THE CANCER PHARMACOLOGY ANNUAL 2

B.A. CHABNER and H.M. PINEDO

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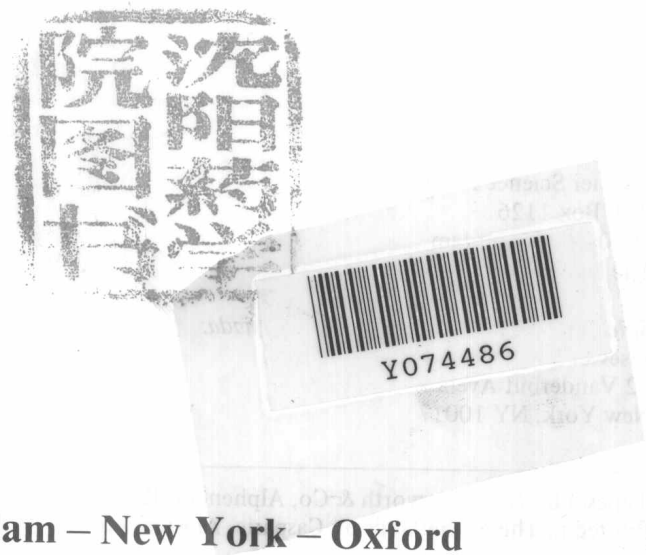

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Introduction

The texts in this volume have appeared in the same format in the Cancer Chemotherapy Annual published in November 1983. The reasons for issuing the present publication is the belief that the information may also serve pharmacological interests as such and that a relatively cheap yearly volume on the latest findings may satisfy the needs of a new audience.

The reviews are compiled on the basis of the information published in articles in 4000 medical, pharmacological and chemical journals screened for the Excerpta Medica Database. Of the approximately 6000 articles published in the period under review only those are discussed in this book which contain essential information on the pharmacological properties of antineoplastic agents. The reader will find all information on basic mechanisms of action, analog developments, assay methods, pharmacokinetics and toxicity. This information should be useful to clinicians, pharmacologists, pharmacists, chemotherapy nurses as well as to the laboratory scientific workers in the field of cancer chemotherapy.

B.A. Chabner and H.M. Pinedo

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1 Antimetabolites

Jacques Jolivet, Gregory A. Curt, Neil J. Clendeninn, Grace C. Yeh and Bruce A. Chabner

INTRODUCTION

1982 was a year of significant advances in understanding the cellular pharmacology, drug interactions, and clinical effectiveness of new and traditional antimetabolites (methotrexate, 5-fluorouracil, and cytosine arabinoside). Among these advances are the following: The importance of methotrexate convertion to polyglutamate forms is now clearly established. Additionally, a thorough study of methotrexate-resistant cells which lack transport capability for this agent has indicated that such cells remain sensitive to lipid-soluble antifolates such as the newly introduced trimetrexate. Leucovorin, the methotrexate rescue agent, enhances 5-fluorouracil toxicity in some cell lines, while uridine protects cells against high-dose 5-fluorouracil toxicity; both interactions await clinical trial.

In cytidine analog pharmacology, the antileukemic drug 5-azacytidine has evoked great interest because of its ability to reduce DNA methylation, and has demonstrated the ability to reactivate fetal hemoglobin genes in patients with thalassemia. Regarding cytosine arabinoside, the facilitated uptake system for this antileukemic drug has been quantitated by specific biochemical probes, and the number of these transport sites has shown a positive correlation with clinical resistance to ara-C. Interest is growing in the use of high-dose ara-C infusions for treating high-risk patients with acute leukemia.

In the field of purine pharmacology, a promising new drug with a unique mode of action, thiazofurane, has reached the stage of clinical trial. The biochemical basis for strongly synergistic action between methotrexate and thiopurines has been clarified. These and other developments will be reviewed in detail in this chapter.

METHOTREXATE

During the past year, research has furthered the understanding of methotrexate. Interest has focused on the intracellular metabolism to polyglutamate

derivatives which are selectively retained. This process appears to be an important determinant of drug effect. Resistance mechanisms have been examined in greater detail at a molecular level. New rescue regimens and new applications of clinical pharmacokinetics have been developed. Promising new antifols have entered clinical trial and offer the hope of improved therapeutics.

