Scientific Research and Essays Vol. 6(30), pp. 6364-6371, 9 December, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1693
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Effect of neutral mutation on HIV-1 drug resistance in highly active antiretroviral therapy

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Accepted 8 November, 2011

Human immunodeficiency virus 1 (HIV-1) drug resistance is a grave problem in highly active antiretroviral therapy (HAART) and neutral mutation is an important factor in the evolution of HIV-1. We consider the process of HIV-1 quasispecies transition in HAART to be transformation among three viral phenotypes: wild-type HIV-1, neutral-type HIV-1 and resistant-type HIV-1. A new model is proposed to study the transition dynamics of resistant-type HIV-1 induced by neutral mutation. The colonized time, the stable time and the stable size of the resistant species are simulated with the model. The results indicate that neutral mutation is closely related to the colonized pattern of resistant-type HIV-1 quasispecies which may lead to important strategies for predicting or checking HIV-1 drug resistance.

Key words: Human immunodeficiency virus -1, neutral mutation, drug resistance, highly active antiretroviral therapy, stability delay, stability.

INTRODUCTION

As highly active antiretroviral therapy (HAART) has become widely adopted, the prognosis is greatly improved in human immunodeficiency virus 1 (HIV-1) positive patients. However, drug resistance became a new obstacle since the advent of HAART. The nature of drug resistance is mutation of amino acid sequence and thus appearance of new phenotype (Jacquescoley, 2007), induced by nucleotide mutation in the region of polymerase and proteinase. In terms of viral ecology, the process of resistance is transition from the steady state of wild-type HIV-1 (before antiretroviral treatment) to the steady state of resistant-type HIV-1. Since the detection of drug-resistance genes is currently unavailable in most developing countries, researchers are looking for an easy and rapid way for predicting HIV-1 drug resistance in order to save human and material resources. Thus, it is important to study the transition mechanism of the steady states of HIV-1 quasispecies in HAART.

Many mathematical models have been proposed for a better understanding of the dynamics of HIV-1 drug resistance since 2000. Sevin et al. (2000) first investigated the relationship between drug-susceptibility phenotype and HIV-1 genotype by applying three different statistical methods---cluster analysis, recursive partitioning and linear discriminant analysis. Later, using a machine learning or regression approach, Beerenwinkel et al. (2002, 2003) obtained concise and easily interpretable models to predict drug resistance from sequence information. Then, two neural network models were constructed based on artificial intelligence (Wang et al., 2003). Furthermore, Wang et al. (2004) used standard stepwise linear regression to construct drug resistance models for seven protease inhibitors and ten reverse transcriptase inhibitors using data obtained from the Stanford HIV drug resistance database. However, these models show limitations in application such as high prediction error. Therefore, more biological information of drug resistance is needed for

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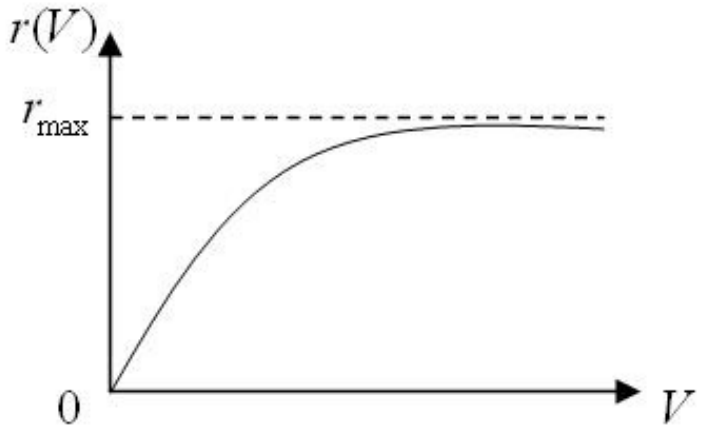

Figure 1. Illustration of the production function

constructing practical models.

