INFLUENZA PANDEMIC H1N1 AND OSELTAMIVIR-RESISTANCE: THE DILEMMA OF TO TREAT OR NOT TO TREAT

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ABSTRACT

Antiviral agents are considered as essentials for control of influenza outbreaks, but it is usually accepted that a massive treatment with antiviral medication should increase frequency of resistant viruses. Due to pandemic influence A, there is a great debate on physicians should prescribe (or not) oseltamivir on demand. We hypothesized that the equations on the origin and fate of drug resistant mutants from population genetics theory can give an unambiguous response to dilemma of to treat or not to treat with neuraminidase inhibitors (NI). During initial influenza outbreak (prior to generalised oseltamivir prescription) most of viruses are oseltamivir-sensitive wild type genotype T^s. However, oseltamivir-resistant genotypes (T^r) arose spontaneously by rare spontaneous mutations (not through specific adaptation in response to drug exposure). Under this oseltamivir-free environment, mutations from T^s to T^r occur recurrently, but T^r alleles are disadvantageous in fitness and a significantly number of these mutants is eliminated sooner or later by natural selection. Thus, an selection-mutation equilibrium is reached when the number of oseltamivir-resistant viruses originated by mutation is equal to the number of oseltamivir-resistant viruses eliminated by selection. Population genetics calculus demonstrates that, i) fitness of resistant viruses is almost similar to fitness of sensitive wild type, ii) Even under the most controlled employ of antivirals the frequency of oseltamivir-resistant viruses will be increased rapidly, and iii) Delays in application of treatments because they could increase frequency of oseltamivir -resistant viruses are not supported by population genetics theory. Epidemiological data on pandemic influenza A in Europe support these three recommendations. European countries where oseltamivir was widely used in promptly

start of treatments show significantly lesser amount of fatal cases per million than countries where oseltamivir treatments are not widely used

INTRODUCTION

The impact of new pandemic influence makes effective measures to control A(H1N1) virus infection a public health priority. On one hand, antiviral agents such as oseltamivir are considered as essentials for control of initial influenza outbreaks caused by new viruses, and many governments have stockpiled NI. On the other hand, it is currently accepted that a massive treatment with antiviral medication (in particular at low doses or short duration) could to increase frequency of oseltamivir -resistant viruses. Only low prevalence of oseltamivir-resistant viruses (<1%) had been detected in circulating viruses prior to generalised use of NI, but the frequency of oseltamivir-resistant viruses increased significantly after May 2008 (>11%) in areas where oseltamivir was widely prescribed [1]. Consequently, there is a great debate on physicians should prescribe (or not) oseltamivir on demand.

HYPOTHESIS

In order to add new knowledge about the dilemma of to treat or not to treat with NI, we make use of the population genetics theory of drug resistant mutants to formulate sound predictions on the origin and fate of oseltamivir-resistant viruses under diverse scenarios of antiviral drug prescription. The population genetics equations give an unambiguous response: even under the most controlled employ of NI the frequency of oseltamivir-resistant viruses will be increased rapidly. Based on these population genetics models we hypothesized that antiviral treatment must be generalised at a good number of population, initiated in patients as soon as possible and to include (if it is possible) the use of two antiviral drugs with different genetic-resistance mechanisms simultaneously (i.e. oseltamivir and zanamivir).

EVALUATION OF THE HIPOTHESIS

In seminal papers, Luria and Delbruck [2] and Lederberg and Lederberg [3] and dozen papers latter, unequivocally demonstrated that drug-resistant cells arose spontaneously by rare spontaneous single mutations that confer drug resistance usually before to drug exposure and not through direct and specific adaptation in response to drug exposure. Recently Hill et al. [4] show that oseltamivir resistance has arisen (as expected) by independent point mutations.

If we let the probability of oseltamivir-sensitive wild type genotipe $(T^s) = p$ and the probability for its oseltamivir-resistant mutant allele $(T^r) = q$, in any virus population $p + q = 1$. Oseltamivir-

resistant alleles *(T^r)* arise recurrently by forward mutation from oseltamivir sensitive wild type wild type alleles *(T^s)* at a mutation rate of u. Reverse mutation from T^r alleles to T^s also occurs at a reverse mutation rate of v. Consequently, a dynamic equilibrium is possible in the viral population when the gain of T^r alleles by forward mutation is the same that the loos of T^r by reverse mutation ($pu = qv$). The probability of oseltamivir-sensitive wild type genotipe in mutational equilibrium (p_e) is $p_e = v / (u+v)$. However, forward mutation is slow and reverse mutation is slower. As a result a mutational equilibrium needs thousand generations to be reached and in the practice natural selection as well as chance (genetic drift) are the pacemakers of gene change within populations [5, 6].

