The Manipulation of Metabolism by Drugs and Nutrients

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SUMMARY

The evidence that the growth of certain types of cancer can be limited by a deficiency of folic acid or depletion of asparagine is convincing enough to impel the search for other nutritional peculiarities which may provide a basis for cancer therapy. The manipulation of metabolism by regulating the supply of nutrients has the potential to reinforce or to counteract the effectiveness of drug treatments. To take advantage of whatever quantitative differences exist, any means of inducing metabolic stress should be evaluated for effect on the viability of cancer cells. The objective of inducing selective metabolic stress is discussed viewing the likelihood that impairment of biochemical functions by drugs may be augmented by specific deficiencies of nutrients, by the administration of toxic metabolites, or by agents capable of stimulating growth. The evidence that some neoplastic cells can revert to a normal state indicates the need to devise experimental systems to evaluate the effect of any agents on the capacity of cancer cells to differentiate.

INTRODUCTION

Many chemotherapeutic agents classed as antimetabolites interrupt biosynthetic pathways or interfere with essential cofactor functions. As a consequence of the presence of such drugs, new nutritional requirements are created. There is ample evidence that specific metabolites can allow the survival of microbial and mammalian cells in vitro in the presence of concentrations of antimetabolites which would otherwise be lethal. A critical review of work in this area of chemotherapy and pharmacology indicates the need for further work to appraise the extent to which such nutrients may vitiate drug action in vivo. Is it possible that the potency or effectiveness of such anticancer drugs could be changed if the availability of metabolites were controlled? Concentrations of drugs and nutrients can be readily changed in culture media, whereas any persistent changes in the serum levels of specific nutrients are counteracted by physiologic regulatory systems intended to maintain homeostasis. To kill cancer cells in the body selectively, however, may require control both of exposure to a drug and the availability of nutrients affecting cellular responses to the drug.

Biochemical technics based on the incorporation of precursors into cellular polymers allow identification of specific functions impaired by antimetabolites. The focus of attention of the biochemist, however, is on the biosynthetic pathway or specific cellular functions affected by the drug. Indeed, cancer chemotherapy has made important contributions to molecular biology by providing drugs such as azaserine, actinomycin D, and analogs of nucleic acid precursors which serve as tools for dissecting metabolic pathways (19). Studies in the field of pharmacology have made real progress in identifying the intracellular sites of drug binding and, in some cases, the specific receptors of potent anticancer agents. The focus of attention of the pharmacologist is on the site of drug action, the pathways of drug metabolism, and the kinetics of drug disposition. Such biochemical and pharmacologic studies reveal information important to any adequate understanding of the action of a chemotherapeutic drug, but they do not deal directly with those factors which may increase or decrease the sensitivity of cells in vivo to anticancer antimetabolites. There is good evidence that anticancer agents exert a metabolic stress which an affected cell may not survive. An excess or a deficiency of any essential metabolite can also exert a metabolic stress. Would it not then be a reasonable objective to achieve any selective metabolic stress on neoplastic cells by manipulating the cellular levels of both drug and related nutrients?

New Nutritional Requirements in the Presence of Drugs

The evidence that nutrients added to culture medium can remarkably change the sensitivity of cells to the inhibitory action of drugs has been recognized for many years. This is the basis for the “product reversal” first observed with bacteria growing in chemically defined media. The subsequent development of the “inhibition analysis” provided a useful means to identify those metabolites which allow growth in the presence of an antibiotic or antimetabolite (21). Such information helps to identify the locus of action of the particular drug. Although the technics are equally applicable, the inhibition analysis has been applied to a lesser extent to the study of drug effects on mammalian cell lines growing in semidefined media. This can be attributed in part to the much higher cost and the longer time required for mammalian cell studies. A number of observations deserve our further consideration of what may happen in vivo, based on the real manipulation of drug response in vitro by controlling exposure of cells to nutrients.

The requirement of microbial and mammalian cells for folic acid can be voided completely if nutrients are provided which are products of reactions dependent upon folate cofactors. Although these observations are not new, their significance is timely. Under circumstances in which there is no requirement for folate, the growth of the cells can be completely insensitive to the presence of folate antagonists. Methotrexate can be added to cultures of cancer cells in amounts sufficient to color...
the medium without impairing their growth if the medium contains thymidine, natural purines, and adequate amino acids (11). We do not know and we need to know the extent to which tissue breakdown products, reflected by elevated uric acid levels in serum, may contribute to the survival of neoplastic cells in patients receiving Methotrexate as part of a therapeutic regimen. Although we tend to think of drug combinations in relation to the emergence of drug-resistant mutant cells, part of the problem may be to devise combinations of drugs to counteract an artificial drug resistance created by the availability of metabolites allowing survival of cancer cells already exposed to Methotrexate.

