Selectivity—Key to Chemotherapy: Presidential Address

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For many years, chemotherapists have sought the touchstone which would turn lead into gold, the magic bullet of Ehrlich which would go directly to the target without harming normal cells. There have been many approaches to this goal, but in essence there have been two main philosophies. One is that you should find the basic biochemical differences between the target and the host, whether the target is a bacterium, a virus, a protozoan, or a cancer cell, and then seek ways to utilize this information. The other approach has been more impatient. It is the one which George H. Hitchings has called "Enlightened Empiricism." It says that we cannot wait until all the biochemical differences are understood. We must design and test compounds on the basis of some reasonable hypothesis, and hopefully we will be able to learn the basis for selectivity as we go along. Some feel that the latter approach is somehow not scientific enough and that there is something a little ignoble about finding a useful chemotherapeutic agent by "accident." But I would like to espouse the idea that basic knowledge and serendipity are not opposites but integral parts of the process of discovery. Serendipity is a gift to be appreciated and treated with the wonder and respect it deserves. It should be regarded not as the end of the search but as the beginning, the important clue which may lead to the solution of the mystery.

How does one begin to unravel the mystery of selectivity? First, one needs a compound which is active in some biological system. Where does one find such a compound? There are two possibilities: synthesis and isolation from natural sources. Synthesis gives us an opportunity for so-called "rational design." We may reason by analogy to compounds already shown to be active. Alternatively, we may choose to mimic some essential metabolite or cell constituent or seek to inactivate some important metabolic pathway. This will hopefully lead to an active compound, although not necessarily a selective one. Next, one needs to evaluate the compounds in some biological systems, preferably in both in vitro and in vivo tests. These are usually limited by the investigator's interests as well as by his facilities. But it is important that the net be spread widely enough to reveal differences between species or between tissues when they exist.

What are some of the bases for selectivity between cancer cells and normal cells? At one time, it was thought that all tumor cells were rapidly dividing, whereas normal cells were not. This would presumably make the tumor cells more vulnerable to inhibitors of nucleic acid synthesis. While this is true for some malignant cells, e.g., leukemic lymphocytes in acute leukemia, it is not true for many solid tumors, which often divide slowly and have a small growth fraction. Moreover, bone marrow and intestinal mucosa are rapidly dividing normal cells and therefore particularly vulnerable to cytotoxic agents. Differences in metabolite pool sizes can influence the effects of antimetabolites in different tissues. Similarly, the level of the target enzyme can be highly important. Thus, cells can become resistant to folate antagonists because of increased levels of DHFR caused by gene amplification. An important reason for selectivity is a difference in the concentration of active inhibitor in the tissue. This difference may be due to selective transport but, in the case of antimetabolites, it may also be due to differences in the levels of anabolic (i.e., activating) and catabolic enzymes. The possibility dearest to the researcher's heart is that of finding isozymes, enzymes performing the same function in different tissues or in different species but which are sufficiently different in substrate specificity or inhibitor selectivity to be chemotherapeutically exploitable.

6-MP was one of the compounds which emphasized for us the importance of activation for the production of antimetabolite effects. It is not 6-MP itself which has antitumor activity but the nucleotides formed from it. When 6-MP was given to a tumor-bearing mouse, and extracts of the tumor were examined, we found, within 20 min, a larger amount of thioninosinic acid, as well as thioxantholic acid and methylthioninosinic acid (Chart 1) (20). These compounds, although formed very rapidly, persisted intracellularly for hours. In fact, methylthioninosinic acid, which is an excellent inhibitor of the first enzyme in the de novo pathway to purine biosynthesis, remained elevated for many hours, while 6-MP itself was disappearing from the plasma at a very rapid rate. It is important to remember, when one considers the pharmacokinetics of compounds such as 6-MP, that it is not the pharmacokinetics of the inactive "prodrug" in plasma which is critical but rather the concentration of the active form of the compound inside the cell.