During the course of a population adapting to its surroundings, the balance between the robustness and the evolvability of its genetic phenotype is a crucial factor. The robustness of genetic phenotype is the invariability of phenotype when the population is faced with a complex or disordered condition, and the evolvability of genetic phenotype is the adaptability of phenotype when the population is under selective pressure. Hu et al. (2009) believe that neutrality and variability are two aspects of evolvability in linear genetic programming. The former makes a system tolerant of mutations and provides a hidden staging ground for future phenotypic changes. The latter produces explorative variations with phenotypic improvements. Furthermore, Draghi et al. (2010) demonstrate that neutral diversity correlates with the adaptability of a population. When the phenotype neighbourhood is less than the fitness landscape, there is a non-monotonic relationship between the neutral mutation rate and the average adaptive time and the intermediate neutral mutation rate induces the shortest time of environmental adaptation. When the phenotype neighbourhood is equal to the fitness landscape, there is a monotonic increasing relationship between the neutral mutation rate and the adaptive time. These results indicate that the neutral mutation is an important factor in virus evolution, which can either impede or facilitate adaptation. Thus, it is necessary to combine the theory of neutral diversity with evolution of HIV-1 quasispecies in HAART.

In this paper inspired by Hu et al. (2009) and Draghi et al. (2010), we will propose a different mathematical model in terms of neutral diversity. The main purpose of our investigations is to find out the effect of neutral mutation in the process of drug resistance for HIV-1 positive patients in HAART and hope to quantize the colonized pattern of resistant-type HIV-1 quasispecies. Concretely, the colonized time, the stable time and the stable size of the resistant-type HIV-1 quasispecies are simulated with the model and the conditions under which the resistant-type HIV-1 completely replace wild-type HIV-1 are obtained which is useful in evaluating different drug therapy regimens during HIV-1 infection.

MATERIALS AND METHODS

Although many intricate models have been developed to simulate HIV dynamics within individual patients (Lou et al., 2004; Li and Ma, 2007; Cai and Li, 2009; Xiao, 2009; Li et al., 2011), the data obtained from the perturation experiments (such as those of drug therapy) may not support the models (Perelson and Nelson, 1999). The following simple model may be more proper to simulate HIV dynamics which is formulated by:

$$\frac{dV}{dt} = r(V) - dV$$

Where $r(V)$ is an undetermined function representing the rate of virus production, d is the clearance rate constant and V is the virus concentration. Clearly, $r(V) = 0$ if the virus is absent and it will gradually increase with the increase of the virus concentration. However, due to the limited number of the susceptible cells and the limitation of the immune response, $r(V)$ will remain at a constant level when the virus concentration is sufficiently large. This means that $r(V)$ will grow in the following pattern (Figure 1).

According to Kribs-Zaleta (2004, 2009), we take the following general saturation function

$$r(V) = r_{\max}\left(\frac{V^n}{A^n + \mathrm{V}^n}\right)^{1/n}$$

to mimic the production function of virus, where $r_{\max}$ is the maxmum of the virus production rate. When $n = 1$, $r(V)$ is the Verhulst (Holling type II) function, A is the corresponding half-