Among the large family of purine and pyrimidine antagonists many examples could be listed relating changes in potency in cell cultures to the presence of specific metabolites. The specific nutritional requirements for thymidine induced by the presence of 5-fluorodeoxyuridine or 5-bromodeoxyuridine has been well documented (24). An extension of this relationship in vitro to similar interaction in vivo was demonstrated dramatically by the prevention of the toxic effects of 5-bromodeoxyuridine when thymidine was administered to patients by arterial infusion (15).

A remarkable change in the sensitivity of mammalian cells in culture to the inhibitory action of asparaginase is related to the amino acid composition of the medium (10). A cell line which grows equally well when supplied with either glutamine, asparagine, glutamic acid, or aspartic acid was cultured with each of these nutrients separately in the presence of asparaginase. The cells were most sensitive to this "drug" when growth depended on asparagine or aspartic acid. In the medium containing glutamine, 200 times more asparaginase was required for inhibition and in the presence of glutamic acid the cells were 2000 times more resistant to the action of the enzyme than in the presence of aspartic acid or asparagine. How would the dose required and the effectiveness of treatment be affected if serum levels of these nutrients could be controlled?

Can Cancer Cells of Any Type Be Starved Selectively?

There is very little direct evidence allowing comparison of the specific nutritional requirements of the different cells and tissues in the body. The cultural conditions for explants of mammalian cells favor, in those cases which are successful, the selection of a few cells from a large inoculum which can tolerate the artificial environment. Thus, similarities rather than differences are emphasized. Important new information may be obtained from attempts to learn the reasons for the low incidence of success in culturing mammalian cells typical of different tissues.

The substantial body of literature concerning nutrition and tumor growth cannot be reviewed here. The effects of individual vitamin deficiencies on hematopoiesis and the growth of experimental tumors has served as a basis for the synthesis of potential antagonists. The evidence that the growth of experimental tumors can be inhibited by dietary deficiencies of riboflavin, folic acid, and pyridoxine implies that the particular tumor may have a higher requirement for the specific vitamin than other tissues of the body. This interpretation must be qualified, however, since the observed effects may be due in part to impaired function of the liver in sustaining the serum levels of other nutrients.

In order to starve any type of cancer cell selectively, there must be nutritional requirements quite different from other tissues of the body. Such a circumstance may at first seem to be quite unlikely. Yet, the evidence indicates that this can occur. The evidence which bears directly on this point concerns the effect of depletion or absence of a nutrient as distinct from the functional deficiencies due to the presence of an antimetabolite within cells. The evidence for the limitation of tumor growth due to folic acid deficiency or asparagine depletion is convincing enough to initiate intensive search for other nutritional peculiarities which may provide a basis for cancer chemotherapy.

Folic Acid Deficiency. Quite different nutritional requirements for two tissues in vivo are shown by the comparative response of the Walker carcinoma 256 and the Murphy-Sturm lymphosarcoma when implanted in opposite flanks of folate-depleted rats (28). The Walker tumor fails to grow under conditions which do not slow the normal growth of the lymphosarcoma. The Walker carcinoma 256 has been studied in our laboratory as an example of a solid tumor naturally refractory to Methotrexate. Even partial depletion of the folic acid content of the diet caused remarkably selective cessation of tumor growth while increase in body weight was maintained (27). This lymphosarcoma was selected for comparison since real regression of established tumors follows treatment with Methotrexate. When the uptake of tritiated folate or Methotrexate was compared, considerably less radioactivity was found in the Walker tumor than in the Murphy-Sturm lymphosarcoma in each case (21). Thus, the capacity for uptake of the vitamin was related to the ability of the tumor to grow in the depleted host. If such information were available for Methotrexate-refractory solid human tumors, then the use of other folate antagonists which can enter cells by different routes of uptake might be desirable since such agents are quite effective as inhibitors of the Walker tumor (20).

Asparagine Deficiency. It is characteristic of tumors that respond to treatment with L-asparaginase that their cells require L-asparagine. The cells of unresponsive tumors and normal cells have no apparent requirement for an exogenous supply of L-asparagine. Although the intravenous injection of this enzyme corresponds to drug treatment, the action on the cancer cells appears to be indirect by depleting the serum of this amino acid, thereby causing selective starvation of tumors for which L-asparagine is an essential nutrient. It has been very encouraging that the studies of experimental leukemias have had good correlation with clinical treatments guided by a test for sensitivity (3, 22). Part of the lesson to be learned from this example may well be that unusual technics are needed to disclose the peculiar nutritional requirements of various types of cancer cells in the body.

Pyridoxine Deficiency. Attempts to impair tumor growth by interfering selectively with pyridoxine metabolism deserve mention as an example of the difficulties in accomplishing this objective and the pitfalls in the interpretation of observations.
Work on this subject was reviewed previously in relation to the extensive studies undertaken in our laboratory (26).

