Nutritional Factors in the Design of More Selective Antitumor Agents

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SUMMARY

Manipulation of nutritional factors, either by supplementation or by creation of a deficiency, has already been found to be effective in the therapy of a few specific neoplasms. Particular examples are the Walker 256 carcinoma, which is sensitive to folate deficiency but not to Methotrexate administration; certain lymphoblastic leukemias which are relatively sensitive to asparagine deficiency; and some lymphomas which are sensitive to riboflavin deficiency. However, the area is as yet relatively unexplored, due partly to the difficulties in obtaining nutritional depletion and due more to lack of knowledge of those specific nutritional requirements by which specific tumors might differ from the requirements of their host.

INTRODUCTION

The successful use of an enzyme, asparaginase, to selectively deplete serum levels of its substrate, asparagine, and thus to produce antitumor effects in acute lymphocytic leukemia has provided a new stimulus for the investigation of other possible methods to obtain selective antitumor responses by depletion of normal nutrients. The rationale for this type of approach lies in the fact that certain tumors appear to have a greater requirement for preformed substances (some of which may not be considered essential) than do host tissues. Nutritional factors may also be important for antitumor selectivity by virtue of the growth behavior of the tumor cell as compared to normal tissue; for example, the tumor cell may not be able to withstand depletion of a particular nutrient for as long a period of time as would normal cells, or it may lack adaptive mechanisms by which normal tissues are able to survive a nutritional depletion. In addition, the neoplastic cell, by virtue of its continued partial differentiation and function, may have very specific requirements for nutrients that may make it even more vulnerable to dietary or biochemically created deficiencies. An example of such a neoplastic cell would be the melanoma.

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The purpose of this communication is to briefly review those studies that deal with therapy of neoplastic cells and that utilize dietary manipulation, either alone or in combination with specific drug therapy. Since in a broad sense, almost all of the available antitumor agents act by creating a deficiency of one of the building blocks necessary for new cell formation, be it protein, RNA, or DNA, we will not attempt to encompass all of these antimetabolite therapies in this discussion. Rather, attention will be focused, on the one hand, on those therapies in which the whole-body availability of a particular normal nutrient is altered and, on the other hand, on those neoplasms which have a specific nutritional dependency which is significantly greater than that of host tissues.

ATTEMPTS TO OBTAIN TUMOR REGRESSION BY DIETARY RESTRICTION

Remarkably little has been published with regard to treatment of experimental animal tumors or of tumors in man by means of dietary restriction. Perhaps this is not surprising, in view of the relative urgency of obtaining rapid tumor regression in most malignant conditions and in the expense and general difficulties of adhering to synthetic diets. Further, these efforts are necessarily directed to obtaining deficiencies in essential amino acids and vitamins and possibly trace metals or essential fatty acids. As might be expected, such deficiencies result in serious host toxicities.

Studies with Amino Acids

Dietary Restriction of Essential Amino Acids. Rose (26) and Cannon (3) first demonstrated that experimental animals and man could be maintained in positive nitrogen balance on a mixture of well-defined compounds. These studies were then followed by the investigations from the laboratories of Greenstein (2, 8, 31) and Skipper and Thomas (29), in which selective amino acid deficiencies were induced in rats and mice respectively, and the effect of these deficiencies on tumor growth was assessed. Sugimura et al. (31) found that when tumor-bearing rats were maintained on a water-soluble, chemically defined diet which was force fed, tumor weight increased at the expense of carcass weight, indicating that this Walker carcinoma was able to grow at the expense of normal tissues. When diets were fed lacking one essential amino acid, individual deficiencies of tryptophan, threonine, isoleucine, and in particular valine led to the death of some of the animals even within an 11-day test period while deficiencies of the
other essential amino acids (i.e., arginine, histidine, lysine, phenylalanine, methionine, and leucine) were better tolerated. Some tumor response, ranging from 15 to 41 percent of control tumor weight, was produced by deficiencies of any of these amino acids except for lysine. However, all animals lost weight (overall weight less tumor weight) except those receiving arginine, lysine, tryptophan, or isoleucine. Since the best tumor-slowing results were obtained with diets lacking isoleucine, methionine, and valine, it is clear that the best therapeutic index, albeit not a very great one, was obtained with isoleucine deficiency. Skipper and Thompson (29) examined the effects of selective amino acid deficiencies on the growth of the Sarcoma 180 neoplasm in mice. Synthetic diets deficient in either valine, leucine, isoleucine, threonine, phenylalanine, histidine, or methionine resulted in a decreased rate of growth of the Sarcoma 180 tumor. Deficiencies of 3 other essential amino acids, tryptophan, lysine, and arginine, were inconsistent in their ability to slow tumor growth. Again, as might be expected, depletion of essential amino acids resulted in rapid loss of host body weight.

