CANCER CHEMOTHERAPY 1981

The EORTC Cancer Chemotherapy Annual 3

Edited by H.M. Pinedo

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Edited by H.M. Pinedo
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Introduction

H.M. Pinedo

The Cancer Chemotherapy Annual seems now definitely to be finding its way to the oncologist's desk. From the present volume it will be clear to the reader that each new annual contains no duplication of references reviewed in its predecessors. This means that in certain instances in which data included in previous volumes have now been updated, the author refers to the respective previous chapters. As I promised last year, we have included again the titles of the articles in the reference lists, which is in keeping with our initial aim. The omission of the reference titles which we had to resort to last year was indeed a drawback for the reader as the title does give him the additional orientation he may need in deciding whether to read the original paper.

Again the authors have succeeded in writing reviews which are entirely up to date. This is partly because of the excellent service of the Excerpta Medica database. I wish to thank all the contributors for adhering to the deadline, which is the only way to secure the timely publication of a book like this. Now that this aim has been attained, the reader is again presented with a most comprehensive and critical review of today's cancer chemotherapy.

When the first volume of this series appeared in 1979, I was asked many times whether such a series of annual reviews was perhaps not too ambitious. It was frequently thought that there was not enough news to justify the publication of a new volume each year. These questions have now faded. On the contrary, it is now rewarding to receive the many letters and reviews conceding that the second annual has proven to fulfill the need of the oncologist to keep abreast of the vast amount of new information which is being published each year.

In most of the fields of oncology some advance in chemotherapy is made each year. Although these advances are not of the same extent for each tumor type, it is invaluable to the oncologist to be able to keep track of gradual changes. Most of the progress is being achieved with new drugs or new combinations of existing drugs. For certain tumor types progress has also been achieved through the introduction of combined-modality treatment, for instance in Stage III and IV ovarian cancer. In contrast, for other tumor types the value of combined-modality treatment is being questioned with the im-

provement of the results which are achieved with one of the modalities formerly used in the combined set-up. An example is the treatment of anaplastic bronchial carcinoma, particularly in cases with extensive disease.

A major development is autologous bone marrow transplantation, the role of which is now also being investigated in patients with solid tumor. This approach may offer the oncologist the possibility of increasing the drug dosage to a level which, under normal conditions, would be supralethal. It will become clear to the reader when going through the chapters that the latter approach still needs to be explored further and has as yet certainly not reached the stage of routine use.

Most interesting is the development of new methods of drug sensitivity testing which are currently under study. The major example is the tumor stem cell assay, originally set up by Salmon and his co-workers. During the past year many centers have included this assay in their research programs. The present state of this art has been updated in this volume by Dan Von Hoff, who has been one of the major contributors to its development. Within the EORTC, Marcel Rozencweig has created this year a 'Stem Cell Assay Club', with the aim to evaluate and improve the quality of the assay through collaboration of all European investigators and a few American friends in this exciting field. It may well appear that the conditions for growing different tumor cells into colonies in the semi-solid medium which is being applied differ widely between tumor types. One may anticipate that each tumor type has its own colony-stimulating factor. Although the initial aim of this research was to evaluate the method as a tool for studying the sensitivity of individual tumors to drugs, the method is presently also being evaluated as a way to screen potential new agents. At the ASCO meeting this year, a long and exciting symposium was devoted to the stem cell assay, reflecting the fact that this particular development has attracted the greatest attention during the past year.

In the first chapter, that on antimetabolites, again a comprehensive review is given of the latest news on this group of agents. It has not been until now, tens of years since 5-fluorouracil was introduced in the clinics, that the mechanism of action of this important drug is being better understood. The reader will find in this chapter also new information on the pharmacology of methotrexate and other antimetabolites.

The chapter on alkylating drugs once more adroitly summarizes within the space of a very limited number of pages literally hundreds of papers which appeared on the subject in only one year. Among the many important observations included in this year's review, the reader will find a report on several patients who developed a Budd-Chiari syndrome during treatment with DTIC.