Since virus populations are enormous, genetic drift has imperceptible effects. However, diverse scenarios of natural selection (as result of to treat or not to treat with Tamiflu) will be examined. First of all we consider the absence of oseltamivir treatment. Most of viruses are oseltamivir-sensitive wild type genotipe T^s. Prior to oseltamivir prescription, less than 1% viruses were oseltamivir-resistant T^r [1]. If we let the fitness of oseltamivir-sensitive wild type genotipe $T^s = w_s$ and the fitness for its oseltamivir-resistant mutant allele $T^r) = w_r$, then in any virus population $w_s > w_r$ (in practice $w_s = 1$ and $w_r = (1\text{-}s)$ where s is the selection coefficient [5, 6]. Under this oseltamivir free environment, mutation from a normal wild-type oseltamivir-sensitive *(T^s)* to a oseltamivir-resistant mutant allele *(T^r)* occur recurrently, but the oseltamivir-resistant alleles are disadvantageous in fitness. Then new mutants arise in each generation, but a good number of these mutants are eliminated sooner or later by natural selection [6, 7]. Thus, at any one time there will be a certain number of oseltamivir-resistant viruses that are not yet eliminated. The balance between the mutation rate (u) and the rate of selective elimination (s) will determine the probability of such mutants (q). An selection-mutation equilibrium is reached when $q_e = u / (u + s)$ [7, 8]. In contrast with mutational equilibrium, needs few generations to be reached due to the high values of selection [5, 6]. Consequently, populations of influenza viruses were in selection-mutation equilibrium prior to antiviral treatment.

CONSEQUENCES OF THE HYPOTHESIS AND DISCUSSION

According to Hurt et al. [1] probability for its oseltamivir-resistant mutants is $q_e \approx 0.01$. If we assume an mutation rate in influenza viruses around 10^{-4} (influenza viruses has very high mutation rates, [9]) then the selection coefficient of oseltamivir-resistant viruses can be estimated in $s = 0.0099$.

This value is not trivial because it demonstrates that even in the absence of oseltamivir, fitness of resistant viruses is almost similar to fitness of sensitive wild type (0.9901 *vs* 1.0000). In contrast, in presence of oseltamivir fitness of wild type sensitive is $w_s \approx 0$, whereas fitness of resistant viruses

is $w_r = 1$. As a result, the frequency of oseltamivir resistant viruses will be increased rapidly even after a very restricted increase in use of oseltamivir. Epidemiological data support this prediction. The frequency of oseltamivir-resistant viruses increased significantly (from less of 1% to more than 11%) after May 2008 in areas where Tamiflu was prescribed [1]. An high proportion of oseltamivir-resistant influenza A viruses (around two thirds) emerged in Norway after a moderate use of Tamiflu [10]. Delays in application of treatments because they could increase frequency of oseltamivir -resistant viruses are not supported by population genetics theory. Even, oseltamivir has been detected at doses until 58 ng L^{-1} in rivers of areas where oseltamivir is being prescribed widely [11]. Therefore, natural reservoirs of influenza virus such as waterfowls are exposed to oseltamivir and could contribute to dissemination of resistant viruses. Nowadays, the probability for oseltamivir-resistant mutant allele $(T^r) = q$ in A(H1N1) virus population is high and probably become more high. However, a treatment with oseltamivir should contribute to reduce viral charge and probably infecting dose in some cases. It has been suggested that infecting doses could to explain different severity cases of influenza A(N1H1) [12] and demonstrated in chickenpox [13]. In addition, recent results shown that oseltamivir-resistant mutant viruses still are strongly inhibited by other neuraminidase inhibitors as zanamivir because resistance is conferred by different mutations [14]. Even assuming a high mutation rate for both oseltamivir-resistant and zanamivir-resistance (around 10^{-4} for each one), a treatment with both antiviral drugs give simultaneously, reduce probability of a double resistant (around 10^{-8}). In addition, fitness of the double resistant mutant (oseltamivir- and zanamivir-resistant) will be less than wild type or than oseltamivir-resistant. Consequently, propagation of double resistant in virus population will be slow.

Taken in account these population genetics principles, we propose that:

1. In patients antiviral treatment should be initiated as soon as possible.
2. In populations antiviral treatment should be generalised, if clinical cases it required, just at the start of pandemic influenza.
3. Influenza treatments must include combination the use of two antiviral drugs simultaneously, which have different genetic basis for resistance (i.e. oseltamivir and zanamivir).

Epidemiological data on pandemic influenza A in Europe seems to support these three recommendations. The European countries where more oseltamivir was prescribed and antivirals are available for sale show a significantly lesser amount of fatal cases (per million) than those of countries where oseltamivir treatments are not widely used (Table 1). For example, in Spain where oseltamivir treatments are reserved only for influenza A patients with radiologically confirmed neumonitis or severely affected hospitalised ones, 271 persons had died because of pandemic influenza (December, 31). It was notice in press that only about 6000 treatments of Tamiflu were

done at middle December [15]. In contrasts, in Germany, where the use of antivirals is more diffused, and with a population greater than Spain, only died 132 patients.

Independently of the severity of pandemic in these countries, possibly different across Europe, these evidences suggest than promptly start of treatment is perhaps the best protection factor for prevention of fatal cases. This therapeutical option could be decide promptly, avoiding a misuse, and attending at the profile of security of these drugs more than the eventuality of emergence of resistance for abuse.

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Table 1. Influenza pandemic mortality in Europe (December 31, 2009) and availability of antivirals*.

Country	Cumulative number of fatal cases	Population (mill.)	Mortality per mill.	Antiviral availability (Oseltamivir on demand)
France (Mainland F.)	198	65	3.05	Yes
Germany	132	82,6	1.6	Yes
Italy	188	60	3.13	Yes
Portugal	70	11,3	6.19	Yes
Spain**	271	46,6	5.82	No until November, 2009
United Kingdom	308	60,6	5.08	No until July, 2009

* Source ECDC
** Antivirals only for SARI or patients with risk factors. In practice, antivirals only for inpatients. Healthy patients with persistence or progression of symptoms are excluded of antiviral therapy in national and regional protocols. Infants below 5 years with ILI are also excluded of treatment.