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Research Article

Purine nucleosides, which accumulate in adenosine deaminase and purine nucleoside phosphorylase deficiency, are toxic to lymphoid cells. Since adenine nucleosides inhibit S-adenosylhomocysteine hydrolase, they could potentially decrease intracellular methionine synthesis. To test this hypothesis, we measured methionine synthesis by the use of [14C]formate as a radioactive precursor in cultured human T and B lymphoblasts treated with varying concentrations of purine nucleosides; 2'-deoxycoformycin and 8-aminoguanosine were added to inhibit adenosine deaminase and purine nucleoside phosphorylase, respectively. In the T lymphoblasts methionine synthesis was inhibited approximately 50% by 10 microM of 2'-deoxyadenosine, adenine arabinoside, or 2'-deoxyguanosine. By contrast, in the B lymphoblasts methionine synthesis was considerably less affected by these nucleosides, with 50% inhibition occurring at 100 microM of 2'-deoxyadenosine and adenine arabinoside; 100 microM of 2'-deoxyguanosine yielded less than 10% inhibition. Adenosine and guanosine were considerably less potent inhibitors of methionine synthesis in both the T and B lymphoblasts. An adenosine deaminase-deficient and a purine nucleoside phosphorylase-deficient cell line, both of B cell origin, exhibited sensitivities to the nucleosides similar to those of the normal B cell lines. In both the T and B cell lines homocysteine reversed the methionine synthesis inhibition induced by the adenine nucleosides and guanosine and largely reversed that induced by 2'-deoxyguanosine. Methionine synthesis from homocysteine generates free tetrahydrofolate from 5-methyltetrahydrofolate, the main intracellular storage form of […]

Decreased Methionine Synthesis in Purine Nucleoside-treated T and B Lymphoblasts and Reversal by Homocysteine

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Abstract. Purine nucleosides, which accumulate in adenosine deaminase and purine nucleoside phosphorylase deficiency, are toxic to lymphoid cells. Since adenine nucleosides inhibit *S*-adenosylhomocysteine hydrolase, they could potentially decrease intracellular methionine synthesis. To test this hypothesis, we measured methionine synthesis by the use of [^{14}C]formate as a radioactive precursor in cultured human T and B lymphoblasts treated with varying concentrations of purine nucleosides; 2′-deoxycoformycin and 8-aminoguanosine were added to inhibit adenosine deaminase and purine nucleoside phosphorylase, respectively. In the T lymphoblasts methionine synthesis was inhibited ~50% by 10 μM of 2′-deoxyadenosine, adenine arabinoside, or 2′-deoxyguanosine. By contrast, in the B lymphoblasts methionine synthesis was considerably less affected by these nucleosides, with 50% inhibition occurring at 100 μM of 2′-deoxyadenosine and adenine arabinoside; 100 μM of 2′-deoxyguanosine yielded <10% inhibition. Adenosine and guanosine were considerably less potent inhibitors of methionine synthesis in both the T and B lymphoblasts. An adenosine deaminase-deficient and a purine nucleoside phosphorylase-deficient cell line, both of B cell origin, exhibited sensitivities to the nucleosides similar to those of the normal B cell lines. In both the T and B cell lines homocysteine reversed the methionine synthesis inhibition induced by the adenine nucleosides and guanosine and largely reversed that induced by 2′-deoxyguanosine. Methionine synthesis from homocysteine generates free tetrahydrofolate from 5-methyltetrahydrofolate, the main intracellular storage form of folate. We conclude that purine nucleoside toxicity may be partly mediated through (*a*) decreased intracellular methionine synthesis, and (*b*) altered folate metabolism.

Introduction

A deficiency of either of two consecutive enzymes of the purine catabolic pathway, adenosine deaminase (E.C. 3.5.4.4.) or purine nucleoside phosphorylase (E.C. 2.4.2.1.) leads to altered immune system function (1, 2). A deficiency of the former enzyme leads to severe combined immunodeficiency disease, a disorder of both T and B cell function with T cell function more profoundly affected, whereas deficiency of the latter enzyme leads to isolated T cell dysfunction. Although much is known about the biochemical consequences of these two enzyme deficiencies, the biochemical basis of purine nucleoside toxicity to lymphoid cells remains disputed.

