Pharmaceutica Analytica Acta
ISSN: 2153-2435

Tarro, Pharm Anal Acta 2016, 7:3
http://dx.doi.org/10.4172/2153-2435.1000469

Short Communication Open Access

Anti-Rhinovirus Activity of Ethyl 4-(3-(2-(3-Methylisoxazol-5-Yl) Ethoxy) Propoxy) Benzoate (EMEB)

Giulio Tarro*

Department of Biology, Center for Biotechnology, Sbarro Institute for Cancer Research and Molecular Medicine, Temple University, Philadelphia, USA

Abstract

The compuond EMEB has got a definite anti-Rhinovirus activity on both HRV14 (group A) and HRV39 (group B). The specific activity is lower than that found for Pirodavir used as a positive control, but, since the cytotoxic activity of EMEB on human HeLa cells is more favourable than that of Pirodavir (50 µg/ml against 3 µg/ml), the final Protection Index is higher for EMEB (> 700) as compared to Pirodavir (250). EMEB seems to be stable in aqueous solutions, since its activity after 10 days was unchanged. When EMEB is challanged with Rhinovirus infected HeLa cells during the whole reproduction cicle, its antiviral activity remains evident and strong even after 18 hours from infection. This fact is important because it means that the compound keeps functioning even when the viral infection is already in progress; this finding makes us to hypothesize that the compound EMEB could act not only as a prophylactic agent against the common cold, but also as a therapeutic drug in patients who already show the disease symptoms (at least within the first 24 hours from the start of symptoms). These last statements must be confirmed with assays on the mechanism of action of the compound, by analysing its adhesion to the cell virus internalization into the cells, the viral uncoating, transcription and translation, and finally on viral morphogenesis.

Keywords: Glyoxal derivative; Rhinovirus; Antiviral activity

Introduction

Common cold is the most widespread illness known. The NIH estimates that, in the United States alone, individuals suffer from more than 1 bilion cold episodes for year. Accordingly, the economic impact of the common cold is enormous. The National Center for Health Statistic estimates that, in 1996, 62 million cases of the common cold in the U.S. required medical attention, resulting in 45 million days of restricted activity and in the loss of 22 million school days. More than 200 different viruses are known to cause the symptoms of the common cold. Rhinoviruses are believed to cause an estimated 30% - 35% of all adult colds. More then 110 distinct rhinovirus types have been identified. Only symptomatic treatment is avaiable for uncomplicated cases of the common cold: bed rest, fluids, gargling with warm salt water, petroleum jelly for raw nose, and aspirin or acetaminophen to relieve headache or fever. It has been shown that compounds of diphenyl-, naftil- and cumaroil-glyoxal are able to stop the infetious process, probably by preventing the penetration of the virus into the cell. If these results are confirmed, glyoxal derivative could be developed as a non-symptomatic drug for the treatment of the common cold. Glyoxal derivative's antiviral activity is currently under in vitro study. Preliminary results indicated the possibility of an interaction of the molecule either with the membrane cell receptors or with the enzymatic systems specific to rhinoviruses at a low dosage. According to the preliminary report, the compound has a toxicity concentration detectable by optical microscope of about 50 mcg/ml while the dosage still able to inhibit the cytophatic effect of the virus (IC50) was shown to be 0.006 mcg/ml [1]. These data give a protection index (calculated as the ratio between toxic dosage and the minimum inhibitory dosage) of approximately 8,500, which is higher than the protection indices of similar anti-rhinovirus compounds described in the literature, such as disoxaril [2]. We are currently conducting studies to determinate the mechanism of action and expect the results of these studies to be released later on [3] (Table 1).

Materials and Methods

Viruses and cell lines

Rhinovirus HRV 14 and HRV 39. Isolated from throat washings of patients with respiratory illness [4-7]. Cell line: Human adenocarcinoma of the cervix (HeLa) cells.