Mechanism of action

Dihydrofolate reductase (DHFR) is the enzyme responsible for maintaining intracellular folate pools in their biochemically active reduced state. By tight binding to DHFR, the folic acid analog methotrexate (MTX) depletes intracellular reduced folates required for single carbon transfer reactions and interferes with *de novo* thymidine and purine synthesis as well as intermediary amino acid metabolism. Of central importance to MTX cytotoxicity is the fact that methylation of dUMP to dTMP, catalyzed by thymidylate synthetase (TS), is the only reaction in which reduced folate is oxidized to inactive dihydrofolate. Thus, the activity of *de novo* thymidylate synthesis is a crucial factor in depletion of intracellular reduced folate pools.

Washtien recently confirmed the importance of TS activity to MTX cytotoxicity in 5 independently derived human gastrointestinal tumor cell lines [1]. Although these 5 tumor cell lines showed similar ability to transport MTX and similar target enzyme (DHFR) levels, they demonstrated a 20-fold variation in TS activity. As expected, the greater the activity of TS in each cell line, the less MTX required for 50% growth inhibition. This study not only demonstrates that there is great heterogeneity in enzyme levels within a given tumor type, but also raises the interesting possibility that direct measurement of fresh tumor cell TS levels might predict individual responsiveness to MTX.

The activity of enzymes involved in intermediary amino acid metabolism may affect the rescue of cells from MTX cytotoxicity by leucovorin (5-formyltetrahydrofolate, 5-formyl-THF). When administered to patients, 5-formyl-THF is converted to 5-methyl-THF, which when used as a cofactor by methionine synthetase is transformed to FH_4, and thereby becomes available to the intracellular pool of reduced folate. Of interest is the finding that methionine synthetase is deficient in a number of tumor cell lines compared to normal tissues. This fact may explain the relative sparing of host tissues despite persistant tumor cell kill observed in high-dose MTX-leucovorin rescue protocols. In support of this hypothesis, Dudman et al. have demonstrated that inhibition of methionine synthetase in cultured human lymphoblast lines by nitrous oxide can impair leucovorin rescue from MTX cytotoxicity [2].

Since MTX is a phase-specific agent, the extent of cytotoxicity is dependent on both the proliferative state of the exposed cell population and the length of that exposure. Using a murine line, O'Keefe et al. have explored the relationship of MTX doses and duration of exposure on cell kill [3]. Duration of exposure appears more important than absolute drug concentration: A 1-log increase in duration of exposure results in nearly a 2-log increase in cytotoxicity, while a 1-log increase in drug concentration results in only a 0.3-log increase in cytotoxicity. These data may be important in devising optimal clinical dosing regimens.

Precisely how MTX kills cells remains uncertain. Proposed mechanisms include direct DNA damage due to dUMP misincorporation (Annual 3) and inhibition of both *de novo* thymidylate and purine synthesis (Annual 4). Taylor et al. have exhaustively studied DNA and RNA synthesis as well as changes in deoxyribonucleotide pool sizes following exposure to MTX with and without exogenous thymidine and/or purine sources [4]. As these authors have previously shown (Annual 4), MTX cytotoxicity does not appear to result from unbalanced growth (excessive RNA content in relation to DNA content); and inhibition of RNA synthesis does not correlate with cell kill. Rather, MTX cytotoxicity correlates best with inhibition of DNA synthesis and, specifically, reduction in dTTP pool size.

Intracellular transport

The intracellular transport of MTX has received attention as a possible determinant of drug resistance (*vide infra*). Further evidence has accumulated to suggest that MTX enters cells via a process in which intracellular anions are exchanged down a concentration gradient to provide energy for cumulative MTX uptake [5]. Intracellular phosphate, the level of which is maintained by a sodium-dependent phosphate transport system, appears to be a principal energy source for transport of MTX [6]. The observed effects of cellular metabolism on MTX transport (net increased uptake in the presence of metabolic inhibitors, net decreased uptake in the presence of glucose or pyruvate) may result from changes in intracellular phosphate concentration.

Liposomes have also been used to encapsulate MTX as a means of overcoming transport resistance. Unilamellar cationic MTX-containing liposomes can partially reverse drug resistance in a human leukemia cell line with defective transport [7].

