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Guanine ribonucleotide depletion in mammalian cells

A target of purine antimetabolites

Binh T. Nguyen, Marvin B. Cohen, and Wolfgang Sadée

Departments of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143, USA

Summary. *In a previous report we demonstrated in mouse lymphoma (S-49) cells that DNA synthesis inhibition resulting from guanine starvation is associated with GTP rather than dGTP depletion. Since several effective anticancer drugs act via guanine depletion, it is important to test whether critical GTP depletion is unique to S-49 cells or also occurs in other cell lines. Mycophenolic acid-induced guanine starvation caused a drastic DNA synthesis inhibition in the human lymphoblastic T leukemia (CEM) and the mouse B leukemia (L1210) cell lines, which was again associated with GTP depletion rather than dGTP depletion. These results suggest that GTP depletion represents a common target of purine antimetabolites in mammalian cells.*

Introduction

Antimetabolites that cause purine starvation represent potent anticancer, antiviral, antipsoriatic, antifungal, and immunosuppressive agents. Early de novo purine biosynthesis inhibition results in a drastic inhibition of DNA synthesis, an effect that we have recently shown to be primarily caused by guanine, rather than adenine nucleotide depletion [3]. Moreover, DNA synthesis inhibition subsequent to guanine starvation in mouse T lymphoma (S-49) cells appeared to be associated with GTP rather than dGTP depletion [2]. The precise function that GTP may serve in DNA synthesis remains elusive at present; however, these results suggest GTP as a major primary target of purine starvation in S-49 cells. Indeed, recent studies on thiazole-C-nucleosides, which have been shown to be highly effective anticancer drugs in animal tumors, demonstrate that their mechanism of action occurs primarily by guanine starvation [4]. In view of the general pharmacological importance of purine antimetabolites the present report addresses the question as to whether selective GTP starvation associated with drastic DNA synthesis inhibition is unique to the mouse T lymphoma (S-49) cells or more generally applicable.

The experimental approach to study selective GTP depletion involves the use of wild-type and corresponding HGPRTase-negative mutant cell lines, guanine biosynthesis inhibition by mycophenolic acid, and rescue from mycophenolic acid toxicity by guanosine (Guo) and deoxyguanosine (dGuo). Relevant biochemical pathways are depicted in Fig. 1; whereas Guo and dGuo were capable of rescuing wild-type S-49 cells from mycophenolic acid toxicity via the HGPRTase pathway to GTP, there was no rescue in the HGPRTase-negative cell line in which GTP pools were unaffected by Guo and dGuo salvage [2]. In contrast, dGuo enlarged the dGTP pools above control in wild-type and mutant cells, indicating that it can selectively restore dGTP but not GTP pools in the mutant line without restoring DNA synthesis inhibition by mycophenolic acid [2]. At higher concentrations dGuo becomes toxic itself because of feedback inhibition of ribonucleotide reductase by elevated dGTP pools [6]. Thus, dGuo cell toxicity at high levels can be taken as evidence that the dGuo to dGTP pathway is intact.

We report here that two additional cell lines (wild-type and HGPRTase-negative), the human lymphoblastic T leukemia (CEM) and mouse B leukemia (L1210) cells, respond to mycophenolic acid and Guo/dGuo in a fashion analogous to that of the mouse T lymphoma (S-49) cell line reported earlier [2].

Materials and methods

Cell culture procedures used here have been described previously [2]. Cell lines were obtained from the Cell Culture

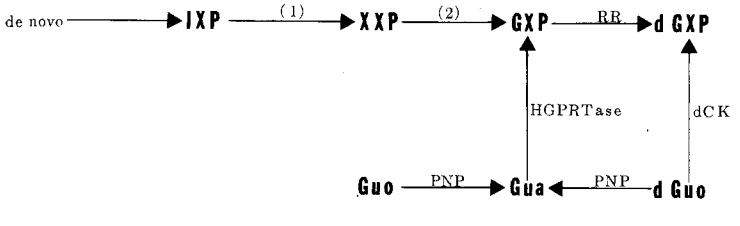

Fig. 1. De novo biosynthesis pathway (inhibited by mycophenolic acid) and salvage pathways of guanine and deoxyguanine ribonucleotide synthesis. De novo pathway: (1) Inosinate dehydrogenase; (2) guanylate synthetase. Salvage pathway: PNP, purine nucleotide phosphorylase; HGPRTase, hypoxanthine-guanine phosphoribosyl transferase (this enzyme was deficient in mutant cells); dCK, deoxycytidine kinase; RR, ribonucleotide reductase; XP is defined as mono-, di-, or triphosphate

Reprint requests should be addressed to W. Sadée

Facility of the University of California at San Francisco. Cell lines used were the human lymphoblastic leukemia (CEM) and HGPRTase-negative mutant (selected against 6 thioguanine, #BU-CEM-HGPRT) lines, and mouse B leukemia (L1210) and HGPRTase-negative mutant (#MJB-MMPR-10) lines.

Cell lines were first tested for response to Guo and dGuo and the ability of Guo and dGuo to rescue from mycophenolic acid toxicity in the wild type. Lack of toxic dGuo effects and lack of Guo/dGuo ($\leq 200\ \mu M$) rescue indicated deficient cell uptake of the nucleosides. Thus, human B lymphoblast (WI-L2) and mouse L-cell lines, wild-type and HGPRT-ase-negative, were excluded from the study.

