Decrease in S-adenosylmethionine synthesis by 6-mercaptopurine and methylmercaptopurine ribonucleoside in Molt F4 human malignant lymphoblasts

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6-Mercaptopurine (6-MP) and methylmercaptopurine ribonucleoside (Me-MPR) are purine anti-metabolites which are both metabolized to methylthio-IMP (Me-tIMP), a strong inhibitor of purine synthesis *de novo*. Me-MPR is converted directly into Me-tIMP by adenosine kinase. 6-MP is converted into tIMP, and thereafter it is methylated to Me-tIMP by thiopurine methyltransferase, an *S*-adenosylmethionine (*S*-Ado-Met)-dependent conversion. *S*-Ado-Met is formed from methionine and ATP by methionine adenosyltransferase, and is a universal methyl donor, involved in methylation of several macromolecules, e.g. DNA and RNA. Therefore, depletion of *S*-Ado-Met could result in an altered methylation state of these macromolecules, thereby affecting their functionality, leading to dysregulation of cellular processes and cytotoxicity. In this study the effects of 6-MP and Me-MPR on *S*-Ado-Met, *S*-adenosylhomocysteine (*S*-Ado-Hcy), homocysteine and methionine concentrations are determined. Both drugs cause a decrease in intracellular *S*-Ado-Met concentrations and an increase in *S*-Ado-Hcy and methionine concentrations in Molt F4 human malignant lymphoblasts. The effects of both 6-MP and Me-MPR can be ascribed to a decreased conversion of methionine into *S*-Ado-Met, due to the ATP depletion induced by the inhibition of purine synthesis *de novo* by Me-tIMP. Both 6-MP and Me-MPR thus affect the methylation state of the cells, and this may result in dysregulation of cellular processes and may be an additional mechanism of cytotoxicity for 6-MP and Me-MPR.

INTRODUCTION

6-Mercaptopurine (6-MP), an analogue of the purine base hypoxanthine, is commonly used in the maintenance treatment of children with acute lymphoblastic leukaemia [1]. Two metabolic pathways contribute to 6-MP cytotoxicity (Scheme 1). Both pathways are initiated by the conversion of 6-MP into thio-IMP (tIMP) by the purine salvage enzyme hypoxanthine/guanine phosphoribosyltransferase [2]. Subsequently, tIMP can either be incorporated into DNA as thioguanine nucleotides [3,4], resulting in DNA damage [5–7] and consequently delayed cytotoxicity [8–10], or tIMP can be methylated to give methyl-tIMP (Me-tIMP). Me-tIMP is a strong inhibitor of purine synthesis *de novo* [11,12], inducing depletion of purine nucleotides. As a consequence, DNA and RNA synthesis become inhibited, resulting also in inhibition of cell growth and cytotoxicity [13,14]. Methylmercaptopurine ribonucleoside (Me-MPR), another purine anti-metabolite, exerts its cytotoxicity after its conversion into Me-tIMP [14,15] by adenosine kinase, an ATP-dependent conversion [16,17].

Methylation of tIMP to Me-tIMP is an *S*-adenosylmethionine (*S*-Ado-Met)-dependent conversion, catalysed by thiopurine methyltransferase [18,19]. *S*-Ado-Met serves as a methyl donor for numerous methyltransferases, including those involved in methylation of proteins, phospholipids and nucleic acids, thereby affecting their functionality [20]. These methyltransferases convert *S*-Ado-Met into *S*-adenosylhomocysteine (*S*-Ado-Hcy), which is further hydrolysed to adenosine and homocysteine by *S*-Ado-Hcy hydrolase [21] (Scheme 1). Regeneration of *S*-Ado-Met occurs by methylation of homocysteine into methionine by the vitamin B_{12} and by methylenetetrahydrofolate reductase-dependent methionine synthase. Methionine is the direct precursor for *S*-Ado-Met synthesis [22]. Alterations in the transmethylation pathway have been found in most tumour cell lines, and methionine dependency is often observed in malignant cell lines [23].

In the present study the effects of various concentrations of 6-MP and Me-MPR on cell growth, cell viability and ATP concentrations were determined in Molt F4 human malignant lymphoblasts. Because the conversion of 6-MP into Me-tIMP consumes *S*-Ado-Met, the concentrations of *S*-Ado-Met, *S*-Ado-Hcy, homocysteine, methionine and Me-tIMP were determined to establish possible effects of 6-MP on the transmethylation pathway. The effects of Me-MPR on these parameters were also determined because Me-MPR is also converted into Me-tIMP, but without the consumption of *S*-Ado-Met.