Chemicals and reagents

The compound that has been studied is Ethyl 4-(3-(2-(3-Methylisoxazol-5-yl) Ethoxy) Propoxy) Benzoate (EMEB). It has been synthetized by Dr Gunter Bartels ASM Germany, on 08/09/2006 and is a derivative of the compounds previously synthetized and found active on Rhinovirus HRV14 and HRV39. The positive control is the Pirodavir (Janssen Pharmaceutics) [8-12].

Assays for antiviral activity against respiratory viruses

Cytopathic effect (CPE) inhibition: This test, run in 96 well Flat-bottomed microplates, will be used for the initial antiviral evaluation of all new test compounds. In this CPE inhibition test, four log10 dilutions of each test compound (e.g. 1000, 100, 10, 1 µg/ml) will be added to 3 coups containing the cell monolayer; within 5 min, the virus is then added and the plate sealed, incubated at 37°C and CPE read microscopically when untreated infected controls develop a 3 to 4+ CPE (approximately 72 h to 120 h). A known positive control drug is evaluated in parallel with test drugs in each test. Follow-up testing

***Corresponding author:** Giulio Tarro, Department of Biology, Center for Biotechnology, Sbarro Institute for Cancer Research and Molecular Medicine, Temple University, Philadelphia, USA, Tel: (+39) 0815750090; E-mail: gitarro@tin.it

Received February 18, 2016; **Accepted** March 15, 2016; **Published** March 18, 2016

Citation: Tarro G (2016) Anti-Rhinovirus Activity of Ethyl 4-(3-(2-(3-Methyl-isoxazol-5-Yl) Ethoxy) Propoxy) Benzoate (EMEB). Pharm Anal Acta 7: 469. doi:10.4172/2153-2435.1000469

with compounds found active in initial screening test are run in the same manner except 8 one-half log 10 dilutions of each compound are used in 4 cups containing the cell monolayer for dilution. The data are expressed as 50% effective concentrations (EC50).

Visual observation: In the CPE inhibition tests, two wells of uninfected cells treated with each concentration of test compound will be run in parallel with the infected, treated wells. At the time CPE is determined micoscropically, the toxicity control cells will also be examined microscopically for any changes in cell appearance compared to normal control cells run in the same plate. These changes may be enlargement, granularity, cells with ragged edges, a filmy appearance, rounding, detachment from the surface of the well, or other changes. These changes are given a designation of T (100% toxic), PVH (partially toxic-very heavy-80%), PH (partially toxic-heavy-60%), P (partially toxic-40%), Ps (partially toxic-slight-20%), or 0 (no toxcity-0%), conforming to the degree of cytotoxicity seen. A 50% cell inhinitory (cytotoxic) (Table 2) concentration (IC50) is determined by regression analysis of these data.

Natural red uptake: This test is run to validate the CPE inhibition seen in the initial test, and utilizes the same 96-well micro plates after the CPE has been read. Neutral red is added to the medium; cells not damaged by virus take up a greater amount of dye, which is read on a computerized micro plate autoreader. The method as described by McManus (Apll. Enviroment. Microbiol. 31:35-38, 1976) is used. An EC50 is determined from this dye uptake.

Viable cell count: Compounds considered to have significant antiviral activity in the initial CPE and NR tests are re-tested for their effects on cell growth. In this test, 96-well tissue culture plates are seeded with cells (sufficient to be approximately 20% confluent in the well) and exposed to varying concentrations of the drug while the cells are dividing rapidly. The plates are then incubated in a CO_2 incubator at 37°C for 72 h, at which time neutral red is added and the degree of color intensity indicating viable cell number is determined spectrophotometrically; an IC50 is determined by regression analysis.

VIRAL INHIBITION %				
Compounds	Time of Challange Hour 0% ± SD*	Hour + 6	Hour + 12	Hour + 18
EMEB 1 µg/ml	99 ± 2	98 ± 3	86 ± 1	76 ± 2
Pirodavir 1 µg/ml	87 ± 2	51 ± 2	< 50	< 50

*SD: Standard Deviation

Table 1: Anti-Rhinovirus activity of EMEB on HRV14 during growth cycle.

