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Mini-review

Rhinovirus chemotherapy

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Human rhinoviruses (HRV), members of the Picornaviridae family, are comprised of over 100 different virus serotypes. HRV represent the single most important etiological agents of the common cold [Arruda, E., Pitkaranta, A., Witek Jr., T.J., Doyle, C.A., Hayden, F.G., 1997. Frequency and natural history of rhinovirus infections in adults during autumn. J. Clin. Microbiol. 35, 2864–2868; Couch, R.B., 1990. Rhinoviruses. In: Fields, B.N., Knipe, D.M. (Eds.), Virology. Raven Press, New York, pp. 607–629; Turner, R.B., 2001. The treatment of rhinovirus infections: progress and potential. Antivir. Res. 49 (1), 1–14]. Although HRV-induced upper respiratory illness is often mild and self-limiting, the socioeconomic impact caused by missed school or work is enormous and the degree of inappropriate antibiotic use is significant. It has been estimated that upper respiratory disease accounts for at least 25 million absences from work and 23 million absences of school annually in the United States [Anzueto, A., Niederman, M.S., 2003. Diagnosis and treatment of rhinovirus respiratory infections. Chest 123 (5), 1664–1672; Rotbart, H.A., 2002. Treatment of picornavirus infections. Antivir. Res. 53, 83–98]. Increasing evidences also describe the link between HRV infection and more serious medical complications. HRV-induced colds are the important predisposing factors to acute otitis media, sinusitis, and are the major factors in the induction of exacerbations of asthma in adults and children. HRV infections are also associated with lower respiratory tract syndromes in individuals with cystic fibrosis, bronchitis, and other underlying respiratory disorders [Anzueto, A., Niederman, M.S., 2003. Diagnosis and treatment of rhinovirus respiratory infections. Chest 123 (5), 1664–1672; Gern, J.E., Busse, W.W., 1999. Association of rhinovirus infections with asthma. Clin. Microbiol. Rev. 12 (1), 9–18; Pitkaranta, A., Arruda, E., Malmberg, H., Hayden, F.G., 1997. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. J. Clin. Microbiol. 35, 1791–1793; Pitkaranta, A., Virolainen, A., Jero, J., Arruda, E., Hayden, F.G., 1998. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. Pediatrics 102, 291–295; Rotbart, H.A., 2002. Treatment of picornavirus infections. Antivir. Res. 53, 83–98]. To date, no effective antiviral therapies have been approved for either the prevention or treatment of diseases caused by HRV infection. Thus, there still exists a significant unmet medical need to find agents that can shorten the duration of HRV-induced illness, lessen the severity of symptoms, minimize secondary bacterial infections and exacerbations of underlying disease and reduce virus transmission. Although effective over-the-counter products have been described that alleviate symptoms associated with the common cold [Anzueto, A., Niederman, M.S., 2003. Diagnosis and treatment of rhinovirus respiratory infections. Chest 123 (5), 1664–1672; Gwaltney, J.M., 2002a. Viral respiratory infection therapy: historical perspectives and current trials. Am. J. Med. 22 (112 Suppl. 6A), 33S–41S; Turner, R.B., 2001. The treatment of rhinovirus infections: progress and potential. Antivir. Res. 49 (1), 1–14; Sperber, S.J., Hayden, F.G., 1988. Chemotherapy of rhinovirus colds. Antimicrob. Agents Chemother. 32, 409–419], this review will primarily focus on the discovery and development of those agents that directly or indirectly impact virus replication specifically highlighting new advances and/or specific challenges with their development.

Keywords: Rhinovirus; Antiviral therapy

Contents

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1. Pathogenesis of HRV-induced colds

A general understanding on the pathogenesis of HRV-induced colds is derived primarily from studies in which colds are experimentally induced in healthy adult volunteers (Couch, 1990; Pitkaranta and Hayden, 1998; Turner et al., 1984). Results from these studies suggest that the transmission of HRV to susceptible individuals is primarily by direct contact but virus can also be spread by small or large particle aerosol (Anzueto and Niederman, 2003). The virus is deposited on the nasal or conjunctival mucosa and is transported to the posterior nasopharynx by ciliated epithelial cells. In the nasopharynx, the virus attaches to epithelial cells via either the intracellular adhesion molecule-1 (ICAM-1 for HRV major serotypes) or low-density lipoprotein (LDL-1 for the HRV minor serotypes) receptors and initiates infection. Examination of biopsy specimens of nasal epithelium by immunofluorescence or in situ hybridization techniques demonstrate infection in a very small number of cells with little change to the morphology or integrity of the nasal epithelium (Couch, 1990; Pitkaranta and Hayden, 1998; Turner et al., 1984). These findings are consistent with the hypothesis that symptoms of the common cold are due to elaboration of inflammatory mediators rather than a direct lytic effect on host cells (Naclerio et al., 1988; Pitkaranta and Hayden, 1998; Turner et al., 1998).

