Pyrimidine and Purine Antimetabolites

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Development of pyrimidine and purine analogs as potential antineoplastic agents evolved from an early presumption that nucleic acids are involved in growth control. Among the first analogs produced and tested for biologic activity were the 5-halogenated pyrimidines: 5-chloro-, 5-bromo-, and 5-iodouracil. Although in original concept these agents were targeted toward the malaria parasite, G. H. Hitchings and his colleague G. B. Elion recognized that these compounds might be valuable in the treatment of cancer, which was correctly perceived as a disease of uncontrolled growth.1,2 These early studies focused primarily on the incorporation of analog nucleic acid bases into ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) of bacteria species.3 Concurrent studies on the metabolic activation of these heterocyclic analogs and their biochemical targets for growth inhibition, as well as the study of resistance to them, afforded many new insights into the intermediary metabolism responsible for the synthesis of DNA and RNA precursors.4 Subsequently, it was recognized that control of these biosynthetic pathways afforded additional targets for therapeutic intervention.

Further development of these analogs was stimulated by the demonstration of quantitative, but not qualitative, differences in the activity of these pathways between normal versus neoplastic tissue. It was also realized that rapid catabolism of these agents to inactive compounds might be valuable in the treatment of cancer, which was recognized as a disease of uncontrolled growth,1,2 These early studies focused primarily on the incorporation of analog nucleic acid bases into ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) of bacteria species.3 Concurrent studies on the metabolic activation of these heterocyclic analogs and their biochemical targets for growth inhibition, as well as the study of resistance to them, afforded many new insights into the intermediary metabolism responsible for the synthesis of DNA and RNA precursors.4 Subsequently, it was recognized that control of these biosynthetic pathways afforded additional targets for therapeutic intervention.

A virtually complete understanding of enzymes involved in the biosynthesis of purine and pyrimidine nucleotide precursors of RNA and DNA is now at hand.5,6 This intricate matrix of metabolic reactions operates under a complex web of positive- and negative-feedback controls. Most purine or pyrimidine analogs are active only after metabolic activation to the nucleotide form, so these fraudulent nucleotides not only may be incorporated but also can mimic the natural effector compounds in regulatory pathways. Alternatively, they may deplete critical intermediates, thereby generating enlarged pools of the natural precursors behind a metabolic block, producing effects that can distort the balance of ribonucleoside and deoxyribonucleoside triphosphates. A target of even greater complexity is the incorporation of triphosphates into DNA or RNA and the subsequent modification of these macromolecules. Subtle differences in the specificity and function of the polymerases generate the selectivity of certain purine and pyrimidine nucleotides as anticancer and, more importantly, antiviral agents.

Demonstrating the inhibition of specific enzyme reactions by analog pyrimidine or purine nucleotides does not ensure that these reactions are rate limiting for tumor growth or responsible for cytotoxicity to either normal or neoplastic tissues. Even though several inhibitory sites have been identified, some having greater apparent sensitivity than others, attribution of a biologic effect to the inhibition of a specific reaction, in general, is difficult. Similarly, analogs may be incorporated into nucleic acids and either inhibit subsequent replication cycles or result in miscoding; however, the impact of these mechanisms may be modified by DNA editing and repair reactions that can minimize, or in some cases increase, the effects of incorporation.

In addition to purine and pyrimidine analogs, other agents have been developed that inhibit biosynthetic reactions leading to the ultimate nucleic acid precursors. These include N-phosphonoacetyl-L-aspartic acid, brequinar, acivicin, and hydroxyurea.

Another factor that may affect the action of nucleoside analogs is the rate and nature of the transport systems for both normal and analog nucleotides in and out of host versus neoplastic tissues. A wide range of nucleoside cell lines have a saturable system that is responsible for the facilitated diffusion of ribonucleosides and deoxyribonucleosides.7 This system essentially equilibrates the cytoplasm with the extracellular milieu. More recently, Na+-dependent active transport systems for purine and pyrimidine nucleotides have been found in a variety of normal tissues.8-10 In neoplastic cell lines and some tumors, the Na+-dependent concentrative mechanisms, if they exist, are nullified by the facilitated diffusion mechanism. These effects are particularly evident with uridine, which is threefold to tenfold more concentrated in some normal tissues and may be responsible for the selectivity of some antimetabolites.

PYRIMIDINE ANALOGS

Pyrimidine analogs include 5-fluorouracil, cytosine arabinoside, 5-azacytidine, and gemcitabine. 5-FLUOROURACIL Background and Properties A major motivation for the development of pyrimidine analogs of uracil was the early observation that preneoplastic rat liver and hepatomas incorporated uracil more actively than did the normal liver.11 Although this may reflect a difference in the relative degradative capacity of these different tissues for uracil, it also provided a focus for the synthetic efforts of Duschinsky and colleagues that led to 5-fluorouracil (5-FU) (Figure 50-1) and a family of related fluorinated pyrimidines.12 This specific site of substitution on the pyrimidine ring was selected because it might inhibit subsequent conversion of a uracil nucleotide to thymine nucleotides. Because insertion of the methyl group occurs on the 5-position, halogen replacement of hydrogen in that position was thought to have a greater chance of inhibiting DNA synthesis and, thus, growth. The selection of fluorine to replace the hydrogen in uracil was based on their similar van der Waals radii (F = 1.47 Å and H = 1.20 Å). Unlike earlier syntheses of halogenated pyrimidines, which involved simple displacement of the hydrogen with the halogens chlorine, bromine, or iodine, 5-FU was originally synthesized from an acyclic precursor. This permitted formation of the corresponding 5-fluoroorotic acid; subsequently, the ribosides and deoxyribosides of 5-FU were prepared (see Figure 50-1). More recently, a direct means of fluorinating 5-FU has been developed that permits positron emission tomography studies with [18F]-5-FU.13

As anticipated, the pKa of 5-FU (8.1) is more acidic than that of uracil (9.6); thus, under physiologic conditions, 5-FU partially exists as an anionic species. This is undoubtedly important to the metabolic activation to the nucleotide form via the orotidylate pyrophosphorylase reaction. This uridylicate analog, 5-fluorouridine monophosphate (5-FUMP), can then substitute for uridine monophosphate (UMP) in a wide spectrum of intermediary reactions. The product of one of these reactions, fluorodeoxyuridine monophosphate (FdUMP), plays a major role by inhibiting displacement of hydrogen from the 5-position of deoxyuridylate and replacing it with a methyl group via a tetrahydrofolate catalyzed reaction (Figure 50-2).14 Many of the properties predicted for 5-FU were seen in early studies of bacterial and model tumor systems, and a
remarkably rapid progression to a clinical trial occurred within 2 years of its synthesis. These early clinical studies showed enough promise in colon cancer and other solid tumors to sustain 40 subsequent years of further development. A primary focus of this research has been to reduce its very real toxicity to a variety of normal tissues, while retaining its antitumor activity. Today, 5-FU remains an important component in the therapy of several of the most common solid tumors, not only as a single agent but also in combination with other chemotherapy agents.

**Cellular Entry and Efflux Mechanisms**

Limited evidence suggests that 5-FU enters cells by a carrier-mediated transport mechanism. Early reports suggested that a specific mechanism for the transport of uracil existed in the intestine; however, these studies used methods that made it difficult to distinguish between transport and metabolism. Evidence has been presented for a nonconcentrative transporter in the Novikoff hepatoma that exhibits competitive kinetics between uracil and 5-FU. Under conditions in which the 5-FU ring is minimally ionized, enhanced entry of 5-FU occurs if cells are preloaded with uracil, which is consistent with a countertransport mechanism. Using standard analytic techniques, no evidence to date suggests that an alteration of 5-FU entry into cells is responsible for either natural or acquired resistance. However, use of more sophisticated methods has revealed a different picture of 5-FU uptake and retention. Using [19F]-5-FU, a difference in the ability of selected tumors to accumulate free 5-FU was noted to correlate with their response to chemotherapy. Extension of these studies to four patients with breast and colon carcinoma indicated a half-life of 0.4 to 2.1 h for free 5-FU in the tumor compared with a plasma half-life of less than 10 to 15 min. Independent studies using gas chromatography/mass spectroscopy (GC-MS) documented free 5-FU concentrations in normal and neoplastic tissue that were at least tenfold higher than those in plasma. This study also revealed that after an initial, rapid clearance from plasma, it was possible to detect a second, longer half-life of approximately 3.5 h. These new observations on the trapping of 5-FU in tumors lend support to the view that 5-FU is transported into the cells by an active transport mechanism as well as by a facilitated diffusion mechanism. Free 5-FU could also be concentrated in the cytoplasm (pH, 7.2) from extracellular spaces of tumors rendered acidic by anaerobic glycolysis (pH, 6.2–7.0) by virtue of ionization trapping of this pyrimidine analog, which has a $p_{K_a}$ of 8.1. An alternative source would be a slow liberation of free 5-FU from nucleotides and nucleic acids; this liberation sustains an intracellular concentration because of the limited efflux of free 5-FU from the cells. This capacity for trapping free 5-FU may serve as a measure of potential clinical response and deserves further study.

In contrast to 5-FU, the entry of fluorodeoxyuridine (FdUrd) (see Figure 50-1) into most neoplastic cells involves the saturable but nonconcentrative mechanism that is responsible for the facilitated diffusion of a wide spectrum of nucleosides. This transporter has been quantified in several cell lines by titration with $p$-nitrobenzylthioinosine. Deletion of this transport mechanism is the basis for resistance to FdUrd or purine nucleoside analogs in at least two cell lines. Such a deletion makes the cells collateralistically sensitive to methotrexate and other inhibitors of thymidylate synthase (TS) because they are unable, or limited in their ability, to salvage thymidine, whether naturally available or administered. Fluorouridine and FdUrd released from 5-fluorouridylic acid and 5-fluorodeoxyuridine by phosphatase action exit the cell via this same facilitated diffusion transporter. Thus, agents that affect this transporter may selectively affect 5-FU cytotoxicity by a differential effect on specific normal or neoplastic cell types. The facilitated diffusion mechanism may play a secondary role in the modulation of 5-FU action in vivo by uridine because this normal nucleoside, but not 5-fluorouridine (5-FUrd) or FdUrd, is actively concentrated by a Na+-dependent system. Neoplastic cells appear to be less capable of this transport and are not protected.

