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Treatment of primary herpes simplex virus infection in guinea pigs by imiquimod

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Abstract

Imiquimod (also known as R-837 and S-26308) is an imidazoquinoline immune response modifier and is available in the US and several other countries for the treatment of external genital warts. Imiquimod has no direct antiviral activity but demonstrates efficacy in several animal models of virus infection. The drug is recognized by antigen presenting cells including monocytes, macrophages, B-cells and dendritic cells and induces these cells to produce cytokines including interferon-α (IFN-α) and others. Imiquimod's ability to inhibit primary lesion development in the guinea pig model of Herpes simplex virus (HSV) intravaginal infection was studied. Imiquimod given intravaginally reduced primary lesions, reduced virus shedding and reduced virus content of spinal cords from HSV infected guinea pigs. A single drug application of 0.5 mg/kg reduced lesion frequency when given between 24 h before inoculation to 16 h after inoculation. A single drug application of 5 mg/kg reduced lesion frequency and severity when administered between 72 h before inoculation to 24 h after inoculation. The antiviral effect resulting from interferon induction in the animal lasts much longer than the drug itself, thus imiquimod is different than drugs having direct antiviral activity. Twice daily drug application for 4 days was effective when initiated up to 72 h after inoculation, however, once lesions began to appear, imiquimod treatment was not able to stop lesion development. Imiquimod treatment inhibited lesion development and/or virus shedding in guinea pigs inoculated with HSV-1, HSV-2 or virus isolates resistant to acyclovir. Imiquimod is currently in clinical trials for treating human HSV infections.

Keywords: Imiquimod; R-837; HSV infection; Guinea pig; Immune response modifier

1. Introduction

Several nucleoside analogs are currently available for the treatment or suppression of Herpes simplex virus (HSV) lesions. Antiviral activity of these drugs is dependent on maintaining a sufficient concentration of active drug in the body to directly inhibit virus replication (De Miranda et al., 1981). However, even continuous treatment does not alter HSV latency once established (King and Galasso, 1982). When drug treatment is stopped, lesions can recur at the same frequency as occurred before drug treatment was started

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(Douglas et al., 1984). Despite antiviral therapy, HSV-2 seroprevalence is increasing and now more than 20% of the US population have antibody to the virus (Fleming et al., 1997). In addition, genital ulcers caused by HSV-2 may enhance the transmission of the human immunodeficiency virus (HIV) (Holmberg et al., 1988). Acyclovir resistant HSV can easily be generated in the laboratory (Coen and Shaffer, 1980) and acyclovir resistant HSV has been isolated from immunosuppressed patients (Burns et al., 1982; Erlich et al., 1988; Chatis and Crumpacker, 1991; Posavad et al., 1997). Clincal isolates resistant to acyclovir are also resistant to penciclovir (Safrin and Phan, 1993).

Interferon-α (IFN-α) has shown some benefit for the treatment of HSV (Shupack et al., 1990; Cardamakis et al., 1998). Imiquimod applied topically induces IFN-α and other cytokines at the treatment site in hairless mice (Imbertson et al., 1998) and in humans (Tyring et al., 1998) which enhance the innate immune response. These cytokines also enhance antigen presentation and stimulate a Th1 immune response (Wagner et al., 1999). The activities of imiquimod have been recently reviewed (Slade et al., 1998; Miller et al., 1999).

Guinea pig infected intravaginally with HSV is an established model of human genital disease (Stanberry et al., 1982). Imiquimod's activities in primary HSV-2 (Harrison et al., 1988) and in recurrent HSV-2 (Harrison et al., 1994) in guinea pigs have been previously reported. This report details the efficacy of imiquimod in different treatment regimens and two different formulations. Treatment of HSV-1, HSV-2 and acyclovir resistant HSV-1 and HSV-2 in this model is also presented.

2. Materials and methods

2.1. Drug preparation

The structure of 3M Pharmaceuticals' drug imiquimod (1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine) previously known as R-837 and S-26308 is shown in Fig. 1. Imiquimod was tested in two different drug preparations. The free base was micronized to an average particle size of 5 microns or less as measured microscopically and was suspended in 5%v/v Tween 80 (Emulsion Engineering, Inc.) and 95% water. The drug suspension, 0.1 ml/dose, was applied intravaginally at the times indicated. A second drug preparation consisted of a cellulose based aqueous gel of 1% micronized imiquimod. This gel remained in place and was easier to administer intravaginally than was the suspension. A cream formulation of imiquimod was also tested. Micronized, gel and cream formulated imiquimod were prepared by 3M Pharmaceuticals Pharmacy R&D group.

