Journal of Theoretical Biology 385 (2015) 66–76

Contents lists available at ScienceDirect

Journal of Theoretical Biology

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

Mathematical modeling of fungal infection in immune compromised individuals: The effect of back mutation on drug treatment

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HIGHLIGHTS

- Fungal infection in an immune compromise host is modeled.
- Resistance to anti-fungal agents and back mutation is considered.
- Administering minimum effective dose is crucial.
- Model suggests back mutation should not affect clinical decisions.

ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form
18 August 2015
Accepted 23 August 2015
Available online 2 September 2015

Keywords:
Susceptible strains
Resistant strains
Fungus
Bifurcations
Stability

ABSTRACT

We present a mathematical model that describes treatment of a fungal infection in an immune compromised patient in which both susceptible and resistant strains are present with a mutation allowing the susceptible strain to become resistant as well as a back mutation allowing resistant fungus to again become susceptible. The resulting nonlinear differential equations model the biological outcome, in terms of strain growth and cell number, when an individual is treated with a fungicidal or fungistatic drug. The model demonstrates that under any levels of the drug both strains will be in stable co-existence and high levels of treatment will never completely eradicate the susceptible strain. A modified model is then described in which the drug is changed to one in which both strains are susceptible, and subsequently, at the appropriate level of treatment, complete eradication of both fungal strains ensues. We discuss the model and implications for treatment options within the context of an immune compromised patient.

1. Introduction

In this paper we model host–pathogen interactions with a forward and backward mutation. We consider the host as an immune compromised patient and consider a general case of a fungal infection. This type of scenario could describe fungal growth in the blood stream in the absence and presence of a fungicidal or fungistatic treatment. This model simulates fungal growth when a subpopulation of the fungus has developed a spontaneous mutation that now allows the fungus to be resistant to the treatment as well as a spontaneous back mutation whereby the resistant fungus again becomes susceptible to treatment. We also consider fungal growth when the treatment is changed such that both populations of fungus are now sensitive to the new pharmaceutical.

This model describes systemic infection with a generalized fungus. The filamentous fungi, *Candida albicans*, is found in normal human skin flora and is the most common strain of pathogenic fungus in humans. *C. albicans* is best known among the general populace as the fungus that causes thrush and topical Candidiasis. These diseases are easily treated topically with drugs such as fluconazole. Additionally, *C. albicans* can spread internally to the lungs or bloodstream. In normal healthy individuals, the immune system can keep this fungus in check and clear it from the bloodstream or lungs. However, when the environment in which *C. albicans* is living is altered, it can spread into a large-scale and sometimes life-threatening infection. Additional fungal systemic infections can be caused by other *Candida* species as well as other fungal genus such as *Apergillus* (Fernandez et al., 2009; Gallien et al., 2008). In immune compromised individuals, such as individuals treated with chemotherapy for cancer, those treated with immunosuppressant drugs to combat organ rejection after

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http://dx.doi.org/10.1016/j.jtbi.2015.08.017
0022-5193/

transplant, or those with acquired immunodeficiency syndrome, the immune system is not capable of clearing a fungal infection (Netea et al., 2008). Instead, the immune system may be able to keep the level of fungus suppressed far enough such that an individual can function and does not show symptoms of a full blown systemic fungal infection. However, at some tipping point, which may be a decrease in immune function or an increase of the fungus cell number, an individual develops a systemic fungal infection. Alternatively, the immune system in an immune compromised individual may not be able to mount any viable fight against a fungal pathogen and then the fungus would quickly multiply out of control.

Eradication of fungal infections is difficult because the fungus is eukaryotic and cannot be treated with conventional antibiotics, which kill bacteria. Thus, most treatments that will kill this eukaryote also will kill the human host cells. Luckily, there are two major differences between fungal cells and its human host, which can be exploited for therapy for fungal infections. In addition, there are two general types of drugs—fungicidal and fungistatic. Fungicidal drugs actually kill the fungus whereas fungistatic drugs prevent the fungus from dividing further.