**Anabolism**

Once inside the cell, 5-FU has 5-fluorouracil (5-FU) and analog structures. Figure 50-1 5-Fluorouracil (5-FU) and analog structures.

![Figure 50-1](image-url)

5'-Deoxy-5-fluorouridine

![Figure 50-2](image-url)

![Figure 50-2](image-url)

Covalent thymidylate synthase-fluorodeoxyuridylate complex; $R = H$ or $CH_2FH_4 = 5$, 10-methylene tetrahydrofolate.
Consideration of 5-FU

Alternative activation routes of 5-FU follow the salvage pathways for uracil and thymine, but these are presumed to be less important in most tissues. The first enzyme in the pathway, uridine phosphorylase, condenses ribose-1-P with uracil or 5-FU in a reaction that energetically favors synthesis; normally this is catalytic in the cell because further reactions such as of PRPP synthesis and phosphatases reduce the concentration of ribose-1-P. The corresponding reaction for thymine uses deoxyribose-1-P, but it is not considered to make a significant contribution to 5-FU activation in current therapeutic regimens. After formation of the nucleoside, phosphorylation by uridine kinase and adenosine triphosphate (ATP) forms 5-fluorouridine monophosphate (5-FUMP) (Figure 50-3). Further phosphorylation of 5-FUMP to 5-fluorouridine diphosphate (5-FUDP) by nucleotide kinase provides a branch point in 5-FU anabolism. Additional phosphorylation of a major portion of 5-FUDP to fluorouridine triphosphate (5-FUTP) provides the substrate for RNA polymerases with consequent incorporation into several forms of RNA. Alternatively, 5-FUDP can be reduced to 5-fluorodeoxyuridine diphosphate (FdUDP), which is hydrolyzed to the monophosphate FdUMP, the covalent inhibitor of thymidylate synthase. Some FdUDP is phosphorylated to fluorodeoxyuridine triphosphate (FdUTP), which is an alternate substrate for deoxynucleotidase (dTTP) in DNA polymerase reactions; however, high deoxynucleotidase triphosphate (dUTP) pyrophosphatase activity converts most of the FdUTP to FdUMP. When 5-FU is incorporated into DNA, uracil N-glycosylase removes it, leaving an apyrimidinic sugar for the process of DNA repair. Errors in this process provide an additional basis for cytotoxicity.

Minor amounts of 5-FUDP sugar derivatives have been detected as anabolic products, but their potential to inhibit cell growth or toxicity has not been documented. In some of the previously discussed reactions, the analog 5-FU nucleotides are better substrates than the corresponding uracil derivatives.

Pharmacokinetics Consideration of 5-FU pharmacokinetics must focus primarily on the balance between anabolism and catabolism. The conversion to nucleotide derivatives is responsible for most, if not all, of its antineoplastic activity, even though it accounts for a very minor portion of the administered drug. Catabolism via the normal degradation pathway for uracil is the immediate fate of more than 80% of an administered dose of 5-FU. Therefore, slight alterations in this pathway can greatly affect the very limited amount that is available for conversion to the nucleotide form.

Because of the appearance of greater variability in response among patients and apparent limited bioavailability (10% to 25%) via the oral route, there has been a long-held recommendation that 5-FU should be administered intravenously (IV). The basis for this apparent poor bioavailability has not been well understood, particularly since the low molecular weight and pKₐ of 5-FU should predict excellent absorption. Recent clinical studies in which 5-FU was administered orally together with ethynyluracil—a potent inactivator of the initial enzyme of the pyrimidine pathway, dihydropyrimidine dehydrogenase (DPD)—have demonstrated that 5-FU in fact has excellent absorption and bioavailability with the variability from patient to patient being due to the variability of DPD levels in the population.

Dosage used clinically in general depends on the schedule of administration. The most common dosage schedules are a monthly course of one dose given on each of 5 days as an IV bolus of 400 to 600 mg/m² or the same dosage given as a single bolus on a weekly basis. The limiting toxic effect of these regimens generally is myelosuppression or mucositis. When continuous IV infusion is employed, higher doses are required (1,000 to 2,000 mg/m²/d) to sustain steady-state concentrations of 5-FU (1 to 5 µM) in plasma adequate to achieve therapeutic effects. With this route, toxicity is most frequently mucositis, with minimal myelosuppression. Several studies have shown that this regimen is superior to the bolus regimen when 5-FU is given as a single agent. Optimal treatment was a 48-hour infusion at weekly intervals, which improved both response and survival. Prolonged infusion of 5-FU for up to 12 weeks at 300 mg/m²/d also produced a better response than the bolus regimen.

The rate of plasma clearance generally is first order with a half-life of 10 to 20 minutes and ranges between 500 and 1,500 mL/min. Above a dosage of 800 mg/m², clearance may decrease rapidly. Because the primary fate of the drug is catabolism, this decreased clearance undoubtedly reflects saturation of these reactions. The circulating concentrations of the initial metabolite, dihydro-5-FU, can be much greater than those of 5-FU, and the fate of this metabolite may affect both the pharmacokinetics and response to 5-FU.

Intraarterial infusion of 5-FU has been used with some success in patients with isolated hepatic metastases. As with systemic therapy, extensive single-pass clearance is achieved (19 to 51%), but saturation of catabolism occurs when doses are elevated. Nevertheless, hepatic 5-FU concentrations considerably in excess of those tolerated sys-
The predominant phosphorylated experimental evidence for its relative stability in humans is the observation that approximately 80% of an oral dose is excreted unchanged in the urine, compared to approximately 5% of a comparable dose of 5-FU.

Recently, to improve the specificity of chemotherapy treatment and reduce the systemic toxicity of the drug, and increase the local concentration of the antineoplastic agent at the tumor site, prodrug-activating gene therapy protocols have been developed to activate fluorouracil into 5-FU by cytosine deaminase.

Cytosine deaminase is not a mammalian enzyme, but it is present in bacteria and fungi and can be utilized to produce high concentrations of 5-FU in tumors through the enzymatic deamination of fluorouracil.56,57 This approach has been initially developed for the treatment of colorectal carcinoma metastatic to the liver, by utilizing a delivery system based on a replication-independent adenoviral vector.68 In vivo and in vitro evaluations of this gene therapy system have shown an increased sensitivity of colon carcinoma cells to fluorouracil exposure. Even if the adenoviral vector carrying cytosine deaminase is transferred to a limited number of the total tumor cells (10%), a bystander effect is observed, likely due to the local diffusion of 5-FU generated in the virus-infected cells.69–72

Catabolic Reactions The primary clearance mode of 5-FU is via catabolism along the degradative pathway for uracil.42 Because the products of this pathway do not absorb ultraviolet light, GC-MS or radioisotopic methods must be employed. The initial reaction is reduction by dihydouracil dehydrogenase. The liver is a major site of 5-FU metabolism, and this is particularly true when the drug is given orally, intraperitoneally, or by intrahepatic arterial infusion. It is now recognized, however, that metabolism in the lung and kidneys may be of equal, or even greater, importance after IV administration.55 These findings have therapeutic relevance because it was previously felt that hepatic metastases might compromise 5-FU clearance and limit dosage.

Marked circadian variations in the metabolism of 5-FU have been detected related to 24-hour cyclic variations in dihydouracil dehydrogenase activity.52,73 These changes are reflected in the inverse variations of plasma 5-FU concentrations during IV infusions in humans.52,74 Means to employ these differences in the design of clinical protocols have been outlined.75 Preclinical data in murine models have indicated that less toxicity was encountered during a circadian infusion when the maximal concentration of 5-FU was programmed to occur at 4 am.76,77 More recent data indicate that if the maximal concentration is programmed for 9 to 10 pm, even less toxicity is observed than with the previous schedule.78,79 Several clinical protocols comparing a continuous flat infusion with the circadian schedule have been conducted with 5-FU alone and in combination with leucovorin and/or platinum derivatives.80–82 A clinical study using a 14-day continuous infusion of 5-FU and leucovorin suggests that circadian administration with a maximal infusion rate at 4 am increases the maximum tolerated dose (MTD) for both agents; 5-FU, 250 mg/m²/d; leucovorin, 20 mg/m²/d.83 In patients who experienced grade 2 or higher toxicities with this schedule, the peak of their circadian infusion was moved to 9 to 10 pm. Decreased toxicity was observed (mostly diarrhea and stomatitis), and the MTD for 5-FU increased to 300 mg/m²/d, a 50 mg increment over the MTD for a flat continuous infusion.84

Dihydropyrimidine dehydrogenase represents the initial rate-limiting step in the catabolism of the pyrimidines uracil, thymine, and 5-FU. More than 85% of an administered dose of 5-FU is eliminated with rapid formation of dihydrofluorouracil.42 In a small percentage of the population, less than 3%, DPD activity is significantly below the average (below 50% of the control mean). This pharmacogenetic condition, which typically goes undetected until administration of 5-FU, can cause very serious life-threatening toxicity in patients following 5-FU-based chemotherapy; the toxicity is due to increased exposure to and activation of the antineoplastic agent.85 The variability in DPD activity in normal tissues of the liver and gastrointestinal tract has been recently linked to the erratic oral bioavailability of 5-FU. DPD inhibitors, such as ethynyluracil, have been recently developed in an attempt to increase 5-FU efficacy and improve oral absorption although unfortunately this has not been associated with equivalence to intravenous 5-FU regimens.86–88

The subsequent metabolic step, catalyzed by dihydropyrimidinidase, yields β-fluorocytidine, yields β-fluorouracil.89 α-Fluoro-β-alanine, the counterpart to the final product of uracil catabolism β-alanine, is the major urinary excretion product of 5-FU.90 In patients with cancer, this has been shown to be conjugated with bile acids and constitutes the primary biliary secretion product of 5-FU.91 It has been suggested that the chenodeoxycholate conjugate may be responsible for the biliary toxicity seen after large-dose, intrahepatic infusion of 5-FU, and cholestasis associated with this conjugate has been demonstrated in isolated, perfused rat livers.91 A summary of 5-FU metabolism is shown in Figure 50-3.

