Antiviral Research, 13 (1990) 61–74
Elsevier

AVR 00398

Comparative studies on the antirhinovirus activity and the mode of action of the rhinovirus capsid binding agents, chalcone amides

Yasuyuki Ninomiya, Nobuo Shimma and Hideo Ishitsuka

Nippon Roche Research Center, Kamakura City, Kanagawa, Japan

(Received 24 July 1989; revision accepted 28 October 1989)

Summary

Studies of various analogs related to the antirhinovirus agent 4′-ethoxy-2′-hydroxy-4,6′-dimethoxychalcone (Chalcone Ro 09-0410) led to the identification of amide analogs that are 4.5 to 10 times more active against human rhinovirus (HRV) in tissue culture as measured by chemotherapeutic indices. Chalcone amides Ro 09-0535, Ro 09-0696 and Ro 09-0881 inhibited viral replication at concentrations as low as <2–3 ng/ml and were cytotoxic between 30 to 50 μg/ml. These compounds bind to HRV and reduce the virus infectivity titers by 3 $\log_{10}$ or greater at 0.5 μg/ml for 60 min similar to Ro 09-0410. These amide analogs competitively inhibited the binding of [^{3}H]Ro 09-0410 to the viral capsid similar to capsid binding antirhinovirus agents, Ro 09-0410, 4′,6-dichloroflavan and WIN-51711. Furthermore, strains of HRV type 2 resistant to each of the above agents showed cross-resistance to all the other agents. These results indicate that the chalcone amides also bind to the same or close-proximity site for the capsid binding antirhinovirus agents, which is on the specific site within the viral capsid protein. However, differences in the degree of the inhibition of [^{3}H]Ro 09-0410 binding, cross-resistance of strains of HRV resistant to the agents and HRV serotype specificity were observed not only between the chalcone amides and the other antivirus agents (Ro 09-0410, 4′,6-dichloroflavan and WIN-51711) but also among the chalcone amides, particularly between Ro 09-0535 and Ro 09-0696. These differences are presumably due to alterations in the binding affinities of compounds as a consequence of variations in the shape and size of the hydrophobic pocket that exists between serotypes including resistant strains.

Antirhinovirus agent; Ro 09-0410; Chalcone amide; Binding site; Drug resistant HRV-2 subline

Correspondence to: H. Ishitsuka, Nippon Roche Research Center, 200 Kajiwara, Kamakura City, Kanagawa, 247 Japan.

Introduction

Many antivirus agents have been reported to be effective against human rhinovirus (HRV) (Ash et al., 1979; Bauer et al., 1984; Ishitsuka et al., 1982a,c; Otto et al., 1985; Sperber et al., 1988; Wikel et al., 1980), which is the major causative agent of the common cold. As reported previously, we synthesized a potent antiviral agent Ro 09-0410 (4′-ethoxy-2′-hydroxy-4,6′-dimethoxychalcone), which is active exclusively against HRV (Ishitsuka et al., 1982a). Ro 09-0410 binds only to HRV at the specific site of the virus capsid and causes contact virus inactivation (Ninomiya et al., 1984). However, neither nasal application of Ro 09-0410 nor its oral prodrug, Ro 09-0415, were effective against experimental HRV infections in volunteers (Al-Nakib et al., 1987b; Phillpotts et al., 1984). Therefore, we have made further attempts to synthesize various analogs related to Ro 09-0410 with the aim of identifying anti-HRV agents that have higher therapeutic ratio. Among them, amide analogs such as Ro 09-0535, Ro 09-0696 and Ro 09-0881 were found to be 4.5 to 12 times more effective against various serotypes of HRV in tissue cultures than either Ro 09-0410 or other agents that bind to HRV capsid protein (Ishitsuka et al., 1984).

In the present report, we investigate antirhinovirus activities of the three amide analogs in terms of inhibition of viral cytopathogenic effect (cpe) and contact virus inactivation, and compared their activities with those of other antirhinovirus agents that bind to HRV capsid protein, 'capsid binding antirhinovirus agents'. Al-Nakib has already reported that these amide analogs inhibit cpe caused by HRV type 2 at quite low concentrations of 0.002 μg/ml (Al-Nakib et al., 1987a). In the previous report (Ninomiya et al., 1985), we showed that antirhinovirus agents such as Ro 09-0410, RMI-15,731 and 4′,6-dichloroflavan bind to or interact with sites which are the same or very close to each other on the capsid protein of HRV type 2. This study provides further support for the hypothesis that all of these compounds bind in the same site within the virus.

Materials and methods

Cells and viruses

HeLa cells (Bristol strain) were grown and used for antiviral assays (Ishitsuka et al., 1982c). HRVs were purchased from the American Type Culture Collection, Rockville, MD, and titrated by a plaque assay method (Ishitsuka et al., 1982c). ^{14}C-labeled HRV type 2 was prepared as described elsewhere (Ninomiya et al., 1984). Subclones of HRV type 2, which are resistant to each of antivirus agents used in this study, were isolated by continuous passage in HeLa cell culture in the presence of subinhibitory concentrations of the antiviral agents.

