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6-Dimethylamino-9-(β-D-arabinofuranosyl)-9H-purine: pharmacokinetics and antiviral activity in simian varicella virus-infected monkeys

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Summary

6-Dimethylamino-9-(β-D-arabinofuranosyl)-9H-purine (ara-DMAP) effectively prevented the development of rash and appreciably reduced viremia in simian varicella virus-infected monkeys. Doses of 100 and 50 mg/kg/day, administered orally, were highly effective. The lowest dose of 20 mg/kg/day was much less effective in preventing moderate viremia. However, the 20 mg/kg/day did prevent the development of rash in two of three monkeys. All three doses of ara-DMAP reduced liver infection as reflected by lower aspartate aminotransferase values in the sera of the African green monkeys. Orally administered ara-DMAP was rapidly absorbed. However, significant variation among individual monkeys in the AUC values, peak plasma levels, and plasma half-lives were observed.

Pharmacokinetics; Nucleoside; Simian varicella virus; Antiviral

Introduction

6-Dimethylamino-9-(β-D-arabinofuranosyl)-9H-purine (ara-DMAP) is a potent, selective antiviral agent against cells infected with human varicella-zoster virus (VZV) (Koszalka et al., 1991). It has a favorable in vivo pharmacokinetic profile in cynomolgus monkeys and was demethylated to the equally potent anti-VZV agent 6-methylamino-9-β-D-arabinofuranosyl-9H-

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purine (ara-MAP) (Lambe et al., 1991; Koszalka et al., 1991).

The favorable half-lives of ara-DMAP and ara-MAP and their minimal substrate activity with adenosine deaminase (Lambe et al., 1991) made them candidates for evaluation against simian varicella virus in vivo. This report describes the in vivo antiviral activity and pharmacokinetics of ara-DMAP in simian varicella virus-infected African green monkeys.

Materials and Methods

Monkeys. African green monkeys, purchased as feral animals, completed a 90-day quarantine period and were determined to be free of antibody to simian varicella virus.

Compounds. Ara-DMAP and ara-MAP were synthesized as described (Koszalka, 1991).

Pharmacokinetics. The elimination rate constant, k, was obtained by log-linear regression analysis of drug or metabolite plasma concentration versus time (Notari 1987). Drug and metabolite half-lives ($t_{1/2}$) after oral administration of ara-DMAP or ara-A were calculated from the equation $t_{1/2} = 0.693/k$. The areas under the plasma concentration time curves (AUC) over 24 h were calculated using the trapezoidal method.

Chromatographic separation of metabolites. Deproteinated plasma samples were analyzed on a reverse-phase HPLC column (Rainin Microsorb, C18, 4.6 × 250 mm 5 μ) (Rainin Inst. Co., Woburn, MA) developed with a linear gradient of acetonitrile (0.5% to 12% over 40 min) in ammonium acetate (pH 4.8, 0.05 M) at a flow rate of 1 ml/min. UV absorbance was monitored at 254 and 280 nm. UV absorbance peak areas were digitized and computer-integrated (Digital Specialties, Chapel Hill, NC). Linearity of the concentration versus UV area standard range was determined at the beginning of these assays. The lower limit of detection was approximately 0.2 μM. Concentration, based on UV absorption, was calculated on the basis of comparison with area/nmol of known concentrations of authentic compounds measured on the same day. Therefore inter- and intra-day variability are not factors in these assays. Parent drug and metabolites were identified on the basis of co-chromatography with pure standards, by their 254/280 nm UV absorbance ratio, and by spectral analysis with a LKB 2140 Rapid Sectral Detector diode array spectrophotometer (Pharmacia LKB, Gaithersburg, MD) in-line with the column effluent.

Blood sampling and preparation. Pairs of African green monkeys, which had been fasted for 12 h, received oral doses of ara-DMAP by gavage at dose concentrations of 100, 50, or 20 mg/kg. Blood samples were drawn predose and

at 0.25, 0.5, 0.75, 1, 2, 3, 4, 8 and 24 h postdose. Blood (2 ml) was drawn from the femoral vein into Vacutainer tubes containing EDTA and 0.05 mmoles deoxycoformycin to inhibit adenosine deaminase. Plasma was collected within 30 min of the time of bleeding and frozen at −20°C. The plasma specimens were shipped on dry ice to the Burroughs Wellcome Co. and stored at −20°C until analysis of ara-DMAP levels. Thawed plasma was deproteinated by centrifugation through Centrifree Micropartition filters (Amicon, Danvers, MA) and then analyzed by HPLC. Therefore the plasma concentration of drug measured represent free drug in the plasma and excludes that bound to plasma proteins.

