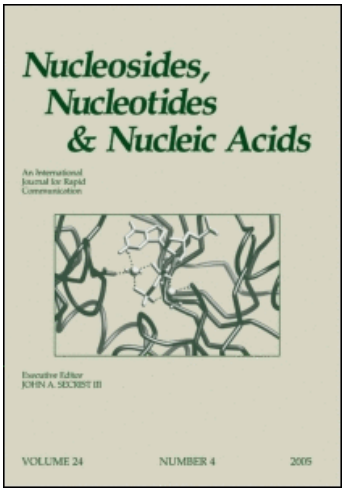

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Studies on 6-Mercaptopurine Cytotoxicity in Molt F4 Human Malignant T-Lymphoblasts

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Studies on 6-mercaptopurine cytotoxicity in Molt F4 human malignant T-lymphoblasts.

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Abstract

In this study the role of methyl-thioIMP (Me-tIMP) formation in 6-mercaptopurine (6MP) cytotoxicity was determined in Molt F4 human malignant T-lymphoblasts. Cytotoxicity of 6MP was increased under conditions where Me-tIMP formation was favored, indicating a role for Me-tIMP in 6MP cytotoxicity. Furthermore, 6MP treatment caused a decrease in concentration of the methyldonor S-adenosylmethionine (S-AdoMet). This could influence many intracellular methylation processes, for example DNA methylation.

Introduction

6-Mercaptopurine (6MP), an hypoxanthine analogue, is used in combination with methotrexate in the maintenance treatment of children with acute lymphoblastic leukemia[1]. The first step of intracellular 6MP metabolism is conversion into thio-IMP (tIMP) by hypoxanthine guanine phosphoribosyltransferase[2]. Thereafter, tIMP can be converted into thioguanine nucleotides, which can be incorporated into RNA and especially into DNA of the tumor cells[3,4]. This pathway is commonly considered as the main mechanism for 6MP cytotoxicity. tIMP can also be methylated to methyl-thioIMP (Me-tIMP) by thiopurine methyltransferase. As in many cellular methylation processes, S-adenosylmethionine (S-AdoMet) is the methyldonor for this reaction[5]. By donating its methyl-group, S-AdoMet is converted into S-AdoHcy. Me-tIMP is a strong inhibitor of the de novo purine biosynthesis, at the PRPP amidotransferase level[6], and inhibition of this route may lead to cytotoxicity for tumor cells[4,7].

In this study the importance of the methylation pathway for 6MP cytotoxicity was studied in Molt F4 human malignant T-lymphoblasts. Conversion of tIMP into thioguanine

nucleotides was inhibited by mycophenolic acid (MPA), an inhibitor of IMP dehydrogenase[8]. The effects of 6MP treatment were assessed, by determining cell growth, cell viability, endogenous nucleotide concentrations and intracellular thiopurine nucleotide concentrations. To establish a possible effect of tIMP methylation on intermediates of the transsulfuration pathway, the effects of 6MP treatment on S-AdoMet and S-AdoHcy concentrations were also determined.

Materials and methods

6MP, MPA, S-AdoMet and S-AdoHcy were obtained from Sigma Chemicals, U.S.A.

The experiments were performed with Molt F4 human malignant T-lymphoblasts. Conditions for cell culture and cell incubation experiments have been described previously[9]. After incubation, cells were harvested and counted with a Coulter Counter. Cell viability was determined by means of the trypan blue exclusion test.

Endogenous nucleotides (di- and triphosphates) and thionucleotides were determined by means of HPLC, according to the method described previously[9,10]. Nucleotides were measured at a wavelength of 254 nm. The concentrations were expressed as pmoles/10^6 cells. tGMP and Me-tIMP were measured at a wavelength of 320 nm and 290 nm, respectively. Concentrations were expressed as pmoles/10^6 cells.

Intracellular concentrations of S-AdoMet and S-AdoHcy were determined by means of HPLC, according to the method described by Molloy et al[11]. Concentrations were expressed as μmoles/10^6 cells.

Results and discussion

6MP cytotoxicity was concentration-dependent (results not shown). Treatment of Molt F4 cells with a combination of 0.5 μM MPA and 2 μM 6MP resulted in an increase of cytotoxicity (FIG. 1). Furthermore, after treatment of the cells with both drugs combined more Me-tIMP was formed than with 6MP alone (TABLE 1), whereas the tGMP concentration with the combination remained lower than with 6MP alone (results not shown). These results indicated a possible role for Me-tIMP in 6MP cytotoxicity, contradicting earlier studies in which the methylation route of 6MP is implicated as a detoxification mechanism[12,13].

