-insoluble cell material was isolated on glass-fibre filters with a Titertek Cell Harvester (Flow), using distilled water for washing [22]. This rapid method gave comparable results to the elaborate conventional method of precipitating in tubes followed by three wash–centrifugation steps with 5% TCA to remove radioactively labelled sugar precursors. Radioactivity was assayed in a Beckmann Betaszint BF 8000 liquid scintillation counter after a period of 4 h solubilisation in 0.2 ml Soluene-100 (Packard, Downers Grove, Illinois, U.S.A.), followed by acidification with 0.1 ml 2 mol/l HCl, to reduce quenching, and addition of 8 ml Opti-fluor (Packard) per filter. Significance levels for differences from control values, and between treatments, were obtained using the Student's *t*-test.

Conversion of radioactive monosaccharides

The possibility that the radioactive monosaccharides were converted to other metabolites, leading to aberrant incorporation values, was checked by HPLC analysis of hydrolysates of acid precipitates from the various incubations. The contents of wells were transferred in duplicate to Eppendorf vials, and were precipitated with 5% ice-cold TCA (final concentration), followed by three wash–centrifugation steps with 5% TCA. Pellets were hydrolysed by the method of Bierhuizen *et al.* [23], and analysed by HPLC using a Lichrosorb.NH2 column (Merck, Darmstadt, Germany) on a Hewlett-Packard 1040A liquid chromatograph (Hewlett-Packard, Palo Alto, California, U.S.A.) with acetonitrile/KH_2PO_4 (85/15%) as eluent according to Blanken *et al.* [24]. Radioactivity was determined in 2-ml fractions after addition of 8 ml Opti-fluor. Pure monosaccharides were used as standards to determine retention times in parallel runs.

Specific radioactivity of nucleotide sugars

Cells were seeded in 24-well plates (Greiner), incubations were identical as described above for 96-well plates except for the volumes, which were five times larger. In order to determine radioactivity incorporated into cellular glycoconjugates, 100 μl aliquots were transferred to 96-well plates in quadruplicate and harvested as described above. Radioactively labelled cells from two pooled large wells were extracted with perchloric acid, and the nucleotide sugars separated by HPLC using a Partisphere Sax column (Whatman, Clifton, New Jersey, U.S.A.) on a Kratos Spectroflow liquid chromatograph (Kratos, Ramsey, New Jersey, U.S.A.) and a KH_2PO_4 gradient (5–500 mmol/l, pH 4.0–4.5) by the method of Pels Rijcken *et al.* [25]. Ultraviolet (UV) absorption was recorded continuously at 262 nm, and peak areas were quantitated by a Hitachi D-2000 chromato-integrator (Merck). Radioactivity in 0.5-ml fractions was determined after addition of 8 ml Opti-fluor. UV peaks were assigned according to retention times of pure standard solutions. Specific radioactivities were calculated by dividing the dpm by the area of the corresponding UV peak. Analyses were performed in triplicate.

DNA flow cytometry

Cells were fixed in methanol at 4°C, washed three times with PBS, treated with RNase (RNase A, which was made DNase-free by heating at 80°C for 10 min, Boehringer, Mannheim,

Germany), 5 Kunitz units per ml for 30 min at 37°C, washed once with phosphate buffered saline (PBS) and stained with propidium iodide (Sigma) 1 mg/ml PBS. DNA flow cytometry was performed with a FACStar plus (Becton Dickinson, Touson, Maryland, U.S.A.) at 488 nm. A scatter window was set to eliminate dead cells (usually about 5%).

Gel electrophoresis

Non- and [^{14}C]GlcN-labelled cells were washed three times with HBSS, and were solubilised in sodium dodecyl sulphate (SDS) buffer, 5 min at 95°C. Material from 1–10 × 10^5 cells was separated on 10% slab gels in the presence of β-mercaptoethanol, using piperazine di-acrylamide (BioRad, Veenendaal, The Netherlands) as a crosslinker [26]. Proteins in the non-labelled cell extracts were stained with BioRad silver stain. [^{14}C]GlcN-labelled glycoproteins in the cell extracts were detected by fluorography by the method of Bonner [27], using 25% isopropanol as a fixative, and impregnation with 2,5-di-phenyloxazole (Merck) for enhancement of the signal on Kodak X-OMAT XAR 2 film (Sigma). The relative intensity of the protein bands was determined by densitometry using a LKB 2222-020 UltroScan XL laser densitometer at 633 nm (Bromma, Sweden).