MATERIALS AND METHODS

Chemicals

6-MP, Me-MPR, *S*-Ado-Met and *S*-Ado-Hcy were obtained from Sigma, U.S.A.

Abbreviations used: 6-MP, 6-mercaptopurine; Me-MPR, methylmercaptopurine ribonucleoside; Me-tIMP, methylthio-IMP; *S*-Ado-Met, *S*-adenosylmethionine; *S*-Ado-Hcy, *S*-adenosylhomocysteine.

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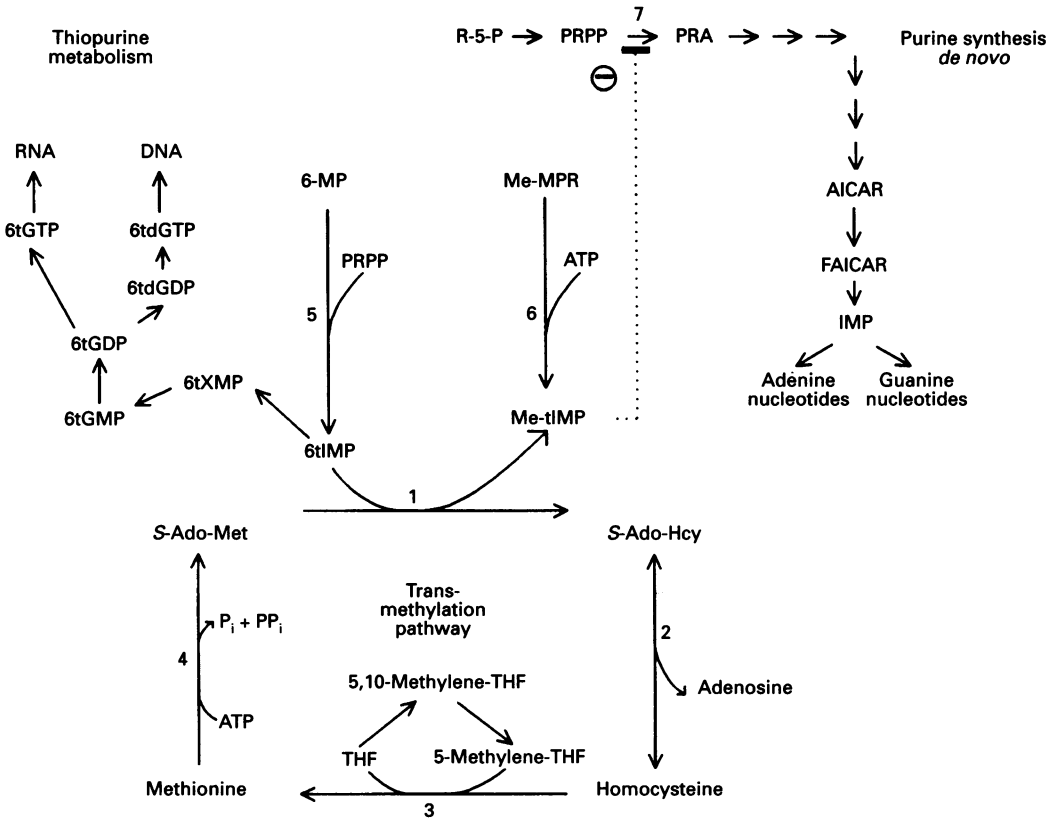

Scheme 1 6-MP metabolism and transmethylation pathway

Enzymes: 1, thiopurine methyltransferase; 2, *S*-Ado-Hcy hydrolase; 3, methionine synthase; 4, methionine adenosyltransferase; 5, hypoxanthine/guanine phosphoribosyltransferase; 6, adenosine kinase; 7, phosphorobosyl pyrophosphate (PRPP) amidotransferase. Abbreviations: t, thio; R-5-P, ribose 5-phosphate; PRA, 5-phospho-β-D-ribosylamine; AICAR, 5′-phosphoribosyl-5-amino-4-imidazole carboxamide; FAICAR, 5′-phosphoribosyl-5-formamido-4-imidazole carboxamide; THF, tetrahydrofolate.

