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Contributions of Pharmacologic Studies of Anticancer Drugs to Their Clinical Trials

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Pharmacologic studies and clinical trials of anticancer agents constitute the major research activities of our group at the University of Texas M. D. Anderson Hospital at Houston. Broadly defined, pharmacology is an experimental science that deals with the interactions between chemical substances and biological systems. Chemical substances are not necessarily drugs, but in our studies we confine ourselves to those that have shown significant anticancer activities in at least some experimental systems. However, "biological systems" include human beings, animals such as dogs and rodents, cultured cells, organ homogenates, and crude enzymes.

Studies of the effects of a chemical on a biological system resulting from the interactions of the former with receptors or target enzymes of the latter, particularly the mechanisms of anticancer action, are often called pharmacodynamics. On the other hand, investigations of the fate of a chemical or a drug falls within the definition of pharmacokinetics, especially if the time course is followed and mathematical modeling is involved.

Because the main purposes of our research are not only to learn the most effective ways of administering the agent under study, but also on

the basis of our work to discover leads that may assist in the development of more efficacious agents, later on I shall briefly discuss with you my thoughts regarding the development of new anticancer agents. At the outset, it should be pointed out that in pharmacologic studies of anticancer agents, the possibility of interactions among the host, the tumor, and the drug must always be considered as a result of the peculiar nature of cancer some unique properties of anticancer drugs.

Moreover, other drugs often interact with anticancer agents to give rise to serious consequences. It suffices to say, at this point, that since cancer patients on chemotherapy are frequently treated with a variety of drugs, one must be on the alert for drug interactions. As a world leader in developing anticancer drugs, obviously Japan is fully aware of the high cost of these endeavors.

In 1978 in the U.S., the average cost for preclinical development of synthetic anticancer agents was well over \$200,000 and that of plant products nearly twice as much. Today the cost would even be considerably higher. But, more efficacious anticancer drugs are nonetheless desperately needed simply because most of the

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currently available ones are fraught with serious shortcomings such as the lack of specificity, the failure to reach certain anatomical sanctuaries, and the natural as well as induced resistance inevitably encountered.

Thus far, most anticancer drugs were discovered by chance or enlightened empiricism. To save time and effort, in short, to be cost effective, it would be desirable to have some intellectual bases and guiding principles to aid in their development.

Unfortunately, these principles do not exist. Almost 50 years ago H. Meyer and E. Overton observed a linear relationship between a standard biological response and some physicochemical properties such as water solubility, oil-water partition coefficient, and the like in a homologous series of aliphatic drug substances. However, it was Professor Corwin Hansch in the U. S. who not only pointed out that the relationship was not always linear but often parabolic but also greatly extended this concept.

Additionally, he introduced several novel ideas and the inclusion of electronic and steric effects. This quantitative structure-activity relationship seems to be applicable to many types of drugs including anticancer agents such as the nitrosoureas¹⁾, in which both the anticancer activity and toxicity of these compounds are parabolically related to their partition coefficients. Although I know no useful new anticancer agents that have ever been designed and synthesized by the Hansch approach, his is nonetheless a signal contribution to the quantitative study of structural-activity in anticancer drugs.

Another approach to develop more efficacious anticancer agents is to modify structurally existing ones, fltorafur and vindesine are examples. However, compared with the parent drug, analogues with even apparently minor structural

modifications often display strikingly different toxicological, pharmacological, and therapeutic properties.

Furthermore, in my view, as far as antimetabolites are concerned, to synthesize exotic novel compounds of this category that differ structurally from the natural counterparts in more than one feature is bound to fail. They are inevitably inactive clinically; arabinosyl-6-mercaptopyrimidine is a typical example. Why is it so? It occurs to me that teleologically as the human race successfully ascends the evolutionary ladder, a critical human enzyme may occasionally accept a fraudulent substrate differing slightly from the natural substrate. But for the human species to survive and compete successfully, our enzyme systems must have become refined and specific in their substrate requirements so that they can readily discriminate against and to reject an obvious bogus imposter.

