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Antiviral Activity of Win 41258-3, a Pyrazole Compound, Against Herpes Simplex Virus in Mouse Genital Infection and in Guinea Pig Skin Infection

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Win 41258-3 (4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3,5-diethyl-1H-pyrazole methane sulfonate) has in vitro and in vivo activity against herpes simplex virus types 1 and 2. In cell culture, a concentration of 2 μg/ml produced a >50% inhibition of plaque formation of herpes simplex virus type 2, and 3 μg/ml produced a 100% reduction of herpes simplex virus type 1. Win 41258-3 was effective against herpes simplex virus types 1 and 2 in mouse genital infection after intravaginal administration. Win 41258-3 was administered to mice at 4 h postinfection with solutions containing 1.25, 2.5, 5, or 10% of the compound in saturated tampons. Therapy resulted in a high survival rate (80 to 100%) of treated animals versus 20 to 30% of placebo-treated controls. Win 41258-3 was also effective in guinea pig skin infection produced by herpes simplex virus type 1. Solutions of 2.5, 5, and 10% Win 41258-3, applied to the skin starting 24 h postinfection, resulted in rapid suppression of development of herpetic vesicles and significant reduction of the virus titers in the lesion sites.

Win 41258-3, a water-soluble pyrazole, is an analog of arildone (Win 38020), which has previously been reported to be an active antiviral agent (see Fig. 1 for structures of these compounds) (1–4, 8, 10). This pyrazole was among the analogs synthesized in our laboratories after arildone showed topical activity in guinea pig skin infection produced by herpes simplex virus type 1 or 2 (HSV-1 or -2).

In vitro evaluation of Win 41258-3 demonstrated that the compound inhibits the cytopathic effect of HSV-1 and -2 in tissue culture. No inhibition, however, could be demonstrated against ribonucleic acid viruses such as influenza viruses A2/Japan 170 and A/PR8, parainfluenza virus type 3, respiratory syncytial virus, or poliovirus types II and III.

Win 41258-3 was evaluated in vivo against HSV-1 and -2 in mouse genital infection by intravaginal and systemic administration and against HSV-1 in guinea pig skin infection by topical application.

MATERIALS AND METHODS

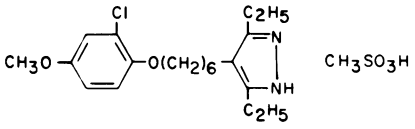
In vitro testing. (i) Compound. Win 41258-3 was synthesized at Sterling-Winthrop Research Institute. It is a white solid, stable to light and heat, and soluble in water. For in vitro testing, the compound was dissolved in dimethyl sulfoxide (DMSO), followed by dilution in appropriate tissue culture medium.

(ii) Cell culture and viruses. Monolayers of BSC-1 (monkey kidney cells) were serially propagated in our laboratory in a modified Eagle medium with minimum essential amino acids and 10% fetal calf serum. Leibovitz L-15 medium supplemented with 5% heated (56°C for 60 min) fetal calf serum was used as maintenance medium.

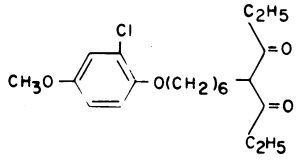
HSV-1, Sheely strain, and -2, Curtis strain, were were obtained from J. O. Oh, Francis Procter Foundation, University of California. Virus pools were prepared in BSC-1 cultures and stored at −90°F. Respective titers: HSV-1 Sheely strain—$\log_{10}$ 6.75 50% tissue culture infective doses per 0.2 ml; HSV-2 Curtis strain—$\log_{10}$ 6.0 50% tissue culture infective doses per 0.2 ml.

