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Antiherpetic activity and mode of action of natural carrageenans of diverse structural types

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Abstract

The λ-carrageenan 1T1, the κ/ι-carrageenan 1C1 and the μ/ν-type 1C3, isolated from the red seaweed *Gigartina skottsbergii*, proved to be potent and selective inhibitors of herpes simplex virus (HSV) types 1 and 2. The antiviral IC_{50} values determined by virus yield inhibition assay in different cell lines ranged from 0.4 to 3.3 μg/ml, and no cytotoxic effects, measured by trypan blue exclusion on stationary or proliferating cells, tetrazolium salt method or cell protein synthesis, were observed. Time of addition and attachment studies suggested that the main target for antiviral action of the three carrageenans was virus adsorption, whereas no effect on virus internalization, or early or late protein synthesis was detected. However, the λ-carrageenan 1T1 was still significantly inhibitory when added any time after adsorption. The pretreatment of virions with the carrageenans showed that 1C1 and 1C3 lacked direct inactivating effect at concentrations near the antiviral IC_{50} but 1T1 exerted virucidal action. The cyclization of 1T1 to afford the derivative 1T1T1 maintained the antiviral activity but eliminated the virucidal properties. Thus, the structure of 1T1 seems to be responsible for its differential behavior from 1C1 and 1C3, probably allowing a more stable binding to HSV, leading to virion inactivation. In contrast, 1C1 and 1C3 fail to bind with high affinity to virus alone, but are able to interfere with the interaction between HSV particles and the cell.

Keywords: Herpes simplex virus; Carrageenans; Antiviral activity; Adsorption inhibitor; Virucidal activity; Sulfated polysaccharide

1. Introduction

Several sulfated polysaccharides, such as heparin, dextran sulfate, pentosan polysulfate, mannan sulfate, sulfated cyclodextrins and others, have been shown to inhibit the replication of

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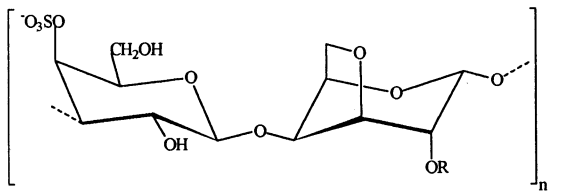
various enveloped viruses, including herpes simplex virus (HSV), human cytomegalovirus (HCMV) and human immunodeficiency virus (HIV) (Witvrouw et al., 1994a). Although mechanistic studies have produced contradictory results (Lederman et al., 1989; McClure et al., 1992), their mode of action has been often attributed to a blockade of the early stages of the virus replication cycle (Baba et al., 1988a; Callahan et al., 1991; Neyts et al., 1992; Jagodzinski et al., 1994).

Carrageenans are sulfated galactans that can be extracted from certain red seaweeds. They comprise a broad range of structures and can be divided into two families: the κ-family, defined by the presence of a C4-sulfate group on the β-D-unit, and formed by κ/ι-carrageenans and μ/ν-carrageenans, and the λ-family, characterized by the sulfate on C2 and constituted by all the variations of the λ-structures (Painter, 1983). In the course of a screening study on the biological properties of marine natural products, diverse carrageenans isolated from the red seaweed *Gigartina skottsbergii* have proved to be potent inhibitors of HSV types 1 and 2 in vitro, and details of the relationship between their structural features and the degree of antiviral activity were reported (Carlucci et al., 1997). Three active carrageenans representative of each structural type (λ-, κ/ι- and μ/ν-carrageenan) (Fig. 1) were chosen for further characterization of their antiherpetic activity and mode of action against HSV-1 infection.