Cells

The experiments were performed with Molt F4 cells, a human T-cell acute lymphoblastic leukaemia cell line. Conditions for cell culture have been described previously [24].

Experimental procedures

At 24 h prior to the start of the experiments, exponentially growing cells were seeded at a concentration of 0.2×10^6 cells/ml. At $t = 0$, 6-MP or Me-MPR were added as a single dose in a small volume (1/100). During the experiments 2 mM glutamine was supplemented every 24 h in order to prevent glutamine exhaustion of the medium [25]. After incubation, the cells were harvested and counted with a coulter counter model S5 (Coulter Electronics, Luton, U.K.). Cell viability was determined by means of Trypan Blue exclusion. Cell growth was determined by counting the cells and correcting cell number for cell viability.

Determination of endogenous nucleotides and thionucleotides

Endogenous nucleotides (di- and tri-phosphates) were determined in 3×10^6 viable cells, according to a method described previously [26], and were measured at a wavelength of 254 nm. Nucleotide concentrations were expressed as pmol/10^6 viable cells. The results were compared by using Student's t test. Thionucleotides were extracted from 10^7 viable cells, by a procedure described previously [24]. Thio-GMP was determined at 320 nm. Me-tIMP was measured at 290 nm. Concentrations were expressed as pmol/10^6 viable cells.

Determination of *S*-Ado-Met and *S*-Ado-Hcy

S-Ado-Met and *S*-Ado-Hcy concentrations were determined by h.p.l.c. in 10^7 viable cells by the method described by Molloy et al. [27]. The cell pellet was resuspended in 200 μl of 25 mM potassium phosphate buffer, pH 6, and proteins were precipitated by adding 20 μl of 50% trichloroacetic acid (BDH, U.K.). The samples were kept on ice for 10 min, and then centrifuged at 13000 *g* for 2 min. Supernatants were collected, and washed with 3×2 vol. of peroxide-free diethyl ether to remove trichloroacetic acid. The samples were flushed with nitrogen, while on ice, and diluted with distilled water to a final volume of 200 μl. Hereafter, *S*-Ado-Met and *S*-Ado-Hcy were separated on a Waters μBondapak C_{18} column (3.9 cm × 30 mm). Separation was performed with a gradient consisting of two buffers. Buffer A consisted of 25 mM potassium phosphate (Sigma, U.S.A.) 10 mM heptanesulphonic acid (Sigma, U.S.A.), pH 3.2. Buffer B consisted of 12.5 mM potassium phosphate, 50% methanol and 10 mM heptanesulphonic acid. The gradient was built up during consecutive time points as follows: start 70% buffer A + 30% buffer B, 11 min; 40% A + 60% B, 19 min; 20% A + 80% B, 21 min; 70% A + 30% B. One run lasted 30 min. The samples were analysed by a h.p.l.c. system, consisting of a Kontron

Table 1 ATP and Me-tIMP concentrations in Molt F4 cells treated with 2 or 10 μM 6-MP

ATP concentration is expressed in pmol/10^6 viable cells, as mean ± S.D. (in parentheses) of 9 experiments: ** $P < 0.01$. Me-tIMP concentrations are expressed in pmol/10^6 viable cells, as mean ± S.D. (in parentheses) of 8 independent experiments.

	ATP			Me-tIMP	
Time (h)	Control	2 μM 6-MP	10 μM 6-MP	2 μM 6-MP	10 μM 6-MP
24	4534 (815)	2493 (454)**	1495 (389)**	128 (66)	372 (94)
48	3345 (760)	2875 (645)	1991 (388)**	136 (108)	677 (207)

autosampler (MS1660; The Netherlands), a Spectra Physics pump (SP8800; Santa Clara, CA, U.S.A.), and a u.v. spectrophotometer from ABI Separations (759A; The Netherlands). The data were processed with Spectra Physics Winner software (Santa Clara, CA, U.S.A.). Concentrations were compared by Student's *t* test.

Determination of extracellular homocysteine

Total homocysteine concentrations were determined in 1 ml of culture medium after treatment of the cells with 6-MP or Me-MPR. Samples were freeze-dried overnight, and thereafter resuspended in 0.2 ml of distilled water. Subsequently total homocysteine was determined by h.p.l.c., essentially by the method described by Fiskerstrand et al. [28]. A programmable sample processor (Gilson, model 232 BIO, Dilutor 401) was used for the automated homocysteine derivatization and sample injection. The pump (SP8800), the integrator (SP4400) and the fluorescence spectrophotometer (Linear Fluor LC 304) were from Spectra Physics. Again the results were compared by Student's *t* test.

