5'-Cholesteryl-phosphorothioate oligodeoxynucleotides: potent inhibition of methotrexate transport and antagonism of methotrexate toxicity in cells containing the reduced-folate carrier

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ABSTRACT

Polyanionic 5'-cholesteryl-phosphorothioate oligodeoxynucleotides of varying polymer length and nucleobase composition were examined for an effect on methotrexate transport via the reduced-folate carrier of L1210 mouse cells. Methotrexate transport was inhibited by each of the oligodeoxynucleotide analogs tested. Inhibition was most pronounced ($IC_{50} = 0.21 \mu M$, standard assay) for a 5'-cholesteryl heteropolymer consisting of 15 phosphorothioate deoxynucleotides with alternating deoxycytosine and deoxyadenosine (Chol-PS-d(CA)₇C). Homopolymers with 15 deoxycytosine (Chol-PS-dC₁₅) or deoxythymidine (Chol-PS-dT₁₅) residues were ~2-fold less inhibitory than Chol-PS-d(CA)₇C. The relative potency of transport inhibition by deoxycytosine oligomers of varying length was: Chol-PS-dC₅ > Chol-PS-dC₁₅ > Chol-PS-dC₂₈ > Chol-PS-dC₃. Substantial inhibition was retained in cells preincubated with inhibitors and washed prior to transport determinations and the inhibitor sensitivity could be increased substantially by reducing the concentration of cells. Mixed competitive and non-competitive inhibition was observed for each analog. In standard high-folate medium, Chol-PS-oligodeoxynucleotides (5.0 μM) had minimal effects on the growth of L1210 cells, but antagonized the cytotoxicity of methotrexate. The response to methotrexate ($IC_{50} = 12 \text{ nM}$) decreased to the greatest extent (20.8-fold) in the presence of Chol-PS-d(CA)₇C ($IC_{50} = 250 \text{ nM}$). Under limiting folate conditions, Chol-PS-d(CA)₇C alone inhibited cells growth by a process which could be reversed by folic acid. The results show that Chol-PS-oligodeoxynucleotides are among the most potent known inhibitors of the reduced-folate carrier. Direct growth inhibition of folate-deficient cells and antagonism of methotrexate cytotoxicity indicate that Chol-PS-oligodeoxynucleo-

tides retain the ability to inhibit the reduced-folate carrier for several days in cultured cells.

INTRODUCTION

Substantial evidence now exists to suggest that oligodeoxynucleotides can, in a sequence-specific manner, inhibit the translation of mRNA into protein (for a review, see ref. 1). However, phosphodiester (PO) oligonucleotides are nuclease-sensitive and are extensively degraded both intracellularly and extracellularly. In contrast, phosphorothioate (PS) oligodeoxynucleotides, which contain a sulfur atom substituted for oxygen at a non-bridging position at each phosphorus atom, are significantly more nuclease resistant (2). Constructs containing the PS linkage have also been utilized successfully as antisense inhibitors of genetic expression (3,4). Phosphodiester and PS oligonucleotides appear to be internalized by cells predominantly via a process of adsorptive endocytosis and pinocytosis (5). Subsequent to uptake, both PO and PS oligodeoxynucleotides come to reside in vesicular structures, probably endosomes and/or lysosomes.

5'-Cholesteryl-modified PS-oligodeoxynucleotides were first synthesized by Letsinger *et al.* (6) and shown to be more active anti-HIV agents than their unmodified congeners (6,7). Subsequently, it was discovered that the 5'-cholesteryl modification was able to increase the intracellular accumulation of oligodeoxynucleotides. The latter occurred by two separate mechanisms: (i) increased adsorptive endocytosis by direct insertion of the cholesteryl moiety into the cell membrane (8); and (ii) binding to low-density lipoprotein (LDL) and internalization via the LDL receptor (8,9). Cholesteryl oligodeoxynucleotides have been suggested to be more potent antisense effectors than their unmodified congeners (8), but unexpected secondary effects on cells have also been observed. For example, Chol-PO-oligodeoxynucleotides activate a cell membrane calcium channel in HL60 cells, which causes a significant increase in intracellular Ca^{++} (10).

The reduced-folate carrier is a major influx route for folate and antifolate compounds in various cultured tumor cells (11-13).

