Evaluation of a θ-Defensin in a Murine Model of Herpes Simplex Virus Type 1 Keratitis

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Purpose. To test the activity of a synthetic θ-defensin, retrocyclin (RC)-2, in a murine herpes simplex virus (HSV)-1 keratitis model.

Methods. The in vitro antiviral activity of RC-2 against HSV-1 KOS was determined by yield reduction and viral inactivation assays. Efficacy in an experimental murine HSV-1 keratitis model was tested using pre- or postinfection treatment with 0.1% peptide in PBS with or without 2% methylcellulose. Viral titers in the tear film were determined by plaque assay.

Results. RC-2 inhibited HSV-1 KOS in vitro with an EC_{50} of 10 μM (~20 μg/mL) in yield-reduction assays, but was not directly virucidal. RC-106 (a less active analogue) did not inhibit HSV-1 KOS in culture. Incubating the virus with RC-2 or applying the peptide in 2% methylcellulose to the cornea before viral infection significantly reduced the severity of ocular disease, but postinfection treatment with 0.1% RC-2 in PBS with or without 2% methylcellulose did not. Viral titers were significantly reduced on some days after infection in the preincubation and prophylaxis groups.

Conclusions. RC-2 was active against HSV-1 KOS in cultures and showed protective activity in vivo when used in a prophylactic mode, but the peptide showed limited activity in a postinfection herpes keratitis model. These findings support data obtained from experiments with HIV-1, HSV-2, and influenza A, indicating that RCs inhibit the entry of viruses rather than their replication. (*Invest Ophthalmol Vis Sci.* 2007;48: 5118–5124) DOI:10.1167/iovs.07-0302

Ocular infection with herpes simplex virus (HSV)-1 is the leading infectious cause of blindness in the United States.[1] Unfortunately, the antiviral agents that are currently available to treat herpetic keratitis are not always effective.[2] Adding steroids to treatment regimens to suppress inflammation has limited efficacy and is associated with side effects.[2,3] For these reasons, there is a need to develop additional therapeutic options for HSV-1 ocular infections.

Endogenous antimicrobial peptides (AMPs) are key components of the innate host defense systems of plants, fungi, invertebrates, and vertebrates.[4] Two families of AMPs predominate in mammals: cathelicidins[5] and defensins.[6,7] The cathelicidin AMPs are structurally diverse, but their biosynthetic precursors share a common 11-kDa "cathelin" domain.[5,6] While some mammalian species (e.g., pigs and cattle) express more than 10 different cathelicidin AMPs, humans express only one: the peptide HCAP-18, also called LL-37.[5]

Defensin peptides comprise three subfamilies, the α-, β- and θ-defensins. Humans express six different α-defensins[6] and up to 30 distinct β-defensins.[8] θ-Defensin peptides are found only in Old World monkeys, gibbons, and orangutans[9] and were selected for this study because they have broad-spectrum antiviral properties.[10–13] θ-Defensins are circular octadecapeptides with six cysteines pairing to form a ladder-like disulfide array that connects their two antiparallel β-sheets.[14] Studies on the three θ-defensin peptides (RTD-1 to -3) isolated from the leukocytes or bone marrow of the rhesus macaque[15–17] revealed that θ-defensin (DEFT) genes are mutated α-defensin (DEFA) genes and that mature θ-defensin peptides arise in vivo by post-translational trimming and ligation of two "demidefensin" precursors.[15,16] The human genome contains multiple θ-defensin (DEFT) genes, including some that are transcribed.[10] However, each human DEFT gene contains a premature stop codon that prevents translation of its expressed mRNA.[9]

The human cornea can express at least three β-defensins. Two of them (HBD-1 and -3) are constitutively expressed.[18–20] In contrast, HBD-2 expression is induced by cytokines and occurs during corneal wound healing.[20,21] Other AMPs detected in human corneal samples include LEAP-1/hepcidin (DTHFPICIFCCGCCHRSSKCGMCCK), LEAP-2 (MTPFWRGVSLRPIGASCRDDSECITRLCRKRRCSLSVA), and HCAP-18/LL-37.[22]

Studies have shown that the θ-defensin retrocyclin (RC)-2 inhibits HSV-1 MacIntyre and HSV-2 G, indicating that RC-2 is a broad-spectrum inhibitor of HSV.[13] The goal of this study was to test the efficacy of a θ-defensin in a murine model of HSV-1-induced keratitis. RC-2, the θ-defensin selected for this study, is identical with RC-1, except for a single gly→arg substitution that enhances its potency against both HIV-1[23] and herpes simplex, type 2.[13] The sequence of RC-2 is based on information encoded in two human θ-defensin pseudogenes. Thus, RC-2 arguably represents a θ-defensin that humans would express if some arboreal ancestor's DEFT gene had not experienced a mutational event resulting in a premature stop codon.[9]

