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Immunomodulating and antiviral activities of the imidazoquinoline S-28463

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Abstract

Recently, a new class of immunomodulating agents, represented by the molecules imiquimod and R-842, has demonstrated potent antiviral and antitumor activities in animal models. In this study, another representative of this class, S-28463 (4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol) was evaluated for its immunomodulating and antiviral activities. S-28463 induced IFN and other cytokines in vivo in mice, rats, monkeys and in vitro in human peripheral blood mononuclear cell cultures. S-28463 showed potent antiviral activity against herpes simplex virus-challenged guinea pigs when given subcutaneously, dermally, or intravaginally 24 h before infection. Antiviral activity in guinea pigs correlated with the induction of serum 2′,5′-oligoadenylate synthetase activity. Thus, S-28463, like the other imidazoquinolines, demonstrates potent antiviral and immunomodulating effects in a number of models.

Keywords: Immunomodulator; Antiviral activity; Cytokine; Interferon;Interleukin

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1. Introduction

Recently, a new class of immunomodulating agents, represented by the molecules imiquimod (R-837, S-26308) and R-842, has demonstrated both antiviral and antitumor activities in animals. Imiquimod was effective against primary herpes simplex virus type 2 (HSV-2) infection (Miller et al., 1985; Harrison et al., 1988; Bernstein and Harrison, 1989) and recurrent HSV in guinea pigs (Bernstein and Harrison, 1989; Harrison et al., 1991, 1994), arbovirus infection in mice (Kende et al., 1988) and cytomegalovirus infection in guinea pigs (Chen et al., 1988).

Imiquimod inhibited the growth of a number of implantable tumors in mice including MC-26 colon carcinoma, Lewis lung carcinoma and FCB bladder tumor (Sidky et al., 1990, 1992b; Borden et al., 1991). In the FCB bladder tumor model, imiquimod actually cured these animals since upon rechallenge they were resistant (Borden et al., 1991).

Imiquimod's activity against both viruses and tumors, is mediated through its ability to induce cytokines. Imiquimod induces interferon-α (IFN) in a number of species including mice, rats, guinea pigs, monkeys and humans (Miller et al., 1986; Gibson et al., 1990; Borden et al., 1991; Imbertson et al., 1992; Reiter et al., 1994) and also tumor necrosis factor-α (TNF), and interleukin 6 (IL-6) in mice (Reiter et al., 1994). Imiquimod, its hydroxylated metabolite R-842 and a more potent analog S-27609 stimulated production of IFN, TNF-α, IL-1α, IL-1β, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) in cultures of human peripheral blood mononuclear cells (PBMC) (Weeks and Gibson, 1993; Gibson et al., 1995; Testerman et al., 1995a). Thus, imiquimod and its analogs can induce not only IFN, but also other cytokines, all of which may contribute to their overall biological activity.

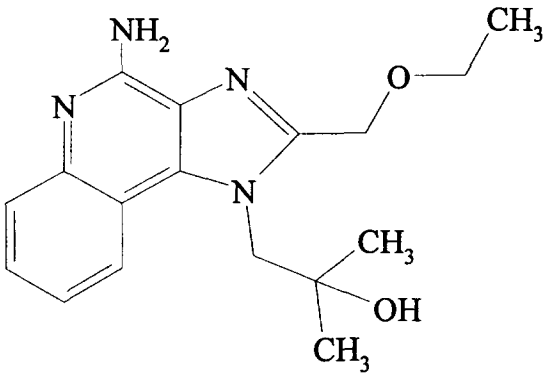
Studies by our group and others indicate that human monocytes are largely responsible for the cytokines produced by human PBMC treated with imiquimod and S-27609 (Gibson et al., 1995); however, B-lymphocytes may also produce IFN in response to imiquimod (Megyeri et al., 1995).

In the current study, we tested another analog of imiquimod, S-28463 (4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol), for its antiviral and immunomodulating properties. Our results demonstrate that S-28463 induces IFN and other cytokines in a number of models including mice, rats and monkeys in vivo and cultures of human PBMC in vitro. S-28463 appears to be 100-fold more potent than imiquimod in protecting guinea pigs from primary HSV infection when given by a number of routes. The antiviral activity of this molecule also correlated with an increase in serum concentrations of 2′,5′-oligoadenylate synthetase (2′,5′-AS) activity.

