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Antiviral Research 47 (2000) 215–220

Antiviral Research

www.elsevier.com/locate/antiviral

Short communication

In vitro activity of pleconaril and AG7088 against selected serotypes and clinical isolates of human rhinoviruses☆

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Received 28 March 2000; accepted 18 May 2000

Abstract

Background: We tested the in vitro activity of pleconaril and AG7088 against five numbered human rhinovirus (HRV) serotypes and 46 clinical HRV isolates of undefined serotype recovered from patients with common colds. Antiviral effect of pleconaril and AG7088 were assessed by cytophathic effect (CPE) inhibition assays in Ohio HeLaI cells using microscopic and spectrophotometric methods. Both compounds were tested at concentrations of 1.0, 0.1 and 0.01 μg/ml. For the numbered HRV serotypes, the median EC_{50} value determined by the microscopic CPE inhibition was slightly better for AG7088 compared to pleconaril ($P = 0.02$) but was similar by spectrophotometric assay ($P = 0.15$). For clinical HRV isolates the median EC_{50} value determined microscopically was 0.01 μg/ml range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.07 μg/ml (range, $< 0.01 - > 1$ μg/ml) for pleconaril ($P < 0.001$). The median EC_{50} value determined by spectrophotometric assay was 0.01 μg/ml (range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.04 μg/ml (range, $< 0.01 - > 1$ μg/ml) for pleconaril ($P < 0.001$). By either the microscopic or spectrophotometric methods the median EC_{50} value of AG7088 was < 1.0 μg/ml for all isolates and was > 10.0 μg/ml of pleconaril for approximately 9% of isolates. In vitro AG7088 appeared to be more potent and to have a broader antirhinoviral spectrum than pleconaril among clinical HRV isolates. The clinical relevance of these in vitro results needs to be determined in controlled clinical trials.

Keywords: Human rhinovirus; AG7088; Pleconaril; Protease inhibitors; Capsid binding agents

1. Introduction

No effective antirhinoviral therapy is currently available for clinical use, but new antiviral agents are in development (Arruda et al., 1997; Turner et al., 1999). Pleconaril is a capsid function inhibitor which targets a conserved hydrophobic pocket of

☆ Presented in part at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 1999.

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0166-3542/00/$ - see front matter
PII: S0166-3542(00)00106-6

a major viral capsid protein called VP-1. Pleconaril inhibits attachment and/or virus uncoating (Arruda et al., 1997).

AG7088 is an irreversible inhibitor of HRV 3C protease responsible for the cleavage of viral polyproteins into essential proteins (Matthews et al., 1999). The coding region of the active site is conserved among HRV serotypes, and in vitro studies have shown that AG7088 is a potent inhibitor of HRV (Matthews et al., 1994; Patick et al., 1999). AG7088 is formulated for intranasal delivery.

Little data on the in vitro activity of AG7088 and pleconaril against clinical HRV isolates are available. We tested the in vitro activity of these two compounds against five selected HRV serotypes and against clinical HRV isolates of undefined serotype recovered from patients with common colds.

2. Methods

Nasopharyngeal washes, performed in adults or children with colds between 1988 and 1998, were first inoculated onto monolayer of human embryonic lung fibroblast cells, rhinovirus was identified by acid liability testing and in the majority of cases also by RT-PCR. Rhinovirus positive nasal washes were frozen at −70°C. For the purpose of this study these specimens were thawed and inoculated onto monolayers of Ohio HeLa-I cells (Arruda et al., 1996), and frozen at −70°C when rhinovirus related cytopathic effect (CPE) of 90% was present. At the time of the antiviral assay these specimens were thawed, centrifuged and supernatant was used for the antiviral assay (Arruda et al., 1992, 1996). In addition to clinical specimens, five selected serotypes representing the two principal groups of HRV receptors (HRV-2, HRV-14, HRV-16, HRV-39 and HRV Hanks) were tested. Pleconaril and AG7088, were diluted in maintenance media at concentrations of 1.0, 0.1 and 0.01 μg/ml and a final concentration of 0.25% dimethyl sulfoxide. The molecular weight of AG7088 is 599 and the molecular weight of pleconaril 381. AG7088 was provided by Agouron Pharmaceuticals. The 50% effective inhibitory concentration (EC_{50}) of AG7088 and pleconaril were determined in CPE inhibition assay on Ohio HeLa-I cells as described (Arruda et al., 1992). For each virus isolate (inoculated at three different dilutions) and compound dilution, drugs were tested in triplicate on plates containing also cytotoxicity controls and inoculum titration (backtiters). Assays were read on inoculum for which backtiters identified a 50% tissue culture infective doses per milliliter ($TCID_{50}$/ml) between 32 and 320. Monolayers were treated sequentially with 0.05 ml of each compound solution, 0.05 ml of virus inoculum, or 0.1 ml of McCoy's media with 2% fetal bovine serum (Hyclone Labs, Pittsburgh, PA) for cell control, and incubated at 34°C in CO_2 incubator. Determination of the proportion of cells with morphologic signs of CPE (ballooning, refractiveness, granularity, shrinkage) and spectrophotometric reading (at 550 nm) were performed as previously described (Arruda et al., 1992, 1996; Ohlin et al., 1994).

