GOODMAN and GILMAN's The Pharmacological Basis of Therapeutics

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In this textbook, reference to proprietary names of drugs is ordinarily made only in chapter sections dealing with preparations. Such names are given in SMALL-CAP TYPE, usually immediately following the official or nonproprietary titles. Proprietary names of drugs also appear in the Index.

Drugs currently used in chemotherapy of neoplastic diseases may be divided into several classes, as shown in Table XIII–1. This somewhat arbitrary classification is used in Chapter 55 as a convenient framework for describing the various types of agents; the major clinical indications for the drugs are listed in Table XIII–1 in order to facilitate rapid reference. Dosage regimens, which are often complex, are discussed under the individual drugs.

Mechanistic classification of these agents is increasingly important, particularly as investigators attempt to utilize this information to design "rational" regimens for chemotherapy. A simplified overview of the sites of action of many of the drugs described in Chapter 55 is shown in Figure XIII–1.

CHAPTER

55 ANTIPROLIFERATIVE AGENTS AND DRUGS USED FOR IMMUNOSUPPRESSION

Paul Calabresi and Robert E. Parks, Jr.

I. Alkylating Agents

History. Although synthesized in 1854, the vesicant properties of *sulfur mustard* were not described until 1887. During World War I, medical attention was first focused on the vesicant action of sulfur mustard on the skin, eyes, and respiratory tract. It was appreciated later, however, that serious systemic intoxication also follows exposure. In 1919, Krumbhaar and Krumbhaar made the pertinent observation that the poisoning caused by sulfur mustard is characterized by leukopenia and, in cases that came to autopsy, by aplasia of the bone marrow, dissolution of lymphoid tissue, and ulceration of the gastrointestinal tract.

In the interval between World Wars I and II, extensive studies of the biological and chemical actions of the *nitrogen mustards* were conducted. The marked cytotoxic action on lymphoid tissue prompted Gilman, Goodman, and T. F. Dougherty to study the effect of nitrogen mustards on transplanted lymphosarcoma in mice, and in 1942 clinical studies were initiated. This launched the era of modern cancer chemotherapy (Gilman, 1963).

In their early phases, all these investigations were conducted under secrecy restrictions imposed by the use of classified chemical-warfare agents. At the termination of World War II, however, the nitrogen mustards were declassified and a general review was presented by Gilman and Philips (1946), and shortly thereafter there appeared summaries of clinical research by Goodman and associates (1946), Jacobson and coworkers (1946), and Rhoads (1946). Recent reviews include those by Colvin (1982), Wheeler (1982), Connors (1983), and Ludlum and Tong (1985).

Thousands of variants of the basic chemical structure of the nitrogen mustards have been prepared. However, most attempts at the rational design of "active-site-directed" molecules have failed, and only a few of these agents have proven more useful than the original compound in specific clinical circumstances (*see* below). At the present time five major types of alkylating agents are used in the chemotherapy of neoplastic diseases: (1) the nitrogen mustards, (2) the ethylenimines, (3) the alkyl sulfonates, (4) the nitrosoureas, and (5) the triazenes.

Chemistry. The chemotherapeutic alkylating agents have in common the property of undergoing strongly electrophilic chemical reactions through the formation of carbonium ion intermediates or of transition complexes with the target molecules. These reactions result in the formation of covalent linkages (alkylation) with various nucleophilic substances, including such biologically important moieties as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. The cytotoxic and other effects of the alkylating agents are directly related to the alkylation of components of DNA. The 7 nitrogen atom of guanine is particularly susceptible to the formation of a covalent bond with both monofunctional and bifunctional alkylators and may well represent the key target that determines the biological effects of these agents. It must be appreciated, however, that other atoms in the purine and pyrimidine bases of DNA—for example, the 1 or 3 nitrogens of adenine, the 3 nitrogen of cytosine, and the 6 oxygen of guanine—may also be alkylated to a lesser degree, as are the phosphate atoms of the DNA chains and the proteins associated with DNA.

Therapeutic Uses and Clinical Toxicity. At present, dacarbazine is employed principally for the treatment of malignant melanoma; the overall response rate is about 20%. Beneficial responses have also been reported in patients with Hodgkin's disease, particularly when the drug is used concurrently with doxorubicin, bleomycin, and vinblastine (Santora and Bonadonna, 1979), as well as in various sarcomas when used with doxorubicin (Costanzi, 1976; Gottlieb *et al.*, 1976). Toxicity includes nausea and vomiting in more than 90% of patients; this usually develops 1 to 3 hours after treatment. Myelosuppression, with both leukopenia and thrombocytopenia, is usually mild to moderate. A flulike syndrome, consisting in chills, fever, malaise, and myalgias, may occur during treatment. Hepatotoxicity, alopecia, facial flushing, neurotoxicity, and dermatological reactions have also been reported.