2. Materials and methods

2.1. Animals

Male CFW mice (6–10 weeks of age), male CD rats (7 weeks of age) and female Hartley guinea pigs (200–300 g) were purchased from Charles River Laboratories,



4-Amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol

Fig. 1. Structure of S-28463, 4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol.

Portage, MI. Stock adult male and female cynomolgus monkeys, *Macaca fascicularis*, weighing between 3 and 10 kg were used for primate studies. All protocols using animals were reviewed by our laboratory animal review committee.

2.2. Reagents

The structure of S-28463, 4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol, is shown in Fig. 1. This compound is a proprietary molecule of 3M Pharmaceuticals and is used as the HCl salt or as the free base. For human PBMC studies, the HCl salt is dissolved in pyrogen-free water or culture medium and stored as a stock solution at 4°C. For dermal and intravaginal studies in guinea pigs, the free base was dissolved in a gel formulation for application. Human, mouse and rat lymphoblastoid IFN (IFN-α), used as standards in the IFN assays, were obtained from the NIH, Bethesda, MD.

2.3. Oral cytokine induction by S-28463 in mice, rats and monkeys

Mice and rats were dosed by oral gavage with the free-base of S-28463 as previously described (Reiter et al., 1994). Blood was collected from mice through the retrobulbar plexus at 1, 2, 4 and 24 h after dosing and serum was obtained and stored at −70°C until assayed for cytokine concentrations. Blood was collected from the tail veins of rats at 1 and 4 h postdose and serum obtained and stored at −70°C.

Monkeys were dosed orally with the HCl salt of S-28463 using a nasogastric tube. Blood was collected at 1, 2, 4, 6, 8, and 24 h postdose. Serum was obtained and frozen at −70°C until it was assayed for IFN concentrations.

2.4. Human PBMC culture

Blood was obtained from healthy volunteers and separated by ficoll hypaque (Sigma Chemicals, St. Louis, MO) density gradient centrifugation as previously described

(Gibson et al., 1995). The mononuclear cells were diluted in RPMI medium containing 10% fetal bovine serum (FBS, Sigma), 2 mM L-glutamine and penicillin/streptomycin solution (RPMI-complete) to 2×10^6 cells/ml and cultured in 6-well tissue culture dishes (Becton Dickenson Labware, Lincoln Park, NJ) with the various stimuli for 24 h. Cell-free supernatants were collected, filter sterilized and frozen at −70°C until they were assayed for cytokine concentrations.

2.5. Cytokine assays

Murine cytokines were assessed according to previously described methods (Reiter et al., 1994). TNF and IL-6 concentrations were evaluated using ELISA kits from Genzyme, Cambridge, MA and Endogen, Cambridge, MA, respectively, and IFN was detected by bioassay. Rat IFN was determined using VSV infection of NRK-49F cells (ATCC, Rockville, MD). Human and monkey IFN were measured by bioassay as previously described (Gibson et al., 1990; Weeks and Gibson, 1993), whereas TNF, IL-1α, IL-1β, IL-6 and IL-8, were measured by ELISA using kits purchased from R&D Systems, Minneapolis, MN (Gibson et al., 1995). IFN results were presented as U/ml and all other cytokine concentrations were expressed in pg/ml.

2.6. In vivo antiviral HSV-2 model in guinea pigs

Female guinea pigs were given S-28463 at various doses either s.c., topically on the back, or intravaginally 24 h before challenge with HSV-2 strain 333 (10^4 plaque forming units (PFU)/0.1 ml). For dermal studies, hair was removed 24 h before application of 100 μl drug to the back of the animal and the site was covered with a Hilltop chamber for 24 h to prevent animals from ingesting the drug. The chamber was then removed and the remaining drug residue was eliminated using soap and water before viral challenge. For intravaginal studies, 100 μl of the gel was applied by syringe and the excess drug was rubbed into the genitalia. Lesions generally appeared 4–5 days after virus inoculation. Animals were scored over the 4- to 5-day period for lesions from 0 to 4, with 0 being no infection and 4 being large ulcers and necrosis. Six to 7 animals per treatment group were used. For each treatment group, the scores for all animals were added together. The data are presented as the percent inhibition of lesion formation when compared to the sham-treated animals