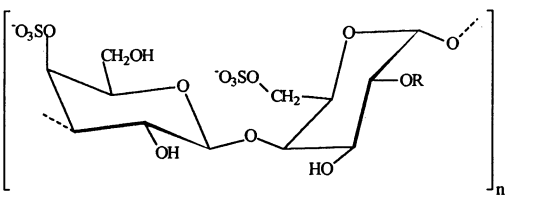
2. Materials and methods

2.1. Compounds

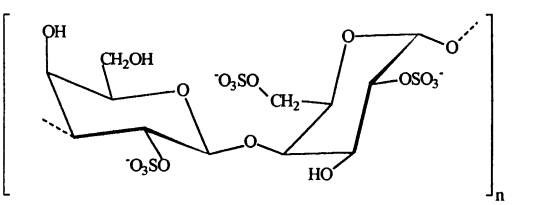
The λ-carrageenan 1T1, its derivative 1T1T1, the κ/ιcarrageenan 1C1 and the μ/ν-carrageenan 1C3 were extracted and purified from cystocarpic and tetrasporic stages of *G. skottsbergii*, a red seaweed collected in Camarones Bay, Chubut, Argentina, as previously described (Carlucci et al., 1997). Briefly, carrageenans were extracted with water at room temperature and were fractionated with KCl. Fractionation of the tetrasporic carrageenan (1T) yielded 1T1, which precipitated between 0.60 and 0.70 M KCl. 1C1 and 1C3 were obtained from the cystocarpic carrageenan (1C); 1C1 precipitated between 0.30 and 0.31 M KCl ,and 1C3 remained soluble in 2.0 M KCl. Alkaline treatment of 1T1 and subsequent fractiona-



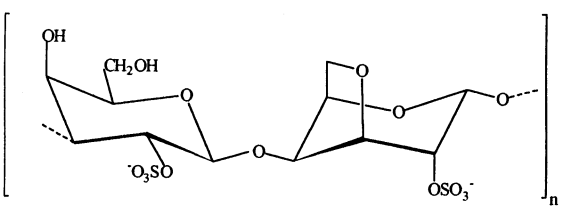
1C1



1C3



1T1



1T1T1

Fig. 1. Chemical structure of the carrageenans isolated from *Gigartina skottsbergii*. 1C1: R = H, κ-carrageenan; R = SO_3^-, ι-carrageenan. In a natural κ/ι-carrageenan, some of the 3,6-anhydro-α-D-galactose units and 3,6-anhydro-α-D-galactose 2-sulfate units are replaced by α-D-galactose 6-sulfate and α-D-galactose 2,6-disulfate units. 1C3: R = H, μ-carrageenan; R = SO_3^-, ν-carrageenan. In a natural partially cyclized μ/ν-carrageenan, some of the α-D-galactose units are replaced by 3,6-anhydro-α-D-galactose and 3,6-anhydro-α-D-galactose 2-sulfate units; 1T1, λ-carrageenan; 1T1T1, cyclized λ-carrageenan.

tion of the product yielded 1T1T1, which was soluble in 2.0 M KCl. Pure structures were determined by methylation analysis and nuclear magnetic resonance spectroscopy. Their chemical structures are shown in Fig. 1. Dextran sulfate with average molecular weight of 8000 (DS8000) was purchased from Sigma-Aldrich (USA). Stock solutions of all compounds were prepared in sterile water.

2.2. Viruses and cells

Vero cells (ATCC) were grown as monolayers in Eagle's minimum essential medium (MEM) (GIBCO, USA) supplemented with 5% inactivated calf serum. For maintenance medium (MM), the serum concentration was reduced to 1.5%. The human diploid foreskin fibroblast cell line PH was provided by Dr G. Carballal (CEMIC, Buenos Aires, Argentina) and propagated in MEM supplemented with 10% fetal calf serum.

HSV-l strain F and HSV-2 strain G were obtained from the American Type Culture Collection; the HSV-1 TK^- strain B2006 was obtained from Prof. Dr E. De Clercq (Rega Institute, Belgium); the HSV-1 strain 1180 BE/94 and the strain 244 BE/94 of HSV-2 were clinical isolates provided by the Instituto Nacional de Microbiologia Carlos Malbran (Buenos Aires, Argentina) (Carlucci et al., 1997).Virus stocks were propagated and assayed by plaque formation in Vero cells.

