New Antifolates: Pharmacology and Clinical Applications

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Key Words. Methotrexate · Trimetrexate · Edatrexate · Piritrexim · ZD1694 · Lometrexol · AG337 · LY231514 · 1843U89

ABSTRACT

Many new antifolate compounds with unique clinical properties are currently in clinical development. Some of these agents have been rationally designed to circumvent known mechanisms of resistance to methotrexate, the most useful and extensively studied antifolate in clinical practice. Resistance to methotrexate can result from decreased active transport into cells, decreased polyglutamation resulting in enhanced drug efflux from cells, mutations in dihydrofolate reductase which reduce drug binding affinity, and increased expression of dihydrofolate reductase due to gene amplification or increased translational efficiency. As a consequence, the newer antifolates may differ from methotrexate because of increased lipid solubility, improved cellular uptake or increased ability to undergo polyglutamation. Several of these newer agents also uniquely target specific folate-dependent enzymes such as thymidylate synthase or glycaminide ribonucleotide transformylase. Antifolates currently in clinical development include trimetrexate, edatrexate, piritrexim, ZD1694, lometrexol, AG337, LY231514 and 1843U89. This report summarizes the basic pharmacology and potential clinical applications of these promising new agents.

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INTRODUCTION

The modern era of antimetabolite cancer chemotherapy began in the late 1940s with the demonstration by Farber and colleagues that the antifolate aminopterin (2,4-diamino,4-deoxyfolic acid) could induce remissions in pediatric acute leukemia [1]. Today, the most widely used antifolate in medical oncology is methotrexate, the N10-methyl derivative of aminopterin, which has documented activity in acute leukemia, non-Hodgkin’s lymphoma, breast cancer, head and neck cancer, choriocarcinoma, osteogenic sarcoma and bladder cancer. Antifolates are also widely used in the treatment of nonmalignant diseases such as rheumatoid arthritis, psoriasis, bacterial and plasmodial infections, and in the opportunistic infections associated with AIDS.

At present, there are a growing number of promising new antifolates in clinical development as antitumor agents [2-4]. Many of these agents have been rationally designed to target specific folate-dependent enzymes or to circumvent known mechanisms of resistance to classical antifolates. The first part of this report describes the basic cellular and molecular pharmacology of the most intensively studied antifolate, methotrexate. For further details on the indications for use, pharmacokinetics, and clinical toxicities of methotrexate, the reader is referred elsewhere [5]. The second part of this paper describes the status and potential applications of several newer antifolates in clinical development. Practicing clinicians need to have a clear understanding of the basic pharmacology of methotrexate in order to better comprehend the unique properties and the promising therapeutic potential of these newer drugs as they become available for clinical use.

METHOTREXATE PHARMACOLOGY

Mechanism of Action

Methotrexate differs from the essential vitamin, folic acid, by the substitution of an amino group for a hydroxyl at the 4-position of the pteridine ring (Fig. 1). This minor structural alteration changes the normal substrate into a tight-binding inhibitor of dihydrofolate reductase (DHFR) [5], the enzyme principally responsible for the maintenance of the intracellular reduced folate pool. Reduced forms of folic acid, called tetrahydrofolates, are essential cofactors which serve as single carbon atom donors in the enzymatic synthesis of thymidylate and purine nucleotides. Tetrahydrofolates are only biologically active in their fully reduced forms. One reduced folate, 10-formyltetrahydrofolate, is responsible for donating single carbon groups in the...
de novo biosynthesis of purine nucleotides in the reactions catalyzed by the enzymes glycaminamide ribonucleotide (GAR) and aminomimidazole carboxamide ribonucleotide (AICAR) transformylases (Fig. 2). Another reduced folate, 5,10-methylenetetrahydrofolate, donates a methyl group to deoxyuridylate (dUMP) during the biosynthesis of thymidylate (dTMP) in the reaction catalyzed by thymidylate synthase (TS). During this process, 5,10-methylenetetrahydrofolate is oxidized to dihydrofolate and must be converted back to its tetrahydrofolate form by DHFR in order to maintain the reduced folate pool. In the presence of ongoing thymidylate synthesis, the inhibition of DHFR leads to the partial depletion of intracellular reduced folates, which can ultimately contribute to the impaired production of essential nucleotide precursors for DNA synthesis.