Materials and Methods

Cell Culture and Virus

The procedures for growing Vero cells and preparing high-titer stocks of HSV-1 KOS have been described previously.[24] Briefly, Vero cells were cultured in Dulbecco's modified Eagle's medium (DMEM) con-

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taining a 5% serum (1:1 mixture of defined supplemented calf serum and fetal bovine serum; Hyclone, Ogden, UT), 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, and 10 mM HEPES (pH 7.4). High-titer stocks of HSV-1 strain KOS were prepared by inoculating Vero cells at a multiplicity of infection (MOI) of 0.1 to 0.01. When 90% of cells showed cytopathic effects, the cells were collected by scraping and centrifugation at 400*g*. The cell pellets were resuspended in one tenth the original volume of medium, frozen and thawed three times to release virus, and finally centrifuged at 2000*g* to remove debris. High-titer virus stocks were stored at −80°C.

Peptides

RC-2 and RC-106 were synthesized at the University of California, Los Angeles (model I 431 A; Applied Biosystems, Inc. [ABI], Foster City, CA), essentially as previously described.[10] The two peptides are identical except for the residues 4 and 10, which are arginines (R) in RC-2 but are tyrosine (Y) and glycine (G) in RC-106 (Fig. 1). RC-106, available in limited quantities, was not included in all studies. Peptide concentrations were determined as described previously.[25,26]

In Vitro Antiviral Activity

The inhibitory effect of RC peptides against HSV-1 KOS was tested in typical yield-reduction assays.[25,26] Briefly, virus at an MOI of 2 was mixed with concentrations of peptide varying from 3.1 to 200 μM and immediately added to Vero cell monolayers. After a 24-hour incubation, the cells were harvested by scraping. They were frozen and thawed three times, and infectious virus titers were determined on Vero cell monolayers. Direct virucidal activity was tested by incubating high-titer virus stocks (1×10^8 PFU/mL) with various peptide concentrations for 1 hour at 37°C. Then, samples were serially diluted and the amount of infectious virus was determined on Vero cell monolayers, as just described.

Cytotoxicity Assay

The toxicity of RC-2 for Vero cells was tested in a dye-reduction assay, as describe previously.[27] Briefly, Vero cells were seeded at a density of 1.5×10^4 cells per well in a 96-well microtiter plate. After incubation overnight at 37°C, 20 μL of medium containing the desired concentration (serial twofold dilution) of RC-2 was added. Control wells received medium only. As a positive control for toxicity, the bKLA peptide, which we previously showed was toxic to Vero cells, was included.[25] After incubating the cells in the presence of peptide overnight at 37°C, 20 μL of cell proliferation assay reagent (Celltiter 96 AQueous One Solution; Promega, Madison, WI) was added to each well in a total volume of 120 μL of culture medium. The plates were incubated for 2 hours at 37°C, and the absorbance at 490 nm was determined in a plate reader (BioTek Instruments, Inc., Winooski, VT).

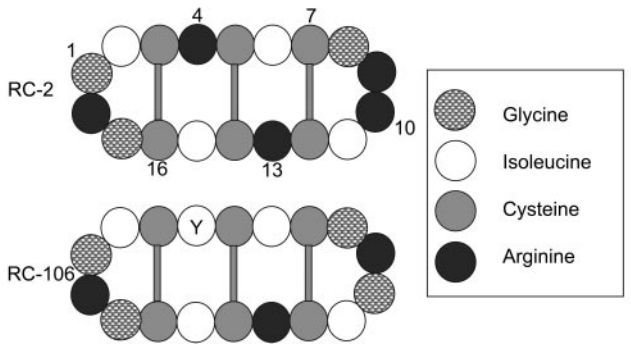

FIGURE 1. Retrocyclin structures. RCs are cyclic, 18-residue peptides with three disulfide bonds and a net positive charge. The structures of RC-2 and RC-106 are shown, with several residues of RC-2 numbered. Y denotes the tyrosine substitution for arginine in RC-106.

All assays were performed in triplicate, and the data are presented as the mean ± SD.

