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Pharmacokinetics and antiviral activity of a novel isonucleoside, BMS-181165, against simian varicella virus infection in African green monkeys

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Summary

A novel nucleoside analog BMS-181165 with potent activity against varicella-zoster virus was tested for efficacy in a simian varicella virus infection in African green monkeys. BMS-181165 was effective in preventing the development of a rash, decreasing the development of viremia and preventing death in infected monkeys when administered orally at 4, 16 or 64 mg/kg/day. The compound is well orally absorbed in monkeys, between 44 to 50% oral bioavailability, and may prove of value in therapy of varicella-zoster infections in humans.

Pharmacokinetics; Antiviral activity; BMS-181165; Simian varicella virus; African green monkey

BMS-181165, [3*S*-{3α(*E*),4β,5α}]-5-(2-Bromoethenyl)-1-[tetrahydro-4,5-bis(hydroxymethyl)-3-furanyl]-2,4(1H,3H)]pyrimidinedione (Fig. 1) is a highly selective inhibitor of varicella-zoster virus (VZV) with ED_{50} values in the 0.03 to 0.3 μM range (Tino et al., 1993) compared to ED_{50} values of 1–3 μM for Herpes simplex, type 1, and greater than 290 for Herpes simplex, type 2, and human cytomegalovirus, respectively. This activity against VZV suggested that the compound could be of value in therapy of VZV infections.

As there is no established animal model for VZV infection, a simian varicella

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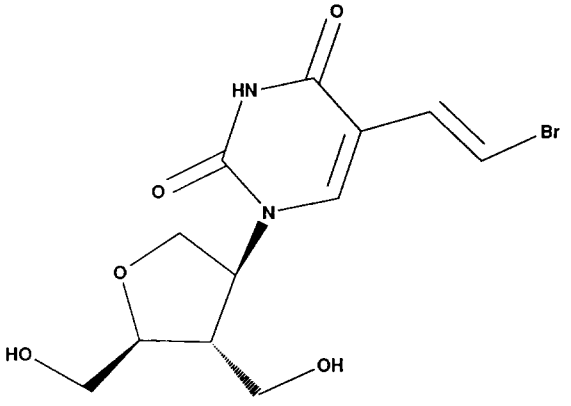

BMS-181165

Fig. 1. Chemical structures of BMS-181165 [3*S*-{3α(*E*),4β,50α}]-5-(2-Bromcethenyl)-1-[tetrahydro-4,5-bis(hydroxymethyl)-3-furanyl]-2,4(1H,3H)]pyrimidinedione.

virus (SVV) infection of African green (vervet) monkeys (*Cercopithecus aethiops*) was utilized. SVV infection of African green monkeys results in severe systemic disease similar to that seen with VZV infections in immunocompromized humans. The model has been used to evaluate agents such as acyclovir (Soike et al., 1981) and BV-ara-U (Soike et al., 1984, 1992) and has been predictive of antiviral efficacy in human VZV infection.

In vitro plaque reduction assays for antiviral efficacy were performed on Vero cell monolayers grown in 24 well plates using standard procedures. Monolayers were infected with 100 PFU of SVV, strain G815, which was also used for the monkey infection study. BMS-181165 was added to triplicate wells in concentrations from 100 to 0.1 μg/ml in half-$\log_{10}$ dilutions. The ED_{50} of BMS-181165 for simian varicella virus was 2.5 μg/ml. In vitro cytotoxicity was evaluated in confluent and in actively dividing Vero cell cultures at concentrations of BMS-181165 from 1000 to 1.0 μg/ml. No cytotoxic effects were observed in either culture at 1000 μg/ml, the highest concentration tested.

The pharmacokinetics of BMS-181165 was investigated in four fasted African green monkeys. After fasting for 12 h, each of the four monkeys was given aqueous solutions of BMS-181165 to provide single oral doses at 2, 8, and 32 mg/kg or a single intravenous dose at 8 mg/kg with a 2 week washout period between each of the doses. Serial blood samples were obtained from each monkey out to 24 h after administration of drug. Plasma was separated by low speed centrifugation and frozen within 30 min of collection. The compound was extracted from plasma using C18 solid phase extraction cartridges, and the sample extracts were analyzed by HPLC on a ZORBAX RX C-18 analytical column with UV detection at 298 nm. The mobile phase consisted of 0.01 M phosphate buffer (pH 7)/methanol/acetonitrile (80:10:10) at a flow rate of 1.3 ml/min. The lower limit of quantitation was 0.02 μg/ml.

