The Effect of 4-Amino-5-imidazolecarboxamide on the Synergistic Antineoplastic Activity of 6-Chloropurine and Azaserine

Several studies have shown that the treatment of transplanted neoplasms with combinations of 6-chloropurine and azaserine causes an inhibition of tumour growth which is greater than that obtained with optimum doses of either agent alone[1-3]. Determination of the metabolic lesions produced by each of these agents in the biosynthetic pathways leading to purine nucleotides indicated that azaserine caused essentially complete blockade of purine nucleotide formation de novo and that chloropurine inhibited the conversion of inosine 5′-phosphate to xanthosine 5′-phosphate[4]. These drug-imposed blocks presumably result in a pronounced decrease in the quantity of guanine nucleotides available for cellular growth and function: the block by azaserine preventing synthesis de novo and that by chloropurine retarding the utilization of the adenine nucleotide pool. Although considerable evidence supports the importance of this mechanism in the synergistic growth-inhibitory activity of this drug combination, data are available which indicate that an additional site of action is also involved[4].

In the present study, the influence of 4-amino-5-imidazolecarboxamide (a compound capable of yielding a terminal intermediate of the purine biosynthetic pathway de novo) both on the synergistic tumour-inhibitory activity of chloropurine and azaserine and on the chloropurine-induced inhibition of inosine 5′-phosphate dehydrogenase has been investigated in order to assess further the importance of this enzymic site to the growth-inhibitory potency of this drug combination.

The effect of 4-amino-5-imidazolecarboxamide hydrochloride (AIC) on the tumour-inhibitory action of combinations of chloropurine and azaserine is shown in Table I. Ha/ICR Swiss mice were implanted intraperitoneally with sarcoma 180 ascites cells as previously described[3]. Therapy was begun 24 h after tumour transplantation and was continued once daily for 6 consecutive days. Neither AIC nor chloropurine alone altered the survival time of tumour-bearing mice. The dose of AIC employed did not significantly affect the prolongation of survival produced by treatments with azaserine. However, the enhancement of the inhibitory action of azaserine induced by chloropurine was antagonized by AIC; in combination an effect comparable to azaserine alone was produced at lower doses of chloropurine, while at the highest dose of the purine analogue, an inhibitory effect greater than that achieved by azaserine alone was obtained in the presence of AIC, a finding that suggests the existence of a competitive relationship between these compounds. In a series of similar experiments, a daily dose of 10 mg of AIC · HCl per kg did not significantly decrease synergic effects on the growth of sarcoma 180 caused by combinations of azaserine (0.2 mg/kg daily) with either 6-thioguanine (0.5 mg/kg daily) or 6-mercaptopurine (40 mg/kg daily).

The effect of AIC on the chloropurine-induced inhibition of polynucleotide guanine biosynthesis (Table II) was determined by the simultaneous administration of chloropurine, AIC and 90 μg of sodium formate-C^{14} ($4.7 \cdot 10^4$ counts/min/μg) by intraperitoneal injection to mice bearing 6-day ascites cell growths. 1 h later, the cells were harvested, and the polynucleotide purines were isolated and analyzed in the manner described by LEPAGE[5]. Sodium formate-C^{14} was selected as the tracer to estimate the effects of AIC on the chloropurine-induced inhibition of inosine 5′-phosphate conversion to guanine nucleotides, since earlier experiments indicated that AIC served as an acceptor molecule for formate, which subsequently appeared in the adenine and guanine of polynucleotides[6]. Thus, AIC served not only to generate inosine 5′-phosphate which competes with 6-chloropurine mononucleotide for the active site of inosine 5′-phosphate dehydrogenase[7], but also to act as an acceptor for the

Table I. Effect of drug therapy on survival time of mice bearing sarcoma 180 ascites cells

