

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

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Abstract

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 Pirodavis used as a positive control, but, since the cytotoxic activity of EMEB on human HeLa cells is more favourable than that of Pirodavis (50 µg/ml against 3 µg/ml), the final Protection Index is higher for EMEB (> 700) as compared to Pirodavis (250). 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 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 billion 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 than 110 distinct rhinovirus types have been identified. Only symptomatic treatment is available 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 cumarol-glyoxal are able to stop the infectious 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 cytopathic 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 synthesized by Dr Gunter Bartels ASM Germany, on 08/09/2006 and is a derivative of the compounds previously synthesized and found active on Rhinovirus HRV14 and HRV39. The positive control is the Pirodavis (Janssen Pharmaceuticals) [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 coupons 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

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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-Methylisoxazol-5-Yl) Ethoxy) Propoxy) Benzoate (EMEB). Pharm Anal Acta 7: 469. doi:10.4172/2153-2435.1000469

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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 microscopically, 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 toxicity-0%), conforming to the degree of cytotoxicity seen. A 50% cell inhibitory (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 (Appl. Environment. 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

Compounds	VIRAL INHIBITION %			
	Time of Challenge Hour 0% ± SD*	Hour + 6	Hour + 12	Hour + 18
EMEB 1 µg/ml	99 ± 2	98 ± 3	86 ± 1	76 ± 2
Pirodavis 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
Pirodavis	3.1	0.012	0.006	250 / > 500

*MNTD: Maximum Non-Toxic Dose

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

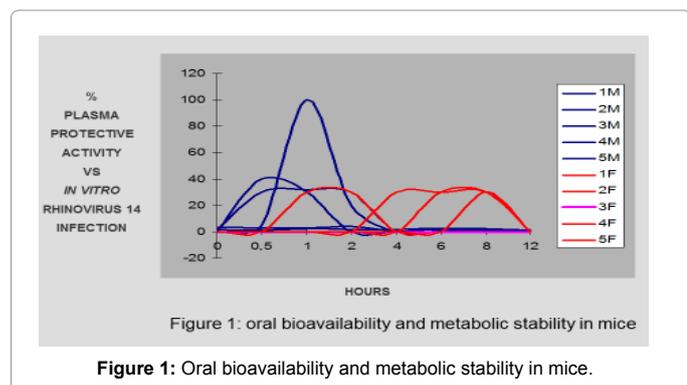


Figure 1: Oral bioavailability and metabolic stability in mice.

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 CO₂ 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.

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 Pirodavis used as positive control, but, since the cytotoxic activity of EMEB on human HeLa cells is more favourable than that of Pirodavis (50 µg/ml against 3 µg/ml), the final Protection Index is higher for EMEB (> 700) as compared to Pirodavis (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 infections 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.

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