Activity of sulfa drugs and dihydrofolate reductase inhibitors against *Candida albicans*

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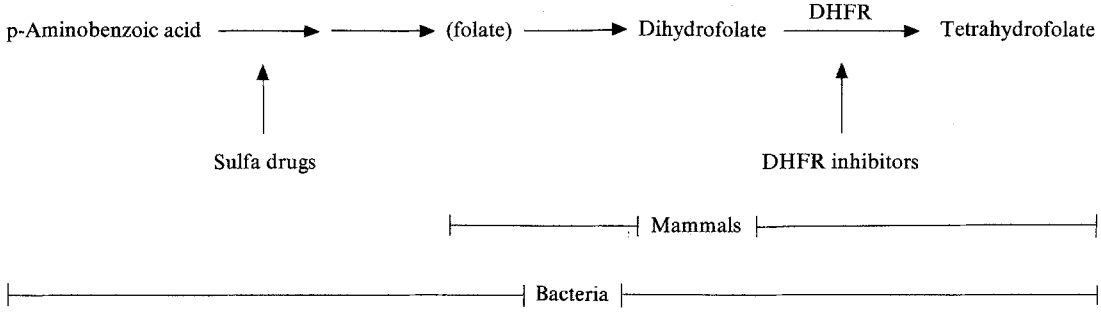
Summary. Growth of *Candida albicans* can be inhibited by sulfa drugs which prevent biosynthesis of folic acid. The dihydrofolate reductase (E.C. 1.5.1.3) inhibitors aminopterin and methotrexate also exhibit anticandidal activity, but trimethoprim does not. Kinetic evaluations with *C. albicans* dihydrofolate reductase indicate that methotrexate and aminopterin are tight-binding inhibitors whereas trimethoprim binds poorly.

Folic acid metabolism in prokaryotes and eukaryotes has long been recognized as an important target for the development of antimicrobial agents. Although non-identical folate pathways exist within these kingdoms, e.g. mammals require an exogenous source of folate whereas most microorganisms synthesize folate de novo, the reduction of dihydrofolate to tetrahydrofolate by dihydrofolate reductase (DHFR) is essential for both (fig.). Trimethoprim, a tight-binding inhibitor of bacterial DHFR[1], is highly active against a wide variety of bacterial species, while pyrimethamine combined with various sulfonamides or sulfones has been used to treat drug resistant malarias[2]. To date, however, no antifolates have been described possessing high activity against *Candida albicans,* though some anti-yeast activity of such compounds has been reported[3,4].

In our studies, we have investigated the anticandidal activity of the DHFR inhibitors-trimethoprim, methotrexate and aminopterin-alone and in combination with sulfa drugs. In addition, we have studied the inhibition characteristics of these antifolates with DHFR isolated from *C. albicans* and bovine liver.

Materials and methods. C. albicans SC 5314, from our culture collection, was grown on a chemically defined medium containing (g/l): glucose, 50; NH_4NO_3, 1.5; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01; thiamine, 0.1; biotin, 0.01 and agar, 15.0. To determine the interaction between the DHFR inhibitors and the sulfa drugs, i.e., sulfamethoxazole and sulfadiazine, or between the sulfas and p-aminobenzoic acid, antibiotic discs (Schleicher & Schuell, New Hampshire) saturated with the desired solutions were placed on the synthetic medium seeded with *C. albicans* SC 5314. The distance between the discs was sufficient to allow the diffusing substances to meet and interact at a point approximately midway between them. Petri dishes were incubated overnight at 37 °C and then examined for inhibition of growth, reversal of growth inhibition, or synergism. For whole cell studies, sulfamethoxazole was dissolved in acetone, whereas sulfadiazine, trimethoprim, aminopterin, methotrexate and p-aminobenzoic acid were dissolved in 0.05 M NH_4OH. Solutions of 5 mg/ml, and less, were prepared. For enzyme studies, DHFR from *C. albicans* was prepared by sonicating cells suspended in 0.05 M phosphate buffer, pH 7.5, for 45 min (cooling provided by an ice bath). After centrifugation, the supernatant was treated with ammonium sulfate. The precipitate resulting from 45–80% saturation contained DHFR which was stable for at least 4 months at −5 °C. Bovine liver DHFR was obtained commercially (Sigma). Both preparations were dissolved in 0.05 M phosphate buffer, pH 7.5, containing 10 mM mercaptoethanol before use. DHFR activity was determined as described by Mathews et al.[5] using a Gilford 250 spectrophotometer. K_m-values were calculated from Lineweaver-Burk plots. K_i-values were determined according to Dixon[6]. Folic acid, dihydrofolate, NADPH, trimethoprim, methotrexate and aminopterin, all from Sigma, were freshly prepared in 0.5 M phosphate buffer, pH 7.5.

Results and discussion. C. albicans can satisfy its need for



Representation of folate metabolism in bacteria and mammals, indicating pharmacological targets.

Kinetic constants for dihydrofolate reductase from various sources

Compound	Kinetic constant	Source of dihydrofolate reductase		*E. coli* MRE 600
		Bovine liver	*Candida albicans*	
NADPH	K_m (M)	2×10^{-5}	2×10^{-5}	1×10^{-5} (ref. 1)
Dihydrofolate	K_m (M)	3×10^{-6}	5×10^{-6}	3×10^{-5} (ref. 9)
Trimethoprim	K_i (M)	2×10^{-4}	2×10^{-4}	4×10^{-9} (ref. 9)
Methotrexate	K_i (M)	5×10^{-8}	2×10^{-8}	6×10^{-9} (ref. 1)
Aminopterin	K_i (M)	3×10^{-9}	2×10^{-8}	N.D.