A good example of the selectivity which can be achieved because of differences in the levels of both anabolic and catabolic enzymes is found in the data of Moore and LePage (43). They administered thioguanine to mice bearing Ehrlich ascites tumor cells and measured the levels of thioguanylic acid and of the catabolic product, thiouric acid, in the tumor cells and in the intestines of the same animals (Chart 2). The tumor cells formed a much higher amount of thioguanylic acid than did the intestine, even though those cells were also rapidly dividing. Moreover, the amount of thiouric acid formed from thioguanine by deamination and oxidation was greater in the intestine. Thus, on the basis of both higher activation and lower degradation, the growth of the ascites tumor was selectively inhibited.

The selectivity which is based on the differences between isozymes is well exemplified by the small-molecule antifolates which show a high specificity for the species source of the DHFR which they inhibit (6, 25, 33). Methotrexate, which is a very close
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ADENOCARCINOMA - 755
6-MP, 25mg/kg, i.p.

Chart 1. Concentrations of ribonucleotides formed from 6-MP in adenocarci
noma 755 at various times following i.p. injection of 6-[35S]mercaptopurine, 25 mg/
kg, into tumor-bearing C57BL mice. Extracts of the tumors were analyzed by high-
pressure liquid chromatography. TIMP, 6-thiosinosinic acid; TXMP, 6-thioxanthoryl
acid; MTIMP, 6-methylthiosinosinic acid; TGMP, 6-thioguanylic acid. Reproduced
from Ref. 20 by permission.

Table 1

<table>
<thead>
<tr>
<th>Inhibitors of DHFR of various species</th>
<th>I_so [10^8 x concentration (M)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Human</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.2</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>180</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>30,000</td>
</tr>
</tbody>
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structural analogue of folic acid, inhibits DHFR from human cells,
*Escherichia coli*, and the malarial parasite *Plasmodium berghei*
to approximately the same extent (Table 1). It has found utility
as an anticancer agent. On the other hand, pyrimethamine (2,4-
diamino-5-chlorophenyl-6-ethylpyrimidine) has a very strong in-
hibitory activity against the malarial DHFR with an I_so of 0.05 x10^-8 M for *P. berghei* but is much less inhibitory to human
or *E. coli* DHFR. Another 2,4-diaminopyrimidine, trimethoprim
[2,4-diamino-5-(3',4',5'-trimethoxybenzyl)]pyrimidine], shows
an even greater degree of selectivity. It inhibits the *E. coli* DHFR
at 0.7 x 10^-8 M but requires a concentration 43,000 times higher
than this to inhibit the human enzyme. While the antifolate activity
of the 2,4-diaminopyrimidines was discovered from studies with
*Lactobacillus casei*, it was the discovery of the antimalarial
activity of pyrimethamine and the wide antibacterial spectrum of
trimethoprim which then led to the investigation and determina-
tion of the differences in the DHFR in various species.

One of the serendipitous findings which led us to a new area
of research was one made by J. J. Marr. He made the observa-
tion that allopurinol was a good inhibitor of the growth of leish-
maniae, *Leishmania braziliensis*, *Leishmania donovani*, and *Leishmania*
*mexicana* (36, 46). Incubation of radioactive allopurinol with *L.
donovani* in culture, followed by high-pressure liquid chromato-
graphy of the cell extracts, produced some amazing findings.
First, there was the production of a large amount of allopurinol
ribonucleotide, something we had never found in mammalian
cells. Moreover, the leishmaniae transformed allopurinol ribonucleo-
tide to the 4-amino derivative, 4-aminopyrazolo[3,4-d]pyrim-
dine ribonucleotide as well as to the di- and triphosphates of the
latter and incorporated the amino compound into RNA (45, 46).
A similar conversion occurred in *Trypanosoma cruzi*, the organ-
ism which causes Chagas’ disease (37). However, the amina-
tion does not occur in mammalian tissues. Enzymic studies
clarified the reasons for these differences. In leishmaniae and
in trypanosomes, there is not only a very large amount of the
enzyme hypoxanthine-guanine phosphoribosyltransferase which
converts allopurinol to its ribonucleotide, but the enzyme utilizes
allopurinol as a substrate much better than does the human
hypoxanthine-guanine phosphoribosyltransferase (62). In addition,
the leishmaniae have a phosphotransferase capable of
converting allopurinol ribonucleoside directly to the ribonucleo-
the adenylosuccinate synthetase of the leishmania has a different tide; this enzyme has not been found in humans (46). Moreover, the adenylosuccinate synthetase of the leishmania has a different substrate specificity and is able to use allopurinol ribonucleotide as a substrate, whereas the mammalian enzyme cannot (56). Thus, several enzymic differences between host and parasite are responsible for the selective toxicity of allopurinol and allopurinol ribonucleoside for the parasite.