One effective mechanism of treating fungal infections is through targeting a membrane constituent found only in fungus, ergosterol. Humans, on the other hand, have cholesterol in their membranes, and so targeting ergosterol or ergosterol biosynthesis is a specific treatment for fungal infection. Fluconazole inhibits the pathway for synthesizing ergosterol by inhibiting the fungal cytochrome P450 enzymes (Ghannoum and Rice, 1999). Ergosterol is a multi-ringed hydrophobic molecule similar in structure to cholesterol, is the major sterol in the fungal membrane, and is required for increased fluidity of fungus membranes; when the synthesis of this molecule is disrupted by fluconazole, precursor molecules of 14-alpha-sterol build up and change the membrane fluidity of the plasma membrane, inhibiting many plasma membrane enzymes (Ghannoum and Rice, 1999). Although fluconazole is readily absorbed in the human gasterointestinal tract, the human P450 enzyme is much less sensitive to fluconazole than the fungal version is, so usually a level of fluconazole that is needed for treatment but is not harmful to the patient can be found (Rex et al., 1995). Thus, the first treatment of a fungal infection usually utilized is fluconazole. Fluconazole is relatively well tolerated and shows few side effects, but these can include potentially deadly negative drug interactions, because fluconazole can inhibit human P450 enzymes as well (Ghannoum and Rice, 1999). Additionally, many cases of resistance have been seen to fluconazole and thus we model resistance in this paper (Rex et al., 1995). These are often seen in immune compromised patients because these patients often are treated prophylactically for months or years (Lawa et al., 1994). Moreover, *Aspergillis* infections do not respond to fluconazole at all, although there are other more broad spectrum triazole anti-fungal drugs such as itraconazole and voriconazole that *Aspergillis* species are often sensitive to Gallien et al. (2008). Additionally, other triazole anti-fungals suffer from the same resistance issues as fluconazole and some species of *Candida* such as *Candida tropicalis* are less sensitive to the triazole drugs (Gallien et al., 2008; Niimi et al., 1999).

When resistance is observed or suspected, other non-triazole, antifungals that target ergosterol are routinely used, especially the fungicidal drug, Amphoterican B. Amphoterican B also targets the ergosterol in fungus plasma membranes. However this drug binds to the ergosterol in the membranes and causes the membrane to develop holes, through which the cytosol leaks out (Ghannoum and Rice, 1999). Amphoterican B is not absorbed in the gut and must be given intravenously in a hospital, as it readily damages internal organs (Ghannoum and Rice, 1999). Resistance to Amphoterican B is much more rare (for example Lawa et al., 1994). However, it does occur and is attributed to the lack of ergosterol in the membrane (for example Nolte et al., 1997). The loss of ergosterol in the membrane is due to mutations in many genes, such as ergosterol biosynthesis pathway enzymes (for example see Kelly et al., 1997).

The other difference between fungal cells and human cells, which can be exploited for therapeutic means, is that fungal cells have a cell wall that helps to regulate cellular osmolarity (Chaffin et al., 1998) and human cells do not. A relatively new fungicidal treatment family for systemic fungal infections is the echinocandin family of drugs such as Micafungin and Caspofungin (Morrison, 2006). This class of drugs inhibits fungal cell wall 1-3-beta glucan biosynthesis thereby causing the fungus to lose its cell wall, triggering rapid lysis in susceptible strains (Deresinski and Stevens, 2003). There have been very few reports of resistance to this antifungal; however, a strain of resistant *Candida* was isolated, which had a point mutation in the 1-3-beta-D-glucan synthase gene (Baixench et al., 2007).

Resistance to antimicrobial therapy by the organism is a normal consequence to antimicrobial therapy and is seen during the treatment of single celled parasites such as those that cause malaria, to fungal infections, to prokaryotic infections by bacteria (Cohen and Tartasky, 1997). Resistance is often caused by random mutation and then selective pressure leading to a population of cells that can now somehow combat the actions of the antimicrobial therapy. Our model does not describe the resistance mechanisms, just that it exists in the general population of fungus before, during and after the treatment and that this resistance is passed down to daughter cells. Resistance can be caused by other mechanisms we do not model here (for example Cannon et al., 2009; White et al., 1998).

Just as forward mutations (such as the mutation of a susceptible strain to a resistant strain) are random events, reversion or back mutation, in which the original trait is restored, are also random events. DNA is constantly being mutated by many processes such as by errors in replication; small molecules, such as oxidants, made by the cells themselves; and UV light. Each cell or strain has a particular mutation rate, usually one in a few hundred thousand to a million or more, which describes how often any given nucleotide might be mutated. Thus, with a large enough population, random mutation can cause back mutation or reversion, and the original trait or phenotype can be restored, such as is seen in Kirsch et al. (1999). Additionally *Candida* are generally diploid, and a forward mutation that causes resistance can be a simple case of up-regulation of a transporter; this phenotype will often revert back to wild type under the appropriate conditions (Claudino and Peixoto, 2009). Therefore we see at a reasonable rate *Candida* strains once resistant to antifungals reverting back to a susceptible strain.

In our previous work (Camacho et al., 2011), we had explored the implications of a fungal strain mutating to a resistant strain and had described the consequences of this to the treatment of systemic infection in an immunocompromised patient. We now add back mutation to the model to determine the physiological consequences of this new DNA mutation. Resistant strains are not necessarily the most biologically fit in a system, as the resistance mutation leads to additional pleiotropic phenotypes. Resistance to anti-fungal pharmaceuticals leads to additional consequences in the cells that are not necessarily conducive to long term survival in the host, such as growth inhibition under different conditions (Anderson et al., 2003), cells that grow more slowly without the drug (Gerstein et al., 2012), cells that are more sensitive to stress (Gerstein et al., 2012), and cells with complex biological limitations, including increased levels of Hsp90 (indicating internal cellular stress), high sensitivity to external stress, and deficiencies in their ability to form filaments and invade tissues (Vincent et al.,