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ACYCLOVIR – A REVIEW OF THE PRECLINICAL AND EARLY CLINICAL DATA OF A NEW ANTIHERPES DRUG

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A review of the literature on acyclovir (Zovirax), a new antiherpes drug, with particular activity against herpes simplex virus types I and II and also against varicella-zoster, Epstein–Barr, cytomegalo and herpes B viruses, is presented. The article deals with 'in vitro' and 'in vivo' efficacy in animals, animal toxicity, latency and resistance, the mechanism of action and early clinical experience.

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INTRODUCTION

The discovery of acyclovir (previously known as acycloguanosine), was first reported by Elion et al. [20] in late 1977. They reported that the studies of Schaeffer et al. [49] had shown that acyclic side chains may substitute for sugar moieties in binding to enzymes. In a continuation of these studies they synthesised the 9-hydroxyethoxymethyl derivatives of adenine, diaminopurine and guanine [48]. These compounds showed activity against herpes simplex virus type 1 (HSV-1) in a plaque reduction assay at a dose required for 50% inhibition (ED_{50}) of 34, 12 and 0.1 μM respectively. Moreover, the guanine derivative was essentially non-toxic to the host Vero cells, showing an ED_{50} of 300 μM and representing a therapeutic index of approximately 3000 in these cells. This compound, 9-(2-hydroxyethoxymethyl)guanine, approved name acyclovir (aciclovir) and registered trade name Zovirax, is the subject of the following review. The discussion will be confined to effects relevant to human virus infections.

IN VITRO ACTIVITY

The activity of acyclovir against HSV-1 in Vero cells was first reported by Schaeffer et al. [48]. They compared the activity with other antiherpes compounds and demonstrated that the ED_{50} for acyclovir was 0.1 μM, but 0.2, 1.0. 1.5, 16 and 57.5 μM for cytarabine, idoxuridine, trifluorothymidine, vidarabine and phosphonoacetic acid respectively. In follow-up studies, Collins and Bauer [11] demonstrated that the sensitivity

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of HSV-1 and HSV-2 were very similar with a range of 0.05–0.14 μM for 10 strains. Using HeLa cells, the ED_{50}s shifted to a range of 0.36–0.74 μM and similar shifts were seen with the other compounds tested. Further studies with HSV have essentially confirmed these findings. However, Crumpacker et al. [15] showed a difference in ED_{50} values for HSV-1 and HSV-2 of approximately 10-fold. Seventeen clinical isolates of HSV-1 were tested in Vero cells and the ED_{50}s ranged from 0.06 to 0.35 μM. However, the ED_{50}s of 10 clinical isolates of HSV-2 ranged from 0.46 to 3.00 μM. De Clercq et al. [18] reported activities for a wide range of antiherpes compounds against 11 strains of HSV-1 and 7 strains of HSV-2. The mean ED_{50} was 0.04 μg/ml for acyclovir against both types. E-5-(2-bromovinyl-2′-deoxyuridine (BVDU) showed a greater activity against HSV-1, mean 0.008 μg/ml, but a lower activity against HSV-2, mean 1 μg/ml. 2′-Fluoro-5-iodoaracytosine (FIAC) had approximately the same activity against HSV-1, mean 0.02 μg/ml, and HSV-2, mean 0.05 μg/ml.

Harmenberg [28] demonstrated the importance of the host cell line used, and this combined with factors such as quantity of virus and number of passages of both virus and host cell line make predicting adequate in vivo blood levels from in vitro data extremely uncertain.

Schaeffer et al. [48] reported that acyclovir was also active against varicella-zoster virus (VZV). Biron and Elion [2] found that the ED_{50}s for six VZV isolates were 1.25–2.50 μM and that in longer term assays [3] they increased to 2.06–6.28 μM. Studies comparing the effect of acyclovir on HSV and VZV in the same system suggest that VZV is 2–8 times less sensitive than HSV [3]. Crumpacker et al. [15] also demonstrated a similar ED_{50} value against VZV of 3.75 ± 1.30 μM.