In mammalian cells, the enzyme folylpolyglutamyl synthase (FPGS) can attach up to six glutamate molecules to the pteridine ring of naturally occurring folates and antifolates, such as methotrexate [5]. This polylguatamimation reaction has several biologically important consequences. First, by increasing the size and ionization state of the methotrexate molecule, it decreases cellular efflux and traps the drug within the cell, thereby prolonging drug
action. Furthermore, the increased propensity of malignant cells to polyglutamate methotrexate compared to normal tissues may also account for some of the drug’s selective cytotoxicity [6]. Methotrexate polyglutamates are direct inhibitors of DHFR and, compared to monoglutamated drugs, have increased potency as inhibitors of other folate-dependent enzymes such as TS [7] and GAR and AICAR transformylases [8]. Polyglutamation of the natural enzyme substrate, dihydrofolate, which accumulates as a consequence of DHFR inhibition, is also an important component of methotrexate action. Because dihydrofolate polyglutamates are, by themselves, potent inhibitors of TS and GAR and AICAR transformylases [9, 10], they further contribute to the impaired production of thymidylate and purine nucleotide precursors. Thus, the inhibition of DNA synthesis by methotrexate is a multifactorial process which results from both the partial depletion of intracellular reduced folates and from the direct inhibition of folate-dependent enzymes by methotrexate and dihydrofolate polyglutamates. The relative contributions of each of these mechanisms toward the generation of methotrexate-induced cytotoxicity can vary in different cancer cell lines. Decreased polyglutamation due to the loss of FPGS activity has been identified as a potentially important mechanism of clinical methotrexate resistance in human soft tissue sarcomas [11]. Furthermore, normal tissues with high FPGS activity, such as the liver, accumulate and retain methotrexate polyglutamates for prolonged periods of time, a process which may be responsible for the occasional hepatotoxicity seen during chronic drug administration.

Although the depletion of thymidylate and purine nucleotides can inhibit DNA synthesis, the precise events leading to methotrexate-induced cell death are still under investigation. Cytotoxic DNA damage may result from the loss of DNA precursors, which leads to ineffective DNA repair and strand breakage. Another consequence of the inhibition of TS is the intracellular accumulation of dUMP, which can be converted into the deoxyribose phosphate nucleotide, dUTP, and misincorporated into DNA [5]. The DNA excision repair enzyme, uracil-DNA-glycosylase, recognizes and removes these uracil residues in DNA leading to further fragmentation and potentially cytotoxic DNA damage. However, the addition of thymidylate alone to methotrexate-treated cells does not prevent cytotoxicity unless a purine source, such as hypoxanthine, is also provided [12]. This further emphasizes the importance of both purine and thymidylate effects in the generation of methotrexate cytotoxicity.

Membrane Transport

Folate transport has been extensively studied because of its importance as a mechanism of antifolate drug resistance. Cellular uptake of methotrexate occurs via the same active transport pathways responsible for the influx of normal physiologic folates. At least two distinct carrier-mediated active transport systems are responsible for the uptake of methotrexate into mammalian cells [13]. One system, called the reduced folate carrier, is a relatively low affinity transporter of both methotrexate and reduced folates with affinity constants in the micromolar range. A second system utilizes a high affinity, membrane-associated, folate-binding protein, called the human folate receptor (hFR) which has affinity constants for reduced folates and folic acid in the nanomolar range. Some tumors, such as ovarian cancers, express high levels of hFR on the cell surface. The relative contribution of these two distinct transport pathways to the uptake of methotrexate in clinical cancer chemotherapy is an area of active research. However, in vitro resistance to methotrexate resulting from the decreased transport activity of one or both of these carrier systems has been reported. Newer, more lipophilic antifolates, such as trimetrexate or piritrexim, are not substrates for either folate transport system and enter cells by energy-independent mechanisms such as passive diffusion. Cell lines resistant to methotrexate because of decreased transport generally retain their sensitivity to these newer lipophilic antifolates. Efflux of methotrexate from the cell is also mediated by several different transport systems, some of which are distinct from the folate uptake pathways [14]. As previously mentioned, drug efflux is greatly influenced by the degree of methotrexate polyglutamation. Methotrexate efflux is not associated with the P-glycoprotein multidrug resistance (MDR) phenotype which confers cross-resistance to numerous other anticancer agents.

