Potent In Vivo Antiviral Activity of the Herpes Simplex Virus Primase-Helicase Inhibitor BAY 57-1293

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BAY 57-1293 belongs to a new class of antiviral compounds and inhibits replication of herpes simplex virus (HSV) type 1 and type 2 in the nanomolar range in vitro by abrogating the enzymatic activity of the viral primase-helicase complex. In various rodent models of HSV infection the antiviral activity of BAY 57-1293 in vivo was found to be superior compared to all compounds currently used to treat HSV infections. The compound shows profound antiviral activity in murine and rat lethal challenge models of disseminated herpes, in a murine zosteriform spread model of cutaneous disease, and in a murine ocular herpes model. It is active in parenteral, oral, and topical formulations. BAY 57-1293 continued to demonstrate efficacy when the onset of treatment was initiated after symptoms of herpetic disease were already apparent.

During the last 50 years, the treatment of herpesvirus infections has been continuously refined. Following the discovery of iodoxuridine in the mid-1950s and its successful demonstration as a topical therapeutic agent for herpes simplex virus (HSV) keratoconjunctivitis, vidarabine was licensed for systemic use and approved for the treatment of HSV encephalitis in 1978. Since it was first approved in 1981, the guanosine analogue acyclovir and later its L-valyl ester prodrug valacyclovir have been widely used in the treatment of HSV infections. Additional compounds used to treat HSV infections are famciclovir, the prodrug of penciclovir; ganciclovir; foscarnet; and cidofovir.

Nevertheless, a high medical need exists for improved antiherpetic drugs for the treatment of severe disease. Encephalitis in newborns, for example, results in 15% mortality, and only 29% of survivors develop normally after acyclovir therapy (22). Also, for patients with less severe disease, an agent that will achieve a better reduction of lesion duration with episodic treatment beyond the 1 to 2 days reduction achieved with current medications is urgently required (16). Furthermore, a drug which continues to show profound efficacy when given at a later stage of herpetic disease would be a new and highly desired standard in the treatment of herpes (10). BAY 57-1293 (N-(5-[(aminosulfonyl)-4-methyl-1,3-thiazol-2-yl]-N-methyl-2-[4-(2-pyridinyl)phenyl]acetamide) (Fig. 1) is a member of the thiazolylsulfonamides, a recently discovered class of non-nucleosidic compounds with potent antitherpetic activity in vitro and in vivo, based on a novel mechanism of action (11a). It inhibited the replication of HSV type 1 (HSV-1) and HSV-2 in Vero cells with a 50% inhibitory concentration (IC50) of 20 nM and a selectivity index of 2,500 and had about equal potencies against different strains and clinical isolates, while under the same assay conditions, acyclovir exhibited an IC50 of 1 μM and a selectivity index of 250. BAY 57-1293 targets the viral primase-helicase complex and inhibits its ATPase activity in a dose-dependent manner with an IC50 of 30 nM. Resistant viral mutants exhibited amino acid substitutions in viral UL5 and/or UL52, which code for components of the viral primase-helicase complex. Accordingly, BAY 57-1293 also is active against acyclovir-resistant mutant strains which carry mutations in the tk or DNA pol genes. The compound showed favorable pharmacokinetics in all species investigated (mouse, rat, and dog), with an oral bioavailability of >60% and an elimination half-life of >6 h. In the study described here we have examined the activities of BAY 57-1293 in various rodent animal models of herpetic disease.

MATERIALS AND METHODS

Virus strains, tissue culture, and animals. The viral strains used in this study were HSV-1F (ATCC VR-733), HSV-1walki (laboratory stock), HSV-2MS (ATCC VR-540), and HSV-2G (ATCC VR-734). Virus stocks were grown on Vero (ATCC CCL-81) cells. After a cytolytic effect was evident, the cells were frozen-thawed several times; the cell debris was removed by centrifugation; and the supernatant was aliquoted, titrated, and kept at −80°C. Mice and rats were purchased from a commercial supplier (M&B A/S, Bomholtvej, Denmark) and kept under standard conditions.