Determination of intracellular methionine

The intracellular methionine concentration was determined in 5×10^6 viable cells. The cells were centrifuged (5 min, 800 *g*), and the cell pellet was resuspended in 0.2 ml of distilled water. The cells were sonicated (3 × 10 s, 15 W, Branson sonifer) and proteins were precipitated with 0.1 ml of 18.8% sulphosalicylic acid (Merck, Germany) with norleucine (600 μM, Merck) as internal standard. The suspension was kept on ice for 10 min. Subsequently, 0.1 ml of buffer (pH 3.4), consisting of 0.2 M lithium citrate, 50 mM citric acid (Merck), 200 mM LiOH (Fluka, Switzerland), 10 mM phenol, 0.2% thiodiglycol and 0.06% HCl (Merck), was added, and the suspension was centrifuged (4 min, 12000 *g*). Distilled water (0.2 ml) was added to 0.2 ml of this suspension, and 0.25 ml was used for determination of methionine. Methionine concentrations were determined by ion-exchange chromatography on an amino acid analyser (Biotronik LC6001) according to the procedure specified by the manufacturer, with some modifications. To increase the sensitivity of the method, the amino acids were derivatized with *o*-phthaldialdehyde (Sigma, U.S.A.), and the methionine–*o*-phthaldialdehyde adduct was quantified with a fluorescence detector (Jasco, model 821-FP). Concentrations were compared by Student's *t* test.

Determination of *S*-Ado-Hcy hydrolase activity

This was performed with 100000 Molt F4 cells. Cells were freeze-dried overnight and then incubated for 20 min with 3.375 μmol of KH_2PO_4 (Sigma, U.S.A.), 3.375 μmol of K_2HPO_4 (Sigma, U.S.A.), 150 nmol of EDTA (Siegfried, Germany), 1.5 nmol of *erythro*-9-(2-hydroxy-3-nonyl)adenine (Burroughs Wellcome, U.S.A.), 0.5 μl of 10% Triton X-100 (Sigma, U.S.A.), 155 nmol of dithiothreitol (Boehringer, Mannheim) and 1.3 μmol of *S*-Ado-Hcy, at 37 °C and pH 7.0. The reaction was terminated by the addition of 8 M $HClO_4$ (BDH, U.K.). The suspension was kept on ice for 10 min. Thereafter, the samples were centrifuged for 3 min and the supernatant was collected and neutralized with 4 M K_2HPO_4. *S*-Ado-Hcy activity was determined by monitoring the formation of adenosine by reversed-phase h.p.l.c., at a wavelength of 254 nm.

RESULTS

6-MP induced a concentration-dependent cytotoxicity in Molt F4 cells. Cell viability of cells treated for 48 h with 0, 2, 5 or 10 μM 6-MP was 96.7%, 75.7%, 45.1% and 35.5% respectively. Me-tIMP was detected in cells treated with 6-MP (Table 1). With 10 μM 6-MP, more Me-tIMP was formed than with 2 μM, and with 10 μM 6-MP the Me-tIMP concentration still increased after 24 h (Table 1). The ATP concentration decreased as a result of treatment with 6-MP (Table 1). Depletion of ATP was more severe with 10 μM 6-MP than with 2 μM. 6-MP also decreased the concentration of *S*-Ado-Met (Table 2). Furthermore, treatment with 5 μM and 10 μM 6-MP resulted in an increase of the concentration of *S*-Ado-Hcy. The decrease in *S*-Ado-Met and increase in *S*-Ado-Hcy resulted in a concentration-dependent decrease in the *S*-Ado-Met/*S*-Ado-Hcy ratio. After treatment with 6-MP excretion of homocysteine was lower than that of untreated cells (Table 2). This was especially obvious 48 h after treatment. The intracellular homocysteine concentration in Molt F4 cells was below the detection limit. *S*-Ado-Hcy hydrolase activity was not directly inhibited by 10 μM 6-MP, 10 μM tIMP or 10 μM Me-tIMP (results not shown). Intracellular methionine concentrations increased after treatment with 5 and 10 μM 6-MP (Table 2), especially between 24 and 48 h. The *S*-Ado-Met/*S*-Ado-Hcy ratio appeared to be correlated with the ATP concentration, whereas the concentration of intracellular methionine appeared to be inversely correlated with ATP (Figure 1).