2.3. Measurement of cell growth and viability

Cellular viability was measured with the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma-Aldrich) (MTT) method in confluent cultures of Vero, and PH cells grown in 96-well plates and exposed during 48 h at 37°C to two-fold dilutions of the carrageenans, with three wells for each dilution. Cytotoxicity was calculated as the cytotoxic concentration 50% (CC_{50}), the compound concentration required to reduce the MTT signal by 50% compared with controls.

The effects of carrageenans on cell proliferation and viability in Vero cells were compared using a cell count assay. Vero cells were seeded in 24-well plates at a density of 5×10^4 cells/well and incubated in growth medium at 37°C. To measure the effects on proliferating and confluent cultures, carrageenans were added 3 h after seeding in growth medium or 2 days after seeding in maintenance medium, respectively. The cells were then incubated with compounds at 37°C for 72 h, at which time the medium was aspirated from the wells, cells were washed and detached with trypsin, and the viable cell number was determined by trypan blue exclusion. The experiment was done twice and duplicate samples for each drug concentration were counted.

2.4. Antiviral assay

Antiviral activity was evaluated by a virus yield inhibition assay. Vero and PH cells were infected at a MOI of 0.5 in the presence of different concentrations of carrageenans (two wells per concentration). After adsorption, cells were washed and refed with MM containing the compound. After 24 h of incubation at 37°C, the medium and the infected cells were frozen and thawed twice, and the virus yields were determined by a plaque assay in Vero cells. The 50% inhibitory concentration (IC_{50}) was calculated as the concentration required to reduce virus yield by 50%.

2.5. Time of addition experiments

The compounds were evaluated in a high MOI time-of-addition assay by virus yield reduction. Vero cells grown in 24-well plates were infected with HSV-1 (MOI 0.5) and the compounds (20 μg/ml) were added either simultaneously with virus (time 0) or at various times after infection and allowed to remain on the cells for the entire assay period (22 h). Then, supernatants and infected cells were frozen and virus yields were quantitated by plaque formation.

The influence of the time of treatment was also determined in a plaque reduction assay. Vero cells grown in 24-well plates were infected with 60 PFU

of HSV-1 under different treatment conditions: exposure to compounds (20 μg/ml) was restricted to the virus adsorption phase only (compound in the inoculum), or to adsorption and post-infection (compound in the inoculum and in the plaquing medium), or to post-infection only (in the plaquing medium). After 48 h incubation at 37°C, plaques were counted and percent inhibition with respect to control cultures without compound was calculated for each treatment.

2.6. Effect of carrageenans on HSV-1 adsorption kinetics

The effect of carrageenans on virus adsorption was measured by two techniques.

1. Infectivity assay of cell-adsorbed virus. Vero cells were infected with HSV-1 at a MOI of 1 in the presence or absence of 20 μg/ml of each carrageenan. After adsorption for varying time periods at 4°C, cells were washed with phosphate buffered saline (PBS) to remove unadsorbed virus, and disrupted by freezing and thawing. The amount of infectious bound virus was then measured by plaque formation.
2. Binding assay of radiolabelled HSV-1 particles. The preparation of radiolabelled HSV-1 and the binding assay have been described previously (Pujol et al., 1996). Briefly, [^{35}S]methionine-labelled HSV-1 (7.3×10^6 PFU/ml and 4×10^6 cpm/ml) and MM or 20 μg/ml of carrageenan were added to Vero cells and incubated at 4°C for 0 or 60 min. Then cells were washed extensively with PBS, lysed with a 0.1 M NaOH solution containing 1% sodium dodecyl sulphate (SDS), and cell-associated radioactivity was determined.

2.7. HSV-I internalization

HSV-1 was adsorbed to Vero cells at a MOI of 1 for 1 h at 4°C. The cells were then incubated at 37°C to allow virus penetration in MM containing, or not, 20 μg/ml of carrageenan for 60 min. After incubation, cultures were washed with PBS and treated with 0.5 mg/ml of proteinase K in PBS to remove external adsorbed virus. Protease treatment was then stopped by adding 1 mM phenyl-methyl-sulphonyl-fluoride in PBS containing 3% bovine serum albumin. Cells were then pelleted and internalized virus was quantitated in the cell pellet by an infectious center assay.