Lethal challenge model in mice and rats. A total of 50 μl of virus suspension (HSV-1walki or HSV-2MS) in ice-cold phosphate-buffered saline (PBS; ~5 × 106 PFU) was applied to the nares of BALB/cABom female mice (weight, 19 g; age, 7 weeks) lightly anesthetized with ether. The injection resulted in a mortality rate of 90 to 100% due to disseminated disease after 6 to 10 days. Ten mice from each group were used. LEW/Mol female rats (weight, 150 to 200 g) were used for the experiments with rats. A total of 200 μl of virus suspension (HSV-1walki) in ice-cold PBS (~2 × 106 PFU) was applied to the nares of rats lightly anesthetized with ether. The injection resulted in a mortality rate of 90 to 100% after 6 to 10 days. Five rats from each group were used.

In all cases, the infected animals were inspected daily for signs of disease (encephalitis, paralysis), and moribund animals were euthanized. Mortality was recorded over a period of 21 days postinfection. All of the compounds tested have sufficient enteral bioavailabilities in mice and rats (5, 11a).

Zosteriform spread model. C57/B16Bom-hr female mice (weight, 18 g; age, 7 weeks) were anesthetized with ether, and the lateral side of the body was scratched 10 times in a crossed-hatch pattern with a 27-gauge needle. A total of 10 μl of virus suspension (1 × 106 PFU of HSV-2G) was applied to the scarified area and rubbed in with a pipette tip. The mice were inspected daily; and disease severity was determined with the following scoring system: 0, no signs of infection visible; 1, vesicle formation; 2, slight zoster spread; 3, formation of large patches
of zoster; 4, confluently infected band; 5, hind limb paralysis; and 6, death. Moribund animals (e.g., those with a score of 5) were euthanized.

Ocular herpes model. BALB/cABom female mice (weight, 19 g; age, 7 weeks) were purchased (M&B A/S) and inoculated 1 week later. The mice were anesthetized with ether, and the right cornea was scratched three times vertically and three times horizontally with a sterile 30-gauge needle. A total of 5 μl of virus suspension (7.5 × 10^6 PFU of HSV-1 or 5 × 10^6 PFU of HSV-2G) was applied to the scarified cornea. The mice were inspected daily for signs of herpes infection (blepharitis, keratitis, encephalitis). Moribund animals were euthanized.

Antiviral compounds and treatment regimen. BAY 57-1293 was synthesized at Bayer AG (Leverkusen, Germany) and micronized with a conventional air-jet mill. Acyclovir was purchased as Zovirax injection flakes (GlaxoWellcome), valacyclovir was purchased as Valtrex film tablets (GlaxoSmithKline), famciclovir was purchased as Famvir (Novartis Pharma), ganciclovir was purchased as Cymeven injection flakes (Roche), and brivudine was purchased as Helpin tablets (Berlin-Chemie). For some experiments valacyclovir which had previously been extracted and purified from Valtrex tablets was used. For oral treatment, the compounds were suspended in 0.5% methylcellulose. Virus plaques were counted after 72 h of incubation.

At day 5 postinfection, BAY 57-1293 (60 mg/kg t.i.d.)-treated and placebo-treated animals were killed and total DNA was prepared from their trigeminal ganglia. As shown in Fig. 3, HSV DNA could be detected by Southern blotting analysis in samples derived from placebo-treated animals. In contrast, no viral DNA could be detected by this method in animals treated with BAY 57-1293. By PCR and reverse transcription-PCR, however, it was possible to detect trace amounts of viral DNA and RNA (data not shown).