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Substrates for this system include the antifolate methotrexate, which upon cell entry binds to dihydrofolate reductase and inhibits cell growth by disrupting one-carbon metabolism. The reduced-folate carrier has been shown to exhibit unusual responses to anions. Chloride, bicarbonate and various other anions are competitive inhibitors of methotrexate transport (14), non-folate anions such as phthalate, phosphate and sulfate are also transported by this system (12,15–17) and anion gradients are the likely driving force for the concentrative uptake of substrates (12,15). These findings support the hypothesis that the reduced-folate carrier mediates an electro-neutral exchange of various anions, and that folate compounds are preferred substrates by virtue of their relatively high binding affinity.

Suramin is a hexaanionic polyarylsulfonate which binds competitively and has a high affinity for the reduced-folate carrier of L1210 cells, but is not transported at an appreciable rate (18). A comparison of structural analogs of suramin indicated that binding diminishes substantially when the large size or multianionic composition of suramin is reduced. These results suggested that other multianionic aromatic compounds such as polynucleotides might also act as inhibitors of this system. In the present study, PS-oligodeoxynucleotides were examined for the ability to inhibit methotrexate transport via the reduced-folate carrier of L1210 cells. A PS-oligodeoxynucleotide of 28 deoxycytidine units was shown to have little effect on methotrexate transport, but potent inhibition was observed with various 5'-cholesteryl-modified PS-oligodeoxynucleotides of varying length and nucleobase composition. Chol-PS-oligodeoxynucleotides also antagonize the toxicity of methotrexate and inhibit cell growth in folate-deficient medium and hence are stable and persistent transport inhibitors which could cause folate deficiency in cells which rely on the reduced-folate carrier for the uptake of folates.

MATERIALS AND METHODS

Chemicals

[3',5',9-³H]Methotrexate (20 Ci/mmol) (Moravak Biochemicals) was purified and stored as described previously (19). Unlabeled methotrexate, (R,S) 5-formyltetrahydrofolate (calcium salt) and HEPES were obtained from Sigma Chemical Co. PS-dC₂₈ (5) and Chol-PS-oligodeoxynucleotides (8) were synthesized and purified by procedures described previously. Stock solutions of Chol-PS-oligodeoxynucleotides (stored at –20°C) were incubated for 1 min at 37°C and then mixed vigorously prior to use.

Cells

L1210 mouse leukemia cells were grown in 500 ml portions of RPMI 1640 medium (Gibco) containing 3% fetal bovine serum (Gibco) and 100 units each of penicillin and streptomycin in sealed 1000 ml flasks in a shaking 37°C incubator. Cells (at a density of 0.9–1.6 × 10⁶/ml) were chilled to 4°C, collected by centrifugation at 4°C (500 g), and washed twice with 50 ml of HBS (20 mM HEPES, 140 mM NaCl, 5 mM KCl, 2 mM MgCl₂, pH 7.35 with NaOH). The final suspension (in HBS) was adjusted to 2.2 ± 0.2 × 10⁷ cells/ml. Protein concentrations were determined by the Biuret method (20) using bovine serum albumin as the standard. The protein content of 10⁷ L1210 cells is ~1.6 mg.

Transport measurements

Methotrexate transport in the standard assay was measured in duplicate samples containing 2.0 ± 0.2 × 10⁷ L1210 cells, 5.0 μM [³H]methotrexate (100 000 c.p.m./nmol), the desired additions and HBS in a final volume of 1.0 ml. Assay mixtures were combined (at 0°C) in 16 × 100 mm glass tubes and the cells were added last. After incubation for 3 min at 37°C, the cells were diluted with 7 ml of ice-cold saline (1 mM Na-phosphate and 160 mM NaCl, pH 7.4 with NaOH), recovered by centrifugation at 1000 g (5 min at 4°C), washed with 5 ml of ice-cold saline, resuspended in 0.5 ml saline, transferred to vials with the aid of two 4 ml portions of ScintiVerse (Fisher) and analyzed for radioactivity. Uptake at 0°C served as the control. Methotrexate transport in the dilute cell assay was performed similarly, except that samples contained 2.0 ± 0.2 × 10⁷ cells, 5.0 μM [³H]methotrexate, the desired additions and HBS in a final volume of 3.0 ml. Results were derived from the combination of two or more separate experiments.