The EC_{50} of the compounds was calculated using the software Dose-Effect Analysis with Microcomputers (Biosoft, Cambridge, United Kingdom). In order to calculate a median EC_{50} value we assigned arbitrary EC_{50} values of 0.005 μg/ml if the obtained EC_{50} was < 0.01 μg/ml and 2.154 μg/ml if this value was > 1.0 μg/ml. These values represent the value obtained if the next lowest drug concentration, or the next highest, would have reach 0 or 100% of inhibition respectively and are estimate of the true EC_{50} value.

Cytotoxicity was evaluated in parallel to antiviral assays on non-growing cells. In addition, inhibition of uninfected cell growth was determined in a separate experiments using the cellular protein adhering dye sulforhodamine B (Skehan et al., 1990; Rollins et al., 1993).

3. Results

3.1. Cellular toxicity

For both compounds altered cellular morphology was observed in uninfected control cells at the highest concentration tested (100 μg/ml). Slight alterations of cellular morphology were observed

at a pleconaril concentration of 10 μg/ml, but no change was observed for both compounds at concentration of 1.0 μg/ml. For growing cells slight toxicity was observed at pleconaril concentration of 10 μg/ml and at AG7088 concentration of 50 μg/ml. The median cytostatic concentration calculated as described above was 30.0 μg/ml for pleconaril and > 100 μg/ml for AG7088.

3.2. Antiviral assays

The median EC_{50} value determined by the microscopic CPE inhibition assay performed on five serotypes was 0.02 μg/ml (0.03 μM) (range, < 0.01–0.03 μg/ml) for AG7088 compared to 0.05 μg/ml (0.09 μM) (range, 0.03–0.07 μg/ml) for pleconaril ($P = 0.02$ for comparison between AG7088 and pleconaril). The median EC_{50} value determined by the spectrophotometric assay was 0.01 μg/ml (0.02 μM) (range < 0.01–0.08 μg/ml) for AG7088 and 0.05 μg/ml (0.08 μM) (range, 0.03–0.07 μg/ml) for pleconaril ($P = 0.15$).

Cytopathic effect inhibition assay was performed on 46 clinical HRV isolates originally recovered from nasopharyngeal washes of patients with common colds (Table 1). The median EC_{50} value determined microscopically was 0.01 μg/ml (0.02 μM) (range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.07 μg/ml (0.17 μM) (range, < 0.01– > 1 μg/ml) for pleconaril ($P <$ 0.001 for comparison between AG7088 and pleconaril). The median EC_{50} value determined by spectrophotometric assay was 0.01 μg/ml (0.01 μM) (range, < 0.01–0.04 μg/ml) for AG7088 compared to 0.04 μg/ml (0.12 μM) (range, < 0.01– > 1 μg/ml) for pleconaril ($P < 0.001$). By either the microscopic or spectrophotometric methods the median EC_{50} value of AG7088 was < 1.0 μg/ml for all isolates and was > 1.0 μg/ml for ten of 46 (22%) isolates with pleconaril. We tested pleconaril at 10 μg/ml for seven of ten of these isolates. Inhibition was not observed in four of these seven isolates by both the microscopic and spectrophotometric methods.

The correlation of EC_{50} values obtained for all strains tested with the two methods, the microscopic and spectrophotometric assays, was high (r-value, 0.82; 95% CI, 0.74–0.88).

4. Discussion

Under these in vitro conditions AG7088 and pleconaril demonstrated comparable antiviral activity against five selected HRV serotypes. However, AG7088 appeared to be more potent and to have a broader antirhinoviral spectrum than pleconaril among clinical HRV isolates. At the concentrations tested, all 46 HRV clinical isolates were sensitive to AG7088 with the two assay end-points used, whereas the EC_{50} values for pleconaril were greater than 10 μg/ml in approximately 9% of isolates. The median EC_{50} values were also significantly different and depending on the method used, were 5–6-fold higher for pleconaril than for AG7088. These results suggest that a small subset of HRV strains circulating in the community lack susceptibility to pleconaril. However, a limitation of our conclusions is that we did not identify the serotypes of these different isolates and we did not performed further characterizations.