The marked lymphopenia and inhibition of tumors induced in experimental animals by simple dietary depletion of pyridoxine serves as an adequate basis for attempts to discover or to synthesize more potent antagonists of this vitamin. Very few drugs among the many which have been tested using Sarcoma 180 caused an incidence of complete regression as high as that observed when this tumor was implanted into mice treated only by feeding a pyridoxine-deficient diet (17). It was soon recognized that the response of the host to the implanted tissue was a major factor contributing to the tumor regression (16). The reasons for the expression of this host response upon modification of pyridoxine metabolism have not been explained. Because of this evidence, however, it was possible to explain an observation which would otherwise be quite perplexing; namely, that the administration of the analog, 4-deoxypyridoxine, to pyridoxine depleted mice reduced the incidence of regression of Sarcoma 180. Since information is needed concerning the suppression of the host response to tumor antigens by drugs which might otherwise be better chemotherapeutic agents, the ability of other drugs to interfere with the regression of Sarcoma 180 has been studied using pyridoxine depleted mice (34).

A major difficulty in the study of pyridoxine antagonists has been the ease with which their antitumor effects are counteracted by the presence of the vitamin B₆ congeners in a normal diet. The potency of 4-deoxypyridoxine against experimental tumors is increased markedly when the animals are fed a pyridoxine deficient diet (26, 30). Homologs of pyridoxine prepared by extending the side chain in the 5-position (14) were more potent than 4-deoxypyridoxine in bacterial tests, but their activity in vivo was disappointing. New antagonists of pyridoxine are needed which can be bound to drug receptors in the manner characteristic of azaserine or Methotrexate.

The experimental observations of the antitumor effects due to interference with pyridoxine metabolism served as a basis for a detailed clinical study of seventeen patients with advanced neoplastic diseases (7). Diets especially prepared to limit vitamin B₆ content were fed for periods of many weeks. Nine of the patients were also treated with 4-deoxypyridoxine. Despite biochemical and clinical evidence of vitamin B₆ depletion, no definite antitumor effect was noted in any of the patients.

Concept of Metabolic Stress

From many biochemical studies on known anticancer agents, we are made aware that the basis for their selective action is related to quantitative differences in the metabolism of the target cells. If chemotherapy could be based on qualitative differences, there would certainly be better potential for highly selective drugs. The fact of the matter is, however, that we must try to take advantage of whatever quantitative differences exist. Such differences are indicated by the examples just mentioned. Many other drugs could be cited with reference to their unique toxicity for certain tissues, reflecting quantitative differences between cells of different types. If our objective is to exert any metabolic pressure which limits the capacity of cancer cells to grow or to survive, then the use of drugs represents one of several means of achieving such selective interference with metabolism.

On a theoretical basis, the effects of inhibiting a specific enzymatic reaction by a drug should be duplicated if the supply of the substrate were restricted. In each case, the formation of the product would be limited. If the effect of the drug is obviated by an exogenous supply of the product of the inhibited reaction, then the effect of the drug should be amplified if the product is unavailable. If the drug accomplishes only partial inhibition or is easily displaced from its receptor, then such conditioning factors may be of critical importance. Thus, viewing the ability of a drug to exert some specific metabolic pressure in the directional sense of the flow of metabolites along metabolic pathways, the manipulation of metabolism by controlling the supply of nutrients has the potential to reinforce or to counteract the drug effect.

A broad view of the various means of inducing metabolic stress to limit viability includes the following different approaches which are applicable to this objective in vivo. These may be considered as adjuncts to the administration of cytotoxic drugs with potential for selective action. (a) Limit the availability of any nutrient for which there is evidence of unusual requirement by individual tumors. (b) Limit the availability of any metabolite which can impair or prevent the cytotoxic action of the drug administered. (c) Administer metabolites known to be cytotoxic in order to create a specific metabolic imbalance. (d) Administer metabolites which can impair drug detoxification. (e) Administer agents capable of stimulating tumor growth or initiating cell division as a means of modifying drug sensitivity related to the incorporation of antimetabolites or drug action related to phases of the cell cycle.

Although such combined treatments cannot predictably lead to greater selectivity, nonetheless the response to such conditions may disclose some of the characteristics of different cells.

Cytotoxicity of Metabolites

Nutritional studies have been concerned primarily with the effects of specific deficiencies and caloric restriction on tumor growth. Less attention has been directed to the antitumor effects of metabolites, including certain amino acids, purines, and vitamins which are known to be toxic when administered to animals in relatively large doses. A brief report published in 1946 (2) indicated that adenosine and guanosine monophosphates inhibited the growth of a transplanted sarcoma. Since these compounds are metabolized and presumably do not enter cells in the nucleotide form, many questions concerning their action remain unanswered. A report from a laboratory in Russia states that mice bearing spontaneous lymphocytic leukemia treated by intubation into the stomach of 50–100 mg per kg of cytosine showed a reduction in leukocytes and prolongation of life (5). Considering the examples of end-product inhibition and regulation by allosteric binding, it is likely that persistent high levels of such metabolites can inhibit essential functions. Since the half-life of injected
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