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The Purine Path to Chemotherapy

GERTRUDE B. ELION

Research on antimetabolites of nucleic acid purines led to drugs for the treatment of acute leukemia (6-mercaptopurine and thioguanine), gout and hyperuricemia (allopurinol), and herpesvirus infections (acyclovir), and for the prevention of organ transplant rejection (azathioprine).

IN 1944, WHEN I JOINED THE WELLCOME RESEARCH LABORATORIES, the state of knowledge of nucleic acids was rather rudimentary. The prevailing theory was that there were two purines and two pyrimidines in each tetranucleotide and that these tetranucleotides were strung together in some fashion, but the sequences were not known. The nature of the internucleotide linkage had not been established and the helical structure of DNA had not yet been proposed.

In 1940 Woods (*1*) and Fildes (*2*) had put forth the antimetabolite theory to explain the action of sulfonamides on bacteria, suggesting that the sulfonamides interfered with the utilization of a necessary nutrient, para-aminobenzoic acid. Hitchings theorized that, since all cells required nucleic acids, it might be possible to stop the growth of rapidly dividing cells (for example, bacteria, tumors, and protozoa) with antagonists of the nucleic acid bases. One might hope to take advantage of the faster rate of multiplication of these cells compared with normal mammalian cells and eventually sort out the biochemical differences between various types of cells by the way they responded to these antimetabolites (*3, 4*). It was my assignment to work on purines, pteridines, and some other condensed pyrimidine systems.

It was, of course, necessary to have some biological systems to determine the potential activities of the new compounds. Essentially nothing was known at that time about the anabolic pathways leading to the utilization of purines for nucleic acid synthesis. A number of catabolic enzymes were known: nucleases, nucleotidases, nucleosidases, deaminases (for guanine, adenine, adenosine, and adenylic acid), xanthine oxidase, and uricase. In 1947 Kalckar described the reversibility of nucleoside phosphorylase (*5*). The enzymes guanase and xanthine oxidase were useful in our laboratory to examine the purines as substrates or inhibitors of these enzymes (*6, 7*). However, it was the microorganism *Lactobacillus casei* upon which we mainly relied. This organism could grow on adenine, guanine, hypoxanthine, or xanthine, provided the pyrmidine thymine was added. It could also synthesize purines and thymine, if given a source of folic acid in the form of liver powder. [The structure of folic acid was not elucidated until 1946 by the Lederle group (*8*)]. Hitchings and Falco had devised a screening test in which it was possible to determine whether a compound could substitute for thymine (*9*) or a natural purine (*4, 10*) or inhibit its utilization, and they could also determine whether a compound was a folic acid antagonist (*11*).

Few chemists were interested in the synthesis of purines in those days and I relied on methods in the old German literature. The transformation reactions were carried out mainly by the methods of Emil Fisher and the syntheses from pyrimidine intermediates by the methods of Traube. The direct replacement of oxygen by sulfur by the method of Carrington (*12*) also proved to be exceedingly useful for synthesizing the mercaptopurines (*13*).

In 1948 we found that 2,6-diaminopurine inhibited the growth of *L. casei* very strongly and that the inhibition was reversed specifically by adenine, but not by the other natural purines (*4, 14*). However, low concentrations of diaminopurine could also be reversed by folic acid, an attribute that diaminopurine had in common with other diaminopyrimidines and diaminopyrimidine condensed systems (*10*). Studies on a diaminopurine-resistant strain of *L. casei* revealed that it grew poorly on adenine as a source of purine. We deduced that adenine and 2,6-diaminopurine must be anabolized by the same enzyme, and that the product of diaminopurine anabolism interfered with purine interconversion (*15*). That enzyme was reported by Kornberg *et al.* in 1955 to be adenylate pyrophosphorylase (adenine phosphoribosyltransferase) (*16*). When

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tested on mouse tumors and the AKR mouse leukemia (*17*) or tumor cells in tissue culture (*18*), diaminopurine was strongly inhibitory. It produced two good clinical remissions in chronic granulocytic leukemia in adults but produced severe nausea and vomiting, as well as severe bone marrow depression, in two other patients (*19*). Interestingly, diaminopurine showed activity against vaccinia virus, a DNA virus, in vitro (*20*), but its toxicity in animals led us to abandon that possible utility.