Virus. Infection of the African green monkeys was accomplished by the intratracheal inoculation of 2.8×10^4 plaque forming units (PFU) of simian varicella virus.

Efficacy study. The protocol for treatment and monitoring simian varicella virus infection has been previously described (Soike et al., 1991). Treatment with ara-DMAP began 48 h after virus inoculation. The drug was administered by gastric tube. Doses of 100, 50, and 20 mg/kg/day were split into two equal doses administered 8 h apart (08.00 and 16.00). Rash development was monitored daily and viremia was quantitated at days 3, 5, 7, 9 and 11 after virus inoculation. Hematology and clinical chemistry tests were performed on day 0, 3, 7, 9 and 11 post-infection. Titers of neutralizing antibody were determined after 14 and 21 days using a plaque reduction assay.

Results and Discussion

Pharmacokinetics. Both absorption and extent of metabolism of ara-DMAP were quite variable in these monkeys after oral administration of 20–100 mg/kg ara-DMAP. Four of the 6 treated monkeys rapidly absorbed the drug and had peak plasma levels of both ara-DMAP and its demethylated metabolite ara-MAP at 30 min to one hour after dosing. The other 2 monkeys showed peak levels of ara-DMAP and ara-MAP in plasma at 4 h or later. Further metabolism of ara-MAP produced hypoxanthine arabinoside in all monkeys. Fig. 1 shows the plasma profiles in all 6 monkeys studied.

Individual variations in ara-DMAP plasma levels are related to the oral bioavailability of the drug. Oral bioavailability is affected both by the rate of absorption of drug and first-pass metabolism in the liver. Since the monkeys had been fasted, variability due to binding to food or to delayed gastric emptying were minimized. However, absorption was still slow in 2 of the 6 monkeys.

It is clear from the plasma profiles in Fig. 1 and from the pharmacokinetic data presented in Table 1 that the extent of demethylation of ara-DMAP is variable between individuals of this species. Ara-DMAP has been shown to be

