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Antiviral Research 25 (1994) 67–72

Antiviral Research

Short Communication

Antiviral capsid-binding compounds can inhibit the adsorption of minor receptor rhinoviruses

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Received 9 February 1994; accepted 2 April 1994

Abstract

The effect of four structurally diverse capsid-binding compounds on the adsorption of seven human rhinoviruses (HRV), representative for both receptor and antiviral groupings was studied using infective center assays. Antiviral compounds studied included a pyridazinamine (R 61837), an isoxazole (WIN 51711), a flavan (4′,6-dichloroflavan) and a chalcone (Ro-09410). Minor receptor viruses studied were HRV 1A, HRV 2 and HRV 29 (antiviral group B), major receptor viruses were HRV 9, HRV 39 and HRV 14, HRV 35 (antiviral group B and A, respectively). The adsorption of four out of the seven serotypes was inhibited by some antiviral compounds, but not by others, indicating that the conformational alterations induced by antiviral compounds can vary considerably within a given serotype, depending on the chemical nature of the antiviral compound used. A correlation between inhibition of adsorption and receptor grouping or antiviral grouping could not be found.

Key words: Rhinovirus; Capsid-binding compound; Adsorption; Receptor group

A variety of structurally unrelated antiviral molecules have been found to inhibit rhinoviral replication by binding to viral capsid proteins (reviewed in McKinlay et al., 1992; Andries, in press). X-ray diffraction analysis of crystals of human rhinovirus (HRV 14 serotype) infused with (oxazolinylphenyl)isoxazoles (e.g., WIN 51711), pyridazinamines (e.g., R 61837) and others, has shown that these capsid-binding compounds bind into a hydrophobic pocket within the eight-stranded β-barrel of VPI, one of the four proteins of the rhinoviral capsid (reviewed in Zhang et al., 1992).

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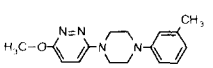
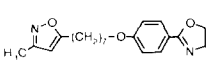
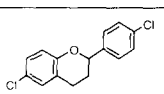
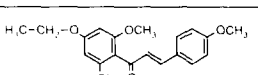
SSDI 0166-3542(94)00038-A

Resistant mutants raised against some of these compounds are cross-resistant to a panel of several other compounds, indicating that they probably all bind to a place corresponding to the hydrophobic pocket in HRV 14, although not necessarily binding to the same amino acids (Andries et al., 1989; Ninomiya et al., 1990). The mechanism of action of many of these capsid-binding compounds involves inhibition of the early rhinoviral replication steps, such as adsorption, penetration or uncoating (Fox et al., 1986; Tisdale et al., 1984; Ishitsuka et al., 1986; Ninomiya et al., 1985).

All but one typed rhinoviruses have been subdivided into a major and a minor group on the basis of their use of one of two possible receptors upon entry into the cell (Abraham and Colonno, 1984). Binding of capsid-binding compounds into the hydrophobic pocket of HRV 14, a major receptor group virus, but not HRV 1A, a minor receptor virus, induces conformational shifts in the viral capsid, resulting in inhibition of attachment of the virion to its receptor (Pevear et al., 1989A). These observations suggested that the effect on viral adsorption may be determined by the receptor specificity serotype studied (McKinlay et al., 1992). In this report, we examined the effect of a series of capsid-binding antivirals on the adsorption of major and minor receptor viruses. Rhinoviruses were also subdivided into two antiviral groups, based on

Table 1
Compound structures and their antiviral activities against selected rhinovirus serotypes

	HRV 1A	HRV 2	HRV 29	HRV 9	HRV 39	HRV 14	HRV 35
 R 61837	0.312	0.039	0.047	0.006	0.375	11.700	0.975
 WIN 51711	3.350	0.658	1.650	3.500	2.475	0.175	0.009
 4',6-dichloroflavan	0.033	0.163	0.002	0.125	0.213	16.000	16.000
 chalcone	0.313	0.010	0.130	0.097	0.036	0.600	0.028

The MIC[a] (μg/ml) is defined as the lowest concentration of compound that protects 50% of the HeLa cells against a cytopathic effect (Andries et al., 1990). MIC values are the median of at least three separate assays. On the average, the upper and lower limits of the 95% confidence intervals ranged between 0.5 and 2.3 times the median MICs.

their differential susceptibility to capsid-binding compounds (Andries et al., 1990). Our selection also included rhinoviruses from antiviral groups A and B.