Mechanisms of Action Experimental evidence has suggested numerous sites for the biologic action of 5-FU (see Figure 50-3). The relative importance of each varies widely among different normal tissues and neoplasms. Commonly, the effects are divided into RNA- or DNA-directed toxicity.

RNA The predominant phosphorylated nucleoside of 5-FU, 5-FUTP, is as good a substrate as uridine triphosphate (UTP) for several RNA polymerase reactions. The degree of 5-FUTP incorporation into RNA bears a direct relationship to its concentration relative to that of the normal substrate UTP. In cell lines, greater incorporation is associated with reduced clono-
genic survival. Very substantial amounts of 5-FU replacement of uracil have been reported in each of the RNA species; the highest degree of incorporation generally is seen in the 4S-RNA. Some evidence suggests that with a given cell type, the proportion of RNA incorporation in different species depends on the available form of the analog, 5-FU versus FdUrd; this result suggests compartmentalization or channeling of the analog en route to incorporation.94

What is less clear about incorporation into RNA is its contribution to cytotoxicity. Earlier studies indicated effects on transfer RNA acceptor activity, miscoding of protein synthesis, and inhibition of the maturation or processing of ribosomal RNA. More recently, attention has focused on the inhibition of processing nuclear RNA to smaller-molecular-weight species. Other post-transcriptional effects of 5-FU include inhibiting polyadenylation of messenger RNA (mRNA) and effects on DNA primase. In some model tumors and tumor lines, there is persuasive evidence that these RNA-directed events can be associated with cytotoxicity, particularly when the effects of extended exposure are monitored.97

Thymidylate Synthase The target site that can be defined most clearly is the covalent inactivation of thymidylate synthase by FdUMP. This fluorinated deoxyuridylate analog is formed via the reduction of FUDP by ribonucleotide reductase and dephosphorylation. Alternatively, it can be formed directly from 5-FdUrd by thymidine kinase when this 5-FU deoxynucleoside is regionally infused. The earliest studies by Umeda and Heidelberger indicated that in selected cell lines growth inhibition could be prevented by thymidine but not by uridine. Direct inhibition of the enzyme responsible for the 1-carbon transfer confirmed this site of action, and subsequent research identified specific steps in the reaction in which a methylene group from 5, 10-methylene tetrahydrofolate is transferred to the 5-position of 2'-deoxyuridylate. These studies elegantly established the formation of a stable ternary complex among the 5'-fluoro analog of deoxyuridylate, the reduced folate derivative, and thymidylate synthase. The obvious consequence of this inhibition is an induced enzyme deficiency, depletion of dTTP, and the accumulation of deoxyuridine monophosphate (dUMP) behind the blockade. More recently, it has been shown that in some tumors or normal tissues the rate-limiting factor in the formation of the abortive ternary complex with FdUMP is availability of the reduced folate derivative. When this cofactor is limiting, it is possible to enhance inhibition by the administration of leucovorin. The consequence of dTTP depletion is generally considered to be unbalanced growth consequent to reduced DNA synthesis. As might be anticipated, this mode of inhibition would be nullified if thymidine were supplied because after phosphorylation by thymidine kinase, it would circumvent the site of inhibition. However, thymidine administration in vivo can actually increase the cytotoxic effects of 5-FU in vivo by inhibiting 5-FU catabolism.111

Recent studies have analyzed a possible correlation between TS expression and therapeutic outcome in colorectal, head and neck, and breast tumors. A retrospective study in patients with rectal carcinoma revealed that the expression of high levels of TS was linked to a significantly reduced disease-free survival, and that the staining intensity of TS monoclonal antibody was stronger in higher-grade, less differentiated tumors. Another study indicated that patients with advanced gastric and colorectal tumors expressing high TS protein did not respond to 5-FU-leucovorin treatment.114 Association between TS level in tumors and response to chemotherapy in colorectal cancer was also seen when a biochemical assay and a reverse polymerase chain reaction (PCR) method were used. Such a correlation was not observed in an immunohistochemical study from Findlay and colleagues on primary colorectal tumors.118 A report on head and neck squamous cell carcinomas also failed to establish a relationship between TS level and patient survival or treatment outcome. A retrospective immunohistochemical study in breast tumor of patients with early-stage breast cancer indicated that high TS expression was associated with a significantly worse prognosis in node-positive but not in node-negative breast cancer patients.120

A more likely explanation for the discrepancies in these studies is that factors other than TS alone may be involved. Thus more recent clinical studies have shown that tumor response and survival are likely determined by multiple factors including TS; DPD, which controls the amount of 5-FU available for anabolism; and various enzymes in the pyrimidine anabolic pathway which control the interconversions of 5-FU anabolites.121

DNA Initially, the incorporation of 5-FU into DNA was not detected, and it was assumed to be prevented by the active dUTP phosphatases that also dephosphorylate FdUDP as it is formed. Small quantities of 5-FU were detected in internucleotide linkages within DNA. Like dUTP, FdUDP, when it is available, is fully active as a substrate for the several DNA polymerases, but a very active glycosylase is present in most cells and excises any 5-FU or uracil that is incorporated in the place of thymine. Mutants have been found that are relatively deficient in this editing function, and it may be that incorporation per se is not the cytotoxic event, but that the excision and repair involving a pyrimidine endonuclease generates opportunities for error-prone repair that might again re-incorporate 5-FU or uracil instead of thymine. Because a considerable accumulation of dUMP occurs behind the blockade of thymidylate synthase, higher concentrations of dUTP are generated. These concentrations and any FdUUTP increase the need for an editing function to remove incorporated uracil. Examination of the kinetics of this excision reaction indicates that uracil is removed as much as 30 times more rapidly than 5-FU.

A similar elevation of dUTP concentrations can be achieved by methotrexate therapy via secondary inhibition of thymidylate synthase. Under these conditions, uracil incorporation into DNA is also increased, and the potential for error-prone repair is enhanced. It is not possible to rank the importance of these different potential mechanisms of cytotoxicity: RNA incorporation, dTTP depletion by thymidylate synthase inhibition, DNA incorporation, or damage to DNA consequent to excision of uracil or 5-FU. In fact, the relative importance of each of these sites may vary in different cell types. In some tumor lines, evidence for high sensitivity to RNA-directed effects is seen by the inability of thymidine to overcome growth inhibition, despite the presence of an active thymidine kinase. In these same lines, uridine rescue is more successful than in others where thymidine effectively prevents cytotoxicity, presumably by repleting dTTP.

Resistance As with most drugs, partial or complete responses of human cancer to 5-FU generally are followed by the eventual regrowth of tumor despite sustained, or even increased, dosages. Understanding some of the factors that contribute to natural or acquired resistance has stimulated several approaches to modulating 5-FU therapy. The most prominent mechanism seen in experimental tumors is reduced anabolism of the analog to nucleotide form. This may reflect altered condensation with PRPP or activation via the two-stage salvage pathway involving ribose-1-phosphate or deoxyribose-1-phosphate and the appropriate nucleoside phosphorylase, with subsequent phosphorylation of the resultant nucleoside by uridine or thymidine kinase. Alternatively, lack of sensitivity has been correlated with an increased disappearance rate of 5-FU nucleotides, which were documented in one case to reflect enhanced nucleotide phosphatase activity. Alterations in the catabolism of 5-FU appear to affect sensitivity and predict responsiveness to the drug. DPD, the rate-limiting enzyme in the catabolism of pyrimidines, regulates the amount of 5-FU available for the activation to nucleotide forms. In hepatocellular carcinomas inherently resistant to fluoropyrimidine-based chemotherapy, DPD activity was found elevated compared to that of normal tissue. DPD activity was also found to predict response to 5-FU in head and neck tumors, and DPD mRNA levels predicted resistance to the drug in colorectal cancer patients. Other well-documented mechanisms of resistance reflect changes in the thymidylate synthase, with reduced affinity for FdUDP or increases in the rate of synthesis and activity of the enzyme, possibly associated with gene amplification or altered enzyme turnover rates. The mode of exposure to the drug can result in the selection of tumor cells with different mechanisms of resistance. Finally, effective deletion of the facilitated diffusion transport of FdUrd has been shown to confer resistance to this 5-FU derivative, but not to 5-FU in a human colon cancer cell line.
Modulation of Therapy: Leucovorin To improve the limited response rate to therapy with 5-FU, a rate of 10% to 25% in the most responsive cancers, various biochemical strategies have been investigated. The degree of 5-FU activation by orotidylate pyrophosphorylase is affected by the available concentrations of PRPP. Because alterations of traffic along both the purine and pyrimidine nucleotide biosynthesis pathways affect the available concentrations of PRPP, several drug or metabolite combinations have been shown to modify the activation of 5-FU, presumably by altering the concentration of this ribose-5-phosphate donor. Others have explored depletion of pyrimidine nucleotides by inhibitors of the de novo synthesis of pyrimidines. A major focus in this area has been enhancing the efficiency with which the covalent complex of FdUMP with the folate cofactor and thymidylate synthase is formed by supplementation with the reduced folate cofactor.

Formation of the ternary complex of FdUMP, thymidylate synthase, and folate coenzymes may be limited by the availability of reduced folates in some cell lines and tumors. To optimize formation of the covalent complex, large doses of leucovorin, or D,L-5-N-formyl tetrahydrofolate, have been employed to saturate target enzymes with L-5-10-methylene tetrahydrofolate via conversion of the l-isomer of leucovorin to 5-methyl tetrahydrofolate.

Sound experimental evidence supports the logic of this approach to modulation. Early studies have demonstrated that optimal 5-FU cytotoxicity in cell lines was achieved only when the cells were supplemented with folates to achieve concentrations much greater than those required for optimal growth. These effects directly related to the quantity of the ternary complex formed within the cells. The importance of sustaining the folate levels to stabilize the ternary complex could be seen in xenografts of human tumors, in which only transient inhibition of thymidylate synthase with 5-FU would be expected unless supplemental reduced folates were present. The importance of polyglutamylation to enhance binding to thymidylate synthase in retaining folates within cells also has been documented, using cells that were defective in polyglutamate synthase.

