Introductory Comments on Selective Biological Effects of Alkylating Agents in Cancer Chemotherapy

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Ever since accepting the invitation to discuss the alkylating agents within the general framework of this meeting—basic considerations in the area of cancer chemotherapy—I have been a bit perplexed as to how to proceed. This is not because there are no basic concepts of alkylating agent action, but rather because there are none which have not been vocalized many times before. In view of this situation I have interpreted the title of this session fairly literally and have decided to outline for you what I believe is the present position of the alkylating agents, basing the conclusions on the results of fairly extensive studies of the activities of these compounds against a wide variety of experimental neoplasms and on observations of their diverse pharmacologic characteristics.

There are many clinical investigators in this audience who have worked extensively with the alkylating agents and who may take exception to some of the comments and conclusions that are to follow. I would like to think, however, that the latter would stimulate something other than an avalanche of criticism. I do not apologize for what some may consider to be a nonbasic approach to the problem of the activity of alkylating agents. Basic considerations have their place. It is always a more rewarding exercise, however, if such considerations revolve about highly reproducible and biologically significant gross effects.

By way of introduction, it should be remarked that the modern interest in alkylating agents as tools in cancer chemotherapy derived its impetus during World War II from the classical toxicologic studies of Gilman and Philips on nitrogen mustard and the follow-ups by Dougherty (in this audience), the late Drs. Rhoads, Wintrobe, and Jacobsen in the area of neoplasia. When Gilman and Philips formally reported their observations on this parent mustard and its simple congeners and took note of their activities against various experimental and human neoplasms, they made a prediction which has become all too true—namely, that within a short period of time there would be a deluge of derivatives comparable to the flood of sulfonamides which followed demonstration of the antibacterial action of sulfanilamide in the mid-thirties. This situation was reached in the mid-fifties, when there were more alkylating agents being synthesized than laboratories to test them. Consequently, many of the examinations were deficient; compound comparisons were essentially nonexistent or, if done, were not meaningful. This background created a serious problem for those who wished to have a sound basis for studying alkylating agents at the clinical level. I have remarked before that selection for clinical use was often based on the decibel system—he who propagandized longest and loudest for an agent found a receptive outlet in some clinical area.

In 1957 the Cancer Chemotherapy National Service Center (CCNSC) became greatly concerned with the plethora of alkylating agents which came from the chemist's workshop, the willingness of some clinical investigators to examine them in patients bearing tumors without having solid guidelines, and the resulting prospects of devoting an unduly large fraction of the limited clinical material to nonproductive efforts. The need for some improved methods for studying the agents at the experimental level and, in particular, for defining comparative activities was clearly recognized. Without being unfair to anyone, it should be noted that in 1957 there were few comparative data and essentially no quantitative data on the activities of members of any class of compounds. One investigator would test alkylating agent A against one tumor, another investigator would test agent B against a second tumor, etc. No one seemed to appreciate the need for determining with precision how different agents compared in terms of activity and tolerability, es-

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pecially whether there was anything unique in their behavior. Little attention was given to the correlations between results in animals and responses of human tumors.

Recognition of this deficient and rather chaotic situation led the CCNSC to ask the group with which I am associated to undertake development of systems which would permit reasonably reliable and precise quantitative assessments of the activities of various alkylating agents against experimental neoplasms and both qualitative and quantitative appraisals of their capacities to evoke untoward reactions in large animals such as the dog and monkey. It was hoped that such work would lead to improved procedures for guiding the efforts of both the synthetic chemist and clinical investigator. We undertook responsibility for this study in 1957. It has grown substantially since that date and at present occupies a very sizable fraction of the effort of our Institute. In a highly informal way I would like to tell you a little about the general design of the various studies and the results which they have produced.

First, I would like to direct your attention to the group of experimental tumors which have been employed in comparing the activities of the various alkylating agents. Since it was understood that my remarks were to be kept informal, I shall use no slides but merely list these tumors on the blackboard. First on the list are three lines of Walker carcinosarcoma 256. These lines have different growth characteristics and significantly different responses to certain alkylating agents. One came from Sloan-Kettering Institute for Cancer Research, a second from the Chester Beatty Institute, and the third from the University of Wisconsin. Each of these tumors has been manipulated as a subcutaneous neoplasm and as a pulmonary tumor developing subsequent to the intravenous inoculation of rats with a fine homogenate. In addition, the Wisconsin tumor, also designated CH, has been handled in the ascites form. This selection has afforded an opportunity to determine whether the location of the tumor has an influence on the comparative activities of different agents.