Animal Inoculation

Four- to six-week-old female BALB/c mice (Harlan Sprague-Dawley, Indianapolis, IN) were used for all studies, and all treatment groups consisted of 10 mice each. The procedures for infecting mice are described elsewhere.[24,28,29] Briefly, the right corneas were scratched three times vertically and three times horizontally with a sterile 30-gauge needle under isoflurane anesthesia. A 5-μL drop of DMEM (2% serum) containing 1.0×10^6 plaque-forming units (PFU) of HSV-1 KOS[24] was applied to the scarified cornea, and the mice were returned to their cages. Four different treatment methods were used, with groups of 10 mice each. Group 1: To test the effect of preincubating virus with peptide, virus was incubated with peptide in DMEM (0.1% wt/vol final concentration) at 37°C for 1 hour and then used to infect mice (1×10^6 PFU/eye). No other treatment was given. Group 2: For postinfection treatment, eye drops (5 μL) containing 0.1% (wt/vol) RC-2 in PBS were administered starting 4 hours after infection and continued four times per day for 7 days. Group 3: The postinfection treatment regimen was repeated using RC-2 (0.1% wt/vol) suspended in PBS with 2% (wt/vol) methylcellulose. Methylcellulose was chosen because it is not toxic and is compatible with aqueous solutions. Group 4: To determine whether RC-2 had prophylactic activity, mice were anesthetized, and the cornea was scarified with a sterile 30-gauge needle. A 5-μL drop of RC-2 in 2% (wt/vol) methylcellulose (0.1% wt/vol final RC-2 concentration) was then applied to the cornea with a micropipette, and the mice were returned to their cages. Ten to 15 minutes later, the mice were reanesthetized, and a 5-μL drop of HSV-1 KOS (1×10^6 PFU) was added to the scarified cornea. The mice in this group received no other RC-2 treatments.

Disease Scoring

The severity of ocular disease was scored as we have described previously.[24,28,29] Briefly, blepharitis was scored: 1+, puffy eyelids; 2+, puffy eyelids with some crusting; 3+, eye swollen shut with severe crusting; and 4+, eye completely swollen shut and crusted over. Vascularization was scored: 1+, <25% of the cornea involved; 2+, 25% to 50% corneal involvement; and 3+, >50% corneal involvement. Stromal disease was scored: 1+, cloudiness, some iris detail visible; 2+, iris detail obscured; 3+, cornea totally opaque; and 4+, corneal perforation. All animal experiments were approved by the UW-Madison Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The disease severity data were analyzed with the Mann-Whitney U test.

Measurement of Ocular Viral Titers

On days 1, 3, 5, 7, 9, 11, and 13 after infection, samples were harvested from the mice as follows. The mice were anesthetized with isoflurane, and the infected cornea was flushed with 10 μL of serum-free DMEM. The rinse was added to 190 μL of serum-free DMEM and stored at −80°C until all samples had been collected. Serial 10-fold dilutions were quantified by using a standard plaque assay on Vero cells.[24] Each group consisted of 10 mice, and significant differences were determined by Student's *t*-test.

RESULTS

Effect of RC-2 on HSV-1 Strain KOS in Culture

RC-2 has been shown to inhibit HSV-1 strain MacIntyre and HSV-2 strain G.[13] Activity against HSV-1 KOS was tested at an MOI of 2 by adding virus to various concentrations of RC-2 or RC-106 and immediately adding the mixtures to Vero cell monolayers. After 24 hours of culture in the presence of the peptides, the infectious virus concentration was measured by