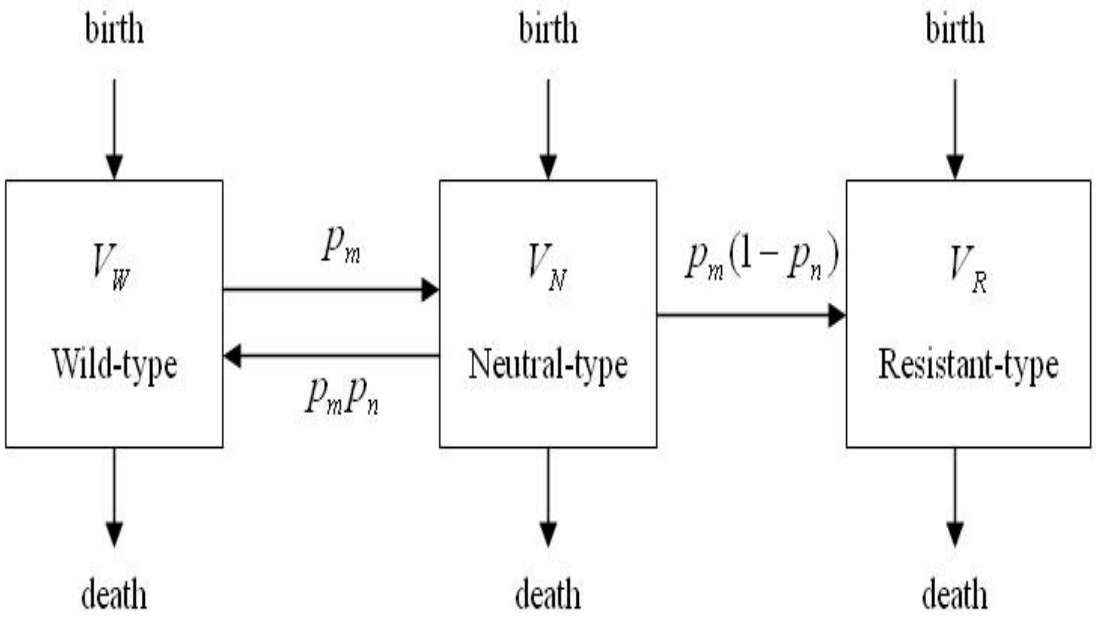

Figure 2. Model dynamics flow-chart.

saturation concentration, that is $r(A) = r_{\max}/2$. Note that the production function $r(V)$ saturates ever more sharply as n increases with the saturation approaching Holling type I as $n \to \infty$. That is, n forms an abstract sharpness measure; high values of n imply that saturation occurs suddenly rather than gradually near the saturation point. For mathematical simplicity we consider only $n = 1$. Therefore, HIV-1 dynamics within individual patients can be described as

$$\frac{dV}{dt} = \frac{r_{\max} V}{A+V} - dV \tag{1}$$

For Model 1, it is easy to know that there are two steady states, $V^* = 0$ (unstable) and $V^* = (r_{\max} - dA)/d$ (stable). Because of the meaning of biology, in the rest of this paper we assume that $A < r_{\max}/d$, that is, the half-saturation concentration is always less than the maximum viral load.

Since virus mutation exists in HAART, according to Draghi et al. (2010), we think that there are three viral phenotypes: wild-type HIV-1, neutral-type HIV-1 and resistant-type HIV-1. Each virus may mutate and each mutation produces a unique genotype. Neutral-type HIV-1 is the intermediate product between the wild-type and the resistant-type virus. Resistant-type HIV-1 must be generated from mutation of the neutral-type and there is transformation between the wild-type and the neutral-type. We assume that a mutation occurs with probability p_m (general mutation rate) and the transition probability from the neutral-type to the wild-type is p_n (neutral mutation rate). Thus, the viral dynamics in HAART can be illustrated by Figure 2. Suppose that the dynamics of each type of HIV-1 follows Model 1. Based on Figure 2, we can formulate the model of HIV-1 mutation dynamics in HAART as the following system of equations:

$$\begin{cases} \dfrac{dV_W}{dt} = \dfrac{r_W V_W}{A_W + V_W} - (d_W + p_m)V_W + p_m p_n V_N, \\ \dfrac{dV_N}{dt} = \dfrac{r_N V_N}{A_N + V_N} - (d_N + p_m)V_N + p_m V_W, \\ \dfrac{dV_R}{dt} = \dfrac{r_R V_R}{A_R + V_R} - d_R V_R + p_m(1-p_n)V_N. \end{cases} \tag{2}$$

Here, r_W, r_N and r_R are the maximum production rate of wild-type HIV-1, neutral-type HIV-1 and resistant-type HIV-1 respectively. A_W, A_N and A_R represent their corresponding half-saturation concentration. d_W, d_N and d_R are their clearance rate constant. p_m and p_n are the general mutation rate and neutral mutation rate. Because of the meaning of biology, all parameters are nonnegative constants. Since both the wild-type and the neutral-type are susceptible to drug, we further assume that $r_W = r_N = r$, $A_W = A_N = A$ and $d_W = d_N = d$ for simplicity.