2.2. Virus assays

All virus titrations and isolations were done by standard methods using Vero cells (ATCC, Rockville, MD CCL 81). Cells were grown using the following materials from Gibco: medium 199, 100 units penicillin/ml, 100 mg stretomycin/ml and heat inactivated 5% fetal calf serum. Vaginal swabs were placed in 1 ml of medium. Dilutions of medium from vaginal swabs and undiluted spinal cord homogenates were titered using Vero cells and medium containing double strength antibiotics, 250 mg Fungizone/ml and no serum. After incubation, cell monlayers were stained with a solution of 20% ethanol and 0.05% crystal violet.

In the Virus Yield Assay, Vero cells were infected with a multiplicity of infection (MOI) of 0.1 plaque forming units (PFU)/cell and incubated for 24 h. The cells were then freeze-thawed

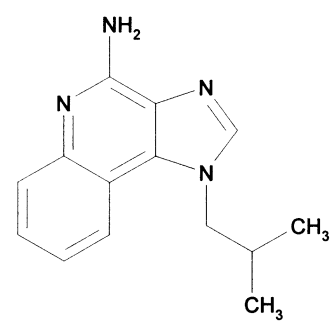

Fig. 1. Imiquimod structure.

and the virus content of the culture was assayed in a standard plaque assay on Vero cell monolayers.

In the Virucidal Assay, the effect of imiquimod was determined by incubating 10^7 PFU of HSV-1 strain F(1) with final drug concentrations of 2, 10 or 50 mg/ml at room temperature. Samples were removed at various times up to 6 h and the virus titer was determined by plaque assay on Vero cells. The virucidal effect of 95% ethanol was assessed as a positive control.

2.3. Virus strains and virus pools

HSV-2 virus strains 333, MS and G and HSV-1 strain F(1) were originally obtained from Dr F. Rapp, Penn State Medical School. Clinical isolates were obtained from Dr M.N. Ellis while at Burroughs Wellcome. The ED_{50} for acyclovir in μg/ml is presented in parenthesis for each isolate. Clinical isolates of HSV-1 tested were BW 1201 (0.16) and BW 9014 (0.24) and acyclovir resistant isolates BW 1209 (16.1) and BW 9013 (40). Clinical isolates of HSV-2 tested were BW 7050 (1.2) and acyclovir resistant isolate BW 7049 (36). Laboratory strains of HSV-1 SC16 (0.02) and TK-altered acyclovir resistant SC16-S1 (2.7) (Darby et al., 1981) were obtained from Jack Hill, Burroughs Wellcome. Virus pools were prepared in Vero cells by inoculating nearly confluent monolayers with a multiplicity of infection (MOI) of about 0.1 PFU/cell. Virus pools were harvested when the cytopathic effect was nearly complete by freeze-thaw and the supernatant was clarified by centrifugation. Virus pools were maintained at −70°C.

2.4. Guinea pig inoculations

Female Hartley guinea pigs (200–250 g from Charles Rivers) were anesthetized with methoxyflurane (Metafane®, Pitman-Moore, Inc.) and the vaginal area was swabbed with a dry cotton swab. The guinea pigs were then inoculated intravaginally with a cotton swab saturated with medium containing HSV. For HSV-2 strain 333, the virus pool contained 1×10^5 PFU/ml and caused lesions in nearly 100% of the control animals. Other virus pools were also titrated for infectivity by intravaginally inoculating guinea pigs with virus dilutions and each pool was then used at the highest dilution that caused lesions in nearly 100% of the animals. The avirulent virus pools were used undiluted at the highest virus titer obtained. Before inoculation, guinea pigs were assigned to groups of 6–10 animals depending on the experiment. Drug suspension or gel (0.1 ml/dose) was applied intravaginally at the times indicated. Efficacy was evaluated by comparing lesion development in drug treated versus vehicle treated animals. External lesions were usually scored for 10 days using the scale: 0, no lesion; 1, redness and swelling; 2, a few small vesicles; 3, several large vesicles and 4, large ulcers and necrosis. Hind limb paralysis was also recorded and animals with paralysis were sacrificed. The maximum lesion score of each guinea pig was used to calculate the percentage lesion inhibition as follows:

$$100 - [(\text{Mean of maximum lesion scores of the treated group/mean of maximum lesion scores of the vehicle control group}) \times 100]$$