The anthracycline research is helping us more and more to understand the mode of action of adriamycin and the biochemical effects which lead to the cardiac toxicity of this drug and its analogs. However, new analogs, such as aclacinomycin A, are being studied, which may appear useful to circumvent this important side effect.

Mitomycin C, an antibiotic which has been known for more than 20 years, has been reintroduced in the clinic, and appears to be a valuable drug for the treatment of several tumor types, mostly in combination regimens. However,

this drug also has some disturbing side effects which should be known to the clinical oncologist. These include pulmonary toxicity; in addition, mitomycin C possibly potentiates the development of adriamycin cardiotoxicity, when used in combination with that drug.

The epipodophyllotoxins VP-16 and VM-26 have emerged as a very active group of new agents. In my previous introduction I already mentioned their activity in several tumor types, but this year it has become very clear that VP-16 is one of the most active drugs in the treatment of testicular teratomas as well. In the very near future, maybe even in the next volume, the readers may find this drug included in first-line combination regimens for teratomas.

The very important drug cisplatin has been the object of further pharmacological studies. The pharmacokinetics of free platinum have become available during the past year. Although methods of administration suitable for use on an outpatient basis have now been described, nephrotoxicity still remains the major side effect of this drug. At the moment clinicians should still be discouraged to use the drug routinely on an outpatient basis. Interestingly, there are now five analogs of cisplatin being examined in Phase I trials in Europe and the United States. Hopefully, one of these drugs will appear to have a better therapeutic index than cisplatin.

This year's annual again features addenda to the chapter on new drugs and that on miscellaneous drugs. One of the important data in the former chapter is that on interferons. These agents, which have caused great excitement and expectations because of the attention they received in non-professional magazines, have given very disappointing results in the treatment of cancer up to now. They have certainly not come up to expectations. In the chapter on new drugs the very latest news on Phase I and II trials has been incorporated, including data reported at the ASCO and AACR meetings held in the spring of this year!

As the antiestrogens have been replacing the estrogens to a great extent in the treatment of breast cancer, relatively much attention has been given to the pharmacology of this very important new group of agents. Similarly, aminoglutethimide, which causes a medical adrenalectomy, is finding a definite role in the treatment of breast cancer.

The chapter on immunotherapy constitutes a very comprehensive review on every aspect of present-day immunotherapy and offers some additional basic information on interferons.

Also in Part II of the book, which reviews publications on the treatment of each individual tumor type, the reader will find most interesting and important developments in each of the chapters.

As one may expect, the chapter on breast cancer is again the longest in this second part. Chemotherapy adjunctive to primary treatment of premenopausal patients with breast cancer continues to give very promising long-term results. Moreover, it would seem that patients with estrogen receptor positive breast cancer may benefit even more if cytotoxic combination chemotherapy is combined with tamoxifen (Fischer et al., *New England Journal of Medicine*, *305*, 1, July 2nd, 1981). Obviously it has not been possible to include this report on adjuvant chemotherapy in this year's review. In

contrast, it appears from several studies that combined hormono-cytotoxic therapy does not increase survival of patients with advanced breast cancer. Next year the reader may expect more news on this exciting topic.

I would like to conclude these introductory pages by once more thanking all the authors for their contributions. This year the pressure on them was even greater than before because the deadline for the submission of the manuscripts was advanced several weeks. Still they were in time! I am also most grateful to my wife, Rita, and to my children for mustering the incredible amount of patience necessary to allow me to devote so many hours to the arduous but challenging exercise of going through all the manuscripts and galley proofs during the past spring.

Contents

1 Antimetabolites

Richard L. Schilsky, Jacques Jolivet and Bruce A. Chabner

METHOTREXATE

During the past year, continued progress has been made in understanding the biochemical pharmacology of methotrexate (MTX) and its intracellular metabolism to polyglutamate derivatives. New insights have been gained into some aspects of MTX membrane transport and into the complexities of drug resistance and drug interactions. Clinically, the application of high-pressure liquid chromatography (HPLC) to measurement of plasma levels of MTX and its metabolites now allows a more precise estimation of the extent of MTX metabolism following drug administration. Other pharmacokinetic studies have focused on the distribution of MTX in the cerebrospinal fluid following high-dose and intrathecal administration. These developments will be reviewed in detail in the following discussion.

