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Brief Communication

Antileukemic Effects of Pseudoisocytidine, a New Synthetic Pyrimidine C-Nucleoside[1]

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SUMMARY

Pseudoisocytidine, a new synthetic pyrimidine C-nucleoside, which might be considered a more stable analog of 5-azacytidine, is active *in vitro* and *in vivo*, i.p. and p.o., against various 1-β-D-arabinofuranosylcytosine-resistant lines of mouse leukemia. This antileukemic activity is blocked by cytidine but not by deoxycytidine or thymidine.

INTRODUCTION

ara-C[2] (6, 19) has been the mainstay of the treatment of AML for many years. Despite the fact that its short plasma half-life (10) and its schedule specificity make its administration somewhat difficult, it has been an integral part of protocols that have produced remissions in 50 to 70% of patients with AML (5, 8). In most cases, however, these remisssions are temporary and the patient's disease eventually develops resistance to ara-C. Another pyrimidine nucleoside, aza-CR, originally synthesized in 1964 by Piskala and Sorm (16), is active in lines of mouse leukemia resistant to ara-C (2) and its toxic and therapeutic effects can be blocked by cytidine (2, 13, 17) but not by deoxycytidine as is the case with ara-C. aza-CR has been demonstrated by Karon *et al.* (12) and McCredie *et al.* (15) to produce remissions in AML resistant to ara-C. aza-CR, however, is a relatively unstable compound chemically and it can also be catabolized in the body by cytidine deaminase and nucleoside hydrolase. Clinically, it has undesirable side effects such as severe nausea and vomiting at therapeutic doses, although this can be diminished considerably by continuous administration of the drug at 150 mg/sq m daily over a 5-day period (18). Whether these side effects are caused by the products of catabolism or the parent compound is not yet certain.

The design of an analog of aza-CR that would be more resistant to both enzymatic catabolism and nonenzymatic degradation (hydrolysis) was undertaken with the hope that such a compound might have a better therapeutic effect with less undesirable side effects. Toward this end, Chu *et al.* (3) recently achieved the synthesis of 5-(β-D-ribofuranosyl)isocytosine (pseudoisocytidine or ψ-isocytidine), a C-nucleoside which may be viewed as a "1-deaza-5-azacytidine" (Chart 1). Studies with ψ-isocytidine against mouse leukemia *in vitro* and *in vivo* are herewith reported.

MATERIALS AND METHODS

The technique for evaluating the chemotherapeutic activity of a drug by its ability to prolong the survival time of mice with transplanted leukemia has been reported previously (1). The experiments described here were done with mouse leukemias L1210, L5178Y, P815, and their respective lines resistant to ara-C or methotrexate in C57BL × DBA/2 F_1 mice. One million leukemic cells suspended in 0.85% NaCl solution were inoculated i.p. into each animal, producing an ascitic leukemia which later progressed to the generalized disease. The mice were divided into groups of 10 animals each, and treatment was initiated 24 hr after the inoculation of leukemic cells and continued once daily or every 4th day to a total of 3 to 10 doses. Compounds were dissolved in 0.85% NaCl solution and injected i.p.

For cell culture studies, a modification of the technique of Fischer (7) was used. The cells were incubated in McCoy's medium with 15% fetal calf serum. The initial inoculum was 40,000 to 60,000 leukemic cells/ml. For growth inhibition studies, 0.1 ml of a 50-fold concentration of the drug in question was added to 5 ml of the cell-containing media. The tubes were set up in triplicate, loosely capped, and allowed to incubate in 5% CO_2 at 37° for 96 hr. Growth to approximately 10^6 cells/ml occurred in the control tubes. The contents of each tube was counted on a Coulter counter and percentage of inhibition of growth was calculated. Cell culture experiments were done with mouse lines L1210, L5178Y, and P815 and their respective sublines resistant to ara-C and on the human leukemic cell line SKL 7 (4).

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[2] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; AML, acute myeloblastic leukemia; aza-CR, 5-azacytidine; i.c., intracerebrally.

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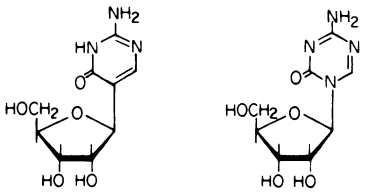

Chart 1. Formula showing the close structural similarity of ψ-isocytidine and Aza-CR.

RESULTS

In Vitro. As shown in Table 1, the ability of ψ-isocytidine to inhibit the growth of mouse and human leukemic cells in culture (concentrations inhibiting to 50% of cells, 0.04 to 3.8 μg/ml) was equal to or perhaps somewhat better than that of aza-CR (concentrations inhibiting to 50% of cells, 0.5 to 3.6 μg/ml).