(iii) Plaque assay. Four-day-old confluent monolayers grown in 60-cm^2 Falcon plastic flasks were used. Nutrient medium was removed, and cultures were infected with approximately 50 plaque-forming units of each virus, contained in 0.5 ml of Eagle minimal essential medium supplemented with 2% fetal calf serum. Virus was allowed to adsorb for 1 h before the addition of agar overlay. Quadruplicate infected monolayers were overlaid with semisolid agar containing equal volumes of 1% Ionagar, L-15 Leibovitz medium, and 5% calf serum. Win 41258-3 was added to the agar overlay to provide the final desired concentration. Cytotoxicity controls consisted of uninfected monolayers overlaid with medium containing various concentrations of Win 41258-3. Cultures were incubated in an inverted position at 37°C and after 3 days were fixed in 10% Formalin (in 2% sodium acetate). The agar overlay was removed, and the monolayers were stained with 0.1% crystal violet. Plaques were counted in each flask, and the average of four culture flasks was established.

Only those compound-treated monolayers showing no evidence of cytotoxicity when examined microscopically after 3 days of incubation were used in interpreting antiviral results.



Win 41258-3: 4-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-3,5-diethyl-1H-pyrazole methanesulfonate



Arildone: 4-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-3,5-heptanedione

FIG. 1. *Structure of Win 41258-3 and arildone.*

In vivo testing: mouse genital infection. (i) Mice. Mice were white, Swiss albino, Blue Spruce females weighing 11 to 13 g. There were 10 mice per group.

(ii) Viruses. Viruses were HSV-1 and -2; pools of viruses were the same as described above under in vitro testing.

(iii) Infection procedure. Animals were infected intravaginally with approximately 100,000 50% tissue culture infective dose of virus per mouse. Virus suspension was delivered into the vagina in cotton tampons (no. 4 dental pellets; Richmond Dental Cotton Co., Charlotte, N.C.) saturated with a virus suspension. The amount of virus was calculated to produce 70 to 100% deaths in untreated animals.

(iv) Medication procedure. Animals were treated intravaginally starting 4 h postinfection with a cotton tampon saturated with the appropriate concentration of drug. The tampon was left inserted for 24 h but was remoistened 6 h after insertion with a second treatment consisting of 0.02 ml of drug solution. Tampons were removed daily and replaced with fresh, saturated tampons, followed by the addition of 0.02 ml of compound 6 h later. The treatment was carried out for 4 days. Mice were kept for 14 days postinfection, and deaths were recorded daily.

(v) Drugs. Win 41258-3 was prepared as a solution in water, phosphate-buffered saline, DMSO, or propylene glycol at various concentrations ranging from 1.25 to 10%. Adenine-9β-D-arabinofuranoside was purchased from Sigma Chemical Co. Drug solutions (10 and 5%) were prepared in DMSO. Disodium phosphonoacetate hydrate was obtained through the courtesy of Abbott Laboratories. 9-(2-Hydroxyethoxymethyl) guanine (acycloguanosine) was synthesized at Sterling-Winthrop Research Institute, and solutions of 2 and 5% of the compound were prepared in water.

The effect of pH of each solution was monitored by adjusting the corresponding placebo vehicle to the same pH of the compound solution. The pH varied from 3.0 for the water solution to 6.0 for the DMSO solution of Win 41258-3.

In vivo testing: guinea pig skin infection. (i) Experimental animals. Guinea pigs were albino, Hartley strain, weighing 350 to 400 g, of either sex.

(ii) Virus. HSV-1, AA strain, was maintained as a tissue culture pool in BSC-1 cells containing $\log_{10}$ 7.25 50% tissue culture infective doses per 0.2 ml. The pooled virus was stored in heat-sealed glass ampoules at −70°C.

(iii) Compound formulation. Concentrations of 2.5, 5, and 10% Win 41258-3 were prepared in a hydrophilic solution of polyethylene glycol, which also served as the placebo.

(iv) Infection. The herpetic skin infection model in guinea pigs was first described by Hubler et al. (6), and its application as a chemotherapeutic tool was demonstrated by Schafer et al. (11). A total of 120 guinea pigs were infected intradermally with HSV-1, AA strain, and divided into four groups of 30 animals (2.5, 5, and 10% Win 41258-3, and placebo controls). An area 12 mm in diameter was marked on one epilated flank of each guinea pig. A 0.05-ml amount of an undiluted virus suspension was placed in the circle and injected intradermally with a Sterneedle vaccination gun (Panray Division, Ormont Drug and Chemical Co., Inc., Englewood, N.J.) activated 25 times through the virus suspension.

