Differential Inhibition of Dihydrofolic Reductase from Different Species

Sir:

One of the key enzymes necessary for cellular reproduction is (dihydro)folic reductase; when this enzyme reduces (dihydro)folic acid to tetrahydrofolic acid, a spate of some 15 enzymic reactions follow which ultimately are involved mainly in purine and pyrimidine biosynthesis (2, 8). Although all types of cells would not be dependent on all 15 enzymic reactions, almost all cells will be dependent upon (dihydro)folic reductase and some of the subsequent reactions, unless the cell can utilize both preformed purine and thymine or their derivatives.

A variety of 2,4-diaminoheterocycles known to be inhibitors of (dihydro)folic reductase (2) are sufficiently species- or tissue-specific to be useful chemotherapeutic agents—for example, amethopterin for leukemia (5), 5-(*p*-chlorophenyl)-2,4-diamino - 6 - ethylpyrimidine (pyrimethamine) (7) and 1-(*p*-chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine (4) as antimalarials, 2,4 - diamino - 5 - (3,4,5 - trimethoxybenzyl)-pyrimidine as an antibacterial (13), and 1-(*p*-chlorophenyl) - 4,6 - diamino - 1,2 - dihydro-(4′ - methylpentamethylene) - *s* - triazine as an anthelmintic (1, 12). The selectivity of amethopterin on certain tissues can be attributed to a difference in the efficiency of the folic acid active-transport system (15). Contrariwise, the malaria parasite presumably has no active-transport system for folic acid; since pyrimethamine enters cells by passive diffusion, the host cells are protected by active-transport of folic acid or leucovorin (6).

For years many investigators in the field of comparative biochemistry have sought to establish that all species use identical biosynthetic pathways and that these enzymes are identical regardless of species. Although the biosynthetic pathways appear to be similar, small differences in specific enzymes are readily apparent. For example, (dihydro)folic reductase from most mammalian cells and *S. faecalis* (16) can utilize folate as the substrate, albeit less efficiently than dihydrofolate. The (dihydro)folic reductases from some species show no perceptible utilization of folic acid, particularly those that synthesize dihydrofolate *de novo*, such as *E. coli* (16) and presumably the malaria parasite (7). Furthermore, some (dihydro)folic reductases can use TPNH, but not DPNH, as the cofactor, whereas others can use DPNH although somewhat less efficiently (9, 10).

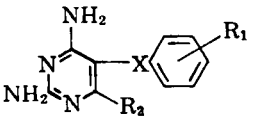
Since these differences in the (dihydro)folic reductases do exist, it would not be beyond the realm of possibility for various reversible inhibitors to show a more potent inhibition of the dihydrofolic reductase from one species compared to another. Such thirty to seventyfold differences have now been observed among the diamino pyrimidines and triazines and these are the subject of this paper.

No large differences were observed in the inhibition of dihydrofolic reductase from pigeon liver or mouse liver (Table I); it might be anticipated that less difference in the dihydrofolic reductases from the same tissue of two different species might be found than from two different tissues of the same species (3). However, large differences were observed in the inhibition of dihydrofolic reductase from pigeon liver and *E. coli*.

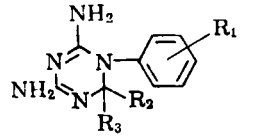
Excluding the 2-chlorophenyl triazine (IX), the structural modifications in Table I change the effectiveness on *E. coli* dihydrofolic reductase by only a factor of 10. In contrast, structural modification can change the effectiveness on pigeon liver dihydrofolic reductase by a factor of 1000. For example, the 3-chlorophenyl derivative (VII) and the 3,4-dichlorophenyl derivative (II, V) are twenty-five to fiftyfold more effective on the pigeon liver enzyme than the corresponding 4-chloro derivatives (III, VI); on the *E. coli* enzyme, the effectiveness is hardly altered. Insertion of an oxygen between the two rings (IV) causes little change on the *E. coli* enzyme, but causes a thirty-two-fold decrease in effectiveness on the pigeon liver enzyme. Thus the 5-phenoxypyrimidine (IV) is thirtyfold more effective on the *E. coli* enzyme than the pigeon liver enzyme; in contrast, the 3-chlorophenyl triazine (VII) is seventy-one-fold more effective on the pigeon liver enzyme than the *E. coli* enzyme.

If the effectiveness of pyrimethamine is primarily due to its inhibition of malaria (*P. berghei*) dihydrofolic reductase, the lack of correlation between the effectiveness of pyri-

TABLE I.—INHIBITION OF DIHYDROFOLIC REDUCTASES BY DIAMINO PYRIMIDINES AND TRIAZINES



Compd.	NSC No.[a]	R_1	R_2	X	—μM Concn. for 50% Inhibition[b,c]— Mouse Liver	Pigeon Liver	*E. coli* B.	Ratio *E. coli*: Pigeon Liver
I	3062	3,4-Cl_2	C_2H_5	...		0.018	0.10	5.5
II	19494	3,4-Cl_2	CH_3	...	0.010	0.032	0.50	16
III[d]		4-Cl	CH_3	...		0.85		
IV	61641	4-Cl	CH_3	O		27	0.90	0.033



		R_1	R_2	R_3				
V	3077	3,4-Cl_2	CH_3	CH_3	0.046	0.015	0.16	11
VI	3074	4-Cl	CH_3	CH_3	0.66	0.44	0.40	0.91
VII	3080	3-Cl	CH_3	CH_3	0.0076	0.0085	0.60	71
VIII	3082	3-Br	CH_3	CH_3		0.0085	0.39	46
IX	3079	2-Cl	CH_3	CH_3		160	200	1.2
X		3-Cl	C_6H_5	H		5.5	1.0	0.15
XI		4-Cl	C_6H_5	H		9.0	3.7	0.41

[a] Ascension numbers of the Cancer Chemotherapy National Service Center; we thank Dr. Harry B. Wood, Jr., for these NSC compounds. [b] The technical assistance of Rita Zielinsk and Bonnie Myers for these assays is acknowledged. [c] An 0.45 to 0.90 saturated ammonium sulfate fraction was used for assay (11) employing 6 μM dihydrofolic acid and 12 μM TPNH in 0.05 M Tris buffer (pH 7.4) containing 10 mM mercaptoethanol and 1 mM Versene; 36 μM TPNH was used for *E. coli*. [d] Prepared by W. H. Myers in this laboratory.

methamine analogs as antimalarials (14) and the dihydrofolic reductases listed in Table I indicate that inhibition of the *P. berghei* dihydrofolic reductase has still a different order of effectiveness; for example, the 3,4-dichlorophenyl analog (I) and the 4-chlorophenyl analog are twenty-fold more effective than the 3-chlorophenyl analog against *P. berghei* in mice.

Whether differential inhibitions will be observed with the dihydrofolic reductases from other species or tissues that can be correlated with chemotherapeutic effectiveness remains to be determined.

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