Antiviral Research 96 (2012) 130–137

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Oseltamivir–zanamivir bitherapy compared to oseltamivir monotherapy in the treatment of pandemic 2009 influenza A(H1N1) virus infections

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ARTICLE INFO

Article history:
Received 7 February 2012
Revised 2 August 2012
Accepted 4 August 2012
Available online 14 August 2012

Keywords:
Oseltamivir
Zanamivir
Combination therapy
Pandemic
Influenza A(H1N1)pdm09 virus

ABSTRACT

Background: The emergence of oseltamivir resistance in 2007 highlighted the need for alternative strategies against influenza. To limit the putative emergence of resistant viruses this clinical trial aimed to evaluate the antiviral efficacy and tolerability of oseltamivir–zanamivir (O + Z) bitherapy compared to oseltamivir monotherapy (O). This clinical trial was designed in 2008–2009 and was conducted during the A(H1N1) influenza virus pandemic in 2009–2010. The A(H1N1)pdm09 viruses were reported to be sensitive to oseltamivir and zanamivir but resistant to amantadine.

Methods: During the pandemic phase in France, adults with influenza-like illness for less than 42 h and who tested positive to influenza A were randomised into treatment groups: (O + Z) or (O). Patients had a nasal wash at day 0, before the beginning of treatment and daily at days 1 to 4. They also had a nasal swab at days 5 and 7 to check for the negativation of viral excretion. Virological response was assessed using the GAPDH adjusted M gene quantification.

Results: Analysis was possible for 24 patients, 12 in the (O + Z) arm and 12 in the (O) arm. The mean viral load decreased at around 1 $\log_{10}$ cgeq/µl per day regardless of allocated treatment group. We could not detect any significant difference between treatment groups in the duration needed to alleviate symptoms. All treatments were well tolerated. No oseltamivir-resistant H275Y NA mutated virus has been detected in patients of both treatment groups.

Conclusions: The sample size of our study is too limited to be fully informative and we could not detect whether combination therapy (O + Z) improves or reduces the effectiveness of oseltamivir in the treatment of influenza A(H1N1)pdm09 virus infection in community patients. Additional studies are needed to improve the antiviral treatment of patients infected with influenza virus.

1. Introduction

The emergence of oseltamivir-resistance in 2007 and the rapid worldwide spread of the pandemic influenza A(H1N1)pdm09 virus have highlighted the need for effective novel antiviral approaches against influenza.

The neuraminidase inhibitors (NAIs) oseltamivir and zanamivir are the recommended antiviral agents against influenza A and B viruses. Amantadine was one of the first antiviral agents used against influenza A viruses. In the last decade there has been a substantial worldwide increase in amantadine-resistance, starting with the seasonal influenza A(H3N2) viruses (Deyde et al., 2007).

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0166-3542/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2012.08.002

When the study was designed, seasonal influenza A(H1N1) viruses were resistant to oseltamivir but mostly sensitive to amantadine. However, as a consequence of the emergence of the A(H1N1)pdm09 virus that carried the S31N mutation in M2 (Dawood et al., 2009), all currently circulating human influenza A viruses are resistant to amantadine (WHO, 2011).

In the winter of 2007–2008, seasonal oseltamivir-resistant A(H1N1) viruses related to the A/Brisbane/59/2007 (H1N1) variant emerged and disseminated in individuals who were not under oseltamivir treatment (Lackenby et al., 2008; Meijer et al., 2009). However, the emergence of these viruses bearing the H275Y NA mutation had a major impact, showing that A(H1N1) viruses may develop the H275Y NA mutation and that the oseltamivir could not be used to treat infected patients. With the displacement of seasonal A(H1N1) by the A(H1N1)pdm09 viruses, all human influenza viruses were sensitive to oseltamivir and zanamivir. However, in order to limit the putative emergence of resistant clones in the context of a A(H1N1) pandemic when the use of NAIs is large, it was important to test possible alternative strategies. Theoretically, antivirals in combination could improve antiviral efficacy, reduce the duration of symptoms, and lower the risk of emergence of antiviral resistance.

The *in vitro* combination of NAIs may be antagonistic because these agents target the same binding pocket in the neuraminidase. However, different routes of administration, orally for oseltamivir and by inhalation for zanamivir, could induce a pharmacologically interesting interaction *in vivo*. During the 2008–2009 season when A(H3N2) virus was predominantly circulating, a clinical trial conducted in France confirmed that the oseltamivir–zanamivir bitherapy was antagonistic (Duval et al., 2010). However, these results may be different for A(H1N1) and A(H3N2) viruses because neuraminidases N1 and N2 show structural differences (Russell et al., 2006). Moreover, in the context of the pandemic it was interesting to study if the results found with the A(H3N2) virus would be confirmed or not with the A(H1N1)pdm09 virus.