Compound	Toxicity MNTD* ug/ml	Anti-viral activity on HRV14 ug/ml	Anti-viral activity on HRV19 ug/ml	Protection index (PI)
EMEB	50.0	0.07	0.07	> 700 / > 700
Pirodavir	3.1	0.012	0.006	250 / > 500

*MNTD: Maximum Non-Toxic Dose

Table 2: Cytotoxic and anti-Rhinovirus activity of compound EMEB and Pirodavir.

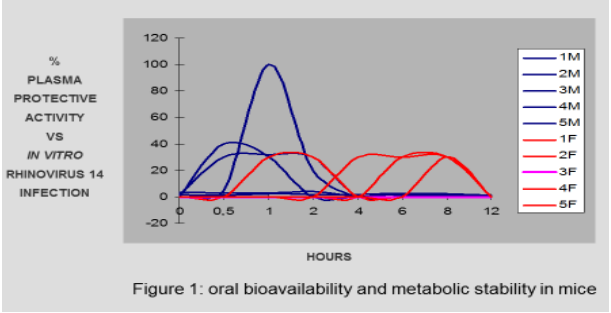

Figure 1: Oral bioavailability and metabolic stability in mice.

Results and Discussion

The compound EMEB has got a definite anti-Rhinovirus activity on both HRV14 (group A) and HRV39 (group B). The specific activity is lower than that found for Pirodavir used as positive control, but, since the cytotoxic activity of EMEB on human HeLa cells is more favourable than that of Pirodavir (50 µg/ml against 3 µg/ml), the final Protection Index is higher for EMEB (> 700) as compared to Pirodavir (250) [13-15].

EMEB seems to be stable in aqueous solutions, since its activity after 10 days was unchanged. When EMEB is challenged with Rhinovirus infected HeLa cells during the whole reproduction cycle, its antiviral activity remains evident and strong even after 18 h from infection [16-18].

This fact is important because it means that the compound keeps functioning even when the viral infection is already in progress; this finding makes us to hypothesize that the compound EMEB could act not only as a prophylactic agent against the common cold, but also as a therapeutic drug in patients who already show the disease symptoms (at least within the first 24 h from the start of symptoms). These last statements must be confirmed with assays on the mechanism of action of the compound, by analysing its adhesion to the cell, the virus internalization into the cells, the viral uncoating, transcription and translation, and finally on viral morphogenesis [19,20].

Conclusion

The current compound EMEB has the requisites for immediate passage to animal trials. No preventive and curative treatment of HRV-related infetions is available. Oral bioavailability and metabolic stability in mice (Figure 1). product in the form of a nasal spray; additional potential for extra-nasal solutions Metabolically stable, more prolonged antiviral activity of capsid-binding inhibitor molecule (18 h vs. 6), 16 times less toxic, superior therapeutic index (approximately 3 times greater), potential for modification into a dual-target compound (capsid and proteases). Predictive modelling studies show strong potential for significant further improvement.

Acknowledgement

The author is very much thankful to the late Dr. Enrico Malizia, professor of pharmacology at the University Rome for his support..

Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this article.

References

1. Sidwell RW, Huffman JH (1971) Use of disposable micro tissue culture plates for antiviral and interferon induction studies. Appl Microbiol 22: 797-801.
2. Sidwell RW, Huffman JH, Khare GP, Allen LB, Witkowski JT, et al. (1972) Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science 177: 705-706.
3. Sidwell RW, Huffman JH, Barnett BB, Pifat DY (1988) *In vitro* and *in vivo* Phlebovirus inhibition by ribavirin. Antimicrob Agents Chemother 32: 331-336.

4. Barnard DL, Huffman JH, Meyerson LR, Sidwell RW (1993) Mode of inhibition of respiratory syncytial virus by a plant flavonoid, SP-303. Chemotherapy 39: 212-217.