Any comparisons between AG7088 and pleconaril need to consider that these drugs have different antiviral mechanisms of action and have different pharmacokinetic characteristics. Pleconaril is an oral compound with good systemic distribution, whereas AG7088 is topically delivered. After a single oral dose of 200 mg or 5 mg/kg of pleconaril the maximum peak plasma or serum concentration of the drug in children was approximately 1.2 μg/ml, a value above EC_{50} values obtained for approximately 80% of clinical isolates tested (Abdel-Rahman and Kearns, 1998, 1999). In adults a single dose of 400 mg give peak plasma levels of 2–2.5 μg/ml (Abdel-Rahman and Kearns, 1999). The EC_{50} values for pleconaril were greater than 10 μg/ml in approximately 9% of isolates. Such plasma concentrations are not achievable with oral pleconaril but animal studies suggest that peak concentrations in nasal epithelium are several fold in excess of those observed in the plasma (Abdel-Rahman and Kearns, 1999). Of note, pleconaril shortens the illness duration in adults with acute upper respiratory tract infection (Hayden et al., 1999). The differences observed in

Table 1
In vitro antiviral effect of AG 7088 and pleconaril against clinical isolates of human rhinoviruses

HRV isolate	EC_{50} values (μg/ml)			
	Microscopic assay		Spectrophotometric assay	
	AG7088	Pleconaril	AG7088	Pleconaril
1	0.02	>10.0	0.02	>10.0
2	0.01	0.02	<0.01	0.02
3	0.02	0.02	0.02	0.02
4	0.01	0.08	0.03	0.14
5	0.03	0.08	0.03	0.10
6	0.02	0.10	0.02	0.03
7	0.02	0.22	0.01	0.13
8	0.04	0.17	0.02	0.10
9	0.01	0.22	<0.01	0.05
10	0.01	2.15	0.03	>10.0
11	0.03	0.03	0.01	0.01
12	<0.01	0.03	<0.01	0.02
13	0.01	0.02	0.02	0.03
14	0.02	0.11	0.01	0.17
15	0.01	<0.01	0.02	<0.01
16	<0.01	<0.01	<0.01	<0.01
17	<0.01	>1.0[a]	<0.01	>1.0[a]
18	<0.01	0.94	<0.01	0.05
19	<0.01	0.11	<0.01	0.14
20	<0.01	>1.0[a]	<0.01	>1.0[a]
21	<0.01	1.75	<0.01	0.54
22	<0.01	0.03	0.01	0.03
23	<0.01	<0.01	<0.01	0.01
24	0.02	0.09	0.02	0.10
25	0.02	0.22	0.02	0.04
26	0.01	0.10	<0.01	0.07
27	0.01	0.02	<0.01	<0.01
28	0.01	0.02	<0.01	<0.01
29	0.01	>1.0[a]	<0.01	>1.0[a]
30	0.02	>10.0	<0.01	>10.0
31	0.02	1.45	0.02	2.59
32	<0.01	0.03	<0.01	0.03
33	0.02	0.02	0.01	0.02
34	0.04	0.01	<0.01	0.03
35	<0.01	0.05	0.01	0.02
36	0.02	0.14	0.02	0.05
37	0.02	0.05	0.01	0.02
38	0.02	0.05	0.01	0.09
39	0.01	>10.0	<0.01	>10.0
40	0.01	0.01	<0.01	<0.01
41	0.02	0.05	0.04	0.07
42	0.02	0.02	0.01	0.01
43	0.02	0.05	0.02	0.04
44	0.02	0.05	<0.01	0.04
45	0.01	>10.0	0.01	>10.0
46	0.02	0.02	0.02	0.03
Median, range	0.01 (<0.01–0.04)	0.07 (<0.01–>1.0)	0.01 (<0.01–0.04)	0.04 (<0.01–>1.0)

[a] Not tested at concentration 10 μg/ml.

our in vitro study suggest that compared to AG7088, pleconaril potentially will not have activity against a small number of circulating rhinovirus strains. Whether this could translate in different clinical efficacy needs to be investigated in comparative clinical trials.