In a civilized society, it is absolutely immoral and unconscionable to experiment with human beings. Therefore, before its clinical trial, a new drug, especially an anticancer drug, must be tested in experimental animals. Usually rodents are chosen because they are economical, consume less food and drug, are relatively pure genetically, easy to keep, and the results speedily available. Earlier Dr. Gordon Zubrod and others noted that those rodent tumors sensitive to chemotherapy all have short doubling times²⁾.

It is instructive that human cancers thus far noted for their sensitivity to chemotherapy also have short doubling times. Unfortunately, these are comparatively rare tumors. Consequently, animal tumors more predicative of the human disease are needed and the U. S. National Cancer Institute has adopted standard preclinical screening systems for this purpose. Even these elaborate screening panels are far from being ideal. In

fact, the majority of agents active against animal tumors fail to show clinical activity.

In Tab. 1 a few of the major reasons for the failure are listed. Comparing the transplantable animal tumor with the human disease, the first strike difference is in the genetic makeup of the host. Relatively genetically pure inbred mice are routinely used for experimental purposes. But human beings are obviously almost never genetically homogeneous. Etiological differences are another consideration.

For example, the murine leukemia L1210 was chemically induced with 3-methylcholanthrene in the spleen and lymph node of the DBA mouse. But the etiology of human acute lymphocytic leukemia is unknown thus, they are comparable only in a limited sense. Moreover, at presentation, the patient's tumor varies widely in age, stage, grade, and metastatic potential. In contrast, in experiments with rodents, similar variations are minor because the tumor is transplanted at the same time.

Additionally, in animal screening drug dosages could be high, commonly to the LD10 and sometimes even higher. Clearly, this is not permissible in man; in Phase I clinical trial the

Tab. 1 Possible Reasons Why Animal Models Fail to Predict Clinical Activities of Anticancer Drugs

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1. Genetic makeup
 2. Pharmacokinetic differences
 3. Etiology
 4. Biochemical and pharmacological considerations
 5. Differences in tumor, age, stage, grade, and metastatic potentials
 6. Drug dosage
 7. Drug interactions
 8. Cell kinetics
 9. Others
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rule of thumb is that the starting dose is 1/10 but certainly less than 1/3 of the LD10 of the drug in the most sensitive species by the same route and schedule.

Further, biochemically and pharmacologically, transplantable animal tumors may be totally dissimilar to the human disease. For instance, the depletion of hypoxanthine-guanine phosphoribosyltransferase has been shown to be a mechanism of resistance in murine leukemia. Likewise, increased dihydrofolate reductase activities in murine leukemia resistant to methotrexate have been demonstrated.

However, unequivocal evidence is scant that similar mechanisms are operative in human tumors resistant to these drugs. Lastly, patients with cancer are seldom treated with one single agent but combinations of anticancer agents plus a variety of other drugs as indicated. As a result, the possibility of drug interactions always exists. But in screening of a new anticancer drug, nothing but the drug is administered and no drug interaction is expected.

Rodents and large animals are widely used for toxicological studies of anticancer drugs as well. For example, dogs and monkeys are useful in predicting marrow suppression, gastrointestinal disturbances and hepatotoxicity in man. On the other hand, they fail to predict renal, cardiovascular, or neuromuscular toxicities³⁾.

Unusual toxicities such as the pancreatitis of L-asparaginase, the myocardial toxicities of doxorubicine, and ototoxicity of cisplatin actually were not detected in animals until after clinical trial has begun. Since the role of the monkey in preclinical toxicological studies of antitumor agents is only secondary and gradually diminishing, nowadays monkeys are not as much used in the U. S. as previously.

The extrapolation of dosages of cancer chemo-

therapeutic agents from one species to another is customarily based on body surface areas, for example, mg/m^2 .