2.8. HSV-1 protein synthesis

Vero cells were mock-infected or infected with HSV-1 at a MOI of 10. At several times post-infection, the cells were pulse-labelled for 2 h with 10 μCi/ml of L-[^{35}S]-methionine (Sp.act. 1031 Ci/mmol; New England Nuclear, USA) in methionine-free MM containing, or not, 20 μg/ml of carrageenan. The cells were then washed with PBS and lysed with sample buffer containing 5% SDS, 2% 2-mercaptoethanol, 10% glycerol and 0.005% bromophenol blue in 0.0625 M Tris–HCl, pH 6.8. Polypeptides were then electrophoresed on 12% polyacrylamide–SDS slab gels and visualized by fluorography.

2.9. Virucidal assay

A virus suspension containing 4×10^4 PFU HSV was incubated with an equal volume of MM with or without carrageenans for 1.5 h at 37°C. The samples were then chilled and diluted to determine residual infectivity by plaque formation.

3. Results

3.1. Effects of carrageenans on HSV-I production and cell viability

In the previous screening studies, the carrageenans 1T1, 1C1 and 1C3 were shown to inhibit plaque formation of various strains of HSV-1 and HSV-2 in Vero cells, including acyclovir-resistant variants and clinical isolates (Carlucci et al., 1997). The antiherpetic activity of these compounds was also evaluated in a more stringent assay by determining inhibition of virus yield in Vero cells after 24 h of infection at a high MOI. As shown in Table 1, the three types of natural carrageenans were very effective in blocking virus production. The IC_{50} values against

Table 1
Anti-HSV-1 activity and cytotoxity of carrageenans in Vero and PH cells

Compound	IC_{50} (μg/ml)[a]		CC_{50} (μg/ml)[b]		SI (CC_{50}/IC_{50})	
	Vero	PH	Vero	PH	Vero	PH
1C1	3.2 ± 0.3	3.3 ± 0.3	>1000	>1000	>312	>303
1C3	0.9 ± 0.3	0.8 ± 0.1	>1000	>1000	>1111	>1250
1T1	0.4 ± 0.1	0.8 ± 0.1	>1000	>1000	>2500	>1250
DS8000	1.8 ± 0.4	0.8 ± 0.2	>1000	>1000	>555	>1250

[a] Antiviral activity was determined by virus yield inhibition assay. Results are presented as the mean ± SD from duplicate independent tests.
[b] Cytotoxicity was determined by the MTT assay.

HSV-1 varied between 0.4 and 3.3 μg/ml, i.e. similar concentration as those previously determined by the plaque reduction assay (Carlucci et al., 1997). When compared with dextran sulfate, a reference substance, two of the compounds (the λ-carrageenan 1T1 and the μ/ν-carrageenan 1C3) were slightly more active. Furthermore, another cell type, such as the diploid human cell line PH, showed a similar level of sensitivity to the antiherpetic action of the carrageenans in a HSV-1 yield reduction assay (Table 1). These data demonstrate that the antiviral action of carrageenans was not mainly affected either by the MOI in the antiviral test or by the host cell origin.

Simultaneously with the virus yield inhibition assay, cytotoxicity was determined by the MTT method in uninfected confluent cultures of Vero and PH cells on the same day as the antiviral activity experiments. As shown in Table 1, the CC_{50} for the three carrageenans was greater than 1000 μg/ml (maximum concentration tested). Thus, the selectivity index (ratio CC_{50}/IC_{50}) in the confluent cultures used in antiviral assays was always greater than 300 (range 303–2500). To further assess the lack of cytotoxicity of these compounds, their effects on cell proliferation of actively growing cells were also evaluated. The viability of proliferating cells after 72 h exposure to the carrageenans added at 3 h after seeding compared well with that obtained in confluent cells treated at 2 days after seeding, since 1T1, 1C3 and 1C1 caused only 26, 16 and 14% inhibition of cell growth, respectively, at a concentration of 500 μg/ml, which is 151–1250 times higher than the IC_{50} for HSV-1, thus confirming the high selectivity of these compounds.

