

daily dosing, for the greater part of the day drug levels in the nose are lower than 10 ng/ml, which is believed to be under the lower limit for full efficacy.

The search continues for a sustained release formulation that would overcome this dosing problem.

Safety and efficacy of intranasal pirodavir (R 77975) in experimental rhinovirus infection. *Antimicrob Agents & Chemother* 1992; 36: 727-732.

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Structural Comparisons of Several Antiviral Agents Complexed with Human Rhinoviruses of Different Serotypes

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Human rhinoviruses (HRV) are the most frequent cause of the common cold. Their RNA genome is surrounded by a nearly spherical coat composed of 60 copies of each of four proteins, VP1 through VP4, arranged with icosahedral symmetry (see Fig.). A 'canyon' circles around each of the fivefold axes (1). It is a depression into which the cellular receptor binds (recently published in *PNAS*, 2). The polypeptide folds of VP1, VP2 and VP3 have a similar topology and form a β -barrel with a hydrophobic interior (1). A variety of capsid binding antiviral agents can bind within the hydrophobic interior of the VP1 β -barrel. In some picornavirus structures, the

pocket is occupied by electron density which is probably a cellular cofactor. This cofactor can be displaced by the antiviral agents and is probably functionally important in the virus life cycle.

There are several classes of antirhinoviral agents. These include the WIN compounds, designed by the Sterling-Winthrop Pharmaceutical Research Division, denoted by a 'WIN' number, as well as compounds designed by Janssen Research Foundation, denoted by an 'R' number. They have proved to be clinically effective and have been the focus of structural studies at Purdue University (reviewed in

3). These and other compounds are being studied structurally in rhinoviruses (at Purdue University and at Rutgers University), in Coxsackieviruses (at Purdue University) and in polioviruses (at Harvard Medical School). The different classes of antiviral agents have different chemical structures, but all are hydrophobic and of similar size. They all bind into the WIN pocket, displacing any natural cofactor that might be there. They have a wide spectrum of activity, including most rhinoviral serotypes, as well as other picornaviruses, and function by inhibiting either attachment to cellular receptors or uncoating.

There are several mechanisms through which the inhibition of uncoating is affected when the antiviral compound binds to the viral capsid (in press, *J. Mol. Biol.*, 4). First, there is increased hydrogen-bonding between VP1 and the neighbouring VP3 due to antiviral-induced conformational changes. If the structure of native HRV14 (which normally contains no cofactor), native HRV1A (which normally does contain a cofactor) and drug-complexes of HRV1A or HRV14 are compared, there are progressively fewer

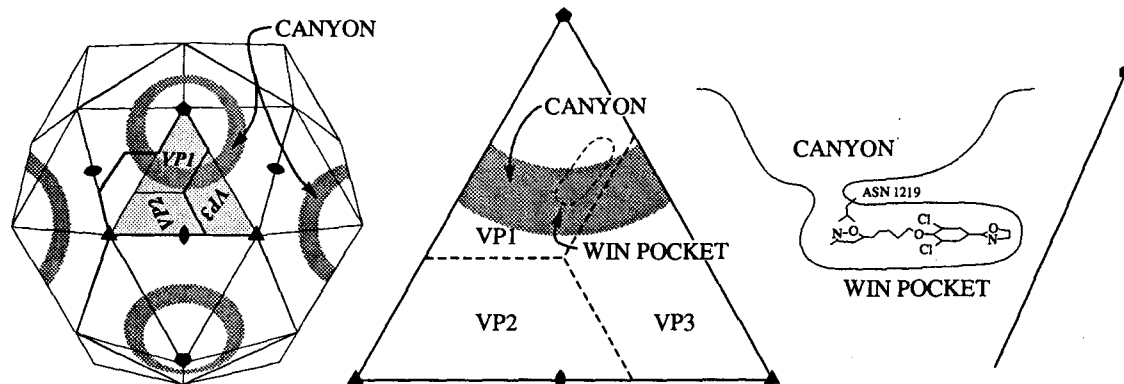


Fig. The rhinoviral capsid and WIN-pocket binding site are shown schematically in progressively greater detail from left to right. Left: the capsid consists of 60 identical triangular units each containing parts of two protomers. Center: within one of the 60 units, the location of the drug binding site is shown within VP1, but close to an adjoining VP3. Right: WIN54954 is shown in the pocket which is underneath the floor of the canyon, the site of receptor attachment.



genomes) and the DNA pol I complex are related by simple exon shuffling suggests that this essential gene of retrotransposons and retroviruses probably predates the prokaryotic-eukaryotic divergence.