Finally, I come to a compound which I would like to discuss in some detail because its selectivity makes it a new and important chemotherapeutic agent against herpesviruses. This compound is ACV (Chart 3). It was synthesized by Schaeffer et al. (54) as a potential antimetabolite, in which the acyclic chain mimics a portion of the sugar moiety of deoxyguanosine. In earlier studies, Schaeffer had found that adenine with this side chain was able to serve as a substrate for adenosine deaminase, indicating that at least one enzyme could mistake the acyclic side chain for a sugar. Since purines and purine nucleosides have been a major interest in our laboratory over the years, testing was available for antitumor, antibacterial, antiparasitic, and antiviral activities. Indeed, 2,6-diaminopurine arabinoside and 1-ß-D-arabinofuranosylguanine had been found to be as active against HSV as 9-ß-D-arabinofuranosyladenine (24). However, the extremely potent inhibition of HSV-1 and HSV-2 by ACV reported by our Wellcome U.K. colleagues, Bauer and Collins, was completely unexpected (11, 23, 54). The IC50 for HSV-1 was 0.1 µM, and for several strains of HSV-2, the virus which causes genital herpes, the IC50 was approximately 1 µM. On the other hand, for the uninfected host Vero cells, the IC50 was 300 µM. This great selectivity of ACV for herpesviruses was also seen for VZV, the type of herpesvirus which causes chicken pox and shingles; the IC50 was 3 to 4 µM for VZV, whereas for uninfected human WI38 cells in which this virus was grown, the IC50 was >3000 µM (3, 11). HCMV was less sensitive than were the other herpesviruses, with IC50 at 115 µM for strain AD169, but still much more sensitive than the host WI38 cell (11). For Epstein-Barr virus, the herpesvirus responsible for mononucleosis, the IC50 was originally reported as 7 µM (10) but, more recently, was found to be 0.3 µM by nucleic acid hybridization (48).

Since selectivity of this magnitude has not been seen previously for DNA viruses such as HSV and VZV, intensive research was undertaken to determine the reason for this unusual selectivity. The first clue came when HSV-infected Vero cells and uninfected cells were incubated with radioactive ACV for 6 h and extracts of both were examined by high-pressure liquid chromatography (23). In the chromatograms of uninfected Vero cells, the radioactivity was essentially all in the form of unchanged ACV. In the HSV-infected cells, 3 new peaks of radioactivity were seen in the mono-, di-, and triphosphate regions of the chromatogram (Chart 4). The identity of these new phosphates was confirmed by enzymatic hydrolysis and rechromatography as being the phosphates of ACV. It was apparent that they were formed specifically in virus-infected cells, with only very small amounts being present in uninfected cells. The question which then had to be answered was, “Which enzymes are responsible?”

The enzyme responsible for the conversion of ACV to ACV-MP was identified by Fyfe as a herpesvirus-specified TK (23, 31). This was surprising since purine nucleosides were not known to be substrates for this enzyme. In fact, ACV is a much better substrate for this viral TK than is guanosine. The cellular TK does not phosphorylate ACV. Thus, the first and rate-limiting step in the activation of ACV to the true antiviral agent, ACV-TP, is a herpesvirus-specific one. The remaining steps, conversion of ACV-MP to ACV-DP and of ACV-DP to ACV-TP, were found to be carried out by cellular enzymes (Chart 5) (40, 41).

ACV-TP, once formed, persists intracellularly for a considerable length of time, even when free ACV is removed from the medium. When HSV-1-infected Vero cells were exposed to 100 µM ACV, 75% of the intracellular radioactivity was still present after 24 h, even though most of the free ACV had been removed from the medium.