Although initial symptoms can appear as early as 10–12 h, most symptoms develop 1–2 days after infection, peak at 2–3 days and then rapidly resolve within one week (Gwaltney, 2002a; Gwaltney et al., 1996; Harris and Gwaltney, 1996; Rao et al., 1995). Symptoms include rhinorrhea, nasal congestion, sore throat, headache, cough, malaise and low-grade fever. The median time to onset of virus shedding into nasal secretions has been reported to be 10 h with peak virus titers occurring 2–3 days after inoculation. In a certain percentage of individuals virus can be detected up to 2–3 weeks after infection. The close temporal association between virus replication and initiation of the inflammatory cascade with symptom development combined with the rapid resolution of illness poses perhaps one of the biggest challenges for antiviral chemotherapy. These kinetics suggest that for an antiviral agent to demonstrate an optimal therapeutic effect it would have to be administered very early in the infection. An alternative approach that combines an antiviral agent with an agent to suppress inflammation has also been described (Gwaltney et al., 2002b) and represents a strategy that may be required.

2. Clinical trial study approaches

An extensive review of the results derived from 241 published and unpublished randomised controlled trials with chemotherapeutic agents for the intervention of HRV-induced upper respiratory disease has been detailed by Jefferson and Tyrrell (2001). Two general study designs have been utilized. These include the experimental human challenge model and natural cold studies (Gwaltney et al., 1996; Rao et al., 1995; Sperber and Hayden, 1988). In the absence of a practical animal model for HRV, the experimental human challenge model has been used frequently to study virus pathogenesis, virus transmission and effects of drugs under controlled conditions. In this model, sero-susceptible human volunteers are inoculated with a defined inoculum of safety-tested HRV and treated with drug either before or after virus challenge. Subjects are isolated in individual rooms and symptom reporting is done under standardized conditions. Objective measures may include levels of infectious virus and virus RNA in nasal washings and mucus weight. Although the number of specific safety-tested HRV serotypes are limited and rates of infection and illness vary from study to study, these models are very effective to establish initial proof of concept for antiviral agents.

Since no sensitive and rapid diagnostic for HRV exist that could allow prospective enrollment of HRV positive subjects, natural cold studies depend on the successful recruitment of individuals who present with specific respiratory symptoms. Because of the wide variation in duration and severity of illness following infection, patient inclusion criteria relating to symptomatology must be carefully considered to optimally detect treatment effects. In addition, it is critical to enrich the population for those infected with HRV. Although infections with HRV occur throughout the year, peaks of HRV-induced illness in the Northern hemisphere are well described in early autumn and spring. HRV are also the most frequently isolated virus during summer months, however, the overall incidence of illness is low (Monto, 2002). Based on this information, natural infection studies in the Northern hemisphere are typically targeted for fall or spring months. Based on the tight association of virus replication, symptom development and subsequent illness resolution it is important that patients are enrolled early (<24 h) following symptom development in order to maximize the opportunity to see treatment effects.

Analyses of virus samples are performed retrospectively to precisely define the HRV positive patient population. The con-

ventional method for detecting and quantifying HRV in nasal samples from study subjects is by virus culture combined in some instances with measure of acid lability (Couch, 1990). The culture-based methods though are typically not sufficiently sensitive or discriminating for rigorous detection. Immunological methods have also been described, e.g. optical immunoassays, but are limited either by the inability to recognize multiple serotypes based on the high variability of the regions utilized for antigen production (e.g. capsid proteins) or when multiserotype recognition can be achieved, by the degree and timing of virus-induced antigens in nasal aspirates (Ostroff et al., 2001). Other more sensitive and specific methods based on RT-PCR have recently been described (Hayden et al., 2003a,b). Going forward, it will be interesting to utilize RT-PCR methodologies to readdress issues relating to HRV pathogenesis, kinetics of virus replication during disease, etiology of HRV in respiratory disease and incidence rates of HRV-induced illnesses.

