CANCER CHEMOTHERAPY 1980

The EORTC Cancer Chemotherapy Annual 2

Edited by H.M. Pinedo

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Contributors

RETO ABELE
Jules Bordet Institute
1, Rue Bordet Héger
1000 Brussels
Belgium

R.W. BALDWIN
Cancer Research Campaign Laboratories
University Park
Nottingham NG7 2RD
U.K.

RICHARD A. BENDER
Department of Biochemistry
Scripps Clinic and Research Foundation
10666 North Torrey Pines Road
La Jolla, CA 92037
U.S.A.

VIVIEN H.C. BRAMWELL
Department of Medical Oncology
University of Manchester
Christie Hospital and
Holt Radium Institute
Wilmslow Road
Manchester M20 9BX
U.K.

RICHARD J. CAMBARERI
Division of Medical Oncology
Vincent T. Lombardi
Cancer Research Center
Georgetown University
School of Medicine
Washington, DC 20007
U.S.A.

BRUCE A. CHABNER
Clinical Pharmacology Branch
Division of Cancer Treatment
National Cancer Institute
Bldg. 10, Rm. 6N 119
National Institutes of Health
Bethesda, MD 20014
U.S.A.

T.A. CONNORS
MRC Toxicology Unit
Medical Research Council Laboratories
Woodmansterne Road
Carshalton, Surrey SM5 4EF
U.K.

STANLEY T. CROOKE
Associate Director of Research and
Development
Bristol Laboratories
P.O. Box 657
Syracuse, NY 13201
U.S.A.

DEREK CROWTHER
Cancer Research Campaign
Christie Hospital and
Holt Radium Institute
Wilmslow Road
Manchester M20 9BX
U.K.

L.H. EINHORN
Medical Oncology Branch
Indiana University
Indianapolis, IN 46223
U.S.A.

HEINE H. HANSEN
Finsen Institute
49 Strandboulevarden
DK-2100 Copenhagen
Denmark

MOGENS HANSEN
Finsen Institute
49 Strandboulevarden
DK-2100 Copenhagen
Denmark

GARY J. HARRIS
Department of Medicine
Division of Oncology
University of Texas Health Center at
San Antonio
San Antonio, TX 78284
U.S.A.

J.C. HEUSON
Jules Bordet Institute
1, Rue Bordet Héger
1000 Brussels
Belgium

JOHN Y. KILLEN
Division of Medical Oncology
Vincent T. Lombardi Cancer Research Center
Georgetown University School of Medicine
Washington, DC 20007
U.S.A.

J. DE KRAKER
Emma Children's Hospital
Spinozastraat 51
1018 HJ Amsterdam
The Netherlands

JOHN KUHN
Department of Medicine
Division of Oncology
University of Texas Health Center at San Antonio
San Antonio, TX 78284
U.S.A.

FRANCO M. MUGGIA
New York University Medical Center
Division of Oncology
550 1st Avenue
New York, NY 10016
U.S.A.

CHARLES E. MYERS
Clinical Pharmacology Branch
Division of Cancer Treatment
National Cancer Institute
Bldg. 10, Rm. 6N 119
National Institutes of Health
Bethesda, MD 20014
U.S.A.

R.J. PARIDAENS
Jules Bordet Institute
1, Rue Bordet Héger
1000 Brussels
Belgium

H.M. PINEDO
Netherlands Cancer Institute
Plesmanlaan 121 and
University Hospital, Free University
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

MIKAEL RØRTH
Finsen Institute
49 Strandboulevarden
DK-2100 Copenhagen
Denmark

MARCEL ROZENCWEIG
Jules Bordet Institute
1, Rue Bordet Héger
1000 Brussels
Belgium

R.D. RUBENS
Guy's Hospital
St. Thomas Street
London, SE1 9RT
U.K.

PH. RÜMKE
Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

PHILIP S. SCHEIN
Division of Medical Oncology
Vincent T. Lombardi Cancer Research Center
Georgetown University School of Medicine
Washington, DC 20007
U.S.A.

WILLIAM R. SHAPIRO
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, NY 10021
U.S.A.

FREDERICK P. SMITH
Division of Medical Oncology
Vincent T. Lombardi Cancer Research Center
Georgetown University School of Medicine
Washington, DC 20007
U.S.A.