Daily dosage (mg/kg)[a]			Average survival (days)	No. of regressions[b]	Av. Δ weight[c] (g)
Azaserine	Chloropurine	AIC · HCl			
0	0	0	13.0	0/25	+ 5.7
0.2	0	0	19.2	0/20	+ 1.6
0.2	0	10	17.8	0/10	+ 4.1
0.2	20	0	25.8	0/10	+ 3.6
0.2	40	0	28.3	2/25	+ 4.2
0.2	60	0	33.5	3/10	+ 3.4
0.2	20	10	19.7	0/10	+ 6.6
0.2	40	10	19.7	0/25	+ 4.4
0.2	60	10	28.4	1/10	+ 6.6

[a] Administered once daily for 6 consecutive days, beginning 24 h after tumor implantation with combination treatments given simultaneously. [b] Tumor-free animals were calculated as 50-day survivors in the determination of the average survival time. [c] Average weight change from onset to termination of drug treatment.

Table II. Effect of 4-amino-5-imidazolecarboxamide on the 6-chloropurine-induced inhibition of incorporation of formate-C^{14} into polynucleotide guanine of sarcoma 180 ascites cells

Dose of AIC · HCl (mg/kg)	% Inhibition of incorporation		
	Dose of chloropurine (mg/kg)		
	20	40	80
0	–	42	–
1.25	46	45	64
5	41	26	57
10	28	30	30
30	0	37	28

Each tumor-bearing mouse was injected with the indicated dose of chloropurine and AIC · HCl, simultaneously with 90 μg of sodium formate-C^{14} ($4.7 \cdot 10^4$ counts/min/μg). 1 h later, the cells were harvested, the polynucleotide guanine was isolated, and its specific radioactivity was determined. Each point represents the average of the results from 4 to 8 animals. The specific activity of the polynucleotide guanine from controls not exposed to chloropurine was about $5.5 \cdot 10^3$ counts/min/μmole; this value was similar in samples from ascites cells of mice that received 1.25 to 30 mg of AIC · HCl per kg.

[1] G. S. TARNOWSKI and C. C. STOCK, Cancer Res. *17*, 1033 (1957).
[2] D. A. CLARKE, H. C. REILLY, and C. C. STOCK, Antibiot. Chemother. *7*, 653 (1957).
[3] A. C. SARTORELLI and B. A. BOOTH, Cancer Res. *20*, 198 (1960).
[4] A. C. SARTORELLI, Prog. exp. Tumor Res. *6*, 228 (1965).
[5] G. A. LEPAGE, Cancer Res. *13*, 178 (1955).
[6] B. A. BOOTH and A. C. SARTORELLI, J. biol. Chem. *236*, 203 (1961).
[7] A. HAMPTON, J. biol. Chem. *238*, 3068 (1963).

formate-C^{14}. The use of other radioactive precursors of purine nucleotides to measure these effects is limited by the dilution incurred by the non-radioactive pools formed from the AIC. The specific radioactivity of both polynucleotide guanine and adenine following administration of formate-C^{14} in the presence of 30 mg of AIC · HCl per kg was comparable to that observed with 1.25 mg of AIC · HCl per kg, a finding which suggested that increased levels of AIC did not result in greater incorporation of this molecule into the polynucleotide fraction. Although considerable variation was obtained in cells from mice treated with chloropurine, the data indicate that increased concentrations of AIC decreased the chloropurine-induced inhibition of polynucleotide guanine formation.