Adenosine deaminase deficiency leads to the accumulation of adenosine (Ado)[1] and 2′-deoxyadenosine (dAdo), whereas purine nucleoside phosphorylase deficiency leads to the accumulation of inosine (Ino), 2′-deoxyinosine (dIno), guanosine (Guo), and 2′-deoxyguanosine (dGuo) (3, 4). In most cell systems Ino and dIno are nontoxic, whereas Ado, dAdo, Guo, and dGuo are potent inhibitors of T and B cell proliferation. Some of the dAdo toxicity relates to the accumulation of dATP, a potent inhibitor of ribonucleotide diphosphate reductase, thus interfering with the synthesis of the other deoxynucleotides required for DNA synthesis (3). This, however, cannot explain all of dAdo's toxicity since pyrimidine deoxynucleosides only partially reverse the toxicity, and, perhaps more important, resting non–DNA-synthesizing lymphocytes are lysed by dAdo (3, 5, 6). Another major mechanism of dAdo toxicity is through inhibition of *S*-adenosylhomocysteine hydrolase (E.C. 3.3.1.1.), a property that is shared by the dAdo

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1. *Abbreviations used in this paper:* Ado, adenosine; dAdo, 2′-deoxyadenosine; Ara-A, adenine arabinoside; Guo, guanosine; dGuo, 2′-deoxyguanosine; Ino, inosine; dIno, 2′-deoxyinosine.

analogue adenine arabinoside (Ara-A) (7, 8). Inhibition of this enzyme causes accumulation of *S*-adenosylhomocysteine and, as shown in Fig. 1, this compound inhibits its own formation from *S*-adenosylmethionine, thus inhibiting most intracellular transmethylation reactions (9). Inhibition of *S*-adenosylhomocysteine hydrolase should also reduce intracellular homocysteine production, and thus methionine synthesis, since the pathway depicted in Fig. 1 is the only mechanism mammalian cells have for generating homocysteine (10).

We therefore measured methionine synthesis in normal T and B lymphoblasts and in B lymphoblasts deficient in adenosine deaminase or purine nucleoside phosphorylase treated with varying concentrations of several purine nucleosides. We used [^{14}C]formate as our radioactive precursor as previously described (11). We found that dAdo and Ara-A were potent inhibitors of methionine synthesis in T lymphoblasts and less potent inhibitors in B lymphoblasts, whereas Guo and dGuo inhibited methionine synthesis only in T lymphoblasts. Homocysteine fully reversed the inhibition induced by dAdo, Ara-A, and Guo but only partially reversed that induced by dGuo.

Methods

Origin of cell lines and cell culture. The two T lymphoblast lines, CCRF-CEM and MOLT4, were derived from acute T cell leukemias and were provided by Drs. Alice Yu and J. Edwin Seegmiller, respectively, both of the University of California, San Diego. Three of the four B lymphoblast lines, MGL8B2 (a normal cell line, from the Genetics Unit of the Massachusetts General Hospital, Boston, MA), 889 (an adenosine deaminase-deficient cell line, from Dr. Seegmiller's laboratory), and 679 (a purine nucleoside phosphorylase-deficient cell line, from Dr. Randall Willis of the University of California, San Diego) were established by Epstein-Barr virus transformation of peripheral blood lymphocytes, whereas the fourth, WIL-2, is a normal splenic-derived line. The two deficient cell lines had <1% of their respective enzyme activities. Cells were routinely grown in RPMI-1640 medium (Gibco Laboratories, Gibco Div., Grand Island, NY) supplemented with 10% fetal bovine serum and 2 mM glutamine in a humified atmosphere of 5% CO_2; they were regularly tested for mycoplasma by a biochemical assay.