When the dosage is so expressed, a one-to-one correspondence between the maximum tolerated dose and the LID₁₀ in several animal species becomes readily apparent⁴⁾. Preclinical studies in experimental animals merely pave the way for later clinical trials that ultimately determine the activities of the agent and the most efficacious way of its use in man.

By general acceptance, clinical trials are divided into 4 Phases, each with a definite purpose. These purposes actually do overlap; for instance, although the Phase I trial is designed primarily to determine clinical toxicity and optimal dosage, any antitumor activities of the agent obviously will not escape notice.

Additionally, in our laboratories pharmacologic studies of antitumor agents are deemed an integral part of their clinical trials. Without any doubt, for the trial to be successful, the clinician should be familiar with certain relevant properties of the agent, especially its disposition and metabolism. To this end, therefore, we routinely conduct pharmacological studies of new agents in animals and man currently with the Phase I trial, sometimes even before.

The usefulness and limitations of the animal model for antitumor screening and toxicological evaluations of new agents have already been alluded to. Animal models are equally invaluable and indispensable in studies of the disposition and metabolism of drugs. The caveat is that models are models, not the real thing. In our experience, pharmacokinetic comparisons of man versus animals, generally drug disposition is qualitatively similar but may be quantitatively different.

Thus, if a drug is excreted in the bile of mice

treated with it, most likely it will be excreted in the bile of man receiving the same drug. On the other hand, drug metabolism may be entirely dissimilar quantitatively as well as qualitatively from one species to another. At conventional doses, methotrexate is metabolized by the rabbit and guinea pig but not by man; the literature abounds with such findings.

The remaining part of this paper will deal with the contributions of pharmacologic studies of anticancer drugs to their clinical trials. To illustrate my points, examples are necessarily taken from our own work on account of my familiarity with it.

For years I have spoken against administering anticancer agents to patients by mouth because of the difficulties to ensure their complete and dependable gastrointestinal absorption. A variety of factors exert influence on drug absorption but few of them are readily controllable. Since with anticancer drugs a slight dose fluctuation may result in drastic changes in cytotoxicity, the so-called steep dose-response, incomplete, erratic, and unreliable drug absorption may either over- or underdose the patient with serious consequences.

Another problem is the first-pass effect following oral administration, that further reduces the bioavailability of the drug. However, oral administration of drugs has many practical advantages and is the usually preferred route if absorption is dependably complete. During the Phase I trial of dacarbazine or DIC, plasma drug concentrations were monitored in a patient who received the agent intravenously and then orally again afterwards⁵⁾. From the areas under the plasma drug concentration versus time curves, evidently, at least in this patient, DIC was incompletely absorbed.

An alternative, non-invasive method to esti-

mate gastrointestinal absorption and bioavailability of a drug is to compare its cumulative urinary excretion of the unchanged drug in the same patient after iv and po administrations. Again, we showed that DIC was incompletely absorbed and its bioavailability was low when given by mouth in the patients studied.¹⁰

Pharmacologically, the body resembles a black box with a very few windows open to the observer for but a fleeting, superficial view of limited perspective. From this, to paint a whole picture of the ongoing events in the body taxes the ingenuity, imagination, and daring of the scientist. In practice, the blood offers the most conveniently accessible window to gain such an insight. Accordingly, a standard pharmacokinetic study protocol inevitably stipulates blood sampling and quantitative analysis of the drug therein at designated intervals.

From these results, we hope to estimate the corresponding drug concentrations in the tumor and certain critical tissues by reasonable inferences. The information so gathered may help the clinician to determine if after appropriate manipulations in dosage and schedule, an effective dose of the chemotherapeutic agent has actually been delivered to the tumor without, at the same time causing drug concentrations in certain susceptible sensitive normal tissues to reach dangerously toxic levels. In reality, it must be emphasized that this is not an easily attainable goal. Assay of plasma drug concentrations provides useful information for other purposes also. In highdose methotrexate therapy, drug toxicity is dependent upon plasma drug concentration; the critical concentration appears to be around 1 μM ⁶.

