The Prediction of Growth-inhibitory Drug Combinations Showing Enhanced Differential Toxicity and Collateral Sensitivity

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SUMMARY

The possible application to chemotherapy of factors affecting the regulatory systems of cells has been considered. The extensive coordinate changes in intracellular enzyme concentrations which result from exposure to compounds interfering with the normal regulatory mechanisms suggest the possibility of combination therapy based upon the selective induction of quantitative changes in enzymatic activities. Such approaches would appear to be useful in circumventing two major problems of chemotherapy: finding sufficient metabolic differences between host and parasite to provide targets for selective drug toxicity, and preventing the eventual development of drug resistance.

It is concluded that a combination of two compounds affecting the same metabolic pathway, one producing feedback inhibition and the other effective through lethal synthesis, will show an enhanced differential toxicity greater than that from either drug alone. Similarly, pairs of compounds in which one member produces enzyme repression and the other is active through lethal synthesis should produce collateral sensitivity and thus prevent the development of drug-resistant populations.

A major limitation in the more effective application of tumor chemotherapy has been the scarcity of differences in the metabolism of host and target cells which are of sufficient magnitude to be utilized as a basis for selective toxicity. Since both genetic theory and the experimental evidence available indicate that the metabolic pathways and the structures of the individual enzymes are the same for tumor and host cells, potential sites of therapy have been restricted to quantitative differences in the relative emphasis of various metabolic pathways. Chemotherapy has been further limited by the refractoriness to treatment which eventually arises from the development of drug-resistant populations of pathogenic cells and organisms. This latter problem now clearly presents a major obstacle in the development of successful antitumor agents as well as in the treatment of microbial parasites.

A consideration of some of the recent findings on the regulatory mechanisms which operate to determine intracellular enzyme concentrations has suggested the possibility of new approaches to these problems. Since these possibilities appear to be byproducts of other work on regulatory mechanisms in this laboratory (28) they are outlined here in the hope that they may be of some usefulness to other investigators.

REGULATORY MECHANISMS

The metabolic regulatory mechanisms involved at the cellular level have been described in detail (10, 21, 29, 32). Although analysis of these mechanisms has been primarily carried out in microorganisms, they appear to be operative at the cellular level in animals as well, as evidenced by a variety of observations in tissue culture systems (4, 8, 18, 24, 42). Those features which are essential to the present discussion are summarized here.

A biosynthetic pathway, leading for example to an amino acid or nucleotide, is subject to two kinds of regulation. In the first type, termed feedback inhibition (34, 37, 43), the final product of a

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pathway acts as an inhibitor of an early step in the reaction sequence. Thus, an increase in the final concentration of the product tends to shut off further synthesis along the pathway, and a decrease in concentration acts to stimulate synthesis. This type of control is clearly able to respond rapidly to a change in the concentration of the final product.

Repression, the second mechanism (6, 25, 39, 44), operates more slowly but is more economical through its conservation of energy and materials. In this case the intracellular concentrations of the enzymes concerned in the synthetic pathway are themselves sensitive to the concentration of the final product. An increase in the concentration of the product tends to shut down the synthesis of these enzymes, and thus indirectly the rate of its own synthesis: inhibition at an early step in synthesis, and repression of the formation of those enzymes specifically involved in its synthesis. These systems are diagrammed in Chart 1. In addition, it is possible to induce a considerable change in the intracellular concentration of an enzyme by affecting factors which regulate other reactions in the same pathway. The chemotherapeutic devices which are described in detail in the following sections in essence represent an attempt to take advantage of this fact.

**Analog**s **Affecting Metabolic Regulation**

Metabolic analogs affecting regulatory processes are not uncommon, and it appears that regulation may provide a fruitful area for the development of new drugs. A number of antimetabolites which mimic both of the regulatory functions of the normal end-product have been described. Among the examples of a feedback-like inhibition which have been reported are the inhibition of anthranilate synthesis, an early step in tryptophan biosynthesis, by 4-methyltryptophan (36), and the inhibition of the condensation of anthranilate and phosphoribosyl pyrophosphate by both tryptophan and 6-fluorotryptophan (27). In pyrimidine biosynthesis Handschumacher and Pasternak (14) have shown that the orotidyl acid decarboxylase of both normal tissues and tumors is inhibited by 6-azauracil ribotide, and Bresnick and Hitchings (3) have demonstrated the inhibition of dihydroorotase in Ehrlich ascites cells by the ribonucleosides and ribonucleotides of cytosine and uracil, as well as by a variety of analogs of these compounds. In the area of purine biosynthesis Gots and Gollub (11) have described a series of purine analogs which strongly inhibit aminoimidazole carboxamide ribotide synthesis, and it is interesting that the relative bacteriostatic activ-

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**CHART 1.**—A precursor, A, is converted by enzymes a, b, and c to a final product, D, which is subsequently used in the synthesis of specific cell products. Compounds B and C are the intermediates formed in the conversion of A to D. The metabolite D may exert two physiological effects which can modify its own rate of synthesis; it inhibits the enzyme a at the first step in the pathway, and it represses the synthesis of enzymes a, b, and c.
ities of these latter compounds paralleled their effectiveness as feedback inhibitors.

