Cancer Chemotherapy and Biological Response Modifiers Annual 10

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Introduction

The year 1987 witnessed further steps in the transition from conventional chemotherapy to biological compounds for the treatment of cancer. The potential of biological agents has never been in doubt, given their unique specificities for host or malignant tissues and their ability to modulate fundamental properties of cells in vitro, such as proliferation, differentiation, metastasis, and immunogenicity. However, the initial experience with the prototype biological, α-interferon was disappointing, in that only a single disease, hairy cell leukemia, responded consistently, and with long-term benefit. To that brief list can now be added chronic myelocytic leukemia, which responds with complete remissions and with disappearance of the malignant Ph^+ clone in a significant fraction of patients, and malignant melanoma, in which 5% of patients achieve clinically valuable complete remissions. But the most important reasons for excitement about biological agents are the antitumor responses generated by interleukin-2 with or without LAK cells (lymphokine-activated killer cells) in renal cell carcinoma and melanoma, and the efficacy of bone marrow colony stimulating factors, G-CSF and GM-CSF, in preventing leukopenia due to chemotherapy. The possibilities of CSFs, as well as the related interleukins (IL-1 and IL-3), are discussed in detail in this edition of the Chemotherapy Annual, and represent a landmark in that the major limiting toxicity of conventional chemotherapy, myelosuppression, may become controllable and avoidable. The potential of CSFs for differentiation of leukemic cells, as suggested by early studies of myelodysplastic syndromes, requires confirmation, as does the ability of IL-1 to modify radiation damage to bone marrow.

Less developed clinically, but no less promising in their potential, are monoclonal antibodies and growth factor-related strategies. Progress toward useful clinical applications seems slow at this point, although perhaps less frustrating when one realizes that the technology for purification and cloning of growth factors and for production of monoclonals has advanced rapidly in the past decade, and that such products as TGF-β, immunotoxins and immunochelates, and growth factor-toxin conjugates, are only now becoming available in clinically useful quantities. Each of these approaches is discussed in detail in the sections on biologicals (Chapters 28–34) and 'Steroid and peptide hormones and growth factors' (Chapter 11). The reader should be alerted to the growing evidence for a role of growth factors, particularly TGF-α and β, and insulin-like growth factor-1 (IGF-1), in mediating the response to estrogens and antiestrogens.

This highlighting of progress in the understanding and clinical application of biologicals does not minimize the substantial progress made in the past year in conventional chemotherapy. Perhaps the most important developments here are the expanding indications for, and improved results of therapy with, VP-16. This agent is now a part of first-line therapy for germ cell tumors of the testis, for small cell carcinoma of the lung, and for diffuse large cell lymphoma and has promising activity in ovarian cancer, childhood acute lymphocytic leukemia, and other diseases. An important new type of multidrug resistance, mediated by mutations in topoisomerase II (the enzymatic target of VP-16, VM-26, m-AMSA, and possibly the anthracyclines), has

been identified in cell lines, and requires investigation at the clinical level. It contrasts with the classical MDR-1 phenotype in that the latter is basically a transport mutant, with an amplified exit pump, the P170 glycoprotein. Considerable progress has been made in understanding MDR-1 at the biological and clinical levels in the past year, as the reader will find in Chapter 9. The amplified P170 has been identified in human renal cell carcinomas, colon carcinomas, and post-therapy in occasional patients with leukemia and lymphoma, but not in small cell lung cancer (Chapter 9). The reader is also alerted to the drug-related chapters that provide important new information on the isolation of the transport protein for folates and antifolates (Chapter 1), the important action of methotrexate and dihydrofolate polyglutamates as direct inhibitors of purine and thymidylate synthesis, and the rate-limiting role of transmembrane transport in the action of cytosine arabinoside (ara-C).

Finally, new active agents have been identified in the past year, including taxol (melanoma and ovarian cancer) and fludarabine (chronic lymphocytic leukemia), and evidence grows for a role of the less cardiotoxic anthracycline analogs epirubicin in non-Hodgkin's lymphoma, breast cancer and ovarian cancer and idarubicin in leukemia and breast cancer. Altogether 1987 was a year of significant accomplishment in cancer chemotherapy.