Antileukemic Drugs

By 1951, we had made and tested over 100 purines in the *L. casei* screen (*21*) and discovered that the substitution of oxygen by sulfur at the 6-position of guanine and hypoxanthine produced inhibitors of purine utilization. 6-Mercaptopurine (6-MP) and 6-thioguanine were tested at the Sloan-Kettering Institute, with whom we had established a collaboration, and were found to be active against a wide spectrum of rodent tumors and leukemias. Of special interest was the finding by Clarke and co-workers (*22*) that 6-MP–treated tumors, although they had not regressed completely in the host mouse, were not transplantable into other mice. After some animal toxicology studies by Philips *et al.* (*23*), Burchenal and colleagues proceeded rapidly to clinical trial with 6-MP in children with acute leukemia (*24*). At that time the only drugs available for the treatment of these terminally ill children were methotrexate and steroids, and the median life expectancy was between 3 and 4 months; only 30% lived for as long as 1 year. The findings that 6-MP could produce complete remissions of acute leukemia in these children, although most of them relapsed at various intervals thereafter, led the Food and Drug Administration to approve the drug for this use in 1953, a little more than 2 years after its synthesis and microbiological investigation. A symposium on 6-MP was held at the New York Academy of Sciences in 1954 (*25*). The addition of 6-MP to the antileukemic armamentarium increased the median survival time to 12 months in these children, and a few remained in remission for years with 6-MP and steroids. This convinced us, as well as many other investigators in the cancer field, that antimetabolites of nucleic acid bases were fruitful leads to follow. Today 6-MP remains one of the dozen or more drugs found useful in the treatment of acute leukemia. With the use of combination chemotherapy with three or four drugs to produce and consolidate remission, plus several years of maintenance therapy with 6-MP and methotrexate, almost 80% of children with acute leukemia can now be cured.

Although we felt we were on the right track in 1952, there were still many unanswered questions. Reversal studies with 6-MP in *L. casei* did not pinpoint antagonism for any single purine. The inhibition was reversed by hypoxanthine, adenine, guanine, and xanthine (*26*). However, studies with a 6-MP–resistant strain of *L. casei* revealed that 6-MP was unable to utilize hypoxanthine for growth (*27*). Again, as with the earlier studies with 2,6-diaminopurine, we concluded that 6-MP and hypoxanthine were anabolized by the same enzyme and that interference with purine interconversions at the nucleotide level was involved (*28*). In 1955 (2 years after the introduction of 6-MP into clinical use), Kornberg *et al.* identified the enzyme that converts hypoxanthine and 6-MP to their respective nucleotides as hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*16*). Also in the mid-fifties, the pioneering work of Greenberg (*29*) and Buchanan (*30*) revealed the pathways of the biosynthesis of purines and the importance of hypoxanthine ribonucleotide (inosinic acid, IMP) as the first purine nucleotide formed in this biosynthetic pathway. It took a number of investigators and a period of years to unravel all of the pathways in which the nucleotide of 6-MP, thioinosinic acid (TIMP), and nucleotides derived from TIMP participated as substrates and inhibitors (*31*) (Fig. 1). Our current knowledge of enzyme inhibition constants and the concentrations of the various nucleotides achieved with the therapeutic regimens indicates that the principal sites of action appear to be feedback inhibition of de novo purine synthesis [particularly by methylthioinosinic acid (MTIMP)], inhibition of inosinate dehydrogenase, and incorporation into DNA in the form of thioguanine (*32*). Selectivity for neoplastic cells probably depends on the levels of the individual anabolic and catabolic enzymes. Catabolic enzyme levels are generally much lower in tumor cells than in normal cells. In addition, mitotic rate, drug transport, and metabolite pool sizes influence selective toxicity.

While these biochemical studies were going on, we studied the metabolic fate of 6-MP, first in mice (*33*) and then in humans (*34*). Pharmacokinetic and metabolic studies were then in their infancy, perhaps because the methodology for separation of metabolites and the counting of radioactive samples as thin films in a flow Geiger counter were so tedious and time-consuming. Nevertheless, using Dowex-1 and Dowex-50 ion-exchange columns and paper chromatography, we investigated the fate of 6-MP in vivo and attempted to discover whether it was possible to modify this metabolism and thus improve the efficacy of 6-MP. We continued to synthesize derivatives of 6-MP and thioguanine and to investigate structure-activity relationships (*35*). Thioguanine, which we had synthesized earlier than 6-MP, was more active but also more toxic (*23*). It was also more difficult to synthesize and, since its mechanism of action appeared to be similar to that of 6-MP, its metabolic fate and clinical activity were explored somewhat later (*36*). Thioguanine later found its main utility in the treatment of acute myelocytic leukemia in adults, in combination with cytosine arabinoside.