Since ACV-TP was a potential antimetabolite, in which the acyclic chain mimics a portion of the sugar moiety of deoxyguanosine, it was of interest in our laboratory over the years, testing was available for antitumor, antibacterial, antiparasitic, and antiviral activities. Indeed, 2,6-diaminopurine arabinoside and 1-ß-D-arabinofuranosylguanine had been found to be as active against HSV as 9-ß-D-arabinofuranosyladenine (24). However, the extremely potent inhibition of HSV-1 and HSV-2 by ACV reported by our Wellcome U.K. colleagues, Bauer and Collins, was completely unexpected (11, 23, 54). The IC50 for HSV-1 was 0.1 µM, and for several strains of HSV-2, the virus which causes genital herpes, the IC50 was approximately 1 µM. On the other hand, for the uninfected host Vero cells, the IC50 was 300 µM. This great selectivity of ACV for herpesviruses was also seen for VZV, the type of herpesvirus which causes chicken pox and shingles; the IC50 was 3 to 4 µM for VZV, whereas for uninfected human WI38 cells in which this virus was grown, the IC50 was >3000 µM (3, 11). HCMV was less sensitive than were the other herpesviruses, with IC50 at 115 µM for strain AD169, but still much more sensitive than the host WI38 cell (11). For Epstein-Barr virus, the herpesvirus responsible for mononucleosis, the IC50 was originally reported as 7 µM (10) but, more recently, was found to be 0.3 µM by nucleic acid hybridization (48).

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μM ACV for 4 h, they accumulated 100 pmol of ACV-TP/10⁶ cells. The cells were then washed and placed in an ACV-free medium, and aliquots were examined periodically for ACV-TP concentration and the Kᵢ for that DNA polymerase. Since inactivation does not occur to a large extent in vivo, e.g., 9-0-D-arabinofuranosyladenine is rapidly deaminated, 5-iododeoxyuridine is reduced and loses its iodo group.

ACV penetrates tissues well and even passes the blood-brain barrier, although the level in the brain is lower than in other tissues (17, 54). In humans, the concentration of ACV in the cerebrospinal fluid following i.v. administration is approximately one-half of the plasma level (4).

Extensive toxicology studies in mice, rats, rabbits, and dogs by a variety of routes of administration showed ACV to be a very safe drug at doses well beyond the anticipated therapeutic range (59–61). It was noncarcinogenic in lifetime studies in mice and rats (60), was nonteratogenic in rats and rabbits (44), and had no effect on neonatal development or reproduction and fertility in rodents (44). It had no effect on the immune response, either antibody or cell-mediated, in mice (50). Plasma levels were monitored throughout the toxicology studies to assure that adequate drug levels had been achieved. In a 1-year study in

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dogs given ACV p.o. daily in 3 divided doses, 60 mg/kg/day were tolerated with no toxicity; plasma levels of 45 to 50 µM were observed 1 h after each dose. If dogs were given 150 mg/kg/day p.o., blood levels greater than 100 µM were obtained, and weight loss and diarrhea were seen within 2 weeks. In 11 different mutagenicity assays in vitro, ACV was negative in 9; it was positive at the TK locus of L5178Y cells at 400 to 2400 µg/ml and produced chromosome breaks in lymphocytes after 48 h of exposure above 250 µg/ml (1.1 mm) (8). In cell transformation systems, it was negative in the C3H/10T½ cells and positive only at 50 µg/ml in BALB/c-3T3 cells.

Therapeutic studies in animals were performed by a large number of investigators. They found ACV to be effective by a variety of routes of administration (i.p., s.c., p.o., topical) against HSV infections: mucocutaneous herpes and herpes encephalitis in mice; genital herpes in mice and guinea pigs; herpetic keratitis in rabbits; and cutaneous herpes in guinea pigs (34, 54). The compound also showed therapeutic activity against the Delta herpesvirus in monkeys, murine cytomegalovirus, and Herpesvirus simiae in rabbits (34). Herpesviruses are known to establish latency in the spinal ganglia of the host following the primary infection. It is this latent virus which is responsible for recurrences. Treatment of mice with ACV very soon after infection with HSV-1 was able to prevent the establishment of latency in about one-half of the animals. However, once latency was established, treatment with ACV did not abrogate the latency (34).

