

In vitro
pharmacodynamic
infection model
(ivpm) analysis



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' Drug resistance follows the drug like a faithful shadow' said by Paul Ehrlich. For the major classes of known antibiotics resistance has developed within few years from the time of clinical introduction of the drug. Sulphonamide resistance was reported in 1939, streptomycin resistance in 1946 and penicillin resistance in 1946 (G. J. Ebrahim, 2010).

Bacterial resistance can be innate or acquired. In innate resistance, a bacterial species may be naturally resistant to a drug before its clinical introduction. Acquired resistance where bacteria that were initially sensitive to a drug become resistant which is more serious. The mechanisms include inactivating enzymes that destroy the drug e. g. β -lactamase produced by several Staphylococci inactivate most penicillins and many cephalosporins. More prominently, the genes for β -lactamases may be chromosomal borne on plasmids or on transposons. Hence, there is possibility for bacteria to share them.

Another mechanism is alteration of binding sites an example of this is tetracycline resistance. In most resistant organisms, the binding sites may be altered so that they no longer have affinity for the drugs and most frequently acquisition of drug-resistant from plasmids or transposons.

Such mobile genetic elements help to extend resistance rapidly among bacteria. The above list makes an interesting variety of mechanisms evolved by micro organism. It is becoming palpable that antimicrobial resistance needs to be viewed as an ecological problem. Little by little, yesterday's possibly frank hopes and the early dream of omnipotent antibiotics have been eroded and progressively replaced with deep distrust.

MRSA Resistance in INDIA

Methicillin Resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen causing momentous morbidity and mortality. In India, the significance of MRSA had been recognized reasonably late and it appeared as a problem in the 80s and in the 90s. Recently epidemic strains of these MRSA are generally resistant to several other antibiotics (K. Rajaduraipandi et al, 2009) Antibiotics can no longer be seen as magic bullets. They have become a vital part of the problem of modern-day Hospital acquired infections.

According to the National Staphylococcal Phage Typing Centre, New Delhi, there is an raise in the occurrence of Methicillin Resistant strains of S. aureus from 9. 83% in 1992 to 45. 44% in 1998. Thus, it is probable that the prevalence of methicillin resistance in community acquired S. aureus strains also varies in different regions (Rahul patil et al, 2005)

Introduction to in vitro Pharmacodynamic Infection Model (IVPM)

Use of the Minimum Inhibitory Concentration (MIC) is the popular technique for assessing the potential therapeutic efficacy of antibiotics, though it is generally agreed that the experimental procedure for MIC determination does not exactly mimic the in vivo situation. The MIC is usually assessed in static conditions, in which the antibiotic at unvarying concentration is in contact with the microorganism for a long time. But the in vivo situation is clearly different, because the antibiotic concentration frequently changes with time. After a single administration, the actual time of contact is

reasonably short, particularly with antibiotics that are rapidly excreted from the body, such as cephalosporins, penicillins, and aminoglycosides.

The development of in vitro kinetic models for evaluation of antibiotic activity has aroused interest because they offer the possibility of imitating the same pharmacokinetics as found in vivo. This would permit investigation into how pharmacokinetic parameters persuade antibacterial activity of Antibacterial agents.

One of the first notable in vitro models to be described in the literature was that of the urinary bladder, developed to simulate conditions of uncomplicated cystitis by O'Grady, 1966, the model was used to study the effects of cycles of ' dilution' and ' micturition' on bacterial growth. The first kinetic model that reproduced plasma levels of antibiotics comparable to those observed in vivo was developed by Sanfilippo & Morvillo in 1968.

A more straightforward model for the simulation of drug distribution processes was adapted by Grasso et al. (1978) to examine the antibacterial activity of antibiotics. The apparatus functioned by first-order dilution techniques. The diluent was pumped from the reservoir into the flask by a peristaltic pump at a steady flow-rate, since the flask was tightly stoppered, the fluid was forced out of it at an equal flow-rate. The fluid coming out of the flask constituted a continuous sample of the culture, on which it was possible to determine the bacterial count and the antibiotic concentration as functions of time.

Murakawa et al. (1980) projected a device, based on the pharmacokinetic two-compartment open model. This model was certainly an improvement in <https://assignbuster.com/in-vitro-pharmacodynamic-infection-model-ivpm-analysis/>

an in vitro simulation of plasma drug levels after iv injection. Interest consequently focused on models that avoided dilution of the bacterial population by the use of a filter membrane (Shah 1980).

Insight into the basic principles used in IVPM

Characteristics of in vitro models

The two major characteristics of in vitro Pharmacodynamic (PD) models are drug exposure and bacterial concentration. The bacterial concentration represents the extent of the PD effect. In static in vitro models, bacteria should be suspended evenly in a culture vessel with invariable antibiotic exposure in the medium. All conditions remain the same over the complete observation period.

The working principle of dynamic models is complex. The idea is to simulate the body clearance or half-life of the antibiotic become conscious in dynamic models by varying drug concentrations. In dilution models the drug concentration in the culture vessel changes via replacement with fresh medium or by simple dilution. Simple dilution means to add a specific volume of medium to the culture vessel. Either (i) medium is added to the input and the outflow is uncontrolled via overflow (or does not exist) or (ii) a pump removes medium from the culture vessel and fresh medium is sucked in from a reservoir by low pressure. In both cases, the drug concentration in the culture vessel will be diluted. The input of medium in dilution models can happen continuously or stepwise, i. e. at intervals.