RESULTS

Metabolic labelling

The ^{3}H-labelled sugars GlcN, Man, Fuc and Gal were used as precursors in the metabolic labelling of cellular carbohydrates of L1210 cells. Label concentrations were not limiting since addition of 0.74–74 kBq of radioactive precursors per well resulted in a nearly linear increase in radioactivity incorporated per 10^6 cells (data not shown). At most, 3% of added ^{3}H-labelled monosaccharide was incorporated by the cells. The sialic acid precursor [^{3}H]ManNAc could not be used in these studies, since the incorporated radioactivity hardly exceeded background counts, even after addition of 37 kBq [^{3}H]ManNAc per well.

Cell growth and inhibition by antimetabolite

The effects of the antimetabolites on both growth and radioactive sugar incorporation varied highly with seeding cell density as is illustrated for 5FU and AraC in Table 1. Seeding cell density had to be below 6 × 10^4 cells/well in order to keep the control cells in exponential growth phase during the 24 h of incubation. Standard seeding density was 4 × 10^4 cells/well, because this was enough to ensure accurate and reproducible measurement of incorporated radioactivity. Antimetabolite concentrations were selected that induced maximal growth inhibition, without affecting viability by more than 5% (Table 2). Delayed toxicity was checked by transferring exposed cells to drug-free medium (4 × 10^4 cells/well). During culturing for another 24 h no significant changes in cell viability were detected by trypan blue exclusion. On the contrary, recovery from growth inhibition (a decrease in doubling time) was observed for the cells previously exposed to 5FU, MTX or AraC (unpublished results).

Effects of antimetabolites on radioactive incorporation into acid precipitates

Large changes from control values were found in the radioactivity incorporated per 10^6 cells, varying both with the antimetabolite and the ^{3}H-labelled monosaccharide used (Table 2). In 5FU- and AraC-treated cells the incorporated radioactivity per 10^6 cells varied by 1.5 to 2-fold for the various ^{3}H-labelled monosaccharides, as compared to control cells. MTX treatment increased the incorporation of radioactivity from [^{3}H]Man, [^{3}H]Fuc, and [^{3}H]Gal by about 1.6-fold compared to control values, but did not affect [^{3}H]GlcN incorporation. 6MP decreased the incorporation of radioactivity from [^{3}H]Man and [^{3}H]Gal to about 80% of the control, but increased [^{3}H]GlcN incorporation by 1.35-fold. 6TG treatment had no significant effect on the incorporation of [^{3}H]Man and [^{3}H]Gal.

Metabolic conversion of ^{3}H-labelled monosaccharides

HPLC analysis of hydrolysed TCA precipitates demonstrated that over 80% of the radioactivity therein was incorporated in the form of the added monosaccharide. [^{3}H]GlcN incorporated for over 85% in [^{3}H]*N*-acetylhexosamine (HexNAc). Five to nine per cent of added [^{3}H]Man was converted to [^{3}H]Fuc. None of the other sugars turned out to be converted to other monosaccarides. No significant differences were found between control and exposed cells.

Specific radioactivity of nucleotide sugars

In the cell, the administered radioactively labelled monosaccharides have to be metabolised to nucleotide sugars, the immediate precursors for glycans. In case an antimetabolite changes the specific radioactivity of these pools, the specific radioactivity of the incorporated sugar in glycan chains will change likewise, resulting in artificially altered incorporation values.

The specific radioactivity of both a purine and a pyrimidine nucleotide sugar were determined to either remain constant or diminish under the influence of antimetabolites (Table 3). This result shows that the antimetabolite-induced increase in radioactivity per 10^6 cells was due to increased incorporation of

Table 1. Effect of L1210 seeding cell density on growth inhibition by 5FU or AraC and concomitant incorporation of radioactivity from [^{3}H]GlcN into acid-precipitable material

× 10^4 cells/well at $t = 0$	Control doubling time (h)	Growth inhibition (%) 5FU	AraC	Incorporation of radioactivity per 10^6 cells Control (dpm ± 1 S.D.)	Incorporation of [^{3}H]GlcN (% of control ± 1 S.D.) 5FU	AraC
2.3	10.7	61	83	92242 ± 6457	144 ± 32	320 ± 58
4.6	12.1	69	79	89321 ± 9825	195* ± 13	206‡ ± 9
6.2	11.7	72	74	81905 ± 13103	206† ± 24	193§‖ ± 13

Values from a representative experiment. Blanks did not exceed 200 dpm per 10^6 cells. Changes were significant at the following levels: * 144–195, $P \leq 0.025$; † 144–206, $P \leq 0.05$; ‡ 320–206, $P \leq 0.005$; § 206–193, $P \leq 0.005$; ‖ 320–193, $P \leq 0.0025$.

