Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Pre-clinical *in vitro* infection models

George L Drusano

Given the current magnitude of the problem of antimicrobial resistance, particularly in the hospital setting, getting new agents to patients at the right dose and schedule is incredibly important. *In vitro* systems such as the 1-compartment model and the hollow fiber infection model (HFIM) can provide valuable information that allows rational decisions to be made which will drive the best choices of dose and schedule. Studies identifying the dynamically-linked index (dose fractionation studies) and the size of the index to obtain a specific amount of bacterial kill can be performed in both systems. Studies to examine dosing regimens that will help suppress resistance emergence are best done in the HFIM.

Address
Institute for Therapeutic Innovation, University of Florida, USA

Corresponding author: Drusano, George L (gdrusano@ufl.edu)

Current Opinion in Pharmacology 2017, **36**:100–106

This review comes from a themed issue on **Anti-infectives**

Edited by **John Rex** and **Sujata Bhavnani**

http://dx.doi.org/10.1016/j.coph.2017.09.011

As we are in the midst of a crisis of antimicrobial resistance, the rapid, safe and efficacious development of new antibiotics is a critical issue. Both the Food and Drug Administration in the United States and the European Medicines Agency for the European Union have recognized the gravity of the situation and have provided a path forward for drug developers, particularly for agents with limited spectra of activity [1,2]. The FDA has had a series of Workshops on this topic and the National Institute for Allergy and Infectious Diseases has had a Workshop specifically devoted to the use of pharmacodynamics approaches for new drug development [3].

In the pre-clinical arena, there are two major areas of investigation where information necessary for new drug development can be generated. These are *in vitro* pharmacodynamic experiments and animal model experiments. In this paper, we will limit the discussion to the former.

There are multiple types of *in vitro* pharmacodynamic systems. Some were described in the early 1970s [4]. Because of space limitations only so-called chemostat-type or 1-compartment systems and the hollow fiber infection model (HFIM) will be examined. The utility of each system, its strengths and weaknesses and the therapeutic question each is most suited to address will be discussed.

In order to understand which system to use for a specific purpose, it is critical to know what pieces of information will allow antibiotic development programs to be de-risked.

What do we need to know?

There are three pieces of information regarding an antimicrobial that allow identification of the proper dose and schedule of drug. These are: first, the delineation of the pharmacodynamically-linked index for bacterial cell kill, second, the relationship between exposure (size of the dynamically-linked index) and the ability to kill bacteria and third, the amount of drug exposure which will counter-select amplification of resistant mutant subpopulations. For the latter, it is important to realize that the dynamically-linked index for bacterial cell kill may be different from the index for resistance suppression.

Once these pieces of data have been identified, it is relatively straightforward to choose an optimal dose and schedule of drug to obtain the best clinical outcomes and to keep the drug clinically useful by suppressing resistance. The dynamically-linked indices drive the choice of dosing interval. For instance, if C_{min}/MIC ratio or time > MIC is the dynamically-linked index, then a relatively short dosing interval will optimize bacterial cell kill. Likewise, if AUC/MIC ratio is best linked, then dosing interval has less impact on outcome (as long the dosing interval does not exceed 24 hours). Once the relationship between the size of the index and either bacterial cell kill or resistance suppression is known, one can simply use a population pharmacokinetic model along with Monte Carlo simulation to determine what fraction of the time a specific drug dose will allow target attainment (e.g. attaining an AUC/MIC ratio of 100 for a stasis endpoint). Different clinical indications will require different targets and, hence, may require different drug doses. Once these pieces of data are identified, the development process can be de-risked.

Chemostat (1-compartment) system versus the hollow fiber infection model

There are advantages and disadvantages for each of the systems. Recognizing the advantages and limitations of each system will drive the choice of the system for

generation of the *in vitro* data that is desired. There is no question that can be posed and answered in the chemostat system that the HFIM cannot. Why, then have the chemostat system? The answer is straightforward: expense. The HFIM is an expensive system to run, whereas costs in the chemostat system are relatively modest. This begs the question of why such an expensive system should be employed. The answer lies in how sure we are of the answer and how frequently we have to perform the experiment.