In the study of the transmission dynamics of resistant-type HIV-1, our focus is on the colonized pattern of resistant-type HIV-1 quasispecies, including the colonized time, the stable time and the stable size of resistant species. Here the colonized time is defined as the time when $V_R \geq 10$, the stable time and the stable size are defined as the time and the size when V_R remains unchanged

Table 1. The sign of $\varphi'_1(V_W), \varphi'_2(V_N)$.

Conditions	Results
$\frac{B_2}{B_1} < A < \frac{r}{d}$	$\varphi'_1(V_W) > 0, \varphi'_2(V_N) > 0$
$A < \frac{B_2}{B_1}, V_W > \sqrt{\frac{B_2 A}{B_1}} - A, V_N > \sqrt{\frac{B_2 A}{B_1}} - A$	$\varphi'_1(V_W) > 0, \varphi'_2(V_N) > 0$
$A < \frac{B_2}{B_1}, 0 < V_W < \sqrt{\frac{B_2 A}{B_1}} - A, 0 < V_N < \sqrt{\frac{B_2 A}{B_1}} - A$	$\varphi'_1(V_W) < 0, \varphi'_2(V_N) < 0$

respectively. Thus, starting from the wild-type population in steady state, we take the initial conditions as an environmental shift time, that is

$$V_W(0) = V_{W0}, V_N(0) = V_R(0) = 0 \tag{3}$$

RESULTS

Standard and simple arguments show that solution of system (2) under initial conditions (3) always exists, and stay positive and bounded.

Existence and stability of equilibria

Because V_R appears only in the third equation of Model 2, we can reduce the system as follows:

$$\begin{cases} \frac{dV_W}{dt} = \frac{rV_W}{A+V_W} - (d+p_m)V_W + p_m p_n V_N \equiv F_1(V_W, V_N), \\ \frac{dV_N}{dt} = \frac{rV_N}{A+V_N} - (d+p_m)V_N + p_m V_W \equiv F_2(V_W, V_N). \end{cases} \tag{4}$$

For (4), the extinct equilibrium $E_0^{'} = (0,0)$ always exists. In order to find possible nonnegative equilibria, we let $F_1(V_W, V_N) = 0, F_2(V_W, V_N) = 0$, and obtain:

$$\begin{cases} V_N = \frac{B_1}{p_n} V_W - \frac{B_2 V_W}{p_n(A+V_W)} \equiv \varphi_1(V_W), \\ V_W = B_1 V_N - \frac{B_2 V_N}{A+V_N} \equiv \varphi_2(V_N), \end{cases}$$

where $B_1 = (d+p_m)/p_m, B_2 = r/p_m$. Note that:

$$\varphi'_1(V_W) = \frac{B_1(A+V_W)^2 - B_2 A}{p_n(A+V_W)^2},$$
$$\varphi'_2(V_N) = \frac{B_1(A+V_N)^2 - B_2 A}{(A+V_N)^2}.$$

After calculation, we obtain the sign of $\varphi'_1(V_W), \varphi'_2(V_N)$, which is listed in Table 1. Furthermore, let

$$k_1 = \varphi_1^{'}(0) = \frac{B_1 A - B_2}{p_n A},$$
$$k_2 = \varphi_2^{'}(0) = \frac{B_1 A - B_2}{A}.$$

According to Table 1, when $A > B_2/B_1$, there is a unique extinct equilibrium $E_0^{'} = (0,0)$ in system (4) if $k_1 \geq 1/k_2$ (Figure 3A); otherwise, there is a coexisting equilibrium $E_+^{'} = (V_W^*, V_N^*)$ besides the extinct equilibrium $E_0^{'}$ (Figure 3B). When $A < B_2/B_1$, $E_0^{'}$ and $E_+^{'}$ always exist (Figure 3C). To formulate the global stability of $E_0^{'}$ and $E_+^{'}$, we need the following lemma.

Lemma

There is no limit cycle in system (4).