Acyclovir 5% ointment (Zovirax®) was used as a reference drug. The two tailed Mann–Whitney rank sum test was used to compare maximum lesion scores between treatment and control groups. The two tailed Fisher's Exact Test was used to compare the proportion of animals with lesions between the treatment and control groups. A P-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Effect of different treatment regimens of imiquimod suspension on primary HSV-2 strain 333

A dose response for imiquimod suspension in 5% Tween 80 was seen for each time after inoculation that treatment was initiated. The longer that initiation of treatment was delayed, the more drug that was needed for lesion inhibition (Table 1). Virus lesions were completely prevented by twice daily treatment for 5 days using 0.3%

imiquimod when initiated at 1 h after inoculation, by 3% imiquimod when initiated at 6 h, by 5% imiquimod when initiated at 24 h and by 10% imiquimod when initiated at 48 h after inoculation. Treatment with the reference drug Zovirax twice daily for 5 days caused complete protection when initiated at 1 h, only slight protection when initiated at 6 h and no protection when initiated at 24 h after inoculation (Table 1).

Inhibition of lesions by imiquimod treatment regimens appears to correlate with a reduction in virus shedding on day 2 after virus inoculation, the normal day of maximum virus shedding (Stanberry et al., 1982). Inhibition of lesions also appears to correlate with a reduction in the number of virus-positive lumbar spinal cord homogenates (Table 1).

3.2. Efficacy of twice daily treatment with imiquimod gel

Twice daily intravaginal application of imiquimod 1% gel at about 5 mg/kg/dose for 4 days resulted in 92% lesion inhibition when treatment was initiated at 24 h after inoculation, 96% when initiated at 48 h and 85% when initiated at 72 h after inoculation. These are all statistically significant at the < 0.05 level when compared to the control group. When initiated at 96 h after inoculation and lesions were beginning to appear, only 19% inhibition was seen in the imiquimod treated group which was not significantly different from the control group.

3.3. Efficacy of single treatment with imiquimod gel

When compared to the gel vehicle treated group, imiquimod 1% gel (about 5 mg/kg) inhibited lesion development when a single treatment was given between 72 h before inoculation or up to and including 24 h after inoculation. Single treatments at either 96 h before inoculation or at 32 h after inoculation were ineffective. When delivered at about 0.5 mg/kg, the imiquimod 0.1% gel effectively reduced lesion development following a single treatment given at 24 h before to 24 h after inoculation (Table 2).

3.4. Efficacy of imiquimod gel formulation on several strains of HSV

Several laboratory HSV strains and clinical isolates of HSV-1 and HSV-2 were inoculated intravaginally in guinea pigs. Imiquimod 1% gel (about 5 mg/kg per dose) and the vehicle were tested for efficacy by initiating twice daily treatment at 24 h after inoculation and continuing for 4 days (Table 3). The listed HSV-1 strains and HSV-2 strains that are resistant to acyclovir were less virulent in the animal model since less frequent and less severe lesions resulted in these vehicle treated groups. No lesions developed in the groups infected with acyclovir resistant HSV-2 strain 333, although 1×10^4 infectious units of virus were recovered from the vaginal swabs of the vehicle group on day 2 after inoculation. Imiquimod treatment reduced virus recovered from the vaginal swabs taken on day 2 after inoculation of most groups, including HSV-2 333 resistant to acyclovir. Imiquimod treatment also significantly reduced the development of lesions when compared to the vehicle control group for all wild type HSV strains and for HSV-2 BW 7049 resistant to acyclovir. The HSV-1 isolates resistant to acyclovir caused only minor lesions in the vehicle group and no lesions in the imiquimod treated group (Table 3).