G. STOTER
University Hospital
Free University
Rm. V-1066 A
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands

SAMUEL G. TAYLOR, IV
Cancer Center
Presbyterian St. Luke's Medical Center
1725 West Harrison Street
Chicago, IL 60612
U.S.A.

DANIEL D. VON HOFF
Department of Medicine
Division of Oncology
University of Texas Health Center at San Antonio
San Antonio, TX 78284
U.S.A.

P.A. VOÛTE
Emma Children's Hospital
Spinozastraat 51
1018 HJ Amsterdam
The Netherlands

PETER H. WIERNIK
National Cancer Institute
Baltimore Cancer Research Program
22 South Greene Street
Baltimore, MD 21201
U.S.A.

S.D. WILLIAMS
Medical Oncology Branch
Indiana University
Indianapolis, IN 46223
U.S.A.

ROBERT C. YOUNG
Medicine Branch
National Cancer Institute
Bldg. 10, Rm. 12N 236
National Institutes of Health
Bethesda, MD 20014
U.S.A.

Introduction

H.M. Pinedo

With the publication of this second Cancer Chemotherapy Annual the unrivalled position of this series is clearly demonstrated. In an almost epic struggle authors and editorial staff managed to work their way through the 6000 publications which appeared in the year preceding their deadline: May 1980. This volume therefore covers the very latest literature and in a most comprehensive way.

This second annual again shows that this series differs from many annual reviews in that it reviews, by far, most articles published in the previous year. Whereas the 1979 volume covered several years and, where considered necessary by the authors, even older literature since this was to form the basis of the series, the present volume concentrates on the very latest literature. The authors have assumed that Annual 1 is available to the reader, and refer regularly to that first volume. The present volume therefore covers mainly publications in 1979 with the addition of a few 1978 papers which, due to 'growing pains', were missed in the previous volume.

The present volume also differs from its predecessor in that it is more complete with regard to the most recent publications. While about 65% of the 1978 literature had been reviewed in the first volume, we are now really approaching completeness, with more than 90% of the 1979 papers being screened by the authors. A total of 6000 articles was published on cancer chemotherapy during the past year. The number of papers selected as references by the authors on the various subjects varies from 50 to 500, depending on the papers available in each field.

In editing this volume the main question in my mind was whether to place emphasis on completeness or on the critique to be made of the literature. Some authors have preferred the first approach, some the second, others a combination of these.

As quite a large number of papers published in 1978 had still to be reviewed, the present volume contains more text pages than the previous one. In order to limit the size and cost of the present book, a choice had to be made between deletion of parts of the text and removing the titles of the articles from the reference lists. The latter choice was made. I admit that article titles in the references are useful to the reader. However, after consul-

tation with the authors, a decision was made to delete the titles primarily to control the price of this book. Next year, with the expected reduction in the number of papers to be reviewed, we shall be able to concentrate entirely on one year (1980), and it is hoped to provide titles in the references again.

As far as the content is concerned, one is amazed at the amount of new information provided by both basic and clinical researches for the clinical oncologist in a single year. The authors have presented these data in a very expert way. Here, I should like to raise the veil on a few interesting developments starting with Chapter 1.

Our knowledge of the experimental and clinical pharmacology of the antimetabolites has increased at its familiar pace. The transformation of methotrexate to polyglutamate forms is beginning to shed a new light on the mechanism of action of this antimetabolite.

The amount of new information on alkylating agents is even greater than in the past, and is presented to the reader in a comprehensive and critical review. Here again the mechanism of action is still receiving full attention from researchers in this field.

The new developments taking place in the field of anthracycline analogs are proving most exciting. These are clearly the result of the continuing efforts being made to get around the obstacle presented by adriamycin cardiotoxicity. Also, the comparable problem of bleomycin lung toxicity has received a great deal of attention. Again, efforts to overcome this side effect have resulted in the development of new analogs.

The epipodophyllotoxins are emerging as a valuable new class of drugs and have shown activity particularly in non-Hodgkin lymphoma, brain tumors, bladder carcinoma and small-cell lung cancer. Little is still known about the clinical pharmacology of this group of drugs, a subject which is receiving the necessary attention from several pharmacologists.

