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Efficacy of BMS-180194 against experimental cytomegalovirus infections in immunocompromised mice

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Abstract

A new antiviral nucleoside, BMS-180194 [1R-(1α,2β,3α)]-2-amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-1,9-dihydro-6H-purin-6-one, is a broad spectrum antiviral agent. The antiviral effectiveness of BMS-180194 against murine cytomegalovirus (MCMV) infection in immunocompromised C57BL/6 mice was investigated and was compared to that of ganciclovir (GCV). LP-BM5 murine retrovirus complex-induced immunocompromised C57BL/6 mice were challenged with MCMV then treated intraperitoneally or per os with various doses of BMS-180194 ranging from 30 to 3 mg/kg/day. When administered intraperitoneally, BMS-180194 was effective against MCMV-mediated mortality in a dose-dependent manner demonstrating a 50% protective dose (PD50) of 3.12 mg/kg/day which was comparable to that of GCV. There was a marked reduction in organ MCMV titers in BMS-180194-treated animals (10–10 000-fold lower than the placebo controls). Similar findings were observed when the compound was administered orally. Interestingly, oral BMS-180194 demonstrated a similar antiviral efficacy as that obtained by the parenteral route of administration suggesting a high oral bioavailability of the compound. Oral ganciclovir treatment, however, required more than a 4-fold higher amount of GCV to confer the same degree of protection obtained by a parenteral route of administration. Oral BMS-180194 was also effective in reducing the organ MCMV titer in genetically severe combined immunodeficient (SCID) mice. The parenteral or oral antiviral efficacy of BMS-180194 was comparable to that of parenteral ganciclovir against MCMV infection in the present study. Doses of BMS-180194 employed in the present study showed no toxicity to mice. These results suggest that BMS-180194 may be of value as an oral antiviral agent for treatment of opportunistic CMV infections in immunocompromised individuals.

Keywords: Murine cytomegalovirus; Cyclobutylguanine; Antiviral; Nucleoside; LP-BM5 retrovirus complex; Immunocompromised host

1. Introduction

Cytomegalovirus infection, like other herpesvirus infections, is common but is usually controlled by the host's immune system in adults.

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However, it may be a causative agent for major life-threatening diseases in immunocompromised individuals, newborns or infants born to infected mothers. HCMV is reported to be responsible for retinitis, pneumonitis, encephalitis, and gastrointestinal diseases especially in immunocompromised HIV-infected patients and in organ transplant recipients (Macher et al., 1983; Snider et al., 1983; Murray et al., 1984; Myers et al., 1986; Navia et al., 1986; Chachoua et al., 1987; Palestine, 1988; Rubin, 1990;).

Currently, intravenous (iv) formulations of ganciclovir (Cytovene, GCV, Syntex) and foscarnet (Foscavir, Astra) are the available therapeutic drugs for CMV infections, including CMV retinitis (Laskin et al., 1987; Walmsley et al., 1988). However, both of these drugs have two major disadvantages. They have to be administered intravenously due to their poor oral bioavailability and both exhibit dose-limiting toxicities. The most often reported dose-limiting side effect of ganciclovir therapy is myelocyte toxicity in which 40% and 20% of patients taking the drug develop granulocytopenia and thrombocytopenia, respectively (Faulds and Heel, 1990). In the patients who are on foscarnet therapy, nephrotoxicity is the major limiting factor.

An orally bioavailable anti-CMV agent with an improved toxicity profile would be of value as a therapeutic agent as well as a prophylactic or maintenance agent. BMS-180194, the active R-isomer of racemic cyclobutyl-G, is a new carbocyclic nucleoside analogue which possesses a broad spectrum of antiviral efficacy (it has been referred to in the literature as SQ 34 514, the active enantiomer of the racemate SQ 33 054, carbocyclic oxetanocin-G, or cyclobut-G). It is active against a variety of herpesviruses including herpes simplex virus-1 and -2 (HSV-1, HSV-2), human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) in culture (Braitman et al., 1991; Clement and Kern, 1991; Field et al., 1990). Importantly, this compound is equally active against ganciclovir (GCV)-resistant and GCV-sensitive clinical isolates of HCMV, and also active against acyclovir (ACV)-resistant clinical isolates of HSV-1, HSV-2 and VZV (Clement and Kern, 1991). Similarly BMS-180194 was also shown to be active against GCV- or foscarnet- or HPMPC-resistant MCMV strains (Smee et al., 1995). This agent is active in animal models of acute disseminated systemic infections of MCMV, HSV-1, and HSV-2 (Braitman et al., 1991).