Studies of the urinary metabolites of 6-MP revealed that extensive metabolic transformations occurred in vivo (*33, 34, 37–39*). The single product present in the highest amount was 6-thiouric acid, formed by the action of xanthine oxidase on 6-MP. In addition, there were various substances in which the sulfur had been methylated, and the methylthio derivative had been oxidized on the sulfur or

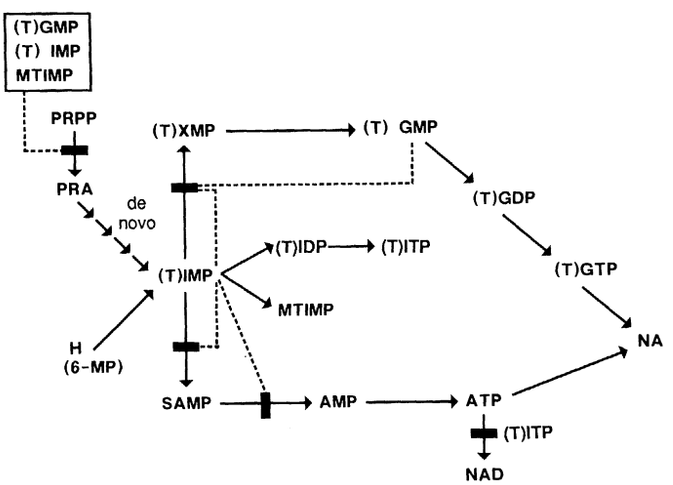

Fig. 1. Pathways in the anabolism of 6-MP and loci of the nucleotides derived from 6-MP. In addition, (T)GDP is converted to d(T)GDP and d(T)GTP for incorporation into DNA. Abbreviations not in the text: PRA, phosphoribosylamine; (T)XMP, thio equivalent of xanthine monophosphate; (T)GMP, (T)GDP, (T)GTP, thio equivalents of guanosine monophosphate, diphosphate, and triphosphate, respectively; (T)IDP and (T)ITP, thio equivalents of inosine diphosphate and triphosphate, respectively; SAMP, adenylosuccinate; AMP and ATP, adenosine monophosphate and triphosphate; NAD, nicotinamide-adenine dinucleotide; NA, nucleic acid.

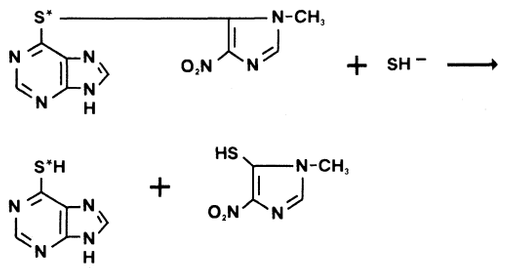

Fig. 2. Reaction of sulfhydryl ion on azathioprine to release 6-MP. [Reproduced from (*39*) with permission copyright 1967 by FASEB.]

on the purine ring. A considerable amount of the sulfur had been removed and converted by oxidation to inorganic sulfate, which also appeared as ethereal sulfates. Very little 6-MP was excreted unchanged.

In an attempt to modify the metabolism of 6-MP, we introduced substitutents at the 2- or 8-positions, or both, of the purine ring and on the ring nitrogens. This led to the loss of antitumor activity, with the exception of the 2-amino-6-mercapto derivative, thioguanine, which we had previously found to have strong antimetabolic activity. Attempts were then made to protect the sulfur from oxidation and hydrolysis by blocking groups that might be removed intracellularly to release 6-MP by, for example, some tumor-specific enzyme. The most successful compound to emerge from this approach was the 1-methyl-4-nitro-5-imidazolyl derivative, the compound now known as azathioprine (Imuran). This compound acts as a pro-drug for 6-MP which, because of the proximity of the ortho-nitro group, is subject to attack by sulfhydryl groups and other nucleophiles (*38–41*) (Fig. 2). In particular, the glutathione present in red cells reacts with azathioprine, releasing 6-MP back into the plasma (*40*). This compound had a better therapeutic index in mice bearing adenocarcinoma 755 and was as active as 6-MP but less toxic (*42*). In patients with leukemia, however, the chemotherapeutic index of 6-MP and azathioprine were similar (*43*).