The annual meeting of the American Association of Cancer Research and that of the American Society of Clinical Oncology offered the participants all the important information on new drugs over the past year. Although the manuscripts for this volume had been submitted prior to these meetings, the Addendum to the chapter on new drugs by Dr. Von Hoff provides a valuable review of the abstracts on this topic published in the Proceedings of the San Diego meeting.

There is an increasing amount of information on steroid receptors and the possible role of steroids as anticancer agents. There is more news on the mechanism of action and the metabolism of the antiestrogens. Besides the well-known indications such as breast cancer, prostate cancer and endometrial cancer, there is an indication that the antiestrogens may acquire a place in the treatment of advanced cases of melanoma, particularly in elderly patients.

A section on immunotherapy was missing from last year's volume. Professor R.W. Baldwin of Nottingham has now joined our group of authors and presents a first review of the immunological approaches to cancer therapy. This chapter therefore includes some older references to provide a firm basis for his next chapter which will deal only with papers published in 1980.

Similar to last year's Annual, the second part of this book is disease-ori-

ented. In this section there is also a newcomer. Dr. Mogens Hansen has taken care of a missing link, namely the chemotherapy of endocrine tumors. He has reviewed the rather limited amount of literature published on this subject to provide the medical oncologist with several methods of treating patients with rare diseases such as apudomas.

There has also been an immense amount of new data on the leukemias and on lymphomas. Besides treatment, a great deal of new information has been reviewed on diagnostic and prognostic factors in the leukemias which are relevant to adequate treatment. While last year's chapter on lymphomas offered little information about mycosis fungoides and related diseases, this year's reviews current methods of treating this disease, which is often considered a no-man's land. It is exciting that the treatment of lymphomas as a whole continues to improve, and this development is now having an impact on national mortality figures in the United Kingdom.

In contrast to the lymphomas, little progress has been made in the chemotherapy of head and neck cancers, although the preliminary results of combined modality treatment with radiotherapy and chemotherapy do seem promising.

On the other hand, the results of chemotherapy of lung cancer continue to improve, particularly for small-cell carcinoma and adenocarcinoma. Many protocols are investigating the value of alternating drugs in small-cell carcinoma, and the role of cisplatin in these combinations. For adenocarcinoma the new vinca alkaloid vindesine has emerged as a promising drug.

Albeit slowly, the treatment of gastrointestinal cancers is making some headway. This is particularly the case for stomach cancer and pancreatic cancer. Many clinical trials are still being performed and it can be expected that therapeutic results will continue to improve in 1980.

While the results of chemotherapy of advanced testicular cancer improved dramatically in previous years, as reported in Annual 1, no further advances were made in 1979. However, a major effort to use combination chemotherapy in an adjuvant setting is proving successful and this should eventually produce a high percentage of cures.

The great successes achieved with combination regimens including cisplatin in ovarian cancer have received a great deal of attention in this volume. Again the importance of staging is emphasized. The availability of the stem-cell assay has opened up great possibilities for research in individualization of treatment. This appears to be one of the main research areas not only for tumors of the ovary but also for others such as brain tumors. In the chapters on both these topics the emerging role of the stem-cell assay is discussed.

The primary treatment of breast cancer apparently should include adjuvant combination chemotherapy. The results of the Milan adjuvant trial look promising for premenopausal women as well as for menopausal women who have received an adequate dosage of the drugs. However, the important role of additional trials should be emphasized since we still do not know the optimal doses, choice of drugs and duration of treatment.

Adjuvant chemotherapy has also proved useful as an integral part of the combined modality approach to the treatment of osteosarcoma. Even if chemotherapy does not seem to prevent the development of lung metastases,

as suggested by certain trials, the number of metastases is reduced, thus making subsequent surgical resection possible, and even cure in many cases.

After completing the editorial work on this volume I was struck by its quality which is even superior to last year's Annual. The book is more complete and more cohesive, most probably as a result of the discussions which the authors and I have had on content and procedures, the improved structure of the literature supply from the Excerpta Medica database and, above all, a gain in control of the enormously complicated flow of the data to be reviewed. It is my privilege to thank my collaborators for their efforts. I trust the results will be appreciated by our colleagues.