Human CMV is highly host-specific that it does not result in a productive infection in other species of animals including commonly used laboratory rodents. Since various host of animals can be infected with a strain of CMV that is indigenous to that species, mice infected with murine CMV, which resembles many characteristics of HCMV, was used to evaluate antiviral agents against HCMV (Hudson, 1979; Kern, 1991). Human cytomegalovirus infection, unlike in immunocompetent adults, causes major life-threatening diseases in immunocompromised individuals. To better correlate animal experimental therapeutic results to treatments for human CMV diseases, it is recommended to evaluate treatments for CMV in immunosuppressed animal models (Laughlin et al., 1991).

Infection of C57BL/6 mice with the LP-BM5 murine leukemia virus complex causes an acquired immunodeficiency syndrome (AIDS)-like disease (MAIDS) which is similar to that observed in humans infected with the human immunodeficiency virus (Pattengale et al., 1982; Mosier et al., 1985; Morse III et al., 1988; Yetter et al., 1988; Hartley et al., 1989). Because of similarities to human AIDS, this MAIDS model has been employed to evaluate agents for treatment of opportunistic herpes simplex virus or cytomegalovirus infections (Gangemi et al., 1989; De Castro et al., 1991). Further, severe combined immunodeficient (SCID) mice, C.B-17-scid/scid, which lack both humoral and cell-mediated immune function due to a severe deficiency in numbers of functional T and B cells have also been used as an immunosuppressed animal model to study opportunistic virus infections (Neyts et al., 1992; Smee et al., 1992).

This study evaluates the therapeutic utility of BMS-180194 in the treatment of an MCMV infection in LP-BM5 murine retrovirus complex-immunosuppressed C57BL/6 mice and in

congenitally immunodeficient C.B-17- scid/scid mouse models which mimic CMV diseases in immunocompromised humans.

2. Materials and methods

2.1. Antiviral agents

BMS-180194, the active R-isomer of cyclobutyl-G, was solublized in phosphate buffered saline (PBS, pH 11) and stored at 4°C for the duration of the treatment. Ganciclovir (Cytovene®; GCV; Syntex, Palo Alto, CA., USA) was purchased from a local pharmacy and prepared according to the package insert.

2.2. Animals and viruses

Sixteen to 18 g female C57BL/6 mice and C.B17-scid/scid (SCID) mice were purchased from Harlan Sprague Dawley Inc., Indianapolis, IN, and Taconic Farms, Germantown, NY, respectively. The Smith strain of MCMV was originally obtained from the American Type Culture Collection, Rockville, MD. Five to 6 week old Swiss Webster mice were inoculated i.p with a sublethal dose of this virus, and their salivary glands were removed between 18 and 21 days post virus inoculation. A 10% wt/vol salivary gland tissue homogenate was prepared and the homogenate supernatant was titrated for the virus on a mouse embryo fibroblast monolayer. This material was used as a stock virus for animal inoculation experiments. The LP-BM5 murine retrovirus complex was obtained from John Bilello, Johns Hopkins University, School of Medicine, Baltimore, MD, propagated and maintained in a persistently-infected SC-1 cell line.

2.3. Cells

A primary mouse embryo fibroblast (MEF) cell line was prepared from 13 to 16 day old C57BL/6 mouse embryos and used within the 5th to 6th passage of the cells. SC-1 cell line, a feral mouse embryo fibroblast, was used for propagation of LP-BM5 virus complex.