3.2. Influence of time of addition of carrageenans on HSV-1 infectivity

In attempts to determine the mode of antiviral action of the carrageenans against HSV-1 replication, time of addition experiments were conducted. In a first experiment, compounds were added either simultaneously or at 1-h intervals after exposure of cells to virus at a high MOI (from 0 to 8 h p.i.). At 22 h p.i., virus yields were determined. As shown in Fig. 2, 1C1, 1C3 and 1T1 all reduced virus yield by 3–4 log units when added during the attachment period of virus with cells (time 0). By contrast, 1C1 and 1C3 were ineffective in cells treated after virus attachment, since virus yields were similar to those obtained in control cultures. However, the λ-carrageenan 1T1 still produced a significant reduction in virus multiplication when added after 1 h of infection and the levels of HSV-1 yield remained 2 log units lower than in the control cultures at any time after compound addition.

To confirm the differential behavior of 1T1, the carrageenans were evaluated in a second experiment, by a plaque reduction assay under three different treatment conditions. A similar level of efficacy (90–98% inhibition) was attained if the compounds were present either only during HSV-1 adsorption or during the whole period of the plaque assay (Table 2). When present only after adsorption, 1C1 and 1C3 were no longer effective,

but again, 1T1 still produced a lower but significant reduction in plaque formation as compared with the control.

3.3. Effects of carrageenans on virus adsorption, internalization and protein synthesis

The inhibition of virus adsorption by the carrageenans was confirmed by evaluating directly the kinetics of HSV cell attachment in the presence of the compounds. As seen in Fig. 3, infectious virus adsorption to Vero cells at 4°C was highly inhibited by treatment with 20 μg/ml of each carrageenan. Furthermore, the carrageenans also inhibited the binding of radiolabelled HSV-1 particles to Vero cells (Fig. 4).

The possible effect of the carrageenans on a subsequent stage of the virus cycle was studied, first analyzing the effect on virus internalization. To this end, virus was adsorbed at 4°C without compound, and then the temperature was raised to 37°C to allow penetration either in the presence or absence of the carrageenans. Neither compound prevented penetration of HSV-1 since the amount of internalized virus, determined as the number of infectious centres after inactivating the adsorbed unpenetrated virus by protease treatment, was similar in carrageenan-treated and control cells.

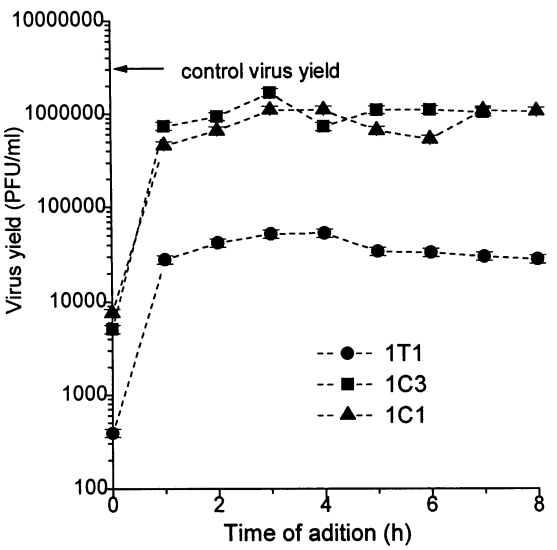


Fig. 2. Influence of time of addition of carrageenans on antiherpetic activity. Vero cells were infected with HSV-1 (MOI 0.5) and compounds (20 μg/ml) were added simultaneously (time 0) or at the indicated times. Virus yields were determined after 22 h of infection. Each point is the mean value of duplicate independent experiments.