Proof: Take a Dulac function $D = 1/(rV_W V_N)$. We have:

$$\frac{\partial(DF_1)}{\partial V_W} + \frac{\partial(DF_2)}{\partial V_N} = -\frac{1}{V_N(A+V_W)^2} - \frac{p_m p_n}{rV_W^2} - \frac{1}{V_W(A+V_N)^2} - \frac{p_m}{rV_N^2},$$

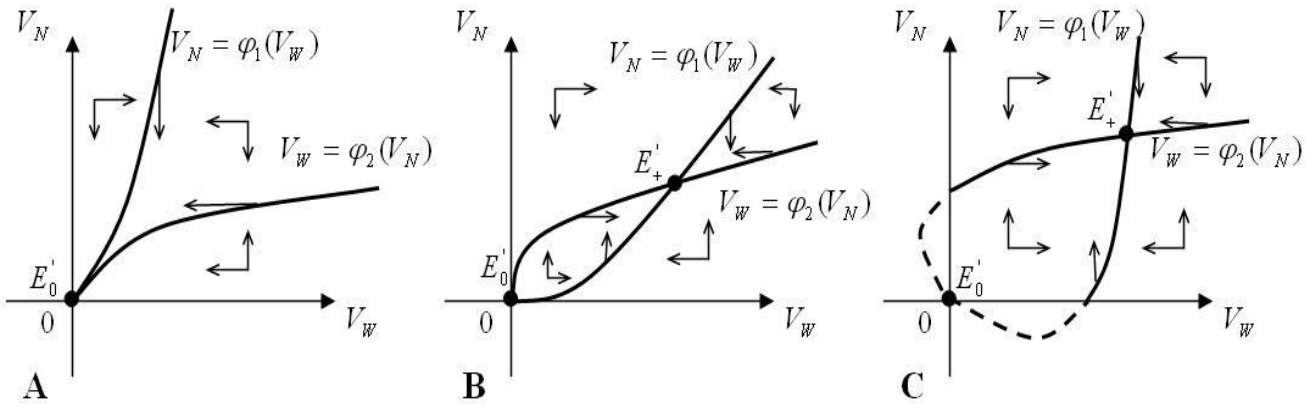

Figure 3. An illustration for the plane phase diagram of system (4). A) $\frac{r}{d+p_m} < A < \frac{r}{d}$ and $k_1 \geq 1/k_2$; B) $\frac{r}{d+p_m} < A < \frac{r}{d}$ and $k_1 < 1/k_2$; and C) $A < \frac{r}{d+p_m}$.

which is negative because V_W, V_N stay positive. This means there is no limit cycle for (4) in the positive cone based on the Bendixson-Dulac theorem.

The combination of Lemma and the plane phase diagram (Figure 3) of system (4) yields the following results.

Theorem 1

For system (4), the following results are true:

(i) The unique extinct equilibrium $E_0^{'}$ is globally asymptotical stable if $p_m > (r - Ad)/A$ and $p_n < ((Ad + Ap_m - r)/Ap_m)^2$ (case A);

(ii) $E_0^{'}$ is unstable and $E_+^{'}$ is globally asymptotical stable if $p_m > (r - Ad)/A$ and $p_n > ((Ad + Ap_m - r)/Ap_m)^2$ (case B) or $p_m < (r - Ad)/A$ (case C).

Now, for Model 2, we can obtain the following results.

Theorem 2

For system (2), the following results are true:

(i) The trivial steady state $E_0 = (0,0,0)$ always exists and is unstable;

(ii) There is a unique boundary equilibrium $E_R = (0,0,(r_R - d_R A_R)/d_R)$ and it is globally asymptotical stable if $p_m > (r - Ad)/A$ and $p_n < ((Ad + Ap_m - r)/Ap_m)^2$ (case A);

(iii) E_R is unstable, and a unique positive equilibrium $E_+ = (V_W^*, V_N^*, V_R^*)$ appears and is globally asymptotical stable if $p_m > (r - Ad)/A$ and $p_n > ((Ad + Ap_m - r)/Ap_m)^2$ (case B) or $p_m < (r - Ad)/A$ (case C).