2.4. Animal immunosuppression and MCMV infection

Murine acquired immunodeficiency syndrome (MAIDS) was induced in female C57BL/6 mice following an intraperitoneal inoculation of 1×10^5 syncytia forming units (sfu) of the LP-BM5 retrovirus complex. Splenomegaly and generalized lymphadenopathy were evident starting 8–12 weeks post virus inoculation. Splenocytes isolated from 13 week post LP-BM5 virus-infected mice were challenged with Con A and LPS. Splenocytes from the age-matched immunocompetent control mice responded well to Con A and LPS challenges demonstrating a stimulation index (SI) of 9.9 and 4.2, respectively. The parallel group of mice infected with LP-BM5 virus failed to respond to either of the two mitogens ($SI < 1.0$) indicating an immunosuppressive state of these cells. This was also reflected in the size of the lymph nodes (LN) measured. The mean of six submaxillary lymph nodes diameter was 8.2 mm in LP-BM5 virus-infected mice while LN in the age-matched uninfected control mice could not be measured (< 1.0 mm). Between 13 and 17 weeks post virus inoculation, when infected splenocytes were unresponsive to mitogenic stimulation, immunocompromised mice were pooled, then randomly selected and each mouse was challenged intraperitoneally with 3.7×10^6 plaque forming units (pfu) of MCMV. This inoculum resulted in 80–100% mortality in LP-BM5-immunosuppressed mice and 0–20% mortality in age-matched immunocompetent control mice. For the MCMV infection in SCID mice, 3.7×10^5 pfu MCMV was inoculated i.p per animal. This resulted in 80–100% mortality in the infected SCID mice with a mean day of death (MDD) of 8 days.

2.5. Drug treatment and antiviral efficacy endpoints

Ganciclovir and BMS-180194 were administered once daily by the intraperitoneal route or by oral gavage for five consecutive days starting 6 h post MCMV challenge. Effectiveness of the compounds were measured in terms of % survival/total number animals treated, an extension of the

mean day of death, and reduction in organ viral titers. Survival studies were carried out for 21 days post virus inoculation. Spleen, liver, and lungs were removed on days 6 or 21 post MCMV challenge for determination of the organ viral titers. A group of 10–15 mice was used for mortality studies and a group of 5–7 mice was used per time point for organ virus titers. Ten percent wt/vol tissue homogenates were prepared from organs removed from the appropriate experimental groups of animals for a virus titration on MEF cells.

2.6. Virus titration

Ten-fold dilutions of tissue homogenates were made and 0.25 ml of each dilution was adsorbed onto a MEF monolayer for 60 min. (24-well cluster plates; Costar, Cambridge, MA). After adsorption, the tissue inoculum was removed and the infected cells were overlaid with Eagle's minimal essential medium (EMEM) supplemented with a 5% fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin/streptomycin/Fungizone® mixture (GIBCO Laboratories, Grand Island, NY) and 1% methylcellulose. The infected cell cultures were incubated in a 37°C, 5% CO_2 incubator for 3 days until viral plaques were visible. Infected cell monolayers were stained with Ziehl-Neelsen carbol fuchsin and virus plaques counted.

2.7. Statistical Analysis

The Fisher exact test (Kleinbaum et al., 1982) was used for the evaluation of percent survival. The Gehan-Wilcoxon test (Lee, 1980) was used for the evaluation of the mean day of death. A P-value of < 0.05 was considered statistically significant. Virus titration data were analyzed using Van der Waerden scores. The analysis was performed using an nonparametric analysis. The Van der Waerden scores are approximate normal scores derived by applying the inverse normal distribution function to the fraction ranks. A standard normal (z) sample statistic was calculated to obtain the two sided P-values.