The laboratory strain of HSV-1, SC 16S1, was resistant to acyclovir but more pathogenic since it caused normal lesions in guinea pigs (Table 4). Lesions caused by the wild type virus, SC-16, were inhibited by acyclovir but the lesions caused by the resistant virus, SC-16S1, were not inhibited by acyclovir. Imiquimod treatment inhibited lesions caused by either the wild type or HSV-1 with altered TK having resistance to acyclovir (Table 4).

3.5. Lack of in vitro antiviral activity of imiquimod

Significant antiviral activity in standard in vitro antiviral assays has not been obtained using imiquimod. The growth rate of Vero cells was somewhat slowed in cultures that contained 5–10 μg/ml of imiquimod and the cells did not grow in

Table 1
Effect of different intravaginal treatment regimens of imiquimod or acyclovir on primary genital HSV-2 strain 333 lesions in guinea pigs

Treatment[a]	Hours delayed[b]	Animals with lesions/total	Mean maximum lesion score (SD)		Percent inhibition	Day 2 virus titer[c]	Spinal cord virus[d]	Percent virus positive[e]
Control	–	8/8	3.9	(0.5)	–	1×10^4	30/36	83
Imiquimod								
0.1%	1	$4/7^{++}$	1.0**	(1.0)	75	6×10^2	2/7	29
0.3%	1	$0/6^{++}$	0.0**	(0.0)	100	ND[f]	ND	ND
0.3%	6	6/7	2.1**	(1.2)	46	ND	ND	ND
1%	6	$2/7^{++}$	0.7**	(1.5)	82	4×10^2	4/7	57
3%	6	$0/14^{++}$	0.0**	(0.0)	100	1×10^2	2/14	14
4%	6	$0/7^{++}$	0.0**	(0.0)	100	<10	0/7	0
1%	24	7/7	2.7**	(1.1)	31	ND	ND	ND
5%	24	$0/7^{++}$	0.0**	(0.0)	100	4×10^3	1/7	14
5%	48	$5/7^{+}$	1.6**	(1.7)	60	ND	5/7	71
10%	48	$0/8^{++}$	0.0**	(0.0)	100	ND	1/8	13
Acyclovir								
5%	1	$0/9^{++}$	0.0**	(0.0)	100	ND	ND	ND
5%	6	8/9	3.3*	(1.4)	15	ND	ND	ND
5%	24	8/8	4.0	(0.0)	0	ND	ND	ND

[a] Treatment was twice daily intravaginal application of 0.1 ml for 5 days using imiquimod suspended in 5% Tween 80 or reference drug, Zovirax, 5% Acyclovir.
[b] Hours after inoculation of initial treatment.
[c] Virus titer in vaginal secretions sampled on day 2 after inoculation was titered on Vero cell monolayers.
[d] Lumbar spinal cords were removed on days 8 or 9 after inoculation, homogenized, and placed on Vero cell monolayers for virus isolation. Numbers indicate the virus positive cultures/total number of spinal cord cultures.
[e] Percent virus positive spinal cord cultures.
[f] Not done.
$^{+}$ Significantly smaller ($P<0.05$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
$^{++}$ Significantly smaller ($P<0.01$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
* Significantly smaller ($P<0.05$) maximum lesion score compared to control group (Mann–Whitney rank sum test).
** Significantly smaller ($P<0.01$) maximum lesion score compared to control group (Mann–Whitney rank sum test).

Table 2
Effect of single-dose treatment with imiquimod gel[a] on primary genital HSV-2 Strain 333 lesions in guinea pigs