Intracellular metabolism

Once being transported into cells, MTX can bind DHFR or be further metabolized to poly-γ-glutamyl derivatives. Not only do MTX polyglutamates (MTX-PGs) retain high affinity for the target enzyme, but new evidence suggests that they are selectively retained by cells.

Jolivet et al. have investigated the profile of MTX-PG formation in three human breast cancer cell lines [8]. Synthesis of polyglutamates was both time and concentration dependent, and there was a correlation between polyglutamate chain length and efficiency of retention. Metabolites with 4 and 5 glutamyl residues (MTX-Glu_4 and MTX-Glu_5) were most avidly retained, while parent drug and polyglutamate derivatives with fewer glutamyl residues effluxed rapidly when cells were incubated in drug-free media. Moreover, the retained polyglutamates continued to inhibit DNA synthesis and cell growth for at least 24 hours after removing intracellular drug. The metabolites thus function as a 'depot' form of drug capable of prolonged antitumor activity. Cell lines which did not form MTX-PGs did not show these prolonged effects, suggesting that defective polyglutamation may be an important determinant of MTX resistance.

Fry et al. have similarly studied MTX-PG synthesis, retention, and target enzyme binding in Ehrlich ascites tumor cells *in vitro* [9]. As described above, MTX-PG formation was time dependent and, once formed, these higher polyglutamates were not only retained intracellularly more efficiently than MTX but could replace MTX already bound to DHFR. Exposure of cells to either vincristine or probenecid could increase MTX-PG formation, presumably by inhibiting MTX efflux and presenting high MTX levels as substrate to the enzyme folyl-polyglutamate synthetase [10].

The cellular pharmacology of MTX-PGs has important implications in the design of 'rescue' regimens. Co-incubation of cells with folinic acid inhibits MTX-PG formation (presumably because of competition for transport and/or folyl polyglutamate synthetase) and is presumably one of the mechanisms by which folinic acid rescues cells from MTX cytotoxicity [11,12].

Mechanisms of resistance

MTX provides a model for the study of mechanisms of cellular drug resistance. Cell lines with defective drug transport, altered DHFR affinity, or elevated DHFR levels have been isolated and the resultant investigations have enhanced our understanding of drug resistance at a molecular level.

Using cultured human T-cell leukemia serially passaged in increasing concentrations of MTX, Ohnoshi et al. developed a MTX-resistant cell line with defective drug transport [13]. Detailed kinetic studies demonstrated that altered transport was due solely to a decreased V_{max} for influx, suggesting a decrease in the number of effective transport sites in the membrane of resistant cells. The MTX-resistant line retained sensitivity to the substituted quinazoline antifolate trimetrexate and the methyl pyrimidine antifol DDMP, both of which are more lipophilic than MTX and might be expected to overcome transport resistance. Interestingly, the cytotoxicity of lipophilic antifols could not be reversed by leucovorin in the resistant line, presumably because of parallel impaired folate transport. These data suggest that combined treatment with lipophilic antifol and leucovorin rescue might be selectively cytotoxic to transport-resistant cells. However, impaired drug transport has yet to be demonstrated as a mechanism of clinical drug resistance.

Other mechanisms for development of MTX resistance include decreased affinity of DHFR for MTX and amplification of the gene coding for DHFR resulting in elevated target enzyme levels. Flintoff et al. have previously described single-step selection of MTX-resistant Chinese hamster ovary (CHO) cells containing an altered DHFR with decreased affinity for DHFR (Annual 4). Further stepwise selection of these cells in progressively higher MTX concentrations resulted in stable amplification of the gene coding for altered enzyme in association with abnormal $2p^-$ and $5q^+$ chromosomes [14]. Wild-type CHO cells similarly selected in a stepwise fashion demonstrated amplification of unaltered enzyme in association with a marker chromosome containing a homogenously staining region (HSR). Restriction blot analysis suggested that no major rearrangement of the gene occurred during the process of amplification.

Cowan et al. have observed structural rearrangement of amplified DHFR

genes in human breast cancer cells made resistant to MTX by stepwise selection [15]. In these cells, amplified DHFR copy number is associated with a marker chromosome containing an HSR. The resulting amplified enzyme appears identical to that in wild-type, MTX-sensitive cells, an observation also reported by others studying gene-amplified KB human cell lines [16].

