Available online at www.sciencedirect.com
SCIENCE DIRECT®
Antiviral Research 65 (2005) 35–43
Antiviral Research
www.elsevier.com/locate/antiviral

The non-nucleoside antiviral, BAY 38-4766, protects against cytomegalovirus (CMV) disease and mortality in immunocompromised guinea pigs

Mark R. Schleiss[a,*,1], David I. Bernstein[a], Michael A. McVoy[b], Greg Stroup[a], Fernando Bravo[a], Blaine Creasy[b], Alistair McGregor[c], Kristin Henninger[d], Sabine Hallenberger[d]

[a] *Division of Infectious Diseases, Children's Hospital Research Foundation, 3333 Burnet Avenue, Cincinnati, OH 45229, USA*
[b] *Division of Infectious Diseases, Virginia Commonwealth University, Richmond, VA, USA*
[c] *Department of Molecular Genetics, University of Cincinnati School of Medicine, Cincinnati, OH 45229, USA*
[d] *Department of Virology, Bayer Pharmaceuticals, Tubingen, Germany*

Received 11 May 2004; accepted 21 September 2004

Abstract

New antiviral drugs are needed for the treatment of cytomegalovirus (CMV) infections, particularly in immunocompromised patients. These studies evaluated the in vitro and in vivo activity of the non-nucleosidic CMV inhibitor, BAY 38-4766, against guinea pig cytomegalovirus (GPCMV). Plaque reduction assays indicated that BAY 38-4766 was active against GPCMV, with an IC_{50} of 0.5 μM. Yield reduction assays demonstrated an ED_{90} and ED_{99} of 0.4 and 0.6 μM, respectively, of BAY 38-4766 against GPCMV. Guinea pigs tolerated oral administration of 50 mg/kg/day of BAY 38-4766 without evidence of biochemical or hematologic toxicity. Plasma concentrations of BAY 38-4766 were high following oral dosing, with a mean peak level at 1-h post-dose of 26.7 mg/ml ($n = 6$; range, 17.8–35.4). Treatment with BAY 38-4766 reduced both viremia and DNAemia, as determined by a real-time PCR assay, following GPCMV infection of cyclophosphamide-immunosuppressed strain 2 guinea pigs ($p < 0.05$, Mann–Whitney test). BAY 38-4766 also reduced mortality following lethal GPCMV challenge in immunosuppressed Hartley guinea pigs, from 83% (20/24) in placebo-treated guinea pigs, to 17% (4/24) in BAY 38-4766-treated animals ($p < 0.0001$, Fisher's exact test). Mortality differences were accompanied by reduction in DNAemia in Hartley guinea pigs. Based upon its favorable safety, pharmacokinetic, and therapeutic profiles, BAY 38-4766 warrants further investigation in the GPCMV model.

Keywords: Guinea pig; Cytomegalovirus; Antiviral therapy; CMV infection; Immunocompromised animal model; Real-time PCR; BAY 38-4766; Placenta

1. Introduction

Infection with human cytomegalovirus (HCMV) is ubiquitous, and generally asymptomatic (Britt and Alford, 1996). Certain patient populations, however, may develop life- and sight-threatening HCMV disease, usually in association with immunosuppression. Patients at risk for HCMV disease include immunocompromised solid organ and bone marrow transplant patients, and individuals with HIV infection (Schleiss, 2002). Congenital infection with HCMV also produces significant morbidity and mortality, and can cause long-term sequelae, including deafness (Demmler, 1996). Although vaccination may ultimately offer promise for protection against HCMV disease, there are as yet no licensed vaccines (Plotkin, 1999). Therefore, antiviral therapy represents the only intervention currently available for prophylaxis and therapy of HCMV in high-risk patient populations.

The first antiviral licensed for HCMV infection was ganciclovir. This nucleoside agent inhibits the viral polymerase, and is efficacious both following intravenous

* Corresponding author.
E-mail address: schleiss@umn.edu (M.R. Schleiss).
[1] Present address: American Legion Chair of Pediatrics, Division of Infectious Diseases and Immunology, University of Minnesota School of Medicine, 420 Delaware St. SE, MMC 296, Minneapolis, MN 55455, USA.

0166-3542/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.antiviral.2004.09.004

administration, or oral administration as the prodrug, valganciclovir (Kimberlin, 2002; Nichols and Boeckh, 2000; Griffiths, 2002). Unfortunately, both drug toxicities (particularly neutropenia) and the emergence of antiviral resistance limit the clinical utility of this agent (Chou, 1999). Alternative therapies, such as foscarnet and cidofovir, are available, but fraught with toxicities, in particular renal toxicity, limiting their usefulness (Schleiss and McVoy, 2004).

Recently, a novel class of non-nucleosidic antivirals has been developed which interfere with cleavage and packaging of viral DNA concatemers during the DNA replication process. One such agent, BAY 38-4766, has been shown to be well-absorbed and safe following oral administration, and is highly active against HCMV in vitro. Based on its favorable pharmacokinetic profile following oral dosing, its activity against HCMV, and its low toxicity, this agent may represent a useful candidate for further clinical trial evaluation in high-risk patients with HCMV disease (Reefschlaeger et al., 2001; McSharry et al., 2001; Weber et al., 2001; Buerger et al., 2001; Evers et al., 2002).