Immunosuppression and Transplantation

In 1958, Robert Schwartz investigated the effect of 6-MP on the immune response, based on the rationale that the immunoblastic lymphocyte formed during an immune response closely resembled leukemic lymphocytes. Schwartz showed that when rabbits were given 6-MP for several days, beginning with the time of injection of a foreign antigen, for example, bovine serum albumin, they were unable to mount an antibody response to that antigen (*44*). With his colleagues, he worked out the importance of drug dose and timing and showed that 6-MP was most effective when treatment was started at the time of antigen administration (*44, 45*). He also demonstrated that animals could be made tolerant to a particular antigen while still retaining immunological reactivity to other antigens (*46*). At Schwartz's instigation, we set up an immunological screening test that consisted of measuring the antibody response of mice to sheep red cells (*47*). It enabled us to identify new active agents, synergistic combinations of drugs, and to show that immunosuppression was greater at higher doses of antigen and of drug.

Roy Calne, a young British surgeon, stimulated by Schwartz's papers, examined the effect of 6-MP on kidney transplant rejection in dogs. He obtained a 44-day survival of a kidney from an unrelated donor in a dog given daily doses of 6-MP (*48*). This was considerably longer than the expected 9- to 10-day graft survival in control animals. When Calne asked us for compounds related to 6-MP to investigate, we suggested that azathioprine might have some advantages. Subsequent studies showed azathioprine to be superior to 6-MP for preventing rejection of canine kidney homografts (*49*). Successful transplantation of kidneys to unrelated recipients became a reality in humans in 1962, with regimens of immunosuppression consisting of azathioprine and prednisone (*50*). By 1977, the Kidney Registry had records of 25,000 kidney transplants done between 1965 and 1972 (*51*), and the numbers have continued to increase yearly (*52, 53*). Today the procedure is considered therapeutic rather than experimental, and the importance of histocompatibility matching is recognized (*53*). Other organ transplants, for example, liver, heart, and lung, have likewise become possible. Other immunosuppressive drugs, for example, cyclosporin, have come into use in recent years, but azathioprine remains a mainstay in kidney transplantation.

The immunosuppressive effects of azathioprine have been studied in a wide variety of immunological systems. The earlier work is reviewed in (*54*) and more recent studies in (*55*). Immunosuppressive drugs have also shown utility in the treatment of autoimmune disease. Remissions with 6-MP, thioguanine, and azathioprine have been reported in autoimmune hemolytic anemia, systemic lupus, and chronic active hepatitis (*54*). Azathioprine is now an approved drug for treatment of severe rheumatoid arthritis (*56*).

Gout and Hyperuricemia

It was time now to try a new approach to the potentiation of 6-MP activity. Since we knew from metabolic studies that 6-thiouric acid was one of the principal products of 6-MP catabolism, it seemed possible that we could interfere with this oxidation by inhibiting the enzyme responsible for it, xanthine oxidase. In the early days of seeking antimetabolites for the natural purines in our laboratory, xanthine oxidase had been one of the test enzymes. Doris Lorz had identified many substrates as well as inhibitors of this enzyme (*7*). These compounds had also been tested on *L. casei* and on animal tumors. To test for xanthine oxidase inhibition in vivo, we chose a compound that had no inhibitory effects on bacteria or tumors, and was nontoxic, but which was a potent inhibitor of xanthine oxidase. This compound was the hypoxanthine analog, 4-hydroxypyrazolo(3,4-*d*)-pyrimidine (allopurinol). When allopurinol was given to mice together with 6-MP, it did indeed inhibit the oxidation of 6-MP and potentiated the antitumor and immunosuppressive properties of 6-MP three- to fourfold (*38, 57*). Moreover, the toxicity of 6-MP to mice appeared to be potentiated only twofold, so that the chemotherapeutic index of 6-MP was increased. With the collaboration of Wayne Rundles, we explored this combination in patients with chronic granulocytic leukemia, in whom the efficacy and metabolism of 6-MP could be investigated. The oxidation of 6-MP to thiouric acid was found to be inhibited in a dose-related manner, and the antileukemic activity of 6-MP was potentiated proportionally (*38, 58–60*). When 300 mg of allopurinol was given together with 110 mg of 6-MP, 6-MP increased fourfold, whereas urinary thiouric acid was four times lower. Later investigations (*61*) showed that the increased activity of 6-MP was accompanied by a proportional increase in toxicity. Thus, although less 6-MP was required to produce an antileukemic effect, the therapeutic index of 6-MP for leukemia remained essentially unchanged.