In the lines of P815 resistant to ara-C, there was a 5- to 10-fold increase in sensitivity to ψ-isocytidine as contrasted to aza-CR, and also as contrasted to the parent ara-C-sensitive P815. On the other hand, the ara-C-resistant line of L5178Y was somewhat less sensitive to both ψ-isocytidine and aza-CR than was the parent line L5178Y. ψ-Isocytidine was somewhat more active than aza-CR against the SKL 7 (4) line of human acute leukemic cells. As can be seen in Chart 2, the inhibitory effect of ψ-isocytidine in concentrations as high as 10 μg/ml against L5178Y *in vitro* was completely blocked by 20 μg of cytidine or uridine per ml but not by 20 μg of deoxycytidine or 3 μg of deoxythymidine per ml. Similar blocking of the inhibition of ψ-isocytidine was observed with cells of ara-C-resistant leukemic lines L5178Y/ara-C and P815/ara-C.

In Vivo. A dose of ψ-isocytidine of 60 mg/kg i.p. on Days 1 through 5 was as active as 150 mg/kg on Days 1, 5, and 9. At these doses, ψ-isocytidine had an approximately equal antileukemic effect against L1210 (Chart 3) or P815 (Chart 4) and a better effect against P815/ara-C (Table 2) than do comparably toxic doses of aza-CR (3 to 5 mg/kg daily for 5 to 10 days). ψ-Isocytidine given i.p. demonstrated activity against P815/ara-C inoculated s.c. but not against the i.c. inoculated leukemia. ψ-Isocytidine administered p.o. at 150 mg/kg daily for 7 days or 100 mg/kg daily for 10 days was more active against P815/ara-C inoculated i.p. than were comparably toxic doses of ara-CR (Table 2).

DISCUSSION

Several C-nucleosides with antitumor effects have been isolated from antibiotic filtrates and tested for antitumor activity by various Japanese investigators. Formycin B has been shown by Ishizuka *et al.* (11) to inhibit the growth of Ehrlich ascites and to increase the life-span of mice with L1210 leukemia by 50% when given i.p. starting 2 hr after i.p. inoculation of 10 cells. Similar antitumor effects have been shown for oxazinomycin (9) and showdomycin (14). Most of these effects were achieved with drugs administered i.p. shortly after small i.p. tumor inoculations and were relatively minor. No clinical therapeutic effect for these naturally occurring C-nucleosides has been reported. ψ-Isocytidine (3), to our best knowledge, is the 1st synthetic pyrimidine C-nucleoside for which antitumor effects have been demonstrated.

ψ-Isocytidine has been shown (C. Chu, K. Watanabe, A. Kyoichi, and J. Fox, personal communication) to be chemically stable at pH 7.4 for 6 days at 22° and at least 3 days at 37°. Preliminary studies (W. Kreis, personal communication) have also shown no deamination on incubation *in vitro* with cytidine deaminase from mouse kidney. These data suggest that, in line with our original design and in contrast to ara-C and aza-CR, ψ-isocytidine is stable against both enzymatic and chemical catabolism. As mentioned above, the antileukemic effects of ψ-isocytidine are blocked by cytidine and

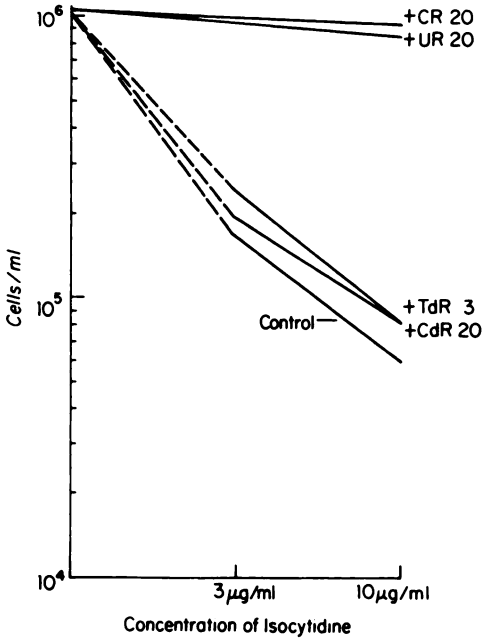

Chart 2. Blocking of the inhibitory effects of ψ-isocytidine by cytidine *(CR)* and uridine *(UR)*, but not by deoxycytidine *(CdR)* or thymidine, *(TdR)*, in cells of mouse leukemia by L5178Y *in vitro*.