Contents

1 Antimetabolites

Bruce A. Chabner

METHOTREXATE (MTX)

The past year has witnessed further significant progress in understanding the pharmacologic action of methotrexate, genetic mechanisms of methotrexate resistance, and the transformation of methotrexate to polyglutamate forms. On the clinical level, we continue to unravel the pharmacokinetics and metabolism of methotrexate and apply this knowledge to the safe use of methotrexate, but remain uncertain as to the relative therapeutic efficacy of high-dose methotrexate, as compared to conventional regimens. These developments will be reviewed in detail in the following discussion.

Mechanism of action

Methotrexate (MTX) is known to exert its biochemical effects through binding to the enzyme dihydrofolate reductase. This binding is extremely tight, but clearly reversible in intact cells. A careful study of purified enzyme from *Escherichia coli* has established that the physiologic enzyme substrate, dihydrofolate, can compete with MTX for binding to the enzyme, although high concentrations of dihydrofolate are required (1000-fold higher than MTX) [1]. Intracellular accumulation of dihydrofolate, resulting from inhibition of reductase, would thus be expected to impede the further binding of MTX to the enzyme; this finding could account for the requirement for excess free intracellular MTX to maintain inhibition of the enzyme. Further studies of this type must be conducted with polyglutamate derivatives of dihydrofolate in order to establish the relative ability of this class of compounds (the actual physiologic intracellular folates) to compete with methotrexate.

The importance of the cellular dUMP pool, and its regulation by deoxycytidylate deaminase, has been emphasized in new studies [2,3] related to the mechanisms of action of MTX. Moran and co-workers [2] have found that the cytotoxicity of MTX, alone or in combination with thymidine (TdR) or with purine nucleosides, could be related to the rate of thymidylate synthesis. Protection from cytotoxicity was correlated with a decreased rate of thymidylate synthesis, and consequently a lower rate of oxidation of

the reduced folate pool. Inhibition of thymidylate synthesis by fluoropyrimidines or depletion of its substrate (dUMP) by any one of several maneuvers diminishes MTX toxicity. It should be noted that the combinations studied (MTX with TdR, or with a purine nucleoside) do not fully reverse MTX toxicity, but the partial reversal in all instances can be explained by effects on the thymidylate synthesis reaction rate. Evidence of this sort clearly indicates a need for more complete information on the regulation of dUMP synthesis and deoxycytidylate deaminase activity in human malignancy. In related work [4], Rode and co-workers have reported evidence that thymidilate synthesis itself may be closely regulated by the intracellular pool of thymidine nucleotides, particularly TMP, providing an additional mechanism for control of the rate of oxidation of reduced folates in the thymidilate synthesis reaction.

The biochemical consequences of MTX treatment for tumor cells have importance not only for understanding its mechanism of cytotoxicity but also for understanding its interaction with other antitumor agents. Cadman and co-workers [5] have shown a highly time-dependent interaction of 5-fluorouracil (5-FU) with MTX, in which pretreatment with the antifolate leads to an inhibition of purine biosynthesis and expansion of the intracellular phosphoribosyl pyrophosphate (PRPP) pool. PRPP is utilized in both purine and pyrimidine biosynthesis to form nucleotides from the various bases, and is likewise required for the formation of FUMP from 5-fluorouracil by the enzyme orotic acid phosphoribosyl transferase (OPRTase). Expansion of the PRPP pool by MTX leads to increased 5-FU activation and synergistic antitumor effects. The use of 5-FU preceding MTX does not allow this interaction, and, in fact, antagonizes the antipurine effects of MTX, leading to less than additive effects. These interactions have been demonstrated for the L1210 murine leukemia cell line [5,6], and are expected to apply to any cells which utilize the OPRTase pathway of 5-FU activation. It must be realized that alternative activation pathways exist for 5-FU, and may be of greater importance in selected tumors.

In addition to these major actions, MTX has interesting and unexpected effects on other cellular processes. The antifolate decreases uptake of glucose and glucose analogs by Ehrlich ascites tumor cells in culture, as an apparent consequence of its inhibition of purine biosynthesis [7]. This action was reversed by hypoxanthine and, it was postulated, may be a result of ATP depletion. The relevance of this action to MTX cytotoxicity was not investigated.