Mechanism of action

Although MTX is well known to produce its cytotoxic effects through binding to the enzyme dihydrofolate reductase (DHFR), recent studies have elucidated the central importance of the thymidylate synthetase (TS) reaction in determining cellular sensitivity to depletion of reduced folates (Annual 2). The *de novo* synthesis of TMP from deoxyuridine phosphate (dUMP), catalyzed by TS, is solely responsible for the depletion of intracellular reduced folate pools which are required for maintenance of purine biosynthesis. Inhibition of TS by fluoropyrimidines or by depletion of intracellular dUMP pools results in a lower rate of oxidation of reduced folates and a relative insensitivity to the effects of MTX. In a careful study Jackson [1] has examined the sequence-dependent biochemical effects of thymidine in modulating MTX cytotoxicity. Pretreatment of cells with thymidine afforded protection from MTX cytotoxicity to both normal and malignant cells. This is mediated by depletion of dUMP pools, which occurs due to inhibition of the enzyme dCMP deaminase by TTP. The decreased rate of the TS reaction which then follows allows preservation of intracellular reduced folates and protection from the antipurine effects

of MTX. By contrast, thymidine was much less effective when administered as a rescue agent following MTX. This appears to be related to the fact that exposure of cells to MTX results in increased cellular dUMP levels which are not significantly diminished by subsequent exposure to thymidine. Utilization of reduced folates then continues and purine synthesis is compromised. Studies of this type clearly indicate that a more complete understanding of the regulation of intracellular nucleotide pools in human malignancy is necessary to fully appreciate the mechanism of action of MTX and other antimetabolites.

Better understanding of the biochemical consequences of MTX administration has also led to a new hypothesis concerning its mechanism of action. In a series of experiments, Goulian et al. [2,3] have demonstrated that exposure of human lymphoblasts to MTX results in elevated intracellular dUTP levels and misincorporation of uracil into DNA. These investigators propose that DNA fragmentation then occurs as a result of excessive activity of the normal excision-repair process. The relative importance of this biochemical lesion in determining the cytotoxicity of MTX remains to be established.

These insights into the biochemical alterations which occur with MTX treatment have also provided a biochemical rationale for the use of MTX in combination with other antimetabolites, particularly 5-fluorouracil (5-FU). Recent studies by Donehower and colleagues [4] and by Benz et al. [5] have demonstrated synergistic cytotoxicity when exposure to MTX precedes 5-FU in human breast and colon carcinoma lines. In both studies, MTX administration following 5-FU resulted in antagonistic effects. These effects are postulated to result from fluoropyrimidine antagonism of MTX's antipurine effects. This hypothesis has been supported by a recent study by Bowen and co-workers [6], who found that fluoropyrimidine pretreatment of Ehrlich ascites tumor cells can antagonize the inhibitory effects of MTX on RNA and protein synthesis. As discussed above, these effects are likely related to fluoropyrimidine inhibition of TS and the resultant preservation of intracellular reduced folates required for other synthetic processes.

The biochemical mechanism by which MTX pretreatment produces synergistic cytotoxicity with 5-FU has been explored by Fernandes and Bertino [7]. These investigators propose that inhibition of DHFR by MTX results in an intracellular accumulation of dihydrofolate polyglutamates, which in cell-free studies were found to foster the formation of tight complexes of 5-FdUMP with TS. This would offset any antagonistic effect which could potentially occur due to a MTX-induced fall in cellular levels of 5,10-CH_2 THF, the usual folate cofactor of TS. MTX pretreatment has also been shown to result in increased cellular 5-phosphoribosyl-1-pyrophosphate pools which may foster incorporation of 5-FU into RNA as well as increase 5-FU activation to 5-FdUMP (Annual 2) [8]. Any or all of these mechanisms may have a role in explaining the schedule-dependent synergism of MTX and 5-FU.