3. Results

3.1. MCMV susceptibility of LP-BM5 virus-induced immunocompromised mice

Since MCMV infection is self-limiting in normal adult mice yet is fatal in immunocompromised mice, it is important to identify a challenge viral dose which would not have a significant effect on the age-matched immunocompetent control mice but would cause a significant mortality in immunocompromised mice. Further, this challege virus dose should allow a window of opportunity to complete the drug therapy regimen in the immunosuppressed mice. Several doses of MCMV ranging from 1×10^5 to 1×10^7 plaque forming units per mouse were tested in normal and immunocompromised groups of mice. Up to 7.5×10^5 pfu per mouse caused no mortality in both groups. Doses of 1.86×10^6 and 3.72×10^6 pfu per mouse resulted in an 80 and 100% mortality with a mean day of death (MDD) of 14.3 ± 5.5 and 6.0 ± 1.4 days post infection (pi), respectively, in the 13–17 week post LP-BM5-infected host. These same doses caused only a 20% mortality in the age-matched immunocompetent control animals with a MDD of 6 and 5 days pi, respectively. We employed $1.86–3.72 \times 10^6$ pfu per mouse as the MCMV challenging dose for subsequent studies in C57BL/6 mice. For SCID mice, an optimal MCMV inoculation dose was determined in a similar manner. Doses of 10-fold increments ranging from 3.7×10^3 to 3.7×10^6 pfu per SCID mouse were tested. All doses resulted in 100% mortality in the infected mice while a MDD of 18.0 ± 0.63, 15.3 ± 1.4, 10.0 ± 1.1, and 5.0 ± 0.63 days pi was observed with challenge virus doses of 3.7×10^3, 3.7×10^4, 3.7×10^5, and 3.7×10^6 pfu per mouse, respectively. An inoculum dose of 3.7×10^5 pfu per animal was selected to allow the completion of 5 day antiviral therapy for the efficacy studies in SCID mice.

3.2. Antiviral efficacy of BMS-180194 by intraperitoneal administration

BMS-180194 demonstrated a similar antiviral activity against HCMV and an enhanced activity

against MCMV in culture when compared to ganciclovir (Braitman et al., 1991). To determine its antiviral efficacy against opportunistic MCMV infection in an immunocompromised animal model, randomly selected LP-BM5-immunosuppressed mice were MCMV challenged i.p. MCMV-challenged immunocompromised mice, ten in an experimental group except for the 3 mg/kg/day group (7 mice), were treated i.p once daily for five consecutive days starting 6 h after MCMV challenge with BMS-180194 or GCV. BMS-180194 was effective in preventing death with a PD50 of 3.12 mg/kg/day (Table 1).

The antiviral efficacy against MCMV-mediated death was dose-dependent. At 30 and 10 mg/kg/day, BMS-180194 protected 100% of the treated animals while 90% of the placebo-treated mice died with a MDD of 7.9 days pi. At 3 mg/kg/day, BMS-180194 protected 43% of the drug-treated mice ($P < 0.05$). In addition, a reduction of three to four orders of magnitude in MCMV virus titers was observed in the spleens and livers of animals treated with 30, 10, or 3 mg/kg/day dose compared to the placebo-treated group. In fact, 100% of the mice (five out of five tested) treated with 30 mg/kg/day showed no detectable level (< 2.69 log10 pfu/gram tissue) of virus in the liver, while three out of the five mice tested exhibited no viruses in the spleens. Because of the rapid progressive nature of MCMV infection model employed in the present study (MDD of 5.5–7.9 days), placebo-treated virus- challenged animals were euthanized and harvested organs for virus titer determination on day 6 post virus inoculation.

The antiviral effectiveness of GCV was comparable to that of BMS-180194 and was dose-dependent. In fact, the survival data with a PD50 of 3.12 mg/kg/day for GCV was identical to that of BMS-180194. Ganciclovir-treated mice also showed a marked reduction in organ virus, however, tissue viral titers of GCV-treated animals appeared to be slightly lower than that of BMS-180194-treated animals in general.

3.3. Oral antiviral efficacy of BMS-180194

An orally active CMV antiviral agent would be of significant therapeutic value. The oral antiviral efficacy of BMS-180194 was determined against MCMV infection in LP-BM5 immunosuppressed animals (Table 2). BMS-180194 was effective orally with a PD50 of 4.1 mg/kg/day which was comparable ($P < 0.05$) to the PD50 obtained with a parenteral route of administration. Eight out of ten treated mice survived at the 15 mg/kg/day dose while six and five out of ten mice in each group survived the infection when treated with 7.5 or 3.75 mg/kg/day, respectively. The drug treatment prevented death and extended the MDD ($P < 0.05$ for all doses) in a dose-dependent manner.

A marked reduction in virus titers was observed in spleens and livers of animals treated with all BMS-180194 doses employed (30 to 3.75 mg/kg/day range) in this study (Table 2). A statistically significant reduction in lung virus titer occurred only at the 30 mg/kg/day dose level.