Table 1

Comparison of the 50% inhibitory concentrations of ψ-isocytidine and aza-CR in various ara-C-sensitive and -resistant lines of mouse and human leukemic cells in vitro

	Concentration inhibitory to 50% of cells (μg/ml)					
	L5178Y	P815	L1210	L5178Y/CA55	P815/ara-C	SKL 7
ψ-isocytidine	0.8	0.5	0.5	3.8	0.04	0.9
aza-CR	1.0	1.9	0.6	3.6	0.5	2.1

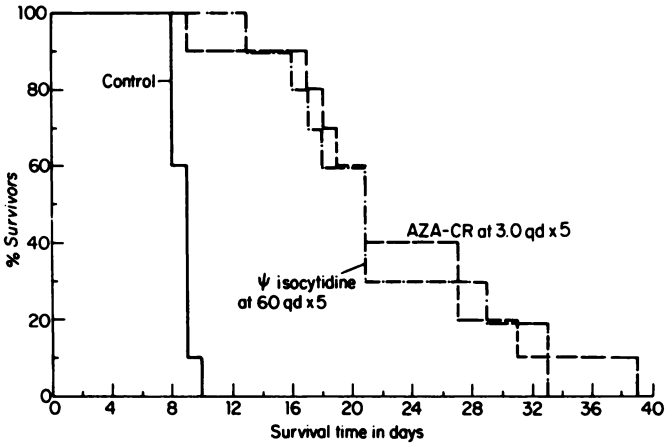

Chart 3. The effects of aza-CR and ψ-isocytidine on survival time of mice with i.p. L1210 leukemia. Drug dosage in mg/kg i.p. daily *(qd)* for 5 days.

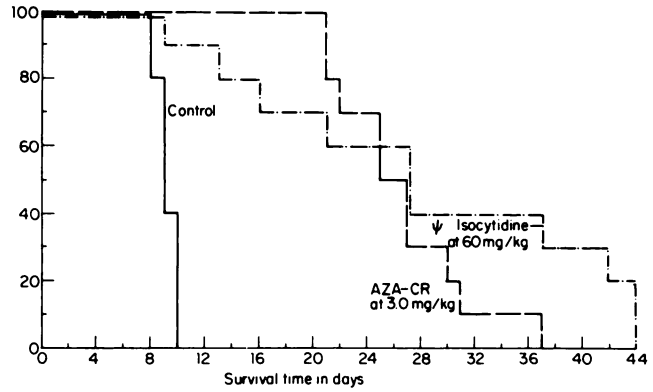

Chart 4. The effects of aza-CR and ψ-isocytidine on survival time of mice with i.p. P815 leukemia. Drug dosage in mg/kg i.p. daily for 5 days.

Table 2

Comparative survival times of mice treated with ψ-isocytidine and aza-CR in ara-C-resistant mouse leukemia

Line	Compound	Dose (mg/kg)	Schedule	Survival time (days)	% increase in life-span
P815/ara-C	Controls			9.3 ± 1.0	
	ψ-Isocytidine	60	Daily for 4 doses i.p.	22.8 ± 4.4	145
	aza-CR	5	Daily for 4 doses i.p.	19.3 ± 5.3	106
P815/ara-C	Controls			10.4 ± 1.6	
	ψ-Isocytidine	150	Every 4th day for 3 doses i.p.	20.8 ± 4.7	100
	aza-CR	10	Every 4th day for 3 doses i.p.	15.3 ± 3.5	47
P815/ara-C	Controls			9.7 ± 0.8	
	ψ-Isocytidine	100	Daily for 10 doses p.o.	15.2 ± 2.6	57
	aza-CR	10	Daily for 10 doses p.o.	12.0 ± 1.1	24
	ψ-Isocytidine	50	Daily for 10 doses i.p.	19.1 ± 1.7	97
	aza-CR	3	Daily for 10 doses i.p.	13.9 ± 1.8	43
P815/ara-C (inoculated s.c.)	Controls			10.5 ± 0.7	
	ψ-Isocytidine	80	Daily for 4 doses i.p.	16.2 ± 4.6	54
	aza-CR	5.0	Daily for 4 doses i.p.	14.6 ± 1.9	39

not by deoxycytidine. Thus it would appear that the mechanism of action of ψ-isocytidine is similar to that of aza-CR. These data and the high activity both *in vitro* and *in vivo* to P815/ara-C demonstrate that 1 mechanism of resistance to ara-C, deletion of the deoxycytidine kinase pathway, does not interfere with the antileukemic activity of ψ-isocytidine. Thus since ψ-isocytidine, a synthetic C-nucleoside analog of 5-azacytidine, has antileukemic effects in mouse leukemia *in vitro* and *in vivo* when administered i.p. or p.o. equal to or better that 5-azacytidine, is metabolically more stable, and has no cross-resistance with ara-C, it might be useful in patients with AML whose disease has developed resistance to ara-C or its analogs. Preclinical pharmacology leading to Phase 1 clinical evaluation would appear indicated.

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