Xanthine oxidase is responsible not only for the oxidation of 6-MP, but also for the formation of uric acid from hypoxanthine and xanthine. Consequently, treatment with allopurinol produces a marked decrease in both serum and urinary uric acid (*58, 60, 62, 63*). This presented the possibility of a unique approach to the treatment of gout and other forms of hyperuricemia.

Allopurinol is not only a potent competitive inhibitor of xanthine

oxidase but it is also a substrate (*64*), its oxidation resulting in the corresponding xanthine analog, oxypurinol (called alloxantine, oxoallopurinol, or oxipurinol in early papers), which is also a xanthine oxidase inhibitor (Fig. 3). Oxypurinol also has the unusual property of binding very tightly to the reduced form of the enzyme, thereby inactivating it (*64, 65*). The enzyme activity can be restored by oxidation, which takes place slowly in the presence of air (*65, 66*). Although allopurinol itself has a short half-life in plasma (about 90 to 120 minutes), oxypurinol has a very long half-life, 18 to 30 hours (*64, 67, 68*), because oxypurinol is reabsorbed in the proximal tubule of the kidney (*69*). Consequently, steady-state levels of oxypurinol are achieved in a few days and uric acid concentrations can be maintained at the desired level by proper dose adjustment (*69*). Because allopurinol is completely absorbed orally, whereas oxypurinol is not, allopurinol remains the ideal pro-drug for oxypurinol.

When xanthine oxidase is inhibited in vivo, the intermediate oxypurines, hypoxanthine and xanthine, do not accumulate in the serum. In fact, their serum levels rise very little during allopurinol treatment (*68, 70*). One reason for this is that both hypoxanthine and xanthine can be reutilized for nucleic acid synthesis via the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*71*). The nucleotides formed, IMP and XMP, are the normal intermediates for adenine and guanine nucleotides (AMP and GMP). Through a process of feedback inhibition IMP, AMP, and GMP can reduce the de novo synthesis of purines by inhibiting phosphoribosylpyrophosphate (PRPP)-amidotransferase (*72*). Thus, the salvage of hypoxanthine and xanthine serves to regulate purine biosynthesis, reducing it when it is excessive. Moreover, when the oxypurines are not reutilized, they are excreted by the kidney by glomerular filtration, since they are not reabsorbed by the kidney tubule to any significant degree (*73*).

Long-term studies with allopurinol in animals and in humans have shown that xanthine oxidase is not induced and that allopurinol is a safe and effective drug for long-term treatment (*74*). A few percent of patients develop a rash when taking allopurinol. Patients with poor kidney function require lower doses of drug because of the pharmacological properties of oxypurinol and its long half-life (*75*).

Allopurinol helps to alleviate several of the clinical problems associated with gout. The hyperuricemia of gout produces deposits of small crystals of uric acid in joints, which result in extreme pain, or large deposits, tophi, which result in gouty arthritis and restricted joint movement. In patients who excrete excessive amounts of uric acid in the urine, urate stones often form in the kidney. With allopurinol it has been possible to prevent and reduce the tophaceous deposits and to prevent urate stone formation (*63, 70, 74, 76*). Likewise, the secondary hyperuricemia that is associated with the therapy of malignancy can be reduced with allopurinol treatment (*77*).

Antiviral Drugs

In 1968, we decided to return to a path that had intrigued us as early as 1948, the path to antivirals. The antiviral activity of 2,6-diaminopurine had been provocative (*20*), although its toxicity had been discouraging. Meanwhile, the pursuit of 6-MP, thioguanine, azathioprine, and allopurinol had occupied 20 years. When it was discovered that arabinosyladenine (Ara-A) inhibited the growth of both DNA and RNA viruses (*78*), the information started a train of thought. Diaminopurine had mimicked adenine in many reactions and could be converted to a nucleoside and nucleotide by adenine-metabolizing enzymes. Moreover, diaminopurine riboside was a poorer substrate for adenosine deaminase than was adenosine. It therefore seemed possible that arabinosyldiaminopurine (Ara-DAP) might persist longer than Ara-A, a compound that is rapidly deaminated in vivo.