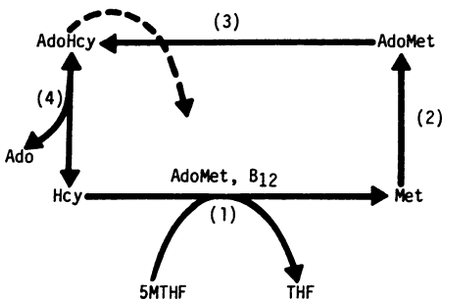

Figure 1. Methionine synthesis and conversion to homocysteine. Reactions: (1) methionine synthetase; (2) methionine adenosyltransferase; (3) methyltransferase reactions; and (4) *S*-adenosylhomocysteine hydrolase. Hcy, homocysteine; Met, methionine; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; 5MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate; and B_{12}, cobalamin. The broken arrow indicates that *S*-adenosylhomocysteine inhibits its own formation from *S*-adenosylmethionine, thereby inhibiting most intracellular methylation reactions.

Methionine biosynthesis assay. Cells were recovered by centrifugation from the RPMI growth medium and resuspended at a density of 1.5 × 10^6/ml in methionine-free modified Eagle's medium (Gibco Laboratories, Gibco Div.) (12) which was supplemented with 10 μM methionine, 100 μM folic acid, 1 μM cobalamin, 10 μg/ml 2-mercaptoethanol, 25 μg/ml human transferrin (one-third iron saturated), and 2 mg/ml bovine serum albumin-fatty acid mixture prepared as previously described (13). At a methionine concentration of 10 μM this totally defined serum free medium supported normal growth of all cell lines for at least 24 h. When experiments were performed at 30 μM methionine in the media, results were similar to those obtained at 10 μM, but the sensitivity of the assay is reduced at the higher concentration because of dilution of the radioactive product. In the homocysteine reversal experiments, D,L-homocysteine thiolactone (Sigma Chemical Co., St. Louis, MO) was added at zero time at a concentration of 200 μM; similar results were obtained when free D,L-homocysteine was prepared fresh by base hydrolysis at 50°C for 6 min.

2-ml aliquots of the cells were placed in 16 × 100 mm glass tubes, flushed with 95% air, 5% CO_2, capped, and allowed to equilibrate at 37°C for 1 h with either 5 μM 2'-deoxycoformycin (supplied by the Drug Development Agency, National Institutes of Health, Bethesda, MD) in the adenine nucleoside experiments or 50 μM 8-aminoguanosine (a generous gift of Dr. Shih-Hsi Chu, Brown University, Providence, RI) in the guanine nucleoside experiments. The former compound is a potent inhibitor of adenosine deaminase, and the latter compound inhibits purine nucleoside phosphorylase; these two nucleosides are not known to be *S*-adenosylhomocysteine hydrolase inhibitors (14), and at these concentrations they did not affect rates of methionine synthesis. Neither 889, the adenosine deaminase-deficient cell line, nor 679, the purine nucleoside phosphorylase-deficient cell line, was pretreated with these inhibitors. The nucleoside to be tested was added and 30 min later rates of methionine synthesis were measured as previously described (11). In brief, [^{14}C]formate (42 mCi/mmol, final concentration 220 μM) or [^{3}H]leucine (50 Ci/mmol, diluted with nonradioactive leucine in the medium to a final concentration of 800 μM), both from ICN Pharmaceuticals, Inc. (Irvine, CA), was added to duplicate tubes and the cells were incubated for 4 h. The reaction was terminated by putting the tubes into ice-cold H_2O; after 5 min the tubes were centrifuged at 500 *g* for 5 min and the medium was discarded.