The terminal half-life and the concentration at time zero C_0 after intravenous administration was estimated by using a polyexponential curve stripping program, RSTRIP (MicroMath, Inc., Salt Lake City, UT). The area under the plasma concentration vs. time curve (AUC) was calculated by the trapezoidal rule using intervals from zero time to the last sampling time point (t_n) which had a quantifiable drug concentration (C_n) (Ghibaldi, 1987). The $AUC_{o\rightarrow\infty}$ was estimated from the sum of $AUC_{o\rightarrow\infty} + C_n/\beta$. The total body clearance was estimated from the dose divided by the $AUC_{o\rightarrow\infty}$ after i.v. administration. The volume of distribution was estimated from the total body clearance multiplied by $t_{1/2}/0.693$. The absolute oral bioavailability of BMS-181165 for each monkey was estimated from the $AUC_{o\rightarrow tn}$ after oral administration (normalized for dose divided by the $AUC_{o\rightarrow tn}$ after i.v. administration.

A summary of the mean pharmacokinetic parameters for BMS-181165 in the African green monkeys are shown in Table 1. The mean maximum plasma concentration (C_{max}) after oral administration at 2, 8, and 32 mg/kg was 0.42, 1.76, and 4.74 μg/ml, respectively, and the mean time to maximum concentration (T_{max}) ranged from 1.4 to 4.7 h. Semilogarithmic plots of the concentration of BMS-181165 after i.v. and oral administration to a single monkey are shown in Fig. 2. Following i.v. administration, the plasma concentration decreased with a terminal half-life estimated to be 2.1 h. The mean plasma clearance and volume of distribution were found to be 0.9 l/h kg and 2.73 l/kg, respectively. The volume of distribution is about 4.6 times that of the total body water (600 ml/kg), which suggests that there is localization of BMS-181165 in the tissues. The absolute oral bioavailability of BMS-181165 ranged from 44 to 55% compared to a mean oral bioavailability of 63% for BV-ara-U in the African green monkey (Soike et al., 1992). The mean AUC after oral dosing at 2, 8, and 32 mg/kg was 1.1, 4.5, and 16.4 μg·h/ml, respectively, indicating that the pharmacokinetics were dose linear ($r = 0.9993$).

Twenty African green monkeys seronegative to SVV were infected by intratracheal administration of 2.5×10^5 PFU of SVV, strain G815 (Soike et al., 1986). Forty-eight hours after virus inoculation treatment was begun by oral gavage. BMS-181165 was administered at 12 h intervals in divided doses of 4, 16 or 64 mg/kg/day to groups of 5 animals, while a placebo group of 5 animals received distilled water. Treatment was continued for 10 days.

Infection was monitored by daily observation for rash which was scored on a

TABLE 1

Summary of mean pharmacokinetic parameters for BMS-181165 in the African green monkey

Dose (mg/kg)	C_{max} (μg/ml)	T_{max} (h)	Half-life (h)	AUC (μg·h/ml)	Clearance (l/h·kg)	V_β[a] (l/kg)	F[b] (%)
8 mg/kg, i.v.			2.1	8.6 ± 0.6	0.90 ± 0.09	2.73 ± 0.16	100
2 mg/kg, po	0.42 ± 0.26[c]	4.7 ± 5.5		1.1 ± 0.1			49 ± 9
8 mg/kg, po	1.76 ± 1.70	1.4 ± 1.8		4.5 ± 0.9			50 ± 13
32 mg/kg, po	4.74 ± 1.21	2.2 ± 2.5		16.4 ± 6.7			44 ± 15

[a]Volume of distribution. [b]Bioavailability. [c]± S.D.

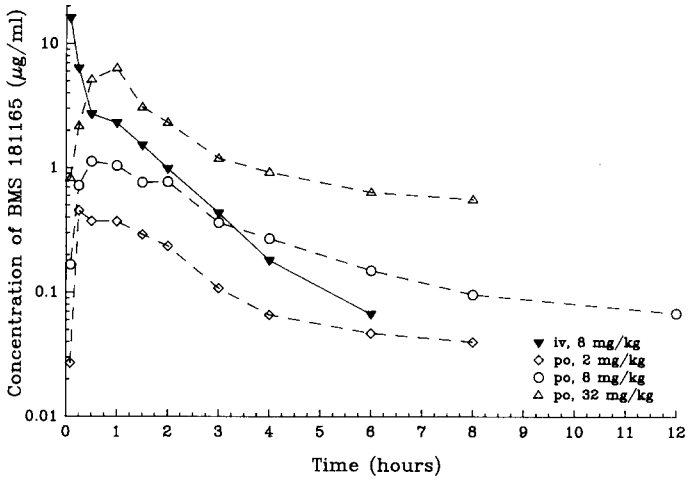

Fig. 2. Concentration of BMS-181165 in plasma of monkey K154 after oral (2, 8 and 32 mg/kg) and i.v. (8 mg/kg) administration of BMS-181165.

scale of ± to 4+ in relation to increased severity (Soike et al., 1986) and by quantitation of viremia in blood specimens collected on days 2, 5, 7, 9 and 11 post-infection. Viremia was measured as previously described (Soike et al., 1992) by co-cultivation of ficoll-hypaque gradient separated lymphocytes with Vero cells. The number of plaques were counted in the monolayers following staining after 5 to 7 days in culture. Antibody titers were determined on sera taken at 14 and 21 days post-infection using a plaque reduction assay (Soike et al., 1986). Blood was drawn at 0, 3, 7, 9 and 11 days post-infection for hematology and clinical chemistry tests.