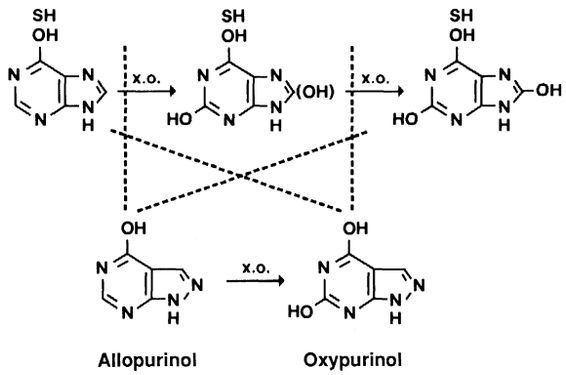

Fig. 3. Pathways in the oxidation of hypoxanthine and 6-MP by xanthine oxidase (x.o.) inhibited by allopurinol and oxypurinol. For 6-MP the intermediate is 8-hydroxy-6-MP; for hypoxanthine the intermediate is 2,6-dihydroxypurine (xanthine).

We synthesized Ara-DAP and, since we did not have a virus laboratory on site at the time, sent the compound to our colleague, John Bauer, in the Wellcome Research Laboratories in the U.K. for antiviral screening. He found the compound highly active against both herpes simplex virus and vaccinia virus, and less cytotoxic to mammalian cells than Ara-A. Thus began our antiviral odyssey. For several years my group pursued studies on the purine arabinosides, exploring structure-activity relationships, seeking better synthetic methods, and doing metabolic studies in mice (*79*). Bauer and Collins studied the activity of these compounds in rabbits and mice (*79*); Ara-DAP was found to be deaminated to arabinosylguanine (Ara-G) in mice, and the guanine derivative (Ara-G) was as active in antiviral as Ara-DAP. In this respect, Ara-G had an advantage over the deamination product of Ara-A, arabinosylhypoxanthine, which had very poor antiviral activity. We were not certain whether this advantage was sufficient to warrant the full-scale development of Ara-DAP.

In 1970, our laboratories moved to North Carolina and Howard Schaeffer joined us as head of the Organic Chemistry Department. He had been studying analogs of adenosine as substrates and inhibitors of the enzyme adenosine deaminase and had examined a variety of acyclic side chains on the 9-position of adenine to determine what changes the enzyme would tolerate in a substrate (*80*). He found that 9-(2-hydroxyethoxymethyl)adenine could serve as a substrate for adenosine deaminase. This suggested that other enzymes might also recognize such a side chain as a ribose and that nucleoside analogs of this kind might have antimetabolite properties.

When the acyclic adenosine analog was tested in the antiviral screen, it showed antiherpetic activity in vitro at about twice the concentration of Ara-A. The antiviral program now concentrated on the acyclic nucleoside analogs, with the syntheses conducted by H. J. Schaeffer and L. Beauchamp, the antiviral testing by D. J. Bauer and P. Collins, and the mechanisms of action, enzymology and in vivo metabolism by my group (*81, 82*). As was the case with the purine arabinosides, the 2,6-diaminopurine analog proved highly active on herpes simplex virus in vivo as well as in vitro. However, unexpectedly, the guanine analog, acyclovir (acycloguanosine, ACV), was over 100 times as active as the diamino compound (*82*). ACV is a metabolic product of the diaminopurine derivative, formed by the action of adenosine deaminase, and is undoubtedly responsible for the antiviral activity of the diamino compound observed in vivo (*83*).

Acyclovir

One of the most intriguing aspects of the antiviral activity of ACV is not only its high potency, but its unusual degree of selectivity (*81, 82, 84, 85*). It is highly active against herpes simplex virus, types 1 and 2, and varicella-zoster virus. It has activity against several other herpes-type viruses, for example, Epstein-Barr virus, pseudorabies, but only slight activity against the human cytomegalovirus (HCMV). It is not cytotoxic to the mammalian cells in which these viruses are grown at concentrations that are hundreds of times greater than those required for antiviral activity. Moreover, the compound is inactive against other DNA viruses, for example, vaccinia, as well as RNA viruses (*82*). It was highly important to determine the reason for this unusual selectivity, since this would undoubtedly offer exploitable information about the herpes viruses. In order to do these biochemical studies effectively, we set up a virus laboratory in-house. Radioactive ACV labeled in the 8-position of the guanine with ^{14}C or with ^{3}H in the side chain was synthesized. Vero cells, uninfected or infected with herpes simplex virus type 1 (HSV-1), were incubated with both types of radioactive ACV for 7 hours. When examined by high-pressure liquid chromatography, the extracts of the uninfected cells showed only the presence of unchanged ACV. In the HSV-1–infected cells three new radioactive compounds were evident (*81*). These products were identified by enzymatic methods as the mono-, di-, and triphosphates (ACV-MP, ACV-DP, ACV-TP) of ACV, and this was later confirmed by comparison with authentic synthetic samples. The enzyme responsible for the conversion of ACV to its monophosphate was isolated, purified, and identified as a herpesvirus-specified thymidine kinase (*81, 86*). While this enzyme had been reported to be formed in herpesvirus-infected cells, it was unexpected that an acyclic nucleoside of guanine could serve as its substrate. A similar enzyme is specified by the varicella-zoster virus. Once the first phosphate has been added, the second phosphate is added by cellular guanylate kinase (*87*) while several other cellular kinases can add the third phosphate (*88*). Since the cellular thymidine kinase cannot use ACV as a substrate, very little ACV-TP is formed in uninfected cells (*81, 89*). The small amount of phosphorylation that occurs in normal cells is due to a 5′-nucleotidase (*90*). The pathways for the formation of ACV-TP are shown in Fig. 4.