Colby et al. [10] showed that acyclovir could reduce the number of Epstein–Barr virus (EBV) genome equivalents per cell in the EBV producer cell line P3HR-1 giving an ED_{50} value of 6 μM. Human cytomegalovirus (CMV) has been reported to be rather resistant to the inhibitory action of acyclovir in vitro [15]. However, Tyms et al. [57] have shown CMV to be sensitive to acyclovir at ED_{50} values of 10.2–77.6 μM. Herpes B virus was inhibited in vitro with 90% inhibition being demonstrated at 4.4 μM [5]. Acyclovir has been shown not to be active against vaccinia, adenovirus type 5, and a range of RNA viruses comprising rhinovirus 1B, Mengo, Semliki Forest, Sindbis, Bunyamwera, yellow fever, measles, respiratory syncytial virus and NWS strain of influenza virus [48].

MECHANISM OF ACTION

Elion et al. [20] in radioactive tracer experiments showed that acyclovir was largely converted to the mono, di- and triphosphates in Vero cells infected with HSV, whereas uninfected cells or cells infected with vaccinia virus did not show significant phosphorylation. Acyclovir triphosphate (ACV-TP) was detectable 2 h after infection and reached a maximum at 8 h. At 0.5 μM, a concentration above that required to suppress HSV plaque formation totally, there was a 40-fold greater concentration of ACV-TP than in the uninfected cells.

In a series of elegant experiments [20,26] the enzyme responsible for the phosphorylation of acyclovir was rather surprisingly found to be the HSV-specified thymidine kinase and not deoxyguanosine kinase. Confirmatory evidence was acquired by using the thymidine kinase-deficient mutant of the KOS strain of HSV which was shown not to phosphorylate acyclovir at a greater rate than normal cells. Using isolated HSV and cellular DNA polymerases [24] the apparent K_i of ACV-TP for the HSV DNA polymerase was 0.08 μM (approximately 1/30th of the concentration for the α-DNA polymerase of the host cell (2.1 μM)). There is evidence that ACV-TP not only inhibits DNA polymerase activity by competition with deoxyguanosine triphosphate but is also capable of preventing further DNA synthesis by being incorporated as a DNA chain terminator [24]. Similar data from experiments with VZV demonstrated that ACV-TP was indeed generated in VZV-infected cells and that the VZV-specified thymidine kinase was responsible for the phosphorylation. The VZV-specified enzyme appears to be somewhat less effective than the HSV-1-specified enzyme [25]. Experiments on the mechanism of the anti-EBV effect would suggest that ACV-monophosphate is only generated at a slow rate in EBV producer cells; however, the di- and triphosphates are generated more effectively than in control cells [9]. Furthermore, the EBV-specific DNA polymerase is sensitive to acyclovir-TP with a K_i of 0.015 μM similar to that of HSV [17].

RESISTANCE

Elion et al. [20] showed that acyclovir was not phosphorylated to its monophosphate by the thymidine kinase-deficient mutants (TK^-) of the KOS strain of HSV-1. Field et al. [23] described the induction of resistance to acyclovir, by passage in acyclovir, due to defective expression of the TK gene. Two separate groups [8,50] demonstrated that resistance to acyclovir could be due to gene differences at either the TK locus or the DNA polymerase locus. It appears to be considerably more difficult to generate DNA polymerase mutants than TK mutants by passage in the presence of acyclovir. Field and Darby [22] demonstrated, however, that TK^- resistant strains were found to be less virulent when injected into mice. Recently, Darby et al. [16] reported the existence of a TK^+ strain with altered substrate specificity that was resistant to acyclovir and still virulent when injected into mice.

The demonstration that resistance development occurs readily in vitro does not necessarily imply that this will be a problem in vivo. The majority of clinical problems are due to reactivation of latent virus. It is unlikely that even if resistance developed on treatment during a clinical relapse the next reactivation would then be with a resistant virus as the template for that virus is believed to be from latent virus in the ganglia. Situations in which resistance could theoretically be a problem are: 1) treatment of a primary infection where establishment of a resistant latent infection might occur; 2) acquisition of a primary infection from an individual on treatment; 3) an immunodeficient patient where chronic viral replication is continuing despite therapy. These hypothetical situations need to be carefully monitored during early experience with the drug.