Oral GCV was also effective in protecting mice from death with a PD50 of 13.4 mg/kg/day. At the 30 mg/kg/day dose, it protected all treated mice while 100% of the placebo infected control mice died with a MDD of 5.5 day pi. At 15 mg/kg/day, five out of nine-treated mice were protected from death with a MDD of 14.3 day pi. In contrast to the parenteral GCV antiviral efficacy (PD50 of 3.12 mg/kg/day), oral administration required greater than 4-fold higher amount of GCV (PD50 of 13.4 mg/kg/day) to protect 50% of the infected mice. Although all mice were protected from death at the top dose tested either via parenteral or oral route, the number of mice with undetectable virus levels in the tissues were higher in the mice given parenteral GCV compared to oral GCV. Virus reduction in the lungs was not as dramatic as it was in the spleens or livers.

3.4. Antiviral efficacy of BMS-180194 against MCMV infection in SCID mice

To demonstrate that BMS-180194 was also effective against an MCMV infection in a congen-

Table 1
Antiviral efficacy of BMS-180194 and GCV on survival and organ virus titers of MCMV-challenged LP-BM5 immunosuppressed C57BL/6 mice following intraperitoneal administration

Compound	Dose(mg/kg/day)	Survival (# live/total)	PD50[a] (mg/kg/day)	MDD[b]	MCMV Titer (Log10 PFU/gram tissue)[c]		
					Spleen	Liver	Lung
BMS-180194	30	10/10*	3.12 ± 0.26	—	3.38 ± 0.43* (3/5)[d]	<2.69 ± 0.00* (5/5)	3.20 ± 0.21* (2/5)
	10	9/9*		—	3.84 ± 0.50* (1/5)	3.43 ± 0.46* (2/5)	3.64 ± 0.34* (1/5)
	3	3/7*		7.0 ± 2.6	4.03 ± 0.19* (0/3)	4.22 ± 0.15* (0/3)	4.13 ± 0.20 (0/3)
GCV	30	10/10*	3.12 ± 0.27	--	2.78 ± 0.10* (3/5)	2.69 ± 0.00* (4/5)	2.85 ± 0.16* (3/5)
	10	9/10*		21.0	3.48 ± 0.36* (2/5)	3.07 ± 0.23* (1/5)	2.92 ± 0.22* (2/5)
	3	3/7*		9.0 ± 3.4*	3.30 ± 0.61* (0/3)	3.85 ± 0.19* (0/3)	3.52 ± 0.17* (0/3)
PBS	placebo	1/10	—	7.9 ± 3.2	6.79 ± 0.20 (0/5)	6.49 ± 0.24 (0/5)	4.82 ± 0.45 (0/5)

Compounds were administered intraperitoneally once a day for five consecutive days starting 6 h post MCMV challenge.
* P-value is <0.05 when compared to placebo virus control group.
[a]PD50, dose which is required to protect 50% of the treated animals.
[b]MDD, mean day of death ± S.D.
[c]Detectable level of assay is 2.69 log10 pfu/gram tissue. Mean ± S.D. of 5 mice except for the 3 mg/kg/day groups ($n = 3$).Organs were harvested on day 21 post MCMV infection. For the placebo animal virus titers, 5 extra mice were included in the group and organs harvested on day 6 post virus challenge prior to their death.
[d]Number in parenthesis, # mice with no detectable level of virus/total tested.

Table 2
Antiviral efficacy of BMS-180194 and GCV on survival and organ virus titers of MCMV-challenged LP-BM5 immunosuppressed C57BL/6 mice following oral administration