	Control group			Imiquimod group				
Time of treatment (h)	Animals with lesions/total	Mean maximum lesion score (SD)		Imiquimod concentration (%)	Animals with lesions/total	Mean maximum lesion score (SD)		Percent inhibition
Pre inoculation								
96	6/7	2.6	(1.5)	1	3/7	1.7	(2.1)	33
72	6/7	2.6	(1.5)	1	$1/7^{+}$	0.1**	(0.4)	94
48	6/7	3.0	(1.7)	1	$0/8^{++}$	0.0**	(0.0)	100
24	8/8	4.0	(0.0)	1	$0/7^{++}$	0.0**	(0.0)	100
Post inoculation								
6	8/8	4.0	(0.0)	1	$0/7^{++}$	0.0**	(0.0)	100
16	7/7	3.4	(1.0)	1	$2/7^{+}$	0.4**	(0.8)	88
24	6/7	3.0	(1.7)	1	2/7	0.9*	(1.6)	71
32	7/7	4.0	(0.0)	1	6/7	3.4	(1.5)	14
Pre inoculation								
48	7/7	4.0	(0.0)	0.1	7/7	4.0	(0.0)	0
24	7/7	4.0	(0.0)	0.1	3/7	0.7**	(0.9)	82
16	5/7	2.9	(2.0)	0.1	$0/7^{+}$	0.0**	(0.0)	100
Post inoculation								
6	5/7	2.9	(2.0)	0.1	1/7	0.4*	(1.1)	85
16	7/7	4.0	(0.0)	0.1	$2/7^{+}$	0.3**	(0.5)	93
24	7/7	4.0	(0.0)	0.1	4/6	2.2*	(2.0)	46

[a] A single intravaginal treatment was given at the indicated time (h) either prior to (pre) or after (post) intravaginal inoculation of guinea pigs with HSV-2strain 333.
$^{+}$ Significantly smaller ($P<0.05$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
$^{++}$ Significantly smaller ($P<0.01$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
* Significantly smaller ($P<0.05$) maximum lesion score compared to control group (Mann–Whitney rank sum test).
** Significantly smaller ($P<0.01$) maximum lesion score compared to control group (Mann–Whitney rank sum test).

Table 3
Effect of imiquimod gel formulation in treating guinea pigs inoculated with different strains of HSV-1 or HSV-2

	Vehicle group				Imiquimod group[a]				
Infecting virus[b]	Animals with lesions/total[c]	Mean maximum lesion score (SD)		Virus titer[d]	Animals with lesions/total	Mean maximum lesion score (SD)		Percent inhibtion	Virus titer
HSV-2 333	6/7	2.0	(1.2)	ND[e]	2/7	0.3**	(0.5)	86	ND
HSV-2 MS	6/7	3.4	(1.5)	ND	$0/8^{++}$	0.0**	(0.0)	100	ND
HSV-2 G	5/7	2.9	(2.0)	ND	$0/8^{++}$	0.0**	(0.0)	100	ND
HSV-2 BW 7050	7/7	4.0	(0.0)	3×10^{2}	$0/7^{++}$	0.0**	(0.0)	100	1×10^{2}
HSV-2 BW 7049 ACV Resistant	5/7	0.7	(0.5)	8×10^{2}	$0/7^{+}$	0.0**	(0.0)	100	8×10^{1}
HSV-2 333 ACV Resistant	0/7	0.0	(0.0)	1×10^{4}	0/7	0.0	(0.0)	–	5×10^{3}
HSV-1 F(1)	2/7	0.3	(0.5)	3×10^{4}	0/7	0.0	(0.0)	100	1×10^{4}
HSV-1 BW 1201	6/7	3.3	(1.5)	3×10^{4}	$0/7^{++}$	0.0**	(0.0)	100	3×10^{3}
HSV-1 BW 1209 ACV Resistant	2/7	0.3	(0.5)	2×10^{5}	0/7	0.0	(0.0)	100	1×10^{5}
HSV-1 BW 9014	7/7	4.0	(0.0)	7×10^{3}	$1/7^{++}$	0.1**	(0.4)	96	2×10^{4}
HSV-1 BW 9013 ACV Resistant	3/7	0.4	(0.5)	7×10^{5}	0/7	0.0	(0.0)	100	3×10^{5}

[a] Intravaginal treatment, about 5 mg/kg, was started 24 h after inoculation and continued twice daily for 4 days.
[b] Guinea pigs were inoculated intravaginally.
[c] Number of animals with lesions/total number in the group.
[d] Virus content of vaginal secretions collected by cotton swab at 48 h after inoculation.
[e] Not done.
$^{+}$ Significantly smaller ($P<0.05$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
$^{++}$ Significantly smaller ($P<0.01$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
* Significantly smaller ($P<0.05$) maximum lesion score compared to control group (Mann–Whitney rank sum test).
** Significantly smaller ($P<0.01$) maximum lesion score compared to control group (Mann–Whitney rank sum test).