Inhibition of DHFR

Methotrexate is a competitive, reversible inhibitor of DHFR which binds tightly to the hydrophobic folate-binding pocket of the enzyme. This noncovalent interaction depends upon the presence of reduced nicotinamide dinucleotide phosphate, a normal cofactor for the DHFR-catalyzed reaction. Methotrexate can be competitively displaced by increased intracellular concentrations of the natural enzyme substrate dihydrofolate. Compared to monoglutamated drugs, methotrexate polyglutamates have slightly greater binding affinity for DHFR due to a slower rate of dissociation [5]. Point mutations occurring both within and outside of the folate-binding pocket can decrease the binding affinity of DHFR for methotrexate. These mutations can cause a decrease in the sensitivity of cells to methotrexate, and thus, they may represent an important mechanism of clinical drug resistance [15]. Naturally occurring DHFR alleles with a decreased affinity
for methotrexate may also account for the rapid development of methotrexate resistance in certain cell lines.

Sensitivity to methotrexate is highly dependent upon the intracellular levels of DHFR enzyme. Methotrexate resistance resulting from DHFR gene amplification has been identified both in cancer cell lines and in clinical tumor specimens [11]. Increased copies of the DHFR gene can exist within the chromosomal DNA as homogeneously staining regions, or they can occur in extrachromosomal fragments called double-minute chromosomes [16]. Cell lines with DHFR amplification in chromosomal DNA are stably resistant to methotrexate. However, cell lines with double-minute DHFR amplification tend to lose their resistance to methotrexate over time in the absence of the ongoing selective pressure of continuous methotrexate exposure.

Acute increases in DHFR expression following exposure to methotrexate may also contribute to methotrexate resistance. The regulation of DHFR protein expression is controlled, at least in part, by an autoregulatory mechanism at the level of mRNA translation [17]. Excess DHFR enzyme binds to its own mRNA and blocks the synthesis of DHFR protein in a negative feedback loop. However, the presence of dihydrofolate, or an inhibitor such as methotrexate, interferes with the binding of DHFR to its mRNA and allows for the continued synthesis of additional DHFR protein. Thus, cells can acutely increase the expression of DHFR under conditions where this is beneficial, such as when excess concentrations of dihydrofolate substrate or a metabolic inhibitor accumulate within the cell. This allows for the maintenance of normal cellular function even in the presence of methotrexate or other antifolates. The relative importance of this process in the development of clinical antifolate resistance must still be determined.

The administration of exogenous reduced folates, such as 5-formyltetrahydrofolate (leucovorin) effectively prevents methotrexate cytotoxicity in mammalian cells. The amount of leucovorin required to prevent severe clinical toxicity in high-dose methotrexate chemotherapy regimens is directly proportional to the amount of methotrexate circulating in plasma [5]. Leucovorin rescues cells by directly competing with methotrexate for cell transport, polyglutamation and binding to DHFR and other folate-dependent enzymes.

**NEW ANTIFOLATE AGENTS**

Our understanding of the basic pharmacology of methotrexate has led to the development of several new antifolate compounds with unique clinical properties and promising therapeutic potential. Many of these agents have been rationally designed to circumvent mechanisms of methotrexate resistance, such as decreased active transport, decreased polyglutamation, DHFR mutations which decrease methotrexate binding affinity, and increased expression of DHFR due to gene amplification or increased translational efficiency. As a consequence, these newer agents may differ from methotrexate because of increased potency, greater lipid solubility, improved cellular uptake, or increased ability to undergo polyglutamation. Several of these newer agents also uniquely target specific folate-dependent enzymes such as TS or GAR transformylase. The characteristics of several of these new antifolate compounds are summarized in Table 1.

**Trimetrexate**

Trimetrexate (Neutrexin™) is a nonclassical, lipophilic, quinazoline derivative which is a more potent inhibitor of DHFR than methotrexate [18]. Trimetrexate was recently approved by the Food and Drug Administration for use in the treatment of *Pneumocystis carinii* pneumonia; however, it has also undergone extensive testing as an anticancer agent.