3. Chemotherapeutic approaches

Chemotherapeutic approaches toward HRV-induced illnesses have been the focus of intense research spanning several decades. Comprehensive reviews on small molecule inhibitors that target specific virus functions including virus attachment, uncoating, virus RNA replication and viral protein synthesis and processing have been extensively detailed (Carrasco, 1994; Patick and Potts, 1998; Shih et al., 2004; Sperber and Hayden, 1988). Antiviral agents directed towards virus attachment, capsid uncoating and 3C protease as well as agents that indirectly impair viral replication (e.g. interferons) are the best studied and will be the primary focus of subsequent sections.

3.1. Cell susceptibility

Interferons mediate antiviral, anticancer and immunomodulatory effects through specific receptor-signal transduction pathways. Several studies have evaluated the efficacy of recombinant interferon alpha 2b in the prevention or treatment of HRV-induced colds (Hayden et al., 1986, 1988; Rotbart, 2002; Sperber and Hayden, 1988). In these studies, intranasal interferon has been shown to be effective in both experimental and natural colds when provided prophylactically but had no little to no effect on the development of infection or symptoms when provided after infection. In many of these studies, interferon was associated with varying side effects including the appearance of blood-tinged mucus, and nasal mucosal bleeding.

3.2. Viral attachment

About 90% of HRV serotypes utilize the ICAM-1 receptor for attachment to susceptible host cells. The strategy of preventing virus attachment by receptor blockade utilizing soluble forms of ICAM-1 (sICAM-1) has been extensively evaluated in both in vitro and clinical studies (Turner et al., 1999; Turner, 2001).

Tremacamra (BIRR-4), developed by Boehringer Ingelheim, has been evaluated in 4 randomized, double-blind experimental infection trials. In these studies, patients received tremacamra formulated either as a nasal spray solution or inhaled powder 7 h before or 12 h after virus challenge and then 6 times a day for 7 days (Turner et al., 1999). Although the magnitude of the effects was small, tremacamra was shown to reduce the severity of experimental colds when given either before or after HRV challenge. Although these result are interesting, potential issues may include the challenge to demonstrate efficacy when provided later than 12 h after infection, possible antibody response to sICAM-1 and poor tolerability and patient compliance associated with a 6 times daily administration. No development currently is reported for these agents.

3.3. Capsid-binding agents

The HRV capsid consists of a densely-packed icosahedral arrangement of 60 protomers, each consisting of 4 proteins (VP1–VP4). VP1, 2 and 3 are located on the viral surface while VP4, located on the inner surface, remains in close association with the RNA core and functions as an anchor to the virus capsid. Elucidation of the solved, three-dimensional crystal structure of HRV reveal a canyon formed by the junctions of VP1 and VP3 (Rossmann et al., 1985). Beneath this canyon, within VP1, lies a pore that leads to a hydrophobic pocket that is occupied by a pocket factor proposed to be a fatty acid. Numerous molecules from diverse chemical classes have been described with in vitro antiviral activity against HRV and that bind in this region (Carrasco, 1994; Shih et al., 2004). Antiviral activity is related to the ability to prevent viral attachment and/or uncoating presumably by preventing capsid destabilization and subsequent release of RNA into the cytoplasm. In vitro, capsid-binders typically are active against most but not all HRV serotypes and possess a wide range of susceptibilities. This variability in susceptibility has served as the basis for one method of classification of HRV serotypes into two different groups, A and B (Andries et al., 1990).

The first capsid-binding compounds that were synthesized and extensively studied include a series of oxazolinyl isoxazoles ('WIN' compounds) from the Sterling Winthrop Pharmaceuticals group. The first compound to advance into clinical trials was disoxaril (WIN 51711). In preclinical studies, disoxaril demonstrated in vitro antiviral activity against both enteroviruses and HRV (Otto et al., 1985) and was shown to reduce mortality in poliovirus 2 infected mice and paralysis in echovirus 9 infected mice (Diana et al., 1985). The subsequent development of crystallurea in human volunteers treated with high doses prohibited subsequent development. The efficacy of orally administered WIN 54954, a next generation compound, was evalauted for the prevention of HRV infection and illness in two experimental studies in which human volunteers were inoculated with either HRV 39 or HRV 23 (Turner et al., 1993). WIN 54954 was administered 3 times before virus challenge (600 mg dose) and 3 times a day on each of the subsequent 5 days. No significant antiviral or clinical effect was detected in either study. Pharmacokinetic studies revealed that on the last day of drug administration, most of the infected subjects had total plasma trough levels (not corrected for protein binding) of WIN 54954 greater than the minimal inhibitory concentration for the appropriate virus but

detectable levels of drug in nasal wash specimens in only a few subjects. These findings suggest that appropriate levels of drug may not have been present at sites of virus replication. Although WIN 54954 did reduce the number and severity of colds when administered prophylactically in humans challenged with coxsackievirus A21 (CAV 21) (Rotbart, 2002), the development of this compound was discontinued primarily due to adverse effects of flushing and rash.