Repressor-like effects are shown by 5-methyltryptophan, which blocks formation of the tryptophan pathway (5), and by 8-azaguanine, which can repress the formation of inosinic acid dehydrogenase in mammalian tissue cultures (20).

REGULATION AND CHEMOTHERAPY

The question to be considered here is whether it is possible, with our present knowledge of metabolic regulation, to construct rules which may be useful in the prediction of drug combinations effective in alleviating either of the two major problems of chemotherapy previously discussed—namely, finding sufficient differences in the metabolism of host and parasite to provide an effective handle for differential toxicity, and preventing the eventual development of drug-resistant populations of pathogenic cells. No attempt will be made in this paper to predict specific agents, but rather to clarify some general principles upon which effective chemotherapeutic combinations might be constructed. The aim is to permit another approach to the rational selection of drug combinations showing enhanced differential toxicity or collateral sensitivity, and thereby decrease our present dependence upon random screening.

Some of the novel implications of using metabolic regulatory devices for chemotherapy become apparent if we attempt to exploit the large changes in enzyme concentrations that may result from the administration of an antimetabolite whose effect is to mimic the normal metabolite in

![Chart 2](chart2.png)

**Chart 2.**—The metabolic pathway is that described in Chart 1. In the first situation an antimetabolite, B*, undergoes a lethal synthesis conducted by enzyme b to the compound C*, which is toxic. If, as in the second situation, an antimetabolite of D, designated D¡, exerts a feedback-like inhibition of enzyme a (indicated by the shaded area), the rate of conversion of A to D will fall and the cell is inhibited by virtue of its inability to synthesize specific products. Initially the rate of conversion of B* to C* will not be affected. However, as indicated in the third situation, if the resulting decline in the concentration of D results in a de-repression of the enzymes, until the total enzymatic capacity of the first step returns to the normal level, the activities of enzymes b and c will be far above normal. As a consequence B* is converted to its toxic product C* at a much more rapid rate, and the concentration and toxicity of C* are increased. In this way the combination of D¡ and C* is considerably more toxic than either alone, but only in the situation where both the A to D pathway is important and the cells are sensitive to C*.
hydrazide (45); the phosphorylations of deoxy-
pyridoxide to a product which competes with
pyridoxal phosphate (38), and of azauridine, which
is itself formed biosynthetically from azauracil
(13); and the incorporation of a wide variety
of purine and pyrimidine analogs into nucleic acids,
presumably through the formation of the corre-
sponding nucleoside triphosphates (9, 15, 22, 23, 41).

FEEDBACK INHIBITION AND LETHAL SYN-
THESIS: THE DEVELOPMENT OF
DIFFERENTIAL TOXICITY

When an antimetabolite mimics feedback inhi-
bition, the inhibition is only transitory where an
enzymatic pathway is sensitive to the repressive
effects of the final product. In the initial stages of
inhibition the concentration of end-product falls,
and the cells become de-repressed. An increased
formation of the enzymes concerned in the path-
way ensues, and their concentration rises until the
effective capacity of the inhibited system begins to
approach that of the original uninhibited system.
These effects have been demonstrated very clearly
by Moyed (26) in the case of 2-thiazoledalanine, a
histidine analog, which shows a feedback-like in-
hibition of an early step in the pathway of histi-
dine biosynthesis.2

2 It is interesting that in this case stable mutations to drug
resistance involve a decrease in sensitivity of the enzymes to
inhibition by both 2-thiazoledalanine and histidine, the conse-
quent loss of feedback control, and an overproduction of histi-
dine, which is excreted (27).

CHART 3.—The basic metabolic pathway is again that described in Chart 1. In the first situation we consider the same anti-
metabolite, B*, which undergoes a lethal synthesis via enzyme b to the toxic product C*. In the second situation a second anti-
metabolite of D1, D2, exerts repressor-like effects on the formation of the enzymes of the A to D pathway. As a result the pro-
duction of specific products is inhibited. As indicated in the third situation, if the mutation to resistance against D1 involves
the loss of repressibility the cell will contain an overabundance of all of the enzymes of the pathway a, b, and c. As a consequence,
the rate of conversion of B* to C* is enhanced, and the concentration and toxicity of C* are increased. In this way a mutation to-
ward resistance against D1 results in an increased sensitivity to B*.
When an early step in a pathway is inhibited and the concentration of the end-product falls, the de-repression which develops, when it is coordinate, serves to increase the level of all enzymes in that pathway. If the cells are simultaneously exposed to a second antimetabolite which is converted to its active form by one of the uninhibited subsequent enzymes of the pathway, the cell becomes increasingly sensitive to the new antimetabolite in the course of overcoming the feedback-like inhibition exerted by the first agent. In this way a judicious selection of compounds should make it possible to take advantage of a combination of metabolic differences between host and target, each of which by itself is not sufficiently different for chemotherapeutic purposes. The operation of this mechanism is diagrammed in Chart 2, and a trivial example may serve to illustrate it further.