Trimetrexate is not a substrate for the common folate active transport systems; however, because of its lipophilicity, it can rapidly enter cells by a nonenergy-dependent process. Cell lines resistant to methotrexate because of deficiencies in folate transport generally retain their sensitivity to trimetrexate. Unlike methotrexate, trimetrexate is a substrate for the P-glycoprotein MDR efflux pump and it may show cross-resistance to natural product anticancer agents [19]. Trimetrexate lacks the glutamic acid side chain and it cannot be polyglutamated by FPGS. As a consequence, despite generating high initial intracellular drug concentrations, trimetrexate is not retained within the cell for prolonged periods of time. Because of this, more prolonged exposures to trimetrexate are necessary to produce optimal antitumor effects [2].

Once within the cell, trimetrexate is a more potent inhibitor of DHFR than is methotrexate [18]. This results in a partial depletion of the intracellular reduced folate pool and the accumulation of dihydrofolate polyglutamates which can further inhibit de novo purine and thymidylate synthesis. In preclinical studies, trimetrexate was active against a broad range of tumors, even in cell lines resistant to methotrexate because of decreased folate transport or decreased polyglutamation. In vitro resistance to trimetrexate can result from the expression of the MDR phenotype [19], enhanced DHFR protein expression [11, 20], DHFR mutations which decrease drug binding [20, 21] and decreased uptake due to a poorly characterized mechanism [22].

The most common schedule of trimetrexate administration is a daily i.v. infusion of 8 to 12 mg/m² for five days, repeated every three weeks; however, short infusions given every several weeks and five-day continuous infusions have also been examined. In trials of trimetrexate in infectious
diseases, oral administration was common, with a reported mean bioavailability of trimetrexate of 44% [23]. Despite its lipophilicity, trimetrexate poorly penetrates into the central nervous system, possibly because of high plasma protein binding (>90%) [24]. Unlike methotrexate, trimetrexate is principally eliminated by hepatic metabolism instead of renal excretion, with a terminal elimination half-life of 15 to 20 h [18]. Less than 5% of the administered drug is excreted unchanged in urine. Some trimetrexate metabolites may also be active against DHFR, but these have not yet been fully characterized [25]. In vitro drug metabolism studies suggest that drug interactions resulting in decreased clearance may occur with cimetidine, ketoconazole and acetaminophen, although the clinical relevance of these observations is unclear [26].

The major dose-limiting toxicity of trimetrexate is noncumulative myelosuppression, primarily leukopenia. Other common toxicities include skin lesions, mild nausea and vomiting, mucositis, hypersensitivity reactions, fever and reversible elevation of the liver transaminases. The most common manifestation of trimetrexate skin toxicity is erythroderma over the neck and trunk [27]. In phase I trials in acute leukemia, abnormal liver function was the dose-limiting toxicity [28]. The ability of trimetrexate to inhibit enzymes involved in histamine metabolism, such as histamine-N-methyltransferase and diamine oxidase, may be responsible for causing relatively rare trimetrexate hypersensitivity reactions [29, 30]. In pharmacodynamic studies of trimetrexate, the area under the concentration-versus-time curve (AUC) correlated with the degree of myelosuppression [31]. In addition, low pretreatment serum protein [32] and albumin levels [33] and the presence of liver metastases [32] all correlated with increased trimetrexate myelosuppression. Given these observations, some caution is warranted in administering trimetrexate to patients with liver dysfunction.

Trimetrexate has undergone broad phase II testing as a single agent in a wide range of histologic tumor types (Table 2). While objective responses have been observed in head and neck cancer (26%) [34], non-small cell lung cancer (14%-19%) [35, 36], urothelial cancer (17%) [37], and breast cancer (5%-15%) [38, 39], the overall activity in most tumors has been disappointing. No responses were
observed in trials of trimetrexate in melanoma [40, 41], leukemia [28, 42], colorectal cancer [43] or pancreatic cancer [44]. A single phase III trial in advanced colorectal cancer randomized patients to two different schedules of trimetrexate or to a third arm of weekly 5-fluorouracil [45]. The administration of trimetrexate at 200 mg/m² i.v. every two weeks generated an overall response rate of only 6%, compared to 18% for 5-fluorouracil. Because toxicities were also greater with trimetrexate, the authors concluded that single-agent trimetrexate had no significant clinical utility in this disease.