Methotrexate also is capable of inducing differentiation of human choriocarcinoma cells in culture [8]. At concentrations which did not fully inhibit DNA synthesis, MTX caused a striking change in morphology from a cytotrophoblastic to syncytiotrophoblastic appearance and increased production of human chorionic gonadotrophin (HCG). These changes were reversed by removal of the drug. While induction of differentiation has been described in experiments with other antitumor agents, these effects have not been observed with MTX in previous work.

Intracellular metabolism

It now seems to be a very likely possibility that methotrexate may undergo transformation to active polyglutamate form(s) in many normal and malignant cells [9,10]. Previous work has shown that glutamyl groups are added to MTX in a γ-peptide linkage in various murine tumor cells, in normal human fibroblasts in culture, and in rat and human liver. The process of polyglutamate formation occurs rapidly in rat hepatocytes [11,12]; addition of one glutamyl residue (MTX + G_1) is detected within 15 minutes, MTX + G_2 by 45 min, and by 12 hrs higher polyglutamates are found in these cells. If the extracellular MTX is diluted by addition of a large volume of medium, the intracellular concentration of MTX falls rapidly, but polyglutamates are retained, indicating either preferential intracellular binding to reductase or a decreased rate of efflux. Experiments with other cell types indicate that polyglutamates do pass the cell membrane, although their exact transport properties have not been clearly defined.

The consequences of polyglutamation are not understood at this time. In cultured human fibroblasts, their formation is associated with a more prolonged inhibition of UdR incorporation into DNA after removal of free extracellular drug [9]; this finding could be the result either of their more avid binding to reductase or greater intracellular retention. MTX + G_1 has 7-fold greater inhibitory activity against thymidylate synthetase (TS) [13], although its K_i is still 3 to 4 logs higher for this enzyme than for dihydrofolate reductase, and the latter is still very likely to be its major intracellular target.

Methotrexate transport

Although the kinetic features of methotrexate transport are well understood, the physical characteristics of the transport system have only recently received attention. McCormick and co-workers [14], using the sulfhydryl-labeling agent N-ethylmaleimide have identified a 56,000 molecular weight protein from L1210 cell membrane which binds to, and is eluted from, a MTX affinity column. Several other higher molecular weight proteins were also eluted. Whether these proteins display the same affinities and specificities as the MTX transport process remains to be established.

An alternative approach to the study of the transport process has been taken by Yang et al. [15], who have observed active transport of MTX by isolated plasma membrane vesicles from L1210 leukemia cells. While this system preserves many of the features of transport in intact cells, its V_{max} and Q_{10} are diminished and the vesicles are less able to concentrate drug from the medium. However, this system does allow the study of transport in the absence of polyglutamation and binding to reductase.

The stereospecificity of the reduced folate transport system, which is shared by methotrexate, 5-methyltetrahydrofolate, and leucovorin, has been studied, with conflicting results, by two different groups. The first reports indicated that the D-diastereoisomer of 5-methyltetrahydrofolate was readily transported and could cause methotrexate efflux from cells by hetero-ex-

change [16]. However, a more recent study by Sirotnak et al. [17] indicates that the L-isomer of leucovorin is 20-fold more effective than the D-isomer in competition with methotrexate for influx; the D-isomer of leucovorin is also ineffective in rescuing cells grown in the presence of methotrexate. These experimental differences in stereospecificity may be the result of differences in the chemical configuration or the purity of the isomers, the specific cell lines studied, or other factors, but have important bearing on the mechanism of leucovorin rescue and require further study.

The possibility of modifying transport by altering the lipid content of the cell membrane has been investigated by Burns et al. [18], who found that a diet high in saturated fats caused a marked increase in membrane fluidity and a decrease in the apparent affinity of the transport system for MTX. These findings indicate that nutritional manipulations, such as i.v. hyperalimentation with diets high in unsaturated fatty acids, might favorably affect transport of the antifolate.