All the Walker tumors, origin or site of development notwithstanding, can be considered as highly sensitive to the alkylating agents. The same may be said of the Yoshida sarcoma and Yoshida hepatoma. The Dunning leukemia is a tumor of intermediate susceptibility. It has been manipulated in both the subcutaneous and ascitic forms. The Novikoff Hepatoma, R-35 adenocarcinoma (subcutaneous and pulmonary forms), Murphy-Sturm lymphosarcoma, LC-18 hepatoma, R-3244 fibrosarcoma, R-3251 lymphosarcoma, R-3259 giant-cell sarcoma, and IRS-9802 spindle-cell sarcoma are all tumors which are comparatively resistant to all alkylating agents. Lymphoma 8, as will be noted later, occupies a special position.

In addition to the above rat tumors, special lines of Walker carcinosarcoma 256, Yoshida hepatoma, and Dunning leukemia resistant to phenylalanine mustard have been used systematically in an effort to determine whether alkylating agents of seemingly different structures have a common mode of action.

The mouse tumors utilized in the systematic study have included Sarcoma 180, both the subcutaneous and ascitic form of Leukemia L1210, Adenocarcinoma 755, the solid and ascitic form of Ehrlich carcinoma EF, and the SAH-I-I adenocarcinoma. The latter tumor has been utilized in both subcutaneous and pulmonary forms.

Each of these tumors has been manipulated in a very precise manner which has yielded highly reproducible growth in untreated animals and equally reproducible responses to standard chemotherapeutic agents. Insofar as ascitic tumors are concerned, these ends have been achieved by the inoculation of carefully measured numbers of tumor cells derived from animals bearing tumors of a very narrow age range. With respect to the subcutaneous and pulmonary neoplasms, inocula of carefully prepared homogenates have been responsible for the high degree of reproducibility.

Altogether 141 different alkylating agents have been subjected to study in one or more of the above neoplasms. Included in these substances were numerous representatives of bifunctional mustards, substituted ethyleneimines, and methanesulfonic acid esters. Twenty typical compounds have been examined against every one of the tumors mentioned above. These agents have been administered via both the intravenous and intraperitoneal routes to animals bearing either recently implanted or well established neoplasms. In most cases a 5-day treatment regimen has been employed. However, single-dose, three-dose, and ten-dose regimens have also been employed. To say that this has provided a mountain of information is something of an understatement.

To supplement this work systematic toxicologic studies have been carried out in both normal rats and mice employing the diverse modalities and routes of drug administration referred to above. The information so obtained, when related to data on activity, has made it possible to calculate therapeutic indices for each agent. Diverse criteria have been employed in developing therapeutic indices for the different tumor systems. The index chosen has depended upon the ultimate

therapeutic response that can be obtained. In all cases the numerator has been the LD_{10}, a figure which is similar to the maximum tolerated or minimal fatal dose. The denominator varies. With highly sensitive tumors or those of intermediate response it is usually an ED_{90}. With more resistant neoplasms it is either an ED_{60} (T/C = 0.4) or an ILS (increase in life span) 60 or 100.

Although these therapeutic indices have a high degree or reproducibility, they are not without error. Great significance cannot be attached to a difference of two in the therapeutic indces of diverse compounds unless such a value has been corroborated many times in side-by-side comparisons.

In addition to these therapeutic evaluations in rodent neoplasms, we have been equally interested in comparing the toxic reactions evoked by diverse alkylating agents in dogs and rhesus monkeys. These studies have been aimed toward determining the relative capacities of repeated doses of these compounds to produce disturbances in CNS function, the hematopoietic system, the gastrointestinal tract, clotting mechanisms, and hepatic and renal function. Histopathologic evaluations have been accorded considerable importance.