(v) Medication. Starting 24 h postinfection, medication was applied five times daily for 4 days by gently massaging approximately 0.2 ml of 2.5, 5, or 10% Win 41258-3 or placebo into the site of infection with a fresh finger cot over rubber gloves for each animal.

(vi) Evaluation of clinical results. The effect of Win 41258-3 on the development of cutaneous lesions was determined daily by examining six animals from each of the four treatment groups. Two parameters were employed to assess the effect of the drug in the skin infection. The lesion scoring was based on severity and ranged from 0 to 3 in 0.5 increments (0, no vesicles, no erythema; 0.5, one to four small vesicles, barely raised; 1.0, one to four raised vesicles, slight erythema; 1.5, one to four large vesicles, pronounced erythema; 2.0, four to ten large vesicles, edematous tissue; 2.5, more than ten large vesicles, partly coalescent, edematous tissue; 3.0, coalescent vesicles, edematous tissue). The animals were examined daily in a blind fashion, and the scores were recorded but not examined by the scorer until the experiment was terminated. The data were analyzed by an analysis of variance. In addition, the effect of drug on virus replication was analyzed.

(vii) Lesion sampling for virus content. Six guinea pigs from each group were euthanized daily for 5 consecutive days starting 24 h postinfection. The infection site was scraped vigorously with a sterile, disposable scalpel, and the recovered material was placed in a Kontes glass tissue grinder tube containing 4.0 ml of Hanks balanced salt solution, in an ice bath. The scrapings were triturated and sedimented by low-speed centrifugation. The opalescent supernatant was divided into three portions and stored at −70°C for virus titer determination and protein analysis.

(viii) Virus assay. Monolayers of BSC-1 cells were prepared in a cluster dish (no. 3506, Costar, Cambridge, Mass.). Each dish contained six wells of 35-mm diameter (10-cm^2 area). The supernatants prepared from skin scrapings were thawed and diluted from 10^{-1} to 10^{-3} in Eagle medium supplemented with 2% fetal

calf serum. A 1-ml amount of each dilution was added to the wells (in triplicate) after the growth medium was removed. The virus was allowed to adsorb for 1 h at 37°C in a 5% CO_2 atmosphere, after which the residual material was removed, and 3.0 ml of a mixture of equal parts of medium 199 supplemented with 5% fetal calf serum and 1% agar (Oxoid agar no. 1), maintained at 43°C, was added. The agar was allowed to gel, and the dishes were then incubated at 37°C in a 5% CO_2 atmosphere for 4 days. At the end of the incubation period, the cells were fixed to the surface of the well with 1.0 ml/well of 1% Formalin containing 0.2% sodium acetate and stored at 4°C for 24 h. The agar was gently removed from the wells, the monolayers were stained with a solution of crystal violet in Formalin, and plaques were counted.

(ix) Protein determination. The protein concentration of samples was determined by the Lowry test (9). Virus content was expressed as plaque-forming units per milligram of protein.

To determine whether or not drug was carried over from treated sites of guinea pig skin to the tissue culture assay system, we used two groups of noninfected animals. The first group received no medication, and the second group was medicated in the same manner as the regular test animals. The highest level of Win 41258-3 (10%) was used to treat the animals. Guinea pigs were scraped on days 2, 3, and 4 of the experiment. The scrapings were triturated as described earlier and tested for antiviral activity against 100 50% tissue culture infective dose of HSV-1.

RESULTS

In vitro antiviral effect. Results shown in Table 1 indicate that Win 41258-3 prevented plaque formation by HSV-1 and -2 in BSC-1 monolayers after 3 days of incubation. A concentration of 2 μg of compound per ml reduced the plaque count of HSV-1 Sheely strain by 33%, and a concentration of 3 μg/ml reduced plaque formation by 100%. Plaque formation of HSV-2, Curtis strain, was also inhibited with 2 μg of Win 41258-3 per ml. A concentration of 2 μg of compound per ml reduced the plaque by 72%, and a concentration of 3 μg/ml reduced the plaque count by 100%. In addition, plaques formed in the presence of 2 μg of compound per ml were significantly reduced in size when compared with controls.