AG7088 is an irreversible peptidomimetic inhibitor of HRV 3C protease, which has a highly conserved active site (Werner et al., 1986; Skehan et al., 1990; Arruda et al., 1992, 1996; Rollins et al., 1993; Ohlin et al., 1994; Abdel-Rahman and Kearns, 1998, 1999; Hayden et al., 1999; Kearns et al., 1999; Patick et al., 1999). Our results showed that AG7088 is active against a wide range of HRV isolates suggesting that the affinity for the binding site is conserved among a wide range of viruses. A similar observation was done in a recent investigation showing that AG7088 was effective against 48 defined HRV serotypes with EC_{50} values comparable to those obtained in our study (Patick et al., 1999). Although these results suggest that primary resistance to this 3C protease inhibitor is uncommon, other RNA viruses such as human immunodeficiency viruses readily develops resistance when exposed to protease inhibitors. Although major differences exist between these two viral infections, whether resistant viruses could be selected by AG7088 treatment should be investigated in further investigations.

Both pleconaril and AG7088 are antiviral compounds effective against a wide range of HRV isolates. The clinical relevance of these in vitro results needs to be determined in controlled clinical trials.

Acknowledgements

We thank Amy K. Patick and Leora S. Zalman for their collaboration and for providing AG7088. We thank also Jack M. Gwaltney Jr. for providing several HRV isolates. Laurent Kaiser is the recipients of a grant of the Division of Infectious Diseases, University Hospital of Geneva, Geneva, Switzerland. This work was supported in part by a grant form Agouron Pharmaceuticals, San Diego, CA.

References

Abdel-Rahman, S.M., Kearns, G.L., 1998. Single-dose pharmacokinetics of a pleconaril (VP63843) oral solution and effect of food. Antimicrob. Agents Chemother. 42, 2706–2709.

Abdel-Rahman, S.M., Kearns, G.L., 1999. Single oral dose escalation pharmacokinetics of pleconaril (VP 63843) capsules in adults. J. Clin. Pharmacol. 39, 613–618.

Arruda, E., Pitkaranta, A., Witek, 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.

Arruda, E., Crump, C.E., Marlin, S.D., Merluzzi, V.J., Hayden, F.G., 1992. In vitro studies of the antirhinovirus activity of soluble intercellular adhesion molecule-1. Antimicrob. Agents Chemother. 36, 1186–1191.

Arruda, E., Crump, C.E., Rollins, B.S., Ohlin, A., Hayden, F.G., 1996. Comparative susceptibilities of human embryonic fibroblasts and HeLa cells for isolation of human rhinoviruses. J. Clin. Microbiol. 34, 1277–1279.

Hayden, F.G., Hassman, H.A., Coats, R., Menendez, R., Bock, T., 1999. Pleconaril treatment shortens duration of picornavirus respiratory illness in adults. In: Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, San Diego.

Kearns, G.L., Abdel-Rahman, S.M., James, L.P., Blowey, D.L., Marshall, J.D., Wells, T.G., et al., 1999. Single-dose pharmacokinetics of a pleconaril (VP63843) oral solution in children and adolescents. Antimicrob. Agents Chemother. 43, 634–638.

Matthews, D.A., Smith, W.W., Ferre, R.A., Condon, B., Budahazi, G., Sisson, W., et al., 1994. Structure of human rhinovirus 3C protease reveals a trypsin-like polypeptide fold, RNA-binding site, and means for cleaving precursor polyprotein. Cell 77, 761–771.

Matthews, D.A., Dragovich, P.S., Webber, S.E., Fuhrman, S.A., Patick, A.K., et al., 1999. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. Proc. Natl. Acad. Sci. US 96, 11000–11007.

Ohlin, A., Hoover-Litty, H., Sanderson, G., Paessens, A., Johnston, S.L., Holgate, S.T., et al., 1994. Spectrum of activity of soluble intercellular adhesion molecule-1 against rhinovirus reference strains and field isolates. Antimicrob. Agents Chemother. 38, 1413–1415.

Patick, A.K., Binford, S.L., Brothers, M.A., Jackson, R.L., Ford, C.E., Diem, M.D., et al., 1999. In vitro antiviral activity of AG7088, a potent inhibitor of human rhinovirus 3C protease. Antimicrob. Agents Chemother. 43, 2444–2450.

Rollins, B.S., Elkhatieb, A.H., Hayden, F.G., 1993. Comparative anti-influenza virus activity of 2′-deoxy-2′-fluororibosides in vitro. Antiviral Res. 21, 357–368.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Institute 82, 1107–1112.

Turner, R.B., Wecker, M.T., Pohl, G., Witek, T.J., McNally, E., St.Georges, R., et al., 1999. Efficacy of Tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. J. Am. Med. Assoc. 281, 1797–1804.

Werner, G., Rosenwirth, B., Bauer, E., Seifert, J.M., Werner, F.J., Besemer, J., 1986. Molecular cloning and sequence determination of the genomic regions encoding protease and genome-linked protein of three picornaviruses. J. Virol. 57, 1084–1093.