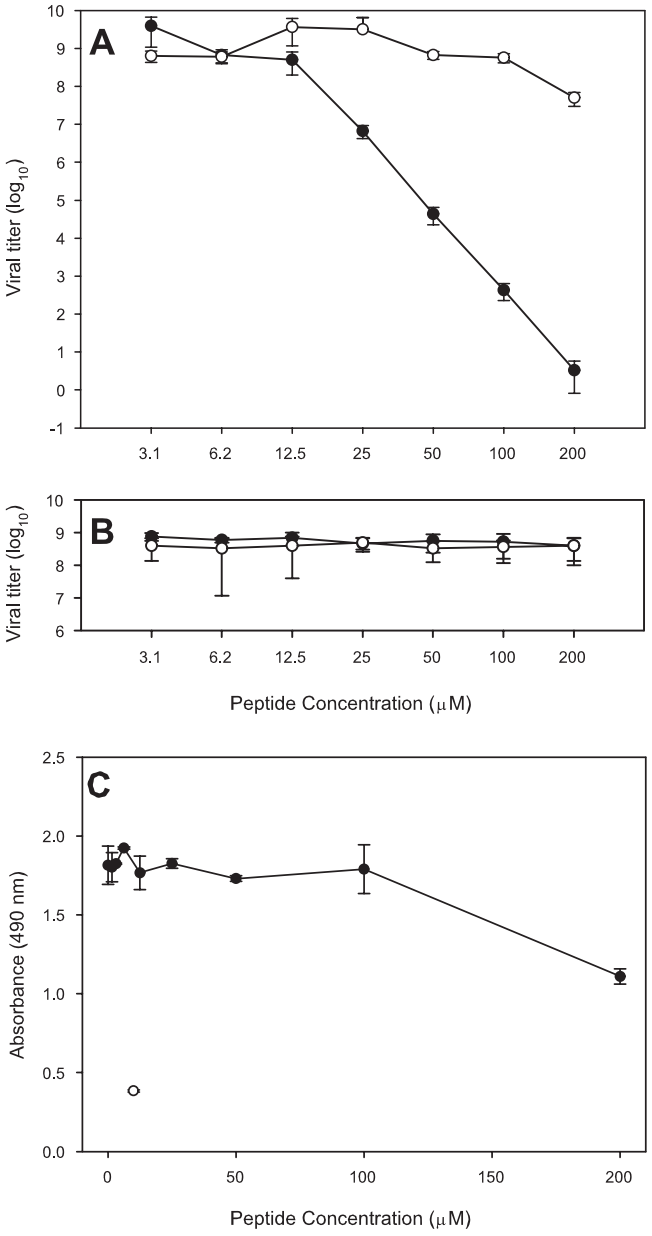

FIGURE 2. In vitro antiviral activity of θ-defensins against HSV-1 KOS. (**A**) Yield-reduction assay in which peptide was mixed with virus immediately before infection and was present at all times. The EC_{50} for RC-2 was 10 μM. RC-106 was inactive. (**B**) Virus-inactivation assay in which peptide was incubated with virus for 1 hour at 37°C and then serially diluted and titered by plaque assay. Both peptides were inactive in this assay. (**C**) Cytotoxicity of RC-2. (**A**, **B**): (●) RC-2; (○) RC-106. (**C**): (●) RC-2; (○) 10 μM bKLA peptide.

titering on Vero cells. RC-2 treatment caused a dose-dependent inhibition of viral replication with an EC_{50} of 10 μM (~20 μg/mL; Fig. 2A). Viral replication was completely prevented by 200 μM RC-2 (~400 μg/mL). In contrast, the EC_{50} for RC-106, a less active control peptide, was ~75 μM (~150 μg/mL). There was an approximately 7-log difference in titers between RC-2 and RC-106 at 200 μM (Fig. 2A). To determine whether RC-2 was virucidal, virus was mixed with various concentrations of the peptides and incubated at 37°C for 1 hour. After serially diluting the samples, their content of infectious virus was determined by plaque assay. As neither RC-2 nor RC-106 inactivated the virus (Fig. 2B), the peptides were not directly virucidal. As shown in Figure 2C, RC-2 was not toxic to Vero cells at concentrations up to 100 μM. A concentration of 200 μM reduced cell viability by 39%. These results suggest that cytotoxic effects do not contribute to the antiviral activity of RC-2.

Effect of Preincubation of Virus with Peptide on Keratitis

Studies have shown that RC-2 interferes with HSV attachment and entry,[13] suggesting that allowing the peptide and virus to interact before or during viral uptake would be effective in preventing disease. To test this notion, virus and peptide (0.1% final concentration in DMEM) were mixed, incubated at 37°C for 1 hour and then used to infect mice without further exposure to peptide. The severity of ocular disease was assessed. Sufficient amounts of the control RC-106 peptide were available for this study, and so mock and RC-106 control groups were included. Preincubation of virus and RC-2 resulted in a significant reduction ($P < 0.05$) in the severity of blepharitis on all days (Fig. 3A). The control RC-106 peptide had some activity but the blepharitis scores for this group were not significantly different ($P > 0.05$) from those in the mock group on all days that were scored. Thus, preincubation of virus with RC-2 was more effective in preventing blepharitis than both the mock and RC-106 treatments.

Preincubating the virus with RC-2 reduced corneal vascularization on days 9 to 13 postinfection (PI), relative to mock-treated virus (Fig. 3B). The differences in severity of vascularization between RC-2 and RC-106 were significant on days 11 and 13 ($P < 0.05$). At no time were vascularization differences between the RC-106 and untreated groups significant ($P > 0.05$).

The differences in stromal disease severity between untreated and RC-2-treated groups were significant ($P < 0.05$) on days 5 to 13 (Fig. 3C). Stromal disease differed significantly between the RC-2 and RC-106 groups on days 9 to 13 ($P < 0.05$). Exposure of virus to RC-106 resulted in significantly less severe stromal disease compared with the untreated group on days 5, 7, and 11 ($P < 0.05$), suggesting that RC-106 was partially effective in reducing stromal disease.

Prophylactic Effect of RC-2

To assess the prophylactic potential of RC-2, we established a mouse model in which RC-2 in PBS with 2% methylcellulose was applied to the scarified cornea before virus was added. As shown in Figure 4, blepharitis and vascularization scores were not significantly (Figs. 4A, 4B) different between the mock- and RC-2-treated groups at any time ($P > 0.05$). The severity of stromal keratitis was lower in the RC-2 group on all days (Fig. 4C) but these differences were only significant on days 3, 5, and 7 ($P < 0.05$; Fig. 4C). These results suggest that RC-2 delays the development of stromal disease in this model. As noted in the Methods section, RC-106 is difficult to prepare, and insufficient amounts were available for this experiment.