2.7. 2′,5′-AS induction in guinea pigs

Female Hartley guinea pigs were dosed buccally/p.o. with the free-base of S-28463. Blood was collected at 24 and 48 h postdose, allowed to clot and serum was collected and frozen at −70°C until assayed for 2′,5′-AS. Serum 2′,5′-AS activity was determined by radioimmunoassay using a kit from Eiken Chemical Co., Ltd., Tokyo, Japan. Briefly, serum samples (50 μl) were incubated with polyinosinic:polycytidylic acid-coated agarose beads at room temperature, after which beads were washed and incubated with buffer containing unlabelled adenosine triphosphate (40 μmol/l). After 1 h at 37°C, ^{125}I-labeled 2′,5′-oligoadenylate and antiserum against 2′,5′-oligoadenylate was added

Table 1
IFN and TNF induction in male CFW mice with S-28463

S-28463 dose [a] (mg/kg)	Cytokine					
	IFN (U/ml) [b]			TNF (pg/ml) [c]		
	1 h	2 h	4 h	1 h	2 h	4 h
10	709	1583	231	1595	5600	297
3	1600	7500	220	–	–	–
1	2000	6050	730	625	1591	0
0.3	3667	8900	577	1363	307	38
0.1	1270	3933	577	1186	548	10
0.03	645	960	830	200	56	10
0.01	625	915	265	118	54	16
0.003	< 150	970	< 150	< 50	< 50	< 50

[a] Male CFW mice were given various doses (0.003–10 mg/kg) of the free base of S-28463 p.o. and serum was obtained from 3 mice at 1, 2 and 4 h postdose. Serum from each time point was pooled for analysis of cytokines.
[b] IFN was analyzed by bioassay as described and results are presented in U/ml.
[c] TNF was analyzed by ELISA as described and results are presented in pg/ml.

and incubated for an additional hour at 37°C. Samples were then centrifuged at 4°C at 2000 *g* for 30 min. Supernatant was removed and radioactivity was counted using a model 5500B gamma counter (Beckman Instruments, Schaumberg, IL). A standard curve using known 2′,5′-oligoadenylate concentrations was made and activity was expressed in pmol/dl. Duplicates for each sample were run and the coefficient of variation was less than 5% for all samples.

3. Results

3.1. Cytokine induction in mice, rats and monkeys by S-28463

Male CFW mice were given various doses of S-28463 p.o. and then serum was collected at 1, 2, and 4 h postdose for each dose level. Serum samples from 3 mice/dose/time point were pooled and analyzed for IFN and TNF (Table 1). Serum from untreated mice in this study contained less than 150 U/ml IFN and less than 50 pg/ml TNF. S-28463 induced a dose-dependent increase in serum concentrations of IFN and TNF. IFN concentrations were observed by 1 h and usually peaked at 2 h after dosing. At 4 h, serum IFN concentrations were generally lower than those seen at 2 h and by 24 h concentrations were not detectable. In contrast, TNF concentrations generally peaked at 1 h, except at the high doses (1 and 10 mg/kg), with concentrations returning to background by 4 h. Both IFN and TNF were detected at dosages ranging between 0.01 and 10 mg/kg. Low concentrations of IFN were also detected at the 0.003 mg/kg dose. IL-6 concentrations were also evaluated, with induction seen as early as 1 h, concentrations peaked at 2 h and generally returned to background by 4 h (data not shown).

Table 2
IFN induction in cynomolgus monkeys by S-28463 [a]

Animal	Dose S-28463 (mg/kg)	Time of blood collection (h)						
		0	1	2	4	6	8	24
Male-1	1.0	0	7	185	140	36	16	0
	0.1	0	0	5	3	2	2	0
	0.01	0	0	0	0	0	0	0
Male-2	1.0	0	140	3,788	4,985	4,985	3,788	959
	0.1	0	0	140	140	107	107	0
	0.01	0	0	0	0	0	0	0
Female-1	1.0	0	0	36	421	37,900	113,600	729
	0.1	0	0	0	2	3	2	0
	0.01	0	0	0	0	0	2	0
Female-2	1.0	0	0	47	1,263	1,662	1,263	185
	0.1	0	0	0	0	0	0	0
	0.01	0	0	0	0	0	0	0

[a] Cynomolgus monkeys were dosed p.o. with a single dose of the free base of S-28463 at 1, 0.1 and 0.01 mg/kg. There was a 2-week wash out between doses. Blood was collected at various times postdose and serum obtained. Serum was frozen at −70°C until assessed for IFN concentrations using the bioassay as described. Results are presented as U of IFN/ml.

