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Effect of amethopterin and vincaleukoblastine on urinary 4-amino-5-imidazolecarboxamide*

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THE ribonucleotide of 4-amino-5-imidazolecarboxamide (AIC) has been established as a key intermediate in purine biosynthesis.[1] AIC, a by-product formed by the breakdown of the ribonucleotide, has been shown to appear in the urine of humans and other mammals.[2–4] In normal individuals, AIC is comparable to creatinine in the constancy of its excretion,[3] suggesting that fundamental homeostatic mechanisms govern the rate of purine biosynthesis and the rate of AIC excretion. In acute leukemia, however, the excretion of AIC is about two times normal,[5, 6] and the recovery of an administered load of AIC is much less than normal.[5] These facts imply a heightened rate of purine biosynthesis in acute leukemia, which is consistent with an increased rate of synthesis of leukocytes.

Folic acid derivatives are required for two steps in purine biosynthesis; one is in the conversion of glycinamide ribonucleotide to α-N-formylglycinamide ribonucleotide, and the other is in the conversion of 4-amino-5-imidazolecarboxamide ribonucleotide to 4-formamidoimidazolecarboxamide ribonucleotide.[7] The folic acid derivatives in each case act as carriers of a formyl residue.

Antifolic acid compounds, such as aminopterin and amethopterin are useful in the treatment of certain neoplastic diseases, particularly acute leukemia. If these drugs act by interfering with purine biosynthesis, one might expect a change in AIC excretion after their administration. This paper reports some preliminary studies on AIC excretion in rats given amethopterin, aminopterin, and vincaleukoblastine, a third compound useful in the treatment of leukemia but with a completely unknown mechanism of action.

Wistar rats, weighing approximately 200 to 250 g, were placed in metabolic cages, allowed food and water *ad lib.*, and the urine collected in dilute acid. Creatinine values on the 24-hr urine specimens were determined by Taussky's modification of the Jaffe reaction and AIC determined by the method previously described.[3] The drugs were administered intraperitoneally, and did not interfere with either creatinine or AIC analyses.

Some representative results on individual rats are given in Table 1. After a single dose of amethopterin (1·5 to 5 mg/kg), both the absolute excretion of AIC and the ratio of AIC to creatinine increased two- to seven-fold. Some 20 rats were studied and each showed this effect. At lower doses the effect was transient, lasting only 1 to 2 days, but at 5 mg/kg the value increased until the third day when both animals succumbed. In another experiment, two rats were given 2·5 mg amethopterin/kg for 2 days and 7 mg/kg on the third. Both rats died on the fifth day, but the AIC excretion did not rise after the third day.

Aminopterin, another antifolic acid compound, was administered to four rats at a dose of 0·2 mg/kg. No effect on either AIC excretion or creatinine excretion was observed at this dosage.

A different effect on AIC excretion was observed when vincaleukoblastine (VLB), an antileukemic drug with an unknown mode of action, was administered (Table 1). Two rats were given 0·5 mg VLB/

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kg, and both the AIC and AIC–creatinine ratio dropped by the second day and remained low until the fifth day.

Folic acid antagonists might be expected to attack purine biosynthesis at two points: one before and one after the formation of AIC ribonucleotide. An increase in AIC excretion could only be expected if synthesis were not too seriously interfered with prior to the AIC ribonucleotide stage, but a blockade established for the further conversion to inosinic acid. This would appear to be the case at single 1 to 5 mg doses of amethopterin/kg. In the two rats given repeated doses of amethopterin, however, AIC ribonucleotide synthesis may have been depressed so that any blockade in the conversion to inosinic acid was not observed.

TABLE 1. AIC AND CREATININE EXCRETION AFTER ADMINISTRATION OF AMETHOPTERIN OR VINCALEUKOBLASTINE

Day	AIC (μg/24 hr)	AIC (μg/24 hr) Creatinine (mg/24 hr)	Day	AIC (μg/24 hr)	AIC (μg/24 hr) Creatinine (mg/24 hr)
1	10, 11	0·9, 1·2	1	11, 13	0·71, 0·93
2	10, 11	0·9, 1·2		0·5 mg vincaleukoblastine/kg	
3, 4	14, 15	0·9, 1·0	2, 3	3·2, 9	0·25, 0·61
	3·0 mg amethopterin/kg		4	4·2, 6·3	0·31, 0·46
5	38, 43	1.9, 2.7	5	9·5, 11·5	0·62, 0·71
6	12, 23	0·9, 1·6			
7	17, 10	1·5, 0·9			

The failure to find a rise in AIC excretion after the administration of aminopterin is in contrast to the findings of Braunshtein and Vilenkina[8] who noted a substantial rise in AIC excretion after doses of aminopterin ranging from 0·125 to 0·48 mg/kg. This difference may be related in some way to the sensitivity of the strains of rats used in the two studies. We did not try larger doses of aminopterin.

The depression of AIC excretion following vincaleukoblastine could indicate an over-all depression of purine biosynthesis. This is also in accord with the results previously reported[5] on AIC excretion in a human leukemic subject; this patient excreted 6·25 mg AIC/day before treatment and 3·50 and 3·34 mg/day after 1 and 2 weeks of treatment with VLB.

It is, of course, not known whether the observed changes in AIC excretion after administration of VLB and of amethopterin are related to the antileukemic action of these drugs. The drop in excretion following VLB may be of particular interest since the mode of action of this drug is unknown.

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Kinsmen Laboratory of Neurological Research,
Department of Psychiatry,
The University of British Columbia,
Vancouver, Canada

P. L. McGEER
E. G. McGEER

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