Subsequent synthetic chemistry efforts led to the discovery of pleconaril (WIN 63843; picovir) an orally bioavailable 5-methyl-oxadiazole analogue with potent broad-spectrum activity against a wide variety of enterovirus and HRV serotypes and clinical isolates (Pevear et al., 1999; Kaiser et al., 2000; Patick et al., 1999). To date, pleconaril represents the first compound to be submitted to the US Food and Drug Administration (FDA) for regulatory approval. In an experimental induced CAV 21 model involving 33 subjects, pleconaril demonstrated statistically significant reductions in virus shedding, nasal mucus production and total respiratory illness symptom scores when administered orally in a 200 mg dose twice a day for 7 days (Schiff and Sherwood, 2000). Pleconaril was subsequently evaluated in two phase II, placebo-controlled, natural cold trials (Hayden et al., 2002) involving 1024 healthy individuals who presented within 36 h of respiratory symptom onset during the months of July–December. In the first trial, subjects received 400 g pleconaril or placebo as either a liquid or tablet formulation 3 times a day for 7 days. Since the number of picornavirus-positive subjects was lower than expected (41% and 43% in first and second trial, respectively), datasets were combined for further analyses. Among the subset of subjects with proven picornavirus infection (42%), pleconaril provided a 1–1.5 day reduction in the time to alleviation of illness when compared with placebo. Pleconaril was generally well tolerated but was associated with gastrointestinal disturbance in all groups who received the liquid formulation and a greater incidence of nausea in groups that received tablets when compared to placebo. In two subsequent pivotal studies, pleconaril produced a moderate reduction of 1 day in the median time to alleviation of illness among 1363 picornavirus-positive subjects (65%) treated with pleconaril compared with placebo (Hayden et al., 2003a,b). Subjects in both studies were enrolled within 24 h of symptom onset. Picornaviral resistance was identified in a total of 24% of patients; 13% of baseline isolates were not susceptible to pleconaril and 11% developed reduced susceptibility (defined as > 10-fold change in baseline value) during the course of the 5 day treatment. The relevance of pleconaril resistance was shown in a subsequent study (Pevear et al., 2005). Patients with virus isolates that had no measurable baseline susceptibility (EC_{50} of virus > 3.8 μg/ml) derived no clinical benefit from pleconaril treatment. Conversely, the emergence of virus with reduced susceptibility during therapy was not associated with measurable adverse clinical consequences (Pevear et al., 2005). In a subsequent 6-week prophylaxis study, pleconaril was found to induce cyp3A4 which led to intermenstrual bleeding and other menstrual abnormalities in women taking estrogen-based oral contraceptives (Hayden et al., 2003a,b; Fleischer and Laessig, 2003). In March 2002, the Antiviral Drugs Advisory Committee of the FDA voted to not recommend pleconaril for approval for the treatment of cold in adults, primarily based on drug interactions, marginal treatment effect and possibility of transmission of resistant virus. Although further development of pleconaril for oral delivery has been discontinued, Schering-Plough, under license from ViroPharma, is developing an intranasal formulation of pleconaril for the potential treatment of the common cold in high-risk populations.

Other capsid-binders that have been well studied include pirodavir (R 77975), a substituted phenoxy-pyridazinamine (Andries et al., 1992), developed by Janssen Research Foundation An earlier compound, R 61837, represents the first compound that demonstrated efficacy in preventing experimental colds in human challenge experiments (Al-Nakib et al., 1989). The safety and efficacy of intranasal pirodavir was evaluated in 4 double-blind, controlled trials of experimental HRV infection (Hayden et al., 1992). In three prophylaxis studies (subjects were inoculated with HRV within 10 min of the second and third doses) pirodavir was shown to be effective in both decreasing infection (defined by viral shedding and/or seroconversion) and development of clinical colds when administered six times a day but not in two additional trials when pirodavir was administrated only three times a day. In a fourth trial pirodavir was able to reduce virus shedding but demonstrated no clinical benefit when administered 24 h after challenge. Overall intranasal pirodavir was well tolerated but was associated with unpleasant taste and nasal dryness. In a subsequent natural infection trial pirodavir was administered six times daily for 5 days to adults who exhibited symptoms of 48 h or less. No significant differences were observed in the resolution of respiratory symptoms or the median duration of illness. There was however a significant differences in the frequency of virus shedding. The reason for the discordance between virus replication and symptomology in these studies is unknown. Development of these compounds has been discontinued.