Rats were given either 0.05, 0.1, or 0.5 mg/kg S-28463 p.o. and blood was collected at 1 h postdose for TNF analysis and 4 h postdose for IFN analysis. Results are presented as the mean of two animals/treatment/time point. S-28463 induced IFN at all doses tested, with the 0.5 mg/kg dose inducing the highest concentrations (19,680 U/ml) and 0.1 and 0.5 mg/kg inducing 2287 and 4372 U/ml, respectively. Higher doses of S-28463 (1 mg/kg) did not increase IFN concentrations, in fact, they were actually lower (3000 U/ml) than those seen with the 0.5 mg/kg dose. TNF concentrations in the serum from rats treated with S-28463 at the 0.5, 0.1 and 0.05 mg/kg dose were 448, 112, and 76 pg/ml, respectively.

Male and female monkeys (two per sex) were dosed orally with S-28463 at 0.01, 0.1, and 1.0 mg/kg. Blood was drawn at various times postdose, serum collected and analyzed for IFN concentrations (Table 2). S-28463 induced a dose-dependent increase in serum IFN concentrations. Concentrations of IFN varied considerably from monkey to monkey. In male monkeys receiving the 1 mg/kg dose, serum concentrations of IFN were detected as early as 1 h postdose and peaked between 2 and 6 h, with concentrations declining by 24 h. In females receiving the same dose, concentrations of IFN were detected in both animals at 2 h, peaking at 6–8 h and gradually declining by 24 h. At the 0.1 and 0.01 mg/kg doses 3/4 and 1/4 animals produced detectable levels of IFN.

3.2. Cytokine induction by human PBMC stimulated with S-28463

Human PBMC were stimulated with various concentrations of S-28463 and cell-free supernatants were collected after 24 h and analyzed for various cytokines (Table 3).

Table 3
Cytokine induction in human PBMC by S-28463 [a]

S-28463 dose (mg/kg)	Cytokine [b]					
	IFN-α	TNF	IL-1α	IL-1β	IL-6	IL-8
10	1,263	4,000	1,498	15,000	13,500	85,350
3	554	4,000	926	13,200	23,000	135,600
1	421	2,996	195	3,608	12,800	162,800
0.3	554	844	133	1,017	5,310	50,690
0.1	1,263	256	7	128	1,211	18,260
0.03	140	41	6	19	143	5,170
0.01	5	8	3	9	20	1,485
0.003	2	10	7	0	20	1,709
0.001	0	30	6	12	41	3,717
0	0	25	2	0	5	1,061

[a] Human PBMC (2×10^6 cells/ml) were cultured with various concentrations of the HCl salt of S-28463. After 24 h of incubation the cell free supernatants were collected, filter sterilized and frozen at −70°C until assessed for cytokine concentrations.
[b] Cytokines were assessed as described. IFN concentrations were determined by bioassay and presented in U/ml, whereas all other cytokines were assessed by ELISA and presented in pg/ml.

Dose-dependent inductions of IFN, TNF, IL-1α, IL-1β, IL-6 and IL-8 were seen. IFN was detected at S-28463 concentrations as low as 3 ng/ml and since concentrations plateaued between 0.1 and 10 μg/ml, a true dose response was not observed. Induction of the other cytokines was first seen between 0.03 and 0.1 μg/ml. Interestingly, the other cytokines exhibited more of a dose-dependent response. Peak IL-1α and IL-1β concentrations were seen at the highest concentration of S-28463 (10 μg/ml), whereas concentrations of TNF, IL-6 and IL-8 peaked at slightly lower concentrations (1–3 μg/ml). Concentrations of IL-6 and IL-8 actually declined at the 3 and 10 μg/ml concentrations. Further studies evaluating other cytokines have found that S-28463 does not induce significant production of the T cell cytokines IL-2, IL-4, or IL-5 (data not shown).