LATENCY

Acyclovir was first shown to provide some protection against the establishment of ganglionic latency by Field et al. [21] in experiments with BALB/c mice. They gave 50 mg/kg per day by intraperitoneal injection for 10 days from the day before virus inoculation. They were unable, however, to effect the virus once latency had been established. Klein et al. [34] produced similar results in hairless mice using 5% topical ointments. They were able to demonstrate complete protection against the establishment of latency when treatment was started 3 h after inoculation. Protection, however, was only partial if treatment was delayed until 24 h after inoculation. They were unable to prevent the establishment of latency by intraperitoneal injection of 25 mg/kg twice daily for 4 days commencing 3 h after inoculation. The same group [35] demonstrated that acyclovir-resistant mutants did not establish an inducible latent infection. Pavan-Langston and Park [43,46] reported that in CD-1 mice a daily dose of 60 mg/kg for 5 days subcutaneously produced partial protection (78%) against latency establishment when given 3 h after inoculation and rather less when instituted 24 h after inoculation (46%). More surprisingly, they demonstrated a progressive decrease in latency following increasing length of therapy starting 3 weeks after inoculation, 100% after 5 days treatment, 33% after 10 days and 12% after 15 days. This appears to contradict the data of Field's [21], Klein's [34] and Blyth's [4].

Blyth et al. [4] demonstrated that the therapy of 100 mg/kg twice daily subcutaneously in Swiss white mice would prevent the induction of a recurrence by sellophane tape stripping, but was unable to influence the established latent infection.

Important differences exist between the different systems, though it can be concluded that acyclovir may prevent the establishment of latency if given up to 24 h after inoculation. Further experimental evidence is required before the effect of treatment on established latency can be conclusively determined. These findings will then need to be confirmed in man during the course of careful clinical studies as the biology may differ in several important respects.

ACTIVITY IN ANIMAL MODELS

Schaeffer et al. [48] reported efficacy in three animal models; herpes simplex encephalitis in the mouse, herpes simplex keratitis in the rabbit and herpes simplex skin lesions in the guinea pig.

Acyclovir has been shown to produce rapid healing of herpes keratitis of the rabbit eye when given as a 3% ointment. It has proved to be either as effective as iododeoxyuridine [1,30,52] and trifluorothymidine [30] or superior to trifluorothymidine [1], vidarabine [1,45] and idoxuridine [42,45]. It has also been shown to be effective if given intravenously 50 mg/kg twice daily for 4 days [1] or 6 days [30]. Protection from a fatal outcome due to encephalitis following inoculation of herpes simplex into the eye has also been demonstrated using both topical [42] and intravenous acyclovir [30,42].

A clear therapeutic effect has been demonstrated in herpes encephalitis in mice using oral doses of 100 mg/kg twice daily for 5 days [49] or continuous oral doses of 400 mg/kg/day for 7 days [32]. Subcutaneous dosing of 100 mg/kg/day for 4 days was also shown to have an effect [44]. Topical therapy has been shown to be highly effective against herpes simplex skin lesions in mice [33,48] and guinea pigs [48]. Acyclovir has also been demonstrated to be effective in herpes B virus infection in the rabbit [5].

PRECLINICAL TOXICITY

The high specificity of acyclovir for the inhibition of herpes DNA replication as compared with normal cells confers a considerable safety margin for its use in the whole animals. Safety studies were first mentioned by Schaeffer et al. [49]. The LD_{50} in mice was reported as greater than 10,000 mg/kg by the oral route and 1000 mg/kg by the intraperitoneal route. Thirty day dosing of 450 mg/kg by the oral route produced no toxicity. Rats tolerated 80 mg/kg/day i.v. for 3 weeks; the only toxicity was related to blockage of nephrons by drug crystals, a physical phenomenon related to the limited aqueous solubility of the drug. Severe clinical effects were observed at 100 mg/kg/day i.v. for 31 days in dogs. The main histological effects were marked hypoplasia of the gastrointestinal mucosa, lymphoid tissue and bone marrow. There were also mild degenerative and regenerative renal changes. Doses of 50 mg/kg/day only produced mild reversible toxicity [56].