3.4. Protease inhibitors

HRV contain a single strand of positive sense RNA 7.4 kb in length that contains a single open reading frame encoding a single polyprotein of 2100–2400 amino acids. Following an initial cleavage by the viral encoded 2A protease to release P1, the majority of subsequent cleavages are mediated by the viral encoded 3C protease (Patick and Potts, 1998). To date, several different compounds which target 3C protease have been described (Patick and Potts, 1998; Shih et al., 2004). Rupintrivir (formerly AG7088), is an intranasally administered, irreversible inhibitor which incorporates an unsaturated ethyl ester Michael acceptor (Dragovich et al., 1999; Matthews et al., 1999). In vitro, rupintrivir has demonstrated potent activity against all HRV serotypes, HRV clinical isolates and enteroviruses tested (Patick et al., 1999; Kaiser et al., 2000; Binford et al., 2005). This broad-spectrum activity together with a narrow range of susceptibilities is consistent with DNA sequence analyses performed on 3C protease-coding gene regions, which demonstrate a significant level of homology in substrate/inhibitor binding regions (Meador et al., 1998; Binford et al., 2005). In a human exper-

imental HRV challenge trial rupintrivir was able to moderate severity of illness and reduce viral load providing proof of concept for the mechanism of 3C protease inhibition (Hayden et al., 2003a,b). In a subsequent natural infection study in patients rupintrivir was not able to significantly effect virus reduction or moderate disease severity and thus was terminated for clinical development (Patick et al., 2005). Subsequent efforts to discover an orally bioavailable inhibitor of 3C protease resulted in the identification of compound 1, also irreversible inhibitor of 3C protease with potent in vitro activity against all HRV serotypes and enteroviruses tested (Dragovich et al., 2003; Patick et al., 2005). In a phase 1 ascending single-dose study in healthy volunteers, compound 1 was shown to be safe and well tolerated with free observed maximum concentrations in plasma higher than the 50% effective concentration required to inhibit 80% of HRV serotypes. Currently, no further clinical development is planned for compound 1.

4. Conclusion

Despite extensive research efforts that have led to the discovery of many potent antiviral agents, no drug today has been approved for the treatment and prevention of HRV-induced illness. There are many factors to consider when trying to understand these shortcomings. In the majority of individuals, HRV-induced colds are mild and self-limiting. This alone dictates that agents must be very safe and tolerable and have a high risk:benefit ratio. Specifically the agent must have limited side effects, have no or low risk of resistance development and must be administered at some acceptable level of frequency (e.g. less than three times a day). To date, no agent has been able to achieve all three criteria and demonstrate appropriate clinical efficacy. Drugs that have been formulated for topical or intranasal administration often have to be provided in multiple daily doses to overcome mucociliary clearance mechanisms that act to clear drug quickly from nasal cavities. For this type of administration the formulation must be carefully considered to allow for appropriate drug deposition and dissolution within the nasal cavity. Physiochemical properties of drugs that allow for prolonged residence time, intracellular accumulation or residual biological activity may be required for appropriate antiviral effect. In addition, although drug levels can be measured in nasal washings and used to predict pharmacokinetics, no good technology exists that allow a direct assessment of drug levels intracellularly at site of virus replication. In these cases, drug efficacy is typically only first realized in trials in infected patients where infectious virus or RNA levels are measured and clinical symptoms can be scored. With orally administered drugs, levels can be easily measured in plasma and these values are assumed to correlate to levels in nasal epithelium. Oral administration is amenable to more convenient dosing regimens and may allow compounds access to additional HRV replication sites that are not available through nasal delivery. However oral delivery may also increase risk of side effects relative to nasal (topical) delivery as was observed in pleconaril clinical studies.

The demonstration of efficacy in clinical studies of natural infection has also been very challenging. Because of the rapid onset of symptoms, patients must be enrolled in trials and initiate antiviral therapy within 24 h to maximize the opportunity for therapeutic intervention. Should an antiviral ultimately be approved for HRV-induced common cold, this will require almost immediate diagnosis and provision of prescriptions to patients by physicians.

Although the hurdles associated with drug approval for HRV-induced illness are significant it is encouraging that drugs developed for HRV also possess broad-spectrum activity against other picornaviruses, including poliovirus and in some instances viruses from other families with homologous proteins, e.g., SARS (Matthews et al., 2004). In these situations it is encouraging to think that drugs developed against HRV may also be able to impact other diseases.

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