Determination of IC_{50}

The IC_{50} was determined as described previously (Ishitsuka et al., 1982a). Briefly, a suspension of HeLa cells (6×10^4 cells) was mixed with virus (about 5×10^3 PFU), and the mixture was immediately plated into a microtest plate which contained the serially diluted compound to be tested. The cells were then cultured at 33°C for 2 to 4 days. Cytopathogenic effect (cpe) was observed after staining the residual cells with crystal violet. The IC_{50} was expressed as the concentration at which cpe was inhibited by 50% as compared with the control.

Virus inactivation assay

HRV was incubated with or without antiviral agent (0.5 μg/ml) at 33°C for the times indicated in the text. Thereafter, the residual virus titer was determined by a plaque assay method (Ninomiya et al., 1984).

Antivirals

Anti-HRV agents, Ro 09-0410 and its amide analogs (Fig. 1), used in this report were synthesized as described by M. Fujiu et al., U.K. patent 79.17975, and by N. Shimma et al., U.K. patent 80.3223, respectively. [4-methoxy-^{3}H]Ro 09-0410 and [4-methoxy-^{3}H]Ro 09-0535 were synthesized respectively from 2′,4-dihydroxy-4′-

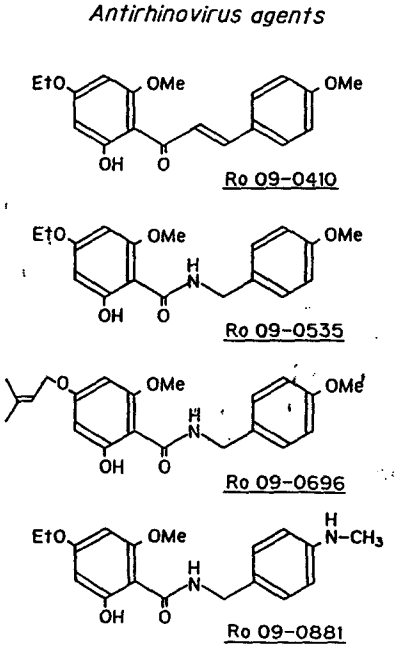

Fig. 1. Chemical structures of antirhinovirus chalcone and its amide analogs.

ethoxy-6'-methoxychalcone and 4-ethoxy-2-hydroxy-6-methoxy-N(p-hydroxy-benzyl)benzamide by methylation in acetone with [methyl-^{3}H]dimethylsulfate (4 Ci/mmol, NET 0.79, N.E.N.) and purified to a single spot by thin-layer chromatography. We synthesized 4',6-dichloroflavan and enviroxime (anti-6-[(hydroxyimino)phenylmethyl]-1-[(1-methylethyl)sulfonyl-imidazole-2-amine) as described elsewhere (Batchelor et al., 1978; Ishitsuka et al., 1982a). RMI-15,731 (MDL-15,731; 1-[5-tetradecyloxy-2-furanyl]-ethanone) (Ash et al., 1979) and WIN-51711 (Disoxaril; 5-[7-[4-(4,5-dihydro-2-oxazolyl)-phenoxy]-heptyl]-3-methylisoxazole) (Ott et al., 1985) were kindly supplied by Merrell Dow Research Institute, Cincinnati, OH and Sterling-Winthrop Research Institute, Rensselaer, NY, respectively.

Sucrose gradient centrifugation

Free [^{3}H]Ro 09-0410 and [^{3}H]Ro 09-0535 were separated from bound agents by gradient centrifugation. Linear gradients containing 15 to 35% sucrose in 0.02 M HEPES buffer (pH 7.2); 1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol were used at 4°C. Gradients of 4.5 ml containing reaction mixture with [^{3}H]Ro 09-0410 or [^{3}H]Ro 09-0535, HRV type 2, and other unlabeled antiviral agents were centrifuged for 70 min at 45000 rpm in a Spinco SW50.1 rotor. The tubes containing the gradients were then punctured at the bottom, and the radioactivity in 4-drop fractions were determined by scintillation counting.

Results

Inhibition of viral cytopathogenic effect by antiviral agents

The IC_{50} of chalcone Ro 09-0410, its amide analogs (Ro 09-0535, Ro 09-0696 and Ro 09-0881), 4',6-dichloroflavan, RMI-15,731 WIN-51711 and enviroxime against various HRV serotypes in HeLa cell cultures were measured. The therapeutic index, the ratio of cytotoxic concentration to the median IC_{50} (Table 1), shows that the chalcone amide analogs were 4 to 12.5 times more effective than their parent compound Ro 09-0410. The chalcone amide analogs have activity with serotype specificity similar to Ro 09-0410, RMI-15,731 and 4',6-dichloroflavan. Except for enviroxime, whose activity does not depend on capsid binding (Ninomiya et al., 1985), these agents are highly active against some HRV serotypes, although they were less active toward others. However, significant differences in the serotype specificity were observed not only between Ro 09-0410 related compounds and the other agents, but also among the more specific group of the amide analogs.