Table 2. Effects of antimetabolites on the incorporation of ^{3}H-labelled monosaccharides into acid-precipitable material of L1210 cells

Antimetabolite	Initial concentration (μmol/l)	Growth inhibition (% ± 1 S.D.)	Relative incorporation of radioactive monosaccharides per 10^6 cells (% of control ± 1 S.D.) [^{3}H]Man	[^{3}H]Fuc	[^{3}H]GlcN	[^{3}H]Gal
5FU	1	74 ± 9	147 ± 18†	198 ± 44¶	188 ± 26§	155 ± 11‖
AraC	0.1	82 ± 9	193¶	211 ± 25¶	188 ± 23‡	177 ± 39‖
MTX	0.01	74 ± 6	162‖	163 ± 27¶	93 ± 12	156 ± 6‡
6TG	0.5	54 ± 7	118 ± 46	n.d.	110 ± 9	112
6MP	10	56 ± 7	85*	n.d.	135 ± 8‡	76*

The data shown (dpm/10^6 cells, % of control) are the mean of at least two experiments performed in quadruplicate. Viability did not decrease below 95%. Per treatment, the significance level of the experiment with the lowest significant difference from the control is given. * $P \leq 0.05$; † $P \leq 0.025$; ‡ $P \leq 0.01$; § $P \leq 0.005$; ‖ $P \leq 0.0025$; ¶ $P \leq 0.0005$; n.d. = not determined. In experiments where the effect of one antimetabolite on several ^{3}H-labelled sugars was determined simultaneously, significant differences between these sugar incorporations could be calculated. At a level of $P \leq 0.025$, the following differences were significant: for 5FU Man/Fuc and Man/GlcN; for AraC Man/Fuc; for MTX Fuc/GlcN and Gal/GlcN; for 6MP Man/Fuc.

Table 3. Effect of antimetabolites on the specific radioactivity of UDP-HexNAc and GDP-Fuc in L1210 cells

Antimetabolite	Relative incorporation of radioactivity from [^{3}H]GlcN (% of control ± 1 S.D.) dpm/10^6 cells	Specific radioactivity UDP-HexNAc (dpm/nmol)	Relative incorporation of radioactivity from [^{3}H]Fuc (% of control ± 1 S.D.) dpm/10^6 cells	Specific radioactivity GDP-Fuc (dpm/nmol)
Control	100 ± 19*	100 ± 10	100 ± 6*	100 ± 5
5FU	191 ± 23	67 ± 14	175 ± 35	87
AraC	207 ± 62	62 ± 5	235 ± 32	104 ± 8
MTX	93 ± 29	57 ± 7	170 ± 13	92
6MP	138 ± 25	73 ± 7	n.d.	n.d.

Data are from two typical experiments each performed in triplicate. * Radioactivities incorporated into TCA-precipitated control cells were 6706 ± 1274 dpm/10^6 cells for [^{3}H]GlcN and 5469 ± 328 dpm/10^6 cells for [^{3}H]Fuc. n.d. = not determined.

the labelled monosaccharides and not to changes in the specific radioactivity of the corresponding precursor pool. Under the conditions used, GDP-Fuc eluted as a single peak, but UDP-GlcNAc and UDP-GalNAc could not be separated; this last peak is further referred to as UDP-HexNAc.

Cell cycle effects and morphological changes

5FU and MTX arrested the cell cycle in the S-phase, AraC and 6TG in the G_2/M phase and 6MP did not significantly change the cell cycle distribution from control values, as was determined by DNA-flow cytometry (not shown). Furthermore, the antimetabolites, except for 6TG, induced morphological changes, such as increases in cell size, increased irregularities of the cell surface, and the appearance of vacuoles when compared to the control population.

Effects on the glycosylation of individual proteins

Fractionation of [^{14}C]GlcN-labelled proteins of control and antimetabolite-treated L1210 cells by SDS–PAGE revealed that almost all glycoproteins were recovered in the four molecular weight regions: 50–110, 110–155, 155–250 and > 250 Kd (Fig. 1). These four regions correspond to the four major peak areas of the radioactive densitographs present in all profiles. The peaks in the densitographs of the protein bands are well recognisable in the peaks and shoulders of the radioactive densitographs. The results obtained for the AraC- and MTX-exposed cells were basically similar to those depicted for 5FU (not shown). In order to approximate the occurrence of changes in glycosylation of individual glycoproteins, we determined the ratios of incorporated radioactivity per protein for the four molecular weight regions from the relative areas of the corresponding parts of the two densitographs (Table 4). Noticeable is the general increase of the carbohydrate/protein ratios in the 155–250 kD region after treatment with antimetabolites. Deviations from control ratios, however, were also found in the other regions in dependence of the antimetabolite used, even in case of 6TG, although no significant 6TG-induced changes were detected in TCA-precipitable material after metabolic labelling (Table 2). This can be explained partly by the increased percentage of non-radioactively labelled protein in 6TG-treated cells (28 vs. 18%). The results clearly show that after antimetabolite treatment the amount of radioactivity incorporated in the various proteins is