Mechanisms of resistance

The exciting work by Schimke and colleagues concerning gene amplification as the mechanism of increased dihydrofolate reductase in resistant cell lines has been extended in the past year. The initial step in the process of development of resistance appears to be the formation of small, paired chromosomal elements termed 'double minutes', which contain the amplified gene sequences [19]. Double minutes are associated with unstable amplification; that is, such cells revert to a normal level of reductase in the absence of methotrexate. Stably amplified genes are integrated into larger chromosomes and appear as new homogeneously staining bands within the chromosomes. The sequential appearance of 5 new chromosomes containing such homogeneously staining regions has been observed in mouse melanoma cells exposed to graded increases in MTX concentration, *in vitro* [20]. The presence of each new chromosome is correlated with stepwise increases in enzyme content, and increasing resistance.

In related work, the Stanford and Yale research groups [21] have devised a method for quantitation of intracellular reductase by using a fluorescein-tagged methotrexate derivative. Tagged cells can be separated by a fluorescence-activated cell sorter, and high-reductase cell lines can be isolated by this technique. The cellular uptake of the fluorescent derivative is slow, requiring approximately 20 hours for saturation of reductase in cells with high enzyme content. This technique could be used to quantitate reductase levels in leukemic cell samples, and to search for the presence of sublines with high reductase content.

While a simple increase in reductase appears to account for resistance in cell lines exposed to graded increases in MTX, alternative explanations, even in the cell lines with increased enzyme, must be considered. Preliminary work indicates that a second species of enzyme of somewhat lower molecular weight (20,000 daltons *vs* 21,000 for the native enzyme) and encoded by a distinct mRNA [22] appears in resistant cell lines. In a second high-reductase cell line, a deficiency in the ability to transport MTX was also found, and

appeared to account for resistance, since hybridization of the resistant cells with a sensitive line yielded a sensitive hybrid with elevated enzyme levels but with an intact transport system [23]. Thus, the role of increased dihydrofolate reductase in MTX resistance is still poorly understood.

Hill and colleagues have examined the effects of transport deficiency on methotrexate uptake in resistant L5178Y cells. They found that the resistant cells take up drug slowly, achieving levels which were 1/6 those of sensitive cells at drug concentrations of 10 μM [24]. Increases in extracellular MTX concentration to 50 μM considerably augmented intracellular MTX, although the steady-state level was still 4-fold less than in the sensitive cells at the same concentration. These studies provide evidence that increasing doses of MTX can drive more antifolate into transport-resistant cells.

Pharmacokinetics

While the basic elements of methotrexate plasma pharmacokinetics have been established for i.v. schedules of administration, the renal handling of this agent is not well understood. In particular, the ability of patients to excrete this agent is not always predictable on the basis of measurement of creatinine clearance. New observations on the renal elemination of methotrexate in the dog and monkey indicate that both species actively secrete the antifolate in the proximal tubule, a process which is blocked by probenecid [25]. The drug is reabsorbed in the distal tubule of the dog, but not the monkey; reabsorption probably occurs as well in man, since values for methotrexate clearance in some patients are lower than creatinine clearance. The reabsorption process is competitively inhibited by folic acid (and likely also by leucovorin). These results in the dog are consistent with earlier evidence for renal secretion and reabsorption of methotrexate in man.

The penetration of antifolates into the central nervous system has become a topic of considerable concern because of the need to prevent central nervous system relapse in acute leukemia. There is now evidence that the permeability of the blood-brain barrier to MTX can be markedly increased by infusion of the carotid artery with hyperosmolar solutions. In the rat, a hyperosmolar arabinose solution (1.6 μM) infused into one carotid artery allowed a 7-fold concentration of methotrexate in the perfused side as compared to the non-perfused side [26]. Since the blood-brain barrier is not intact in most brain neoplasms, this type of approach probably has limited significance for solid tumors. Its application to the prophylaxis of leukemic meningitis is also problematic in that carotid artery catheterization would be required.

McVie and colleagues have evaluated the oral absorption of MTX [27,28]. They have found that oral MTX is better absorbed when given in oral doses of 25 mg q.i.d. as opposed to single doses of 100 mg q.i.d., the latter regimen resulting in only one-half the AUC (area under the curve, or plasma concentration x time) as compared to the former. In comparing an oral dose of 50 mg/m^2 with an identical i.v. bolus dose, they found a consistently smaller AUC after oral administration. Both studies re-emphasize the variable and limited bioavailability of oral MTX as compared to i.v. drug.