Partial Reversal of Toxic Effects of 5-Azacytidine by 6-Azauridine in Normal Mice

A recently synthesized antimetabolite[1], 5-azacytidine, has been shown to possess a potent antileukaemic action against lymphoid leukaemia of AKR inbred mice. It has been observed further that its toxic effects are directed – at least in mice – primarily against the lymphatic system, especially thymus, and against bone marrow myeloid cells. On the other hand, it has been reported that bacteriostatic action of 5-azacytidine can be prevented by addition of uracil, uridine and cytidine. This finding has suggested the application of uridine and cytidine in normal AKR mice to study the effects of these substances on toxic manifestations of 5-azacytidine; the results obtained prompted further investigation with 6-azauridine, which is a known uridine antimetabolite of extremely low toxicity.

The animals used in these experiments were 2-months-old female inbred AKR mice. Leukaemia was transferred to them by means of subcutaneous inoculation of 10^7 leukaemic cells.

For the study of toxic effects in normal mice, the number of blood leucocytes was determined by hemocytometer counts. The brush-smeared bone marrow preparations stained with May-Grünwald-Giemsa stain were evaluated for the percentage of myeloid elements and for

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Table I. The partial reversal of toxic effects of 5-azacytidine in normal mice by uridine and cytidine

Mouse no.	Substance administered[a]	Body weight g	No. of blood leucocytes/mm³	% of bone marrow segments and stabs	Occurrence of atypical bone marrow cells	Thymus weight mg
		before and 24 h after treatment				
1	5-AzCR	22/21	7,900/ 6,800	17 (30)[b]	+++	44 (90)[b]
2	5-AzCR	23/21	8,200/ 5,900	14.5	+++	42
3	5-AzCR	23/22	8,400/ 5,600	14.5	+++	43
1	Cytidine + 5-AzCR	22/22	7,000/ 9,000	17	±	52
2	Cytidine + 5-AzCR	22/22	12,700/16,100	18	±	54
3	Cytidine + 5-AzCR	22/22	9,700/10,000	22	±	61
1	Cytidine	23/22	8,600/10,600	26.2	0	76
2	Cytidine	23/24	7,400/10,600	27.6	0	96
1	Uridine + 5-AzCR	22/22	8,000/11,400	14.3	±	69
2	Uridine + 5-AzCR	23/24	17,900/12,400	20	±	62
3	Uridine + 5-AzCR	22/22	9,500/11,800	24	0	61
1	Uridine	23/23	6,200/ 9,700	32	0	60
2	Uridine	24/24	8,500/ 8,800	23	0	85
3	Uridine	22/22	9,700/ 7,700	27.6	0	75

[a] 5-AzCR and nucleosides were administered at the level of 50 μg and 5 mg (2.2 mg/kg and 220 mg/kg) in 0.2 ml saline on 3 consecutive days i.p. The mice were examined before application of the drugs and 24 h afterwards. [b] Normal values in AK mice (23 g) treated with physiological saline (0.2 ml i.p.) on 3 consecutive days.

Table II. The partial reversal of toxic effects of 5-azacytidine in normal mice by 6-azauridine

Mouse no.	Substance administered[a]	Body weight g	No. of blood leucocytes/mm³	% of bone marrow segments and stabs	Occurrence of atypical bone marrow cells	Thymus weight mg
		before and 24 h after treatment				
1	5-AzCR	19/19	7,100/ 4,600	6.4 (20)[b]	++	34 (70)[b]
2	5-AzCR	20/20	12,800/ 5,000	5.0	+++	35
3	5-AzCR	20/20	11,500/ 6,400	5.2	+++	36
4	5-AzCR	20/20	15,400/ 7,400	10.5	++	34
1	6-AzUR + 5-AzCR	20/21	10,500/ 8,200	14.1	0	55
2	6-AzUR + 5-AzCR	20/20	5,300/ 5,500	9.5	±	48
3	6-AzUR + 5-AzCR	20/20	16,700/10,700	9.4	±	48
4	6-AzUR + 5-AzCR	20/20	5,500/ 8,100	6.0	±	40
1	6-AzUR	20/21	9,900/16,000	16.7	0	62
2	6-AzUR	20/20	8,300/11,000	21.9	0	57
3	6-AzUR	20/21	9,300/10,300	12.3	0	54
4	6-AzUR	20/20	10,700/11,200	15.5	0	62

[a] 5-AzCR and 6-AzUR were administered at the level of 50 μg and 5 mg (2.5 mg/kg and 250 mg/kg) dissolved in 0.2 ml physiological saline on three consecutive days i.p. The mice were examined before application of the drugs and 24 h afterwards. [b] Normal values (mice weighing 20 g) in animals treated with saline (0.2 ml i.p.) on 3 consecutive days.

the presence of atypical cells[2] by counting 500 cells in each instance.