MTX transport

Although the characteristics of membrane transport of MTX have been well described, the energetics and cell-cycle dependence of this process remain subjects of continued interest. Chello et al. [9] have recently described changes in the kinetics of MTX transport which occur as cells progress from the logarithmic to the plateau phase of growth. In studies of L1210 murine leukemia cells, these investigators demonstrated a 3-fold increase in the V_{max} for influx along with a 50% decrease in the efflux rate for exponentially growing compared to stationary-phase cells. These reciprocal changes resulted in a 5- to 6-fold increase in steady state levels of exchangeable MTX in the rapidly proliferating cells. These findings may, in part, explain the increased sensitivity of rapidly growing cells to inhibition by MTX. In addition, they again raise controversial questions concerning the presence of single- or multiple-membrane carrier systems for reduced folates which require additional experimental approaches.

The importance of the extracellular ionic environment in modifying MTX membrane transport has been examined in several recent studies. Fry and colleagues [10] have demonstrated that when Na^+ is replaced by K^+ in the extracellular fluid, MTX influx is reduced by 27% and efflux is reduced by 53% in Ehrlich ascites tumor cells. In L1210 cells, Henderson and Zevely [11] have shown that extracellular Cl^- and $PO_4^=$ competitively inhibit MTX uptake but that Mg^{++} enhances influx. Studies of this type, although of little clinical relevance, may provide some insight into the electrochemical processes involved in the coupling of energy to MTX transport. The role of cyclic AMP as an organic anion which regulates MTX transport has been investigated by White and co-workers [12]. In contrast to earlier studies (Annual 1), White et al. were unable to demonstrate any consistent relationship between cellular cAMP levels and MTX influx in Ehrlich ascites tumor cells. Neither increases in cAMP induced by cholera toxin nor decreases induced by ascorbic acid were accompanied by changes in MTX influx. Thus, the importance of cyclic nucleotides as mediators of MTX transport remains unclear.

Interest in the role of bile salts as inhibitors of MTX uptake has been fostered by the recent studies of Gewirtz and colleagues [13,14]. These investigators first defined the characteristics of MTX transport in freshly isolated rat hepatocytes [13]. In these cells, MTX uptake is mediated by both high- (K_m = 5.9 μM) and low-affinity transport routes, which are energy- and Na^+-dependent but apparently distinct from the transport routes utilized by physiologic reduced folates. Subsequent studies [14] demonstrated that bile salts inhibit both the high- and low-affinity MTX transport systems and that cholate, taurocholate, and deoxycholate reduce MTX polyglutamation as well as influx. The clinical relevance of these findings remains to be established. However, the data raise the possibility that MTX hepatotoxicity may be ameliorated by expansion of the bile salt pool.

Regardless of the experimental conditions, an accurate assessment of MTX transport characteristics requires the use of chemically pure drug. Traditional concepts of MTX uptake hold that there is an extremely rapid component of

uptake related to cell surface adsorption which may account for up to 10% of apparent total drug uptake. Kamen and associates have recently challenged this view [15]. These investigators have demonstrated that ^{3}H-p-aminobenzoyl glutamate, present as an impurity in ^{3}H-MTX preparations, is rapidly taken up by both sensitive and transport-resistant L1210 cells and may account for as much as 5% of apparent MTX uptake. This impurity could be responsible for the apparent early surface binding of ^{3}H which, in the past, has been assumed to be MTX.

Intracellular metabolism

Recent studies have clearly demonstrated that MTX is converted to polyglutamate derivatives by a variety of normal and malignant tissues. Synthesis of these compounds, which are derived by the sequential addition of glutamyl residues to MTX in a γ-carboxyl linkage, has now been described in human liver, bone marrow, fibroblasts, and cultured human breast cancer cells as well as in a number of normal and malignant animal tissues [16–19]. Although initially thought to be an inert intracellular storage form for MTX, Schilsky et al. [17] and Galivan [18] have clearly shown that MTX polyglutamates bind rapidly to DHFR intracellularly. These findings support previous studies which demonstrated that accumulation of MTX polyglutamates is associated with inhibition of DNA synthesis and cytotoxicity [20].