B.A. Chabner
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Contents

CHAPTER 1

Antimetabolites

CARMEN J. ALLEGRA, JEAN L. GREM, GRACE CHAO YEH and BRUCE A. CHABNER

Over the past year antimetabolite research has continued to advance our understanding of the mechanisms by which these agents produce metabolic inhibition and, ultimately, cell death. Research endeavors, focused on the translation of in vitro technology into the investigation of the determinants of cytotoxicity and resistance in clinical specimens, are of paramount importance. The study of relevant parameters in human tumor tissues has been initiated by a number of research groups and has led to the definition of some clinical response predictors for several of the antimetabolite agents. Further understanding of the metabolic perturbations resulting from antimetabolite treatment, both in vitro and in vivo, is critical for the optimal clinical application of these agents in combination with other antineoplastics and biochemical modulators. The reports presented in this year's Annual represent evidence for the advances in our understanding of response determinants in man.

METHOTREXATE

Mechanism of action

Methotrexate (MTX) is a potent inhibitor of dihydrofolate reductase (DHFR). Inhibition of this enzyme initiates a series of intracellular events that culminate in the cessation of de-novo synthesis of purines and thymidylate. Presumably cell death follows in the wake of nucleotide depletion and, perhaps, due to the genetic injury produced by the misincorporation of the expanded pool of uridine nucleotides into DNA (Annuals 3 and 7). While folate depletion secondary to DHFR inhibition could produce metabolic inhibition through substrate depletion, investigations into the precise mechanisms underlying inhibition of nucleotide synthesis suggest that folate depletion is only partial. Baram and coworkers, using purified myeloid precursor cells from normal human bone marrow, found that 65%–70% of the folate substrate required for de-novo purine synthesis (10-formyltetrahydrofolate) was preserved in cells treated with 1 μM MTX for up to 12 hours [1]. These authors also report a rapid expansion of dihydrofolate and a novel physiologic folate, formyldihydrofolate, in the treated myeloid cells. These findings are in concert with those described in human breast cancer cells (Annual 9). Inhibition of de-novo purine synthetic activity and of myeloid colony formation was closely associated with the time course of oxidized folate generation. As these oxidized folates are potent inhibitors of the folate-dependent enzymes of de-novo purine synthesis, the authors concluded that oxidized folates (dihydrofolate and 10-formyldihydrofolate) play an important role in direct enzymatic inhi-

bition of this metabolic pathway. The MTX polyglutamates that also form over time and are inhibitors of purine and thymidylate synthetic enzymes (Annual 9) were also felt to be important in the overall inhibition of synthetic function, but their contribution was not further defined. An elegant paper by Matherly et al [2] examined the folate pools in MTX-treated murine leukemia cells using labeled leucovorin. These studies mirror the above study in that preservation of 10-formyltetrahydrofolate (70%) was also demonstrated along with marked increases in dihydrofolate (10-fold) with antifolate exposure. While the evidence strongly supports direct enzymatic inhibition of the purine pathways, these authors suggested that thymidylate synthase was inhibited predominantly through folate depletion (5-10-methylenetetrahydrofolate) with contributions from MTX polyglutamates. Further assessment of the mechanism of thymidylate synthase inhibition awaits accurate measurements of the 5-10-methylenetetrahydrofolate cofactor pool. A report on the mechanism of purine inhibition by Allegra and coworkers lends additional evidence that in human breast carcinoma cells the dihydrofolate polyglutamates that accumulate behind an inhibited DHFR are the critical metabolic inhibitors responsible for the direct inhibition of the folate-dependent enzymes of purine synthesis during antifolate treatment [3]. In this report a direct correlation was made between purine synthetic activity and the levels of intracellular dihydrofolate modulated by fluoropyrimidine and antifolate exposures. These reports together suggest that, while DHFR inhibition is critical, the event responsible for nucleotide depletion is direct inhibition of the folate-dependent enzymes of purine and thymidylate synthesis by the oxidized folates and MTX polyglutamates. This provides additional rationale for the development of folate analog inhibitors of these alternate enzyme targets. Also, the greater formation of MTX polyglutamates in malignant versus normal tissues may contribute to the selectivity of MTX cytotoxicity as well as the selectivity and competitive nature of leucovorin rescue.

While non-DHFR targets are attractive for antifolate development, inhibitors of DHFR are the most valuable types of antifolates in antineoplastic and antimicrobial therapy. A novel photoaffinity analog of MTX was developed by Price and coworkers and applied to the study of the enzyme structure of murine DHFR [4]. These authors concluded that the analog specifically bound to residues 63–65 (lys-asn-arg) that are homologous with avian DHFR and represent, in part, the locus of the enzyme active site. Such analogs will be useful for aiding structure activity investigations of inhibitors of DHFR.