Microscopic observation of uninfected monolayers treated with 2 to 5 μg of compound per ml over a 3-day period for cytotoxicity showed normal cells comparable to the control cultures. Cells treated with 6 μg of Win 41258-3 per ml showed slight protoplasmic granulation and were excluded from evaluation.

The antiviral activity of Win 41258-3 and its effect on the growth of primary cultures of mouse embryo fibroblasts have recently been studied by K. S. Kim, Department of Virology, New York State Institute for Basic Research in Mental Retardation (personal communication). These studies showed that Win 41258-3, added at concentrations of 1 to 4 μg/ml at the time the cultures were initiated, had no adverse effect on cell growth after 4 days of exposure to the drug. At the end of the 4-day period, all cultures treated with Win 41258-3 had an average cell count equal to or greater than that of the control cultures. It was also shown that Win 41258-3 had no effect on the transport and incorporation of ^{3}H-amino acids in cultures of mouse embryo fibroblasts, F-5000, LLCMH$_2$, and in Vero cells.

In vivo: mouse genital infection. Sham-treated animals developed severe vaginitis, followed by a fatal ascending paralysis, usually developing 4 to 5 days postinfection. Seventy to one hundred percent of the mice died of encephalitis between 6 and 14 days postinfection. At the end of the 14-day postinfection period, the survival rate of HSV-2-infected animals treated with 10% Win 41258-3 in water, phosphate-buffered saline, or DMSO ranged from 80 to 100% (Table 2). A dose response in all solubilizing vehicles was observed (Pancic et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S302, p. 262). Win 41258-3, solubilized in water, was also tested by intravaginal administration against HSV-1, Sheely strain, and found to be effective. The results are shown in Table 2.

Statistical analysis of data shown in Table 2 indicates that the 50% effective dose for the aqueous solution of Win 41258-3 is a 2.5% concentration of drug; it is 3.4 and 6.4% for DMSO and phosphate-buffered saline solutions, respectively.

The effect of a delayed treatment with Win 41258-3 was examined by starting therapy at 6, 8, and 24 h postinfection. It can be seen in Table 3 that a 6-h delay of treatment had an effect on the survival rate similar to the usual results of previous studies where a 4-h postinfection period preceded medication. However, a delay of 8 h resulted in reduced survival. A 24-h delay in initiating therapy rendered the compound ineffective.

A test for the presence of virus in the vaginal tract was conducted after treatment with various concentrations of Win 41258-3 or placebo by daily sampling. In these experiments, groups of mice were infected with HSV-2, Curtis strain, and treated with solutions containing 1.25, 2.5, 5, or 10% Win 41258-3 administered intravaginally in saturated cotton tampons, starting at 4 h postinfection. Samples were obtained from days 1 through 7 postinfection by daily vaginal washings. Washings were done each morning, before the insertion of fresh tampons, by gently introducing 1.5 ml of tissue culture medium M-199

TABLE 1. *Reduction of HSV-1 or -2 plaque formation in BSC-1 (monkey kidney) cells treated with Win 41258-3*

Virus	Win 41258-3 (μg/ml)	Plaque count per plate: 1	2	3	4	Avg	% Reduction
HSV-1 Sheely	2	60	71	54	69	64	33
	3	0	0	0	0		100
	4	0	0	0	0		
	5	0	0	0	0		
	6	—[a]	—	—	—		
	0 (Control)	90	103	100	92	96	
HSV-2 Curtis	2	16	17	15	14	16[b]	72
	3	0	0	0	0		100
	4	0	0	0	0		
	5	0	0	0	0		
	6	—	—	—	—		
	0 (Control)	56	58	53	61	57	

[a] —, Cytotoxic.
[b] Plaque size reduced.