If modulation by leucovorin in human disease is to be successful, the enhancement of ternary complex formation must be selective for tumor tissue. In a murine tumor model, leucovorin expanded the reduced folate pools in the tumor but not in bone marrow. This result was consistent with the antitumor effect seen without increased host toxicity. In other model systems, however, a consistent improvement in the therapeutic index is not seen. Because of the enhanced inhibition of thymidylate synthase when prior supplementation with leucovorin is employed, the dose of 5-FU must be reduced by approximately 20%. Under these conditions, diarrhea and mucositis remain the dose-limiting toxicities.

A wide range of clinical studies have generally confirmed the increased rate of response to 5-FU therapy in colorectal cancer when supplemented by leucovorin. Evidence for increased survival in these trials is limited, however. In breast and stomach cancers, the response rate in patients who are not previously treated with 5-FU appears to be increased by the addition of leucovorin; data for other diseases are insufficient to draw conclusions. The generally favorable results obtained in these studies have led to a rather universal addition of leucovorin to 5-FU trials of combination with other drugs. Particularly promising are three studies combining 5-FU-leucovorin with cisplatin in head and neck cancer. Despite these positive results, carefully controlled studies are needed to ensure the validity of this mode of modulation, particularly as other new drugs and modulators are combined with 5-FU-leucovorin regimens.

Oral Prodrug of 5-Fluorouracil: Capecitabine A recent metaanalysis of infusional versus bolus 5-FU has concluded that protracted low-dose infusion of 5-FU has resulted in a higher response rate, 22% versus 14%, with improvement in survival. However, the long-term delivery requires a surgically implanted venous access and the use of an infusion pump. The administration of oral 5-FU could reduce the cost of treatment and be more convenient to the patient. Its oral use has been hampered by an incomplete and variable bioavailability. Over the past several years several oral fluoropyrimidines have been evaluated clinically. Although several had potentially desirable pharmacologic attributes, only capecitabine has received Food and Drug Administration (FDA) approval in the United States.

Capecitabine is an orally administered fluoropyrimidine carbamate prodrug that is activated to 5-FU by three sequential enzymatic steps. First, hepatic carboxyesterase hydrolyses the N-pentyl carbamate chain to form 5’-deoxy-5-fluorocytidine, which is then deaminated to 5’-deoxy-5-fluorouridine 5’S-d5-FUR by cytidine deaminase; then thymidine and uridine phosphorylases hydrolyze 5’S-d5-FUR to produce 5-FU. The higher phosphorolytic activity expressed in human tumor tissue compared to that of the surrounding normal tissue has been suggested to result in selective activation and an improved therapeutic index. A higher concentration of 5-FU (2.9-fold) has been demonstrated in colorectal tumor specimens when compared to adjacent normal tissue of patients who received oral capecitabine 5 to 7 days prior to surgical removal of the tumor. Capecitabine is typically administered bid at a total daily dose of 2,000–2,500 mg/m2 over 14 days. This dose generates plasma peak levels 2 hours after administration, comparable to the ones achieved with a continuous intravenous infusion of 300 mg/m2/d of 5-FU. Toxicities were also similar to a continuous 5-FU infusion, with diarrhea, mucositis, and hand-foot syndrome. The toxicities are for the most part tolerable, in particular at the 2,000 mg/m2/d dose, and reversible after a short interval off therapy.

Capecitabine was initially approved for the treatment of metastatic breast cancer resistant to chemotherapy containing both paclitaxel and antracyclines. In this patient population, an 18.5% response rate was observed.

Figure 50-4 Structure and metabolic activation of capecitabine. Adapted from Xeloda product information, Roche USA.
Subsequently, capecitabine was approved for use in advanced colorectal cancer based on demonstrated equivalence in Phase III studies to intravenously administered 5-FU-leucovorin (Mayo regimen). It is of interest that coadministration of leucovorin was not required to obtain a comparable effect.

Most recently capecitabine has been approved for combined use with Taxotere in advanced breast cancer. The desirable effect of this combination may be due to upregulation of thymidine phosphorylase by Taxotere. Several other chemotherapy agents in addition to radiation are now recognized to upregulate thymidine phosphorylase, thereby increasing selective intratumoral activation of capecitabine.

**Cytosine Arabinoside**

**Background**

Cytosine arabinoside, or cytarabine or ara-C, is a nucleoside analog of deoxycytidine that was first synthesized in 1950 and introduced into clinical medicine in 1963. One of the most important drugs in the treatment of acute myeloid leukemia, it is also active against acute lymphocytic leukemia and, to a lesser extent, is useful in chronic myelocytic leukemia and non-Hodgkin lymphoma. It has not been proven to be particularly useful in the treatment of nonhematologic neoplasms. Myelosuppression and gastrointestinal epithelial injury are the primary toxic effects of ara-C. Using high-dose ara-C regimens, additional toxic effects such as intrahepatic cholestasis and central nervous system (CNS) toxicity are frequently observed. This toxicity could be due to its impact on mitochondrial DNA synthesis in nonproliferating tissues.

**Metabolism**

Cytosine arabinoside is rapidly deaminated by cytidine deaminase to a much less active compound, arabinosyluracil (ara-U). Ara-C enters cells through a carrier-mediated process or by simple diffusion. At low concentrations of ara-C (< 2 μM), the carrier-mediated process predominates. The efficiency of this transport process depends on the binding affinity of ara-C for the carrier, the number of carrier molecules in the membrane, and the presence of competing nucleosides sharing the same system. After entering the cells, it is metabolized primarily by the enzymes that normally metabolize deoxycytidine or, in some instances, cytidine (Figure 50-5).

The enzyme that is responsible for cytosine arabinoside monophosphate (ara-CMP) synthesis is cytoplasmic deoxycytidine kinase. Mitochondrial deoxyuridine monophosphate kinase, which can phosphorylate deoxycytidine and thymidine, does not efficiently phosphorylate ara-C. The activity of the cytoplasmic deoxycytidine kinase is higher in the S phase of the cell cycle. The amount of ara-CMP formed depends on the relative activity of cytoplasmic deoxycytidine kinase and cytidine deaminase. Tetrahydrouridine is a potent inhibitor of cytidine deaminase, with a Ki value of 10−8 M. Potentiation of the cytotoxic effect of low ara-C concentrations by tetrahydrouridine underscores the role of cytidine deaminase in ara-C metabolism. The enzyme responsible for conversion of ara-CMP to cytosine arabinoside diphosphate (ara-CDP) is cytidylyluridilate-deoxycytidylyltransferase (CMP-UMP-dCMP) kinase. There are two forms of this enzyme, and both are capable of phosphorylating ara-CMP. It has been suggested that ara-CMP could be deaminated to uracil arabinoside monophosphate (ara-UMP) by dCMP deaminase. Whether this pathway is functional in cells is questionable, however, because ara-CMP is a very poor substrate for dCMP deaminase compared to dCMP.

Several mammalian cell lines are partially resistant to ara-C because of a decreased activity of dCMP deaminase. Enzymes responsible for the phosphorylation of ara-CDP to cytosine arabinoside triphosphate (ara-CTP) are nucleoside diphosphate (NDP) kinases. There are multiple species of NDP kinase activities in human cells; many of them belong to the nm23 gene family. Whether a preference exists for one isoform over another in the phosphorylation of ara-CMP is unclear, but the formation of ara-CTP correlates in human cells incubated with ara-C has been reported. The enzyme that catalyzes this reversible process is phosphohydrolase chicken or cytidyltransferase. Both cytidine diphosphate (CDP) choline and deoxy-cytidine diphosphate (dCDP) choline serve as donors of the phosphorylcholine moiety in phosphatidylcholine synthesis; how ara-CDP choline participates in or interferes with this reaction is not clear.

Major attention has also been focused on the incorporation of ara-CTP into DNA in competition with thymidine triphosphate (dCTP). Elongation of DNA by polymerase α is considerably retarded by the incorporation of ara-CMP, whereas no significant impact on elongation by DNA polymerase β could be seen after incorporation of a single ara-C nucleosome residue. However, neither polymerase alone could appreciably elongate the DNA if two consecutive ara-CMP residues were incorporated. Thus, the behavior of ara-CTP on DNA polymerase is not only phosphorylation-dependent but also sequence-dependent.

**Mechanism of Action**

The primary action of ara-C is inhibition of nuclear DNA synthesis. Mitochondrial DNA synthesis is not affected by ara-C, even at concentrations 10 times greater than that required to inhibit cell growth by 50%. The possibility remains, however, that the functional nature of mitochondrial DNA may be compromised through incorporation of ara-C internally.

Three mechanisms have been suggested to account for the inhibition of nuclear DNA synthesis by ara-C. The relative importance of each mechanism may depend on the intracellular concentration of ara-CTP. The first mechanism is inhibition of the initiation of new replication units in chromosomes consequent to the incorporation of ara-C into DNA. The second mechanism is the retardation of DNA-chain elongation because of the incorporation of ara-C into DNA. This effect is DNA polymerase- and sequence-dependent, as discussed earlier. Reactions catalyzed by DNA polymerase α, and perhaps DNA polymerase δ, are more susceptible than other DNA polymerase activities. The third mechanism, which may become important only when a high-dose ara-C protocol is used, is the inhibition of DNA primase. Ara-CTP can inhibit the formation of the RNA oligomer required for the initiation of DNA synthesis with K, values of 25 to 125 μM (depending on the template being used). Although there is no evidence that ara-CMP can be incorporated into an RNA oligomer in vitro, it has been found that some of the ara-C that is associated with DNA is alkaline labile. This indicates the possibility that ara-C is incorporated into the RNA primer of DNA and requires further investigation.

In general, the inhibition of cell growth correlates well with the degree of the incorporation of ara-C into cellular DNA. The majority of incorporated ara-CMP is in internucleotide linkage in DNA. The relative ratio of ara-C in internucleotide, compared with chain-terminal positions, depends on the concentration of ara-C; the higher the concentration of ara-C to which the cells are exposed, the lower the relative amount of internucleotide ara-C residues. This could result from the higher probability of consecutive ara-CMPs being incorporated into DNA, which stops further DNA-chain elongation catalyzed by DNA polymerase α and DNA polymerase β. The amount of ara-CMP that is incorporated into DNA also depends on the relative ratio of ara-CTP to dCTP. Decreases in the intracellular pool of dCTP can increase the amount of ara-CMP that is incorporated. Exonucleases such as the recently identified TREX 1 or 2 and even p53 could remove ara-C incorporated in terminal positions to limit the cytotoxic effects.