Currently, trimetrexate is undergoing clinical testing in combination with other anticancer agents such as cisplatin [46, 47], etoposide [47], 5-fluorouracil [48, 49] and cyclophosphamide [50]. In preclinical studies, trimetrexate plus 5-fluorouracil and leucovorin demonstrated equivalent or better antitumor activity compared to methotrexate plus 5-fluorouracil and leucovorin in human leukemia cells [51]. The trimetrexate combination has a theoretical advantage of avoiding competition between methotrexate and leucovorin for active transport. In a promising clinical study, trimetrexate plus 5-fluorouracil and leucovorin generated response rates of 20% in previously treated patients with gastrointestinal cancer [48]. Further testing of this combination is ongoing.

Because they lack an active folate transport system, the opportunistic pathogens, Pneumocystis carinii and Toxoplasma gondii are resistant to classic antifolates such as methotrexate. In contrast, the greater lipophilicity of trimetrexate allows it to penetrate these organisms and inhibit their growth. The administration of trimetrexate, in combination with leucovorin to reduce host toxicity, can effectively treat Pneumocystis pneumonia in AIDS patients [52]. Trimetrexate therapy is currently recommended for patients with Pneumocystis pneumonia who are either unable to tolerate, or are resistant to, front-line therapy with trimethoprim-sulfamethoxazole. Trimetrexate is also being clinically tested in the prevention of graft-versus-host disease when given in combination with cyclosporin to bone marrow transplant patients [53].

**Edatrexate**

Edatrexate (EdAM) is a classic antifolate inhibitor of DHFR which structurally resembles methotrexate except for the substitution of carbon for a nitrogen at the N¹⁰ position. The cellular pharmacology of EdAM is similar to that of methotrexate, with the exception of slightly greater overall potency. Like methotrexate, EdAM is rapidly transported into cells by the reduced folate carrier at rates which are 2- to 12-fold higher than methotrexate [54, 55] and, once within the cell, EdAM is a more efficient substrate for polyglutamation by FPGS [56, 57]. EdAM polyglutamates are more potent inhibitors of DHFR than are methotrexate polyglutamates; however, EdAM is a somewhat less active inhibitor of TS [57]. As expected, the mechanisms of resistance to EdAM are similar to those observed with methotrexate, with the exception of slightly greater overall potency. Like methotrexate, EdAM is rapidly transported into cells by the reduced folate carrier at rates which are 2- to 12-fold higher than methotrexate [54, 55] and, once within the cell, EdAM is a more efficient substrate for polyglutamation by FPGS [56, 57]. EdAM polyglutamates are more potent inhibitors of DHFR than are methotrexate polyglutamates; however, EdAM is a somewhat less active inhibitor of TS [57]. As expected, the mechanisms of resistance to EdAM are similar to those observed with methotrexate, and they include decreased active transport by the reduced folate carrier, decreased polyglutamation by FPGS, enhanced expression of DHFR enzyme, and DHFR point mutations which reduce antifolate binding affinity [57, 58]. Because of its enhanced transport and polyglutamation characteristics, EdAM demonstrated a better therapeutic index than methotrexate in preclinical studies [56], making it a very promising drug for clinical development.

The most common schedule of EdAM administration is an 80 mg/m² i.v. infusion every week. In rats, about 30% of

<table>
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<th>Complete responders</th>
<th>Partial responders</th>
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*Randomized phase III study arm
EdAM is protein-bound with biliary excretion accounting for most of the drug’s clearance [57]. In man, EdAM has a short initial half-life of 13 min [59] and a longer terminal half-life of 7 to 11.9 h [59, 60], with renal excretion of unchanged drug ranging from 13% to 55% [59]. In preliminary studies, EdAM demonstrated saturable, nonlinear, Michaelis-Menten pharmacokinetics [61]. The clinical toxicity profile of EdAM is similar to that of methotrexate, with mucositis and stomatitis being the most common dose-limiting symptoms. Other toxicities included myelosuppression, nausea and vomiting, diarrhea, skin rash, fatigue, mild alopecia, pneumonitis and reversible elevation of the liver transaminases.