TABLE 1

Antirhinovirus activity and chemotherapeutic index

Serotype	Antiviral activity IC_{50} (μg/ml)						
	Ro 09-0410	Ro 09-0881	Ro 09-0535	Ro 09-0696	4′,6-Dichloroflavan	Enviroxime	WIN 51711
2	0.0084	0.0052	0.0040	0.0014	0.066	0.018	0.22
3	0.21	3.0	2.6	>4.0	>4.0	0.051	–
9	0.048	0.032	0.012	0.0058	0.34	0.073	>4.0
14	0.19	0.90	1.1	>4.0	>4.0	0.020	0.28
15	0.076	0.013	0.017	0.0096	0.020	0.0068	–
16	0.043	0.012	0.0032	0.0058	0.0071	0.018	>1.0
21	0.0051	0.0037	<0.0005	0.0006	0.16	0.024	0.70
25	0.13	0.033	0.046	<0.0005	0.018	0.015	0.4
30	0.014	0.011	0.0075	0.0095	0.022	–	–
31	0.64	0.25	0.32	0.0098	0.0044	0.019	–
36	0.014	0.080	0.038	0.0086	0.24	0.013	–
49	0.0058	0.0037	0.0018	0.0009	0.058	0.015	0.87
Median IC_{50} (μg/ml)	0.045	0.022	0.014	0.0072	0.062	0.018	0.70
Cytotoxicity[a] (μg/ml)	18	43	30	29	25	3.6	4–8
Chemotherapeutic index[b]	400	1910	2070	4030	400	200	6–11
Solubility[c]	<1	160	1	<1	1	not tested	not tested

[a]The 50% cytotoxic concentration, at which growth of HeLa cells for 3-day culture periods was inhibited by 50%, was determined.

[b]Cytotoxic concentration/median IC_{50}.

[c]For a solubility testing compounds were suspended in water (10 mg/ml) and stirred for 80 min, and then those dissolved in water were measured. For antiviral and cytotoxicity testings compounds were dissolved in 5% DMSO in ethanol (0.1–10 mg/ml) and then diluted with media.

TABLE 2

Effect of water solubility of drugs on their virus inactivation activity

HRV serotypes	Drugs[a]	Reduction of virus titer (log_{10} pfu/ml)		
Exp. 1		Incubation time (min)		
		20	60	
HRV-2	Ro 09-0410	1.82	> 2.73	
	Ro 09-0535	1.25	1.41	
	Ro 09-0881	1.20	1.75	
HRV-30	Ro 09-0410	ND[b]	1.48	
	Ro 09-0535	ND	2.13	
	Ro 09-0881	ND	2.20	
Exp. 2		Incubation time (min)		
		15	30	60
HRV-2	Ro 09-0410	0.02	0.08	0.17
	Ro 09-0881	0.31	1.10	1.83
Exp. 3		Incubation time (min)		
		20	40	60
HRV-30	Ro 09-0410	0.06	0.15	0.12
	Ro 09-0881	0.60	1.39	2.55

[a]Drugs were dissolved in EtOH (Exp. 1) or suspended in water (Exp. 2 and 3) to a concentration of 1 mg/ml. Then the drugs were diluted with assay medium to a final concentration of 0.5 μg/ml.
[b]Not done.

Effects of water solubility on the virus inactivation activity

The water solubility of Ro 09-0410 and the amide analogs was examined. As Table 1 shows, Ro 09-0881 is the most soluble in water among them. The most interesting feature of chalcone Ro 09-0410 was its ability to inactivate HRV (Ishitsuka et al., 1982a; Ninomiya et al., 1984). In order to see how the solubility affects the activity, we next examined Ro 09-0410 and Ro 09-0881 for their activity in a rapid virus inactivation assay. In this experiment the agents, which were dissolved in ethanol or suspended in water (1 mg/ml), were diluted to 0.5 μg/ml with MEM, and their capacity of virus inactivation was immediately assessed. As Table 2 shows, although Ro 09-0410 rapidly inactivated HRV when the ethanol solution was used, that suspended in water was inactive due to its poor solubility in water. Similar results were obtained with the chalcone amide analogs Ro 09-0535 and Ro 09-0696 (data not shown). On the other hand, Ro 09-0881 rapidly inactivated HRV even when the suspension in water was tested. These results indicate that Ro 09-0881 is more bioavailable in the nasal cavity than the other agents when intranasally applied as a suspension in physiological solutions.