TABLE 2. *Effect of intravaginally administered Win 41258-3 on the survival of mice infected with HSV-2 or -1*

Treatment[a]	Virus	Daily dose (%) (twice a day)	14-Day survival	% Survival	ED_{50}[b] (95% confidence limits)
Win 41258-3 in water	HSV-1	10	7/10	70	
		5	6/10	60	7.3% (4.6 to 23%)
		2.5	4/10	40	
		1.25	3/10	30	
		0	3/10	30	
	HSV-2	10	9/10	90	
		5	9/10	90	
		2.5	7/10	70	2.5%(0.59 to 3.5%)
		1.25	4/10	40	
		0	2/10	20	
Win 41258-3 in phosphate-buffered saline		10	8/10	80	
		5	5/10	50	Linear estimate 6.4%
		2.5	2/10	20	
		1.25	5/10	50	
		0	2/10	20	
Win 41258-3 in 90% DMSO		10	10/10	100	
		5	6/10	60	Linear estimate 3.4%
		2.5	4/10	40	
		1.25	6/10	60	
		0	3/10	30	

[a] Treatment was started 4 h postinfection with tampons saturated in various concentrations of Win 41258-3 solubilized in water, phosphate-buffered saline, or 90% DMSO.
[b] 50% effective dose is based on a probit regression analysis of percent survival corrected for the observed 0 dose response:

$$\text{Corrected \% survival} = \frac{\text{Daily dose \% survival} - \text{0 dose \% survival}}{100 - \text{0 dose \% survival}} \times 100$$

into the vagina with a syringe and recovering the washes in sterile vials. The washings were frozen at −70°C and assayed in BSC-1 cells.

Results shown in Table 4 indicate that virus was not detected in washings from any animals treated with 10% Win 41258-3 on days 1 through 7. This group showed 100% survival at the end of 14 days postinfection. In groups of mice treated with 1.25, 2.5, or 5% Win 41258-3, virus was detected in one animal in each group on days 2, 2, and 1, respectively. At the end of 14 days, these groups had survival rates of 50, 70, and 80%, respectively. In the placebo-treated group, virus was found in 5 out of 10 animals on day 4 postinfection, and by day 7 postinfection all 10 animals had died.

The comparative effect of four antiherpetic agents—Win 41258-3, adenine arabinoside, acycloguanosine, and phosphonoacetic acid—administered intravaginally in mouse genital infection, is shown in Table 5. Each compound produced a significant increase in the survival rate of mice after 4 days of therapy, started 4 h postinfection. The effect of Win 41258-3 compares very well with that of the other three agents. Statistical analysis of data compared the effect of each compound with the corresponding placebo vehicle used to administer each compound. All three agents were significantly active when compared with their corresponding placebos. No significant increase in the survival rate of mice in the placebo group could be shown attributable to the varied pH of the placebo vehicles.

TABLE 3. *Comparison of the effect of early and delayed intravaginal treatment of herpetic infection in mice with Win 41258-3 solubilized in water*

Treatment	Daily dose (%) (twice a day)	% Survival at (h):		
		6[a]	8	24
Win 41258-3 in water	10	90	50	0
	5	70	70	0
	2.5	20	70	0
	1.25	10	30	10
Placebo control		20	20	10

[a] Time postinfection that treatment was initiated.

In vivo: guinea pig skin infection. (i) Effect of 2.5, 5, and 10% Win 41258-3 on vesicle development. The effect of 5 and 10% of the compound on the development of herpetic lesions was observed after 24 h of therapy, and the effect of 2.5% was observed after 48 h. Reduction of the size of lesions, drying of the lesions, and absence of formation of new vesicles was observed in drug-treated animals in contrast to placebo-treated controls, in which vesicles gradually increased in size and number. The difference between the pyrazole-treated animals and placebo controls was more evident with each day of therapy. On the last observation day (day 5 postinfection), vesicles had completely dried on all drug-treated animals. The skin was either completely clear or showed only slight scabbing at the site of lesions, whereas in placebo-treated animals lesions were large, moist, and in some animals, coalescing into a single, large lesion. A statistically significant difference at the 1% level was recorded from day 2 through day 5 in animals treated with 5 or 10% of drug, and from day 3 in those treated with 2.5%. The only significant difference between groups treated with 5 and 10% was that lesion scores in animals treated with 10% were uniformly lower than those in animals treated with 5%, and the curve (Fig. 2) was flatter.