In phase II testing, EdAM produced encouraging objective responses in breast cancer (17%-41%) [62-65], non-small cell lung cancer (7%-32%) [66-69], malignant mesothelioma (18%) [70], head and neck cancer (6%-38%) [71-74], and soft-tissue sarcomas (6%-14%) [75, 76] (Table 3). Its activity was particularly promising in malignant fibrous histiocytomas, inducing partial responses in five of seven patients treated (71%) [76]. A recent, large, randomized phase III trial compared weekly EdAM to weekly methotrexate in 273 chemotherapy-naive patients with advanced squamous cell carcinoma of the head and neck [74]. Overall objective response rates were similar on either arm: 21% for EdAM and 16% for methotrexate. However, EdAM produced more severe toxicities including stomatitis, alopecia, and a high incidence (28%) of a toxic dermatitis which responded to prednisone therapy [77]. Four toxic deaths also occurred on the EdAM arm, compared to only one on methotrexate. Thus, although both antifolates demonstrated modest activity in this disease, single-agent EdAM clearly did not fulfill its earlier promise of an enhanced therapeutic index compared to methotrexate.

Table 3. Phase II trials of edatrexate

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<td>0</td>
<td>[152]</td>
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<tr>
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<td>5%</td>
<td>0</td>
<td>[153]</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0%</td>
<td>0</td>
<td>[154]</td>
</tr>
<tr>
<td>Small cell lung</td>
<td>33</td>
<td>0%</td>
<td>0</td>
<td>[155]</td>
</tr>
<tr>
<td>Colorectal</td>
<td>14</td>
<td>0%</td>
<td>0</td>
<td>[156]</td>
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<td>[157]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>16</td>
<td>0%</td>
<td>0</td>
<td>[158]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>15</td>
<td>0%</td>
<td>0</td>
<td>[159]</td>
</tr>
<tr>
<td>Central nervous system glioma</td>
<td>16</td>
<td>0%</td>
<td>0</td>
<td>[160]</td>
</tr>
</tbody>
</table>

*Randomized phase III study arm
NS = not specified

Many of these combination regimens combine EdAM with leucovorin rescue [80, 84, 90] or oral cryotherapy [81, 82] in an attempt to decrease the incidence of stomatitis. The combination of EdAM and paclitaxel is particularly promising because of nonoverlapping drug toxicities and preliminary high response rates of 33% in patients with mixed solid tumors [89] and 66% in advanced breast cancer patients [88].

Piritrexim

Piritrexim (BW301U) is a lipophilic antifolate which is similar to trimetrexate. Piritrexim is not a substrate for the folate active transport systems; instead, it rapidly enters cells via an energy-independent process. Like trimetrexate, it is effective against cancer cells resistant to methotrexate because of transport defects [91]. Piritrexim is not polyglutamated by FPGS, but it is a potent, direct inhibitor of DHFR and can also inhibit purine and thymidylate synthesis by causing the accumulation of dihydrofolate polyglutamates. Resistance to piritrexim can result from increased DHFR expression [92], mutations in DHFR which decrease drug-binding affinity [93] and increased efflux due to expression of the MDR phenotype [94]. Cross-resistance to other lipophilic antifolates, such as trimetrexate, has been identified.
in some cell lines [22, 95]. In animal studies, piritrexim was active against a broad range of tumors, with evidence of high tissue penetration due to its lipophilicity [96].

In clinical studies, piritrexim has been principally developed as an oral lipophilic antifolate. The most commonly tested oral dose has been 25 to 50 mg three times a day for five days repeated every three weeks. Daily i.v. infusions for five days and weekly infusion schedules have also been explored. In humans, oral absorption is rapid, with an overall bioavailability of 75%-95%, with some interpatient variation [97, 98]. The terminal half-life following oral administration is 4.5 to 5.6 h [98, 99], with hepatic metabolism being the primary route of drug clearance. The most common dose-limiting toxicity is myelosuppression, with mucositis occurring somewhat less often. Other toxicities include stomatitis, skin rash, nausea and vomiting, severe phlebitis when given intravenously, fatigue, elevated liver transaminases, and pneumonitis [100].