Table 4
Effect of imiquimod or acyclovir in treating guinea pigs inoculated with HSV-1 TK variant

Infecting virus	Vehicle[a]			Imiquimod[a]				Acyclovir[b]			
	Animals with lesions/total[c]	Mean maximum lesion score	(SD)	Animals with lesions/total	Mean maximum lesion score	(SD)	Percent inhibition	Animals with lesions/total	Mean maximum lesion score	(SD)	Percent inhibition
HSV-1 SC 16	8/8	4.0	(0.0)	$1/8^{++}$	0.5**	(1.4)	88	$0/9^{++}$	0.0**	(0.0)	100
HSV-1 SC 16S1 ACV Resistant	8/8	4.0	(0.0)	$1/8^{++}$	0.4**	(1.1)	91	8/8	4.0	(0.0)	0

[a] Intravaginal treatment with vehicle or 1% imiquimod cream (about 5 mg/kg) was started 24 h after inoculation and continued twice daily for 4 days.
[b] Intravaginal treatment with Zovirax, 0.1 ml, was started at 6 hours after inoculation and continued twice daily for 5 days.
[c] Number of animals with lesions/total number in the group.
$^{+}$ Significantly smaller ($P<0.05$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
$^{++}$ Significantly smaller ($P<0.01$) proportion of animals with lesions compared to control group (Fisher's Exact Test).
* Significantly smaller ($P<0.05$) maximum lesion score compared to control group (Mann–Whitney rank sum test).
** Significantly smaller ($P<0.01$) maximum lesion score compared to control group (Mann–Whitney rank sum test).

cultures that contained 30 μg/ml. HSV-1 induced cytopathic effects in Vero cells were not inhibited by non-cytotoxic concentrations of imiquimod. The lack of antiviral activity was confirmed by virus yield reduction assays in which imiquimod at non-cytotoxic concentrations did not reduce virus production by HSV-1 infected Vero cells.

There was no direct virucidal activity of imiquimod. Very high final concentrations of imiquimod (50, 10 or 2 mg/ml) did not reduce the infectivity of HSV-1 after a 6 h incubation period at room temperature. As a control, virucidal activity of 95% ethanol was demonstrated by the complete loss of infectivity within 5 min incubation.

4. Discussion

Traditionally, most antiviral drugs have been discovered by their ability to inhibit virus replication and cytopathology in vitro. The first imidazoquinoline we synthesized and tested showed weak antiviral activity in vitro but prevented lesions when tested in the guinea pig model of genital HSV infection. Further testing in vitro showed the weak antiviral effect was due to cell toxicity. However, these results led to further analog synthesis which led to imiquimod, a drug with activity in animal models of virus infection but no in vitro antiviral activity. Subsequent studies showed that imiquimod induced IFN-α and other cytokines and that IFN-α was at least partially responsible for the antiviral activity.

In the guinea pig model, imiquimod treatment reduced virus shedding within one day after drug treatment. An association was seen between reduced virus shedding on day 2 after inoculation, reduced virus in the spinal cord on day 8 or 9 and reduced primary genital lesions seen during the 10 days following inoculation. These reductions were most notable when drug treatment was initiated either before inoculation or up to 24 h after inoculation. However, genital lesions were significantly reduced even when twice daily drug treatment was initiated at 72 h after inoculation. Occasionally, urinary retention and/or hind limb paralysis developed in these animals whose drug treatment was started at 72 h, but few or no lesions appeared. These results are consistent with and extend those reported by Miller et al. (1985), Harrison et al. (1988) who reported results of imiquimod 5 mg/kg started at 12 h after inoculation and Bernstein and Harrison (1989) who reported results of imiquimod 5 mg/kg started at 36 h after inoculation. Once lesions began to appear, about 96 h after inoculation, imiquimod was unable to modify lesion development and a normal primary lesion episode resulted. However, the combination of topical imiquimod and i.p. acyclovir at 60 mg/kg day^{-1} was shown to reduce primary disease in guinea pigs when started after lesions developed (Bernstein et al., 1993a).