Selected representatives for the minor receptor group and antiviral group B were HRV 1A, HRV 2 and HRV 29. Major receptor group viruses included were HRV 14, HRV 35, belonging to antiviral group A, and HRV 9 and HRV 39, belonging to antiviral group B. The origin of viruses and antiviral compounds has been described (Andries et al., 1990). MIC assays in HeLa cell cultures were used to assess the antiviral potency of the selected compounds (Andries et al., 1988). The structures of the selected antiviral compounds and their MICs obtained against the seven selected rhinoviruses serotypes are listed in Table 1.

Infective center assays were used to study the effect of antiviral compounds on the virus adsorption step (Andries et al., 1992). Briefly, a washed cell suspension, obtained by EDTA treatment of HeLa Ohio cell monolayers, was resuspended to a final concentration of 6×10^6 cells/ml, and cooled at 4°C for at least 2 h. Virus incubated for 60 min at 33°C with different concentrations of antiviral compounds was cooled to 4°C, and subsequently added to the HeLa cell suspension. To prevent the virus from entering the cells, virus-drug mixtures were allowed to adsorb to cells for 60 min at 4°C. After the adsorption period, free virus and compound were removed by washing three times with cold phosphate-buffered saline. Sequential 10-fold dilutions of the cells were made at room temperature and a 0.2 ml vol. of each dilution was plaqued on 10-cm diameter petri dishes with HeLa cell monolayers. After an incubation period of 3 to 5 days, depending on the serotype, plaques were fixed and stained with a crystal violet solution.

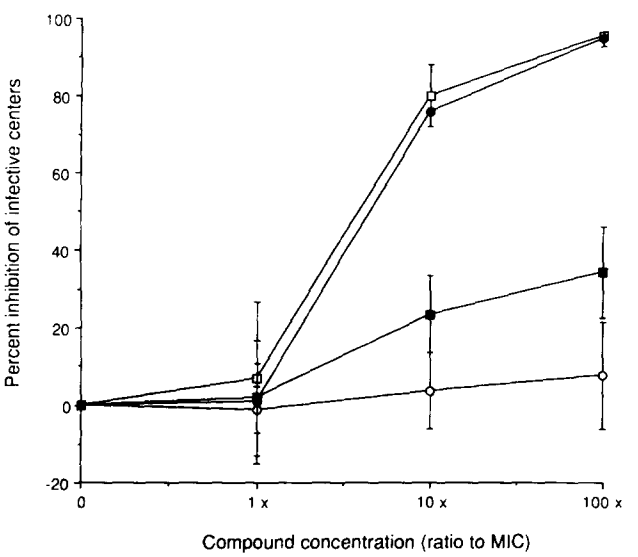


Fig. 1. Effect of capsid-binding compounds R 61837 (■), WIN 51711 (○), 4′,6-dichloroflavan (□) and chalcone (●) on the adsorption of HRV 29. Infective center assay data were plotted as percentage inhibition relative to the no drug controls. The concentrations used are ratio's to the MIC (e.g., 10 MIC is 10 times the MIC of this particular antiviral against this particular serotype) (for MIC values see Table 1).

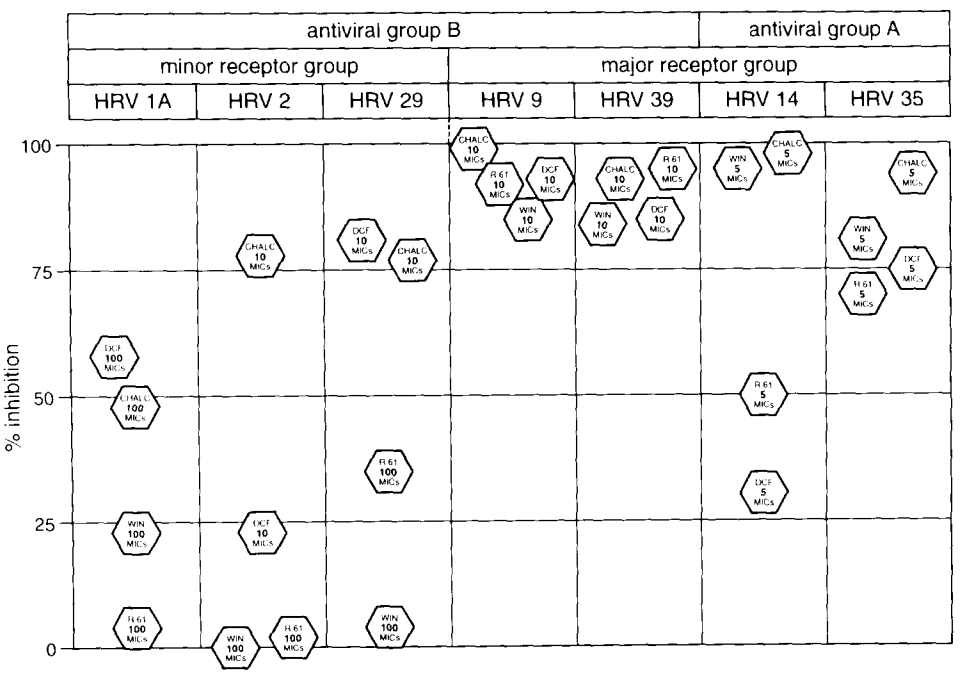
Inhibition of adsorption of minor receptor group viruses, antiviral group B