The randomised trial was then conducted during the A(H1N1)pdm09 virus pandemic in France, to compare the oseltamivir–zanamivir (O + Z) combination therapy with the oseltamivir monotherapy (O), in terms of antiviral efficacy, resolution of symptoms, tolerability, and the prevention of oseltamivir-resistance emergence.

2. Patients, material and methods

This phase II clinical trial was a multicentered, randomised, and unblinded study of two parallel groups.

2.1. Study population

The study targeted adult out-patients of both sexes, aged 18 to 64 years, with influenza-like illness (ILI) for less than 42 h, not vaccinated against influenza in the year of the study and who tested positive for influenza A (QuickVue® Influenza A+B test). Patients also had to be free from chronic diseases, have medical insurance, and give informed written consent. Exclusion criteria were pregnancy (a pregnancy test was performed before inclusion), lactation, lack of effective contraceptive methods, ongoing chronic obstructive pulmonary disease (COPD), asthma, renal failure, epilepsy, confusional state, hallucinations, severe uncontrolled psychotic or neurotic state, depression with antidepressant treatment, congestive cardiac insufficiency, peripheral oedema, orthostatic hypotension and a hypersensitivity to one component of the study drugs. Use of drugs like nasal topics, corticosteroids, immunosuppressive drugs, neuroleptics or antiemetics was not permitted during the study.

Patients were recruited by general practitioners in the community (in Lyon and Paris, France) during the peak circulation of the influenza A(H1N1)pdm09 virus.

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Lyon Ethics Committee (*Comité de Protection des Personnes Lyon B*) on 9 September 2009.

2.2. Study protocol

Randomisation was performed after checking eligibility, obtaining patient consent, and collecting baseline data. A permuted-block algorithm was used for randomisation. Concealed allocation was performed by calling the coordination centre. Patients were randomised into two groups according to the antiviral treatment they received: the oseltamivir–zanamivir (O + Z) bitherapy or the oseltamivir (O) monotherapy.

Oseltamivir (Tamiflu®) was administered orally at the recommended dosage of 75 mg, two times a day for 5 days (150 mg per day). Zanamivir (Relenza®) was administered by inhalation with the Diskhaler system, at the recommended dosage of two inhalations of 5 mg, two times a day for five days (20 mg per day). For the combination therapy both drugs were given concomitantly: there was no more than a few minutes of delay between the oral administration of oseltamivir and the inhalation of zanamivir. Oseltamivir was provided for free by Roche SA. Zanamivir was purchased from GlaxoSmithKline. Drugs were packaged by Creapharm SA.

Specimens were collected at patients' homes by a study nurse. Specimens consisted of a nasal wash within two hours after the first visit (H0), and every 24 h until 96 h after treatment start. The nasal washes were obtained by instilling 2.5 ml of physiological saline solution in each nostril and then aspirating the nasal secretions with a silicone pipe connected to a vacuum pump. To ensure good viral conservation, the viral transport medium (Sigma Virocult®, Medical Wire & Equipment Co.) was aspirated and mixed with the nasal wash. Then, the mixture was kept at 4 °C during transport to the virological laboratory. Subsequent specimens consisted of nasal swabs (Virocult®, Medical Wire & Equipment Co) performed on days 5 and 7. We changed from nasal washes to nasal swabs to alleviate the sampling performed in patients; we first assumed that the viral load would be near zero on these days and nasal swabs were done to check for the negativation of viral load.

Baseline data included the patient's medical history and influenza symptoms. Follow-up data included the evolution of symptoms, and compliance to treatment (assessed by pill count by number of study days). A follow-up visit was performed on day 6 to assess a potential carry-over effect.

Data were collected on Case Report Forms by the investigators, and entered into a database using Clininfo SA software (Clininfo SA, 99 rue de Gerland, 69007 Lyon, France).

2.3. Laboratory procedures: virological analysis

Nasal washes and nasal swabs were added to a viral medium culture for a final volume of at least 1.5 ml. Then the samples were divided into aliquots and frozen at −80 °C. All the samples for an individual were then tested in a same assay run for quantification.

RNA was extracted from 200 μl of nasal wash without antibiotics using the automated NucliSens easyMAG system (Biomerieux). Elution of the extracted nucleic acids was performed in 70 μl.

Influenza A virus was detected and quantified using a real time reverse transcription quantitative polymerase chain reaction (rt RTqPCR) on the influenza A M gene as described previously (Duchamp et al., 2010). The results were expressed in $\log_{10}$ copies of RNA genome equivalent/μl of nasal wash or nasal swab (abbreviated