Growth studies

The growth response of L1210 cells to methotrexate and the antagonistic effects by Chol-PS-oligodeoxynucleotides were determined in 96-well plates by the procedure of Mosmann (21). Wells contained initially 1000 cells and the desired additions in 150 μl of RPMI 1640 medium containing 5% fetal bovine serum and antibiotics. Chol-PS-oligodeoxynucleotides were added from stock solutions (500 μM) containing 50% ethanol. Plates were developed after 4 days (50–80% confluency) using a tetrazolium dye assay and were quantitated in a plate reader at 570 nm. Data points represent the mean of at least two separate experiments performed in duplicate.

The effect of Chol-PS-oligodeoxynucleotides on the growth of L1210 cells in limiting-folate medium was determined similarly, except that folate-free RPMI medium (Gibco) was employed and 5 nM (R,S) 5-formyltetrahydrofolate was added as the source of folate. Intracellular folates were depleted by growing the cells in limiting folate medium for at least 12 generations prior to use. The results are the mean of quadruplicate determinations.

RESULTS

Effect of Chol-PS-oligodeoxynucleotides on methotrexate transport in L1210 cells

Various chol-PS-oligodeoxynucleotides were synthesized and examined for an effect on methotrexate transport via the reduced-folate carrier of L1210 cells. This transport system is present at a high activity and accounts for nearly all uptake of methotrexate in L1210 cells (12,19,22). The time dependence for the uptake of [³H]methotrexate at 37°C using our standard assay is illustrated in Figure 1 for control cells (no addition), and cells with added Chol-PS-deoxycytosine oligomers of varying length. Each of these compounds substantially reduced the rate of methotrexate uptake and the onset of inhibition occurred rapidly since inhibition was independent of incubation time. Inhibitor effectiveness increased with decreasing chain length from 28 to 15 to 5 deoxycytosine residues.

The potency and extent of inhibition by each of the various 5'-cholesteryl derivatives was investigated by measuring methotrexate transport at various inhibitor concentrations. Results with five of these compounds are shown in Figure 2. Cholesteryl

Figure 1. Time dependence for the uptake of [3 H]methotrexate by L1210 cells in the absence and presence of Chol-PS-oligodeoxynucleotides. C5, 1.0 μ M Chol-PS-dC₅; C15, 1.0 μ M Chol-PS-dC₁₅; C28, 1.0 μ M Chol-PS-dC₂₈. Standard assay; [3 H]methotrexate concentration, 5.0 μ M.

oligomers of deoxycytosine with 3, 5, 15 and 28 residues are compared with the 15mer Chol-PS-d(CA)₇C. The most potent inhibitor in the deoxycytosine series was Chol-PS-dC₅, followed by compounds with 15, 28 and 3 deoxycytosines respectively. In each case, the extent of inhibition was complete or nearly complete at high concentrations of inhibitor. The most potent inhibitor overall was Chol-PS-d(CA)₇C, which exhibited an IC₅₀ value of 0.21 μ M. IC₅₀ values for inhibition by the various cholesteryl derivatives are listed in Table 1. Inhibition studies were also performed with a PS-oligodeoxynucleotide (PS-dC₂₈) lacking the 5'-cholesteryl modification. PS-dC₂₈ produced a relatively weak inhibition which reached only 35% at a concentration of 20 μ M.

Table 1. Relative effectiveness of Chol-PS-oligodeoxynucleotides as inhibitors of methotrexate transport under standard and diluted-cell conditions

Inhibitor	IC ₅₀ for half-maximal inhibition (μ M)	
	Standard assay	Diluted-cell assay
Chol-PS-dC ₃	2.2	0.65
Chol-PS-dC ₅	0.30	0.12
Chol-PS-dC ₁₅	0.52	0.22
Chol-PS-dC ₂₈	1.1	0.45
Chol-PS-d(CA) ₇ C	0.21	0.072
Chol-PS-dT ₁₅	0.45	0.29

IC₅₀ values were determined from plots of transport versus inhibitor concentration (see Fig. 2). The standard and diluted-cell assay varied only in the concentration of cells. The standard assay contained 2.0×10^7 cells, varying concentrations of inhibitor and 5.0 μ M [3 H]methotrexate in 1.0 ml buffer, whereas the diluted-cell assay contained inhibitor, 5.0 μ M [3 H]methotrexate and the same number of cells in 3.0 ml buffer. Transport in control samples without inhibitor (9 ± 1 pmol/min/mg protein) was the same in both assay systems.

