Chemical Action and Pharmacology of Methotrexate, Azathioprine and Cyclophosphamide in Man

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With few exceptions, almost every anticancer drug has been shown to have immunosuppressive properties. My role in this symposium is to review with you the pharmacology of three of these drugs that are currently most actively used in the treatment of nonneoplastic disease, namely methotrexate, azathioprine and cyclophosphamide.

GENERAL CONSIDERATIONS

One of the useful concepts that has emerged in recent years in understanding the action of these chemotherapeutic agents in cells is the concept of *cycle active* and *noncycle active* drugs. This concept, based upon the work of Bruce and colleagues (1), states that certain drugs, primarily alkylating agents (and x-ray therapy), will kill cells (assuming the drug is delivered to the cell in sufficient concentration and the cell is *biochemically sensitive* to the agent) whether or not they are *in cycle*—ie, in the process of replicating or not, while certain other agents, primarily antimetabolites, will not be able to kill cells (even if the drug is present in high concentration and the cell is biochemically sensitive), if the cell is not in cycle. Since the cell cycle may be divided into a G_1 (Gap 1), S (DNA synthesizing), G_2 (Gap 2) and M phase (mitosis), it is clear that a cycle active agent may kill a cell in any part of the cycle; the antimetabolites such as methotrexate, appear to act only on cells going through the S phase of the cycle. As cell numbers of a tumor increase, or in any normal stem cell population, such as bone marrow, gastrointestinal mucosa or in a nonstimulated lymphocyte population, many of the cells are not in cycle, but are in a so called *prolonged* G_1, sometimes called a G_0 state. These cells are not affected, therefore, by exposure to cycle active agents, but may be killed by drugs such as alkylating agents or x-ray, although even these agents seem to kill cells in cycle to a greater degree than cells not in cycle. The implications of these considerations are considerable in regard to scheduling of drugs. In the case of rapidly proliferating neoplasms (eg, acute lymphatic leukemia or Burkitt's lymphoma), high-dose, intermittent use of cycle active agents will provide the greatest therapeutic index, since during the short period of time the normal (bone marrow and gastrointestinal mucosa) proliferating population is exposed to the drug, most of the stem cells of these populations will be in so called G_0, while the rapidly dividing tumor cell populations with short generation times will have most of its stem cells in cycle. These considerations may also apply to the situation in which lymphocytes are stimulated to divide; a phase of replication exists that appears to be quite vulnerable to inhibition by S-phase inhibitors (2).

The three drugs that will be discussed in some detail are methotrexate, azathioprine and cytoxan; the former two drugs are *cycle specific,*

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Supported by US Public Health Service Research Grants CA-08010 and CA-08341.

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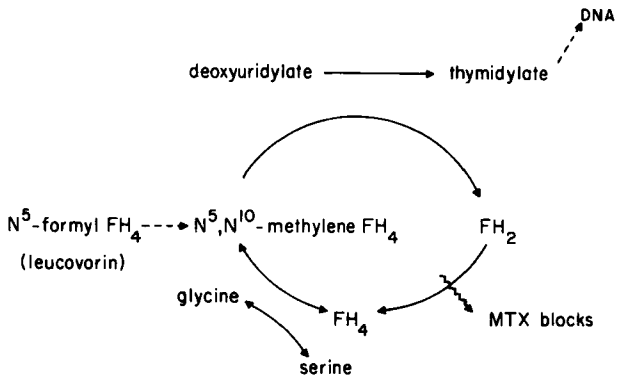


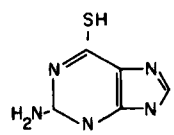
Fig 1. Methotrexate site of action and leucovorin rescue. FH_2, dihydrofolate; FH_4, tetrahydrofolate.

S-phase inhibitors, while the latter drug has properties of both cycle and noncycle specific drugs. Therefore, dose scheduling of all of these drugs may be of importance.

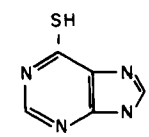
Methotrexate

Aminopterin (4-aminopteroylglutamic acid) was the first antimetabolite found to be useful in treating neoplastic disease. Since 1947 its value in the treatment of neoplastic diseases (eg, psoriasis) has been well documented (3). During the past decade, methotrexate (4-amino-N^{10}-methylpteroylglutamic acid) has supplanted aminopterin in the clinic, because of studies in experimental tumors demonstrating methotrexate to have a better therapeutic index. These antagonists bind to the enzyme dihydrofolate reductase, and thus inhibit the conversion of folic acid and dihydrofolic acid to tetrahydrofolic acid (FH_4) (4). FH_4 is the active coenzyme form of folic acid and acts to transfer one carbon (formyl, methylene, methyl) groups in several important biosynthetic reactions, notably the synthesis of both thymidylic acid and the purine ring. By blocking thymidylic acid synthesis, the pyrimidine found in DNA but not in RNA, methotrexate ultimately blocks DNA synthesis and cellular replication (Figure 1).