Therefore, in many centers, plasma drug concentration is routinely monitored during high-dose methotrexate therapy to forestall toxicity.

Recently, in adult patients with relapsed acute leukemia treated with high-dose arabinosylcytosine at 3 g/m² infused iv over 12 hr for 6 to 12 doses, Dr. William Plunkett and coworkers of our institution showed that the likelihood of achieving complete remission was critically dependent on the intracellular ara-CTP concentration at the end of the 12-hr dosing interval as well as on the elimination rate of ara-CTP from circulating blasts and the extent of inhibition of DNA synthesis.

Since the critical cellular ara-CTP concentration appeared to be approximately 75 μM , based on cellular ara-CTP pharmacokinetics of the first dose, they were able to design the dosing interval to maintain the cellular ara-CTP concentration above this value with encouraging response⁷. This represents a first attempt to apply the results of cellular pharmacokinetic studies to the clinical trial of an old agent in a novel therapeutic application.

In our experience, with few exceptions, the plasma drug concentration versus time curves could best be fitted with an open 2-compartment model that allows the computation of pertinent pharmacokinetic parameters. These parameters are useful and helpful to the clinician in the design and conduct of clinical trials, provided, naturally, the inherent limitations of the model are realized. Vinblastine is moderately active clinically against advanced breast cancer. Because the drug is extensively taken up by platelets and not completely available to the tumor, we thought that the therapeutic index of vinblastine might be enhanced by continuous iv infusion.

Pharmacokinetic studies were conducted in conjunction with the trial; vinblastine was administered by 5-day iv infusion at 1 to 2 mg/m² daily. The pharmacokinetics of vinblastine in 12

patients thus treated have been determined⁸⁾; one of them together with 2 others also received vinblastine by bolus administration at 7.5 mg/m². From these parameters it is readily apparent that in the 4 patients who partially responded to the therapy the plasma half-lives of vinblastine during the terminal phase were longer and the total clearance values were smaller than those in the non-responders, including the 3 patients who received the drug by bolus.

The results are highly suggestive, namely, to elicit a favorable response, vinblastine concentration in the body must be kept at a certain level for a certain period of time. Admittedly, this was a limited study and more patients are needed for confirmation.

At any rate, the findings are interesting and encouraging, and demonstrate the role of pharmacologic studies in clinical trials. As another example, Dr. Michael Rosenblum and colleagues at our institution, found that when the Y-interferon was administered by iv bolus, its disappearance from the serum in terms of antiviral activity was exponential, with a half-life of a few minutes. However, the half-life proportionally increased whereas the total clearance likewise decreased with increasing dose.

Since γ -interferon is a potent inducer of serum β_2 -microglobulin protein, the latter was therefore measured after γ -interferon administration by different routes. By either intramuscular or iv bolus administration, no induction of serum β_2 -microglobulin protein was apparent. In contrast, by continuous iv infusion of 4×10^6 units hourly for 6 hr after a loading dose of 3×10^6 units, daily for 5 to 10 days, induction of serum β_2 -microglobulin was evident⁹⁾.

It is reasonable, therefore, that γ -interferon should be administered by continuous iv infusion to achieve the best effect.

Finally, a few words should be said about the relevance of metabolic studies of anticancer agents to the clinical trials. Based on our experiences, some generalizations on the metabolism of anticancer agents may be made. First, steroids and drugs with unusual structures are metabolized by hepatic microsomal enzymes; for example, prednisone, cyclophosphamide and dacarbazine.

Second, antimetabolites are biotransformed like their natural counterparts. Third, as a consequence, biotransformation of anticancer drugs is not necessarily detoxification. Besides hepatic microsomal enzymes and enzymes necessary for normal biochemical and physiological functions of the body, extrahepatic enzymes may also be involved in drug metabolism, including that of anticancer agents.