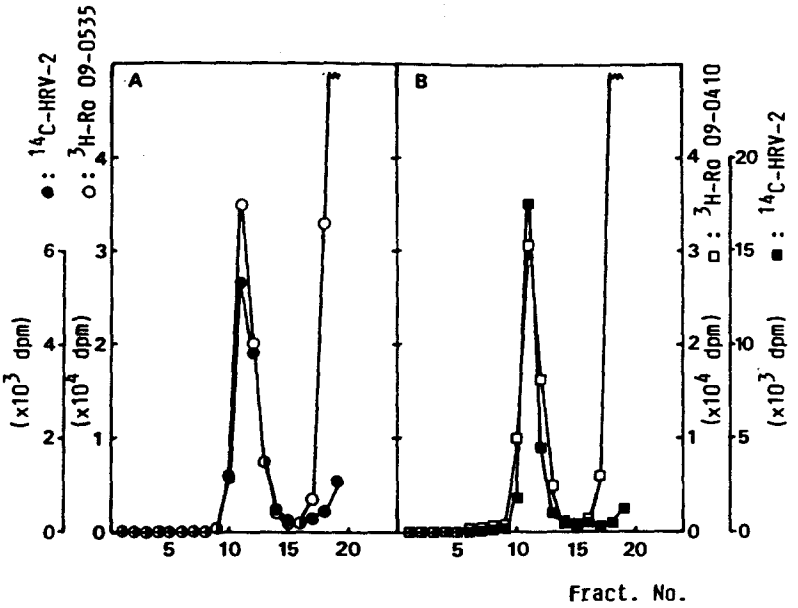

Fig. 2. Specific binding of [^{3}H]Ro 09-0410 and [^{3}H]Ro 09-0535 to [^{14}C]HRV type 2. [^{14}C]HRV type 2 (1.4×10^{9} pfu/ml, 1.9×10^{5} dpm/ml) was incubated with [^{3}H]Ro 09-0410 (306 ng/ml, 2.8×10^{6} dpm/ml) or [^{3}H]Ro-0535 (284 ng/ml, 2.6×10^{6} dpm/ml) at 33°C for 60 min and subjected to centrifugation in a sucrose gradient.

Specific binding of [^{3}H]Ro 09-0410 and [^{3}H]Ro 09-0535 to [^{14}C]HRV

Next we examined whether the chalcone amide analogs show the activity by exactly the same mode of action as Ro 09-0410 does. After incubation of [^{14}C]HRV-2 with [^{3}H]Ro 09-0410 or [^{3}H]Ro 09-0535 at 33°C for 60 min, the amount of the ^{3}H-labeled compounds bound to the virus was assayed by centrifugation through a linear sucrose gradient. As Fig. 2 shows, distinct peaks of [^{3}H]Ro 09-0410 and [^{3}H]Ro 09-0535 were observed at the position of the virus particles, indicating that Ro 09-0535 binds to the virus as Ro 09-0410 does.

Competition of the antiviral agents with Ro 09-0410 for the Ro 09-0410 binding site on HRV type 2

As reported previously, [^{3}H]Ro 09-0410 binds to the native virion of HRV-2 at a specific capsid site, and the binding was inhibited by excess amounts of Ro 09-0410 and other agents with similar characteristics, such as 4',6-dichloroflavan and RMI-15,731 (Ishitsuka et al., 1982a; Ninomiya et al., 1985). In the present study we made the same experiments with the chalcone amide analogs. When HRV type 2 was incubated with [^{3}H]Ro 09-0410 in the presence of an excess amount of unlabeled Ro 09-0410 (38-fold), the binding of the radioactive compound was inhibited by 85–89% (Fig. 3, both panels). Binding was also inhibited by an excess