ACV-TP is a more potent inhibitor of the herpes virus DNA polymerases than of cellular DNA polymerase-α (*91*). The quantitative aspects of these differences in the amounts of ACV-TP formation and DNA polymerase inhibition (K_i value) by ACV-TP in virus-infected cells and uninfected cells are illustrated in Fig. 5 (*92*). Moreover, ACV-TP serves as a substrate for the herpes virus DNA polymerase, but chain termination occurs when it is incorporated, because of the absence of the 3′-hydroxyl group needed for chain elongation (*84*). Thus, only very small fragments of viral DNA are formed (*93*). In addition, ACV-TP not only inhibits, but also inactivates the viral DNA polymerase after the formation of the enzyme–template–ACV-MP complex (*94*). This inactivation does not occur with cellular DNA polymerase.

The high selectivity of ACV for those herpes viruses that induce a herpes-specified thymidine kinase is evident. This enzyme is a very useful tool for determining the structural requirements for other potential substrates of this enzyme (*95*). Its absence or alteration also explains the resistance of some herpes virus isolates to ACV (*96*). On the other hand, transfection of cells with a portion of the herpesvirus genome containing the HSV thymidine kinase gene transforms normally resistant cells to ACV-sensitive cells (*97*). Resistance to ACV may also result from mutations in the viral DNA polymerase (*96*).

Epstein-Barr virus and HCMV infection do not induce a specific kinase capable of phosphorylating ACV. However, the DNA polymerase of Epstein-Barr virus is exquisitely sensitive to the small amount of ACV-TP formed in EBV-infected cells (*98*). Interestingly, although HCMV-infected cells do not phosphorylate ACV to any extent, they do phosphorylate the closely related ACV derivative, ganciclovir (formerly called BW B759U, DHPG, and 2′NDG), which has an extra hydroxymethyl group on the side chain. Ganciclovir has proven to be a much better inhibitor of HCMV replication than ACV (*99*).

Investigation of the pharmacokinetics and metabolism of ACV in several animal species revealed only two metabolites, the 2-carboxymethoxymethylguanine (CMMG) and 8-hydroxyacyclovir (*100*). In humans, CMMG accounted for 9 to 14% of an intravenous dose of ACV, while essentially none of the 8-hydroxy metabolite appeared in the urine (*101, 102*). In other species there were somewhat increased amounts of both metabolites (*100, 102, 103*). ACV has a plasma half-life of approximately 3 hours in humans (*104*), so that an intravenous infusion is generally given every 8 hours. Because ACV is excreted at about twice glomerular filtration rate and has a limited solubility in water (2.5 mg/ml at 37°C), it is administered as a 1-hour intravenous infusion rather than as a bolus injection. The compound is also active topically and orally. Although the oral bioavailability is limited, the blood levels attained by oral administration of ACV are adequate for therapeutic efficacy against herpes simplex and varicella-zoster viruses (*105*). The compound is distributed in all body tissues and crosses the blood-brain barrier (*82, 100, 106*). Toxicology studies in mice, rats and dogs showed the drug to be nontoxic at doses well above those required for therapeutic efficacy (*107*).