Compound	Dose (mg/kg/day)	Survival (# live/total)	PD50[a] (mg/kg/day)	MDD[b]	MCMV Titer (Log10 PFU/gram tissue)[c]		
					Spleen	Liver	Lung
BMS-180194	30	10/10*	4.1 ± 0.47	—	2.38 ± 0.47* (3/5)	2.54 ± 0.36* (2/5)	2.91 ± 0.47* (1/5)
	15	8/10*		14.5 ± 9.2*	2.99 ± 0.54* (2/5)	3.54 ± 0.51* (1/5)	4.38 ± 0.47 (0/5)
	7.5	6/10*		10.8 ± 6.2*	3.08 ± 0.41* (1/5)	3.25 ± 0.50* (1/5)	4.01 ± 0.32 (0/5)
	3.75	5/10*		8.2 ± 3.8*	3.41 ± 0.44* (1/5)	4.21 ± 0.34* (0/5)	3.97 ± 0.44 (0/5)
GCV	30	10/10*	13.4 ± 0.44	—	3.20 ± 0.45* (1/5)	3.33 ± 0.41* (1/5)	3.76 ± 0.45 (0/5)
	15	5/9*		14.3 ± 5.9*	2.26 ± 0.35* (3/5)	3.43 ± 0.44* (1/5)	3.63 ± 0.51* (1/5)
	7.5	2/9		11.6 ± 4.1*	3.04 ± 1.36* (1/2)	4.12 ± 0.48* (0/2)	4.71 ± 0.33 (0/2)
	3.75	2/9		7.7 ± 4.2	2.4 ± 0.71* (1/2)	2.49 ± 0.81* (1/2)	3.55 ± 0.15* (0/2)
PBS	placebo	0/8	—	5.5 ± 0.53	7.16 ± 0.23 (0/5)	6.99 ± 0.24 (0/5)	4.77 ± 0.25 (0/5)

Compounds were administered orally once a day for five consecutive days starting 6 h post MCMV challenge.
*P-value is <0.05 when compared to placebo virus control group.
[a]PD50, dose which is required to protect 50% of the treated animals.
[b]MDD, mean day of death ± S.D.
[c]Detectable level of assay is 1.69 log10 pfu/gram tissue. Mean ± S.D. of 5 mice except for the GCV 7.5 and 3.75 mg/kg/day groups ($n = 2$). Organs were harvested on day 21 post MCMV infection. For the placebo animal virus titers, 5 extra mice were included in the group and organs harvested on day 6 post virus challenge prior to their death.
[d]Number in parenthesis, # mice with no detectable level of virus/total tested.

Table 3
Antiviral efficacy of BMS-180194 and GCV on organ virus titers of SCID mice challenged with MCMV following oral administration

Compound	Dose (mg/kg/day)	MCMV titer (log10 pfu/gram tissue)[a]		
		Spleen	Liver	Lung
BMS-180194	30	4.4 ± 0.16*	3.8 ± 0.80*	3.6 ± 0.33*
	10	4.4 ± 0.23*	5.2 ± 0.82*	4.1 ± 0.30*
	3	4.9 ± 0.34	5.4 ± 0.74*	4.8 ± 0.16
GCV	30	3.8 ± 0.21*	4.4 ± 0.87*	4.1 ± 0.14*
	10	5.1 ± 0.63	5.1 ± 0.78*	4.7 ± 0.15
	3	5.8 ± 0.68	5.8 ± 0.54	5.1 ± 0.22
PBS	placebo	5.7 ± 0.24	6.6 ± 0.42	5.1 ± 0.25

Compounds were administered orally once a day for five consecutive days starting 6 h post MCMV challenge.
*P-value is <0.03 when compared to placebo virus control group.
[a]Each titer is a mean of 6–7 mice ± S.D. Organs of all groups were removed on day 6 post MCMV challenge prior to their death. Detectable level of assay is 1.69 log10 pfu/gram tissue.

itally immunocompromised host, we employed severe combined immunodeficient (SCID) mice. The effectiveness of BMS-180194 was determined by monitoring changes in tissue virus titers (Table 3). Major organs were removed for tissue virus titers on day 6 post MCMV inoculation before any death occurred in any of the experimental groups. When a 30 or 10 mg/kg/day dose was administered orally, a significant decrease in virus titer was evident in all organs examined. A statistically significant reduction was only evident in the liver in the 3 mg/kg/day dose group, nevertheless, the spleens and lungs also demonstrated a notable reduction in viral titers at this dose. Oral GCV treatment with a 30 mg/kg/day dose showed a significant reduction ($P < 0.03$) in virus titers in all organs tested. However, unlike BMS-180194, a statistically significant reduction was only evident in livers in the 10 mg/kg/day dose group while there was no significant decrease in virus titers in the 3 mg/kg/day dose group when administered orally.