(ii) Effect of 2.5, 5, and 10% Win 41258-3 on virus titer in the skin. The effect of all three concentrations of compound on virus titers in the infected sites is shown in Fig. 3. Results indicate that all three concentrations reduced the virus content in lesions when compared with placebo-treated controls. A statistically significant difference at $P = 0.01$ was found on each day starting with day 2 through day 5 between

TABLE 4. *Effect of Win 41258-3 on virus growth in the vagina of mice infected with HSV-2, Curtis strain*[a]

Medication	Day of sampling					
	1	2	3	4	6	7
Placebo	1/10[b]	2/10	4/10	5/10	3/6[c]	All dead
10% Win 41258-3	0/10	0/10	0/10	0/10	0/10	0/10
5% Win 41258-3	1/10	0/10	0/10	0/10	0/8	0/8
2.5% Win 41258-3	0/10	1/10	0/10	0/10	0/10	0/8
1.25% Win 41258-3	0/10	1/10	0/10	0/10	ND[d]	0/7

[a] Medication was administered topically with drug-imgregnated tampons at 4 h postinfection.
[b] Numerator, number of mice with recoverable virus; denominator, number of mice sampled.
[c] Denominator of <10 indicates death of mice; a denominator of 6 indicates that four mice died.
[d] Not determined.

TABLE 5. *Comparative effect of Win 41258-3, adenine arabinoside, acycloguanosine, and phosphonoacetic acid on the survival of mice after intravaginal therapy of herpes genitalis (HSV-2)*

Compound	Daily dose (%) (twice a day)	14-Day survival	Comparison vs. appropriate control[a]	% Survival
Win 41258-3 in propylene glycol	2	9/10	($P = 0.029$)	90
	5	9/10	($P = 0.029$)	90
Placebo–propylene glycol		4/10		40
Adenine arabinoside in DMSO	5	8/10	($P = 0.035$)	80
	10	9/10	($P < 0.01$)	90
Placebo–DMSO		3/10		30
Acycloguanosine in water	2	6/10	($P < 0.01$)	60
	5	9/10	($P < 0.01$)	90
Phosphonoacetic acid in water	2	9/10	($P < 0.01$)	90
	5	9/10	($P < 0.01$)	90
Placebo–water		0/10		0
Infected (not treated)		2/10		20

[a] Fisher's exact test, 1 tail probability—see reference 5, p. 96.

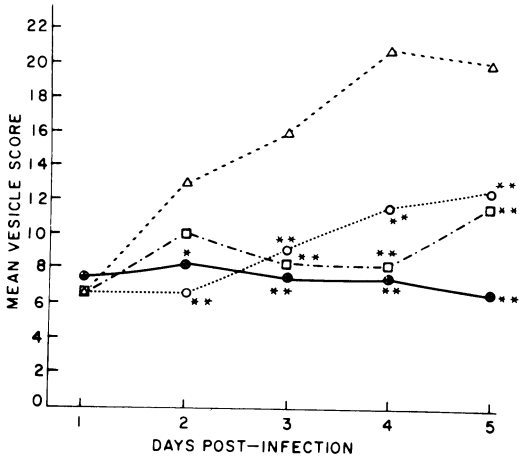


FIG. 2. *Effect of 10, 5, and 2.5% Win 41258-3 in polyethylene glycol on development of herpetic vesicles in guinea pigs infected intradermally with herpesvirus hominis, type 1, AA strain; medication was applied topically five times daily for 4 days starting 24 h postinfection. Symbols: △, placebo; ⊗, 10% Win 41258-3; ○, 5% Win 41258-3; □, 2.5% Win 41258-3; *, significant, P = 0.05; **, significant, P = 0.01.*

animals treated with 10% Win 41258-3 and placebo controls. In animals treated with 5% Win 41258-3, a statistically significant difference of $P = 0.05$ was observed between the treated and placebo groups on day 2, and statistically significant difference of $P = 0.01$ was observed thereafter. Animals treated with 2.5% Win 41258-3, although showing lower titers than placebo controls on days 2 and 3, did not demonstrate a statistically significant difference at the 1% level until days 4 and 5 postinfection. In addition, as shown in Fig. 3, the 10% concentration of Win 41258-3 had consistently and sometimes significantly lower virus titer than the 5% concentration (Pancic et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, A5, p. 1).