<table>
<thead>
<tr>
<th>Compound</th>
<th>HSV-1</th>
<th>HSV-2</th>
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<tbody>
<tr>
<td>BAY 57-1293</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ganciclovir</td>
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<td>1.0</td>
</tr>
<tr>
<td>Valacyclovir</td>
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<td>15</td>
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<td>16</td>
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<tr>
<td>Brivudin</td>
<td>37</td>
<td>&gt;60</td>
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Effects of BAY 57-1293 in a rat lethal challenge model. In order to ensure that the superiority of BAY 57-1293 is not restricted to the mouse, we established a rat lethal challenge model using Lewis rats and HSV-1walki. The results of a typical experiment are shown in Fig. 4. BAY 57-1293 exhibited profound antiviral activity in this model as well, and no toxic effects of BAY 57-1293 were apparent upon gross inspection. While the ED$_{50}$ of BAY 57-1293 was similar in the rat and in the mouse, valacyclovir showed decreased activity in the rat (Fig. 4).

Activities of BAY 57-1293 with once-daily dosing. Once-daily dosing of valacyclovir is successfully used as treatment for the suppression of genital herpes (15). We investigated whether once-daily dosing of BAY 57-1293 would suffice to protect animals in the HSV-2 murine lethal challenge model in comparison to the activity of valacyclovir. For both BAY 57-1293 and valacyclovir, the ED$_{50}$ increased by approximately a factor of 6 with the once-daily dosing regimen compared to that with the t.i.d. dosing regimen. Accordingly, in the once-daily dosing regimen, BAY 57-1293 clearly retained its superior activity compared to the activity of valacyclovir (Fig. 5a).

The detection in serum of antibodies which recognize a given pathogen is widely used as a means of diagnosis of a history of infection with that pathogen. Furthermore, it has previously been shown that treatment with acyclovir reduces anti-HSV antibody titers in serum (1). Therefore, at 4 weeks after infection we assessed the HSV-neutralizing activity in the serum of surviving mice that had been treated once daily from day 0 to day 4 postinfection with either 60 mg of valacyclovir per kg or escalating doses of BAY 57-1293. Figure 5b demonstrates that neutralizing anti-HSV antibody titers were higher in animals treated with 60 mg of valacyclovir per kg than...
in animals treated with 4 mg of BAY 57-1293 per kg. This is in accordance with the finding that valacyclovir-treated animals suffered from a higher HSV burden than the BAY 57-1293-treated animals.

Activities of BAY 57-1293 in the murine zosteriform spread model mimicking recurrent cutaneous herpetic disease. Intradermal infection of mice at the flank leads to local virus replication followed by the entry of virus into the nerves innervating the skin and spread to the ganglion. After replication in the ganglion, the virus disseminates along the sensory nerves to cause infection 5 to 10 days later in the whole innervated dermatome, thereby generating a zosteriform lesion (18). This spread from the sensory ganglia to the innervated skin is a model for recrudescent disease. In order to more closely mimic the clinical situation, in which a patient usually sees a doctor only when herpes vesicles had already developed (Fig. 6a), animals infected with HSV-2 by dermal scarification developed progressive zoster lesions starting 3 to 4 days postinfection and later succumbed to a lethal encephalitis, such that all placebo-treated animals died by day 8 postinfection (Fig. 6b and c). Animals treated orally with 60 mg of valacyclovir per kg t.i.d. from day 3 to day 7 postinfection showed a healing of lesions, leading to a minimal disease score at day 12, after which, however, the animals started to show symptoms of encephalitis, and all animals died by day 19 postinfection. Such a rebound infection was also apparent in animals treated with 240 mg of valacyclovir per kg t.i.d., although to a lesser extent (three animals survived) (Fig. 6b and c). In marked contrast to the results obtained with valacyclovir, treatment with either 15 or 60 mg of BAY 57-1293 per kg t.i.d. resulted in a sustained therapeutic effect. After treatment was stopped, only one animal developed encephalitis and 90% of the animals survived (Fig. 6b and c).