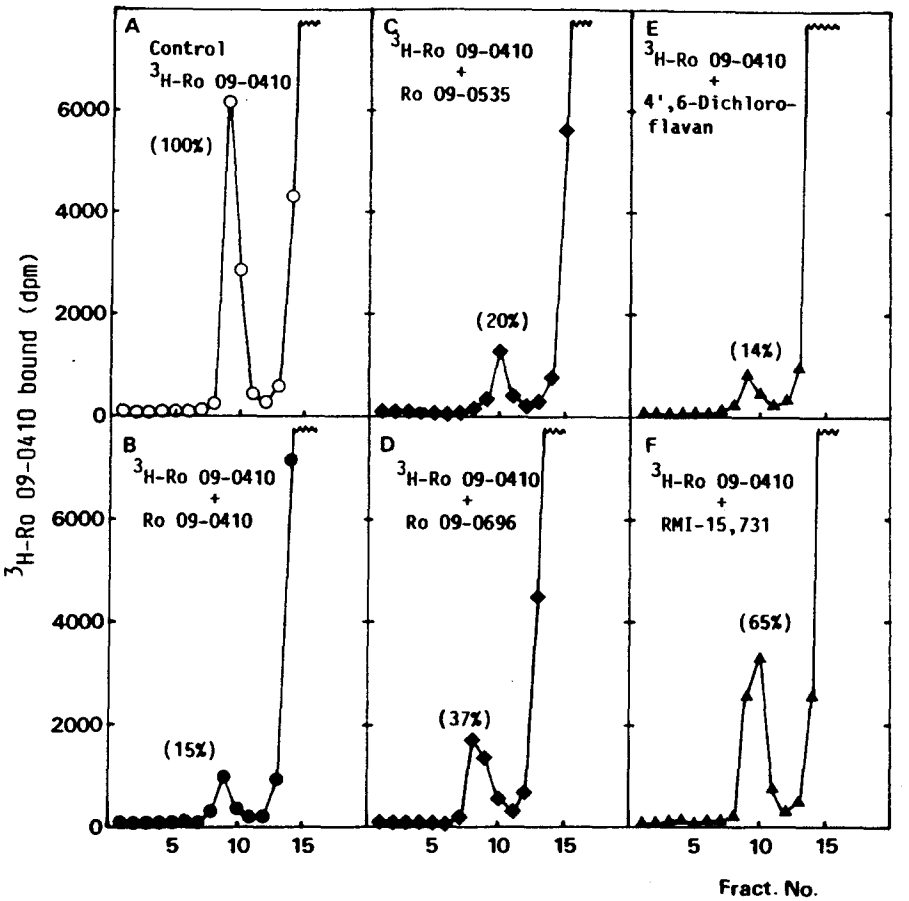

Fig. 3. Inhibition of binding of [^{3}H]Ro 09-0410 to HRV type 2 by antirhinovirus agents. Top panel: HRV type 2 (8.3×10^7 pfu/ml) was incubated with [^{3}H]Ro 09-0410 (36 ng/ml, 5×10^5 dpm/ml) and the unlabeled antirhinovirus agents (1.4 μg/ml) at 33°C for 60 min. The mixture was assayed by centrifugation through a linear gradient. The amount of [^{3}H]Ro 09-0410 bound to HRV was expressed as a percentage of the control (without unlabeled antirhinovirus agents) in parentheses. Bottom panel: the experiment has been done as described above except for the amount of HRV type 2 that was used (1.6×10^8 pfu/ml) and the incubation time (90 min).

amount of Ro 09-0535 or 4′,6-dichloroflavan, and was inhibited to a lesser extent by excess amount of Ro 09-0696, WIN-51711, or RMI-15,731. These results suggest that the binding sites for Ro 09-0410 are the same as those for the chalcone amide analog Ro 09-0535, and 4′,6-dichloroflavan. Whether the differences in ability of Ro 09-0696, RMI-15,731 and WIN-51711 to compete with Ro 09-0410 for binding are significant and reflect differences in the binding site or nature of drug binding, must await further study.