Probably because of these discouraging results, and the difficulties previously mentioned in producing a selective amino acid deficiency, relatively few studies of this nature have been performed in humans suffering from malignancies. One pertinent study is that of Demopoulis (4), who has treated patients with advanced malignant melanoma by means of a diet low in phenylalanine and tyrosine; his rationale is that the large quantities of active tyrosinase in these tumors are important for respiration of the tumor cell. Limitation of the substrate, tyrosine, might then limit tumor growth. He noted tumor regressions in 3 of 5 patients treated with a low phenylalanine-tyrosine diet; these responses were correlated with a decrease in serum levels of these amino acids. However, most patients could not remain on the diet, and he concluded that this diet was “cumbersome, complex, and unpalatable, making its application difficult.” This study, unfortunately, does not establish the antitumor effectiveness of this therapy since the numbers of patients studied are few, and a control series was not employed. The same might be said of the even more limited studies of Lorincz et al. (19), who treated a few patients with gynecologic malignancies by using this diet, and of Yuki et al. (32), who treated patients with chronic monocytic leukemia by a low phenylalanine diet. Because of the difficulties involved with this diet, Demopoulis has shifted his attention to inhibition of tyrosinase activities in melanoma cells by the use of specific inhibitors to accomplish the same purpose.

Restriction of Nonessential Amino Acids. In addition to the 10 amino acids discussed above which are considered essential for the growth of animals, optimum growth of young mice (29) or young rats (8) requires supplementation with other “nonessential” amino acids. In man, histidine is considered a nonessential amino acid, in contrast to its absolute requirement in other animal species. The chemically defined diet used by Greenstein et al. (8) therefore included, in addition to the essential amino acids mentioned, the nonessential amino acids proline, tyrosine, glutamate, aspartate, glycine, cysteine, serine, and alanine, which are required for optimum growth. Of interest also is the finding that many tissue culture lines require amino acids additional to those required by the whole animal (5). Such amino acids are glutamine (or high concentrations of glutamic acid), cystine, glycine, serine, and, in some lines, asparagine. Cell lines are known that (a) can synthesize either asparagine or glutamine, (b) can partially synthesize asparagine or glutamine, and (c) cannot grow on aspartic acid or glutamic acid even in high concentrations and must be supplied with asparagine or glutamine. An interesting phenomenon has been described by Eagle et al. (5) in cells in culture. This is that, at certain critical population densities, certain metabolites are no longer required by particular cell lines. An explanation for this has been found in the observation that, at sufficiently high population densities, the concentrations of these respective metabolites in the medium can be raised to levels effective for cell growth.