Activities of BAY 57-1293 for topical treatment of mucocutaneous and ocular herpes. Treatment of recurrent mucocutaneous HSV infections in immunocompetent patients with topical antiviral formulations is widespread, although it is of only limited clinical benefit (13). In order to test for the topical effectiveness of BAY 57-1293, we assessed its activity in the murine zosteriform spread model using topical treatment with an ethanol-based formulation. Topical 2% BAY 57-1293 was compared with 2% acyclovir. The results are depicted in Fig. 7a. Topical BAY 57-1293 was highly effective and appeared to be more active than topical acyclovir.

The efficacy of BAY 57-1293 as a topical treatment for ocular HSV infections was assessed with an ophthalmic formulation. Animals were infected (Fig. 7b) via the scarified cornea and treated with eyedrops. All placebo-treated animals exhibited signs of disease (blepharitis, keratitis) at day 4 postinfection, developed encephalitis, and died by day 7 postinfection. Treatment with 2% topical acyclovir suppressed
disease progression during treatment. When treatment was terminated, however, signs of HSV infection became apparent and 3 of 10 animals (Fig. 7b) succumbed to infection. In contrast, treatment with 2% topical BAY 57-1293 prevented herpetic disease in all animals for the duration of the experiment (16 days). Among the animals infected with HSV-2, 9 of 10 animals treated with acyclovir died, while only 1 of 10 animals treated with BAY 57-1293 succumbed to infection (not shown).

Pathogenicities of HSV strains resistant to BAY 57-1293. Acyclovir resistance in HSV in most cases is associated with a deficiency in viral thymidine kinase (7). Such thymidine kinase-deficient HSV strains have been shown to be nonpathogenic in animal models (14). Mutant HSV strains, resistant to BAY 57-1293, exhibit mutations in UL5 and UL52, which code for components of the viral primase-helicase complex (11a). In order to study whether a BAY 57-1293-resistant viral mutant also exhibits altered pathogenicity in vivo, BALB/c mice were infected intranasally with $3 \times 10^4$ PFU of HSV-1F, HSV-1F resistant to acyclovir, and HSV-1F resistant to BAY 57-1293 (mutation in UL5 K356N). As shown in Fig. 8, wild-type HSV-1F and the BAY 57-1293-resistant mutant exhibited almost equal pathogenicities, while the acyclovir-resistant mutant was nonpathogenic, as judged by animal survival after infection. Other BAY 57-1293-resistant mutants might behave differently.

![Diagram](http://aac.asm.org/)

**FIG. 6.** Zosteriform spread model. Comparison of BAY 57-1293 with valacyclovir in the zosteriform spread model. C3H/TifBom-hr female mice were dermally infected with $10^6$ PFU of HSV-2G, and the disease score (the mean for 10 animals per group) was subsequently recorded on a daily basis by use of the scoring system described in Materials and Methods. Delayed oral treatment (t.i.d.) with 15 and 60 mg of BAY 57-1293 per kg and 60 and 240 mg of valacyclovir per kg was commenced on day 3 postinfection and continued until day 7 postinfection. (a) Zosteriform lesions for typical animals at the start of treatment (day 3 postinfection) and after 4 days of treatment (day 6 postinfection) with placebo, valacyclovir, or BAY 57-1293. Ten animals from each group were used. (b) The mean disease score for each treatment group was calculated at different times postinfection and plotted versus the time postinfection. The cumulative disease scores were calculated and compared between treatment groups by one-way analysis of variance. In order to account for multiple tests, the resulting $P$ values were adjusted by the Bonferroni-Holm method. Treatment with 15 mg of BAY 57-1293 per kg t.i.d. was more effective than treatment with 240 mg of valacyclovir per kg t.i.d. ($P < 0.001$). (c) Survival curves for the various treatment groups.