Table 2
Influence of the treatment time on the anti-HSV-1 activity of carrageenans[a]

Time of drug treatment	Percentage inhibition (mean value ± SD)		
	1C1	1C3	1T1
During virus adsorption	97 ± 4	90 ± 6	90 ± 5
After virus adsorption	25 ± 8	16 ± 5	79 ± 9
During and after virus adsorption	98 ± 2	93 ± 2	97 ± 6

[a] Vero cells were infected with approximately 60 PFU of HSV-1 in the absence or in the presence of 20 μg/ml of 1Cl, 1C3 or 1T1. After 60 min adsorption at 4°C, unadsorbed virus was removed and the cells were overlaid with MM containing 0.7% methylcellulose with or without compound, and further incubated at 37°C for 2 days. Each value represents the mean of independent duplicate assays ± standard deviation.

The effect of carrageenans on protein synthesis in HSV-1-infected cells was studied next. Vero cells were mock-infected or infected with HSV-1, carrageenans were added after infection, and protein synthesis was then analyzed by polyacrylamide gel electrophoresis. The patterns revealed no inhibition of protein synthesis in carrageenan-treated mock-infected cells, confirming the lack of toxicity of these compounds, whereas in HSV-1-infected cells treated with the compounds during the labelling period at 2–4, 4–6 or 6–8 h p.i., no alterations in viral protein synthesis were observed (data not shown), indicating that neither early nor late HSV-1 protein synthesis was affected by any of the carrageenans.

3.4. Direct action of carrageenans on cells and virions

As no effect of 1T1 on post-binding stages of the HSV-1 replicative cycle was detected but virus yield was significantly reduced when this car-

rageenan was added after adsorption (Fig. 2), the possibility that the compound acted directly on the virus particles or on the virus-infected cells was investigated. When the polysaccharides were incubated with the cell monolayers for 2 h at 37°C and then removed before virus infection, none of the carrageenans was effective in reducing the input HSV-1 titer (data not shown), suggesting that these drugs failed to interact with the target cell. By contrast, preincubation of the virus with the carrageenans resulted in a concentration-dependent reduction of remaining infectivity (Table 3). A striking difference in the level of virucidal activity among the different types of carrageenans was observed: compounds 1C1 and 1C3 had no significant direct inactivating effect on HSV-1 virions at concentrations near the IC_{50} as determined by virus yield reduction assay, whereas 1T1 exerted virucidal activity even at a dose lower than 1 μg/ml. In fact, the IC_{50} values for virucidal activity calculated from data shown in Table 3 were > 50, 27.9 and 0.5 μg/ml for 1C1, 1C3 and

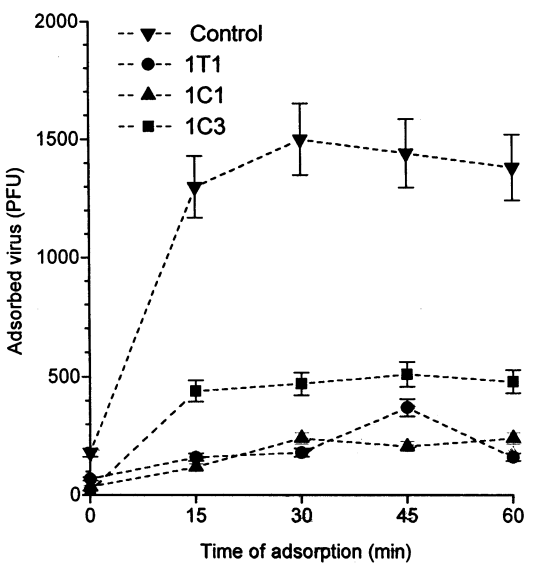


Fig. 3. Effect of carrageenans on HSV-1 adsorption kinetics. Vero cells were incubated during 0, 15, 30, 45 and 60 min at 4°C with HSV-1 (MOI 1) in the absence or presence of 20 μg/ml of each carrageenan. Cells were then washed and disrupted. Cell-bound infectious virus was determined by plaque assay. Each point is the mean value of duplicate independent experiments.