Figure 2. Response of [3 H]methotrexate transport in L1210 cells to varying concentrations of Chol-PS-oligodeoxynucleotides. C3, Chol-PS-dC₃; C5, Chol-PS-dC₅; C15, Chol-PS-dC₁₅; C28, Chol-PS-dC₂₈; CA15, Chol-PS-d(CA)₇C. Standard assay; [3 H]methotrexate concentration, 5.0 μ M.

Table 2. Effect of exposure conditions on the sensitivity of methotrexate transport to inhibition by Chol-PS-dC₁₅ and Chol-PS-d(CA)₇C

Inhibitor	IC ₅₀ for half-maximal transport inhibition (μ M)		
	Standard assay	Preincubated	Washed
Chol-PS-dC ₁₅	0.52	0.60	0.76
Chol-PS-d(CA) ₇ C	0.21	0.23	0.20

IC₅₀ values for half-maximal transport inhibition by Chol-PS-dC₁₅ and Chol-PS-d(CA)₇C were determined under standard assay conditions (see Fig. 2): in cells with no treatment (Standard Assay); in cells preincubated with inhibitor for 5 min at 37°C prior to substrate addition and transport determination (Preincubated); or in cells preincubated with inhibitor for 5 min at 37°C, diluted with 7 ml of HBS, recovered by centrifugation, resuspended in 1.0 ml HBS containing 5.0 μ M [3 H]methotrexate and assayed for transport by the standard procedure (Washed).

The inhibition of methotrexate transport by Chol-PS-oligodeoxynucleotides was investigated further by varying the assay conditions. In a diluted assay in which the cell concentration was reduced 3-fold, the ability of Chol-PS-oligodeoxynucleotides to inhibit methotrexate transport increased substantially (Table 1). The extent of this increase was in the range of 2- to 3-fold. In the standard assay, preincubation of the cells with varying concentrations of Chol-PS-dC₁₅ or Chol-PS-d(CA)₇C prior to substrate addition had little or no effect on the IC₅₀ for half-maximal inhibition and a wash step after preincubation of the cells with inhibitor produced only a small reduction (1.5-fold) in transport inhibition by Chol-PS-dC₁₅ and had no effect on inhibition by Chol-PS-d(CA)₇C (Table 2).

The kinetics of transport inhibition were investigated by performing influx measurements at constant inhibitor levels and varying concentrations of [3 H]methotrexate. Double-reciprocal plots of these data are shown in Figure 3 for control cells and cells

Figure 3. Double-reciprocal plot of transport versus [^3H]methotrexate concentration in the absence and presence of Chol-PS-oligodeoxynucleotides. C5, 0.25 μM Chol-PS-dC₅; C15, 1.0 μM Chol-PS-dC₁₅; C28, 1.0 μM Chol-PS-dC₂₈. Transport units, pmol/min/mg protein. Standard assay.

containing a fixed concentration of three inhibitors. In the control, the calculated K_m for half-maximal influx (4.6 μM) and the $V_{\max}$ (17 pmol/min/mg protein) were comparable to previous values reported in the same buffer (23). Data derived in the presence of Chol-PS-dC₅ (0.25 μM), Chol-PS-dC₁₅ (1.0 μM) or Chol-PS-dC₂₈ (1.0 μM) produced linear responses and extrapolated lines which intercepted the x- and y-axes at points different from the control. The positions of these intercepts gave both a decreased $V_{\max}$ and an increased K_m for each compound and hence were indicative of mixed competitive and non-competitive kinetics. The same results were obtained with Chol-PS-dC₃, Chol-PS-d(CA)₇C and Chol-PS-dT₁₅ (data not shown).

Effect of Chol-PS-oligodeoxynucleotides on methotrexate toxicity in L1210 cells

The potent inhibition of methotrexate transport by Chol-PS-oligodeoxynucleotides suggested that the latter compounds might compromise the toxicity of methotrexate by inhibiting drug entry. Growth studies were therefore performed in the standard medium (containing 2.2 μM folate) in which the toxicity of L1210 cells to methotrexate was measured over a 4 day period in the presence of a fixed concentration (5.0 μM) of each of the cholesteryl derivatives. Methotrexate toxicity in control cells ($\text{IC}_{50} = 12 \text{ nM}$) was decreased by Chol-PS-dC₁₅ and Chol-PS-d(CA)₇C (Fig. 4) and by each of the other Chol-PS-oligodeoxynucleotides (Table 3). Toxicity shifted to the greatest extent (20.8-fold) in the presence of Chol-PS-d(CA)₇C (Fig. 4), the most potent transport inhibitor (Table 1), whereas the smallest effects were observed with Chol-PS-dC₃ and Chol-PS-dC₂₈, the least active transport inhibitors. Chol-PS-oligodeoxynucleotides added alone (at 5.0 μM) produced minimal effects on cell growth (Table 3).