Intracellular transport

The transport of MTX in mammalian cells is a saturable, temperature-dependent, energy-requiring, carrier-mediated event. The precise number of carriers and characterization of the transport systems for folate and antifolate compounds remains an area of continued investigation and controversy. A series of carefully executed studies by Henderson et al [5] illustrates that in a human lymphoblastoid cell line (CCRF-CEM) a single shared carrier could account for all of the influx of MTX, folate and 5-methyltetrahydrofolate over a wide range of concentrations (5 nM–50μM) and that no other high- or low-affinity systems capable of transporting these agents could be found in these cells. Other investigators described an additional low-affinity, high-capacity transport system for folic acid in murine leukemia cells [6]. The potential merits of a complete understanding of these transport systems cannot be understated as new drug developments and strategies to circumvent resistance mechanisms rely heavily on these investigations.

Specific efflux studies using murine leukemia cells directed at 1 of 3 previously described MTX efflux routes (bromosulfophthalein-sensitive efflux) suggest that this may be a route shared by cyclic nucleotides. Correlations were made between the ability of certain compounds, including prostaglandin A_1 and probenecid, to co-inhibit MTX and 3',5'-cyclic AMP efflux [7]. These studies have potential clinical importance as they may suggest ways to increase transport into malignant cells in an effort to enhance the cytotoxicity and selectivity of MTX. Of central importance to this issue is the identification and characterization of the protein responsible for mediating MTX transport. Several research groups over the past several years (Annuals 7–9) have reported the isolation and characterization of membrane-bound and cytosolic folate binding proteins from mammalian cells. The question remains which of the various proteins capable of binding folates is(are) responsible for the actual transmembrane transporting process. An elegantly performed study by Price and Freisheim [8] makes use of a photoaffinity analog of MTX to label and characterize the transport protein in murine L1210 cells. Their study illustrates that the analog is transported with a K_t of approximately 500 nM by a 46–48 kd membrane-bound protein. Both transport and protein binding of the analog are inhibited by MTX to similar degrees in a concentration-dependent fashion. Neither transport nor protein labeling was demonstrated on mutant cells incapable of MTX transport. Finally, internalization, i.e., true transmembrane transport rather than simple membrane binding, was shown by the labeling of 2 cytosolic proteins (38 kd and 21 kd (DHFR)) by the affinity probe when experiments were conducted at 37°C. The study suggests a role for the membrane-bound protein in MTX transport and that the smaller cytosolic protein represents a part of the membrane-bound protein required for internalization; however, no precursor/product relationship was demonstrated. Additional studies with murine leukemia cells provide evidence that the rate of translocation of the folate transporter may be influenced by exposure to MTX or the reduced folate, leucovorin [9]. In these studies, the treatment of tumored mice with MTX, with or without leucovorin, enhanced the V_{max} of MTX transport in the tumor cells by 3–5-fold. The authors concluded that an increased rate of transporter translocation accounted for the increased V_{max}, as no change in transporter amount had occurred.

Intracellular metabolism

A critical determinant of the cytotoxicity and selectivity of MTX action is the formation of intracellular polyglutamates of MTX. These forms have a prolonged intracellular half-life (Annuals 5 and 6) and may potently inhibit a variety of folate-dependent enzymes other than DHFR (Annuals 8 and 9). The formation and quantitation of these metabolites in vivo is of central importance. In monkeys treated with chronic (1 yr) weekly MTX, more than 80% of the total tissue MTX was in the form of polyglutamates (Glu_{3-7}) [10]. Importantly, the brains of the treated animals were found to be more than 90% depleted of total folates, a condition that may explain the neurologic abnormalities associated with chronic MTX therapy. Acute lymphoblastic leukemias are generally quite responsive to MTX treatment. A possible explanation for this sensitivity was suggested by investigating the ability of lymphoblasts harvested from patients with leukemia to polyglutamate MTX [11]. In addition to finding a high rate of polyglutamation, the lymphoblasts were noted to form predominantly long chain-length derivatives (up to the hexaglutamate).

A variety of biochemical agents that can positively modulate the therapeutic index of MTX