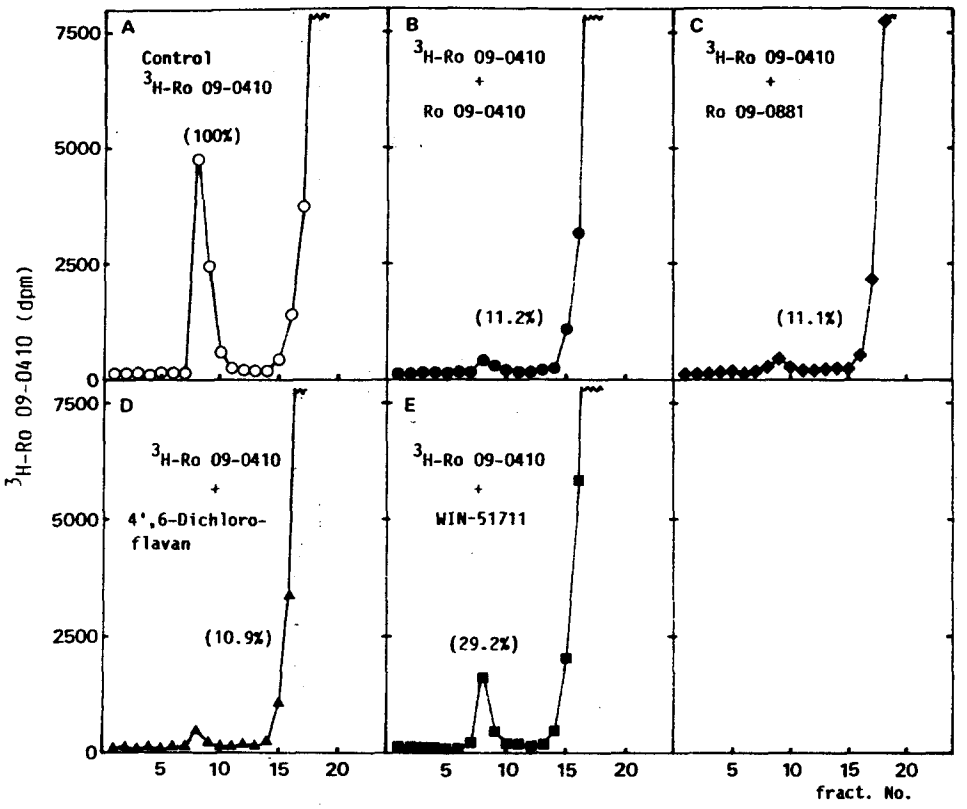

Fig. 4. Binding of [^{3}H]Ro 09-0410 to original HRV type 2 and subclones resistant to Ro 09-0410, Ro 09-0535, and Ro 09-0696. Original HRV type 2 and drug resistant subclones (4.8×10^7 pfu/ml) were incubated with [^{3}H]Ro 09-0410 (306 ng/ml, 2.8×10^6 dpm/ml) at 33°C for 60 min. The mixture was subjected to centrifugation in a sucrose gradient.

Studies on HRV type 2 subclones resistant to the individual test agents

HRV type 2 subclones resistant to antivirus agents, which interacted with the virus capsid, were acquired by serial passages of the virus in the presence of sub-inhibitory concentrations of the agents. These subclones were demonstrated to reduce the binding capability of [^{3}H]Ro 09-0410 (Fig. 4). Furthermore, HRV type 2 subclones resistant to Ro 09-0410 showed cross-resistance to Ro 09-0535 and Ro 09-0696 as well as to 4',6-dichloroflavan and WIN-51711, and vice versa, though chalcone amide analog Ro 09-0696 was again a little different in the pattern of cross-resistance to the other agents (Table 3). These results suggest that all of these compounds bind in the same hydrophobic pocket, but the potentially different amino acid substitutions responsible for drug resistance, combined with varying structures of the compounds tested, are likely to result in altered binding affinities, and hence, the observed differences in antiviral activity.

TABLE 3

Cross-resistance of HRV type 2 subclones to antiviral agents

HRV subclone resistant to antiviral agent[b]	Antiviral activity IC_{50} (μg/ml) (relative resistance)[a]				
	Ro 09-0410	Ro 09-0535	Ro 09-0696	4′,6-Dichloroflavan	WIN-51711
Original HRV-2	0.0055 (1)	0.0018 (1)	<0.0018 (1)	0.10 (1)	0.22 (1)
NR-410^r	4 (730)	4 (2200)	0.4 (>240)	4 (40)	>4 (>18)
NR-535^r	0.87 (160)	0.87 (480)	0.049 (>27)	4 (40)	ND
NR-696^r	0.87 (160)	2.67 (1500)	4 (>2200)	4 (40)	ND
NR-DCF^r	0.15 (27)	0.15 (83)	0.15 (>83)	>4 (>40)	ND

[a]The degree of increase of the drug resistance as compared with the susceptibility of the original strain was expressed as fold increase in the parentheses.
[b]NR-410^r, NR-535^r, NR-696^r, and NR-DCF^r are subclones resistant to Ro 09-0410, Ro 09-0535, Ro 09-0696, and 4′,6-dichloroflavan, respectively.
ND, Not done.

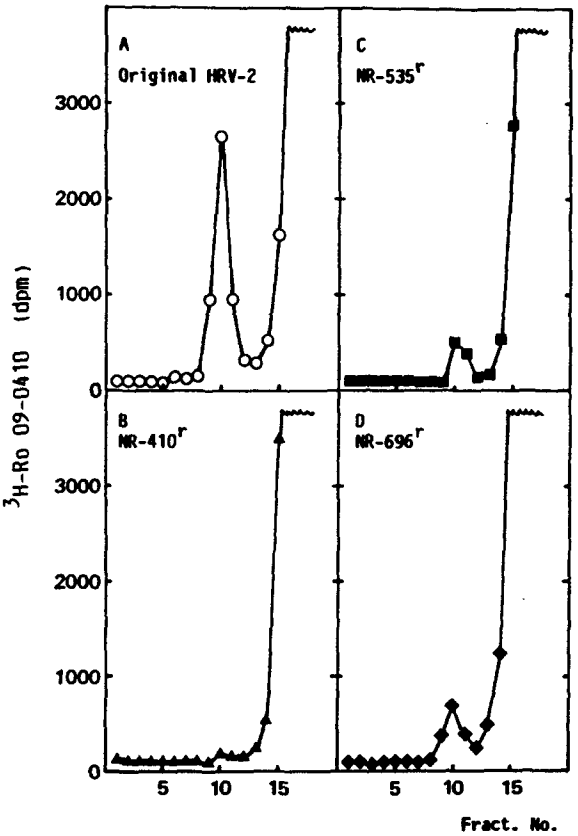

Fig. 4. Binding of [^{3}H]Ro 09-0410 to original HRV type 2 and subclones resistant to Ro 09-0410, Ro 09-0535, and Ro 09-0696. Original HRV type 2 and drug resistant subclones (4.8×10^7 pfu/ml) were incubated with [^{3}H]Ro 09-0410 (306 ng/ml, 2.8×10^6 dpm/ml) at 33°C for 60 min. The mixture was subjected to centrifugation in a sucrose gradient.