Among other potential targets, ara-CTP is not a potent inhibitor of ribonucleotide reductase, a key enzyme early in the course of dCTP formation. Ara-CTP can act in lieu of dCTP to activate dCMP deaminase for the deamination of dCMP to dUMP, the substrate for dTMP synthesis. Because ara-CMP is a poor substrate for
The mechanism of action for ara-C may be dosage-dependent. At noncytotoxic concentrations, ara-C can cause human promyeloblast HL-60 cell lines to differentiate. It has been suggested that the success of low-dose ara-C therapy in patients with myelodysplastic syndrome may result from the differentiation effects of ara-C. When given to patients with leukemia, high doses of ara-C cause rapid tumor-cell lysis. Whether additional mechanisms of ara-C also play important roles in this protocol is unclear. In patients who receive high doses, the concentration of ara-U, the deamination product of ara-C, can exceed 100 μM in plasma. The high concentrations of ara-U may act in concert with ara-C, and it also may affect cell growth by mechanisms that have not yet been established.

Mechanism of Resistance Cells could become resistant to ara-C because of (1) decreased activities of the carrier for ara-C transport and for cytoplasmic deoxycytidine kinase, (2) increased catabolism of ara-C through the action of cytidine deaminase, (3) increased formation of dCTP by ribonucleotide reductase and NDP kinase, or (4) decreased activity of dCMP deaminase, which could lead to increased competition between dCTP and ara-CTP for incorporation into DNA. An increased activity of 3' to 5' exonuclease, which could remove ara-CMP from the DNA-chain terminus, has also been suggested.

5-Azacytidine Background 5-Azacytidine (5-AC) was first synthesized in 1963, and it was later isolated as a natural product from fungal cultures. The clinical utility of this cytidine analog is primarily in the treatment of acute myelocytic leukemia and myelodysplastic syndrome where in low dose, it is able to cause partial or complete differentiation in hematopoiesis of the majority of patients; occasionally, clinical response has been observed in patients with solid tumors. This compound can promote the expression of genes that are suppressed by hypermethylation. This activity suggested use of 5-AC in genetic diseases, such as sickle cell anemia and thalassemia, but its usefulness in treating these diseases has been limited by its bone marrow toxicity and concerns over its carcinogenic potential. The major toxicity of 5-AC is leukopenia and, to a lesser degree, thrombocytopenia. Hepatotoxicity has also been reported, particularly in patients with pre-existing hepatic dysfunction.

Metabolism The replacement of carbon by nitrogen in position 5 of the cytidine heterocyclic ring results in a marked chemical instability. The product of the ring opening, N-formylaminoribofuranosyl guanylurea, may recycle to form the parent compound, but it is also susceptible to further decomposition. This tendency to decompose not only may play a role in its mechanism of action but also is troublesome in its clinical use. Although 5-AC can be deaminated by cytidine deaminase to 5-azauridine (5-AU), a less toxic compound, the efficiency of this deamination by cytidine deaminase is less than that of cytidine. Nevertheless, inhibition of the deamination by tetrahydrouridine can enhance 5-AC toxicity. 5-AC enters mammalian cells by a facilitated nucleotide transport mechanism that is shared with other nucleosides. The initial step in its activation is the conversion to 5-azacytidine monophosphate (5-ACMP) by uridine-cytidine kinase. 5-ACMP is further phosphorylated to 5-AC di- and triphosphate by CMP-UMP-dCMP kinases and nucleoside diphosphate kinases, respectively. 5-AC triphosphate, which for several hours is the predominant metabolite in cells treated with 5-AC, can be incorporated into RNA, but its pathway for incorporation into DNA is not well defined. 5-azacytidine dephosphorylation (5-ACDP) is likely reduced by ribonucleotide reductase to the corresponding deoxynucleotide diphosphate. This diphosphate is phosphorylated by nucleoside diphosphate kinases to 5-azadeoxycytidine diphosphate (5-AdCTP), which can be efficiently incorporated into DNA by DNA polymerases α and β. The incorporated 5-azadeoxycytidine monophosphate (5-AdCMP) at the 3' terminus of DNA has less effect on subsequent DNA-chain elongation than the incorporated ara-CMP at the 3' terminus of DNA. 5-azadeoxycytidine (5-AdC) also is stabilized against hydrolytic degradation by incorporation into DNA, which could result, in part, from hydrophobic shielding of the triazine ring from water and other polar nucleophiles within the DNA double helix.

A summary of 5-AC metabolism is shown in Figure 50-6. 5-AC is most cytotoxic to cells in the DNA-synthetic phase of the cell cycle, but the exact mechanism of its cytotoxic action has not been well established. It could inhibit both DNA and RNA synthesis. Incorporation into RNA can inhibit the processing of ribosomal RNA from higher-molecular-weight species, disassembly of polyribosomes, and markedly inhibit protein synthesis. Incorporation into DNA also could inhibit DNA synthesis. One important, well-documented effect is the inhibition of DNA methylation because of steric hindrance of binding with DNA-methyltransferase After incorporation. The methylation of cytosine residues in DNA is responsible for the inactivation of specific genes; thus, treatment of cells with 5-AC leads to reduced levels of cytosine methylation and enhanced expression of selected genes that are normally suppressed. At minimally cytotoxic concentrations, 5-AC stimulates the differentiation of some tumor cell lines in culture, and it has been suggested for the treatment of genetic diseases that are associated with hypermethylation in myelodysplastic syndrome, as well as for virus-associated cancers in combination with antiviral compounds.

Mechanism of Resistance Cells can become resistant to 5-AC by the reduction or elimination of uridine-cytidine kinase. Decreased nucleoside transport by the facilitated diffusion mechanism also can decrease sensitivity to 5-AC, and cytosine deaminase may play an important role in cell sensitivity as well. In animal models, tumor cells that are resistant to ara-C because of the deletion of cytoplasmic deoxycytidine kinase activity—a frequent mechanism of cellular resistance to ara-C—are more susceptible to 5-AC than is the parent tumor line. Sequential treatment with ara-C and then 5-AC deserves further study, particularly in patients who become refractory to ara-C.

Gemcitabine Background 2', 2'-difluoro-2'-deoxycytidine (dFdC) is a deoxycytidine analog with two fluorine atoms in the 2' position of the sugar moiety (Figure 50-7). First synthesized in 1986, this molecule was initially developed as an antiviral agent because of its potent inhibitory activity against both DNA and RNA viruses. Subsequently, its broad spectrum of activity in murine tumors and human tumor xenografts led to evaluating this antineoplastic activity in clinical trials.

dFdC was approved by the FDA in 1996 as a first-line treatment for patients with locally advanced or metastatic adenocarcinoma of the pancreas. For the first time, approval was granted on the basis of clinical benefit response as the main clinical end point for assessing the drug's effect. Subsequently, dFdC has received FDA approval for the treatment of inoperable, locally advanced or metastatic non–small cell lung cancer, in combination with cisplatin. dFdC has been found active as a single agent in the first-line treatment for breast cancer and has been combined with cisplatin in patients pretreated with anthracycline and taxane.

Activity has also been shown in bladder cancer and in ovarian cancer. dFdC has demonstrated a promising effect in non-Hodgkin lymphoma and in Hodgkin disease, and in patients with relapsed or refractory cutaneous T-cell lymphoma.
The dose-limiting toxicity of dFdC in both single-agent and combination studies has been mild to moderate myelosuppression. The non-hematologic toxicity was mild, with nausea, vomiting, occasional skin rash, alopecia, and pneumonitis. Rare occurrences of hemolytic-uremic syndrome have been reported.229-237

Metabolism 2', 2'-difluoro-2'-deoxycytidine requires phosphorylation by deoxycytidine kinase to exert its cytotoxic activity (see Figure 50-7). The major intracellular metabolite is 2', 2'-difluoro-2'-deoxycytidine triphosphate (dFdCTP); lesser amounts of the monophosphate (dFdCMP) and the diphosphate (dFdCDP) are also present.232 The cellular elimination of dFdCTP was investigated in several human cell lines: CCRF-CEM, K562, and A2780.233,234 Elimination of dFdC follows a biphasic course, with a short initial half-life followed by a second, slower phase of degradation. The biphasic elimination of dFdC differs from the linear monophasic kinetic that is exhibited by the triphosphate of ara-C,235 arabinosyladenine,236 and arabinosyl-2-fluoroadenine.237

Deoxycytidine deaminase inactivates dFdC to 2', 2'-difluoro-2'-deoxyuridine (dFdU), which has no antitumor activity.232 The monophosphate of dFdC also can be deaminated to the uracil derivative, 2'-difluoro-2'-deoxyuridine monophosphate (dFdUMP) by deoxycytidylate deaminase.238

Pharmacokinetic studies during phase I clinical trials have shown a very rapid half-life (8 minutes) for dFdC because of deamination over a wide range of dosages.239 The deamination product, dFdU, which is the only metabolite present in the urine, exhibits a biphasic elimination from plasma, with a long terminal phase of 14 hours. The concentration of dFdCTP in mononuclear cells increases in proportion to the dose of dFdC infused, up to 250 mg/m². Above this dose, the process shows saturation in accumulation of the triphosphate derivative.

Mechanism of Action 2', 2'-difluoro-2'-deoxycytidine exerts its inhibitory activity on DNA synthesis through several distinct mechanisms. The accumulation of dFdCTP causes a reduction in the deoxyribonucleotide pools in both CCRF-CEM and HT-29 human tumor cells.240,241 This reflects a direct inhibition of ribonucleotide reductase, caused mainly by dFdCDP; however, dFdCTP was not as inhibitory of the partially purified enzyme.240 Another important mechanism is the incorporation of dFdCTP into DNA; dFdCTP competes with dCTP for incorporation into the C sites of DNA catalyzed by DNA polymerases α and ε. The primer extension pauses one deoxynucleotide after dFdCMP incorporation.242 Moreover, the exonuclease activity of polymerase ε was unable to excise nucleotides from DNA containing dFdCMP at either the 3’ end or at an internal position.242 The cytotoxic activity of dFdC strongly correlates with the amount of monophosphate that is incorporated into cellular DNA.