Effect of Postinfection Peptide Treatment on Ocular Disease

We next examined whether topical treatment with RC-2 applied after infection prevented the development of keratitis. Topical treatments with a 0.1% solution of peptide in PBS were initiated 4 hours after infection and continued four times per day for 7 days. On various days, the severity of blepharitis, corneal clouding, and corneal neovascularization were assessed. Postinfection treatment with RC-2 in PBS alone did not significantly reduce the severity of ocular disease (data not shown). We reasoned that delivery of RC-2 in PBS alone might lead to inefficient delivery or rapid flushing of the drug from

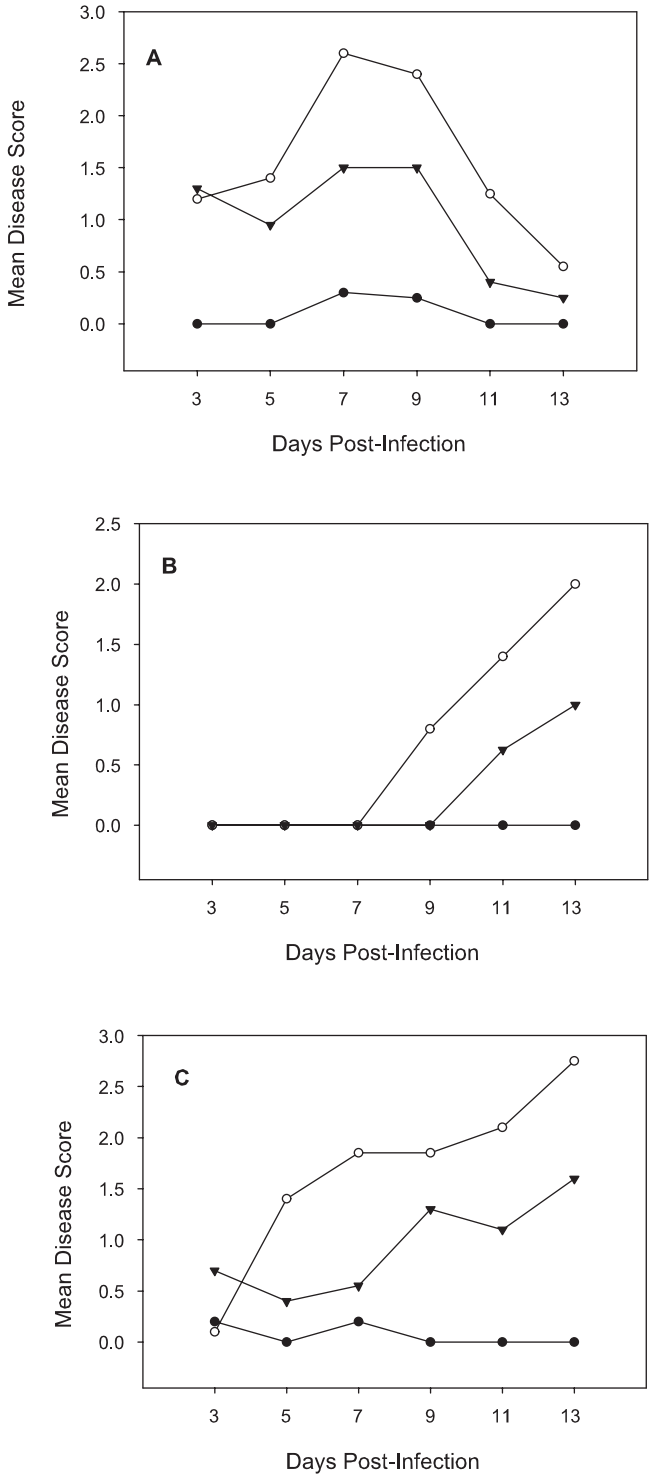

FIGURE 3. Incubation of virus with RC-2 before infection reduced the severity of HSV-1 ocular disease. Virus was incubated with peptide (0.1% wt/vol) for 1 hour at 37°C before infection of the mice. No subsequent exposure to the peptide occurred. At various times after infection, the severity of ocular disease was determined. (**A**) Blepharitis; (**B**) corneal vascularization; (**C**) stromal disease. Each group consisted of 10 mice. (●) RC-2; (▼) RC-106; (○) HSV-1 KOS only.

the cornea, and so we repeated the postinfection therapy using RC-2 in PBS with 2% methylcellulose. The results are shown in Figure 5. There were no significant differences in the severity of blepharitis, vascularization, or stromal keratitis between the