Discussion

Studies of various analogs related to chalcone Ro 09-0410 led to the identification of agents that are more active against HRV than the parent compound and other antirhinovirus agents, that bind to the capsid protein of HRV, such as RMI-15,731, 4',6-dichloroflavan and WIN-51711. Amide analogs of Ro 09-0410, such as Ro 09-0535, Ro 09-0696 and Ro 09-0881, are active exclusively against various serotypes of HRV at concentrations as low as a few ng/ml, but they were not active against DNA or other RNA viruses such as adeno, vaccinia, polio, ECHO and influenza viruses in cell cultures (data not shown). These amide analogs showed contact virus inactivation to a similar extent at 0.5 μg/ml. The potent activity of the same amide analogs has also been demonstrated by Al-Nakib and Tyrrell with a viral cpe assay using HRV types 2 and 9 (Al-Nakib et al., 1987a).

We have investigated Ro 09-0410 as an agent for the treatment of the common

cold. However, its nasal administration was ineffective in experimental HRV infection in volunteers (Al-Nakib et al., 1987b). Ro 09-0410 is not practical to use because of its low solubility in water (less than 1 μg/ml). Direct contact of the virus to capsid binding agents is essential for their antiviral activity. The agents with poor water solubility should be applied as a suspension in physiological solution in such a way that they rapidly dissolve in the nasal cavity so as to cover the nasal mucosal membrane all over and to inactivate HRV before the virus enters the mucosal cells. In this context, high solubility in water of capsid binding agents is one of requisite characteristics for their clinical application to the nasal cavity. The chalcone amide Ro 09-0881 was designed to have a higher solubility in water and showed the activity in the rapid virus inactivation assay even when its suspension in water was tested (Table 2). Ro 09-0881 will be useful for the treatment of the HRV infection via nasal application.

We previously reported that Ro 09-0410, RMI-15,731 and 4′,6-dichloroflavan bind to HRV at some specific sites on the virus capsid and make the virus inactive (Ninomiya et al., 1985). The binding occurred in a rapid and irreversible fashion, though this inactivation of the virus was completely restored to the original level by extraction of the agents with chloroform (Ishitsuka et al., 1982a; Ninomiya et al., 1984, 1985). The ability of virus adsorption to HeLa cell was not changed by the binding of Ro 09-0410 to HRV-2 (data did not show). The agents that bind to the HRV capsid stabilize the virus particles in such a way that the virus does not proceed to the uncoating process (Ninomiya et al., 1984; Sperber et al., 1988). The present study showed that the chalcone amides and another antirhinovirus agent, WIN-51711, bound to sites that are for Ro 09-0410 or very close to each other on a specific pocket within the capsid protein. Rossmann and his colleagues clarified by X-ray crystallographic structural analysis that WIN-51711 binds to a hydrophobic pocket within the β-barrel of VP1, one of the four HRV capsid proteins (Smith et al., 1986). Our observation that the binding of Ro 09-0410 to HRV was inhibited also by excess WIN-51711, 4′,6-dichloroflavan and the amide analogs (Fig. 3, both panels) and the fact that a subclone resistant to Ro 09-0410 was cross-resistant to WIN-51711 (Table 3) suggest that Ro 09-0410, all three amide analogs, RMI-15,731, and 4′,6-dichloroflavan also bind to sites in the same pocket within VP1. Ro 09-881 with hydrophilic nature could bind to the hydrophobic pocket, indicating that 4-aminomethyl position of this compound does not greatly affect the binding to the pocket. In fact, chemical moieties at 4-position of the chalcone amides did not substantially affect the activity as compared with those at different positions (data not shown).

Thus, these antirhinovirus agents bind in the same hydrophobic pocket within VP1, although the precise position and orientation within the pocket may vary. They inhibit the uncoating of HRV possibly by stabilizing the viral capsid protein and thereby preventing any conformational changes required for the release of viral RNA (Ninomiya et al., 1984, 1985). However, these agents appear to have some different modes of interaction with the site(s) in the pocket as shown in the present study. Differences in the degree of the inhibition of [^{3}H]Ro 09-0410 binding, the cross-resistance of strains of HRV resistant to the agents, and HRV serotype

specificity were observed not only between Ro 09-0410-related agents and the other capsid binding agents but also among the specific group of chemicals, Ro 09-0410 and its amide analogs. The differences in the mode of the binding to or interaction with the agents active on capsid protein remain to be elucidated. Structural analysis of the interaction of the agents at sites within VP-1 by using new technologies such as recombinant DNA and computer graphics will provide useful informations not only for elucidation of antiviral actions of the agents and the mode of viral uncoating mechanisms, but also for identification of more active agents against HRV.