Figure 4. Growth response of L1210 cells to varying concentrations of methotrexate in the absence and presence of 5.0 μM Chol-PS-dC₁₅ (C15) or Chol-PS-d(CA)₇C (CA15).

Effect of Chol-PS-oligodeoxynucleotides on the growth of L1210 cells in folate-deficient medium

The combination of transport inhibition from influx measurements and antagonism of methotrexate toxicity from growth experiments indicated that Chol-PS-oligodeoxynucleotides are potent and persistent inhibitors of the reduced-folate carrier. A continuous block of the reduced-folate carrier should also lead to folate deficiency, but L1210 cells grown in standard medium (2.2 μM folate) contain substantial amounts of stored folates which would support growth for several generations subsequent to a block in transport. Moreover, L1210 cells contain a second transport system which alone is able to fulfill the cellular folate requirements at high external folate concentrations (19). Therefore, it was not unexpected that Chol-PS-oligodeoxynucleotides had little effect on cell growth in the standard medium (Table 3).

Table 3. Effect of Chol-PS-oligodeoxynucleotides on the toxicity of methotrexate in L1210 cells

Inhibitor	Growth inhibition (%)	IC_{50} for methotrexate (nM)	Reduction in toxicity (-fold)
None	0	12	1.0
Chol-PS-dC ₃	3	16	1.3
Chol-PS-dC ₅	2	36	3.0
Chol-PS-dC ₁₅	2	38	3.2
Chol-PS-dC ₂₈	7	23	1.9
Chol-PS-d(CA) ₇ C	7	250	20.8
Chol-PS-dT ₁₅	13	24	2.0

Relative growth responses and IC_{50} values for half-maximal growth inhibition by methotrexate were determined in 96-well plates inoculated with a constant level of cells (1000), the indicated Chol-PS-oligodeoxynucleotide (5.0 μM) and varying concentrations (2–1000 nM) of methotrexate.

L1210 cells adapted to limiting concentrations of folate became sensitive to growth inhibition by Chol-PS-d(CA)₇C (Table 4). Inhibition by 5.0 μ M Chol-PS-d(CA)₇C was the highest (76%) in cells incubated for the shortest time interval (68 h). Thereafter, inhibition declined gradually to 70 and 41% (at 92 and 116 h respectively). Reversal of growth inhibition could be achieved at each time interval by the addition of 2.2 μ M folate.

Table 4. Effect of Chol-PS-d(CA)₇C on the growth of L1210 cells in folate-deficient medium

Addition	Relative growth (% of control)		
	68 h	92 h	116 h
None	100	100	100
Chol-PS-d(CA) ₇ C	24	30	59
Chol-S-d(CA) ₇ C + folic acid	68	71	85

Relative growth responses were measured in limiting folate medium using 96-well plates as described in the Materials and Methods. Cells were adapted to folate-free RPMI-1640 medium containing 5 nM 5-formyltetrahydrofolate, added at 3000, 1000 or 400 per well in the presence of the indicated additions and incubated for the indicated times (h) at 37°C in a CO₂ incubator. Chol-PS-d(CA)₇C, 5.0 μ M; folic acid, 2.2 μ M.

DISCUSSION

5'-Cholesteryl-PS-oligodeoxynucleotides represent a new and potentially useful class of modified PS-oligonucleotides. Because of several possible mechanisms of internalization (8,9), these compounds have improved potential for intracellular and intracytoplasmic delivery. However, oligonucleotides can bind non-specifically to cells (24,25) and this binding is enhanced by modification with cholesterol (8). The significance of non-specific binding is not clearly understood, but an increase in the intracellular concentration of Ca⁺⁺ is clearly one of the consequences of non-sequence specific binding of Chol-PO (but not Chol-PS) homopolymers to the surface of HL60 cells (10). Chol-PS-oligodeoxynucleotides are also potent, non-sequence specific inhibitors of HIV-1 replication (7). The latter inhibition may occur at the level of the HIV-1 reverse transcriptase, but increased binding to the cell surface could also be a factor, since Chol-PS-oligodeoxynucleotides are, on a length-for-length basis, more potent inhibitors of HIV-1 induced syncytia formation than are unmodified congeners (7).