Proof: The existence of E_0 is clear. The Jacobian matrix of system (2) evaluated at E_0 is given by

$$J_{E_0} = \begin{bmatrix} \frac{r}{A} - d - p_m & p_m p_n & 0 \\ p_m & \frac{r}{A} - d - p_m & 0 \\ 0 & p_m(1-p_n) & \frac{r_R}{A_R} - d_R \end{bmatrix}$$

Note: $A_R < r_R/d_R$. We have $r_R/A_R - d_R > 0$ and thus E_0 is always unstable based on the Routh-Hurwitz criterion.

When $p_m > (r - Ad)/A$ and $p_n < ((Ad + Ap_m - r)/Ap_m)^2$, Theorem 1 shows that

$V_N(t) \to 0$ as $t \to +\infty$. Using Markus theorem (Hsu et al., 1978), we know the limit equation of the third equation of Model 2 is

$$\frac{dV_R}{dt} = \frac{r_R V_R}{A_R + V_R} - d_R V_R$$

as $t \to +\infty$. Based on this limit equation, combining Theorem 1 and the result that E_0 is always unstable, we can conclude that equilibrium E_R is globally asymptotical stable in this case.

When $p_m > (r - Ad)/A$ and $p_n > ((Ad + Ap_m - r)/Ap_m)^2$ or $p_m < (r - Ad)/A$, Theorem 1 shows that $V_N(t) \to V_N^*$ as $t \to +\infty$. Using Markus theorem (Hsu et al., 1978) again, we know the limit equation of the third equation of Model 2 is

$$\frac{dV_R}{dt} = \frac{r_R V_R}{A_R + V_R} - d_R V_R + p_m(1 - p_n)V_N^*$$

as $t \to +\infty$. For this limit equation, it is clearly to obtain a positive steady state V_R^* exists and is stable. Thus, E_+ appears and is globally asymptotical stable in these cases. The proof is completed.

Numerical simulations

In this subsection, we will use numerical simulations to verify the afore-mentioned analytical results and explore the colonized pattern of resistant-type HIV-1 quasispecies with respect to different values of p_m, p_n including the colonized time, the stable time and the stable size of resistant species. First, we select the following dimensionless parameter values:

$$r_W = r_N = r_R = 100, A_W = A_N = 200, A_R = 100, d_W = d_N = 0.1, d_R = 0.08. \quad (5)$$

Based on Theorem 2, we take $p_m = 0.5, p_n = 0.02$ and then the conditions of case A are satisfied. Thus, resistant-type HIV-1 will completely replace wild-type HIV-1 which is illustrated in Figure 4A. Take $p_n = 0.2$. If $p_m = 0.5$ or $p_m = 0.3$, then the conditions of case B or C are satisfied. This means that all HIV-1 quasispecies will tend to a coexisting steady state which is illustrated in Figure 4B or C.

Next, when the neutral mutation rate p_n is changed from 0.01 to 0.99, we will study the colonized pattern of resistant-type HIV-1 under different general mutation rate ($p_m = 0.5$ in Figure 5A and $p_m = 0.3$ in Figure 5B).

The first panel of Figure 5A and B shows that there is a convex line for the stable size of resistant species in the main which means that the intermediate neutral mutation rate corresponds to the largest stable size of resistant species despite the value of the general mutation rate. However, the small general mutation rate corresponds to the early peak for the neutral mutation rate. Since the conditions of case A in Theorem 2 are satisfied when the neutral mutation rate is sufficiently small, there is a fixed stable size in the first panel of Figure 5A which also verifies the analytical result. The second panel of Figure 5A and B indicates that there is a different pattern for the stable time of the resistant species. When the general mutation rate is large, the stable time of the resistant species increases if the conditions of case A are satisfied. After that, it first decreases and then increases as the neutral mutation rate increases in the main. Otherwise, when the general mutation rate is small, the stable time of the resistant species increases constantly as the neutral mutation rate increases in the main though fluctuations and ladders appear. The third panel of Figure 5A and B shows that the colonized time of the resistant species increases slowly when the neutral mutation rate is small and then increases fast when the neutral mutation rate is large despite the value of the general mutation rate.