4. Discussion

Our study demonstrated that BMS-180194 is an effective antiviral agent against MCMV infection in immunocompromised animal models. Similar antiviral efficacy was observed for BMS-180194 and GCV when they were administered parenterally. Oral administration of BMS-180194 was comparable in efficacy to that obtained by parenteral administration (PD50 of 4.1 mg/kg/day p.o versus 3.12 mg/kg/day i.p) suggesting that the compound is orally bioavailable in mice. In contrast, greater than a 4-fold higher amount of GCV given orally was required to confer the same degree of protection obtained by parenteral administration (GCV PD50 of 13.4 mg/kg/day p.o versus 3.12 mg/kg/day i.p). BMS-180194 administered orally was superior to oral GCV treatment in this infection model (PD50 of 4.1 mg/kg/day versus 13.4 mg/kg/day, respectively).

BMS-180194 and GCV, in general, were similar in their capability in reducing tissue virus titers by several orders of magnitude. However, it appeared that BMS-180194 was more effective in reducing organ viral titers when given orally. In contrast, GCV-treated animal tissue viral titers appeared slightly lower than that of BMS-180194 when given parenterally. Nevertheless both compounds were comparable in effectiveness in extending the mean day of death when administered orally.

It was previously reported that BMS-180194 was active against HCMV and MCMV in vitro as well as in primary MCMV infections in vivo (Braitman et al., 1991). It was well absorbed upon oral administration with an oral bioavailability of 80% in mice based on urinary recovery data. In contrast,

oral bioavailability of GCV was determined to be significantly lower (28%) in a concurrent study (unpublished data). It is interesting to point out that the calculated bioavailability of the two compounds based on parenteral and oral PD50 results in the current study correlated well with the bioavailability calculated based on urinary excretion data (BMS-180194; 76% vs 80% and GCV; 23% vs 28%, respectively).

Currently, intravenous ganciclovir and foscarnet are the two available therapeutic drugs for severe CMV-mediated diseases in immunocompromised individuals including patients with acquired immunodeficiency syndrome, undergoing organ transplantations, or receiving chemotherapy. Poor oral bioavailability is a major drawback for the clinical use of both of these drugs and they also have dose-limiting toxicities. De Miranda et al. (1986) reported that a single dose of 10 mg/kg GCV resulted in a 6% oral bioavailability. Repeated oral GCV administrations of 10 and 20 mg/kg given every 6 h resulted in 4.6% and 3% bioavailability, respectively, in patients with AIDS and CMV retinitis based on urinary excretion (Jacobson et al., 1987). In light of Jacobson's finding of reduced recovery of GCV upon oral administration of an increased dose, it appears that oral therapy employing larger doses of GCV may not be without difficulties.

In contrast, in the first human pharmacokinetic and bioavailability study of BMS-180194, Petty et al. (1994) reported that oral bioavailability of BMS-180194 was 40% with a possible saturable absorption. In a double-blind placebo-controlled safety and pharmacokinetic study employing single dose levels of 0.3, 1.0, 3.0, 6.0, and 10 mg/kg BMS-180184 involving eight asymtomatic HIV- and CMV-seropositive subjects per designated dose, Petty reported that all doses of BMS-180194 employed were well tolerated. In addition, they observed no drug-related granulocytopenia or thrombocytopenia in these patients. Granulocytopenia and thrombocytopenia are the most often reported dose-limiting side effect of GCV which affects 40% and 20% of patients taking the drug, respectively (Faulds and Heel, 1990).

In addition to its high oral bioavailability and its lack of observable myelosuppression in patients at the doses tested, an additional characteristic should also make BMS-180194 a valuable CMV antiviral drug. Increased incidences of virus resistance against approved antiviral agents is a significant concern in antiviral therapy. It is important to point out that BMS-180184 had a comparable efficacy against HCMV clinical isolates to that of GCV and it was also equally effective against GCV-resistant clinical HCMV isolates in vitro. Smee et al. (1995) evaluated several CMV antiviral compounds in culture to determine their cross-resistance. He found that cyclobutylguanine (BMS-180194) was the only agent which was equally effective against wild-type as well as GCV-, foscarnet-, or HPMPC-resistant strains of MCMV.

Our present findings suggest that BMS-180194 may be of value in the clinic as an oral CMV therapeutic antiviral agent. In addition, it may have utility as an oral prophylactic agent for CMV infections in various immunocompromised patients in the clinic.

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