Since, by necessity, polymerases represent one of the most ancient classes of proteins, the finding of a significant modular element in their construction implies that this approach to protein evolution is almost as old as life itself. This is good news for those who desire to predict protein structure from primary sequence data, since the structural possibilities are probably more finite than would otherwise be expected.

This analysis also demonstrates that a proper understanding of these structural and evolutionary relationships can provide a novel view of the mechanistic aspects of polymerase and nuclease activity in general, and RT in particular.

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POINTERS

Pirodavis, a Broad-Spectrum Antirhinoviral Drug

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Rhinoviruses are considered to be the cause of common cold in about 50% of cases. Pirodavis (R 77975) (see Fig.), developed at the Janssen Research Foundation, Belgium, is the follow-up of prototype compound R 61837: one of a series of pyridazinamines with activity against human rhinoviruses.

Similar to other synthetic antirhinovirals (dichloroflavan; chalcone; WIN 51711), the pyridazinamines bind into a specific hydrophobic pocket within the capsid protein VP1, beneath the canyon floor of rhinoviruses, preventing viral attachment and/or uncoating. So far, only the Janssen compounds have provided clinical benefit in an experimental human rhinovirus challenge model (1, 2).

Two distinct groups (designated A and B) of rhinoviruses have been described on the basis of their susceptibility profiles to a range of representative capsid-binding antiviral compounds. Pirodavis has high

activity against serotypes from both antiviral groups (3). It inhibits the replication of 80% of serotypes at concentrations of 0.1 µg or less per ml.

Pirodavis is available as a nasal spray containing 5 mg/ml R 77975 and 100 mg/ml hydroxypropyl-β-cyclodextrine. The clinical efficacy of pirodavis has been tested in experimentally induced rhinovirus infections, in which volunteers (generally students) are sequestered in a hotel and inoculated intranasally with a particular rhinovirus serotype. In a series of studies performed at the University of Virginia by Professor Fred Hayden, rhinovirus serotypes RV 39 or RV Hank's were used (2). All studies were placebo-controlled. Originally, pirodavis was given 6 times daily, and later a reduced dosage of 3 times daily was attempted. In most studies the drug was given prophylactically, i.e. before inoculation with rhinovirus. Treatment generally lasted 5 days with post-treatment follow-up for 3 more

days. In one study, pirodavis was given therapeutically to subjects with naturally occurring common colds (6 times daily, starting within 48 hours of occurrence of symptoms) (4).

In all studies the severity of cold symptoms was noted by the volunteers in a daily diary. Nasal secretions were measured by weighing paper handkerchiefs. Colds were identified by the Jackson criteria, i.e. development of a symptom score of at least 5 and nasal discharge on at least 3 days or the opinion of the volunteer that he had a cold. Serum neutralizing antibodies were measured in blood samples taken before and 3 weeks after rhinovirus challenge. Viral shedding was determined in daily nasal wash samples throughout the treatment period and 3 days afterwards.

In a prophylactic study using serotype RV Hank's, pirodavis reduced the number of Jackson colds and the severity of respiratory symptoms (2). In prophylactic as well as therapeutic studies, a reduction in viral shedding was the most prominent and consistent effect displayed by pirodavis. This property seems dose-related, since a mean reduction of about 30% in virus shedding was obtained with 3 times daily dosing, vs. 60% with the 6 times daily dosing regime.

The discrepancy in performance on clinical symptomatology vs. virological parameters remains an unsolved issue. A possible explanation was found in the results of a recent trial (unpublished), in which several bio-adhesive nasal delivery systems were evaluated in the search for a formulation that would allow a reduced