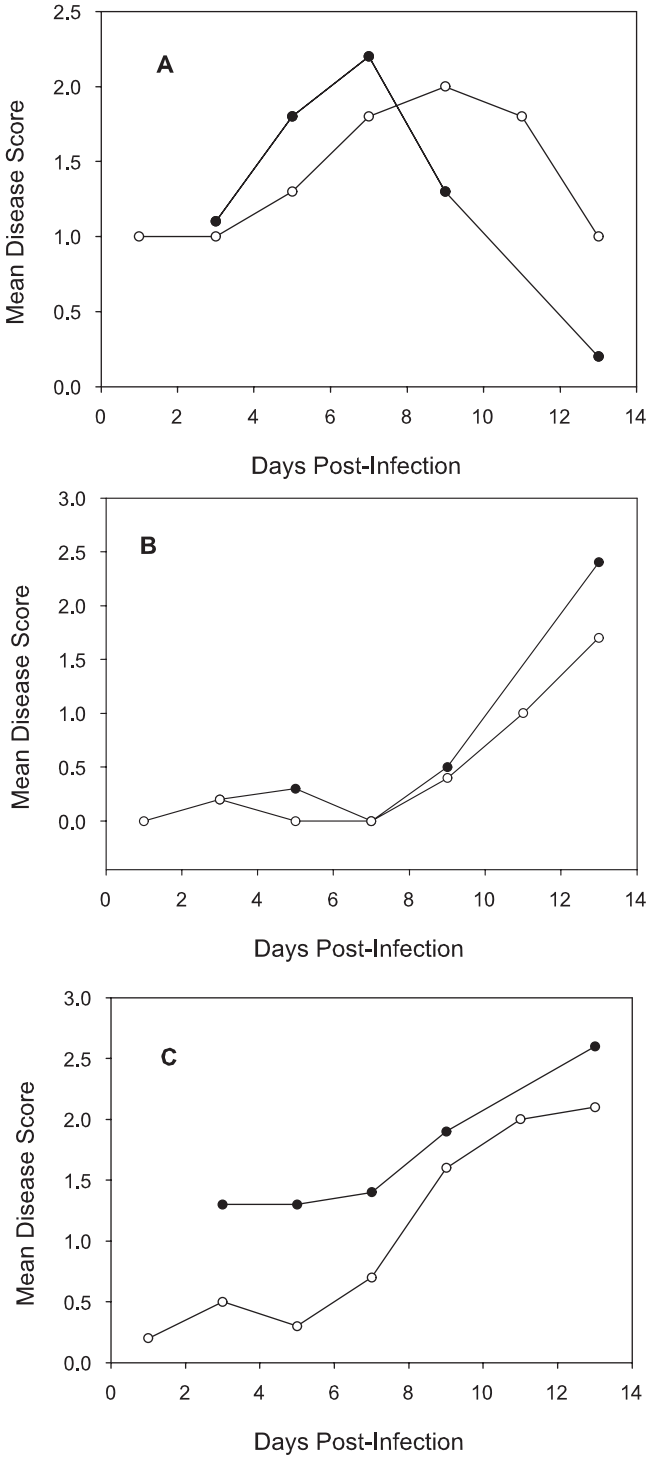

FIGURE 4. The effect of RC-2 in 2% (wt/vol) methylcellulose on HSV keratitis when placed on the cornea before infection. Mice were anesthetized with isoflurane, the right corneas were scarified, and 5 μL of either 2% (wt/vol) methylcellulose/PBS or 0.1% (wt/vol) RC-2 in 2% methylcellulose/PBS was applied to the cornea. Ten to 15 minutes later, the eyes were infected with 1×10^6 PFU of HSV-1 KOS. (**A**) Blepharitis; (**B**) corneal neovascularization; (**C**) stromal keratitis. Each group consisted of 10 mice. (●) Methylcellulose/PBS only; (○) 0.1% (wt/vol) RC-2 in methylcellulose/PBS.