No toxicity was demonstrated in the rabbit eye by either systemic or topical administration [1]. Furthermore, acyclovir has been shown not to inhibit the healing of artificial wounds in the rabbit eye, a system in which vidarabine, vidarabine monophosphate, idoxuridine and trifluorothymidine all inhibit healing [37]. Acyclovir did not inhibit blastogenic or cytotoxic responses induced by phytohaemagglutinin, pokeweed mitogen or concanavalin A [54].

INTRODUCTION TO MAN

Following an extensive evaluation of the toxicity of acyclovir in cell systems and animals, it was decided that it was safe to cautiously expose man to the drug. One of the main problems that had emerged during the animal studies was the variability between species in absorption of acyclovir from the gut. It was, therefore, important to evaluate intestinal absorption in man.

Studies were carried out in normal volunteers raising the dose from an initial 5 mg up to 200 mg five times a day for 5 days. The results of these studies confirmed that the drug was only partially absorbed from the human intestinal tract and the maximum absorption appeared to be dose-limited. From these studies, bearing in mind problems of patient compliance, a dose of 200 mg five times a day appears to produce optimal blood levels. The mean peak blood level rose to around 4 μM, a concentration over 10-fold higher than the ED_{50} for HSV in vitro [7].

Careful pharmacokinetic studies have also been carried out by the intravenous route both in normal volunteers and in volunteer patients. In a normal volunteer study, 50 mg was given by bolus intravenous injection (over 2 min) at a mean dose of 0.73 mg/kg. Pharmacokinetic parameters were calculated from the plasma decay curve. A mean plasma half-life of 2.9 h was observed, renal clearance exceeded creatinine clearance, suggesting that tubular excretion contributed, in addition to glomerular filtration, to the elimination of acyclovir and the majority of the drug was recovered in the urine [6]. These findings in normal volunteers have been essentially confirmed in patients. De Miranda et al. [19] demonstrated dose-independent kinetics at doses ranging from 0.5 to 5.0 mg/kg infused over 1 h. Peak plasma level after a 1 h infusion of 5 mg/kg was 33.7 ± 7.1 μM and the half-life ranged from 2.2 to 5 h. Again total body clearance was high confirming the findings in normal subjects. Urinary recovery ranged from 30% to 69% and small amounts of metabolite 9-carboxymethoxymęthyl-guanine were demonstrated in the urine. The same group [60] have recently reported studies in patients who received full therapeutic courses of the drug up to doses of 15 mg/kg every 8 h for 5 days. Mean peak plasma levels in this study were 28.8 ± 1.9, 51.6 ± 2.7 and 61.1 ± 5.1 μM following 1 h infusions of 5, 10 and 15 mg/kg respectively. Trough levels 7 h after the end of infusion were 3.0, 6.4 and 7.4 μM. Plasma half-lives varied from 2.7 to 3.9 h.

CLINICAL STUDIES

Ophthalmic

The first indication in man that acyclovir could achieve antiviral effects came from a study reported by Jones et al. [29]. He carried out a double-masked placebo-controlled trial in patients with dendritic ulcers. Each patient received minimal wipe debridement of their ulcer followed by either active or placebo ointment five times per day for 7 days. They were then carefully observed for early recrudescence occurring within 7 days of debridement. There were seven recrudescences in 12 patients receiving placebo and none in the 12 patients receiving acyclovir. This result was statistically significant.

Several reports have now appeared of further trials with 3% acyclovir ophthalmic ointment in patients with dendritic ulcers. Coster et al. [14] demonstrated that acyclovir ointment was at least as good as idoxuridine ointment, and Collum et al. [12] showed a clear superiority of acyclovir ointment over idoxuridine. McGill et al. [39] have shown superiority over vidarabine (Ara A). However, La Lau et al. [36], in a study with fewer patients, reported no significant advantage over trifluorothymidine, although the trend favoured acyclovir. Acyclovir has been shown to penetrate into the aqueous humor when applied as the 3% ophthalmic ointment. The levels obtained are well in excess of in vitro antiviral levels [47].