The cells that had been incubated with the [^{14}C]formate were extracted for 30 min in ice-cold 5% TCA, heated at 80°C for 30 min, and then cooled on ice for an additional 30 min. The resulting protein precipitate, free of nucleic acids, was washed three times in ice-cold 10% TCA, resuspended in 0.4 ml of ultrapure 6 N HCl, and hydrolyzed at 110°C for 20 h. The HCl was evaporated under vacuum at 70°C and the resultant hydrolysate was resuspended in 40 μl of H_2O. A 20-μl sample was spotted on cellulose thin-layer chromatography sheets (Eastman Kodak Co., Rochester, NY), and methionine and its oxidation products, methionine sulfoxide and methionine sulfone, were separated from serine and cysteine and its oxidation products, cystine and cysteic acid, in a phenol/ethanol/H_2O/NH_4OH (65:20:20:2) one-dimensional solvent system. Since serine condenses with homocysteine to yield

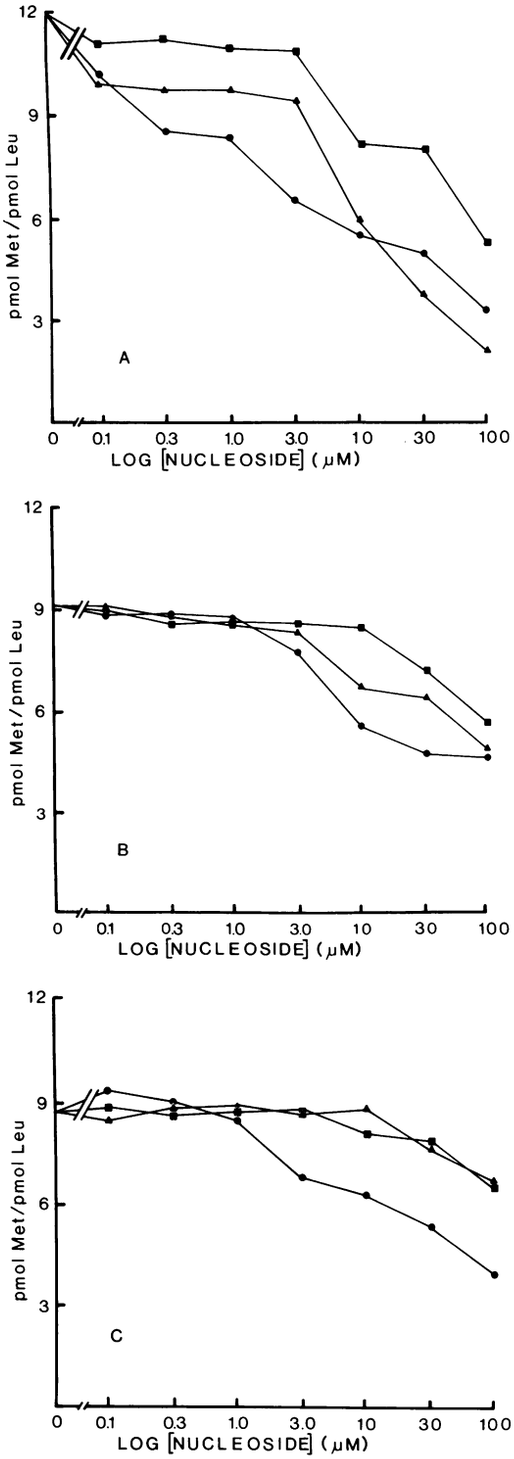

Figure 2. Methionine synthesis in (*A*) the T lymphoblast line CCRF-CEM; (*B*) the B lymphoblast line MGL8B2; and (*C*) the adenosine deaminase-deficient B lymphoblast line 889 at varying concentrations of Ado (■), dAdo (▲), and Ara-A (●). Methionine synthesis was measured in intact cells by following the incorporation of [^{14}C]formate into methionine residues of cellular protein as described fully in Methods. Each point is the mean of at least three independent experiments performed in duplicate.

cystathionine, which is converted to cysteine, any radioactivity that appears in cysteine actually originates from serine. The nonradioactive marker amino acids were visualized under ultraviolet light after the plates were dipped into a 0.01% fluorescamine (Sigma Chemical Co.) acetone solution. The appropriate spots were cut out of the chromatograph and counted at 95% efficiency in a toluene-based scintillation fluid containing 4 g/liter of PPO and 0.05 g/liter of POPOP.

