Folate Antagonists as Therapeutic Agents

1

Biochemistry, Molecular Actions, and Synthetic Design

Edited by

F. M. SIROTNAK
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VOLUME 1

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Folate Antagonists as Therapeutic Agents

VOLUME 1

Biochemistry, Molecular Actions, and Synthetic Design

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Preface

It is apparent that folate antagonists have now assumed major importance in the treatment of a variety of human disorders. Their use derives from the large number of conceptual advances made over the past three decades in our knowledge of folate metabolism and its role in macromolecular synthesis and the subsequent discovery of a number of agents that selectively antagonize this metabolism in target cell populations. In view of this progress, it seemed highly desirable to bring together the vast literature on this topic in the form of a cohesive and comprehensive treatise.

Advances in our knowledge of folate metabolism and its antagonism over the years have relied upon studies in both microbial and mammalian systems with cumulative impact, not only on the treatment of diseases of microbial origin, but also on the treatment of proliferative disorders, namely cancer and psoriasis. Therefore, we sought to organize these volumes according to the various disciplines from which contributions have originated rather than in a disease-oriented manner. In this way we believe we have more effectively integrated the important concepts that have arisen from a highly diverse group of studies in different experimental systems. Accordingly, in Volume 1 we included chapters dealing with molecular aspects of the enzymology and regulation of the metabolism of folate compounds, the mode of action of various categories of folate antagonists, approaches to their synthetic design and membrane transport, and acquired resistance. In Volume 2, chapters are included that focus on the net biochemical and cytotoxic effects of folate antagonists, probable bases for their selectivity, and the consequence of their interaction with other antimetabolites. The remaining chapters deal with the broad range of pharmacological and therapeutic properties of the various folate antagonists in experimental animal models and in patients.

In organizing the format for these volumes we have attempted to develop a balanced and comprehensive view of active research in the various areas covered. As such, we hope that these volumes will provide an authoritative reference source and prove to be of value to laboratory and clinical investigators in the field

of chemotherapy. We also hope that the broad range of coverage provided will be of interest to students, researchers, and medical practitioners in the fields of infectious, tropical, and psoriatic disease and oncology.

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Introduction

Folate metabolism represents an attractive target for chemotherapy, since it plays a key role in the biosynthesis of nucleic acid precursors. It is of interest, therefore, to note that in the less than four decades since the identification of folic acid as a vitamin, agents that antagonize its metabolic role have been discovered that are effective, not only against proliferative disorders such as cancer and psoriasis, but also against a variety of microbial diseases. This area of chemotherapy is also unique in that inhibitory effects of folate antagonists on a single target, dihydrofolate reductase, can impact macromolecular biosynthesis and cytoxicity at a number of metabolic steps involving folate-dependent biosynthetic reactions. However, as documented in various chapters of these volumes, it is now becoming clear that direct effects on the folate-dependent reactions themselves may account for a good measure of the total antifolate effect observed in some target cells under certain circumstances.

The penultimate question pertaining to any chemotherapeutic strategy relates to the manner in which adequate therapeutic selectivity can be achieved. This is realized in the case of bacterial and plasmodial disease by a rare example of differential inhibition by "small-molecule" antifolates of the primary enzyme target, dihydrofolate reductase. The basis of selectivity during therapy of neoplastic disease with antifolates, when obtained, may actually be derived on a multifactoral level, including sites unrelated to this primary enzyme target. Evidence is discussed in these volumes for an emerging role of mediated membrane transport and intracellular metabolic disposition in the form of polyglutamylation. To what extent additional metabolic factors may contribute should become clear in the not-too-distant future.

From an examination of the documentation provided in these volumes, it is apparent that significant advances have been made in the use of folate antagonists for the treatment of a number of human diseases. It is also clear that advances will continue to be made in a number of these clinical areas. To a large measure these advances derive from the vast amount of information now available as to the biochemical and pharmacological actions of these agents. These data have

provided much needed direction for new drug design. With this impetus, improved methods of chemical synthesis have brought forth a number of new agents with exciting potential for more effective clinical application. Finally, a review of the wealth of information provided in these volumes makes it eminently clear that progress made in this area of therapeutic research will provide a useful model for similar efforts toward more rational drug design with other categories of agents, particularly in the area of neoplastic disease. It is our hope that the coverage provided will serve investigators in this and related fields who continue to strive for more improved chemotherapy of human disease. Finally, the editors gratefully acknowledge their indebtedness to the many contributors who made the publication of these volumes possible.

F. M. Sirotnak

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CHAPTER ONE

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I. Introduction

A dietary factor, subsequently identified as folic acid, proved to be effective in curing megaloblastic anemia of pregnant women in India (Wills *et al.*, 1937). Thus did folate studies begin. Folates are synthesized by plants, and folic acid was first isolated from spinach (Mitchell *et al.*, 1941). Many bacteria, including *Escherichia coli,* also synthesize folates, but others such as *Lactobacillus casei, Streptococcus faecium,* and *Pediococcus cerevisiae* resemble animals in having a nutritional requirement for folates. Microbial and animal models were vital in elucidating the nature and metabolic significance of these substances.