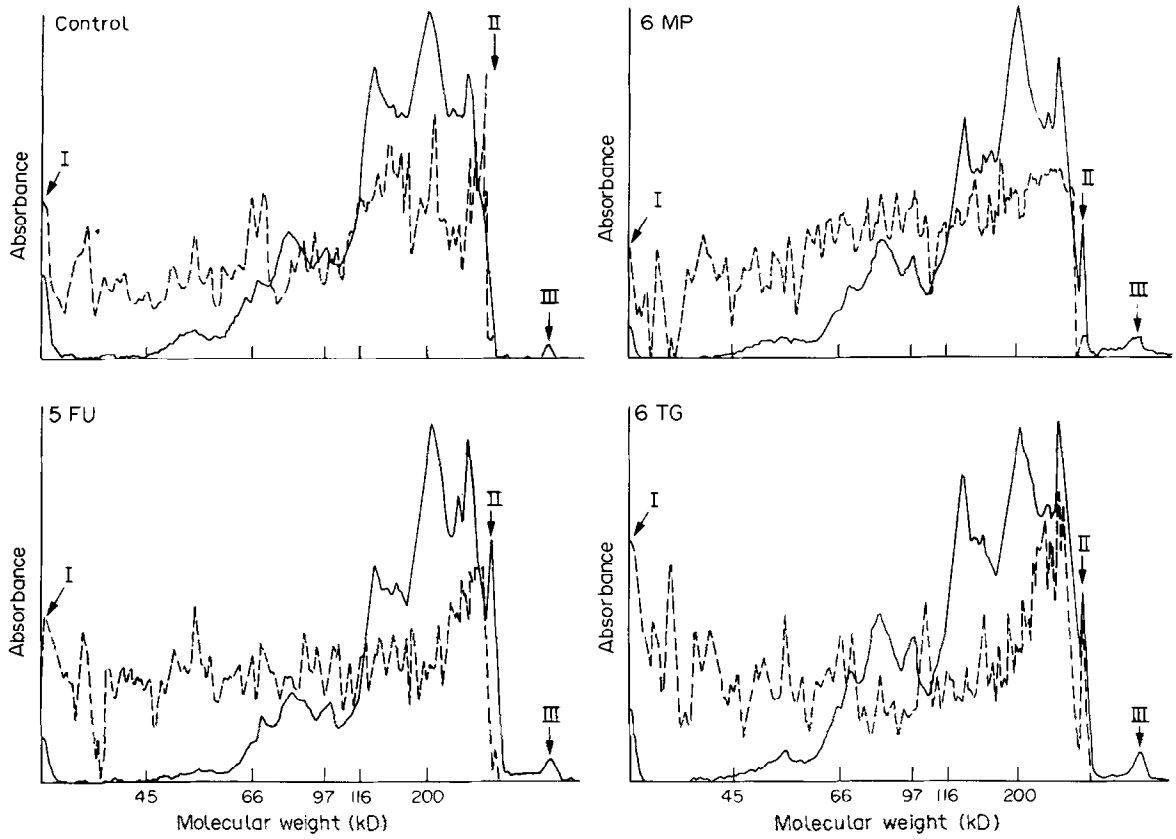

Fig. 1. Densitographs of SDS–PAGE gels of (glyco)proteins of control and antimetabolite-treated L1210 cells. Glycoproteins are identified by the densitographs of fluorographs of SDS–PAGE gels containing [^{14}C]GlcN-labelled cell extracts (full lines). The broken lines represent the densitographs of gels stained for protein. Care was taken that the gels for the fluorographs contained equal amounts of radioactivity and that the gels for the protein stain contained equal amounts of protein in each lane. Positions I, II and III indicate, respectively, the front, the top of the running gel and the top of the stacking gel.