The cells that had been incubated with the [^{3}H]leucine were extracted for 30 min in ice-cold 10% TCA, the precipitate was collected on GF/C filters (Whatman Laboratory Products Inc., Whatman Paper Div., Clifton, NJ), and the radioactivity was counted at ~35% efficiency. The incorporation of both the [^{14}C]formate and the [^{3}H]leucine into protein was linear to at least 4 h; duplicate values agreed to within 10%. Since we measured newly synthesized methionine incorporated into protein, the data are expressed as picomoles of [^{14}C]formate incorporated into methionine per picomole of [^{3}H]leucine incorporated into cell protein. This also allows for compensation of different rates of protein synthesis between cell lines.

Measurement of cell protein. Protein was measured according to Lowry et al. (15).

Results

Effects of Ado, dAdo, or Ara-A on methionine synthesis. Ado, dAdo, and Ara-A inhibited methionine synthesis in the T and B lymphoblasts (Fig. 2). Since similar patterns of sensitivity to these three adenine nucleosides were found in the two T lymphoblast lines, CCRF-CEM and MOLT4, and in the two B lymphoblast lines, MGL8B2 and WIL-2, only the data on CCRF-CEM (Fig. 2 *A*) and MGL8B2 (Fig. 2 *B*) are shown. The adenosine deaminase-deficient lymphoblast line 889, a B cell line, exhibited a pattern of sensitivity (Fig. 2 *C*) similar to that observed in the two normal B lymphoblast lines.

All three adenine nucleosides inhibited methionine synthesis more potently in the T lymphoblasts than in the B lymphoblasts. At a concentration of 0.1 μM, dAdo and Ara-A inhibited methionine synthesis by ~20% in the T lymphoblasts, whereas similar inhibition in the B lymphoblasts required 3 μM of Ara-A and 10 μM of dAdo. Moreover, the concentrations at which methionine synthesis was decreased by 50% of the control value (i.e., the ID_{50}) were remarkably different between the two cell types. Thus, the ID_{50} was <10 μM for either dAdo or Ara-A in the T lymphoblasts, and it was >100 μM for these nucleosides in the B lymphoblasts. Ado, compared with dAdo and Ara-A, was a less effective inhibitor of methionine synthesis, and only at concentrations >10 μM in the T lymphoblasts and 30 μM in the B lymphoblasts did it significantly inhibit methionine synthesis. At 30 and 100 μM, dAdo and Ara-A decreased protein synthesis, as measured by leucine incorporation into TCA-precipitable material, by ~30% in the T

lymphoblasts. Thus, the absolute inhibition of methionine synthesis at these two concentrations of these nucleosides is even greater than that shown in Fig. 2 *A* since the data are expressed as picomoles of methionine incorporated per picomole of leucine incorporated into protein. Ado, at any concentration, did not decrease protein synthesis in either the T or B lymphoblasts.

Effects of Guo, dGuo, Ino, and dIno on methionine synthesis. Both Guo and dGuo inhibited methionine synthesis (Fig. 3) in the T lymphoblasts. (Again, only the data on CCRF-CEM are shown but similar results were obtained with MOLT4.) Guo inhibited methionine synthesis in a pattern similar to that of Ado (compare Figs. 2 *A* and 3, solid squares), and dGuo inhibited methionine synthesis in a pattern similar to that of dAdo (compare Figs. 2 *A* and 3, solid triangles). In the B lymphoblasts, Guo at concentrations as high as 100 μM did not inhibit methionine synthesis (data not shown), whereas dGuo at 100 μM inhibited methionine synthesis by <10% (Fig. 4, open circles). In the purine nucleoside phosphorylase-deficient line 679, a B cell line, Guo again did not inhibit methionine synthesis (data not shown), and dGuo inhibited methionine synthesis in a way similar to that in MGL8B2 (Fig. 4, solid circles). At 30 μM dGuo inhibited protein synthesis by ~25% and at 100 μM by ~50% in the T lymphoblasts; it did not inhibit protein synthesis in the B lymphoblasts. Guo did not decrease protein synthesis in either the T or B lymphoblasts.