Objective responses to oral piritrexim have been reported in urothelial cancer [101], head and neck cancer [102, 103] and melanoma [104] (Table 4). An interesting attempt to alternate piritrexim with methotrexate in head and neck cancer patients did not demonstrate any greater activity than with methotrexate alone [105]. Combinations of piritrexim with cisplatin, 5-fluorouracil, and leucovorin have also been tested with promising results in head and neck cancer patients [106].

Piritrexim has also been studied in the treatment of non-malignant diseases such as psoriasis [107, 108] and Pneumocystis pneumonia [109]. Overall, the principal advantages of piritrexim over trimetrexate are its availability as an oral agent and its lack of a histamine effect [2], which may lower the incidence of skin reactions and hypersensitivity. However, whether piritrexim has any therapeutic advantage over more established antifolates such as methotrexate or trimetrexate must still be proven.

ZD1694

ZD1694 (Tomudex™) is a quinazoline-derived antifolate which specifically inhibits TS. It was originally developed as a water-soluble analog of CB3717, the first quinazoline-derived TS inhibitor to be tested clinically [110]. CB3717 was active in phase I studies, but it produced unpredictable renal- and hepatotoxicity. The poor solubility of CB3717 in acid solutions probably caused the drug to precipitate within the renal tubules. In contrast, ZD1694 is more soluble at low pH and has not demonstrated significant renal toxicity in current clinical trials.

ZD1694 is actively taken up into mammalian cells by the reduced folate carrier and by the hFR-mediated active transport systems [111]. It is a better substrate for FPGS than is CB3717, and, once polyglutamated, is over 100-fold more potent an inhibitor of TS than the monoglutamated drug [112]. While ZD1694 binds to the folate-binding pocket on the TS protein, it does not inhibit DHFR or GAR and AICAR transformylases; instead, it acts as a pure TS inhibitor. ZD1694 cytotoxicity can be reversed by the addition of thymidine or by the simultaneous administration of leucovorin. Resistance to ZD1694 can result from decreased reduced folate carrier transport [113], decreased FPGS activity [114] or TS gene amplification [115].

The most commonly used dose of ZD1694 is 3 to 4 mg/m² administered i.v. every three weeks. In animals, the principal routes of elimination are hepatic metabolism and biliary excretion. In man, ZD1694 pharmacokinetics are characterized by a triexponential model with a long terminal half-life of 50 to 100 h [116]. In phase I studies, a syndrome of severe fatigue and malaise was dose-limiting, with neutropenia being somewhat less common [116, 117]. Other toxicities included diarrhea, stomatitis, nausea and vomiting, reversible elevation of liver transaminases, skin rash, fever and mild alopecia.

Objective clinical response rates of about 25% were reported in phase II trials of ZD1694 in patients with colorectal [118] and breast cancers [119] (Table 5). Slightly lower response rates were seen in non-small cell lung cancer [120] and ovarian cancer [121]. Because of the very promising activity in advanced colorectal cancer, randomized phase III trials are in progress comparing ZD1694 to 5-fluorouracil and leucovorin [122].

Lometrexol

Lometrexol is an analog of tetrahydrofolate that contains carbon atoms substituted for nitrogen at the N³ and N¹⁰ positions. Lometrexol is a specific inhibitor of GAR transformylase [123] with no inhibitory activity against DHFR or TS. Thus, its cytotoxicity is exclusively due to the inhibition of de novo purine synthesis.