Me-MPR is also converted into Me-tIMP, resulting in inhibition of purine synthesis *de novo* and ATP depletion. However, for this conversion no *S*-Ado-Met is required. Therefore, the effects of Me-MPR on the intermediates of the transmethylation pathway have also been determined. Me-MPR was also cytotoxic for Molt F4 cells, with maximal cytotoxicity at 0.5 μM Me-MPR. The viability of cells treated for 48 h with 0, 0.2, 0.5 or 10 μM Me-MPR was 96.7%, 80.4%, 61.4% and 58.7% respectively. Very high concentrations of Me-tIMP accumulated in cells treated with Me-MPR (Table 3). After 24 h of treatment with 0.2 μM Me-MPR, the Me-tIMP concentration resembled that of cells treated with 10 μM 6-MP (Tables 1 and 3). Me-MPR (0.2 μM) resulted already in maximal inhibition of purine syn-

Table 2 Intracellular concentrations of *S*-Ado-Met, *S*-Ado-Hcy and methionine, the *S*-Ado-Met/*S*-Ado-Hcy ratio, and extracellular concentrations of homocysteine after treatment with various concentrations of 6-MP or Me-MPR

S-Ado-Met and *S*-Ado-Hcy concentrations are expressed as μmol/10^7 viable cells. Methionine concentrations are expressed as pmol/10^6 viable cells. Homocysteine concentrations are expressed as μmol/l. Values are means ± S.D. of 3 independent experiments: * $P < 0.05$; ** $P < 0.01$.

	Time (h)	Control	2 μM 6-MP	5 μM 6-MP	10 μM 6-MP	Control	0.2 μM Me-MPR	0.5 μM Me-MPR	10 μM Me-MPR
S-Ado-Met	24	4.8 (0.4)	3.2 (1.0)**	2.4 (0.3)**	2.3 (0.4)**	4.8 (0.4)	2.7 (0.06)**	3.1 (0.6)**	2.7 (0.4)**
S-Ado-Hcy		0.7 (0.3)	0.8 (0.2)	1.4 (0.08)**	1.5 (0.5)*	0.6 (0.4)	0.6 (0.05)	1.1 (0.3)	1.0 (0.3)
S-Ado-Met/*S*-Ado-Hcy		6.3 (1.7)	3.9 (1.6)*	1.7 (0.09)**	1.5 (0.3)**	6.3 (1.7)	4.4 (0.3)*	2.9 (0.3)**	2.7 (0.5)**
S-Ado-Met	48	4.2 (1.0)	2.8 (0.3)*	2.5 (0.2)**	2.4 (0.1)**	4.2 (1.0)	3.3 (0.1)	2.1 (0.2)*	2.3 (0.5)**
S-Ado-Hcy		0.3 (0.07)	0.5 (0.3)*	0.9 (0.04)**	1.4 (0.5)**	0.3 (0.07)	0.4 (0.01)*	0.8 (0.3)**	1.0 (0.3)**
S-Ado-Met/*S*-Ado-Hcy		13.3 (6.1)	5.8 (2.2)*	2.9 (0.1)**	1.9 (0.6)**	13.3 (6.1)	8.0 (0.2)	2.7 (0.9)**	2.3 (0.5)**
Methionine	24	186 (31)	325 (52)	383 (13)*	481 (16)*	188 (3.0)	387 (1.0)**	438 (8.0)**	380 (17)**
	48	100 (17)	181 (38)	657 (123)*	778 (11)**	122 (42)	153 (21)	337 (1.0)*	454 (6.0)**
Homocysteine	24	18.7 (1.5)	15.3 (0.2)	14.4 (0.07)	16.4 (0.4)	19.6 (0.5)	12.6 (0.3)**	10.7 (1.7)	11.1 (0.8)*
	48	33.4 (0.4)	18.4 (2.7)*	14.7 (2.8)*	15.4 (1.3)*	34.0 (1.3)	14.0 (0.0)**	11.8 (0.9)**	11.2 (0.0)**