**DISCUSSION**

BAY 57-1293 showed excellent antitherpetic potency in a murine model of disseminated herpes, with an ED$_{50}$ of 0.5 mg/kg t.i.d. Five other antiviral compounds currently used for the treatment of HSV infections exhibited markedly lower activities in this murine model. The rank order of the potencies...
was BAY 57-1293 > ganciclovir > valacyclovir > famciclovir > acyclovir > brivudine. The ED_{50} published in the literature vary according to the model and treatment regimen used. The superiority of ganciclovir over acyclovir, as evident from our work, is well documented in various murine models in the literature (3, 9, 11). The activity of valacyclovir versus that of famciclovir has been a matter of debate (21), with a superiority of famciclovir being seen by some (20) but not by others (12). The reduced efficacy of famciclovir against HSV-2 compared with that against HSV-1, as also apparent from our data, however, is in accordance with results obtained in in vitro plaque reduction assays (8). The poor oral activity of brivudine, despite its strong in vitro activity against HSV-1, has also been described previously (9). The failure of brivudine in the HSV-2 lethal challenge model is reflected by its poor in vitro activity against HSV-2. These comparisons provide support for our model and the apparent superiority of BAY 57-1293 against HSV-1 and HSV-2 in vivo.

BAY 57-1293 was also markedly more active than valacyclovir (on a weight basis) in the rat (Fig. 4). Our ED_{50} of ~100 mg/kg t.i.d. for valacyclovir is in good accordance with the findings presented in a recent report in which only acyclovir doses greater than 100 mg/kg were able to reduce mortality in a rat model of HSV infection (2).

BAY 57-1293 was also applied topically with good results (Fig. 7a and b). We expect BAY 57-1293 to show efficacy in clinical trials of topical HSV treatment superior to those of the compounds currently used, whose therapeutic benefits in topical formulations have been questioned (13, 19).

Our data show that HSV infection is very susceptible to treatment with BAY 57-1293. Early treatment of primary herpetic disease with current antivirals in humans does not appear to reduce the level of latent DNA and thereby influence the frequency and severity of subsequent recurrent infections. In this regard, it is especially interesting that in contrast to treatment with valacyclovir, no detectable HSV replication took place in the neuronal tissue of BAY 57-1293-treated animals (Fig. 2). Pharmacokinetic measurements confirmed that the thiazolylsulfonamides are able to pass the blood-brain barrier.

BAY 57-1293 is efficacious even when treatment is initiated after the onset of symptoms in the murine zosteriform spread model of cutaneous infection (Fig. 6). This is of utmost importance for episodic treatment in recurrent herpetic disease when the patient initiates treatment only after first symptoms are apparent. The ability of BAY 57-1293 to be efficacious when treatment is delayed or the viral load is increased is also essential for successful treatment of life-threatening herpes infections like herpes encephalitis and disseminated herpes. In that regard, combination therapy with current nucleosidic DNA polymerase inhibitors and the nonnucleosidic primase-helicase inhibitor BAY 57-1293 is also an option.

The superiority of BAY 57-1293 in vivo can be explained by its inherently strong antiviral activity, with an IC_{50} in cellular assays of HSV replication of 0.02 μM, compared to an IC_{50} of 2 μM for acyclovir (the difference was even more pronounced when the multiplicity of infection was increased) and its favor-
able pharmacokinetic profile (oral bioavailability, >60%; half-life, >6 h) (11a). In addition, its physicochemical parameters (e.g., partition coefficient between egg-lecithin and PBS, 1,590) allow penetration of the blood-brain barrier and its use in topical formulations.

Even high doses of BAY 57-1293 were well tolerated in the animal experiments described here. As a primary sulfonamide, however, BAY 57-1293 is a weak inhibitor of mammalian carbonic anhydrase (IC50, 2 to 5 μM).

In summary, we showed that the thiazolylsulfonamide BAY 57-1293 is highly efficacious in the treatment of HSV-1 and HSV-2 infections in a variety of animal models and is superior to the drugs currently in use. Thiazolylsulfonamides therefore should have the potential to become new standards in the treatment of HSV disease.

ACKNOWLEDGMENTS


REFERENCES