Me-tIMP is an inhibitor of de novo purine biosynthesis. Inhibition of this metabolic route upon treatment of Molt F4 cells with 2 or 10 μM 6MP was reflected by a decrease of the purine nucleotide concentrations (TABLE 2). Both adenine and guanine nucleotide concentrations returned to control value after 48-h treatment with 2 μM 6MP. Treatment

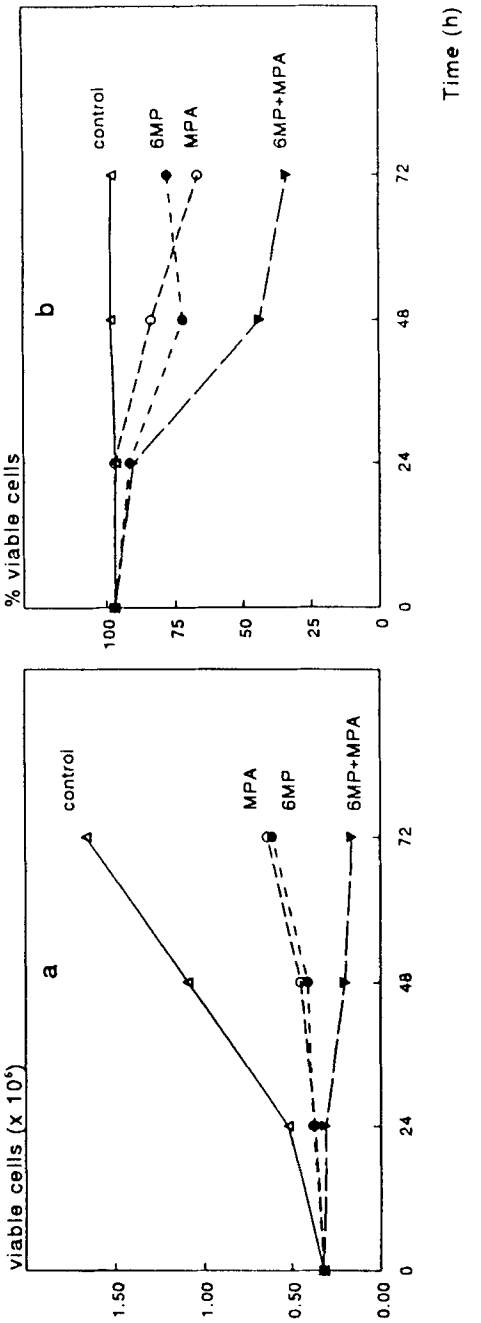

Fig. 1 Cell growth (1a) and cell viability (1b) of Molt F4 cells treated with 2 μM 6MP, 0.5 μM MPA or a combination of both. The results of 1 experiment are shown. Similar results were obtained in 4 other experiments.

TABLE 1 Me-tIMP concentration in Molt F4 cells treated with either 2 or 10 μM 6MP alone, or in combination with MPA (expressed as pmoles/10^6 cells, mean with standard error of 5 independent experiments).

	2 μM		10 μM	
MPA	-	+	-	+
t = 2	18 ± 4	16 ± 7	37 ± 19	39 ± 14
t = 24	92 ± 25	152 ± 14	354 ± 110	380 ± 66
t = 48	58 ± 23	371 ± 43	584 ± 213	740 ± 101

Table 2. Purine nucleotide concentrations of Molt F4 cells treated with 2 or 10 μM 6MP, expressed as percentage of untreated cells (mean with standard error of 5 independent experiment). The purine nucleotide concentrations of Molt F4 before treatment were 4750 ± 640 and 890 ± 120 pmoles/10^6 cells for adenine and guanine nucleotides, respectively.

	Adenine nucleotides		Guanine nucleotides	
	2 μM	10 μM	2 μM	10 μM
t = 2	99 ± 6	74	57 ± 2	34 ± 4
t = 24	59 ± 16	40 ± 10	73 ± 18	79 ± 25
t = 48	98 ± 22	63 ± 6	125 ± 19	80 ± 18

with 10 μM 6MP led to a persistent decrease of guanine nucleotides, reflecting a more severe inhibition of de novo purine biosynthesis as a result of the higher concentration of Me-tIMP obtained with 10 μM 6MP (TABLE 1).

S-AdoMet concentration decreased from 5.0 to 2.3 μmoles/10^6 cells as a result of a 24-h. treatment with 2 μM 6MP, whereas the concentration of S-AdoHcy increased from 1.1 to 2.1 μmoles/10^6 cells. S-AdoMet is the methyl-donor for many cellular methylation reactions,whereas S-AdoHcy is an inhibitor of S-AdoMet mediated mehylation reactions[14]. S-AdoMet is involved in methylation of DNA[15], which is one of the mechanisms regulating gene expression[16]. Therefore, the decrease of S-AdoMet and increase of S-AdoHcy induced by 6MP treatment may affect the methylation state of DNA, and may thereby deregulate gene expression. Whether this may have a therapeutic impact remains to be elucidated.

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