Studying combinations of antibacterial agents

When study of combination of antibiotics with different half-life in an in vitro model in order to achieve different half-lives for both drugs, the drug with the longer half-life (drug B) has to be supplemented into the central compartment constantly to replace what has been over-eliminated due to the too-high clearance of the system (Jurg blaser, 1985)

A number of factors are important in designing such studies:

Characteristics of the model

Characteristics of the model play impact on the results that are obtained, such as whether the antibiotic is removed by dilution or dialysis, the inoculum density, and the growth phase of the isolate.

Doses simulated

The advantage of using pharmacological dose simulations is that it is clear that the pharmacodynamic parameter related to outcomes will be relevant to human dosing.

Susceptibilities of the strains used

If strains are representative of the native population and a single dosing simulation is used, this often produces insufficient range in the pharmacodynamic parameters to produce useful analysis.

Classification of various In vitro Pharmacodynamic Models

Static models

Static models being made up of closed culture vessel. These vessels are available in many shapes, such as tubes, flasks, cell culture flasks or spinner flasks, and may be made of glass or polystyrene.

Pharmacodynamic Models

In dilution model a central compartment contains the bacteria in medium. Fresh medium is periodically added and at the same time the same volume of used medium is removed, leading to a stepwise decrease of the drug and a removal of the bacteria. Continuous simple dilution (without filters) model consists of a flask containing the bacteria in culture vessel, a reservoir and a waste container. Fresh medium is constantly pumped from the reservoir into the flask and used medium leaves dynamic dilution models without bacterial loss.

Stepwise simple dilution in a stepwise simple dilution model the medium is not removed from the central compartment. Fresh medium is added regularly and the drug concentration decreases over time, in relation to the increase in the volume of the medium. Concurrently, bacteria will be diluted; hence, bacterial concentrations have to be adjusted correctly.

In stepwise substitution with filters model ('syringe model') where the drug concentration is declined by stepwise substitution, but the bacterial loss is prohibited by a filter. A syringe needle is fixed into a cell culture flask containing bacteria and medium. The needle is joined with a filter unit and a

syringe. Used medium removed at regular intervals from the cell culture flask and replaced by fresh medium.

Advantages and disadvantages of IVP

In vitro systems have many characteristics which make them excellent experimental platforms, namely, flexibility, adaptability, relatively low cost, good correlation with animal and human data and they are without the ethical drawbacks of animal work.

Generally, in vitro models have numerous advantages compared with in vivo animal studies. They are more flexible and adaptable to diverse conditions, and are less cost and resource-intensive. Additionally, the relatively high inoculum and volumes in in vitro models permit better studies of resistance, because of the higher mutation frequency compared to animals. The PK properties of the drug of interest can be used in an in vitro model and the time course of an antimicrobial agent can be monitored precisely.

On the other hand, in vitro models need unique conditions, such as a temperature-controlled environment, and the risk of contamination of the culture vessel. Since in vitro models cannot imitate all in vivo conditions, such as immunological factors such as host defense mechanisms, the pathology of the infection, and the virulence and metabolic behavior of a pathogen, the derived Pharmacodynamic parameters cannot directly be transferred to the in vivo situation. The in vivo growth environment is dissimilar from the in vitro one. This may lead to phenotypic differences between bacteria grown in vitro and in vivo. In general, in vitro bacterial growth is more rapid than that in vivo.

Susceptibility breakpoints used in IVPM

In spite of the form of results, interpretation of criteria has a obvious impact on drug use and resulting policy. The in vitro antibacterial effects of β -lactams, clindamycin and macrolide antibiotics are normally considered to be Time dependent ($T > MIC$). Aminoglycosides may be classified as concentration-dependent antibiotics. These drugs kill bacteria much faster in an in vitro model, and rising concentrations relative to the MIC will increase the rate of bacterial killing. $C_{max} : MIC$ the most commonly used parameters, which are time above the MIC ($T > MIC$), ratio of peak concentration and MIC (C_{max}/MIC), and ratio of 24-h area under the curve and MIC (AUC/MIC).

There has been significant change and controversy over the type of information given by Antimicrobial Susceptibility Tests (ASTs). In an age of increasing antimicrobial resistance, Pharmacodynamic tool holds great potential for understanding resistance and support rational decision-making concerning the in vitro susceptibility testing and breakpoints.

Animal models provide comparable growing conditions for bacteria, closely mimicking the characteristics of a human infection, and the endpoint of an infection is clearly defined (cure or death) and similar to that in humans. The major disadvantage of animal models is differences in the Pharmacokinetic such as metabolism, which limit or demand complicated scaling methods for transferring data from animals to humans. In contrast, in vitro models can imitate human Pharmacokinetic and are thus better suitable for the investigation of antibacterial activity of antibiotics. Further, they permit resistance analyses, determination of time kill behavior, and the recognition

and optimization of Pharmacokinetic Pharmacodynamic indices and breakpoints.

In conclusion, simple tests of efficacy using in vitro models are easy to carry out, provide descriptive data that gives limited information, but which can have important value. The Pharmacokinetic and the Pharmacodynamic, are characteristics of an antibacterial agent and should be considered in the progress and forecast of the efficacy of the antibacterial therapy. New developments unite the ideas of a one-compartment dilution model with filters and a two compartment dialysis model, resulting in a computer-controlled semi-automated in vitro model for industrial use. In future, this trend of combining models for diverse purposes, as well as automation, might direct to more frequent use and, ultimately, they might become an intrinsic part of drug discovery and development.