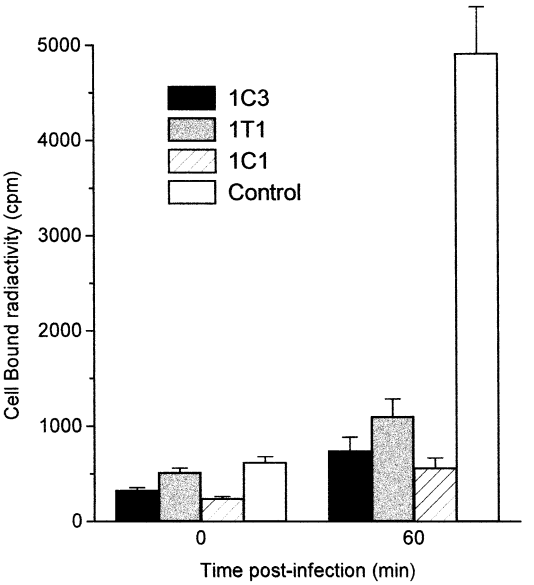


Fig. 4. Effect of carrageenans on binding of radiolabelled HSV-1. Vero cells were incubated during 0 and 60 min at 4°C with L-[^{35}S]-methionine-labelled HSV-1 in the absence or presence of 20 μg/ml of each carrageenan. Cell-associated radioactivity was then determined. Each value is the mean of two independent experiments.

1T1, respectively. The comparison of these data with the IC_{50} corresponding to virus yield inhibition assay (Table 1) indicated that the concentration of 1C1 and 1C3 required to inactivate HSV-1

Table 3
Effect of carrageenans on HSV-1 inactivation[a]

Concentration (μg/ml)	Residual infectivity (% control)			
	lC1	1C3	1T1	1T1T1
0	100	100	100	100
0.5	91 ± 4	81 ± 1	51 ± 3	95 ± 4
2.0	78 ± 1	70 ± 6	32 ± 5	93 ± 1
10.0	70 ± 1	61 ± 5	23 ± 4	76 ± 5
25.0	64 ± 5	52 ± 9	18 ± 1	63 ± 3
50.0	56 ± 3	39 ± 7	17 ± 6	16 ± 4

[a] Samples of HSV-1 strain F containing 4×10 PFU were incubated at 37°C for 1.5 h with different concentrations of the compounds, and remaining infectivity was determined by plaque formation. Results are expressed as 100× compound-treated virus titer/control virus titer (mean value of two independent experiments ± SD).

virions by pretreatment before infection was $>15.6–31$ times higher than the concentration required to reduce virus yield when the compounds were added at the time of virus adsorption. By contrast, the IC_{50} values for 1T1 in both experimental conditions were similar (0.5 vs. 0.4 μg/ml). In addition, the virucidal properties of 1T1 were assayed for other strains of HSV-1 and also for HSV-2: the IC_{50} for virucidal activity of 1T1 against HSV-1 1180 BE/94 strain, TK^- HSV-1 B2006 strain, HSV-2 G strain and HSV-2 244 BE/94 strain were 0.4, 0.8, 0.5 and 0.6 μg/ml, respectively, whereas the corresponding values of IC_{50} for virus yield inhibition assay were 0.3, 0.3, 0.4 and 0.6 μg/ml.

The structural differences between 1T1, and 1C1 and 1C3, may be responsible for its differential mode of action, allowing a more stable binding of 1T1 to HSV-l, thus leading to virus inactivation. This hypothesis was confirmed by analyzing the biological properties of a chemically modified derivative of 1T1. The alkaline treatment of 1T1 produced the cyclization of the α-D-galactose 6-sulfate and 2,6-disulfate units to 3,6-anhydro-D-galactose and 3,6-anhydro-D-galactose 2-sulfate units, affording the cyclized derivative 1T1T1 (Carlucci et al., 1997). This compound maintained the antiviral activity of 1T1 as measured by a virus yield inhibition assay in Vero cells with an IC_{50} value against HSV-1 of 0.3 μg/ml, but the virucidal properties were significantly diminished (Table 3) and the IC_{50} of 1T1T1 determined by direct virion inactivation was 30.4 μg/ml.