The HGPRTase activity in the cell lines CEM and L1210 was determined by measuring the conversion of ^{14}C-hypoxanthine to ^{14}C-IMP in cell extracts [1, 5]. While the HGPRTase-activity was 1.29 and 0.37 nmole per 10^5 cells/min in CEM and L1210 cells (wild-type), respectively, no enzyme activity was detectable in the mutant lines. Finally, sensitivity of the selected cell lines towards mycophenolic acid was determined to assure that wild-type and mutant cell lines are equally responsive to guanine starvation. There was no difference between the growth inhibition by mycophenolic acid of CEM [IC_{50} (24 h) 0.7 μM] and L1210 [IC_{50} (24 h) 1.0 μM] wild-type and mutant cell lines.

Rescue experiments were performed at 2.0 μM (CEM) and 2.5 μM (L1210) mycophenolic acid over 24 h. These concentrations completely prevented cell growth.

Results and discussion

The effects of Guo and dGuo on cell growth in the absence and presence of mycophenolic acid are shown in Fig. 2 (CEM) and Fig. 3 (L1210). Guo did not significantly inhibit cell growth of either wild-type or mutant cell lines, while dGuo caused dose-dependent inhibiting of the cell growth of both wild-type and mutant cell lines. Mycophenolic acid toxicity could be prevented by Guo in wild-type cells (approximately 85% and 100% reversal in CEM and L1210, respectively). The optimum concentration of dGuo (10 μM in CEM and 60 μM in L1210) prevented the toxicity of mycophenolic acid to the extent of 52% in CEM and 78% in L1210 wild-type cells. With a higher concentration, dGuo caused its own inhibitory effect on the cell growth, similar to that observed in the absence of mycophenolic acid, thereby superseding the reversal of mycophenolic acid toxicity. In contrast to the rescue of wild-type cells, neither Guo nor dGuo was able to prevent the toxicity of mycophenolic acid in the HGPRTase-negative cell lines, although dGuo was taken up as evidenced by its toxicity to these cells at higher levels. Therefore, in the HGPRTase cell lines the biochemical pathway from dGuo to GTP was absent, while the pathway to dGTP was intact.

These observations were identical to those obtained with mouse T-lymphoma (S-49) cells. In the presence of mycophenolic acid and dGuo, GTP pools were depleted, while dGTP pools were levated in the mutant cell line; furthermore, DNA

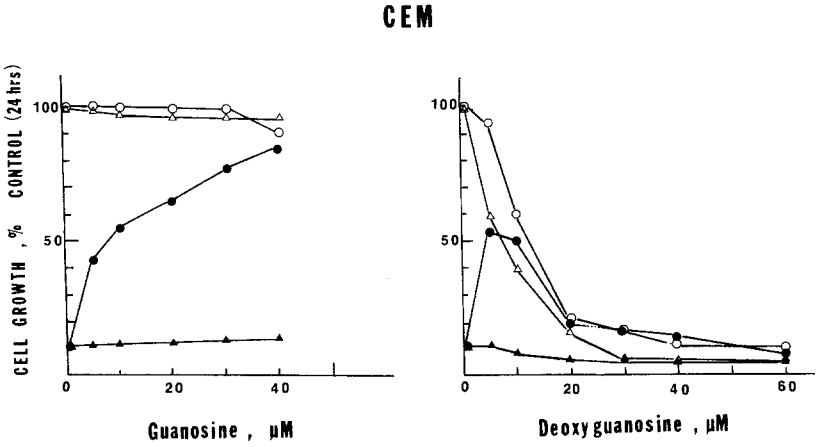

Fig. 2. Prevention of mycophenolic acid toxicity by guanosine or deoxyguanosine in CEM cell lines. Wild-type (*circles*) and HGPRTase-negative (*triangles*) cells were incubated with increasing concentrations of guanosine or deoxyguanosine for 24 h, in the presence (●, ▲) and absence (○, △) of 2.0 μM mycophenolic acid. The experiment was repeated twice with similar results

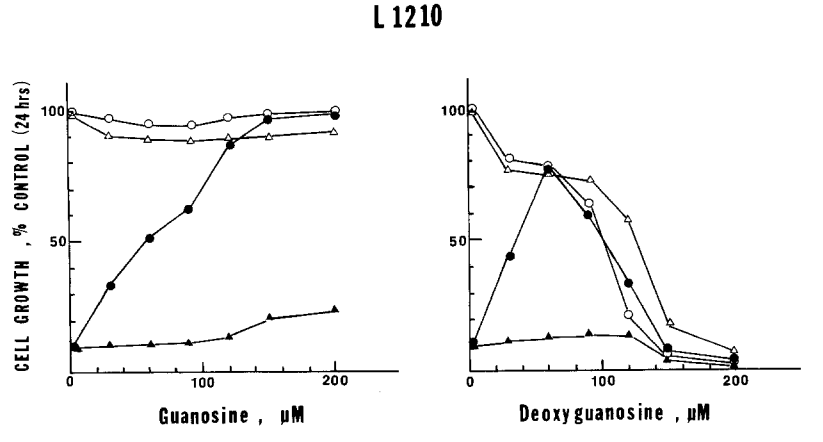

Fig. 3. Prevention of mycophenolic acid toxicity by guanosine or deoxyguanosine in L1210 cell lines. Wild-type (*circles*) and HGPRTase-negative (*triangles*) cells were incubated with increasing concentrations of guanosine or deoxyguanosine for 24 h, in the presence (●, ▲) and absence (○, △) of 2.5 μM mycophenolic acid. The experiment was repeated twice with similar results

synthesis and cell growth were not restored by a return of the dGTP pools to or above control levels [2].

Our previous conclusion, that the depletion of GTP rather than dGTP limits cell growth and is associated with DNA synthesis inhibition, can now be extended to two additional mammalian cell lines, namely human CEM and mouse L1210. Thus, GTP depletion may represent a general target of purine starvation. It is possible that the GTP pool serves as a sensor by which cells determine whether or not environmental conditions are favorable for DNA replication and growth.

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