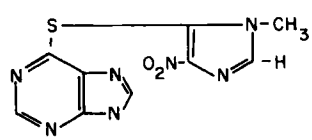
Leucovorin (citrovorum factor, N^5-formyltetrahydrofolic acid), a stable reduced form of the vitamin folic acid, can act as an antidote to the effects of methotrexate by providing cells with a reduced folate source, thus bypassing the meth-



6-THIOGUANINE



6-MERCAPTOPURINE



AZATHIOPRINE

Fig 2. Structure of 6-mercaptopurine, 6-thioguanine and azathioprine.

otrexate-induced block in coenzyme synthesis (5). The compound also acts as an antidote by competing with methotrexate for uptake into cells (6). If administration of leucovorin is delayed for an appropriate time after methotrexate action, it can prevent the toxic effects of methotrexate, but not its therapeutic effects on certain tumors or the immune response (7).

Methotrexate is not appreciably metabolized in man, although it is in some other species, so adequate renal function is the critical factor in avoiding prolonged, severe toxicity. In one study the plasma half-life of methotrexate was increased from the normal expected value of 2.5 hours to 24 hours when a test dose of methotrexate was given to a patient with polyarteritis with a creatinine clearance of 15 mg/min (8). The drug binds to dihydrofolate reductase enzyme in the liver and kidney, and appears to be retained by these organs for several weeks (9).

Azathioprine

This antimetabolite was designed by Hitchings and Elion and coworkers (10) to block the rapid oxidation and methylation of 6-mercaptopurine (6 MP) by attaching an imidazole ring to the sulfur atom at the 6 position in the molecule (Figure 2). However, sulfhydryl groups present in plasma and cells (as on glutathione, cysteine or proteins) react rapidly with azathioprine to generate 6 MP (Figure 3). It is still an unresolved question whether azathioprine, in man, has significantly better immunosuppressive activity than 6 MP. There is evidence in rodent experimental systems that azathioprine is more potent on a molar basis, suggesting that the pharmacology of the 6 MP generated is changed, or that azathioprine has some independent action. In man it seems clear that it does have a better therapeutic index than 6 MP as an immunosuppressive agent—ie, azathioprine can be used in effective doses for a longer period of time than comparably effective doses of 6 MP with less risk of thrombocytopenia or leukopenia.

The exact mechanism of action of purine analogs in inhibiting cell replication is still not clear, although several sites of actions have been elucidated (11). As shown in Figure 3, 6-thioinosinic acid (S-IMP), formed by the action of hypoxanthine phosphoribosyl transferase, is the active form of 6 MP. The nucleotide suppresses several steps in the synthesis of adenine and guanine (Figure 4), preventing interconversion of purine bases, especially inosinic to guanylic acid (GMP). S-IMP also inhibits the first step of de novo biosynthesis, mimicking the effect of

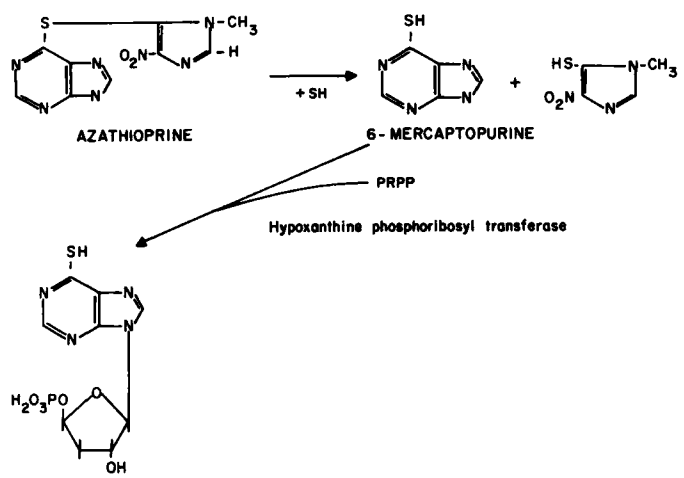


Fig 3. Conversion of azathioprine to 6-mercaptopurine and 6-thioinosinic acid.