Neither Ino or dIno at concentrations as high as 100 μM inhibited methionine synthesis in either the T or B lymphoblasts (data not shown). Since both of these compounds serve as ready sources of hypoxanthine to the cell, these experiments indicate that increased cellular availability of purine bases does not affect the one-carbon pool which is labeled with [^{14}C]formate.

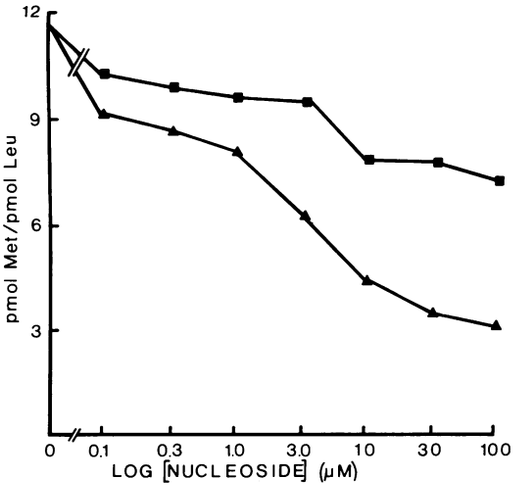

Figure 3. Methionine synthesis in the T lymphoblast line CCRF-CEM at varying concentrations of Guo (■) and dGuo (▲). Methionine synthesis was measured as in the legend to Fig. 2; each point is the mean of at least three independent experiments performed in duplicate.

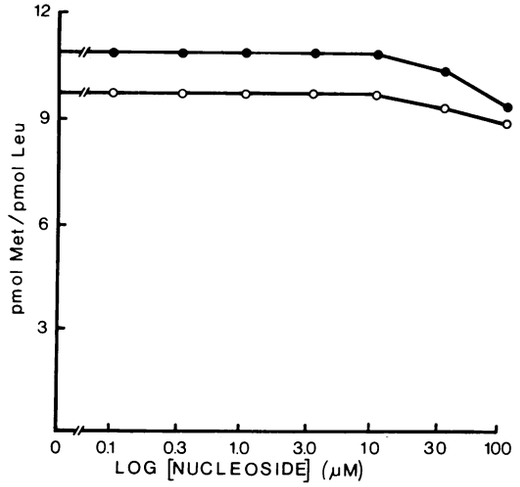

Figure 4. Methionine synthesis in the B lymphoblast line MGL8B2 (○) and the purine nucleoside phosphorylase-deficient B lymphoblast line 679 (●) at varying concentrations of dGuo. Methionine synthesis was measured as in the legend to Fig. 2; each point is the mean of at least three independent experiments performed in duplicate.

Reversal of Ado, dAdo, and Ara-A inhibition of methionine synthesis by homocysteine. In the absence of purine nucleosides, 200 μM D,L-homocysteine thiolactone increased methionine synthesis in CCRF-CEM by 40% and in MGL8B2 by 44%, from ~11 to 16 and from ~9 to 13 pmol methionine/pmol leucine incorporated into protein, respectively (Table I). This modest increase in methionine synthesis suggests that at 10 μM methionine, the concentration used in these experiments, the cell's generation of homocysteine may be submaximal. Thus, when exogeneous homocysteine is provided a small boost in methionine synthesis occurs.

When homocysteine thiolactone was added to either T or B cells treated with all concentrations of Ado, dAdo, or Ara-A, the nucleoside-induced inhibition of methionine synthesis was fully reversed (only the two highest concentrations of the nucleosides, 10 and 100 μM, are shown in Table I). In the presence of homocysteine, Ado at concentrations <10 μM, and dAdo and Ara-A at all concentrations actually increased the incorporation of newly synthesized methionine into protein. Thus, at 100 μM of either dAdo or Ara-A the picomoles of methionine incorporated into protein per picomole of leucine incorporated increased significantly ($P < 0.05$, t test), from ~16 to 21 and from ~13 to 17 in CCRF-CEM and MGL8B2, respectively (Table I). Ado at concentrations of ≥10 μM did not increase methionine incorporation into protein.