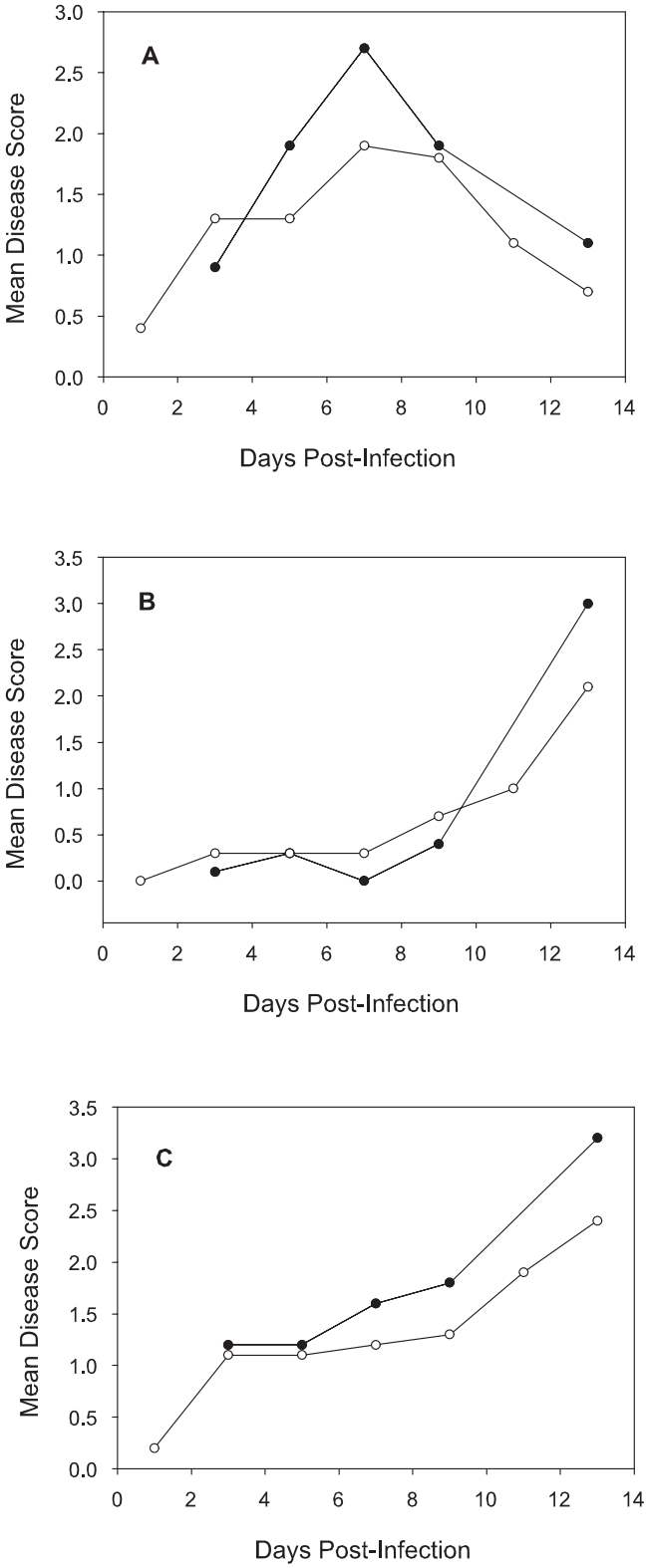

FIGURE 5. The effect of postinfection treatment with RC-2 in PBS with 2% methylcellulose on HSV-1 ocular disease. Mice were infected with 1×10^6 PFU of HSV-1 KOS in 5 μL of DMEM and then treated with RC-2 (0.1% wt/vol) in PBS with 2% methylcellulose four times per day for 7 days. (**A**) Blepharitis; (**B**) corneal neovascularization; (**C**) stromal keratitis. Each group consisted of 10 mice. (●) Methylcellulose/PBS only; (○) 0.1% (wt/vol) RC-2 in PBS with 2% (wt/vol) methylcellulose.

vehicle only and RC-2 treatment groups at any time ($P > 0.05$). We conclude that RC-2 treatment was ineffective against HSV-1-induced keratitis when applied after infection with the formulation used and drug concentration tested.

Effect of RC-2 on Viral Titers In Vivo

To determine whether the reduced disease severity in the mice infected with virus preincubated with RC-2 was reflected in ocular viral titers, tear film samples were collected at intervals, and infectious virus was titered on Vero cell monolayers. As shown in Table 1, viral titers in the RC-2 group were approximately 3 logs lower than in the control or RC-106-treated groups on day 1 ($P < 0.05$). On days 5 and 7, the titers in all three groups were essentially equivalent. By day 9, the virus had been cleared in all three groups. Viral titers were also measured in mice treated with RC-2 prophylactically. The titers in the RC-2 group were approximately five times lower on day 1 after infection ($P < 0.05$). On day 7, viral titers dropped below the limit of detection in the RC-2 prophylaxis group compared with the mock treated mice (1.9×10^1 PFU). Virus cleared completely in both groups on day 9 after infection. These results are consistent with a modest in vivo antiviral effect in the prophylactic model.

DISCUSSION

Certain α-, β-, and θ-defensins show activity against diverse human viruses in cell culture, including HIV, HSV-1, and HSV-2, vaccinia virus, adenovirus, and influenza A.[10–13,30–34] However, much less is known about their in vivo effects in animal models of viral infection. Recently, Hazrati et al.[35] demonstrated that intravaginal application of human α-defensins protect mice from infection in a model of genital HSV-2 infection. Cole et al.[36] reported that RC-101 prevents HIV-1 infection of an organlike construct of human cervicovaginal tissue. RC-101, an $arg_9 \rightarrow$lys analogue of RC-1, is identical with RC-2 in 16 of 18 residues, differing only in that residue 9 of RC-101 is lysine and residue 10 is glycine, whereas both of these are arginine in RC-2.[36]

In this study, we examined the activity of a synthetic θ-defensin, RC-2, in a mouse model of HSV-1 keratitis. RC-2 was effective in cell culture against HSV-1 KOS, did not directly inactivate the virus, and was not toxic to Vero cells at concentrations up to 100 μM. Comparing the efficacy of RC-1 and RC-106 indicated that the anti-HSV-1 activity of RC-2 was sequence specific. Significant protection ensued when the virus was incubated with RC-2 before the mice were infected or when RC-2 in 2% methylcellulose was placed on the abraded cornea before viral infection. In contrast, the topical application of RC-2 in 2% methylcellulose after infection failed to reduce the severity of HSV keratitis.