Table 4. Protein-bound [^{14}C]GlcN to protein ratios in various molecular weight regions of cell extracts from control and antimetabolite-treated L1210 cells

Treatment	Ratio of relative surface areas in molecular weight regions of the densitographs of protein-bound [^{14}C]GlcN and protein (Fig. 1)*			
	50–110 kD	110–155 kD	155–250 kD	> 250 kD
Control	0.8	1.5	1.8	1.6
5FU	0.5	1.8	3.2	2.1
AraC	0.6	2.0	2.7	1.9
MTX	0.7	1.8	2.4	1.5
6MP	0.9	1.7	2.3	1.7
6TG	1.5	2.4	2.1	1.5

* Surface areas within the molecular weight regions were expressed as percentages of the total surface area under each densitograph in Fig. 1.

different from the control. Taken together these observations indicate that the exposure of L1210 cells to the various antimetabolites, under non-lethal conditions, has resulted in changes in the glycosylation of individual glycoproteins. Furthermore, these conditions apparently induce alterations in the relative occurrence of several (glyco)proteins, since the protein patterns also appeared to be affected by the treatment of the cells with antimetabolites.

DISCUSSION

This study shows that under non-lethal conditions various antimetabolites can induce changes in the glycosylation of glycoproteins of murine L1210 leukaemia cells. The effect is dependent on the antimetabolite, the radioactively labelled sugar precursor and the seeding cell density. The changes inflicted upon L1210 cells by antimetabolites do not only show on a molecular level, but are also reflected in changes of the appearance of the cells, enforcing the suggestion of concomitant changes in cell function.

The observed changes in incorporation of ^{3}H-labelled monosaccharides after exposure to 5FU, AraC, MTX and 6MP reflect changes in cellular glycosylation for the following arguments: (i) when the effect per antimetabolite is evaluated, there are significant differences in the percentages of label incorporation, relative to control values, between the various monosaccharides used (Table 2). In case of an aselective stimulatory effect on protein synthesis the ratio between these percentages would have been approximately one. (ii) Changes in the specific radioactivities of the nucleotide sugars UDP-*N*-acetylhexosamine (HexNAc) and GDP-Fuc, were not responsible for the effects of the antimetabolites on the incorporation of [^{3}H]GlcN and [^{3}H]Fuc, respectively. On the contrary, in most cases the specific radioactivity of the nucleotide sugar was lower than in control cells, leading even to an underestimation of the antimetabolite-induced increase in incorporation of the corresponding sugar into the cells. (iii) Metabolic conversion of radioactive label

could be excluded as a major source of difference, since incorporation of radioactivity into other sugars appeared to be minimal. (iv) Molecular changes in glycosylation were indicated to occur in individual glycoproteins, as determined by SDS–PAGE (Fig. 1, Table 4).

Our findings further substantiate indications obtained by several other groups, in *in vitro* and *in vivo* studies, that antimetabolites can influence the glycosylation of cells [8]. Kessel [18] reported 5FU-induced changes in metabolic incorporation of radioactive sugars in L1210 cells, and also observed a 2-fold increase in [^{3}H]GlcN incorporation. However, in contrast to our results, a 30% reduction was reported in incorporated radioactivity from [^{3}H]Fuc. This inconsistency might be the result of the use by Kessel of a glucose-free medium [11] in combination with a lower concentration (0.1 μmol/l) of 5FU, and a lower seeding cell density (1.3×10^5 cells/ml).

Complex mechanisms of action have to be involved in the antimetabolite-induced changes in glycosylation. Direct effects of the antimetabolites on their analogue nucleotide sugars are not very likely as the pyrimidine analogues 5FU and AraC and the purine analogue 6MP influenced both the incorporation of [^{3}H]Gal and of [^{3}H]Man. [^{3}H]Gal is incorporated via the pyrimidine nucleotide UDP and [^{3}H]Man via the purine nucleotide GDP. The observed changes in distribution of the cell population over the phases of the cell cycle can not explain the majority of the changes in incorporation of radioactively labelled monosaccharides. 6MP did not affect the cell cycle pattern, but did change the incorporation of labelled monosaccharides. On the contrary, 6TG did induce a G_2/M phase arrest but no changes in label incorporation from Gal and Man. AraC also induced a G_2/M phase arrest and did affect label incorporation from all monosaccharides used. Fietkau *et al.* [28] have reported that AraC introduced a block in L1210 cells at the G_1/S boundary. However, they studied the drug *in vivo*, using high doses that caused over 50% cell death. For MTX and 6TG the observed cell cycle effects are in accordance with the literature [4, 29].

In chemotherapy with antimetabolites many target and non-target cells will be exposed to similar concentrations as those used in these studies. Therefore, it is tempting to speculate that the cell surface glycosylation of those cells will be affected correspondingly to the changes found for the L1210 leukaemia cells. Phenotypical changes in glycosylation are of importance for processes like adhesion, invasion and metastasis [12–15]. Further studies are needed to investigate whether *in vivo* antimetabolites actually induce such changes in the phenotype of normal or tumour cells, and whether that process contributes to the side-effects of this type of therapy.

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