Human tumor cells can also acutely increase DHFR levels when exposed to MTX, even without evidence for gene amplification [17]. The rapid elevation of enzyme is not due to stabilization by MTX as had previously been conjectured, but appears to be mediated at the level of protein translation. This phenomenon could contribute to MTX resistance by preventing the accumulation of free drug which is critical for MTX cytotoxicity.

It has now been demonstrated that gene amplification may be a determinant of clinical drug resistance. A small-cell lung cancer cell line isolated from a patient in relapse following high-dose MTX treatment demonstrated amplification of the DHFR gene, and elevated DHFR levels [18]. This phenotype was associated with double-minute chromosomes, a karyotype previously described in murine tumors with unstable MTX resistance (Annual 4). Indeed, during serial passage in drug-free media, the cell line lost double-minute chromosomes, amplified genes, and drug resistance – the first demonstration of this phenomenon in a human tumor cell line. This demonstrates that clinical drug resistance may be unstable and underscores the relevance of cytogenetics and molecular biology to cancer chemotherapy.

Clinical pharmacokinetics

The usefulness of pharmacokinetics as a tool capable of predicting individual patient toxicity to cancer chemotherapy was first appreciated in protocols using high-dose MTX (HD-MTX) with leucovorin (CF) rescue. More recent studies have attempted to predict patients at risk for toxicity by studying the kinetics of elimination of a small test dose of MTX before initiation of HD-MTX regimens. Using a preliminary MTX test dose of 50 mg/m^2, Favre et al. were able to identify a subset of patients with head and neck carcinomas with impaired MTX excretion who were more likely to develop toxicity following HD-MTX treatment [19]. Since toxicity was further correlated with poor response to treatment, the test-dose analysis has the potential to select patients most likely to benefit from HD-MTX/CF protocols. Using a smaller test dose of 10 mg/m^2, Kerr et al. were able to predict infusion plateaus during treatment as well as 24-hour post-infusion levels [20]. Interestingly, MTX clearance did not correlate well with creatinine clearance. Together these studies suggest that analysis of test-dose kinetics may allow identification of high-risk patients as well as appropriate pretreatment dose modifications.

Alternate rescue schedules have been used with some clinical success. In cells with intact thymidine salvage pathways (such as normal host cells), thymidine can partially reverse MTX toxicity. Under these conditions, rescue can be further augmented by supplying low concentrations of CF. It appears that CF rescue is non-competitive with MTX in cells capable of utilizing exogenous thymidine. In cells with impaired thymidine salvage, CF rescue remains com-

petitive with MTX even if thymidine is supplied. Since some evidence suggests that tumor cells are deficient in thymidine salvage (Annual 4), Bruno et al. have used thymidine with low-dose CF to rescue patients receiving HD-MTX [21]. Although host toxicity was observed in this protocol, the responses observed in tumors not normally responsive to MTX (e.g., adenocarcinoma of the colon) suggest an improvement in therapeutic index.

Physiologic drug barriers can also be useful in the design of rescue regimens. Mehta et al. have employed a combination of intrathecal MTX with intravenous CF in patients with meningeal disease [22]. Although CF and its principal metabolite 5-methyl-THF were able to penetrate into CSF, these levels were 100- to 1000-fold lower than the MTX levels, precluding rescue at the site of disease. Conversely, although MTX administered intrathecally was detectable in blood, these levels were 5- to 100-fold lower than those of rescue compounds, precluding systemic toxicity. It should be noted that clearance of MTX from CSF in patients with overt CNS disease is slower, presumably because of abnormal transport of MTX out of the CSF [23].

Renal excretion is the major determinant of MTX clearance, so that impaired renal function can markedly potentiate toxicity. An anephric patient receiving only 10 mg/m^2 of MTX developed profound toxicity [24]. Despite aggressive hemodialysis, drug levels could not be effectively reduced. Animal studies have demonstrated that in the absence of renal clearance, enterohepatic circulation becomes the major determinant in prolonging drug half-life [25]. Under these circumstances, drug can be effectively cleared by either biliary diversion [25] or binding in gut with activated charcoal [26].

New antifolates

A new generation of antifols is currently under design, evaluation, and entry into clinical trial with the aim of improving therapeutic index and overcoming drug resistance. These compounds differ from MTX in their ability to traverse membranes, profile of tissue distribution, and binding characteristics to target enzyme.