Acyclovir has now been in clinical use for about 8 years and it seems appropriate to mention some of the areas in which it has decreased suffering and saved lives. First episodes of genital herpes infections are characterized by pain, prolonged viral excretion, and slow healing of lesions. ACV, given by either the intravenous or oral routes, produces a significant alleviation of symptoms; it decreases the median time of viral shedding from 13 days to 1 or 2 days, and

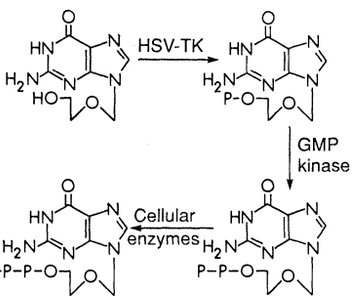

Fig. 4. Pathways for the formation of ACV mono-, di-, and triphosphates. [Reproduced from (*84*) with permission copyright 1982 by American Journal of Medicine.]

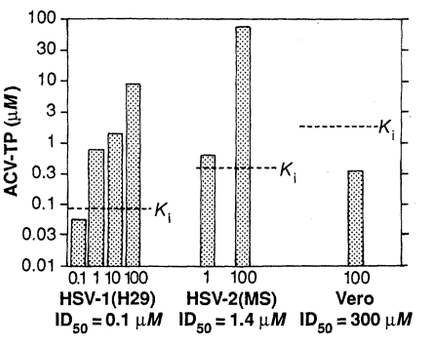

Fig. 5. Amounts of ACV-TP formed in uninfected, HSV-1– and HSV-2–infected Vero cells in the presence of varying micromolar concentrations of ACV. HSV strains are in parentheses. Dotted lines represent the K_i values for the individual DNA polymerases. ID_{50} values are concentrations for 50% inhibition of plaque formation. [Reproduced from (*92*) with permission copyright 1986 by Elsevier Science Publishing Company.]

Table 1. Effect of intravenous ACV in HSV infections in immunocompromised patients.

Patients (*n*)	Median duration in days (ACV/placebo)		
	Virus shedding	Pain	Healing
All (97)	2.8/16.8	8.9/13.1	13.7/30.1
Bone marrow transplants (34)	3/17	10/16	14/28

shortens the time to healing by about 50% (*108*). Recurrent episodes of genital herpes are generally much shorter and less severe than the initial one. Therefore, the benefits of ACV treatment appear to be less dramatic. However, in a study in which patients initiated oral therapy, there was a significant difference in new lesion formation, 23% on placebo, 6.5% on ACV (*109*). For patients with frequently recurring genital herpes, for example, once a month, of several years' duration, it has been possible to decrease the frequency of recurrence to a mean of 1.8 episodes during the first year and 1.4 episodes during the second year by oral prophylaxis with ACV (*110*). The percentage of patients having no recurrences in a year of oral prophylactic therapy was 45 to 50% (*111*).

Herpes zoster, commonly known as "shingles," is caused by a reactivation of latent varicella-zoster virus. It produces severe pain during the acute 2- to 3-week episode, as well as a post-herpetic neuralgia months later in about 10% of patients. ACV causes a significant decrease in the duration of acute pain but has little effect on the post-herpetic neuralgia (*112*). In immunocompromised individuals, herpes zoster can produce serious sequelae in the form of progressive skin dissemination and visceral disease. The latter can be fatal in a small percentage of patients. Intravenous treatment with ACV has effectively prevented this dissemination (*113*).

In two studies that involved 97 immunocompromised patients (*114*) with a variety of diseases and 34 bone marrow transplant recipients (*115*), the effects of intravenous ACV treatment on viral shedding, pain, and time to healing were highly significant (Table 1). In bone marrow transplant patients and in leukemic patients, prophylaxis with ACV has made it possible to prevent the reactivation of latent virus during therapy so that the patients can remain free of herpetic episodes during the period of maximum immunosuppression (*116*). The treatment of herpes encephalitis, a frequently fatal disease, with intravenous ACV has been successful in a large percentage of patients, if begun before the patient is comatose (*117*).

In addition to the clinical utility of ACV, the lessons learned from its development have proven to be extremely valuable for future antiviral research. In-depth studies of mechanisms of action have led to a better understanding of the enzymatic differences between normal and virus-infected cells. It has given impetus to the search for other viral-specific enzymes, which are capable of therapeutic application.

In this attempt to cover 40 years of research on purines and purine analogs, I hope that I have successfully conveyed our philosophy that chemotherapeutic agents are not only ends in themselves but also serve as tools for unlocking doors and probing Nature's mysteries. This approach has served us well and has led into many new areas of medical research. Selectivity remains our aim and understanding its basis our guide to the future.

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