Recently, Dr. John Mieyal and collaborators of the Case Western Reserve University made the very interesting discovery that the human hemoglobin sometimes behaved in vitro like a monooxygenase in that it could hydroxylate aniline¹⁰⁾; a finding later confirmed by Dr. Tomoda and coworkers of Kanazawa University¹¹⁾ and a few others elsewhere. We extended this to anticancer agents, and were able to show that the human erythrocytes oxidized in vitro, 6-mercaptopurine to 6-thiouric acid, methotrexate to 7-hydroxymethotrexate (Fig. 1), and removed the O-methyl group from the radiosensitizer misoidazole (Fig. 2). If confirmed in vivo, metabolism of anticancer drugs by erythrocytes may exert critical influence on the clinical efficacies of many anticancer drugs.

Earlier I have mentioned drug interactions. Now I wish to illustrate this with an example from one of our recent investigations. Diaziquone, or AZQ, is a promising new anticancer agent that may potentially penetrate into the central ner-

Fig. 1 7-Hydroxylation of methotrexate by human RBC lysate.

Fig. 2 O-Demethylation of misonidazole by human RBC lysate.

vous system. Patients participating in AZQ trial sometimes received cimetidine for peptic ulcer either pre-existing or steroid-induced.

Since cimetidine can inhibit microsomal drug metabolism in man and since AZQ was partly biotransformed by hepatic microsomal enzymes, we investigated the effects of cimetidine on the clinical pharmacokinetics of AZQ. AZQ was administered iv at 1-12 mg/m² to 15 patients with cancer; 4 additionally received oral cimetidine at 300 mg every 6 hr. AZQ was assayed by HPLC with [¹⁴C] AZQ as a marker.

In 11 patients who received AZQ only, the harmonic mean terminal plasma half-life of AZQ was 46 hr. In 4 patients who also received cimetidine, this was significantly lengthened to 125 hr. The AZQ total clearance correspondingly decreased from 200 ml/kg/hr to one-half of this value. Cimetidine also caused the apparent volume of distribution of AZQ to increase somewhat from 14 to 17 l/kg, and the 24-hr cumulative urinary excretion to decrease slightly from 4.3 to 3.6%. Apparently cimetidine interferes with AZQ clearance probably by inhibiting hepatic microsomal enzymes involved in AZQ metabolism.

Because more often than not patients with cancer receive a number of different kinds of drugs, the clinician must be constantly on the alert for possible drug interactions that may lead into drastic changes in pharmacokinetics. The above is only one of several instances in which we observed such drug interactions. Time and again modifications of drug metabolism have been proposed to enhance the efficacy of anticancer agents.

If an agent is activated in vivo metabolically by hepatic microsomal enzymes, cyclophosphamide for instance, wouldn't it be possible to make the drug more effective by stimulating its

metabolic activation with phenobarbital, for example? This and similar attempts have actually been made but all to no avail as predicted by us.

A moment's reflection would at once convince one that such attempts are bound to fail because their net effect is to increase the cytotoxicity of the antitumor agent to the tumor tissue as well as to the sensitive normal tissues without enhancing the specificity.

Frequently, neoplasia exerts a profound influence on drug metabolism; in other words, compared with normal subjects, patients with cancer often metabolize a drug quantitatively differently. In our work with ¹⁴C-labeled dacarbazine, we showed that the metabolic degradation of the side chain dimethyl groups to carbon dioxide was greatly reduced in mice bearing the L1210 leukemia although we are uncertain of the clinical significance of this finding. We further showed that the average half-life of antipyrine was about 11 hr in 7 normal subjects; in comparison, it was 14 hr in 13 patients with various kinds of cancer and in 9 patients with advanced cancer. Most strikingly, in 5 patients with acute leukemia and hepatitis, the plasma antipyrine half-life was nearly doubled to 30 hr.

In summary, the importance of pharmacologic studies of anticancer drugs in relation to their clinical trials has been discussed. Examples mostly from our research have been cited our contention that pharmacokinetic and metabolic investigations of anticancer drugs constitute an integral part of their clinical trials.

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