BMS-181165 at doses as low as 4 mg/kg/day was highly effective in reducing rash as noted in Table 2. Placebo-treated control monkeys each had appreciable 3+ to 4+ rash indicating moderately severe to severe rash persisting for several days. Minimal rash was seen for only 1 day in 2 of 5 monkeys treated with 4 mg/kg/day of BMS-181165 and 1 of 5 monkeys treated with 16 mg/kg/day. Rash was completely prevented at a dose of 64 mg/kg/day.

Viremia exceeded 1000 PFU per ml of blood in each of the 5 control monkeys and was seen on two sampling days (days 7 and 9) in 4 of the 5 monkeys. Viremia was greatly reduced in monkeys in each of the three treated groups. Although minimal numbers of plaques were seen in cultures of lymphocytes from the BMS-181165-treated monkeys, a dose effect was observed with lesser numbers appearing with higher doses.

BMS-181165 treatment prevented death from systemic infection with SVV. Four of the 5 control monkeys died with systemic simian varicella, while none of the BMS-181165-treated animals died. In addition, antibody titers to SVV decreased with increasing doses of BMS-181165 suggesting reduced antigenic load with higher doses of compound. The general clinical appearance was good in all of the BMS-181165-treated monkeys. Hematology and clinical chemistry

TABLE 2

Effect of oral treatment with BMS-181165 upon rash, viremia and mortality in African green monkeys infected with simian varicella virus

Treatment	Monkey number	Peak rash[1]	Days P.I. of peak rash	Peak viremia[2]	Days P.I. of peak viremia	Death (days P.I.)	Antibody titer[3]
Control:	M605	3+	10, 11	>1000	7, 9	12	
dist. H_2O,	M588	4+	11	>1000	7, 9	11	
p.o., b.i.d.	M591	4+	10, 11, 12	>1000	7, 9	14	
	M606	3+	9, 10	>1000	7	-	1:1280
	M602	4+	9, 10	>1000	7, 9	10	
BMS-181165:	M601	±	11	30	7		1:320
4 mg/kg/day,	M590	None		44	7		1:80
p.o., b.i.d.	M592	None	-	66	7	-	1:80
	M589	±	11	27	7		1:160
	M596	None		8	7	-	1:160
BMS-181165:	M607	None		2	9		1:20
16 mg/kg/day,	M593	None	-	6	9	-	1:20
p.o., b.i.d.	M594	±	10	8	7		1:20
	M598	None		25	7	-	1:160
	M595	None	-	1	9	-	1:10
BMS-181165	M605	None		3	7	-	1:40
64 mg/kg/day,	M588	None	-	0		-	1:20
p.o., b.i.d.	M591	None	-	1			1:10
	M606	None		0	-	-	1:10
	M602	None		0	-	-	1:10

[1]Rash was scored daily on a scale of ± to 4+ in relation to increased severity.
[2]Viremia is expressed as the mean number of plaques developing in two 25 cm^2 culture flasks of Vero cells after cocultivation with peripheral blood lymphocytes separated from 2 ml of heparinized blood.
[3]Antibody titer at 21 days post-infection is expressed as the dilution of serum inhibiting >80% of the plaques present in control cultures without serum in a plaque reduction assay.

tests were unremarkable in the treated animals. Behavior and general activity were normal in the BMS-181165-treated monkeys and food consumption was unimpaired.

The oral efficacy of BMS-181165 against SVV infection in African green monkeys is excellent. At the lowest level administered, 4 mg/kg/day, the compound inhibited development of a rash, decreased the viremia and prevented death associated with SVV infection in these monkeys. BMS-181165 had an oral bioavailability of between 44 to 50% in the monkey compared to 63% for BV-ara-U (Soike et al., 1992). Sherman et al. (1990) reported that approximately 46% of a single dose of orally administered BV-ara-U was excreted in the urine in humans. As BMS-181165 was well absorbed in the monkey, the compound may be orally absorbed in humans.

Acyclovir is effective in therapy of VZV infections in normal and immunocompromized patients (Dunkel et al., 1991, Shepp et al., 1986). Acyclovir has been reported to be effective in SVV infection of monkeys when given parenterally at 100 mg/kg/day (Soike et al., 1981). BMS-181165 was effective when given orally at 4 mg/kg/day and is approximately 10-fold more active then acyclovir against SVV and around 2–10-fold more active than

acyclovir against VZV (Tino et al., 1993). Thus BMS-181165 may prove of value in therapy of VZV infections in humans.

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