Folate derivatives are coenzymes for the transfer, oxidation, and reduction of single-carbon units used for the biosynthesis of thymidylate, purine nucleotides, methionine, serine, glycine, and many other compounds (Blakley, 1969). In addition, folates play a structural role in some bacteriophages (Kozloff *et al.*, 1979a) and serve as chemoattractants in slime molds (DeWit *et al.*, 1983) and *Paramecium* (DiNallo *et al.*, 1982).

Folate biochemistry has always been closely associated with the development of outstanding chemotherapeutic agents. Sulfonamides block the biosynthesis of folates and selectively inhibit the growth of many microorganisms (Woods, 1962). Methotrexate (Johns and Bertino, 1982) and trimethoprim (Harvey, 1982; Hitchings, 1983) block folate coenzyme metabolism and are useful antitumor and antimicrobial agents, respectively.

The following is a sampling of the many reviews, monographs, and symposium volumes available in this area: Wolstenholme and Cameron (1954), Stokstad (1954), Rabinowitz (1960), Huennekens (1968), Blakley (1969), Bertino (1971), Rader and Huennekens (1973), Pfleiderer (1975), Broquist *et al.* (1977), Botez and Reynolds (1979), Kisliuk and Brown (1979), Kisliuk (1981), Goldman *et al.* (1983), and Blair (1983). This chapter emphasizes developments in the chemistry, enzymology, and metabolism of folate coenzymes.

II. Chemistry

A. Introduction

The structure of H_4PteGlu (tetrahydrofolate) is shown in Fig. 1. It consists of a 2-amino-4-oxy-6-methylenetetrahydropteridine linked to *p*-aminobenzoic acid,

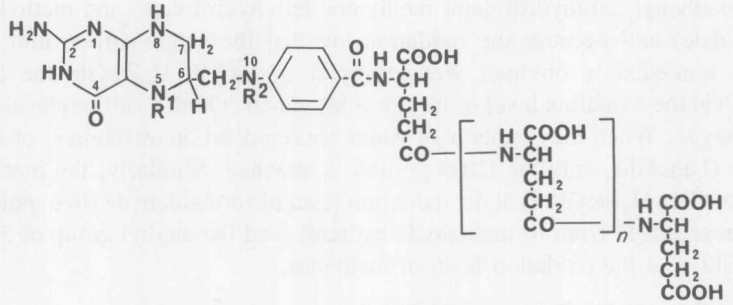

FIG. 1. Structure of tetrahydrofolic acid with γ-linked glutamic acid residues attached.

which is in turn linked to the α-amino group of L-glutamate. The single-carbon units either are carried on the N-5 or N-10 position, or form a bridge between N-5 and N-10 (Table I). These single-carbon units are found at three levels of oxidation corresponding to formic acid, formaldehyde, or methanol.

Derivatives of H_4PteGlu are easily confused because of their similar names

TABLE I

TETRAHYDROFOLATE DERIVATIVES

Name (see Fig. 1 for structure)	R^1	R^2	Abbreviation	Oxidation level of single-carbon unit
5,6,7,8-Tetrahydrofolate	H	H	H_4PteGlu[a]	—
5-Methyl 5,6,7,8-tetrahydrofolate	CH_3	H	5-CH_3-H_4PteGlu	Methanol
5-Formyl 5,6,7,8-tetrahydrofolate[b]	CHO	H	5-CHO-H_4PteGlu	Formate
5-Formimino-5,6,7,8-tetrahydrofolate	HC=NH	H	CHNH-H_4PteGlu	Formate
10-Formyl 5,6,7,8-tetrahydrofolate	H	CHO	10-CHO-H_4PteGlu	Formate
N-5—N-10 Bridge forms				
5,10-Methylene 5,6,7,8-tetrahydrofolate	—CH_2—		CH_2-H_4PteGlu	Formaldehyde
5,10-Methenyl 5,6,7,8-tetrahydrofolate	—CH=		CH-H_4PteGlu	Formate
Oxidized forms				
7,8-Dihydrofolate	$\Delta^{5,6}$		H_2PteGlu	—
Folate	$\Delta^{5,6}$ and $\Delta^{7,8}$		PteGlu	—

[a]Pte, Pteroic acid, or *p*-[(2-amino-4-hydroxy-6-pteridylmethyl)amino]benzoic acid. The total number of glutamate residues is indicated by a subscript; that is, H_4PteGlu$_3$ (Fig. 1) denotes tetrahydropteroyl triglutamate.

[b]Also called folinic acid, leucovorin, citrovorum factor, or CF.