Reversal of Guo and dGuo inhibition of methionine synthesis by homocysteine. The Guo-induced inhibition of methionine synthesis in CCRF-CEM was fully reversed by the addition of

Table I. Reversal of Ado, dAdo, and Ara-A Inhibition of Methionine Synthesis by Homocysteine in CCRF-CEM and MGL8B2

Purine nucleoside concentration	CCRF-CEM				MGL8B2			
	Ado		dAdo or Ara-A		Ado		dAdo or Ara-A	
	−Hcy	+Hcy	−Hcy	+Hcy	−Hcy	+Hcy	−Hcy	+Hcy
μM								
0	11.4±1.6	16.0±2.3	11.4±1.6	16.3±2.1	9.1±0.8	13.1±1.8	9.1±0.8	13.6±1.9
10	8.6±1.3	17.3±2.6	5.7±1.6	21.1±3.1	8.5±1.8	13.8±1.8	6.1±1.1	15.9±1.7
100	4.9±0.6	15.3±1.8	2.7±0.3	20.5±3.2	5.6±0.9	12.6±1.9	4.7±0.8	17.2±2.4

Rates of methionine synthesis were measured as in the legend to Fig. 2. D,L-homocysteine thiolactone (Hcy) was present at a concentration of 200 μM. The data are expressed as picomoles of methionine per picomole of leucine incorporated into protein and are the mean±SD of at least three independent experiments.

homocysteine thiolactone (Table II). Again, as for dAdo and Ara-A, in the presence of homocysteine, Guo at all concentrations caused a small but statistically significant ($P < 0.05$, t test) increase in newly synthesized methionine incorporated into protein over that without the nucleoside. The dGuo-induced inhibition of methionine synthesis, on the other hand, was fully reversed only at nucleoside concentrations of ≤1 μM. At dGuo concentrations of ≥10 μM only partial reversal occurred; at these relatively high dGuo concentrations protein synthesis was significantly inhibited and thus some of this reversal is fictitious. Nonetheless, even at these high dGuo concentrations, homocysteine induced at least a twofold increase in methionine synthesis when expressed as picomoles synthesized per milligram of cell protein.

Discussion

These studies demonstrate that adenine and guanine nucleosides and deoxynucleosides inhibit methionine synthesis in cultured T and B lymphoblasts. The deoxynucleosides were more potent inhibitors of methionine synthesis than were the corresponding nucleosides, and T lymphoblasts were more sensitive to this methionine synthesis inhibition than were B lymphoblasts. These data, therefore, correlate with the results of growth studies that show that deoxynucleosides are more toxic to cultured lymphoblasts than are nucleosides and that T lymphoblasts are more sensitive to this toxicity than are B lymphoblasts (16–18). Moreover, since the adenine nucleosides inhibited methionine synthesis in both the T and B lymphoblasts and the guanine nucleosides inhibited methionine synthesis only in the T lymphoblasts, the data may provide a biochemical basis to explain the clinical differences between adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency.

Table II. Reversal of Guo and dGuo Inhibition of Methionine Synthesis by Homocysteine in CCRF-CEM

Purine nucleoside concentration	Guo		dGuo	
	−Hcy	+Hcy	−Hcy	+Hcy
μM				
0	11.5±1.8	16.2±2.3	11.5±1.8	16.2±2.3
1	9.6±1.9	22.8±3.4	8.1±1.5	21.1±3.5
10	7.9±1.3	22.4±2.9	4.4±0.6	13.0±1.9
100	7.3±1.4	21.0±3.0	3.1±0.5	11.2±1.7

Rates of methionine synthesis were measured as in the legend to Fig. 2. D,L-homocysteine thiolactone (Hcy) was present at a concentration of 200 μM. The data are expressed as picomoles of methionine per picomole of leucine incorporated into protein and are the mean±SD of at least three independent experiments.