Considerable controversy exists concerning the ability of polyglutamates to leave the cell. Studies in rat hepatocytes [13] and cultured human fibroblasts (Annual 2) suggest that these derivatives are retained intracellularly and could thereby play an important role in maintaining inhibition of DNA synthesis even in the absence of extracellular MTX. A recent report by Poser et al. [20], however, demonstrates that, in drug-free medium, efflux of MTX and its polyglutamates from L1210 cells proceeds at essentially equal rates and that MTX polyglutamates can be detected in the efflux medium following the addition of DHFR, which serves to protect these compounds from hydrolysis. It appears, then, that intracellular retention of MTX polyglutamates may vary considerably among different cell types.

Little information is currently available concerning those conditions which might enhance or inhibit MTX polyglutamation. Mammalian folyl polyglutamate synthetase has recently been characterized by McGuire et al. [21]. This enzyme, purified from rat liver, requires K^{+} and ATP and is highly specific for L-glutamate. Virtually all naturally occurring folates, as well as MTX, were found to be suitable substrates, suggesting that administration of reduced folates, such as leucovorin, along with MTX could inhibit MTX polyglutamate synthesis. Indeed, Rosenblatt and co-workers have recently shown this to be the case in cultured human fibroblasts [22]. Exposure of cells to MTX in the presence of either leucovorin or 5-methyltetrahydrofolate resulted in a marked inhibition of MTX polyglutamate synthesis. It is unclear, however, whether this resulted from inhibition of MTX uptake by the cells or from inhibition of MTX metabolism. If subsequent studies demonstrate the latter to be true, then inhibition of MTX polyglutamation could be yet another mechanism of leucovorin 'rescue'.

Mechanism of resistance

Overproduction of DHFR as a result of gene amplification appears to be an important mechanism of MTX resistance in cell lines exposed to stepwise increases in MTX. Several recent studies have now demonstrated that production of altered DHFR molecules may also occur under these selective conditions. Goldie and co-workers [23] have produced two resistant lines of L5178Y cells which contain high levels of wild type DHFR as well as small amounts of an MTX-insensitive enzyme which binds the drug with a K_i of only $3-7 \times 10^{-4}$ M. Similarly, in Chinese hamster ovary cells, Flintoff and Essani [24] have used a two-step selection process to produce resistant cells with high levels of a low affinity DHFR. Resistant Chinese hamster lung fibroblasts which contain an altered DHFR of lower molecular weight than the wild type enzyme have also been produced [25,26]. The low molecular weight enzyme species is encoded by a specific mRNA transcribed from a distinct amplified portion of the genome. The binding characteristics of this altered reductase species await definition.

Whether these types of resistance are relevant to human malignancy and to the clinical use of MTX and other antifolates is currently unknown. Cells which are resistant to MTX by virtue of an increase in DHFR levels should, theoretically, be resistant to other antifolates which act by inhibition of this enzyme. This has been shown to be true for L1210 cells with high DHFR levels which are resistant to both MTX and diamino-dichlorophenyl-6-methyl pyrimidine (DDMP) [27,28]. Conversely, MTX-resistant cells with altered transport properties but wild type reductase levels are inhibited by lipid-soluble antifols such as DDMP and the mono- and dibutylesters of MTX [29]. Transport resistance may also be overcome by the use of MTX-polylysine conjugates which effectively penetrate the cell and exert a cytotoxic effect after intracellular liberation of free MTX [30]. The use of antifols with different transport properties in combination could then be a means of overcoming the drug resistance conferred by changes in membrane transport characteristics.

A theoretical advantage to the use of high-dose MTX regimens is the possibility that transport resistance may be overcome by diffusion at high extracellular drug concentrations. High-dose administration may not, however, overcome resistance which is due to overproduction of DHFR. Bruckner et al. [31] have recently reported studies on an L1210 cell line which contains a 20-fold increase in DHFR yet was only partially inhibited by MTX concentrations up to 10^{-3} M. By contrast, wild type cells were sensitive to drug concentrations as low as 10^{-7} M. Further, in the resistant cells, both protection and rescue by leucovorin were greater than in the sensitive cell line. An explanation of these findings is not immediately apparent but could involve a change in the affinity of the excess DHFR for MTX, an acquired alteration in membrane transport characteristics, or an abnormality of intracellular polyglutamation of MTX.