Limitations to the chemostat (1-compartment) system

The first issue that should be addressed is one of system dilution. The 1-compartment system dilutes the drug so that the correct 'half-life' can be achieved. However, to attain the correct half-life, the end point (bacterial burden) is also diluted. Consequently, the observed effect can be biased. However, the magnitude of the bias is influenced by the half-life of the drug that is being simulated. An agent with a one-hour terminal half-life (e.g. a Type-2 carbapenem such as imipenem, meropenem or doripenem) will have a substantial impact on bias relative to a drug with a longer half-life (note that 69.3% of the system volume needs to be replaced per hour to obtain a one hour half-life). As half-life lengthens, the impact on bias lessens. Actually, there is a mathematical correction for the dilution introduced [5]. However, this correction has been very rarely, if ever, employed in recent years.

The other issue regarding dilution is that it is not specific with regard to the bacterial population. Less-susceptible subpopulations are lost along with susceptible populations. Some agents (e.g. fluoroquinolones) induce error-prone replication, which is fully stochastic [6]. The time of production of a resistant isolate is unknown. Depending on the dilution rate, the less-susceptible organism may be washed out and suboptimal information regarding resistance will be generated from the experiment.

Yet another issue with the 1-compartment system relates to the formation of microcolonies on the walls of the system. This has been described by Haag *et al.* [7]. This can alter the conclusions to be drawn from the experiment. It is possible that this may also occur with the HFIM, but has not been adequately described. Both of the issues noted above (dilution and adherent bacteria) have been well described in an analysis by Keil and Wiedermann [8].

Finally, as a generalization, the 1-compartment system is somewhat time-delimited. Most laboratories generate data over a period of one to two days. There have been published data that demonstrate longer experimental times [9], but these reports are less common. Also, there are variants of this system that commonly go for longer periods. An example is the endocardial vegetation model developed by the laboratory of Rybak *et al.* [10]. As an aside, this model would likely be more reflective of therapy of endocarditis if there was a longer period of time when the simulated vegetations were allowed to exist without therapy before the introduction of drug. The period where there was no drug would allow a closer representation to true endocarditis vegetations. This was highlighted in this reference in that there was a major decrease in daptomycin bactericidal activity with the lag period before drug introduction.

The length of the experiment can be a crucial issue, particularly for the delineation of issues regarding resistance emergence. Sometimes (most frequently) if the bacterial burden is large enough, there will be pre-existing less-susceptible organism subpopulations. This depends upon whether the total bacterial burden exceeds the inverse of the mutational frequency to resistance. As a caveat, however, this number will depend heavily upon the drug concentration in the resistance subpopulation-selecting plate. As a rule of thumb, the selecting drug concentration should be just below the MIC change for the first resistance mechanism (e.g. efflux pump over-expression, porin down-regulation, stable de-repression of an ampC-type β-lactamase).

HFIM issues and limitations

Many of the limitations of the 1-compartment model do not apply to the HFIM. There is no direct dilution of the bacterial burden, as the organisms are maintained in a separate compartment (the hollow fiber cartridge). The dilution occurs outside of this system to allow the attainment of the desired concentration-time profile. There have been no delineations of the same problems seen by Haag *et al.* [7]. The duration of the experiment is virtually unlimited, as durations of 14 days to 28 days have been published [11,12] and durations of six months have been performed in our laboratory (data not shown). Further, delineation of the relationship between drug exposure and resistance amplification has been straightforwardly analyzed [11,12]. It is important to recognize that there is a modest lag time between the concentration in the central compartment and the concentration in the HFIM. This has been looked at extensively and equilibrium is quickly established. Because it is plastic and not glass, the HFIM requires extensive evaluation regarding binding of the drug to the system. There is one other issue with which care must be taken. It is likely [11] that enzymes that degrade antimicrobials (e.g. an ampC β-lactamase) can be concentrated over time in the peripheral compartment of the HFIM. A cartoon of the HFIM is displayed in Figure 1 and the hydrolysis of an antimicrobial is displayed in Figure 2, panels a–h. Only in the experiment with the addition of NXL-104 (a β-lactamase inhibitor now known as avibactam) do we see that the actual target concentrations of cefepime are achieved and maintained.