The mechanism of the observed decrease in methionine synthesis induced by the adenine nucleosides is presumably through their inhibition of *S*-adenosylhomocysteine hydrolase, which causes a decrease in intracellular homocysteine production (Fig. 1). The data support this hypothesis because (*a*) Ara-A is a more potent inhibitor of *S*-adenosylhomocysteine hydrolase than is dAdo (7) and in the studies reported here was a more potent inhibitor of methionine synthesis, and (*b*) homocysteine fully reversed the adenine nucleoside-induced inhibition of methionine synthesis. Guo and dGuo do not inhibit purified human placental *S*-adenosylhomocysteine hydrolase (19), nor do they inhibit the enzyme in erythrocyte lysates (20). Our data, however, suggest that these guanine nucleosides do inhibit the enzyme in intact lymphoblasts, because homocysteine fully reversed the Guo-induced inhibition of methionine synthesis and partly reversed the dGuo-induced inhibition. It appears that dGuo may have an additional mechanism in inhibiting methionine synthesis.

By inhibiting *S*-adenosylhomocysteine hydrolase the nucleosides interrupt the cycle depicted in Fig. 1 in which methionine is converted to homocysteine, via *S*-adenosylmethionine and *S*-adenosylhomocysteine, and is ultimately regenerated by methionine synthetase. German et al. recently showed that this cycle is very active in lymphoblasts since ~20% of the intracellular methionine traverses this cycle at any time

(21). Our data support the quantitative importance of this cycle. In the presence of exogeneous homocysteine the purine nucleosides caused an ~25% increase in newly synthesized methionine incorporated into protein; this is probably because the interruption of the cycle by purine nucleosides caused methionine to be shifted from participation in the cycle to the amino acid pool used for protein synthesis. Unlike the other nucleosides, Ado, at high concentrations, did not increase the incorporation of newly synthesized methionine into protein. This probably shows that at these high Ado concentrations much of the exogenously supplied homocysteine reacted with the Ado to form *S*-adenosylhomocysteine, effectively decreasing the amount of homocysteine available for methionine synthesis and subsequent incorporation into protein.

The normal plasma methionine concentration ranges from 6 to 30 μM (22–24). Since the purine nucleosides decreased methionine synthesis equally at 30 and 10 μM of methionine in the media, decreased methionine synthesis may occur clinically when *S*-adenosylhomocysteine hydrolase is inhibited. Thus, decreased intracellular methionine synthesis would be expected in children with either adenosine deaminase deficiency or purine nucleoside phosphorylase deficiency and in patients treated with Ara-A since in all three clinical settings *S*-adenosylhomocysteine activity is markedly reduced (19, 20, 25, 26). Cautious administration of methionine might prove beneficial in these disorders.

Besides the potential starvation of the cell for methionine, the purine nucleoside-induced inhibition of methionine synthesis demonstrated in these studies could have another major detrimental effect. The methionine synthetase reaction is the only way the cell can regenerate free tetrahydrofolate from 5-methyltetrahydrofolate (27) (Fig. 1). 5-Methyltetrahydrofolate is the main intracellular storage form of folates, normally accounting for ~80% of all cellular folates (28); moreover, its formation from 5,10-methylenetetrahydrofolate is essentially irreversible in vivo (27). Thus, inhibition of methionine synthesis could lead to trapping of folates as 5-methyltetrahydrofolate, much as occurs in cobalamin deficiency. In this regard it is interesting that at least one adenosine deaminase-deficient child and apparently all of the purine nucleoside phosphorylase-deficient children had megaloblastic anemia (2, 29, 30). If folate trapping is shown to occur in purine nucleoside-treated cells, then folinic acid, much as it is used to limit methotrexate toxicity, might also be useful in these immunodeficiency states.

Acknowledgments

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