Dietary deletions of glutamic acid, glutamine, aspartic acid, and other nonessential amino acids did not significantly affect growth of the ascitic form of the mouse-born Sarcoma 180 tumor (29). This is not surprising since the whole animal is able to carry out the de novo synthesis of these nonessential amino acids and would probably supply enough of the respective amino acid for tumor cell growth. Thus, strict selective deletion of these nonessential amino acids has not yet been adequately tested and should still be a profitable area for future exploitation by chemotherapeutic, if not dietary, means. But enzymatic or biochemical approaches designed to deplete plasma stores of one or more of these amino acids may need to be employed. As in the case of asparagine depletion, only those cells (hopefully normal) that have, or induce, the capacity to synthesize the relevant nonessential amino acids will survive. In this regard, the successful use of glutaminase to deplete tumor-bearing animals of glutamine is a lead worthy of continued investigation (7, 14). Enzymatic deletion of serum levels of other amino acids, in particular, serine and/or glycine, should also be possible. Clearly there is a need here to identify appropriate human tumors that might be affected by such amino acid deficiencies.

Use of Amino Acid Analogs. Except possibly for the glutamine antagonists azaserine and diazo-oxo-norleucine (DON), amino acid analogs have not proven to be clinically useful for the treatment of neoplastic diseases (18). More work is clearly needed to obtain analogs of these amino acids (nonessential for the host) which may be essential for tumor cell growth (25). It is likely that the tumor cell may be vulnerable to such an approach since it may possess specific uptake mechanisms for those amino acids which it is unable to synthesize de novo. Analogs which compete for the transport of these amino acids might be useful in producing an intracellular deficiency of the amino acid in question.

Effect of Vitamin Deficiencies on Neoplastic Growth

Dietary deficiencies of folic acid, of pyridoxine, or of riboflavin have all been shown to result in unequivocal inhibition of neoplastic growth. In each case there is attendant host toxicity, also due to the deficiency; in many cases the toxicity is tolerable. The induction of deficiencies of other vitamins has not yet met with real success in cancer chemotherapy but should be further pursued. For example, there is
little information as to the effect of vitamin B₁₂ deficiency on tumor growth. This partly reflects the fact that, outside of pernicious anemia, it is difficult to obtain a pure B₁₂ deficiency, even in experimental animals; moreover, it may take as long as two years to deplete the large, tightly bound body stores of this vitamin, and hence the mere dietary exclusion of B₁₂ is insufficient to cause its deficiency within the time period relevant to cancer chemotherapy. Again, a real chronic deficiency of this vitamin would result in neurotoxicity, presumably independent of its expected effects on rapidly replicating tissues. Therefore, the most valuable future approach in the application of B₁₂ deficiency to cancer chemotherapy might well be to attempt the combination of deficiency together with antimetabolite therapy directed specifically against those B₁₂-dependent reactions which are peculiar to replicating tissues rather than to nervous tissue. An example of the former reactions would be the B₁₂-dependent methyltransferase required for methionine biosynthesis (20). Despite many attempts, there has not yet been described any biologically effective antimetabolite to vitamin B₁₂.

In contrast to the situation with vitamin B₁₂, folic acid can be easily eliminated from the diet, and the body stores can be depleted of folic acid in about 90 days (12). The most notable toxic manifestation of folic acid deficiency is a megaloblastic macrocytic anemia; other manifestations include some mental impairment, glossitis, and occasional diarrhea. Since an extremely effective deficiency of the folate coenzymes can be induced by administration of a folate antagonist (e.g., Methotrexate), there have been few studies of the effects of dietary deficiency per se as an antineoplastic therapy in man. However, this should not be neglected. At least one experimental tumor, the Walker carcinoma 256, has been found to be inhibited by a folate-deficient diet but not by Methotrexate (27).

The explanation of this finding is believed to be that the uptake of Methotrexate is very poor into the Walker carcinoma, and hence it is poorly affected by the antagonist. By analogy, those human neoplasms which are resistant to folic acid antagonists, and whose resistance (either natural or acquired) is the result of ineffective drug transport, might be sensitive to dietary deprivation of this vitamin. However, the mechanisms of Methotrexate resistance in man have yet to be fully elucidated.