Much of the antiviral effect of imiquimod in animals is likely due to induction of IFN by the drug. Imiquimod given intravaginally induced higher serum levels of IFN in guinea pigs than resulted from the primary infection with HSV-2 (Harrison et al., 1988). The IFN induced by imiquimod in guinea pigs is resistant to heat and pH 2 and is thus consistent with type 1 interferon (unpublished results). Interferon induction by imiquimod has been demonstrated in mice, rats, guinea pigs, monkeys and humans (Miller et al., 1999). The antiviral effect of imiquimod in mouse models of Rift Valley Fever and Banzi virus was largely neutralized by antibody to mouse type 1 IFN (Kende et al., 1988). Other cytokines may also be induced by imiquimod and may play a role in reducing the symptoms of virus infections.

Part of the antiviral effect of IFN is thought to be mediated through induction of 2′5′ oligoadenylate synthetase (2′5′ AS) (Kerr and Stark, 1992). Imiquimod causes elevations in serum 2′5′ AS when given orally to the species where IFN induction has been demonstrated (Miller et al., 1999). In guinea pigs, 3 mg/kg of imiquimod induced similar serum levels of IFN when the drug was administered intravaginally or orally (Miller et al., 1986). Guinea pigs and humans have a similar time course after imiquimod administration orally in that drug levels peak in about 2 h and are not detected at 12 h. Interferon peaks about 8 h and is not detected at 24 h and 2′5′ AS is elevated at 24 h and remains elevated through 72–96 h after drug administration (Miller et al., 1999). This

period of elevated 2′5′ AS correlates with the duration of protection seen in this study following a single treatment with imiquimod. This cascade of activity suggests that imiquimod administration two or three times per week might be protective if started before infection is established. Three times per week is the preferred regime for the topical application of imiquimod 5% cream (Aldara™) to patients with external genital warts. Persistence of antiviral activity beyond the time of an effective concentration of the drug is seen for IFN and for drugs that induce IFN such as imiquimod. This persistence of activity is not seen for drugs having direct antiviral activity. Twice daily treatment was used in some of the guinea pig model experiments but this dosing frequency may not have been necessary.

Imiquimod also inhibits recurrent HSV-2 lesions in the vaginally infected guinea pig model, both during the treatment period and after treatment has stopped (Harrison et al., 1994). Treatment for 21 days starting on day 14 after inoculation significantly inhibited recurrent lesions during treatment and during the 5 weeks of observation after treatment was stopped. However, the number of animals with detectable latent HSV by cocultivation of ganglia or spinal cord was not reduced by drug treatment (Harrison et al., 1994). The prolonged effect after drug treatment is likely due to increased cellular immunity to HSV antigens and HSV infected cells (Bernstein and Harrison, 1989; Harrison et al., 1991; Bernstein et al., 1993a; Harrison et al., 1994). In addition, imiquimod can act as a vaccine adjuvant for a HSV glycoprotein vaccine in this model and can be given either prophylactically or therapeutically (Bernstein et al., 1993b, 1995). The fact that many people are infected with HSV, have an antibody response to HSV, carry HSV in their ganglion but do not have recurrent lesions implies the immune response can prevent lesion development. The role of cellular immunity in controlling HSV-2 lesions in humans is being defined (Koelle et al., 1998). A single patient case history of imiquimod's ability to stop recurrent genital HSV lesions has recently been reported (Christensen and Hengge, 1999).

Acyclovir resistant HSV is recognized as a significant clinical problem, especially in immunosuppressed patients (Erlich et al., 1988; Posavad et al., 1997). Estimates of 50–90% of HIV patients are coinfected with HSV-2 and HSV shedding is greater in those infected with HIV (Augerbraun et al., 1995; Schacker et al., 1998). An increase in HSV-2 strains resistant to acyclovir is complicating the treatment of HSV in HIV patients. Thus, HSV infection is a major health problem for HIV patients. In addition, acyclovir resistant genital HSV has been reported in an immunocompetent patient (Kost et al., 1993).

In summary, in the guinea pig model imiquimod significantly inhibited virus production and lesion development caused by HSV-1, HSV-2 and by virus isolates resistant to acyclovir. The antiviral effect of a single imiquimod treatment persists for 3 days suggesting an effective treatment regime is two or three drug applications per week. The activity of imiquimod in the HSV infected guinea pig model suggests that imiquimod may be useful in treating patients with HSV infections.

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