3.3. Protection of guinea pigs from primary HSV infection with S-28463

Guinea pigs were treated with a single dose of S-28463 s.c. (0.003–0.3 mg/kg) 24 h before challenge with HSV-2 and evaluated for lesions over the next 4–5 days. Results in Fig. 2 showed that S-28463 totally protected guinea pigs at doses ranging from 0.03 to 0.3 mg/kg, whereas at 0.003 and 0.01 mg/kg S-28463, only partial inhibition of lesion formation was observed (30 and 50%, respectively). All of the animals receiving virus alone demonstrated lesions scoring a 4 in this assay.

In the next studies, guinea pigs were given a single dose of a gel formulation of S-28463 (0.01–1%) applied dermally to the dorsal skin or intravaginally in 0.1-ml volumes 24 h before HSV-2 infection. Animals were evaluated for prevention of primary HSV-2 infection. Data are presented as percentages of lesion inhibition when compared to the animals receiving virus alone (Fig. 3). The 0.5 and 1.0% formulations totally protected animals regardless of the site of application. In contrast, the 0.05 and

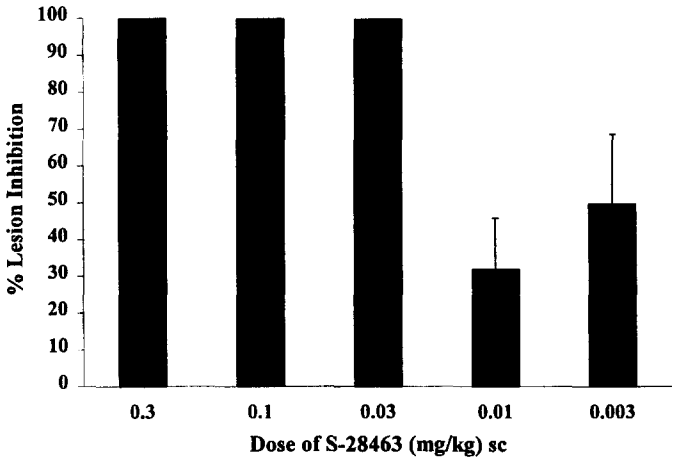


Fig. 2. Inhibition of HSV-2 infection in guinea pigs by S-28463. S-28463 (HCl salt) was given at various doses s.c. in 0.1-ml volumes to female guinea pigs 24 h before infection with HSV-2. HSV-2 was inoculated intravaginally (10^4 PFU/0.1 ml). After 4–5 days, animals were scored for lesion formation as described. Six animals per treatment group were evaluated. Data are presented as the mean percentage of lesion inhibition. All virus-infected controls scored a 4 in this assay.

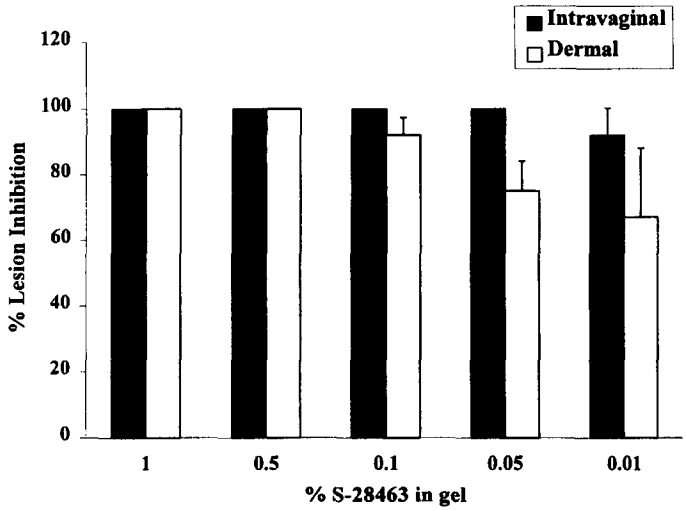


Fig. 3. Inhibition of HSV-2 infection in guinea pigs by dermal and intravaginal application of S-28463 gel. A gel formulation of S-28463 (HCl salt) was given at different concentrations in 0.1-ml volumes to female guinea pigs 24 h before infection with HSV-2. The gel was applied dermally to the back of animals or applied intravaginally. HSV-2 was inoculated intravaginally (10^4 PFU/0.1 ml) and after 4–5 days animals were scored for lesion formation as described. Six animals per treatment group were evaluated. Data are presented as the mean percentage of lesion inhibition. All virus-infected controls scored a 4 in this assay.