Pharmacokinetic studies of ACV in humans, following a 1-h infusion, revealed that the drug has a half-life in plasma of approximately 3 h (18, 34). The maximum concentration of ACV achieved is directly proportional to the dose given, up to 15 mg/kg, as is the area under the plasma concentration-time curve. The drug is excreted almost entirely in the urine at a rate about two thirds the glomerular filtration rate, indicating tubular secretion in the kidney. Because ACV has a limited solubility in water (1.2 mg/ml at room temperature and 2.4 mg/ml at 37°C), bolus injections of ACV may lead to rapid excretion of the drug and may result in crystalluria and kidney dysfunction. Consequently, 1-h infusions at 8-h intervals are recommended, with the maintenance of good hydration (4, 15).

Since it would obviously be desirable to have a p.o. form of ACV for use in the outpatient population, the pharmacokinetics of the p.o. drug was investigated. It had been determined earlier that p.o. absorption of ACV varied with the species (17). It was very good in the dog but was progressively worse in mice, humans, rats, and monkeys. In humans, approximately 15% of the p.o. dose is absorbed. However, if the compound is given every 4 h, steady state peak plasma levels of approximately 2 µM are achievable with 200 mg/dose and 6 µM is seen with 600 mg/dose; the trough levels are about 1 and 4 µM, respectively (15). In some of the clinical studies to be reported below, doses of 200 mg of p.o. ACV were given 4 or 5 times a day.

Finally, we must ask the question, "How effective is ACV as an antitherpetic agent in humans?" Time does not permit an extensive survey of the clinical results, so that only some representative studies will be presented. Symposia held in Washington, DC, in 1981 (34) and in London in 1983 (27) presented a large number of clinical papers. New papers are appearing rapidly, and some studies still remain to be uncoded. All of the studies which I shall describe here were double blind and placebo controlled.

Genital herpes infections, caused mainly by HSV-2, but some by HSV-1, are the cause of a great deal of mental and physical suffering. During the first episode of genital herpes, viral shedding is prolonged, and the median time for the healing of lesions is 2 to 3 weeks. A comparison of the parameters of viral shedding, duration of pain, and time to healing in 4 different studies is shown in Table 3. In 2 of these studies, the drug was given i.v. by infusion every 8 h (12, 42); in 2, it was given p.o. (5, 47). In all studies, there was a significant decrease in the duration of viral shedding and healing time compared with the placebo group; the effect on duration of pain was more variable. The effect of the route of administration on the response of initial genital herpes to ACV has been summarized by Corey et al. (12). As might be anticipated, the order of efficacy was i.v. > p.o. > topical, although the topical ointment did decrease the duration of viral excretion by 56% and shortened healing time by 29% (12, 13).

In recurrent genital herpes infections, the viral shedding and healing times are generally much shorter than in the first episode. Consequently, the effect of ACV treatment is not as striking. Thus, in the study by Nilsen et al. (47) with p.o. treatment of recurrent disease, the mean duration for viral shedding was 1 day for the ACV-treated group and 2 days for placebo, and for healing time it was 5 days for the ACV-treated group and 6 days for placebo. When this is compared with Line 3 of Table 3, it is apparent that the ACV-treated group responded similarly in initial and recurrent episodes, but the placebo groups were very different in the 2 cases. One aspect of recurrent disease in which p.o. ACV therapy made a highly significant difference was the frequency and incidence of new lesion formation. In a study in which patients initiated p.o. therapy as soon as they had the first symptoms of a recurrence, only 6.5% developed new lesions after they started taking ACV, whereas 23% continued to develop new lesions on placebo (51).