Because the association of MTX with DHFR is reversible, the enzyme becomes available for folate reduction and cytotoxicity is reversed in the absence of free drug to replace drug which has dissociated from it. New 'site-directed' antifols have been designed to overcome this phenomenon. These drugs retain high affinity for DHFR, and, once bound to enzyme, alkylate nearby amino acids, resulting in irreversible binding.

Rosowsky et al. have synthesized a MTX analog in which glutamic acid is replaced by iodo-acetyl-L-lysine [27]. After binding DHFR, the drug alkylates histidine via the substituted moiety, irreversibly inactivating enzyme. Similarly, Corbett et al. have described a new triazine antifolate, NSC 127755, which is similar in structure to Baker's antifol [28]. This drug is also an irreversible inhibitor of DHFR, specifically alkylating enzyme through a sulfonyl fluoride group. The compound is highly active against an ovarian tumor in which both MTX and Baker's antifol have only marginal activity and has entered Phase I trial.

FLUOROPYRIMIDINES

Mechanisms of action

5-Fluorouracil (5-FU) and 5-fluorodeoxyuridine (5-FdUrd) are prodrugs which must be activated intracellularly to 5-FdUMP, which inhibits thymidylate synthetase (TS); 5-FUTP, which is incorporated into RNA and alters its function; and 5-FdUTP, which is incorporated into DNA in certain cell lines. The relative contribution of TS inhibition and RNA incorporation in the drug's action varies among different cell types, while the importance of the recently described DNA incorporation remains uncertain. The relative activities of the activating enzymes (Fig. 1) in different cells determine in part the drug's action mechanism, while deletions of certain of these enzymes may be responsible for episodes of drug resistance. In two recently studied lymphocyte cell lines, 5-FU-sensitive B lymphocytes (LAZ-007) anabolized 5-FU through orotic acid phosphoribosyltransferase, and uridine and thymidine phosphorylases, while less sensitive T cells (CCRF-CEM) could only activate 5-FU through orotic acid phosphoribosyltransferase [29]. In other studies, fluoropyrimidine-activation pathways were studied in L1210 and P388 cells made resistant to 5-FU, 5-FdURD, and 5-fluorouridine (5-FURD) by a one-step mutation and selection procedure [30]. Cells resistant to 5-FU had lower orotic acid phosphoribosyltransferase activity, while cells resistant to 5-FdURD and 5-FURD had markedly lower activities of thymidine and uridine kinase, respectively [31]. Another 5-FU-resistant P388 cell line, established in mice after prolonged intraperitoneal treatment with the drug, also showed decreased activities of phosphoribosyltransferase and uridine kinase [32].

In other studies, the activity of 5-FU against four murine colonic adeno-

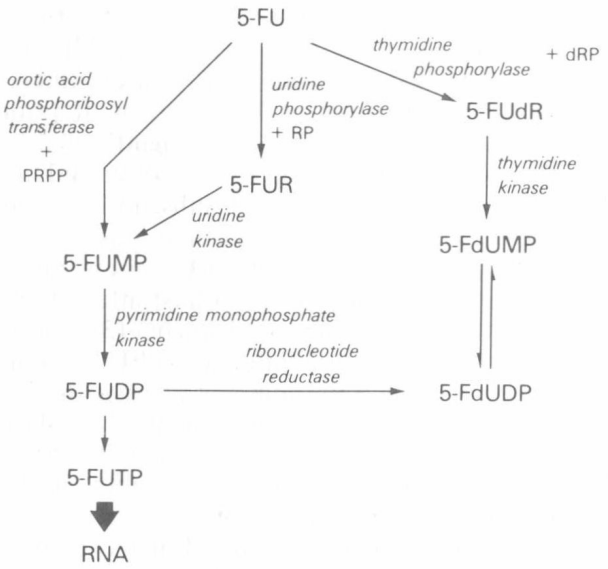

Fig. 1. Metabolic pathways of 5-fluorouracil activation.

carcinomas was correlated with basal pool sizes of phosphoribosyl pyrophosphate (PRPP), the cofactor used by orotate phosphoribosyltransferase to activate 5-FU [33] (Fig. 1). Pool sizes were 2- to 4-fold higher in the 2 sensitive tumors and were related to higher PRPP synthetase activities and higher orotate phosphoribosyltransferase activities after 5-FU treatment than in the resistant lines. Tumor cell resistance to 5-FdURD, a drug activated mainly to 5-FdUMP, also depends on the intracellular levels of that anabolite's target, thymidylate synthetase. The *in vitro* sensitivity of five human gastrointestinal cell lines to 5-FdURD was inversely proportional to their TS levels, with cells containing the highest enzyme level being the most drug resistant [1].