Ideally, candidate antiviral therapies for HCMV should be evaluated in animal models prior to human clinical trials. However, since CMVs are species-specific, preclinical efficacy evaluation of candidate HCMV antivirals unfortunately cannot easily be performed in animal models. This necessitates the study of CMVs of other species, in order to identify promising treatments in animal models which might prove to be of value for HCMV disease. The pathogenesis of guinea pig cytomegalovirus (GPCMV) infection shares many similarities with HCMV, including the clinical manifestations of pneumonitis and disseminated visceral disease, particularly evident in immunocompromised animals. Additionally, in contrast to the CMVs of most small animals, the guinea pig CMV (GPCMV) crosses the placenta, causing infection in utero, thus making the GPCMV model uniquely useful for antiviral evaluations which target the fetus (Bia et al., 1983). These features of the GPCMV model have proven to be very useful in the study of a number of experimental antivirals (Aquino-de Jesus and Griffith, 1989; Li et al., 1990; Feng et al., 1992; Bourne et al., 2000). In view of the antiviral activity which BAY 38-4766 exhibits against HCMV, and in light of the efficacy of the agent observed against disseminated disease in mice experimentally infected with murine cytomegalovirus (MCMV; Weber et al., 2001), these studies were undertaken to evaluate the in vitro and in vivo activity of BAY 38-4766 against GPCMV.

2. Materials and methods

2.1. Virus and cells

GPCMV (strain no. 22122, ATCC VR682) was propagated on guinea pig fibroblast lung cells (GPL, ATCC CCL 158) and maintained in F-12 medium supplemented with 10% fetal calf serum (FCS, HyClone Laboratories),

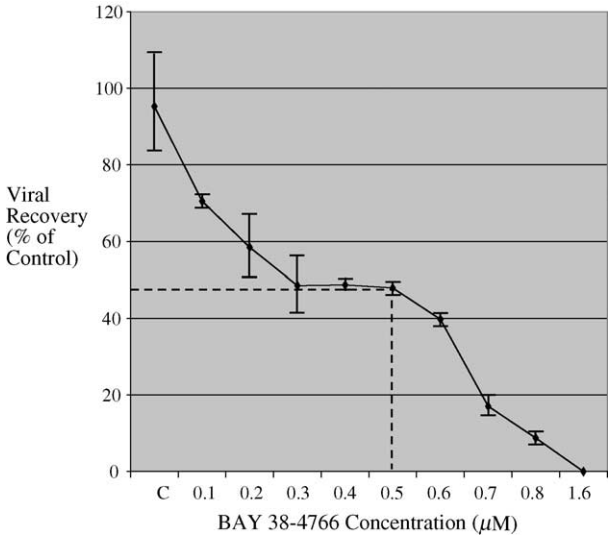

Fig. 1. Antiviral activity in vitro of BAY 38-4766 against GPCMV. Graphical depiction of IC_{50} determination (plaque reduction assay), as defined in Section 2. Range of concentrations (μM concentrations), horizontal axis. C, control inoculum of eGFP-expressing GPCMV. Data points represent mean (±S.D.) of plaque counts from four independent wells. Data are representative of four independent experiments. IC_{50} for BAY 38-4766 against GPCMV was determined to be 0.5 μM.

10,000 IU/I penicillin, 10 mg/l streptomycin (Gibco-BRL) and 7.5% $NaHCO_3$ (Gibco-BRL). An eGFP-expressing recombinant GPCMV, with replication kinetics identical to wild-type (ATCC) virus, was prepared as described elsewhere for in vitro antiviral testing (McGregor and Schleiss, 2001). Salivary gland workpools of GPCMV were prepared for in vivo studies as described elsewhere (Bia et al., 1983).

2.2. Analysis of in vitro activity of BAY 38-4766 against GPCMV

The compound, BAY 38-4766, was provided by Bayer® Pharmaceuticals (Tubigen, Germany). Compound was solubilized in DMSO, and diluted in F-12 media for in vitro testing, and in tylose (as described below) for in vivo administration. For in vitro testing, twofold dilutions of drug were made in F-12 media for determination of IC_{50} by plaque reduction assay, and EC_{90} and ED_{99} by yield reduction assay. The drug concentration determined to produce a 50% reduction in plaques was defined as the IC_{50}. Briefly, GPL cells were seeded in 24-well plates. Confluent cell monolayers were infected with 80–100 plaque-forming units (pfu) per well of eGFP-expressing GPCMV. After a 1.5-h adsorption period, a 0.5% methylcellulose-F12 media overlay containing dilutions of drug was added. Plaques were counted by fluorescence microscopy after 72–96 h of incubation. The number of plaques in treated wells was expressed as a percentage of untreated virus control and plotted against drug concentration (Fig. 1). Yield reduction assays were performed as described elsewhere (Hu and Hsiung, 1989). Briefly, the definitions employed were based on the drug concentration