Ophthalmic toxicity

Morgan et al. [40] reported that adverse effects seen in a toxicity tolerance study appear to be due to ointment base. To date only mild transient stinging on application of the ointment and punctate keratitis, which has not been considered severe enough to withdraw treatment from the patient, have been reported [14,29].

INTRAVENOUS STUDIES

Several anecdotal reports have now appeared in the literature claiming that acyclovir is effective in herpes simplex pneumonia following bone marrow transplantation [27], orolabial and oesophageal herpes simplex and varicella-zoster infections in the immune compromised [38,41,51,55,58] and varicella pneumonia [59]. These anecdotal reports need to be treated with caution. A wide-ranging clinical trial programme is now underway to attempt to define the clinical role of intravenous acyclovir.

SYSTEMIC TOXICITY

In general, the drug has been used without serious adverse effects [31]. Two cases in whom blood urea increased during therapy were reported by Selby et al. [51]. Both of these patients received 5 mg/kg every 8 h for 5 days. Reduction in drug dose and an increase in water intake resulted in the returning towards normal of their blood urea. Several further cases have come to our attention where blood urea and creatinine have risen abruptly following intravenous bolus injections of 5 mg/kg and, more particularly, 10 mg/kg. Experience in the United States at these doses where the drug has been given by slow infusion over 1 h has shown no abnormal changes in these parameters. One case has been reported by Whitley et al. [60] at a dose of 15 mg/kg. Another case has recently been reported to us where a patient being treated at 10 mg/kg by slow infusion over 1 h also showed a modest rise in blood urea; however, this case was special in that the patient was being intentionally dehydrated as part of the treatment for herpes encephalitis.

The observations of renal toxicity have been unusual in that blood urea and creatinine rise rapidly within 24–48 h of starting therapy and in some cases have been observed to return towards normal despite continuing therapy. All cases have returned to pretreatment levels following cessation of therapy.

It has become clear that in animals at least the mechanism of nephrotoxicity is by the development of drug crystals in the renal tubules. This can clearly be shown to be dose-related, and it is our belief that this is the mechanism of the problem in man and that in a satisfactorily hydrated patient with normal renal function doses of 10 mg/kg given by slow intravenous infusion over 1 h at 8 hourly intervals should not produce this problem. Considerable care needs to be exercised in the therapy of dehydrated patients or patients with abnormal renal function. In any event, all patients treated with intravenous acyclovir should be carefully monitored for abnormal renal function.

Several other adverse events have occurred during therapy, but no other clear pattern of drug-related toxicity has emerged. In particular, patients with impaired bone marrow function appear to tolerate acyclovir well without further depression of their haematological indices [51].

TOPICAL ACYCLOVIR

Recently, reports of trials of topical acyclovir (5% acyclovir in polyethylene glycol base) have appeared. Spruance et al. [53] reported the results of a trial in herpes libialis, where 172 clinical case records and 100 virological case records were analysed. A statistically significant effect on virus excretion, both when analysed by the rime of virus excretion and reduction in titre of virus, was observed. No effect, however, was seen on the clinical course of the disease when comparing active drug with placebo. Corey et al. [13] reported on a trial in genital herpes which demonstrated a significant clinical effect in primary disease, but only a clear virological effect in recurrent disease. The results are encouraging, as these diseases have been particularly resistant to a number of other topical agents previously tested, and suggest that when treatment is started earlier, at the time of the onset of symptoms or signs, clear clinical benefit may occur even in recurrent episodes. Trials are now underway where therapy will be initiated by the patient and the results of these are awaited.

CONCLUSIONS

Acyclovir has proved to be a highly active drug against herpes simplex virus, and is also active against varicella-zoster, Epstein–Barr, cytomegalo and herpes B viruses in vitro and, where animal models exist, in vivo. The prevention of latent infection and eradication of established latency due to these viruses remain a controversial issue.

The ophthalmic ointment is clearly effective in man against herpes simplex infections of the eye. An extensive clinical trial programme is currently in progress, and it remains to be seen whether acyclovir will be shown to be an effective drug for the systemic and topical treatment of both mucocutaneous and systemic infections by viruses of the herpes group. However, early data are encouraging and acyclovir does appear to be a safe drug when used as recommended.

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