The incorporation of fluoropyrimidines into RNA has been increasingly recognized as an important factor in the drug's activity in recent years, although the exact mechanism by which this leads to cytotoxicity has not been determined. Studies in a human colon carcinoma cell line (HT-29) suggest that fluoropyrimidine incorporation into nuclear RNA (nRNA) might be an important determinant of cytotoxicity since the amount of nRNA incorporation directly correlated with cell death, while ribosomal RNA processing, DNA, and total RNA synthesis were not significantly impaired by 5-FU exposure in this cell line [34].

The incorporation of fluoropyrimidines into DNA was first detected 2 years ago in L1210 cells exposed to 5-FUdR (Annual 4). This finding has been extended in the past year to (1) cultured human lymphoblasts in which both FdUMP and dUMP were identified in DNA of cells treated with 5-FUdR [35], (2) MCF-7 human breast cancer cells in which DNA incorporation was seen after exposure to either 5-FU or 5-FUdR [36], and (3) HeLa S_3 cells after 5-FUdR exposure [37]. These findings imply that the enzymes responsible for preventing this incorporation, UTPase (converts dUTP and 5-FdUTP to dUMP and 5-FdUMP, respectively) and uracil-DNA glycosylases (excise uracil and 5-FU containing bases from DNA) (Annual 3), have insufficient activity in these cell lines to prevent DNA incorporation from the large dUMP and 5-FdUMP pools which accumulate after TS inhibition [35]. Interestingly, excision of 5-FU bases from DNA was accelerated by MTX treatment prior to 5-FUdR exposure by an unknown mechanism [38]. The significance of fluoropyrimidine incorporation into DNA in the action of fluoropyrimidines is still uncertain, although drug excision from DNA and subsequent DNA fragmentation [37] may contribute to the toxicity of these compounds.

In most of the previous reports, the cytotoxic activity of 5-FU was examined against tumor growing *in vitro*. A recent study indicates that host interactions may be important in antitumor responses to 5-FU obtained *in vivo* [39]. Rats given small intradermal 5-FU doses prior to implantation of 5-FU-resistant carcinomas subsequently responded to systemic 5-FU administration with 20% of the animals achieving cure. A similar effect was also obtained when MTX was used both as the sensitizing and antitumor agent. There was no cross-sensitization between the two agents. An antibody to 5-FU was detected in the sera of the 5-FU-sensitized animals, suggesting the antitumor effects somehow resulted from a combination of the 5-FU antibody and the drug's cytotoxic effects. This interesting new approach deserves further investigation.

Drug interactions

Leucovorin

Thymidylate synthetase is inhibited by 5-FU when the enzyme forms a tight complex with 5-FdUMP and d-L-5-10-methylene tetrahydrofolate (CH_2FH_4). 5-FU resistance in certain cell lines has been attributed to insufficient intracellular CH_2FH_4 (Annual 4). Leucovorin (1-L-5-formyl-tetrahydrofolate), a stable form of reduced folate used clinically in rescue regimens with MTX, can be transformed intracellularly to other reduced folates, including CH_2FH_4. Waxman and Bucknér have shown that leucovorin increases 5-FU antitumor effects against Friend murine erythroleukemia cells [40]. Menadine and α-tocopherol (vitamin E), 2 oxidizing agents, also increased drug efficacy, possibly by increasing intracellular transformation of 1-L-5-methyl tetrahydrofolate (CH_3FH_4) to CH_2-FH_4. An early clinical trial has examined the combined use of 5-FU (370–400 mg/m^2/day) and high-dose leucovorin (200 mg/m^2/day) given simultaneously for 5 days every 21 days in 35 patients with advanced colorectal and gastric carcinomas [41]. Although the combination showed signs of promising activity, the small number of patients and lack of a control arm without leucovorin led to inconclusive results. A randomized trial is needed to better evaluate this combination.