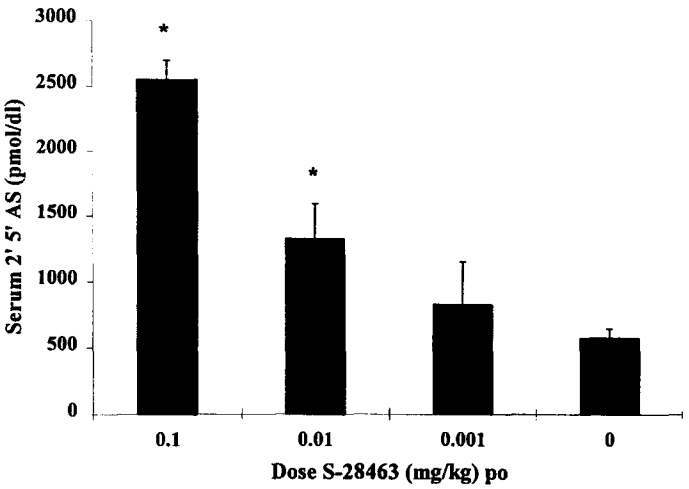


Fig. 4. Induction of serum 2′,5′-AS activity in the serum of guinea pigs treated orally with S-28463. Female guinea pigs were given the free-base of S-28463 p.o. at different dosages (0.001–0.1 mg/kg). Animals were bled 24 h after treatment. Data represent the mean concentrations of 3 individual animals ± S.E.M. * $P < 0.05$.

0.1% formulations totally protected animals when given intravaginally, but gave only partial protection when applied dermally to the dorsal skin (75 and 92%, respectively). The 0.01% gel was only partially protective for both application sites showing 92% protection when given intravaginally and 67% when given dermally. Thus, S-28463 is effective in protecting guinea pigs from primary HSV-2 infection when given prophylactically by a number of routes.

3.4. Induction of serum 2′,5′-AS concentrations in guinea pigs by S-28463

Guinea pigs were administered the free base of S-28463 buccally/p.o. at dosages of 0.001, 0.01, or 0.1 mg/kg. Twenty-four hours after administration, serum 2′,5′-AS was determined (Fig. 4). S-28463 induced a dose-dependent increase in serum 2′5′-AS concentrations with significant induction ($P < 0.05$) at the 0.01 and 0.1 mg/kg doses which increased concentrations 2- and 4-fold, respectively. The 0.001 mg/kg dose did not significantly ($P > 0.05$) elevate 2′,5′-AS concentrations in comparison to controls. At 48 h after administration of the 0.01 and 0.1 mg/kg doses, elevation in 2′,5′-AS was 2-fold higher than in untreated animals (data not shown). In short, antiviral activity in guinea pigs correlated with increased concentrations of serum 2′,5′-AS.

4. Discussion

Recently, a novel class of imidazoquinoline represented by the molecules imiquimod, R-842 and S-27609 have been shown to have potent antiviral, antitumor and immunomodulating activities. Another member of this family of molecules, S-28463,

shares many of these same properties. Like imiquimod, S-28463 has been found to protect guinea pigs from primary HSV-2 infection when given 24 h before infection, but S-28463 appears to be about 100-fold more potent than imiquimod (Miller et al., 1985). S-28463 is also capable of inhibiting HSV-2 infection in guinea pigs when given dermally or intravaginally. Intravaginal application required 10-fold less drug than dermal application in the guinea pig HSV-2 model to give complete protection. These findings indicate that application of this drug at the site of infection is more effective than application by the dermal route, and is possibly the result of better absorption by the vaginal route. The drug caused only slight weight loss when given subcutaneously at high doses and no weight loss when given topically or intravaginally.