Incorporation of dFdC into RNA has been detected in murine colon 26-10 cells as well as human A2780 and CCRF-CEM cells.243 Although the extent of this incorporation was two- to tenfold less than that into DNA, it may play a role in cytotoxicity.

Inhibition of ribonucleotide reductase could have a self-potentiation effect on the inhibitory activity of this drug. The activity of deoxycytidine kinase, which is required for the phosphorylation of dFdC, is regulated by dCTP levels; therefore, a decrease in dCTP pools likely will lead to increased dFdC activation.232 dCTP also is required as an activator of dCMP deaminase, an enzyme that is critical for the catalolysis of dFdC nucleotides; thus, a reduction in dCTP could slow the deamination process and prolong the half-life of dFdC nucleotides.238 Finally, dCTP competes with dFdCTP for incorporation into DNA by polymerases α and ε, and lower dCTP levels also could enhance dFdC incorporation into DNA as well as increase its inhibitory effect on cell proliferation.242

To date, two examples of resistance to dFdC have been reported.244,245 Human ovarian carcinoma A2780 cells that were exposed to increasing concentrations of dFdC became highly resistant to the drug, cross-resistant to ara-C and 2-chlorodeoxyadenosine, and modestly resistant to doxorubicin, vincristine, and cis-platinum. Resistant cells did not possess deoxycytidine kinase activity; therefore, they were not able to phosphorylate dFdC as well as the other two nucleoside analogs. Western blot analyses of the cell extract using a polyclonal, anti-deoxycytidine kinase antibody could not detect this protein in the resistant subline. Another mechanism of resistance has been recently reported. Human KB tumor cells could become resistant to dFdC as the result of increased expression of the M2 unit of ribonucleotide reductase. Resistance leads to elevated activity of the same enzyme, as well as an augmented intracellular dCTP pool, which could prevent the phosphorylation of dFdC by deoxycytidine kinase.245

PURINE ANALOGS

The original syntheses of purine antimetabolites focused on isosteric replacement of oxygen, carbon, or nitrogen in the purine ring, and they were predicated on the same logic as that used for pyrimidines.23,246 C-N or O-N substitutions gave 8-azaguanine and 2-6-diaminopurine. The first clinically useful agent, however, was 6-mercaptopurine (6-MP),247 in which the 6-OH of hypoxanthine was replaced with a thiol group (Figure 50-8). Subsequently, the equivalent analog of guanine, 6-thioguanine, was prepared.248 Two glutamine analogs, 6-diazo-5-oxo-L-norleucine and azaserine, also made major contributions to our understanding of the purine biosynthesis pathways during that period, but these were not found to be clinically useful.249 Studies of these initial analogs established many of the relevant issues addressed in the subsequent development of purine and pyrimidine analogs.250-252

By the identification of metabolites and characterization of resistance mechanisms, early studies with 6-MP in model systems quickly demonstrated the dependence of the inhibitory activity on metabolic conversion to the corresponding analog nucleotide analogs.198 Equally important to the activity of many purine analogs has been an understanding of the catabolic reactions that limit their availability. Of particular relevance are xanthine oxidase, which inactivates 6-MP and thioguanine,251 and adenosine deaminase,253 which is the target for deoxycoformycin and limits the action of arabinosyl adenosine.

Two more recently developed purine analogs, acyclovir and ganciclovir, are acyclic nucleoside derivatives and valuable antiviral agents. Along with arabinosyl adenine, these agents are activated by kinase reactions, but they exert their effects on the same spectrum of biochemical reactions as exerted by purine base analogs. Their role in cancer therapy remains to be established.

![Figure 50-7](https://example.com/figure507.png) Structure, metabolism, and actions of 2', 2'-difluoro-2'-deoxycytidine (dFdC) and its nucleotides. Dashed lines indicate inhibitory actions. Modified from Heinemann et al. dU = deoxyuridine; MP, DP, TP = mono-, di-, and triphosphate; CDP = cytidine diphosphate.

![Figure 50-8](https://example.com/figure508.png) Purine antimetabolites.
6-Mercaptopurine (6-MP) was among the first purine analogs that demonstrated antineoplastic activity, and it remains useful in the treatment of acute leukemia.\textsuperscript{254} This derivative of hypoxanthine is a relatively insoluble, amphoteric compound that is stable, except in alkaline solutions. Metabolic activation primarily occurs by reaction with PRPP via hypoxanthine-guanine pyrophosphorylase (HGPRT) to form 6-MP riboside 5'-phosphate, more properly called thiopurine monophosphate (TIMP).\textsuperscript{255}

TIMP is believed to exert its major effect on purine nucleotide metabolism by inhibition of the first step in purine biosynthesis, the formation of 1-NH\textsubscript{2}-ribose-5-PO\textsubscript{4}, via a pseudofeedback inhibition in which TIMP mimics the regulatory action of adenine or guanine nucleoside monophosphates.\textsuperscript{256-258} An early precursor of purine biosynthesis, 5-amino imidazole-4-carboxamide, which can be converted to the corresponding ribonucleotide, protects cells in culture against the inhibition of growth by 6-MP. This finding is consistent with the view that the primary action is limitation of an early step in de novo synthesis. TIMP also blocks the subsequent metabolism of inosinic acid, the initial purine nucleotide, to adenylic acid by inhibiting adenosylsuccinate synthase.\textsuperscript{255} Similarly, synthesis of guanine nucleotides is reduced by inhibition of the oxidation of inosinic acid to xanthyllic acid. TIMP is not incorporated into nucleic acids as such, but minor amounts are converted to thioguanic acid, which is incorporated into both RNA and DNA. It has not been established, however, that this incorporation is significant to the toxic or antineoplastic actions of 6-MP.\textsuperscript{1} TIMP was recently shown to be a potent inhibitor of DNA exonuclease, which could excise ara-CMP from terminal DNA. This may partly explain the synergistic interaction of 6-MP and ara-C.\textsuperscript{259} A summary of 6-MP metabolism is presented in Figure 50-9.

6-Mercaptopurine is generally administered orally (90 mg/m\textsuperscript{2}) for several weeks. Absorption is variable, incomplete, and associated with a half-life of 20 to 45 min in plasma, where it is minimally bound to serum proteins.\textsuperscript{260} The rapid turnover largely results from oxidation by xanthine oxidase, which converts it to inactive thiouric acid, the primary urinary excretion product.\textsuperscript{261} In patients who are receiving allopurinol to control uricemia, the dose of 6-MP must be reduced by approximately 75% because drug catabolism is sharply reduced with the attendant risks of toxicity.\textsuperscript{262,263} No selective advantage in tumor therapy is achieved by this combination. Another metabolite, the \(S\)-methyl derivative of 6-MP, is found in cells as methyl mercaptopurine ribonucleotide, where it inhibits purine metabolism; it is excreted in urine as methyl mercaptopurine riboside.

The dose-limiting toxicity of 6-MP is myelosuppression. It is slow in onset, 2 to 4 weeks, and rapidly reversed after the dose is either reduced or discontinued.\textsuperscript{264,265} All formed elements (thrombocytes, granulocytes, and erythrocytes) can be affected. Although gastrointestinal mucositis or stomatitis is minimal, approximately 25% of treated patients experience nausea, vomiting, and anorexia, and a small number display hepatotoxicity.\textsuperscript{266}

Therapeutic action depends on the formation of the nucleotide 6-MP ribonucleoside monophosphate. In experimental tumor systems, resistance commonly is associated with a decreased rate of activation to the nucleotide form, resulting from deletion or modification of HGPRT activity. Limited studies in humans, however, suggest that resistance is caused by increased activity of a 5'-phosphatase that limits the concentration and duration of intracellular 6-MP ribonucleotide.\textsuperscript{267}

6-Mercaptopurine is effective in combination with prednisone for inducing remission in children with acute lymphoblastic leukemia. Currently, it is a regular component of consolidation and maintenance therapy for this disease. It also is of some value in adult acute lymphocytic leukemias. It no longer is commonly used in myeloid leukemias of adults, but it does have modest activity in combination therapy.

Although many 6-MP derivatives have been synthesized and evaluated in model systems, only one, azathioprine, is available at present. This methyl-nitro-imidazole derivative of the thiol group on 6-MP is cleaved in vivo, presumably by thiols, to liberate 6-MP. It generally is not used in cancer therapy, but it remains an important element of immunosuppressant therapy for allograft transplantation and selected autoimmune states.\textsuperscript{268}

Thioguanine (see Figure 50-8) is the 6-thiol derivative of guanine corresponding to 6-MP and also depends on activation via HGPRT.\textsuperscript{254} Unlike 6-MP, however, di- and triphosphates of thioguanine ribonucleotide are formed and incorporated into RNA. After conversion to thioguanine deoxynucleotide triphosphate, it can substitute for deoxyguanosine triphosphate (dGTP) in DNA polymerase reactions.\textsuperscript{269} This incorporation is thought to be the primary mechanism of cytotoxicity.\textsuperscript{270} Thioguanylate monophosphate is the predominant acid-soluble nucleotide, but it does not appear to exert the major effects on de novo purine synthesis that have been observed with 6-MP nor to deplete pools of normal purine nucleotides.

Like 6-MP, thioguanine, after deamination to thioxanthine by guanase, is readily catabolized to thiouric acid by xanthine oxidase. \(S\)-methylation also is observed, yielding \(S\)-methyl-thioguanine and thioxanthine.\textsuperscript{271} Dethiolation contributes to metabolism as well, as evidenced by the urinary excretion of 35\(^{S}\)-SO\textsubscript{4} after administration of 35\(^{S}\)-thioguanine. The primary use of thioguanine is in acute myeloid leukemia, where it may be combined with arabinosyl cytosine. Recent studies question its value in this disease, however.\textsuperscript{272,273} A summary of thioguanine metabolism is presented in Figure 50-9.