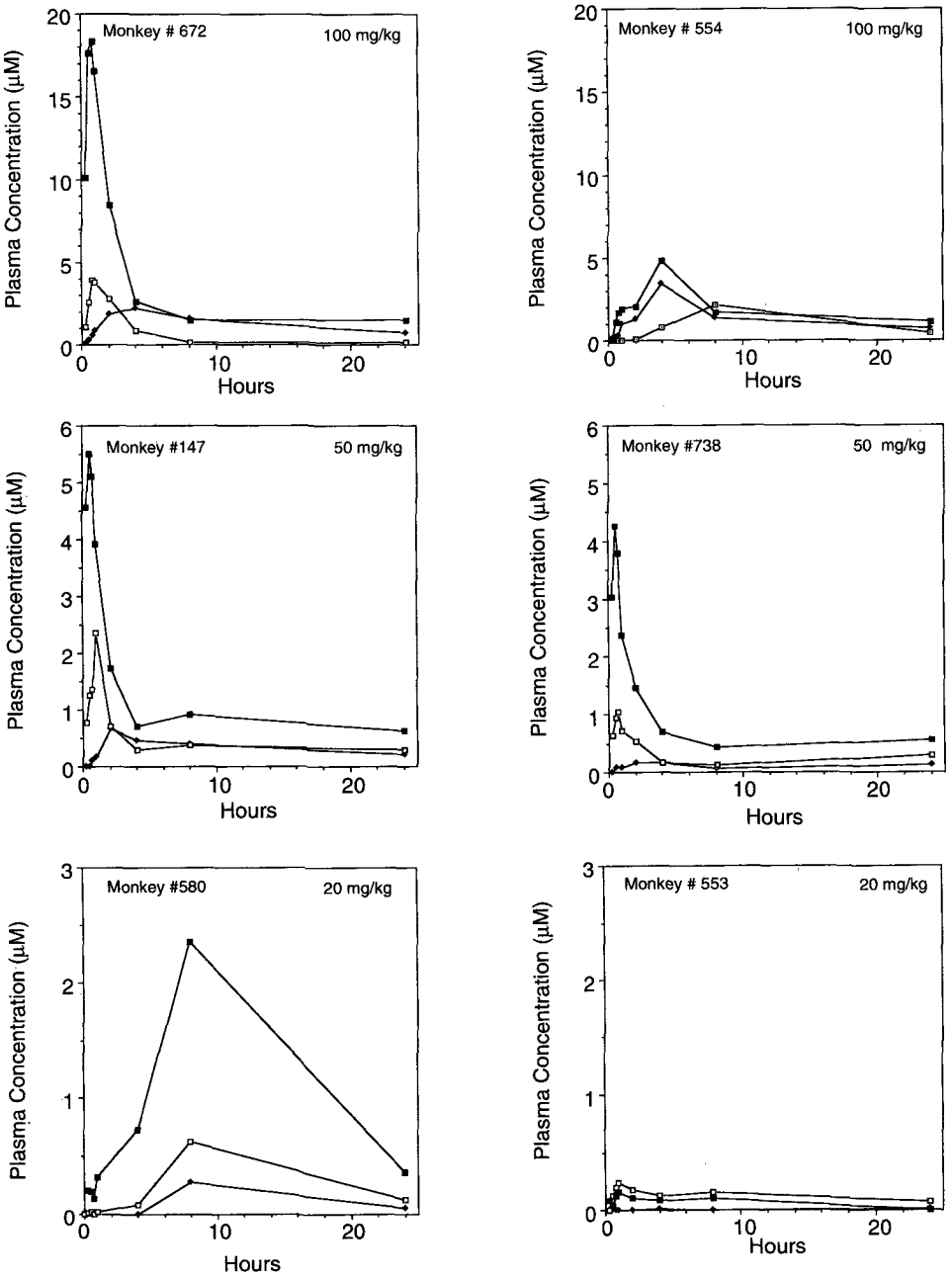

Fig. 1. Pharmacokinetic plasma profile of ara-DMAP administered orally to African green monkeys at 20, 50, and 100 mg/kg. ■, ara-DMAP; □, ara-MAP; ◆, ara-H.

demethylated to ara-MAP by liver microsomal enzymes (Lambe et al., 1991). These enzymes are known to be genetically variable and susceptible to induction of enzyme activity (Goodman et al., 1985). Demethylation of 6-dimethyl-amino-9-(3-amino-3-deoxy-β-D-ribofuranosyl) purine (puromycin aminonucleoside), a compound similar to ara-DMAP, was affected by compounds which stimulate or inhibit liver microsomal enzymes (Mazel et al., 1966).

Table 1 shows observed peak plasma concentrations (C_{max}), elimination half-life ($T_{1/2}$), and area under the plasma concentration-time curve (AUC) of ara-DMAP, ara-MAP and hypoxanthine arabinoside for all monkeys studied. The C_{max} and AUC values for ara-DMAP were higher than those for ara-MAP in monkeys dosed with either 100 or 50 mg/kg. This was also true for one of the two monkeys dosed with 20 mg/kg. The AUC for hypoxanthine arabinoside was substantially lower than that for ara-DMAP in all cases. In cynomolgus monkeys, dosed intravenously with ara-DMAP, the AUC for hypoxanthine arabinoside was essentially the same as that for ara-DMAP (Lambe et al., 1991). Unpublished results for this laboratory show that cynomolgus monkeys orally dosed with 50 mg/kg of ara-DMAP also showed greater plasma levels and AUC's for hypoxanthine arabinoside than in these green monkeys.

Antiviral activity. Data in Table 2 show that the three control monkeys

TABLE 1

Pharmacokinetics of oral 6-dimethylamino-9-(β-D-Arabinofuranosyl)-9H-purine in African green monkeys
Monkeys were given a single oral dose of ara-DMAP by gavage

ara-DMAP[a] (mg/kg)[c]	Metabolite	Plasma peak[b] (μM)	AUC (μM/h)	$t_{1/2}$ (h)
100	ara-DMAP	18.4	45.5	1.1
	ara-MAP[a]	3.9	11.3	1.5
	ara-H[a]	2.2	13.3	
100	ara-DMAP	4.8	23	2.7
	ara-MAP	3.4	15.5	3
	ara-H	2.1	6.6	
50	ara-DMAP	5.5	12.8	0.9
	ara-MAP	1.4	4.4	1.3
	ara-H	0.7	3.3	
50	ara-DMAP	4.3	9.4	0.6
	ara-MAP	1.1	2.7	1.2
	ara-H	0.2	0.9	–
20	ara-DMAP	0.2	0.8	1.5
	ara-MAP	0.2	1.2	3.4
	ara-H	0	0	
20	ara-DMAP	2.4	7.9	*d
	ara-MAP	0.6	1.6	*
	ara-H	0.3	0.6	

[a]ara-DMAP: 6-dimethylamino-9-(β-D-arabinofuranosyl)-9H-purine; ara-MAP: 6-Methylamino-9-(β-D-arabinofuranosyl)-9H-purine; ara-H: Hypoxanthine arabinoside; [b]concentration of drug free in the plasma; [c]two animals were studied at each dose; [d]levels still rising at 8 h.