The reduced-folate carrier from L1210 cells exhibits a high sensitivity to inhibition by Chol-PS-oligodeoxynucleotides. Anionic charge had been recognized in prior inhibitor studies (14) as a primary determinant for inhibitors of this transport system, but additional structural features affect transport inhibition by Chol-PS-oligodeoxynucleotides. The cholesteryl portion of these inhibitors is a dominant binding element since an oligodeoxynucleotide lacking the cholesteryl group (PS-dC₂₈) is a relatively poor transport inhibitor. Polydeoxynucleotide length also contributes significantly to binding since inhibition in a series of Chol-PS-oligodeoxycytosine compounds differed up to 10-fold with variations from 3 to 28 deoxynucleotides (Table 1). Inhibitor potency was not proportional to polymer length since Chol-PS-

dC₅, the most effective inhibitor in the deoxycytosine series, was more potent than shorter (Chol-PS-dC₃) or longer (Chol-PS-dC₁₅) homopolymers. Nucleobase composition had the least effect on binding (2- to 3-fold), as judged by a comparison of affinity for Chol-PS-d(CA)₇C and two other oligomers of the same nucleotide length (Chol-PS-dC₁₅ and Chol-PS-dT₁₅) (Table 1). The present results contrast with the effect of Chol-PS-oligonucleotides on HIV-1 reverse transcriptase activity and induced syncytia formation, which increase with increasing oligomer length (7).

The low IC₅₀ values for Chol-PS-d(CA)₇C in the standard assay (0.21 μ M) and the dilute-cell assay (0.072 μ M) define an inhibitor with a relatively high affinity for the reduced-folate carrier. Comparably high inhibition has been observed with relatively few compounds. The latter include the *N*-hydroxy-succinimide ester of methotrexate (IC₅₀ = 0.02 μ M), an irreversible inhibitor (26) and two reversible inhibitors, a fluorescein derivative of methotrexate (K_i = 0.28 μ M) (27) and suramin (K_i = 0.15 μ M) (18). Inhibition by the latter compounds has been compared in anion-deficient buffers which lack competing chloride and bicarbonate ions and produce inhibition constants 7- to 10-fold lower than in saline buffers.

Kinetic studies of transport inhibition by Chol-PS-oligodeoxynucleotides were consistent with a complex process. Purely competitive inhibition was eliminated by several criteria. Mixed kinetics were observed in double-reciprocal plots of transport versus substrate concentration for each inhibitor (Fig. 3) and inhibition could not be reversed by a wash step in cells treated with inhibitor prior to transport analysis (Table 2). The substantial increase in inhibitor sensitivity that occurred with decreasing cell concentration (Table 1) also supports the notion of complicated inhibition kinetics. The mechanism for improved inhibition upon cell dilution is unclear, but a direct involvement of the hydrophobic cholesteryl groups on these inhibitors appears likely. Based on prior reports that cholesteryl oligodeoxynucleotides bind tightly to cell membranes (8), inhibition of methotrexate transport may be initiated by and result from a rapid and quantitative binding to the plasma membrane. The decline in IC₅₀ value upon cell dilution might then be a consequence of an increase in the amount of total inhibitor bound per cell.

The development of folate deficiency represents a possible non-antisense mechanism for cell growth inhibition by cholesteryl-modified oligodeoxynucleotides. Since mammalian cells require an external source of folate and membrane-associated carriers to move folates into the cytoplasm, folate transport systems are possible sites for interrupting the bioavailability of folates. Chol-PS-oligodeoxynucleotides are candidates for causing folate deficiency since these compounds are potent inhibitors of an uptake system for folate compounds (the reduced-folate carrier) and remain active for several days in cultured cells. Persistence of Chol-PS-oligodeoxynucleotides was indicated by the ability of these compounds not only to antagonize methotrexate toxicity in cells grown in standard medium with excess folate (Table 3) but also to cause folate deficiency in cells grown in a limiting folate medium (Table 4). These combined observations indicate that L1210 and other cells which acquire folate compounds solely via the reduced-folate carrier could become folate deficient during prolonged exposure to cholesteryl-modified antisense oligodeoxynucleotides.

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