References

Ahmad, A.L.M., Dowsett, A.B. and Tyrrell, D.A.J. (1986) Synergism between anti-rhinovirus antivirals: various human interferons and a number of synthetic compounds. Antiviral Res. 6, 241–252.

Al-Nakib, W. and Tyrrell, D.A.J. (1987a) A 'new' generation more potent synthetic antirhinovirus compounds; comparison of their MICs and their synergistic interaction. Antiviral Res. 8, 179–188.

Al-Nakib, W., Higgins, P., Barrow, I., Tyrrell, D.A.J., Lenox-Smith, I. and Ishitsuka, H. (1987b) Intranasal chalcone Ro 09-0410, as prophylaxis against rhinovirus infection in human volunteers. J. Antimicrob. Chemother. 20, 887–892.

Ash, R.J., Parker, R.A. and Mayer, G.D. (1979) RMI-15,731 (1-[5-tetradecyloxy-2-furanyl]-ethanone); a new antirhinovirus compound. Antimicrob. Agents Chemother. 16, 301–305.

Batchelor, J.R., Bauer, D.J. and Hudson, H.F. (1978) U.K. Patent 10251 March.

Bauer, D.J., Selway, J.W.T., Batchelor, J.R., Tisdale, M., Caldwell, I.C. and Young, D.A.B. (1984) 4′,6-Dichloroflavan (BW683C); a new antirhinovirus compound. Nature (London) 292, 369–370.

Ishitsuka, H., Ninomiya, Y., Aoyama, M., Shimma, N., Kamata, M., Fujiu, M. and Suhara, Y. (1984) Potent antirhinovirus agents, *N-p*-methoxybenzyl benzamide derivatives. 6th Int. Cong. Virology Abstr. p. 34–16.

Ishitsuka, H., Ninomiya, Y., Ohsawa, C., Umeda, I. and Suhara, Y. (1982a) Direct and specific inactivation of rhinovirus by chalcone Ro 09-0410. 22, 617–621.

Ishitsuka, H., Ninomiya, Y., Ohsawa, C., Ohiwa, T., Fujiu, M., Umeda, I., Shirai, H. and Suhara, Y. (1982b) New antirhinovirus agents, Ro 09-0410 and Ro 09-0415. p. 1083–1085. In: P. Periti and G.G. Grassi (Eds.), Current Chemotherapy and Immunotherapy. Proc. 12th Internat. Congr. Chemother. Vol. 2. American Society of Microbiology, Washington, D.C.

Ishitsuka, H., Ohsawa, C., Ohiwa, T., Umeda, I. and Suhara, Y. (1982c) Antipicornavirus Flavone Ro 09-0179. Antimicrob. Agents Chemother. 22, 611–616.

Ninomiya, Y., Aoyama, M., Umeda, I., Suhara, Y. and Ishitsuka, H. (1985) Comparative studies on the modes of action of the antirhinovirus agents Ro 09-0410, Ro 09-0179, RMI-15,731, 4′,6-dichloroflavan, and enviroxime. Antimicrob. Agents Chemother. 27, 595–599.

Ninomiya, Y., Ohsawa, C., Aoyama, M., Umeda, I., Suhara, Y. and Ishitsuka, H. (1984) Antivirus agent, Ro 09-0410, binds to rhinovirus specifically and stabilizes the virus conformation. Virology 134, 269–276.

Otto, M.J., Fox, M.P., Fancher, M.J., Kuhrt, M.F., Diana, G.D. and McKinlay, M.A. (1985) In vitro activity of WIN-51711, a new broad-spectrum antipicornavirus drug. Antimicrob. Agents Chemother. 27, 883–886.

Phillpotts, R.J., Higgins, P.G., Willman, J.S., Tyrrell, D.J.A. and Lenox-Smith, I. (1984) Evaluation of the antirhinovirus chalcone Ro 09-0415 given orally to volunteers. J. Antimicrob. Chemother. 14, 403–409.

Smith, T.F., Kremer, M.J., Luo, M., Vriend, G., Arnold, E., Kamer, G., Rossmann, M.G., McKinlay, M.A., Diana, G.D. and Otto, M.J. (1986) The site of attachment in human rhinovirus 14 for antiviral agents that inhibit uncoating. Science 233, 1286–1293.

Sperber, S.T. and Hayden, F.G. (1988) Chemotherapy of rhinovirus colds. Antimicrob. Agents Chemother. 32, 409–419.

Wikel, J.H., Paget, C.J. and Chamberlin, J.W. (1980) Synthesis of syn and anti isomers of 6-[(hydroxyimino)phenyl]-methyl-1-[(1-methylethyl) sulfonyl]-1H-benzimidazole-2-amine: inhibitors of rhinovirus multiplication. J. Med. Chem. 23, 368–372.