TABLE 2

Effect of varying doses of 6-dimethylamino-9-(β-D-arabinofuranosyl)-9H-purine on the development of rash, viremia and antibody titers in African green monkeys infected with simian varicella virus

Treatment group[a]	Monkey number	Appearance of rash[b]: days post-infection								Viremia[c]: mean PFU on days P.I.					Antibody titer[d]	
		7	8	9	10	11	12	13	14	3	5	7	9	11	14 days	21 days
Control PBS	I911	–	1+	3+	3+	4+	4+	4+	3+	4	76	>1000	>1000	3	1:160	Dead
	I900	–	1+	2+	2+	3+	3+	3+	±	4	119	736	2	1	⩾1:320	⩾1:640
	I912	±	±	2+	3+	3+	3+	3+	1+	2	145	459	1	0	⩾1:320	⩾1:640
100 mg/kg/d	I903	–	–	–	–	–	–	–	–	1	2	2	1	1	1:80	1:160
	I909	–	–	–	–	–	–	–	–	1	14	28	0	0	1:80	1:80
	I899	–	–	–	–	–	–	–	–	5	10	42	1	0	1:160	1:160
50 mg/kg/d	I910	–	–	–	–	–	–	–	–	1	1	0	0	0	1:40	1:320
	I908	–	–	–	–	–	–	–	–	1	1	11	0	0	1:20	1:40
	I897	–	–	–	–	–	–	–	–	0	2	1	0	0	1:40	1:80
20 mg/kg/d	I907	–	–	–	–	–	–	–	–	1	4	20	1	0	1:20	1:80
	I902	–	–	–	–	–	–	–	–	9	28	132	3	0	1:320	1:320
	I898	1+	2+	2+	2+	–	–	–	–	2	34	155	0	0	1:320	1:640

[a]Treatment began two days after virus inoculation and continued for 10 days. Doses were divided in half and given orally two times daily; [b]rash scored in relation to severity on a scale of 1+ (minimal) to 4+ (severe); [c]mean number of viral plaques developing in paired Vero cell flasks inoculated with lymphocytes separated from 2 ml heparinized blood on Ficoll-Hypaque gradients; [d]dilution of serum neutralizing 80% or more of simian varicella virus plaques in a plaque reduction assay.

developed rashes of moderately severe to severe intensity after infection. Viremia was also marked in each of the three control monkeys. One control monkey died on the 20th day post-infection of complications related to simian varicella infection.

Rash was completely prevented in monkeys treated orally with 50 or 100 mg/kg/day of ara-DMAP. In the group receiving 20 mg/kg/day, two monkeys did not show any signs of rash development while the third had a rash of moderate severity (Table 2).

A substantially reduced viremia was observed in the monkeys receiving 100 or 50 mg/kg/day, compared to the viremia observed in the untreated monkeys. A moderate viremia was detected in 2 of the 3 monkeys treated with 20 mg/kg/day. A minimal viremia occurred in the third monkey. In each case, the viremia observed was substantially lower than that seen in the 3 control monkeys. Antibody titers were slightly lower in some of the ara-DMAP-treated monkeys, presumably reflecting lowered antigenic stimuli due to virus inhibition.

All monkeys tolerated all doses of the drug well. Hematology values were within normal limits with only the infected control monkeys showing reduced thrombocyte counts and increased white blood cell numbers (data not shown). Clinical chemistry values were generally normal. Elevations in aspartate aminotransferase (AST) values observed in the control monkeys reflects the hepatic infection by simian varicella virus. Only minimal increases in AST values occurred in monkeys treated with ara-DMAP suggesting that ara-DMAP protected against hepatic infection.

6-Dimethylamino-9-(β-D- arabinofuranosyl)-9H-purine effectively prevented rash and appreciably reduced the viremia occurring in response to the simian varicella virus infection. Doses of 100 and 50 mg/kg/day were highly effective while the lowest dose of 20 mg/kg/day was much less effective in preventing moderate viremia. The variation in viremia observed in monkeys treated with the same concentration of ara-DMAP is not surprising when considered in light of the variation seen in the pharmacokinetic studies. Although plasma levels of drug were not measured in the infected monkeys, the pharmacokinetics reported here indicate that wide variations in drug levels should be expected.

Acknowledgements

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