The reduction in viral titers on day 1 with subsequent recovery to the levels seen in the control groups when virus was preincubated with RC-2 suggests that viral replication was delayed. Such a delay could allow time for host defenses to be activated, leading to reduced disease severity. Previous work using trifluorothymidine[28] and a peptidomimetic antiviral[29] revealed that a reduction in the severity of ocular HSV infection could result from either reducing viral titers by as little as 1 log or by enhancing clearance of virus from the eye. The data obtained for RC-2 reinforces the conclusion that it is not necessary to inhibit viral replication completely to achieve a significant therapeutic effect.

Several possibilities could explain the ineffectiveness of topical RC-2 when applied after infection. In studies with other viruses (e.g., HIV-1, influenza A, and HSV-2), RCs and other θ-defensins acted primarily as viral uptake inhibitors and had

TABLE 1. Ocular Viral Titers

Sample	Days Postinfection				
	1	5	7	9	11
Preincubation of virus and peptide*					
KOS	$1.1 \pm 1.7 \times 10^5$	$1.5 \pm 2.6 \times 10^4$	$5.0 \pm 6.1 \times 10^2$	0	0
RC-2	$2.2 \pm 2.8 \times 10^2$†	$1.4 \pm 2.3 \times 10^4$	$1.8 \pm 3.4 \times 10^2$	0	0
RC-106	$1.1 \pm 2.6 \times 10^5$	$6.0 \pm 4.6 \times 10^3$	$1.7 \pm 2.8 \times 10^2$	0	0
Prophylaxis model‡					
Methylcellulose only	$2.5 \pm 3.3 \times 10^5$	$3.6 \pm 3.1 \times 10^3$	$1.9 \pm 4.2 \times 10^1$	0	0
RC-2 in methylcellulose	$4.7 \pm 2.4 \times 10^4$†	$3.8 \pm 2.9 \times 10^3$	0	0	0

Data are expressed as the mean ± SD.

* Virus incubated with peptide (0.1%) for 1 hour at 37°C before infecting mice.

† $P < 0.05$ RC-2 vs. KOS or RC-106; $P < 0.05$ RC-2/methylcellulose vs. methylcellulose alone. Student's *t*-test.

‡ Methylcellulose only or methylcellulose with RC-2 (0.1%) added to scarified cornea 10 minutes before virus.

little effect after the virus had entered the cell.[10,12,13,37] It is possible that using higher peptide concentrations or a vehicle or delivery system other than methylcellulose would have afforded more promising results. In addition, little is known about the uptake and distribution of peptides after topical corneal application; thus, further formulation and pharmacokinetic studies are needed. We are currently testing several other antiviral peptides[4,25,27] in animal models to determine whether one of these may perform better than RC-2.

Animal-derived AMPs other than defensins can also inhibit HSV in cell culture, and some of these may deserve further testing. Among them are a 23-amino-acid analogue of melittin,[38] an 18-residue peptide derived from the apolipoprotein A-I sequence,[39] a 33-amino-acid peptide homologous to the heptad repeat region of bovine herpes virus type 1 glycoprotein B,[40] a series of cationic α-helical peptides,[41] lactoferrin, and peptides released from the amino terminus of lactoferrin by proteolytic digestion.[42,43] To date, no publications describe the effects of these peptides on HSV in animal models.

After this project was initiated, a study that examined the effects of various defensins on in vitro and in vivo HSV-2 infections became available.[35] That study compared the ability of nine human defensins (HD) to protect against HSV-2 infection. It was found that noncytotoxic concentrations of all six human α-defensins (HNP1 to 4, HD5, and HD6) and of human β-defensin-3 inhibited HSV infection, but that two other β-defensins, HBD1 and -2, lacked protective activity. HNP-4, HD6, and HBD3 acted primarily by preventing binding and entry, whereas HNP-1 to -3 and HD5 inhibited postentry events as well, affording protection even when added several hours after entry. Of note, human cervical epithelial cells that were incubated with HNP-1 or HD5 accumulated these peptides intracellularly. Overall, these findings demonstrate that various human defensins act at different (and often multiple) steps in the HSV life cycle. Although defensins that are highly effective as viral entry inhibitors may be optimal choices for disease prophylaxis, defensins such as HNP-1 to -3 or HD5 may be better choices for treating ongoing corneal infection.

Our observations that RC-2 had an effect only when preincubated with the virus or was present on the cornea before infection is consistent with data showing that defensins act to block attachment and/or entry of HSV-1 into cells. When RC-2 was given therapeutically after infection, we saw no effect, suggesting that RC-2 would not be useful for postinfection treatment unless the activity could be improved. As we have mentioned, additional pharmacokinetic and formulation studies are needed and could result in improved activity. Based on our results, the most effective use of RC-2 would be as a prophylactic agent to block HSV-1 corneal infection.

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