II. Antimetabolites

FOLIC ACID ANALOGS

METHOTREXATE

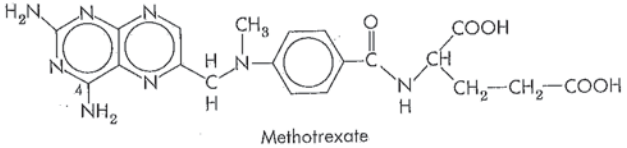
This class of antimetabolites not only produced the first striking, although temporary, remissions in leukemia (Farber *et al.*, 1948) but also includes the first drug to achieve cures of choriocarcinoma in women (Hertz, 1963). The attainment of a high percentage of permanent remissions in this otherwise-lethal disease provided great impetus to chemotherapeutic investigation. Interest in folate antagonists has increased greatly with the introduction of "rescue" technics that employ leucovorin (folinic acid, citrovorum factor) and/or thymidine to protect normal tissues against lethal damage. These methods permit the use of very high doses of folate analogs such as methotrexate and extend their utility to tumors such as osteogenic sarcoma that do not respond to lower doses.

Methotrexate has also been used with benefit in the therapy of *psoriasis*, a nonneoplastic disease of the skin characterized by abnormally rapid proliferation of epidermal cells (McDonald, 1981). Additionally, folate antagonists are potent inhibitors of some types of immune reactions and have been employed as *immunosuppressive agents*, for example, in organ transplantation. (For recent reviews, *see* Symposium, 1981b; Chabner, 1982c; Johns and Bertino, 1982; Jackson, 1984.)

Structure-Activity Relationship. Folic acid is an essential dietary factor from which is derived a coenzyme, tetrahydrofolic acid, and a group of structurally related derivatives; these are concerned with the metabolic transfer of one-carbon units. A detailed description of the biological functions and therapeutic applications of folic acid appears in Chapter 57.

Although there are many metabolic loci where folate analogs (antifols) might act, the enzyme dihydrofolate reductase (DHFR) is the primary site of action of most analogs studied to date (*see* Figure 57–1). This enzyme has been purified from a number of species. Important structural differences among the various enzymes have enabled the design of important therapeutic agents for the treatment of bacterial and malarial infections (*see* discussion of trimethoprim, Chapter 49; pyrimethamine, Chapter 45). These inhibitors have much greater activity against the bacterial and protozoal DHFRs than they do against the mammalian enzyme. Such developments have introduced a new level of sophistication into the science of chemotherapy and suggest the possibility of developing new analogs of folate that have unique advantages for the chemotherapy of neoplastic diseases.

Because folic acid and many of its analogs are very polar, they cross the blood-brain barrier poorly and require specific transport mechanisms to enter mammalian cells. Once in the cell, additional glutamyl residues are added to the molecule by the enzyme folylpolyglutamate synthetase. Intracellular methotrexate polyglutamates have been identified with as many as five glutamyl residues. Since these polyglutamates cross cellular membranes poorly, if at all, this serves as a mechanism of entrapment and may account for the prolonged retention of methotrexate in tissues such as liver. Evidence indicates that polyglutamylated folates have substantially greater affinity than the monoglutamate form for enzymes such as thymidylate synthetase. Other findings indicate that distinct differences exist in the folate influx system in certain tumors in comparison with normal tissues (*e.g.*, bone marrow). Novel folate antagonists have been devised to attempt to exploit these differences. The analog 10-deaza,10-ethyl aminopterin is transported into many tumor cells much more



Methotrexate

efficiently than into normal tissues, is polyglutamylated, and is an excellent inhibitor of DHFR. This promising new compound will be evaluated clinically in the near future (Sirotnak, 1983). In efforts to bypass the obligatory membrane transport system and facilitate penetration of the blood-brain barrier, a number of lipid-soluble folate antagonists have been synthesized; several of these are in the early stages of clinical trial (Johns and Bertino, 1982; Jackson 1984). (For recent reviews, *see* Chabner, 1982c; Goldman *et al.*, 1983; Hitchings, 1983; Jolivet and Chabner, 1983; McGuire *et al.*, 1983; Sirotnak, 1983; Cadman, 1984; Jackson, 1984.)

