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Euro Biopharma 2018: 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

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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 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 Defence 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 contested 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 theorize 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 declarations must be confirmed with assays on the mechanism of action of the combination, by analysing its adhesion to the cell virus internalization into the cells, the viral uncoating, transcription and translation, and finally on viral morphogenesis.

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 normal cold in the U.S. required medical attention, resulting in 45 million days of confined 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 simple cases of the common cold: bed rest, fluids, gargling with warm salt water, petroleum jelly for raw nose, and aspirin or acetaminophen to reduce headache or fever. It has been shown that compounds of diphenyl-, naftil- and cumaroil-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.

Materials & 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).

Assays for antiviral activity against respiratory viruses

Cytopathic effect (CPE) inhibition: This test, run in 96 well Flatbottomed 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 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.

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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 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 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].

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.