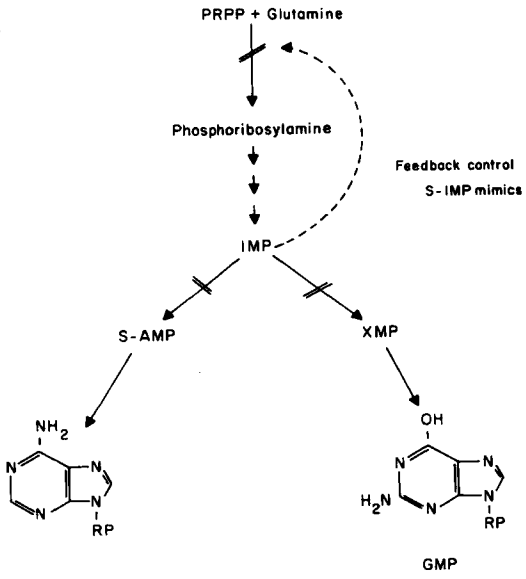


Fig 4. Sites of inhibition of purine biosynthesis by 6-thioinosinic acid. PRPP, phosphoribose pyrophosphate; IMP, inosinic acid; S-AMP, adenylosuccinic acid; AMP, adenylic acid; XMP, xanthylic acid; GMP, guanylic acid; S-IMP, thioinosinic acid.

inosinic acid, a *feedback* regulator of this step. In addition, a small amount of 6 MP is incorporated into RNA and to DNA in the form of thioguanine (12). The latter compound, also a potent immunosuppressive agent, is inactivated via intestinal guanase, and as a result has little gastrointestinal toxicity (13). Thioguanine may be a useful alternative to 6 MP or azathioprine if these compounds cause dose limiting gastrointestinal toxicity. Another important difference between thioguanine and 6MP and azathioprine is that thioguanine is inactivated primarily by deamination, while 6 MP and azathioprine are converted to thiouric acid by the enzyme xanthine oxidase. Therefore, concomitant use of allopurinol, a xanthine oxidase inhibitor used to decrease uric acid formation, does not necessitate a reduction in the dose of thioguanine as it does with the other two purine derivatives. The dose of 6 MP and azathioprine should be reduced to one-third or one-fourth of its usual level if allopurionol is given. Since some drug is excreted unchanged by the kidney, renal insufficiency may also require that lower doses be used. In the anuric patient, doses may have to be decreased to one-half (14).

Cyclophosphamide

The alkylating agents are a group of drugs that have broad clinical use in the treatment of neoplastic disease. Nitrogen mustard (HN 2), one of the earliest cancer chemotherapeutic agents to be used in man, has immunosuppressive properties, but cyclophosphamide, a newer alkylating agent, is of particular interest in this regard. Cyclophosphamide is one of the most powerful immunosuppressive agents in use.

Cyclophosphamide, a cyclic mustard, must be *activated* before it can effect cellular metabolism (Figure 5). This feature allows it to be used safely by mouth, and intravenously, where extravazation outside the vein does not produce tissue necrosis as with nitrogen mustard (15). The nature of the active metabolite appears to have recently been identified (16). This conversion occurs primarily in the liver, via the microsomal activity, and thus, drugs such as phenobarbital increase the rate of activation of cyclophosphamide to its active metabolite. In addition, the recent demonstration that *inacti-*

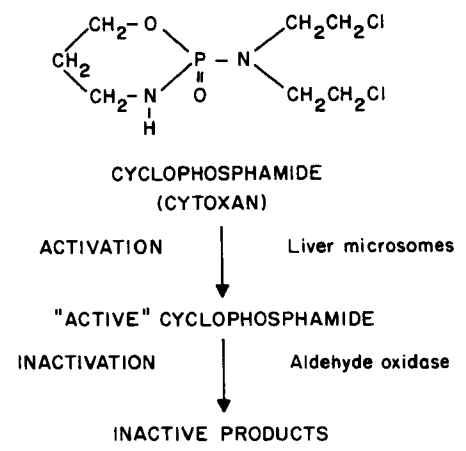


Fig 5. Conversion of cyclophosphamide to its active metabolite and its subsequent inactivation.

vation of the active metabolite appears to be via the well known liver enzyme aldehyde oxidase, indicates that other commonly used drugs such as the phenothiazines, which inhibit this enzyme activity may also modify the rate of cyclophosphamide activity by decreasing the rate of oxidation of the active product (17).

CONCLUDING REMARKS

It may be noted from this brief survey that a fair amount of information has accumulated in regards to the pharmacology of these three important immunosuppressive agents. Relatively little attention has been given to the mechanism of action of these compounds on the immune response nor to the effect of various dose schedules. The use of leucovorin as an antidote to selectively improve the therapeutic index of methotrexate as an immunosuppressive agent is also worthy of further evaluation (7).

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