5. Barnard DL, Hill CL, Gage T, Matheson JE, Huffman JH, et al. (1997) Potent inhibition of respiratory syncytial virus by polyoxometalates of several structural classes. Antiviral Res 34: 27-37.

6. Huffman JH, Sidwell RW, Barnard DL, Morrison A, Otto MJ, et al. (1997) Influenza virus-inhibitory effects of a series of germanium and silicon-centered polyoxometalates. Antiviral Chem and Chemother 8: 75-83.

7. Barnard DL, Bischofberger N, Kim CU, Huffman JH, Sidwell RW, et al. (1997) Acyclic phosphonomethylether nucleoside inhibitors and respiratory viruses. Antiviral Chem and Chemother 8: 223-233.

8. Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, et al. (1999) Antiviral activities of biflavonoids. Planta Med 65: 120-125.

9. Barnard DL, Sidwell RW, Xiao W, Player MR, Adah S, et al. (1999) 2-5A-antisense inhibition of respiratory syncyntial virus replication: Effects of oligonucleotide structure modifications and RNA target site selection. Antiviral Res 41: 119-134.

10. Sidwell RW, Smee DF (2000) *In vitro* and *in vivo* assay systems for study of influenza virus inhibitors. Antiviral Res 48: 1-16.

11. Yasuda S, Huffman JH, Smee DF, Sidwell RW, Miyata K (2000) Spectrum of virus inhibition by consensus interferon YM643. Antivir Chem Chemother 11: 337-341.

12. Smee DF, Huffman JH, Morrison AC, Barnard DL, Sidwell RW (2000) Cyclopentane neuraminidase inhibitors with potent *in vitro* anti-influenza virus activities. Antimicrob. Agents Chemother 45: 743-748.

13. Barnard DL, Stowell VD, Seley KL, Hegde VR, Das SR, et al. (2001) Inhibition of measles virus replication by 5'-nor carbocyclic adenosine analogues. Antivir Chem Chemother 12: 241-250.

14. Song GY, Paul V, Choo H, Morrey J, Sidwell RW, et al. (2001) Enantiomeric synthesis of D- and L-cyclopentenyl nucleosides and their antiviral activity against HIV and West Nile virus. J Med Chem 44: 3985-3993.

15. Morrey JD, Smee DF, Sidwell RW, Tseng C (2002) Identification of active antiviral compounds against a New York isolate of West Nile virus. Antiviral Res 55: 107-116.

16. Smee DF, Bailey KW, Morrison AC, Sidwell RW (2002) Combination treatment of influenza A (HINI) virus infections in cell culture and in mice with the cyclopentane neuraminidase inhibitor RWJ-270201 and ribavirin. Chemotherapy. 48: 88-93.

17. Sidwell RW, Smee DF (2003) Viruses of the Bunya- and Togaviridae Families: Potential as bioterrorism agents and means of control. Antiviral Res 57: 101-111.

18. Smee DF, Morrison AC, Barnard DL, Sidwell RW (2002) Comparison of colorimetric, fluorometric, and visual methods for determining anti-influenza (HINI and H3N2) virus activities and toxicities of compounds. J Virol Methods 106: 71-79.

19. Barnard DL, Xu Ze, Stowell VD, Yuan H, Smee DF, et al. (2002) Coumarins and pyranocoumarins, potential novel pharmacophores for inhibition of measles virus replication. Antivir Chem Chemother 13: 39-59.

20. Zhang N, Chen HM, Sood R, Kalicharran K, Fattom AI, et al. (2002) *In vitro* inhibition of the measles virus by novel ring-expanded ('fat') nucleoside analogues containing the imidazo[4,5-e]diazepine ring system. Bioorg Med Chem Lett 12: 3391-3394.

Citation: Tarro G (2016) Anti-Rhinovirus Activity of Ethyl 4-(3-(2-(3-Methylisoxazol-5-Yl) Ethoxy) Propoxy) Benzoate (EMEB). Pharm Anal Acta 7: 469. doi:10.4172/2153-2435.1000469