Assaying of the skin scrapings of noninfected animals treated with 10% Win 41258-3 or the nontreated ones showed that no inhibition of the virus growth could be achieved when skin scrapings from either group were used. The lowering of the virus titers was achieved in the treated skin and cannot be attributed to the transfer of drug into the tissue culture assay system. Oral or subcutaneous administration of Win 41258-3 at doses of 100, 200, or 400 mg/kg daily starting at 4 h postinfection and continuing for 7 days had no effect on the survival rate of mice.

DISCUSSION

Antiviral evaluation of Win 41258-3, a water-soluble pyrazole salt, against HSV-1 and -2 in mouse herpes genitalis has demonstrated that early topical treatment (up to 6 h postinfection) prevents development of vaginitis, paralysis, and death in a majority of the treated animals. Daily sampling of vaginal contents from day 1 through

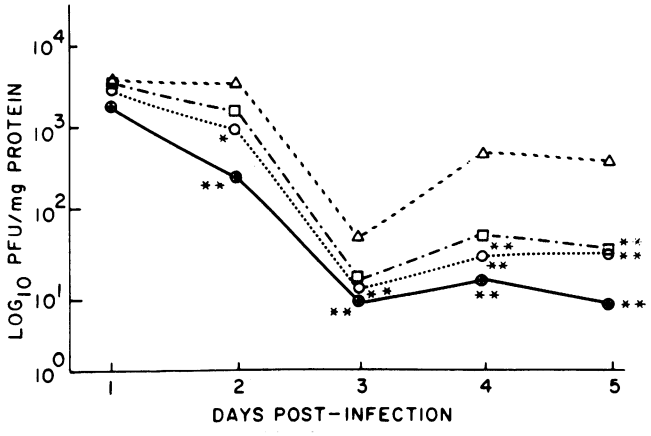


FIG. 3. *Effect of 10, 5, and 2.5% Win 41258-3 in polyethylene glycol on growth of virus in guinea pig skin from animals infected intradermally with herpesvirus hominis, type 1, AA strain; medication was applied topically five times daily for 4 days starting at 24 h postinfection. Symbols: △, placebo; ⊕, 10% Win 41258-3; ○, 5% Win 41258-3; □, 2.5% Win 41258-3; *, significant, P = 0.05; **, significant, P = 0.01. PFU, Plaque-forming units.*

day 7 postinfection showed that virus could not be recovered from animals treated with 10% solution of Win 41258-3, and only occasionally was virus found in animals treated with lower concentrations. The application of Win 41258-3 in saturated cotton tampons left in the vagina for 24 h appears to be an effective topical treatment and decreased the need for the frequent manipulations of animals necessary for multiple daily treatments.

Intravaginal therapy with Win 41258-3 in four different vehicles resulted in high survival rate of mice and compared well with other known antiherpetic agents. Delaying of therapy up to 24 h postinfection resulted in ineffective therapy.

Topical application of the drug in guinea pig skin infection resulted in a rapid suppression of the development of herpetic vesicles and a significant reduction of the virus titer in the lesion sites when therapy was initiated 24 h postinfection. We do not have an explanation for the decrease in virus titer seen in placebo-treated animals on day 3, followed by a rise in titer on day 4 as shown in Fig. 3. We have, however, occasionally seen similar occurrences in other experiments in which different vehicles were used to deliver the compound and in infected, nonmedicated animals.

Thus, two different animal models provide evidence that this drug may be of value in the treatment of herpetic infections in humans.

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