Another vitamin deficiency shown to have profound effects on tumor growth in experimental animals is the deficiency of pyridoxine (vitamin B₆). Growth of Sarcoma 180 in mice has been shown by Mihich and Nichol (21) to be moderately inhibited by a pyridoxine-deficient diet. The subject has been previously reviewed and will be covered in more detail in the accompanying paper by Nichol.

A specific riboflavin-deficient liquid diet is relatively easily obtained, but if it is used alone the production of riboflavin deficiency is slow. The orally administered riboflavin analog galactoflavin is well absorbed and is able to displace whole-body riboflavin, particularly from the liver, into the urine of rodents and of man (15, 17). The resultant riboflavin deficiency in man is characterized by some weight loss, glossitis and cheilosis, seborrheic dermatitis, and a normocytic, normochromic anemia characterized by erythroid hypoplasia and reticulocytopenia. It is notable that the leukocytes and platelets are not affected and, in the rat, the formation of antibodies has been shown to be unimpaired (16, 30). Riboflavin deficiency has been shown to cause marked shrinkage of 6C3HED lymphosarcoma tumors implanted in C3H mice and, indeed, to result in a 30% complete cure rate. Remission of miscellaneous spontaneous human tumors has as yet been poorly documented, but striking remissions have been obtained in patients suffering from lymphoma and from lymphosarcoma and who have been treated by a riboflavin-deficient diet together with galactoflavin (M. Lane, personal communication).

Since this form of therapy requires some weeks to months to become effective, and since it is relatively tolerable, it may prove suitable for the maintenance of remission in those diseases in which it is effective, but this possibility has not yet been assessed. Riboflavin deficiency and its manifestations can be readily reversed by riboflavin administration, and the mechanism of action of galactoflavin appears to be simply as a displacing agent; no nucleotides of galactoflavin, which could be analogs of the flavin coenzymes, can be detected in the tissues following galactoflavin administration. Therefore, it appears that some lymphomas and lymphosarcomas may be more sensitive to riboflavin deficiency than are host tissues, including thrombocytes, leukocytes, and antibody-producing organs. In these neoplasms, then, induced riboflavin deficiency may be a means for obtaining more selective antineoplastic activity.

USE OF NUTRITIONAL MANIPULATION TOGETHER WITH ANTIMETABOLITE THERAPY

In a sense, antimetabolites create either a relative deficiency or a relative ineffectiveness of a particular essential metabolite, especially if it is an essential amino acid or vitamin. However, the use of an antimetabolite differs broadly from that of a deficient diet in the following respects. First, the effects of the antimetabolite are seen more rapidly; secondly, the antimetabolite might be more selective in that it might inhibit only one or a few of the several reactions for which the respective metabolite is essential. It follows that there will be specific situations in which the use of an antimetabolite is able to enhance a therapeutic regimen which is essentially dependent on dietary deprivation and that, conversely, there will be situations in which dietary manipulation is able to broaden and enhance the effects of an antimetabolite.

An example of the former use of the combination of an antimetabolite with dietary restriction is that of galactoflavin. As has been mentioned above, this analog of riboflavin is able to increase the urinary excretion of riboflavin, and so to enhance the deficiency of a restrictive diet to the point where riboflavin deficiency is therapeutically effective for chemotherapy of some forms of cancer. Examples of the second use of this type of combination have not yet been developed for the chemotherapy of humans, perhaps because of the difficulties of dietary restriction. However, they should be given further consideration. For example, it might be possible to create a more effective folate deficiency by the combination of dietary restriction with antifolates: and, again, it has been...
shown for the Sarcoma 180 tumor of mice, that supplementation of a partially purified diet with adenine reduces the antitumor effectiveness of purine inhibitors (28).

Since the induction of a dietary deficiency may require several months, such an approach might be more effective for the long-term maintenance of a remission than it would be for the initial induction of remission.