Allopurinol (4-hydroxyprazolo-3,4-d-pyrimidine) is an important adjuvant to antineoplastic therapy (Figure 50-10). This agent and its primary metabolite, oxyipurinol, are potent inhibitors of xanthine oxidase.\textsuperscript{274,275} As such, they limit the formation of uric acid from the degradation of purine nucleotides and nucleic acids. It is interesting to note that oxyipurinol is formed by the target enzyme xanthine oxidase and is a potent inhibitor of this enzyme. In addition to this mechanism, allopurinol has been shown to inhibit purine nucleotide biosynthesis by feedback inhibition of the first reaction in the pathway and to deplete pyrophosphoryl ribose-5-PO\textsubscript{4}, presumably by formation of the corresponding allopurinol and oxyipurinol ribonucleotides.\textsuperscript{276} These nucleotides are inhibitors of orotidylate decarboxylation as well, and they
result in the excretion of urinary orotate and orotidine.277 These actions may relate to the ability of allopurinol to selectively reduce the toxicity of 5-FU to some normal tissues, as described previously.

Although it was originally synthesized as an antineoplastic agent, allopurinol is widely used in the treatment of hyperuricemia that is associated with gout and other metabolic disorders.278 Certain neoplastic states, particularly lympho- and myeloproliferative diseases, also generate hyperuricemia, and allopurinol is an effective means to avoid the associated episodes of gout or uric acid nephropathy.279 This is particularly important in leukemias, lymphomas, and in patients with other bulky diseases when chemotherapy produces rapid tumor lysis and its attendant release of purine bases from the nucleic acids.

The elevation of hypoxanthine and xanthine concentrations in plasma by the inhibition of xanthine oxidase is less dangerous than elevated levels of uric acid. This is because these purines are more soluble and less likely to form stones or cause gout. Nevertheless, it generally is recommended that patients who are treated with allopurinol for hyperuricemia also be hydrated and alkalinized when uric acid concentrations rise significantly.

Oral doses of 300 to 800 mg/d have been recommended and generally are well tolerated. Skin rashes and gastrointestinal disturbances are common and of increased frequency and severity when the allopurinol is given together with ampicillin, but these effects rarely limit therapy.280 Severe drug-induced fever, vasculitis, and blood dyscrasias of a hypersensitive nature have infrequently occurred.281 Because allopurinol also reduces the rate of metabolic inactivation of oral 6-MP and azathioprine, doses of these purine antimetabolites must be reduced by 50% to 75% to avoid excessive toxicity.260 Oxidation by xanthine oxidase is the primary route of allopurinol metabolism and the relevant site of action, but allopurinol also can inhibit the metabolism of drugs, such as cyclophosphamide, by the mixed function oxidases.282

**Deoxycoformycin** Background Deoxycoformycin (pentostatin) is a natural product first isolated in 1974 from the culture of *Streptomyces antibioticus* (see Figure 50-9).283 Its structure mimics the transitional-state form of adenosine in an adenosine deaminase–catalyzed reaction, and it is one of the most potent inhibitors of adenosine deaminase (Ki 5 x 10⁻¹⁰–10⁻¹² M depending on the source of the enzyme).284 Because adenosine deaminase is not essential for cell growth in culture, this compound did not show antitumor activity in preclinical screenings.

The initial clinical development of deoxycoformycin centered on its activity as an adenosine deaminase inhibitor for the potentiation of adenosine arabinoside, which also was deaminated by adenosine deaminase to yield less toxic compounds. During early Phase I studies, the profound lymphotoxic effect of deoxycoformycin was noted. Other studies described a congenital syndrome of severe combined immunodeficiency associated with low or undetectable levels of adenosine deaminase in lymphocytes,285 and these results suggested the importance of adenosine deaminase in lymphocyte function, leading to intensive development of deoxycoformycin as a single agent for the treatment of lymphoproliferative diseases.

The most responsive tumor identified is hairy cell leukemia, in which durable remissions are achieved in over 90% of patients with a relatively brief course of treatment.286,287 Other responsive lymphoid diseases include chronic lymphocytic leukemia and prolymphocytic leukemia, mycosis fungoides, and acute T-cell leukemia/lymphoma.288,289 Considerable variation exists in the susceptibility of patients to deoxycoformycin toxicity. This includes immunosuppression,290,291 CNS disturbances, impaired renal function, conjunctivitis, and muscle and joint pain. Impaired renal function and poor performance status place patients at high risk for toxicity, even with low dosages of this drug.

**Metabolism** Deoxycoformycin enters the cell through the facilitated-diffusion nucleoside carrier. It can be phosphorylated to mono-, di-, and triphosphate nucleotides, and significant incorporation into DNA, but not RNA, has been observed.286 Adenosine kinase and deoxyctydine kinase283 do not appear to be responsible for the initial phosphorylation, but reversal of the 5'-nucleotidase reaction is a potential basis for nucleotide formation. Definitive statements cannot be made about the enzymology of deoxycoformycin metabolism at this time.
Mechanisms of Action and Resistance

The primary site of action is the inhibition of adenosine deaminase. Because of the inhibition of adenosine deaminase in vivo, deoxyadenosine and adenosine cannot be catabolized efficiently. Consequently, deoxyadenosine-phosphorylated metabolites accumulate in many types of cells. This imbalance in deoxynucleotide pools is known to be toxic to cells, and the antitumor activity of deoxycoformycin may result from the combination of direct effects of deoxycoformycin and its metabolites as well as the expanded pools of deoxyadenosine.

The failure of deoxyadenosine to accumulate in cultures treated with deoxycoformycin is the reason deoxycoformycin was not identified as a potential antitumor compound in cell culture systems. The degree of deoxyadenosine triphosphate (dATP) accumulation correlated well with cell death caused by deoxycoformycin. Thus, dATP, which is known to be an allosteric inhibitor of ribonucleotide reductase, could result in growth inhibition by the generation of an imbalance of deoxynucleotide triphosphate pools. However, additional sites of action for both deoxycoformycin and deoxyadenosine are suggested by the observation that deoxycoformycin and deoxyadenosine are cytotoxic to nondividing cells, which do not require the function of ribonucleotide reductase. One potential site is the depletion of nicotinamide adenine dinucleotide (NAD) in deoxycoformycin- and deoxyadenosine-treated cells. NAD is required for poly-ADP ribosylation, a reaction that is essential to maintain the integrity of DNA and its repair process. Depletion of NAD could reduce the capacity for DNA repair, a constant process in cells, and cause DNA breaks as well as cell death. Inhibition of S-adenosyl homocysteine hydrolase by deoxyadenosine. Inhibition of this enzyme decreases the capacity of cells to perform transmethylation, a reaction that is critical for certain macromolecular functions. This mechanism does not require deoxyadenosine to be phosphorylated, and it may play an important role in the toxicity of deoxycoformycin to non proliferating tissues, such as in the liver and CNS. The activation of mitochondrial-dependent apoptosis through the interaction of Apf and dATP could also contribute to its activity.

Deoxycoformycin and deoxyadenosine also decrease ATP levels in some cell systems. In mice, hemolysis after treatment with deoxycoformycin is related to ATP depletion. Deoxycoformycin has also been shown to form phosphorylated metabolites that can be incorporated into DNA; whether these metabolites contribute to deoxycoformycin action, however, is not clear.

The mechanism of resistance to deoxycoformycin has not been defined because deoxycoformycin is not cytotoxic in cell culture. The action of deoxycoformycin in vivo results from the combined action of deoxycoformycin and deoxyadenosine, so the mechanism of cellular resistance to deoxyadenosine should be applicable. This could include adenosine kinase deficiency or altered quality or quantity of ribonucleotide reductase.

**Fludarabine Background**

In the search for more effective compounds than adenine arabinoside (ara-A, vidarabine), which has limited clinical usefulness because of its rapid deamination by adenosine deaminase, 2-fluoroadenosine arabinoside (9-β-D-arabinofuranosyl-2-fluoroadenine) was synthesized. It has been found to be relatively resistant to adenosine deaminase and has impressive antitumor activities in vivo as well as in cell culture. Its limited solubility and consequent difficulties in formulation led to the synthesis of a prodrg, the 5'-monophosphate of 2-F-ara-A (Fludara IV).

Fludara IV entered clinical trials in 1982, and it is one of the most active agents in the treatment of chronic lymphocytic leukemia. A high level of activity also has been observed in a variety of indolent lymphoproliferative neoplasms, including low-grade non-Hodgkin lymphoma, cutaneous T-cell lymphoma, macroglobulinemia, and hairy cell leukemia. In contrast to these leukemia cells, epithelial crypt cells from mouse intestine possess only a low-affinity system, and this difference in transport could be partly responsible for the favorable therapeutic index of 2-F-ara-A against sensitive tumor cells in mice. In future human studies, the potential role of transport systems in determining the sensitivity to 2-F-ara-A should be considered. Once 2-F-ara-A is taken up by cells, it is phosphorylated to 2-fluoroadenine arabinoside monophosphate (2-F-ara-AMP) and 2-fluoroadenine arabinoside diphosphate (2-F-ara-ADP), not like ara-A as a substrate of adenosine kinase, but by cytoplasmic deoxycytidine kinase. Tumor cells lacking cytoplasmic deoxycytidine kinase are resistant to F-ara-A. Intracellular F-ara-AMP can be further phosphorylated to the diphosphate F-ara-ADP, but it is not clear which enzyme is responsible for this reaction. AMP kinases likely may be responsible for the further phosphorylation of F-ara-AMP to F-ara-ADP. Nucleoside diphosphate kinases may be the predominant enzyme species responsible for the formation of F-ara-ATP from F-ara-ADP. F-ara-ATP can be incorporated into DNA in competition with dATP by DNA polymerases. Although DNA polymerases α, β, δ, and γ are all capable of using F-ara-ATP as a substrate, DNA polymerase α has a greater affinity for F-ara-ATP than do other DNA polymerases. Once F-ara-AMP is incorporated into the terminus of the growing DNA chain, the next step of elongation is retarded, regardless of which DNA polymerase is employed.