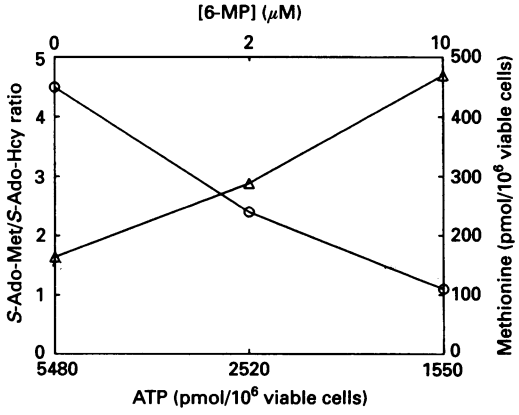

Figure 1 Correlation between ATP concentration and the methionine concentration (△) and the *S*-Ado-Met/*S*-Ado-Hcy ratio (○) after treatment of Molt F4 cells with 6-MP for 24 h

The results of one experiment are shown. Similar results were obtained in two other experiments.

thesis *de novo* during the first 24 h, as indicated by the depletion of the ATP pool (Table 3). Me-MPR also induced a decrease in the *S*-Ado-Met concentration, and an increase in *S*-Ado-Hcy, resulting in a decreased *S*-Ado-Met/*S*-Ado-Hcy ratio (Table 2). Again, extracellular homocysteine diminished as a result of treatment with Me-MPR as compared with untreated cells (Table 2). This effect was more pronounced after Me-MPR treatment than after 6-MP treatment. Despite its resemblance to adenosine, Me-MPR did not directly inhibit *S*-Ado-Hcy hydrolase activity when it was added at a concentration of 10 μM (results not shown). The methionine concentration also increased as a result of treatment with Me-MPR (Table 2). The effects of Me-MPR on *S*-Ado-Met, *S*-Ado-Hcy, methionine and homocysteine were comparable with those of 6-MP.

DISCUSSION

Extensive research has been performed to elucidate the mechanisms by which 6-MP exerts its cytotoxic effects on tumour cells. Most attention has been focused on incorporation of 6-MP into DNA as thioguanine nucleotides [4,8,29], whereas the methylation pathway for 6-MP metabolism was considered to be a detoxifying process after low-dose oral maintenance treatment, and therefore negatively affecting therapy [30,31]. However, our earlier studies using concentrations of 2 μM and 10 μM 6-MP indicated that the methylation route of 6-MP contributed to cytotoxicity by induction of inhibition of purine synthesis *de novo* [14,24,32]. We now postulate a new mechanism by which formation of Me-tIMP from 6-MP may also disturb various cellular processes, thereby inducing additional cytotoxicity.

Treatment of Molt F4 human malignant lymphoblasts with 6-MP results in a depletion of *S*-Ado-Met and an increase in *S*-Ado-Hcy, leading to a concentration-dependent decrease in the *S*-Ado-Met/*S*-Ado-Hcy ratio (Table 2). Theoretically, the depletion of *S*-Ado-Met can be ascribed to two mechanisms. First, the 6-MP metabolite tIMP is methylated by thiopurine methyl-

Table 3 ATP and Me-tIMP concentrations in Molt F4 cells treated with 0.2, 0.5 or 10 μM Me-MPR

ATP concentration is expressed as pmol/10^6 viable cells; mean ± S.D. (given in parentheses) of 3 independent experiments. ** $P < 0.01$. Me-tIMP concentrations are expressed as pmol/10^6 viable cells; ± S.D. (given in parentheses) of 8 independent experiments.

		ATP			Me-tIMP		
Time (h)	Control	0.2 μM Me-MPR	0.5 μM Me-MPR	10 μM Me-MPR	0.2 μM Me-MPR	0.5 μM Me-MPR	10 μM Me-MPR
24	4236 (502)	1469 (352)**	1958 (339)**	1802 (363)**	424 (34)	1381 (208)	4563 (1271)
48	4048 (259)	1668 (698)**	1845 (404)**	2145 (273)**	224 (1)	1332 (192)	4193 (799)

transferase with S-Ado-Met as methyl donor, thereby consuming S-Ado-Met. However, with 2 μM 6-MP less Me-tIMP is formed as compared with 10 μM 6-MP (Table 1), whereas S-Ado-Met is equally depleted at these two concentrations of 6-MP after 48 h (Table 2). Secondly, as a result of 6-MP treatment and subsequent Me-tIMP formation, the purine synthesis *de novo* is inhibited, resulting in a depletion of ATP (Table 1). ATP is also required as a substrate in the formation of S-Ado-Met from methionine by methionine adenosyltransferase (Scheme 1). So, depletion of ATP hampers the conversion of methionine into S-Ado-Met. This mechanism could also account for the observed decrease in S-Ado-Met, and would explain the increase in the methionine concentration (Table 2).