Pyrimidines

Uridine (UR). Uridine can rescue mice from the lethal toxicity of 5-FU, possibly by increasing UTP pools and competing more efficiently with 5-FUTP for RNA incorporation [42,43]. After a single i.p. injection of 800 mg/kg 5-FU, mice were rescued by a five-day infusion of UR (1–10 g/kg/day) started 24 hours after 5-FU administration [42]. Similar schedules of thymidine or deoxyuridine could not prevent 5-FU lethal toxicity. Martin et al. have identified two uridine schedules which improve 5-FU's therapeutic index in colon-tumor-26-bearing mice: (1) UR 800 mg/kg q2h × 3 doses starting 2 hours after 5-FU 200 mg/kg followed 18 hours later by 800 mg/kg q2h × 4 doses, and (2) 2 doses of UR 3500 mg/kg, the first given 2 hours after 5-FU, separated by an 18-hour interval [43]. Both schedules led to millimolar concentrations of UR for 6 to 8 hours on the day of 5-FU administration and the following day. Even better results were obtained when uridine rescue was given after a combination of 5-FU, PALA, and MMPR. Uridine resulted in faster clearance of 5-FU from RNA of both tumor and bone marrow but enhanced recovery of DNA synthesis only in bone marrow. The biochemical mechanisms underlying this interaction are unknown.

Allopurinol

The rationale behind HPP-5-FU combinations was detailed in Annual 3. In the past year, additional experimental [44] and clinical [45–47] studies with this combination have failed to show any therapeutic advantage over 5-FU alone.

Methotrexate (MTX)

Many experimental and clinical studies of the MTX-5-FU combination have been published in the past year. Cadman's group has reported yet another example of *in vitro* synergism between the two drugs in the human mammary carcinoma cell line 47-DN [48]. As in their previous studies, this interaction was accompanied by increased intracellular PRPP levels and 5-FU anabolism. Other investigators, however, were unable to show drug synergism in the treatment of human colon adenocarcinoma xenografts in nude mice [49] or various murine tumors in mice [50]. In clinical trials of the combination [48,51–53], responses and toxicity varied with the different treatment schedules used. Twenty mg/kg MTX followed by 600 mg/m^2 5-FU infused over the next 21 hours along with low-dose leucovorin (10 mg/m^2 i.v. × 4 doses) was the most toxic regimen, but yielded a 41.7% response rate in 29 patients with metastatic colorectal cancer [52]. By comparison, the administration of an i.v. bolus of 250 mg/m^2 MTX followed in 1 hour by 5-FU 600 mg/m^2 by i.v. bolus and leucovorin 10 mg/m^2 24 hours later × 8 doses caused little toxicity and only one partial response in 16 patients with advanced colorectal cancer [51]. Clearly, more work needs to be done to find the MTX and 5-FU administration schedule with the best therapeutic index.

N-(Phosphonacetyl)-L-aspartate (PALA)

PALA and 5-FU have synergistic antitumor activity *in vitro*. This interaction was thought to result from PALA's inhibition of *de novo* pyrimidine synthesis, which leads to increased 5-FUTP formation and RNA incorporation (Annuals 3 and 4). Recent work suggests that enhanced 5-FdUMP synthesis and TS inhibition might also play a role in the synergism [54]. Smaller pyrimidine nucleotide pools compete less efficiently with 5-FU for the activating enzymes and lead to the greater 5-FdUMP formation and lesser dUMP accumulation observed following TS inhibition. Both factors contribute to a more complete inactivation of the enzyme. Unfortunately, clinical trials of the combination have yielded disappointing results so far [55], and the eventual usefulness of this drug combination remains uncertain.

Pharmacology

The administration of 5-FU by prolonged infusions has continued to be studied in the past year. While one advantage of this mode of administration is its reduced myelosuppression, a recent study suggests that the drug might also be more effective in producing direct cytotoxic effects when given in this fashion [56]. In various epithelial cancer cells studied *in vitro*, 1-hour 5-FU incubations with drug levels achievable *in vivo* after i.v. bolus administration were less cytotoxic than prolonged (3–7 days) exposure to low (0.5–1 μg/ml) drug concentrations. These concentrations were maintained without myelosuppression in patients with advanced carcinomas receiving 5-FU 20–25 mg/kg/day by continuous infusion for five days [57]. The importance of