Ideally, one would prefer to prevent the recurrence of genital herpes rather than to treat it. Several prophylactic studies have been done with patients in whom symptoms recur frequently, i.e., every 3 or 4 weeks for a period of years (19, 58). In one multicenter study involving 140 patients, one group received placebo, one received 200 mg ACV twice a day, and one received 200 mg 5 times a day for 4 months (19). Almost all (94%) patients in the placebo group had recurrences every month, as they had previously. In the ACV-treated groups, about 10% of the patients had one recurrence during the first week, similar to the placebo rate, but thereafter the recurrence rate was very low, and 70% never recurred during this 4-month period (19). Interestingly, 200 mg b.i.d. was about as effective as 200 mg 5 times a day in preventing recurrence. Breakthrough occurrences in ACV recipients were of shorter duration and were associated with less viral shedding than were recurrences in placebo recipients. When
treatment was stopped, episodes occurred at their previous frequency, indicating that latent virus had not been affected.

Herpes zoster, commonly known as shingles, is a recurrence of VZV which had been latent. It is characterized by acute pain, before as well as after the formation of a vesicular rash which has a dermatome distribution. In normal, nonimmunosuppressed individuals, the vesicles crust and heal in 2 to 3 weeks, but pain in the form of a postherpetic neuralgia may persist for a period of months or even years in about 10% of patients. Treatment with i.v. ACV has produced a significant shortening of the healing time and has prevented new lesion formation. It has also caused a more rapid subsidence of acute pain (2), although it did not appear to have an effect on the incidence of postherpetic neuralgia.

One of the most serious complications of herpes zoster occurs in immunocompromised patients, such as those with transplants, leukemia, immunodeficiency disease, or cancer which has been treated with radiation and chemotherapy. In such individuals, there is a tendency toward progressive skin dissemination and the development of life-threatening visceral disease. In a study conducted by Balfour et al. (1), ACV was effective in preventing the development of visceral disease in all 52 immunocompromised patients with herpes zoster, whereas 4 of 42 patients on placebo developed visceral disease and 3 of these died. Only one of 52 given ACV i.v. had progressive skin dissemination, compared with 7 of 42 given a placebo.

The severity of herpes simplex virus infections in immunocompromised patients can be a serious medical problem. Virus continues to be shed for a long time, healing is slow, and pain is persistent. Sometimes lesions in the mouth spread to the throat and cause a serious esophagitis. The therapeutic effects of i.v. infusions of ACV have been particularly striking in such cases. In patients (Chart 6) given infusions of ACV, 2.5 mg/kg 3 times a day for 1 week, virus excretion from lesions decreased very rapidly compared with that in the placebo-treated group (39).

In 3 studies (Table 4) which involved a group of 97 immunocompromised patients with a variety of diseases or transplants (39), 34 bone marrow transplant recipients (63), and 10 cardiac transplant patients (7), the effects of ACV treatment on duration of pain and time to healing were highly significant.

It is obvious that in patients whose immunological status is severely depressed it would be preferable to prevent herpesvirus infections if possible. The first prophylactic trial in such patients was done by Saral et al. (53) in bone marrow transplant recipients. The patients chosen for the study were all seropositive for HSV antibodies. Therefore, they all could be presumed to harbor latent virus. Since bone marrow transplantation requires that the patients' immune response first be completely suppressed, one could expect recurrence of HSV infections. One group of 10 patients was given ACV i.v. from 3 days before marrow transplantation until 14 days posttransplant; 10 others were given placebo. In the placebo group, 7 of 10 developed herpesvirus infections during these first 2 weeks; none of the ACV-treated patients did. One ACV recipient shed virus during the 2 weeks but developed no lesions. When the drug was discontinued, 5 of the ACV-treated group developed p.o. HSV lesions during the period of 3 to 53 days (mean, 23.5 days) after cessation of therapy, but these episodes were generally mild. As bone marrow engraftment occurs and the immune response becomes functional again, patients are better able to cope with viral infections.