To discriminate between both mechanisms, we studied the effects of Me-MPR. Since Me-MPR is converted directly into Me-tIMP by adenosine kinase, no consumption of S-Ado-Met occurs, whereas inhibition of purine synthesis *de novo* does occur. Furthermore, conversion of Me-MPR into Me-tIMP by adenosine kinase consumes ATP, and Me-MPR competitively inhibits adenosine kinase. Both these mechanisms also contribute to the ATP depletion that results from Me-MPR treatment. In this way we can discriminate between the effects of S-Ado-Met consumption by methylation of tIMP into Me-tIMP versus decreased S-Ado-Met synthesis as a result of ATP depletion. Overall, the effects of Me-MPR closely resemble those of 6-MP. This demonstrates that the decreased S-Ado-Met concentrations are caused by reduced synthesis due to decreased ATP levels.

The observed increase in the S-Ado-Hcy concentration (Table 2) cannot be explained by decreased synthesis of S-Ado-Met. Conversion of S-Ado-Hcy into homocysteine and adenosine by S-Ado-Hcy hydrolase is a reversible reaction, with its equilibrium strongly in favour of S-Ado-Hcy formation, and an excess of adenosine induces accumulation of S-Ado-Hcy [21]. Since Me-MPR is an analogue of adenosine, administration of Me-MPR could lead to the same effect, thereby inducing accumulation of S-Ado-Hcy and decreasing formation and excretion of homocysteine. However, no direct inhibition of S-Ado-Hcy hydrolase activity was observed after addition of 10 μM Me-MPR to the cell lysates, indicating that the increase in S-Ado-Hcy cannot be ascribed to direct inhibition of S-Ado-Hcy hydrolase activity by Me-MPR. 6-MP, tIMP and Me-tIMP did not inhibit S-Ado-Hcy hydrolase either, when added at a concentration of 10 μM. So, neither Me-MPR and 6-MP nor their metabolites directly inhibit S-Ado-Hcy hydrolase activity. Another explanation for the accumulation of S-Ado-Hcy and the decrease in homocysteine would be an adenosine-induced inhibition of S-Ado-Hcy hydrolase. Whether intracellular accumulation of adenosine occurs after treatment of Molt F4 cells with 6-MP or Me-MPR remains to be elucidated. No increase in extracellular adenosine concentrations was observed after the addition of 0.5 or 10 μM Me-MPR to Molt F4 cells (E. H. Stet, R. A. De Abreu, J. P. M. Bökkerink, T. M. Vogels-Mentink, L. H. J. Lambooy and F. J. M. Trijbels, unpublished work). However, adenosine deaminase activity is present in the culture medium, resulting in degradation of adenosine.

The observed effects of 6-MP and Me-MPR on the transmethylation pathway may contribute to our knowledge of 6-MP cytotoxicity. S-Ado-Met is the methyl donor for numerous methyltransferases, and is involved in methylation of, for example, nucleic acid, proteins and phospholipids [20], whereas S-Ado-Hcy is a potent inhibitor of most S-Ado-Met-dependent methyltransferases [21,33]. So the Ado-Met/Ado-Hcy ratio is a major determinant of the transmethylase activity [34]. Compounds which influence pool sizes of S-Ado-Met and S-Ado-Hcy, and therefore the ratio of S-Ado-Met/S-Ado-Hcy, may directly influence cellular methylation reactions, for instance methylation of DNA [23]. This may induce differentiation in several tumour cell lines [35,36]. Furthermore, methylation of DNA is thought to play a major role in gene regulation [23,37], and aberrant methylation patterns exist in the DNA of tumour cells [9,37–41]. Therefore, tumour cells may be especially sensitive to depletion of S-Ado-Met.

In conclusion, both 6-MP and Me-MPR may function as inhibitors of intracellular transmethylation reactions, due to a decreased S-Ado-Met formation, thereby affecting cellular mechanisms on many different levels. Therefore, further research is warranted, possibly leading to a better understanding of the importance of the methylation route for 6-MP cytotoxicity.

This research is supported by the Dutch Cancer Society, Queen Wilhelmina Fund (Grant NUKC 89-13).

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Received 9 February 1994/ 4 May 1994; accepted 21 June 1994