4. Discussion

The diverse structural types of natural carrageenans assayed in this study exhibit potent and specific inhibitory activity against HSV-1 infection and replication. According to antiviral and cytotoxic determinations, the most effective carrageenans were the λ-type 1T1 and the μ/ν-carrageenan 1C3, with selectivity indices greater than 1000 in both human- and monkey-derived cells (Table 1). In fact, 1C3 is at present the first partially cyclized μ/ν-carrageenan shown to have antiviral properties.

In recent years, several classes of sulfated polysaccharides have been reported to have antiviral activity, particularly against HSV, HIV and CMV. The compounds tested included sulfated mannans, glucans, galactans, fucans (McClure et al., 1991; Koizumi et al., 1993; Beutler et al., 1993; Damonte et al., 1994; Witvrouw et al., 1994b; Kolender et al., 1997), and also κ-, ι- and λ-carrageenans (Gonzalez et al., 1987; Nakashima et al., 1987; Baba et al., 1988b; Girond et al., 1991; Hamasuna et al., 1993) from commercial origin or extracted from natural sources. However, based on our studies, some considerations can be put forward suggesting possible advantages of the carrageenans obtained from *G. skotsbergii*. First, they had no detrimental effects on cell viability or growth in stationary and proliferating cells, after continuous incubation with each of the compounds, even at the highest concentrations tested. Second, the substantial increase in MOI (approximately 1000–10 000 times) required for the virus yield experiments in comparison with plaque reduction assays did not greatly alter the antiviral effectiveness of the three types of carrageenans analyzed, in contrast with results obtained with other antiviral drugs (Harmemberg et al., 1985; Nakashima et al., 1989; Shigeta et al., 1995). Third, carrageenans lack anticoagulant properties, as demonstrated in a previous paper (Carlucci et al., 1997).

Mode of action studies are consistent with the carrageenans having a main effect on the attachment of virus to the cells. Adsorption of HSV-1 is primarily mediated by the envelope glycoprotein gC, which binds to heparan sulfate residues present on the proteoglycans on the surface of target cells (Herold et al., 1991; Shieh et al., 1992; Spear, 1993; Trybala et al., 1994; Tal-Singer et al., 1995). Although, at present, the receptor-binding site of gC is not totally elucidated, there is a cluster of basic amino acids in the vicinity of a very hydrophilic region near the N-terminus of gC, which could be considered as the heparan sulfate-binding domain (Trybala et al., 1994; Tal-Singer et al., 1995). This region in the N-terminal portion, shared in common by other members of the gC protein family in α-herpes viruses (Flynn et al., 1993; Liang et al., 1993), is perhaps the site with which the carrageenans may interact. How

ever the interaction of 1T1 seems to be somewhat different from that of 1C1 and 1C3, in that the λ-carrageenan 1T1 was able to inactivate virion particles at concentrations near the virus yield-inhibitory IC_{50}, whereas for the other two carrageenans, virucidal activity was only observed at concentrations highly exceeding the antiviral concentrations. The divergence in chemical structure among the carrageenans can explain their different behavior. Three main dissimilarities in the primary structure of 1T1 as compared with 1C1 and 1C3 have been described (Fig. 1; Carlucci et al., 1997): the molar ratio of galactose to 3,6-anhydrogalactose is 1:0.03, 1:0.64 and 1:0.37 for 1T1, 1C1 and 1 C3, respectively; the position of the sulfate group in the β-D-galactose residues is on C-2 in 1T1 and on C-4 in 1C1 and 1C3; and the content of α-D-galactose 2,6-disulfate residues is higher in 1T1. At present, it is difficult to ascertain which (or all) of these chemical differences is responsible for the particular behavior of 1T1 in its interaction with HSV-1.

In conclusion, the carrageenans 1C3 and 1T1 have been shown to be antiherpetic compounds, lacking cytotoxicity and anticoagulant action. Further studies relating to the bioavailability, pharmacokinetics and antiviral efficacy of these compounds in animals have been initiated to determine their potential role as clinical drug candidates against HSV infections.

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