What information has been provided by this variety of investigative work? Insofar as therapeutic studies are concerned, it is clear that the site of development of the tumor, the route of administration of the compound, and the frequency of dosage are important determinants of activity. It is equally apparent that in susceptible tumor systems these variables may determine the ranking of the agents. However, in no case except one was a situation encountered in which a totally inactive compound against sensitive tumors was active against an insensitive or only slightly susceptible neoplasm. The single exception was a small group of methanesulfonic acid esters which had activity against Lymphoma 8 and no activity against the Dunning, Yoshida, and Walker neoplasms. Thus, in general, there was a very orderly behavior of given alkylating agents against the different neoplasms. The most effective agents against the more susceptible tumors had some activity against the insensitive. The less effective agents against the susceptible tumors were ineffective against the insusceptible neoplasms.

Insofar as quantitative differences in activity were concerned, there were a number of compounds which had therapeutic indices of one or less—i.e., activity was purchased only at a prohibitive cost in toxicity or no activity could be demonstrated. In a few instances therapeutic indices of 10, 20, or 40 were obtained, signifying very substantial differences between effective and toxic doses. In all the systems but Lymphoma 8 two compounds stood out as superior to all others: these were cyclophosphamide and *p*-L-phenylalanine mustard. It is both unfortunate and perplexing that the superiority of these compounds against transplantable tumors has not found a reflection in the clinical efficacy of these agents.

The L-phenylalanine-mustard-resistant lines of the Walker carcinosarcoma, Yoshida hepatoma, and Dunning leukemia have been used to determine whether diverse types of alkylating agents (mustard derivatives, substituted ethyleneimines, and methanesulfonic acid esters) have a common type of activity. The results of these studies have without exception shown that each of these classes of compounds is without effect on these resistant neoplasms. These findings suggest strongly that, irrespective of their structural variability, there is a common type of antitumor activity among the diverse alkylating agents. This conclusion should be borne in mind by those who are easily persuaded that a change in "carrier group" is likely to yield an alkylating agent with novel activity.

One other area of investigation which has received more than passing attention is combination alkylating agent-nonalkylating agent therapy. For the most part, work has been focused on the less sensitive rodent tumors (Dunning leukemia, R-35 adenocarcinoma, Murphy-Sturm lymphosarcoma, Leukemia L1210, and SAH-I-I adenocarcinoma) and on *p*-L-phenylalanine mustard and cyclophosphamide administered in combination with 6-mercaptopurine, 5-fluorouracil, methotrexate, hydrocortisone, and colchicine. In no case was there a significant gain from conjoint use of these agents over the simple sum of their individual activities. In some instances there was a distinct loss because of enhanced toxicity.

The large animal toxicity studies have shown that there are remarkable similarities in the reactions of dogs and monkeys. In general, the responses of the dogs are slightly more severe, but the differences are not consistent in all cases. There are some rather striking qualitative differences in the toxic reactions evoked by the diverse alkylating agents. The N-oxide congener of nitrogen mustard, known as Nitromin, has a greater CNS-stimulating activity than has any agent other than chloroquine mustard. In general, methanesulfonic acid esters of the Myleran type have greater effects on the myeloid elements of bone marrow than do mustards and ethyleneimines and more cumulative toxicity. The simpler esters, such as methyl and ethyl ethane sulfonate,

are exceptions. These compounds exhibit little toxicity for the hematopoietic system but have substantial effects on liver and kidney. The phenylalanine mustard isomers have a greater capacity than other mustards to induce hemorrhagic lesions in the skin, subcutaneous tissues, and abdominal and thoracic viscera. Apart from these differences, there is very little to choose among the alkylating agents.

One may ask what significance these various findings have insofar as the future of the alkylating agents is concerned. It seems to me that one of two conclusions is inescapable: Either the tumor systems employed represent the wrong choices for demonstrating novel activity or the point of diminishing returns has been reached insofar as development of agents with novel activity is concerned. Personally, I lean toward the latter alternative.

It does not follow from the above that all work on alkylating agents should come to a halt, although further intensive efforts at the synthetic level do not seem warranted. Alkylating agents do have significant activities against certain human neoplasms and lesser activities against others. The problem, then, seems to be one of filling in the gaps in knowledge of the clinical effectiveness of known compounds and especially to define via more precise clinical studies how to employ most effectively those compounds that are already at hand.

With these few rambling reflections I would like to return to the Chairman the responsibility for bringing these viewpoints into perspective with those of the other members of this conference.