Although no definitive explanation of these results can be given, a number of possibilities exist. One is that exposure of these cells to large quantities of AIC results in an increase in the metabolic pool of inosine 5′-phosphate, a metabolite which serves to protect the enzyme inosine 5′-phosphate dehydrogenase from the inhibitory effects of the nucleotide of chloropurine[7]. That increasing levels of AIC did not increase the labeling of polynucleotide guanine by formate-C^{14} would suggest that intracellular control mechanisms maintain the relative size of precursor pools of guanine nucleotides at a relatively constant level in the presence of a large amount of AIC in cells not exposed to chloropurine; however, it is possible that in the presence of available AIC, significant increases in the intracellular concentration of inosine 5′-phosphate did occur in cells treated with the purine analogue. Alternatively, AIC may decrease chloropurine-induced inhibition of guanine nucleotide synthesis either by competing with chloropurine for the available supplies of phosphoribosylpyrophosphate, thereby limiting the amount of the active inhibitory form (i.e., chloropurine ribonucleoside 5′-phosphate) present at the enzymatic site or by competing for transport. A decision between these alternatives must await further evidence. Nevertheless, the results obtained support the concept that the blockade of inosine 5′-phosphate dehydrogenase activity by 6-chloropurine is associated with the ability of this compound to enhance the antineoplastic properties of the glutamine antagonist azaserine[8].

Zusammenfassung. 4-Amino-5-imidazolcarboxamid hat eine antagonistische Wirkung auf die synergistische Tumorhemmung, die sich aus der gemeinsamen Anwendung von 6-Chlorpurin und Azaserin ergibt. Dies steht im Einklang mit der Fähigkeit des 4-Amino-5-imidazolcarboxamids, auch die Hemmung des Einbaues von C^{14}-Format in das Guanin von Polynucleotiden, durch 6-Chlorpurin hervorgerufen, teilweise zu unterbinden.

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On the Loss of Mesodermal Competence of the *Triturus* Gastrula Ectoderm in vivo

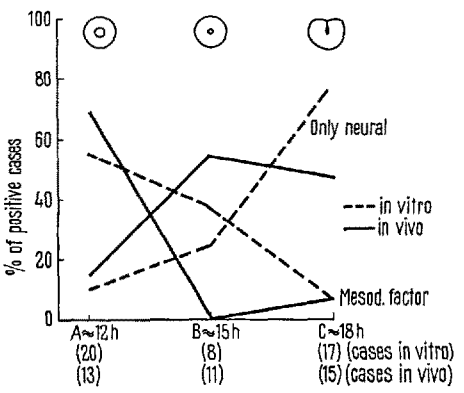
In an earlier work[1] I was able to show, by cultivating pieces of ectoderm of young *Triturus vulgaris* gastrulae as isolated vesicles for various lengths of time and then inserting a heterogeneous inductor (kidney or bone marrow) into these explants, that the mesodermal competence, i.e. the capacity to react to mesodermalizing stimuli, is lost earlier than the neural competence. The present experiments were carried out in order to see whether the intact ectoderm of an old gastrula reacts to the inducing influence of bone marrow in a manner different from that of isolated ectoderm of corresponding age.

Material and methods. For all operations the sandwich technique was used. Small pieces of alcohol-fixed (70% alcohol for 3–48 h in a refrigerator) bone marrow from the femur of young male guinea-pigs were used as inductors. After 8–10 days of cultivation in Holtfreter saline with a double phosphate buffer[2], the explants were fixed in Bouin, stained and histologically examined.

The age of the donor gastrulae was determined partly in hours, taking as starting point Harrison stage 10+ (blastopore straight or slightly curved), partly from the developmental stage only. The explants were divided into four series as follows: A, 10–12 h (Harrison stage 12); B, 13–15 h (Harrison stage $12^1/_2$); C, 16–18 h (Harrison stage 13); D, 20–24 h (Harrison stage 14–15).

Results and discussion. The results are summarized in the Table.

Of the 24 control explants without an inductor (6 for series A, 10 for B, 5 for C, and 3 for D), only one (D) showed any inductions: an archencephalon, evidently



The differential loss of the neural and mesodermal competence of *Triturus* gastrula ectoderm in vitro[1] and in vivo. Mesod. factor: cases with spinocaudal and/or deuterencephalic inductions. Only neural: cases with only archencephalic or unspecific neural (neuroid) inductions.

[1] A. LEIKOLA, Ann. Zool. Soc. 'Vanamo' *25*, 2 (1963).
[2] E. M. DEUCHAR, J. exp. Biol. *30*, 18 (1953).