In a similar prophylactic study in leukemic patients, where ACV was given i.v. for 32 days at a dose of 250 mg/sq m every 8 h, none of 14 developed HSV lesions; whereas in the placebo-treated group, 11 of 15 had HSV infections (52). Prophylaxis of bone marrow transplant recipients with p.o. ACV, 200 mg every 6 h from 8 days before to 35 days after transplantation, resulted in no HSV lesions in 20 patients treated with ACV, while 13 of 19 developed HSV lesions in the placebo group (32). Of interest also was the fact that no ACV-treated patients had HCMV infections during the period, whereas 7 of 19 in the placebo group did. This is rather surprising because ACV is not very effective against HCMV once the infection is acute and has developed into a cytomegalovirus pneumonia. After treatment was stopped, there were breakthroughs of HSV lesions in the ACV group (7 of 20) as well as in the placebo group (11 of 19) during the following 2-month period.

In addition to the many other clinical studies which have been reported with ACV, there are ongoing double blind trials in herpes encephalitis and neonatal herpes patients i.v. ACV with vi darabine. Studies with p.o. ACV in mononucleosis and in herpes zoster are also in progress. Recently, we have been fortunate in finding a prodrug of ACV, 2-amino-9-(2-hydroxymethyl)purine, which is absorbed p.o. much better than is ACV itself and which is converted in vivo almost completely to ACV (35). This is now entering clinical trial.

One of the problems which still exists is that of latency. We have not yet found a way to abolish it or to prevent it. Apparently, the latent virus is not replicating and therefore is protected from the effects of drugs which inhibit viral multiplication. Another potential problem is that of resistance. Fortunately, this has not been a clinical problem to date. In the laboratory, it is possible to produce ACV-resistant strains which are deficient in viral TK, have a TK of altered specificity, or have an altered DNA polymerase (9, 26, 34, 55). Although many hundreds of herpesvirus infected patients were given ACV, we have not found a single case of ACV resistance in any of these patients.

**Table 4**

| Table 4 Effect of i.v. ACV in herpes simplex infections in immunocompromised patients |
|------------------|-----------------|-----------------|----------------|
|Patients          | Virus shedding  | Pain            | Healing        |
|Bone marrow transplants | 3/17           | 10/16           | 14/28          | 63 |
|Cardiac transplants | 3/4/12         | 9/5/21          | 20/28          | 7  |
|Ref.              | 14/28           | 20/28           | 39             |    |
|All               | 2.8/16.8        | 8.9/13.1        | 13.7/20.1      | 39 |

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isolates from ACV-treated patients have been examined, only a few resistant strains have been found, and these have come from heavily immunosuppressed patients (14, 34). These strains have all been ones deficient in the HSV-TK, and patients have improved clinically in spite of the lower sensitivity. Strains of herpesvirus which are TK negative have been shown in animal models to be less neurovirulent (26, 34) and generally do not become latent. Therefore, it is not surprising that, in those patients who develop a less sensitive virus on treatment with ACV, recurrences due to latent virus have the original high sensitivity.

Cytomegalovirus remains a problem because of its low sensitivity to the available antiviral drugs. We do presently have a derivative of ACV, which has an additional hydroxymethyl group on the side chain, which inhibits HCMV in vitro with an IC50 of 2 to 3 μM. This promising compound, 9-[(1,3-dihydroxypropoxy)methyl]guanine (BW B759U), will soon be in clinical trial.

It is clear that selective agents for the herpesviruses are now a reality. Their selectivity is based on differences between viral-specific enzymes and host enzymes. It gives impetus to the search for other viral-specific enzymes which can be exploited therapeutically. Hopefully, cancer-specific enzymes or receptors can be identified which will be targets for selective growth inhibitors.

In closing, I wish to espouse the concept that ignorance of the law is no excuse. We must certainly continue to pursue basic research and to probe Nature for her undiscovered laws, but let us not ignore the clues along the way. Chemotherapeutic agents are not only ends in themselves, they are also beginnings. They are the tools which help us to answer the whys and the wherefores. Unless we use drugs as tools, we will have thrown away the keys and gone searching for new locked doors. Each door we open may lead to a new maze, but an unlocked door leads nowhere. Let us not rely on the excuse that we do not yet know enough to design selective chemotherapeutic agents. Until that time comes, in the words of Admiral Farragut, "Damn the torpedoes. Full speed ahead." Selectivity must be our goal and understanding its basis, our guide to the future.

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