The partial reversal of toxic effects of 5-AzCR by cytidine and by uridine is evident from Table I. Both metabolites in 5-AzCR treated normal mice diminish the loss of thymus and of body weight, the depletion of blood leucocytes and of bone marrow myeloid cells as well as the occurrence of atypical bone marrow cells[2].

In further studies, experiments were designed to disclose whether 6-azauridine would have the same effect as uridine and cytidine. The partial reversal of toxic effects of 5-AzCR by 6-azauridine in normal mice is shown in Table II. The mice used in this instance were of lower average weight by about 2-3 g than in the preceding experiment. This may be the reason for the smaller extent of protection afforded by 6-azauridine in the case of myeloid bone marrow cells. All of the remaining experimental results are very similar to those given in Table I.

The biochemical mechanism of action of 6-azauridine has been elucidated to a considerable degree[3]. 5-Azacytidine is known to undergo phosphorylation and incorporation into various mouse ribonucleic acids[4]. In vitro, in appropriate concentrations, it increases the number of chromosomal breaks[5]. Its cytological effects on lymphocytes and especially on their nuclei have been described elsewhere[6]. The blocking of its action by uridine and cytidine is probably due to the ability of these nucleosides to compete with 5-azacytidine for phosphorylation and for subsequent incorporation into cell nucleic acids.

Mammalian cells, however, do not incorporate 6-azauridine, and for this reason it seems that the reversal effect of 6-azauridine in alleviating the toxicity of 5-azacytidine is mainly due to the competition of both substances at the level of kinase system.

To our knowledge, the reversal of toxic effects of one pyrimidine antimetabolite by another pyrimidine antimetabolite has not been reported up to now. This phenomenon may be of considerable interest for the chemotherapy of cancer.

Zusammenfassung. Die partielle Blockierung der toxischen Effekte von 5-Azacytidin in normalen Mäusen mit Uridin, Cytidin und 6-Azauridin wird beschrieben. Die Hemmung der toxischen Effekte eines Antimetaboliten durch einen anderen kann für die Krebschemotherapie von Bedeutung sein.

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Recombination Among UV-induced Adenine-1 Mutants of *Schizosaccharomyces pombe*

Intragenic recombinational mapping has been reported for several of the genes controlling adenine biosynthesis in the fission yeast *Schizosaccharomyces pombe*[1-10].

The present study was carried out using ten adenine auxotrophs of independent origin, kindly provided by Professor U. Leupold. These ten mutants are located at the adenine-1 locus and had been isolated following irradiation of the wild-type strain, of h^+ mating type, with UV-light (Leupold[11]). Adenine-1 mutants are blocked at one of the early steps in purine biosynthesis, before the formation of 5-aminoimidazole ribonucleotide (AIR), and do not accumulate a red pigment when grown on media containing limiting adenine[2,11].

The isolation numbers of the ten UV-induced adenine-1 mutants are 3, 25, 40, 51, 107, 153, 169, 199, 233, and 249. Mutants 169 and 199 are temperature-sensitive auxotrophs.

The ten mutants were tested for their ability to recombine, in an attempt to produce an intragenic recombination map of the adenine-1 locus.

The results of the crosses[1,12] involving all the possible pairwise combinations of the ten adenine-1 mutants in h^+ and h^- mating types are given in the Table. Recombination frequencies are expressed as numbers of adn^+ recombinants per 10^6 viable ascospores plated on minimal medium agar (MMA). It was impossible to determine frequencies of adn^+ recombinants in the crosses involving mutant 233 with mutants 25, 51, 107, 169, and 199 due to the growth of mainly unstable complementing diploids and aneuploids on the MMA plates (Leupold[12]).

The results obtained show quite clearly that the ten UV-induced adenine-1 mutants tested represent damage at ten distinct sites within the adenine-1 locus. Seven of the ten are clustered towards one end of the locus, two (numbers 233 and 3) are close together in the central part of the adenine-1 locus, and mutant 40 is located towards the other end of the locus. Additional UV-, spontaneous-, and diethyl sulphate-induced mutants also belong mainly to the same cluster containing the seven mutants (25, 51, 107, 153, 169, 199, and 249) tested in the present study, or to a central cluster which includes mutants 3 and 233 of the ten tested here (Leupold[12]). Taken together with

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