Nutritional supplementation may be used to augment cancer chemotherapy in at least two ways. First, a serial administration of a potent antimetabolite, followed by the natural metabolite as an antidote, may be used to create a severe nutritional deficiency for a defined period of time. This approach is worth consideration in the therapy of those neoplasms which are characterized by a short generation time and rapid growth rate. In this circumstance, the growth of the neoplasm might be arrested more than that of slower-growing normal tissues, and a greater therapeutic index could be obtained if the period in which the antimetabolite is effective were related to the generation time of the neoplasm. This approach has been used to enhance the therapeutic index of Methotrexate in the chemotherapy of both experimental tumors (6) and of spontaneous human tumors (22).

The stable, reduced folate, 5-formyltetrahydrofolate (Leucovorin) is able to bypass the metabolic block imposed by Methotrexate at the level of the enzyme dihydrofolate reductase, and it is also able to compete with Methotrexate for its cellular uptake (23). Administration of sufficient Leucovorin is able to antidote Methotrexate and to rapidly end the period in which the latter is effective. This “rescue” technic has also been used to antidote cytosine arabinoside (24) and asparaginase (J. Holland, personal communication) therapy, in these cases by the use of deoxycytidine and asparagine respectively.

A second way in which nutritional supplementation could be used to enhance the efficacy of an antimetabolite would be by manipulation of normally operative metabolic controls. For example, the methylation of homocysteine for the biosynthesis of methionine requires methyltetrahydrofolate, which in turn is formed by the reduction of methylentetrahydrofolate. Preliminary evidence suggests that the utilization of one or other, or both, of these folate coenzymes may be affected by administration of methionine (1). In preliminary experiments, we have attempted to enhance the antineoplastic efficacy of Methotrexate by administration of methionine; some small enhancement was observed but this could not be clearly separated from the toxicity due to methionine, which was also observed. Another example of this approach would depend on application of the phenomenon of “thymineless death.” Cell kill, attributed to this phenomenon and obtained by therapy with either Methotrexate or fluorodeoxyuridine, can be increased by provision of adequate nutrients for protein and purine biosynthesis. Indeed, in the clinic it is frequently observed that those patients who are undernourished respond poorly to antineoplastic chemotherapy when compared with those patients who are well nourished (11).

Another possible approach to improving results obtained with antimetabolic therapy is the use of specific nutrients to increase the rate of proliferation of malignant cells, thus making them more vulnerable to “kill” by particular drugs. For example, it has been observed that folic acid (10) or methionine (9), when administered to patients with chronic myelocytic leukemia, resulted in a marked increase in the peripheral leukocyte count. In a sense, this circumstance may be comparable to adding new nutrients to a “plateau” phase culture, thus allowing the growth rate of the culture to increase. Studies from this (13) and other laboratories indicate that rapidly proliferating (log phase) cells are more vulnerable to “kill” by inhibitors of DNA synthesis.

CONCLUSIONS

The use of induced dietary deficiencies to treat malignancy in man has not been well explored for the reasons discussed; namely, expense, the long time necessary to obtain the deficiency, patient acceptance, and host toxicities. The addition of a specific antimetabolite to the diet which is deficient in the relevant metabolite may allow a more rapid onset of the deficient state. Deficiencies of certain vitamins, in particular folic acid, vitamin B₁₂, pyridoxine, and riboflavin, have been identified as potential areas that may be worth further exploitation for more selective antineoplastic effects. Methods to deplete body stores of certain nonessential amino acids, in particular asparagine, glutamine, serine, glycine, and cysteine are also worthy of further investigation. Other promising approaches for improvement of the results of drug therapy of malignancy are the use of specific nutrients to enhance the metabolic block created by an antimetabolite, the use of a specific metabolite to “rescue” normal cells but not tumor cells, and finally the use of a nutrient or nutrients to stimulate tumor growth so as to obtain an increased sensitivity to inhibitors of DNA synthesis.

REFERENCES


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