In addition, F-ara-A also has been shown to be incorporated into RNA, but which RNA polymerase is responsible has not been established. The incorporation of F-ara-A into poly (A1) RNA was 12-fold greater than that into poly (A) RNA. A summary of the metabolism of 2F-ara-A is shown in Figure 50-11.

Investigations of F-ara-A as a modulator of ara-C therapy are currently underway. When F-ara-A is given before ara-C, an increase in the accumulation of ara-C-TP occurs in leukemic lymphocytes. This modulation of ara-C anabolism probably results from an indirect effect of F-ara-C-TP on deoxycytidine kinase that relates to a reduction in the deoxynucleotide pools regulating the enzyme. It also may reflect a direct effect by F-ara-C-TP on the activity of deoxycytidine kinase. The in vitro accumulation of ara-C-TP also has been shown in the lymphocytes of patients with chronic lymphocytic leukemia treated with this sequential combination. The results of a clinical study in individuals who are refractory to F-ara-A therapy show partial or minor responses in approximately 35% of patients.

**Mechanism of Action**

The major site of growth inhibition by F-ara-A is the inhibition of DNA synthesis. Treatment of cells with F-ara-A is associated with the accumulation of cells at the G1–S-phase boundary and in the S phase; thus, it is a cell cycle S-phase–specific drug. Incorporation of the active metabolite F-ara-ATP retards DNA chain elongation. The degree of incorporation of the analog nucleotide depends not only on the type of DNA polymerase but also on the amount of intracellular dATP that competes with F-ara-ATP for incorporation. Among DNA polymerases in human cells, polymerase α, which is the critical enzyme in nuclear DNA synthesis, is more susceptible to the incorporation of F-ara-ATP. A consequence of this analog nucleotide incorporation is the retardation of DNA-chain elongation.

![Figure 50-11](image-url)
The rationale for the development of 2-chlorodeoxyadenosine (Cl-dAdo, cladribine) was that the death of lymphocytes in patients with adenosine deaminase deficiency was associated with the accumulation of deoxynucleotides. This deoxycytidine analog was selected for its resistance to adenosine deaminase. Its specific action on lymphoid cells is attributed to the high level of deoxycytidine kinase and low 5'-nucleotidase activity in these cells. This compound is highly cytotoxic to a variety of cell lines in culture, and it has potent antileukemic activity in mice. Cl-dAdo was shown to have potant and lasting effects in the treatment of low-grade B-cell neoplasms, such as chronic lymphocytic leukemia, non-Hodgkin lymphoma, and hairy cell leukemia. In addition, Cl-dAdo has demonstrated clinical activity against acute myeloid leukemia in children, including those with leukemic blast cells in the CNS and in T-cell lymphoproliferative disorders. The spectrum of clinical activity is similar to that of Fludara IV; however, a few patients who do not respond to F-ara-A are sensitive to Cl-dAdo. The major toxicity encountered is bone marrow suppression that is associated with severe infections. The degree of suppression relates to the rate of administration, cumulative dose, and tumor burden at the start of therapy.

**Metabolism**

The mechanism of transport for cladribine into a variety of human hematopoietic cell lines was explored using nucleoside transport inhibitors, such as dipyriramole and nitrobenzyl thioinosine (NBTI). The transport mechanism appears to be different in different cell lines, an observation based on their differential response to nucleoside transport inhibitors. Both NBTI-sensitive and NBTI-insensitive nucleoside transporters are involved. Once Cl-dAdo enters cells, it can be phosphorylated by deoxycytidine (dCyd) kinase to 2-chlorodeoxyadenosine mono (2Cl-dAMP), which subsequently is phosphorylated to 2-chlorodeoxyadenosine diphosphate (2Cl-dADP) and then to 2Cl-dATP. The enzymes involved, however, are not established. As 2Cl-dATP, it can be incorporated into DNA through the action of DNA polymerases competing with dATP. The structure and metabolism of 2-chlorodeoxyadenosine are shown in Figure 50-12.

**Mechanisms of Action and Resistance**

2Cl-dAdo can inhibit DNA synthesis in growing cells as well as DNA repair in resting cells. When growing cells were treated with 2Cl-dAdo, an accumulation of cells in the S phase was observed, suggesting that inhibition of DNA synthesis could be responsible for the cell-killing effect of the drug. The active metabolite is 2Cl-dATP, which can compete with dATP to be incorporated into the 3'-end of the growing DNA chain. Elongation beyond the incorporated analog was significantly retarded, and this could partly contribute to its inhibitory activity against DNA synthesis. Furthermore, 2Cl-dATP is a potent inhibitor of ribonucleotide reductase.

Levels of intracellular deoxynucleoside triphosphates were found to decrease in cells after exposure to 2Cl-dAdo, which also could contribute to its antitumor activity.

The mechanism of resistance is not clear, but it could be similar to that of 2F-ara-A. Although 2F-ara-A and 2Cl-dAdo share many similar features, there are differences in metabolism and mechanisms of action. Recently, it was suggested that Ca²⁺-sensitive mitochondrial events could play an important role in 2-Cl-dAdo cytotoxicity.

**Hydroxyurea Background**

Although hydroxyurea was first synthesized in 1869, its biologic activity was not recognized until 60 years later, when it was discovered that hydroxyurea could produce leukopenia, anemia, and megaloblastic changes in the bone marrow of rabbits. This simple molecule has been evaluated in a number of types of cancer, but its principal uses are in myeloproliferative diseases. Currently, it is an initial therapy of choice for chronic myelogenous leukemia; it also is used as therapy for polycythemia vera and hypereosinophilic syndrome. Activity against solid tumors has been demonstrated, but in these cases, it generally is used in combination with other anticancer agents or with radiation. A recent report also indicated the ability of hydroxyurea to inhibit human immunodeficiency type I DNA synthesis in activated blood lymphocytes, either alone or in combination with zidovudine or dideoxynosine, suggesting a possible antiviral application for this compound. However, clinical studies did not support this approach for the treatment of AIDS patients.

Hydroxyurea can be taken orally, and the half-life in plasma is approximately 4 hours. It readily crosses the blood-brain barrier. It is excreted predominantly in urine, but the interpatient variability is significant. The full extent and significance of hydroxyurea metabolism in humans has not been well established. It can be degraded by intestinal bacterial urease to form hydroxylamine, which can interact with acetylcoenzyme A to form acethydroxamic acid; this metabolite is found in the plasma of patients receiving hydroxyurea therapy.

The dose-limiting toxicity of hydroxyurea is myelosuppression. This results from inhibition of DNA synthesis in bone marrow. Toxicity begins within 2 to 5 days, and its duration is short once the drug is discontinued. Gastrointestinal side effects are frequently seen but rarely require discontinuation of therapy at the doses commonly used. Some dermatologic changes, such as hyperpigmentation, can also occur in patients after extended therapy.

**Cladribine Background**

Cladribine (2Cl-dAdo) is a potent inhibitor of ribonucleotide reductase, the key enzyme responsible for the formation of dATP. This causes a decrease of deoxynucleotides in 2F-ara-A- treated cells, which enhances the incorporation of F-ara-ATP into DNA. This may be considered to be “self-potentiation” of the inhibition of DNA synthesis by F-ara-ATP. In addition, F-ara-ATP was found to be an inhibitor of DNA primase, which is responsible for Okazaki fragment synthesis; another important step in DNA synthesis. The inhibition of RNA primer formation for DNA synthesis by F-ara-ATP was recently demonstrated as well, but the inhibition of Okazaki fragment formation by F-ara-ATP could conceivably play a role in the inhibition of DNA synthesis by F-ara-A. In addition, F-ara-A can inhibit mitochondrial DNA synthesis at concentrations similar to those that cause cytotoxicity; however, such inhibition does not affect cell growth for several cell generations. Thus, the cytotoxicity of F-ara-A, which usually is estimated by the continuous exposure of cells to drugs for three to four generations, likely does not result from the inhibition of mitochondrial DNA. Also, it has been reported that incubation of normal lymphocytes for 24 hours with 10 μM, but not 1 μM, caused a decrease in both cytoplasmic NAD and ATP concentrations that could be correlated with a decrease in cellular viability. The mechanism for the depletion of NAD and ATP by F-ara-A is not clear, and whether the inhibition of mitochondrial DNA synthesis by F-ara-A or depletion of NAD and ATP is responsible for the delayed onset of F-ara-A toxicity observed clinically has not yet been established.

Resistance to F-ara-A may occur because of decreased uptake, lack of deoxycytidine kinase, increased intracellular concentration of dATP, decreased susceptibility to the activity of ribonucleotide reductase, decreased affinity of DNA polymerase for F-ara-ATP, or increased efficiency of the removal of F-ara-ATP from the 3' terminus where incorporated into DNA. The potential role of the 3' and 5' exonuclease activities of DNA polymerase D and other 3' and 5' exonuclease activities in removal of incorporated F-ara-AMP remains to be defined as a possible mechanism of resistance.
Mechanism of Action and Resistance

Hydroxyurea is considered to enter cells by passive diffusion. It inhibits cellular DNA synthesis through the inhibition of ribonucleotide reductase, which is the key enzyme responsible for the synthesis of deoxynucleotides, the building blocks of DNA. The substrates for this reaction include the four ribonucleoside diphosphates and the diphosphonucleotides of fluorouridine, azacytidine, and thioguanosine, namely 5-FUDP, 5-aza-CDP, and 6-thio-GDP. The activity of ribonucleotide reductase is highly regulated by the intracellular concentration of ribonucleoside and deoxyribonucleoside triphosphates. Two models, sequential and intercalating, have been proposed for the interplay of ribonucleotide reductase and deoxyribonucleoside triphosphates. The metabolites of deoxynucleoside analogs, such as 2F-ara-ATP and ara-ATP, are potent inhibitors of this enzyme as well. The activity of this enzyme plays a key role in controlling the intracellular concentrations of deoxynucleotide triphosphates; thus, it can influence the activation or incorporation of deoxynucleoside antimetabolites, such as ara-C, 5-FUdR, and 5-fluorodeoxyuridine. The activity of this enzyme is partially prevented by ferrous iron. The mechanism of action of this enzyme is hypothesized, alternating the use of hydroxyurea with antimitabolites, such as 6-thioguanine, warrants further clinical exploration in the treatment of cancer.

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