Imagine a cell carrying out a rapid rate of nucleic acid synthesis and also dependent upon a pyridine nucleotide-linked metabolic process. This cell would be especially sensitive to a combination of antimetabolites in which the first antimetabolite inhibited an early step in pyrimidine biosynthesis and the second, by the action of a later enzyme in the pyrimidine pathway, underwent a lethal synthesis to an antagonist of pyridine nucleotide function. A cell whose rates of pyrimidine synthesis and utilization were slow to begin with could not respond by forming excessive amounts of the enzyme required for lethal synthesis, since the ultimate rate of product formation cannot exceed its rate of utilization; and a cell able to form the enzyme, but less dependent upon pyridine nucleotide function, would be relatively insensitive to the toxic product. Thus, the enhanced sensitivity of the target cells depends upon a particular combination of metabolic requirements, and the possibility is increased of distinguishing the target cells from a variety of host cell types, some of which may possess either but not both of these characteristics in a single cell.

Repression and Lethal Synthesis: The Development of Collateral Sensitivity

If a feedback-like inhibition and lethal synthesis are the two modes of action for a pair of antimetabolites, we are led to expect an increase in differential toxicity. By contrast, if repression and lethal synthesis are the sites of action for two antimetabolites, we should expect to observe collateral sensitivity. Collateral sensitivity has been defined as an increase in the sensitivity to one agent as a consequence of mutation toward resistance against another (35).

When a cell is presented with an antimetabolite which mimics the action of an end-product in repression, the levels of the enzymes concerned in the synthesis of the product fall and the cell is inhibited by virtue of an insufficient supply of the end-product. Mutations toward resistance against drugs with repressor-like activity characteristically involve the entire loss of repressibility, high concentrations of the biosynthetic enzymes, and an excessive production of the final product. Cohen and Jacob (5) have shown that the tryptophan pathway is no longer repressible in mutants resistant to 5-methyltryptophan and that a similar loss of repressibility of the methionine pathway occurs in mutants resistant to nor-leucine; and excessive amino excretion has been observed by Adelberg (1) in mutants resistant to a wide variety of amino acid antagonists.

Thus, when a mutation occurs which confers upon a cell resistance to a repressor-like drug through the loss of repressibility, an overproduction of the enzymatic pathway will result. If one of the enzymes of the de-repressed pathway is capable of a lethal synthesis, we would expect the mutated cells to become highly sensitive to the antimetabolite undergoing this lethal synthesis. In this way a single mutation which makes the cell more resistant to one agent simultaneously makes it more sensitive to another. The expected selection of mutants resistant to an antimetabolite (the repressor-like analog) is therefore prevented in cell populations which are simultaneously exposed to both agents. Mutated cells which are resistant to the repressor-like analog are selected against because they carry an obligatory enhanced sensitivity to the agent undergoing lethal synthesis.

This sequence of events has been diagrammed in Chart 3. In terms of the example used previously of a cell with a high rate of nucleic acid synthesis and dependent upon a pyridine nucleotide-linked metabolic process, the pair of compounds used could be, for example, a repressor of pyrimidine nucleotide synthesis and a pyridine analog giving rise to an inhibitor of pyrimidine nucleotide function.

General Considerations

No attempt has been made here to predict specific pairs of compounds effective in chemotherapy; however, many individual examples of each of the types of antimetabolite action described are already known. No pair of antimetabolites, effective in combination and whose members
are known to act in one of the configurations described here, has yet been identified; however, in at least one case behavior reminiscent of these mechanisms has been reported. Bacterial strains resistant to purine analogs, such as 6-mercaptopurine, develop heightened sensitivity to analogs of thiamine pyrimidine.

Attention should be drawn at this point to Danielli’s (7) interesting discussion of the problem of selective toxicity, in which it is emphasized that the greater the number of biochemical parameters upon which toxicity rests, the greater will be the selectivity of a combination of agents. His paper anticipates the concept developed here by suggesting that advantage be taken of the enzymes which are formed adaptively as a detoxification mechanism in drug resistance through administering a compound which would be converted by these same enzymes to a toxic product.

The optimal choice of a potentially susceptible pathway and the appropriate lethal synthesis depend to a large degree upon the particular host-parasite relationship in question and upon the biochemical properties of the metabolic pathway and the intermediates involved. It should be emphasized that, although this discussion has been oriented in terms of the cancer problem, to the extent of their validity the concepts developed should be equally applicable to all host-parasite relationships whether the parasite is microbial, protozoan, or neoplastic.

It is clear that a preliminary search for compounds effective through the mechanisms which have been discussed must proceed by constructing analogs which possess a particular physiological action, rather than for their individual therapeutic effectiveness; and human tissue culture material would appear to be the favored test system for the development of such antitumor agents. Indeed we shall expect that many compounds which might be effective in combinations of the types described here would have little or no therapeutic usefulness by themselves.


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