Lometrexol is actively transported into mammalian cells by the reduced-folate carrier and the hFR-mediated carrier transport systems [55]. More than any other antifolate,

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Patient number</th>
<th>Response rate</th>
<th>Complete responders</th>
<th>Partial responders</th>
<th>Reference number</th>
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<tr>
<td>Urothelial</td>
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<td>10</td>
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<tr>
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<td>6</td>
<td>[102]</td>
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<td>23%</td>
<td>2</td>
<td>5</td>
<td>[104]</td>
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<td>Sarcoma</td>
<td>20</td>
<td>24%</td>
<td>6</td>
<td>6</td>
<td>[103]</td>
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<td>2</td>
<td>[161]</td>
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<td>20</td>
<td>100%</td>
<td>0</td>
<td>3</td>
<td>[163]</td>
</tr>
<tr>
<td>Central nervous system glioma</td>
<td>20</td>
<td>9%</td>
<td>1</td>
<td>1</td>
<td>[164]</td>
</tr>
</tbody>
</table>
lometrexol is critically dependent upon polyglutamation by FPGS for its cytotoxic activity [124]. Impaired polyglutamation, as a consequence of decreased FPGS activity, is the primary mechanism of lometrexol resistance [125]. A unique alteration which can also contribute to decreased lometrexol sensitivity is the enhanced activity of gamma-glutamyl hydrolase, the enzyme responsible for removing polyglutamyl residues from folate molecules [125, 126]. Alterations in lometrexol transport are much less important in determining drug responsiveness compared to polyglutamation [127].

Lometrexol has commonly been given as an i.v. infusion on a weekly or daily (for 3 days) schedule. In phase I clinical trials, severe cumulative thrombocytopenia was observed [128, 129]. Other side effects included mucositis, stomatitis, neutropenia and anemia. Coadministration of folic acid or leucovorin appears to alleviate the severe toxicities and most clinical studies are now combining lometrexol with folic acid. However, a potential concern is whether the addition of exogenous folates may diminish therapeutic activity as well as clinical toxicity.

Other Agents in Clinical Trials

Two new nonclassical TS inhibitors are the antifolates AG337 and AG331 [130, 131]. These lipophilic, nonpolyglutamated agents were rationally designed based upon x-ray crystallographic modeling of the TS enzyme. In preliminary phase I studies of AG337 given as a five-day continuous infusion, the dose-limiting toxicity was myelosuppression [130]. Other toxicities included mucositis, stomatitis, neutropenia and anemia. Coadministration of folic acid or leucovorin appears to alleviate the severe toxicities and most clinical studies are now combining lometrexol with folic acid. However, a potential concern is whether the addition of exogenous folates may diminish therapeutic activity as well as clinical toxicity.

Thus, LY231514 is a multitargeted agent similar to methotrexate. In phase I studies, the primary dose-limiting toxicity was neutropenia, with other toxicities including diarrhea, mucositis, rash, fatigue, anorexia, and nausea and vomiting [133, 134]. Phase II clinical trials of LY231514 are in progress.

1843U89 is a benzoquinazoline-derived TS inhibitor [135] currently in phase I clinical trials. It is rapidly taken up into mammalian cells by both of the major folate active transport systems [111] and, once within the cell, it is polyglutamated by FPGS to the diglutamate level [135]. This compound is unique in that both the mono- and diglutamated forms of the drug are potent, noncompetitive inhibitors of mammalian TS. In preclinical studies, oral folic acid effectively prevented gastrointestinal toxicity of 1843U89, but did not diminish antitumor activity, suggesting an enhanced therapeutic index [136]. Phase I clinical trials of this combination are under way.

CONCLUSIONS

Methotrexate’s actions at the cellular and molecular level have served as a paradigm for the rational design of many new antifolate compounds. In addition to the agents described here, many additional antifolates with specific and unique pharmacologic properties are currently in preclinical stages of development, some of which will be entering into clinical testing in the near future. Thus, nearly 50 years after their first use as anticancer agents, the antifolates remain a diverse and growing class of drugs with great promise and potential for improving our ability to treat a broad range of human diseases.

ACKNOWLEDGMENTS

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REFERENCES


Takimoto


27 Weiss RB, James WD, Major WB et al. Skin reactions induced by trimetrexate, an analog of methotrexate. Invest New Drugs 1986;4:159-163.


39 Dawson NA, Costanza ME, Korbzun AH et al. Trimetrexate in untreated and previously treated patients with metastatic


41 Iscoe NA, Eisenhauer EA, Bodurtha AJ. Phase II study of trimetrexate in malignant melanoma: a National Cancer Institute of Canada Clinical Trials Group study. Invest New Drugs 1990;8:121-123.