DISCUSSION

Note that the complexity of HIV-1 drug resistance in HAART. We introduce a novel mathematical model to study the transition dynamics of resistant-type HIV-1 induced by neutral mutation. Based on the theoretical analysis and numerical simulations, we find the following predictive conclusions: 1) From Theorem 2, we know that resistant-type HIV-1 will completely replace wild-type HIV-1 if the general mutation rate is sufficiently large ($p_m > (r - Ad)/A$) and the neutral mutation rate is sufficiently small ($p_n < ((Ad + Ap_m - r)/Ap_m)^2$). Otherwise, all types of HIV-1 will coexist within the host. Thus, once mutation occurs, the resistant-type will certainly colonize in patients and the general mutation rate and neutral mutation rate will affect the pattern of colonization. 2) From Figure 5 we can conclude that the balance between the robustness and the evolvability of the genetic phenotype of HIV-1 is a crucial factor for invasive mutant species to adapt to its surroundings. The neutral mutation rate is closely related to the colonized time, the stable time and the stable size of resistant HIV-1 species which can either impede or facilitate adaptation. These results coincide with the main results in Draghi et al. (2010).

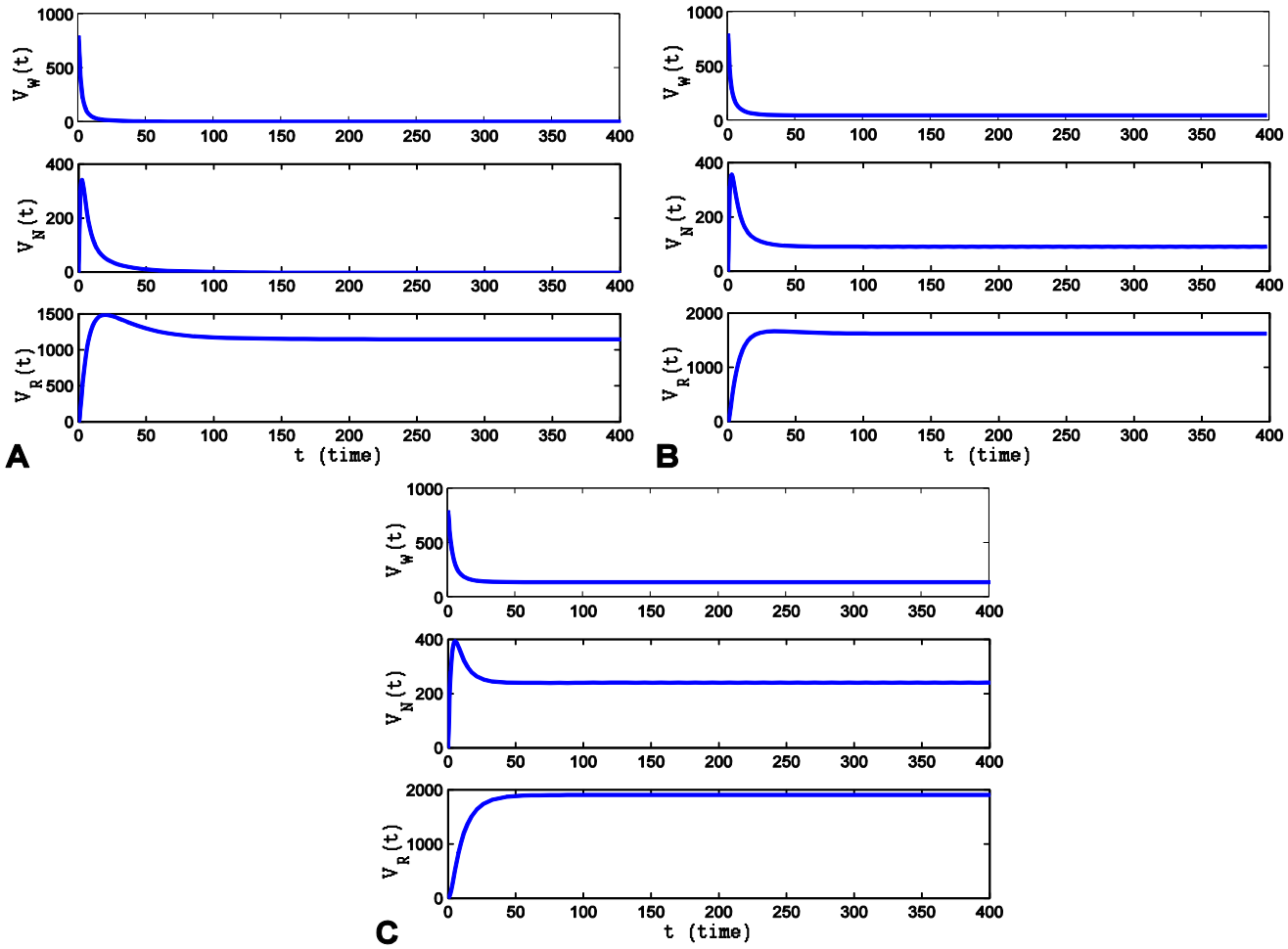

Figure 4. Numerical solutions of Model 2. Here, A) $p_m = 0.5, p_n = 0.02$, B) $p_m = 0.5, p_n = 0.2$, C) $p_m = 0.3, p_n = 0.2$ and other parameters are listed in (5). The initial conditions are $(V_W(0), V_N(0), V_R(0)) = (800,0,0)$.

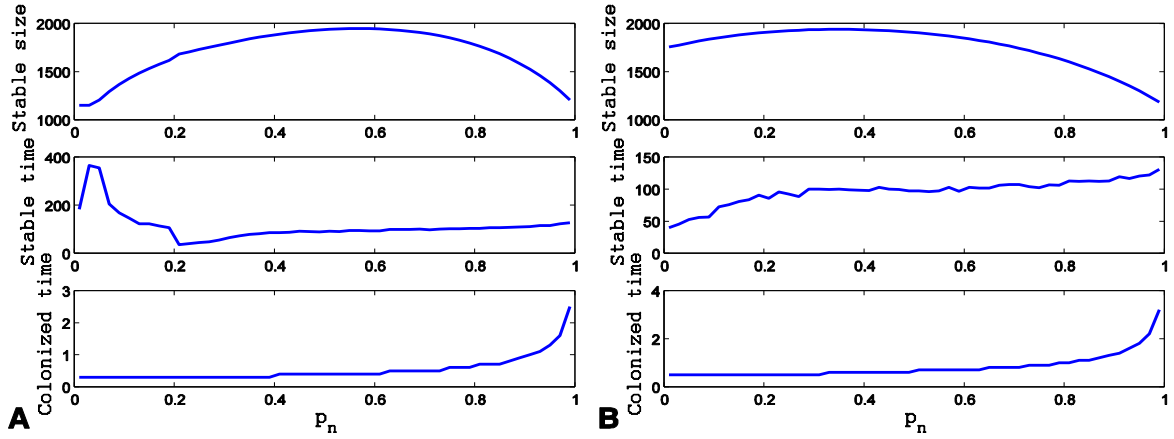

Figure 5. Illustration of the colonized pattern of resistant-type HIV-1. Here, $p_m = 0.5$ in (A), $p_m = 0.3$ in (B) and other parameters are the same as in Figure 4.

Since mathematical models can at best approximate the behavior of real biological systems, the results presented here extend those findings to virulence evolution of HIV-1 in its hosts and maybe it is useful to allow clinician to make more accurate and reliable inferences in HAART. However, since there is no experimental data at present, the parameter values in our simulations are artificial. This is what we shall do in future work to overcome the limitaton.

ACKNOWLEDGEMENTS

This work is supported by the National Natural Science Fund of P. R. China (No. 30770555) and the Natural Science Foundation Project of CQ CSTC (2010BB5020).

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