A dose-response curve of a typical infectious center experiment is given in Fig. 1. At concentrations 10 times the MIC of chalcone and 4′,6-dichloroflavan, the adsorption of HRV 29 was inhibited by about 80%, and at 100 times the MIC, the inhibition was complete. In contrast, similar concentrations of WIN 51711 had no effect on adsorption, and a concentration 100 times the MIC of R 61837 was needed to achieve some inhibition (32%).

Addition of 10 times the MIC of 4′,6-dichloroflavan during an infectious center experiment affected the adsorption of HRV 2 by 23% (Table 2). At a similar concentration, inhibition of adsorption by chalcone was almost complete (78%). Adsorption of HRV 2 was not inhibited at 100 times the MIC of WIN 51711 and R 61837 (0% and 2%, respectively).

Both chalcone and 4′,6-dichloroflavan inhibited the adsorption of HRV 1A at concentrations 100 times the MIC to a similar degree (48% and 58%, respectively). At similar concentrations, the adsorption was slightly inhibited (23%) by WIN 51711, while R 61837 had no effect (4%) (Table 2).

Table 2
Inhibition of adsorption of seven rhinovirus serotypes by four capsid-binding compounds, R 61837 (R61), WIN 51711 (WIN), 4′,6-dichloroflavan (DCF) and chalcone (CHAL)



Infective center assay data are expressed as percentage inhibition relative to the no drug controls.

Inhibition of adsorption of major receptor group viruses, antiviral group B

All compounds tested, at a concentration as low as 10 times the MIC, had a substantial effect on the adsorption of HRV 9 and HRV 39 (Table 2).

Inhibition of adsorption of major receptor group viruses, antiviral group A

WIN 51711 and chalcone, at a concentration as low as 5 times the MIC, almost completely inhibited the adsorption of HRV 14 and HRV 35. R 61837 and 4′,6-dichloroflavan had the same effect on HRV 35, but inhibited the adsorption of HRV 14 incompletely at similar concentrations. Higher concentrations of these compounds could not be tested because of their high MICs against this virus (Table 1).

From a comparison between Table 1 (inhibition of replication) and Table 2 (inhibition of adsorption), it is clear that the inhibition of adsorption can not be the sole mechanism of action of the compounds studied. Inhibition of penetration and uncoating have indeed been described for several capsid-binding compounds inhibiting particular serotypes (reviewed in Andries, in press).

Our results obtained with the adsorption inhibition assay agree with those reported by others, using the same viruses and the same classes of compounds. The adsorption of HRV 14, but not HRV 2 was reported to be inhibited by WIN 51711 (Pevear et al., 1989a). WIN 54954, a related substituted oxazoline, was tested against 3 major (HRV 14, 39, and 89) and 3 minor receptor viruses (HRV 1A, 2, and 49). Inhibition of adsorption of all major viruses was observed, while no effect was observed on the adsorption of the minor receptor viruses (Pevear et al., 1989b). R 77975, a substituted pyridazinamine, inhibited the adsortion of HRV 9, a major receptor virus, but not HRV 1A (Andries et al., 1992).

In our study, chalcone and 4′,6-dichloroflavan did in some cases inhibit the adsorption of minor receptor viruses. The adsorption of 4 out of the 7 serotypes studied was inhibited by some antiviral compounds, but not by others. It has already been shown that a particular compound can induce different conformational changes in different rhinovirus serotypes (reviewed in Zhang et al., 1992; Kim et al., 1993). Our results indicate that the conformational alterations that are induced by antiviral compounds can also vary considerably within the same serotype, depending on the nature of the antiviral compound used.

We conclude that the presence of an effect on viral adsorption is determined by the nature of both the viral serotype and the antiviral compound, and is not solely determined by the receptor grouping. A correlation with the antiviral grouping could also not be found. More detailed knowledge of the molecular interactions and conformational changes within the hydrophobic pocket of minor receptor serotypes is necessary to explain why some compounds inhibit adsorption while others do not.

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