Mechanism of Action. To understand the mechanism of action of folate analogs such as methotrexate, it is necessary to appreciate the complexities of the metabolism of folate cofactors and their multiplicity of functions; this is discussed in Chapter 57. To function as a cofactor in one-carbon transfer reactions, folate must first be reduced by DHFR to tetrahydrofolate (FH_4). Single-carbon fragments are added enzymatically to FH_4 in various configurations and may then be transferred in specific synthetic reactions. A key metabolic event is catalyzed by thymidylate synthetase and involves the conversion of 2-deoxyuridylate (dUMP) to thymidylate, an essential component of DNA. The methyl group transferred to the uracil moiety of dUMP is donated by $N^{5\text{-}10}$-methylene FH_4. Significantly, this carbon atom is transferred to the pyrimidine ring at the oxidation level of formaldehyde and is reduced to methyl by the pteridine ring of the folate coenzyme; the result is the formation of dihydrofolate (FH_2). Thus, to function again as a cofactor, FH_2 must first be reduced to FH_4 by DHFR. Inhibitors with a high affinity for DHFR prevent the formation of FH_4 and cause major disruptions in cellular metabolism by producing an acute intracellular deficiency of folate coenzymes. The folate coenzymes become trapped as FH_2 polyglutamates, which cannot function metabolically. One-carbon transfer reactions crucial for the *de-novo* synthesis of purine nucleotides and of thymidylate cease, with the subsequent interruption of the synthesis of DNA and RNA (as well as other vital metabolic reactions).

Understanding of these events enables appreciation of the rationale for the use of thymidine and/or leucovorin (N^5-formyl FH_4; folinic acid) in the "rescue" of normal cells from toxicity caused by drugs such as methotrexate. Leucovorin is a fully reduced, metabolically functional folate coenzyme; it enters cells via the specific carrier-mediated transport system and is convertible to other folate cofactors. Thus, it may function directly, without the need for reduction by DHFR in reactions such as those required for purine biosynthesis. On the other hand, thymidine may be converted to thymidylate by thymidine kinase, thus bypassing the reaction catalyzed by thymidylate synthetase and providing the necessary precursor for DNA synthesis.

An important feature of the binding of active folate antagonists with DHFRs is the very low inhibition constants observed (on the order of 1 nM). Covalent bonds are not involved in the enzyme-inhibitor interactions despite the high affinity of the antagonists for the protein. Substantial progress has been made in defining the chemical basis for the binding of methotrexate to DHFR (*see* Matthews *et al.*, 1978; Chabner, 1982c).

As with most inhibitors of cellular reproduction, a selective effect on neoplastic cells is obtainable to only a partial extent with methotrexate. Folate antagonists kill cells during the S phase of the cell cycle, and evidence indicates that methotrexate is much more effective when the cellular population is in the logarithmic phase of growth, rather than in the plateau phase. Because it is also capable of inhibiting RNA and protein synthesis, however, methotrexate slows the entry of cells into S phase and its cytotoxic action has been referred to as "self-limiting" (Skipper and Schabel, 1982).

Mechanism of Resistance to Antifolates. Although evidence is incomplete, three biochemical mechanisms of acquired resistance to methotrexate have been clearly demonstrated: (1) impaired transport of methotrexate into cells, (2) production of altered forms of DHFR that have decreased affinity for the inhibitor, and (3) increased concentrations of intracellular DHFR. It has been known for years that blood elements with marked increases in the activity of DHFR appear within days after treatment of patients with leukemia with single doses of methotrexate. This may reflect induction of new enzyme synthesis, temporary elimination from the marrow of cells that are susceptible to the drug because of low enzymatic activity, or protection of DHFR against catabolic degradation by intracellular proteases. It is well established that the enzyme, complexed with methotrexate, undergoes conformational changes that render it remarkably resistant to proteolysis.

Of special interest is the phenomenon of gene amplification and its relationship to acquired resistance to methotrexate and, perhaps, other cytotoxic agents. Methotrexate-resistant cell lines have been isolated that have several hundredfold more DHFR than do wild type cells because of comparable increases in the mRNA specific for the enzyme. This is due to the occurrence in these resistant cells of increased numbers of copies of the gene for DHFR. (For further discussion, *see* Schimke *et al.*, 1978; Bertino *et al.*, 1983; Stark and Wahl, 1984.)

Various therapeutic tactics have been recommended to avoid selection of resistant cells. The use of high doses of methotrexate with leucovorin "rescue" may permit the intracellular accumulation of methotrexate in concentrations that inactivate DHFR even when the enzyme is present at markedly elevated levels. Alternation of treatment with methotrexate with other active therapeutic agents that function by different mechanisms is another way to attempt to kill cells that are resistant.

General Toxicity and Cytotoxic Action. The actions of 4-amino analogs of folate in animals have been studied extensively.

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