All assays were performed at 25 °C in 0.05 M phosphate buffer, pH 7.5 in 10 mM mercaptoethanol. Inhibition studies were carried out using 0.10 mM NADH and 0.10 mM dihydrofolate as substrates. All values were corrected for blank rates observed in the presence of NADPH alone or dihydrofolate alone.

folic acid by utilizing simple precursors, as evidenced by its ability to grow on a defined medium consisting of glucose, ammonium nitrate, salts, thiamine, biotin and agar. Although sulfa drugs can inhibit growth, inhibition is reversed by p-aminobenzoic acid, indicating that the sulfa drugs interfere in folate biosynthesis. No potentiation of this bioactivity was observed in the presence of trimethoprim, which itself lacked anticandidal activity. In contrast, aminopterin and methotrexate, also inhibitors of bacterial DHFR, were active against *C. albicans*. Moreover, these compounds did show synergy with the sulfa drugs.

Since the lack of activity of trimethoprim could be due to permeability barriers, subinhibitory concentrations of amphotericin B (0.5 µg/ml) or econazole (0.1 µg/ml) were incorporated into the agar to increase the cellular permeability[7,8]. Trimethoprim was still without effect. Also, the activities of aminopterin and methotrexate were not noticeably enhanced by the permeabilizing agents. These data suggest that penetration into the cell is not a limiting factor for either the active compounds or trimethoprim, a compound of approximately 0.6 the molecular weight of the other 2 antifolates.

The affinity of trimethoprim for DHFR was then studied. As shown in the table, trimethoprim has poor affinity for the candidal and mammalian enzymes, in contrast to the enzyme from *Escherichia coli*. Aminopterin and methotrexate, on the other hand, act as tight-binding inhibitors in all the systems tested. These data, therefore, are sufficient to explain the lack of trimethoprim activity against *C. albicans*.

Because of the resemblance to mammalian enzyme, *C. albicans* DHFR is not an appealing target for chemotherapeutic purposes. However, the metabolic steps involved in folate biosynthesis may be exploitable in seeking an anticandidal agent.

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The application of sodium deoxycholate and Sephacryl-200 for the delipidation and separation of high density lipoprotein

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Summary. A method to remove lipids from human plasma high-density lipoprotein (HDL) was developed. The procedure required column chromatography on Sephacryl-200 in the presence of the bile salt sodium deoxycholate. The lipid free protein obtained retained the immunological properties of the native HDL.

Previous procedures for preparing soluble apo-HDL depend on delipidation of HDL by extraction with organic solvents[2]. The methods led to incomplete recovery of the apo-protein because some of the polypeptide chains of HDL were preferentially solubilized in the organic solvents[3]. Studies on the effects of detergents on low-density lipoproteins (LDL) showed that 4 detergents could displace all the lipid from LDL and the soluble apo-LDL could be isolated by gel filtration[4]. Best results were obtained with the bile salt sodium deoxycholate (NaDOC)[4]. With NaDOC the apo-LDL did not undergo conformational changes, and the detergent could be removed completely from the apo-protein[4].

In connection with the work in this laboratory on the

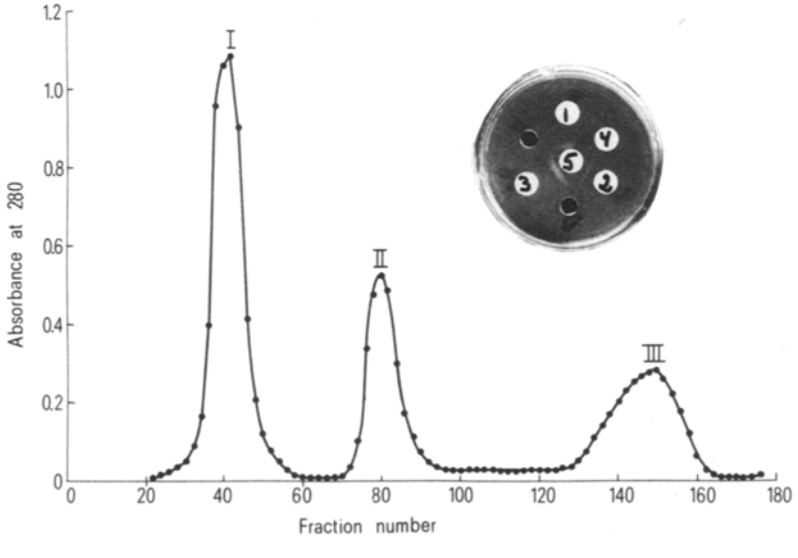

Figure 1. Elution profile of human plasma lipoprotein. Lipoproteins quantitatively removed by ultracentrifugation of plasma at d1.225 were applied to a Bio-Gel A-5 m agarose column 2.5 cm × 90 cm, and eluted in 0.15 M NaCl-0.01% EDTA at 12 ml/h, 2 ml/fraction at 10 °C. Immunodiffusion of various HDL preparations. 1. Native HDL; 2. apo-A; 3. top fractions of protein peaks III; 4. top fraction of protein peak I or II; 5. anti-Apo A.