2′,5′-AS is an enzyme induced by IFN, and has been implicated in IFN antiviral activity (Kerr and Browne, 1978; Beglioni, 1979). Recently, it has been shown that imiquimod induces increased 2′,5′-AS in PBMC and serum of healthy individuals that were dosed orally with imiquimod (Borden et al., 1991; Wick et al., 1991; Imbertson et al., 1992; Witt et al., 1993). A number of studies have demonstrated a good correlation between elevated serum concentrations of 2′,5′-AS with concentrations found intracellularly (Shindo et al., 1988; Okuno et al., 1991; Bruchelt et al., 1992). Since the serum assay measures formation of 2′,5′-oligoadenylate, it was possible to measure enzyme activity in other species. We investigated the potential for S-28463 to induce increases in serum concentrations of this enzyme in guinea pigs and did show increases in serum concentrations of 2′,5′-AS when given buccally. The background 2′,5′-AS activity in guinea pig serum is much higher than that reported for humans (Okuno et al., 1991; Imbertson et al., 1992), which could be due to a higher level of constitutive enzyme in guinea pigs, or to other proteins in guinea pig serum that may affect the assay. Dermal application of S-28463 also induced an increase in serum 2′,5′-AS in guinea pigs (personal observation), indicating that systemic effects of S-28463 could be obtained by this route. The induction of serum 2′,5′-AS by S-28463 may play a role in the antiviral activity of this compound against HSV-2 infection of guinea pigs.

Much of the antiviral and antitumor activities of imiquimod and other imidazoquinolines have been attributed to the induction of IFN (Kende et al., 1988; Sidky et al., 1992a,b). Some of the antiviral and antitumor activity could in part be mediated by TNF since it and other cytokines are induced in mice by imiquimod and S-28463 (Reiter et al., 1994). Kinetics for cytokine induction by both imiquimod and S-28463 are similar in mice for both compounds, S-28463 appears to induce serum concentrations of IFN and TNF at doses approximately 100-fold less than imiquimod.

In rats, dosages of S-28463 as low as 0.05 mg/kg were capable of inducing serum IFN and TNF which is about 100 times lower than an equally effective dose of imiquimod (Weeks and Reiter, 1989). Induction of IFN and TNF by S-28463 was dose-dependent, with the highest concentrations being seen at the 0.5 mg/kg dose. Higher doses of S-28463 have been evaluated and TNF concentrations continue to rise with the increasing dose, whereas, IFN concentrations show no increase at higher S-28463 doses and actually show a slight reduction. At high doses of S-28463 > 10 mg/kg significant weight loss is observed and this could be due in part to TNF being produced.

In cynomolgus monkeys, S-28463 was effective at inducing serum concentrations of

IFN with the 1 mg/kg dose inducing serum concentrations of IFN in all 4 animals. Kinetics of IFN induction using the 1 mg/kg dose of S-28463, were similar to those seen in mice. At the 0.1 mg/kg dose of S-28463, 3 of 4 animals produced detectable concentrations of IFN in the serum; whereas, at the 0.01 mg/kg dose of S-28463, only one of the monkeys had detectable IFN concentrations in its serum at any time point. It is intriguing that this was the same monkey that responded so vigorously to the 1.0 mg/kg dose. Single doses of imiquimod at 10–30 mg/kg are required to induce detectable concentrations of IFN-α in the serum of monkeys (Gibson et al., 1990). Since 3 of 4 monkeys responded at the 0.1 mg/kg dose, it appears that at least 100-fold less S-28463 than imiquimod is required to stimulate IFN production in this species.

Human PBMC have been shown to produce IFN-α and other cytokines in response to imiquimod, R-842 and S-27609 (Weeks and Gibson, 1993; Gibson et al., 1995; Testerman et al., 1995a). Results in this study demonstrate that S-28463 is also effective in this model at inducing a similar profile of cytokines; however, S-28463 is at least 100 times more potent than imiquimod (Gibson et al., 1995). Previous studies indicate that the monocyte/macrophage is the major cell responsible for IFN and the other cytokines produced in response to imiquimod and S-27609 (Gibson et al., 1995) and this is also seen when using S-28463 as the stimulus (data not shown). Purified monocytes produced IFN, TNF and IL-6 in response to S-28463; whereas, lymphocytes did not.

In conclusion, S-28463 exhibits potent antiviral activity in a guinea pig model of HSV-2 which correlates with the induction of serum 2′,5′-AS. The in vivo antiviral activity of S-28463 also correlates with the induction of IFN and other cytokines. Indeed, S-28463 induces cytokines in a number of models including mice, rats, monkeys and human PBMC cultures with its potency in all these models appearing to be about 100-fold greater than that seen with imiquimod.

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