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The analysis of dose-response curve from bioassays with quantal response: Deterministic or statistical approaches?

G. Mougabure-Cueto[a,*], V. Sfara[b]

[a] Centro de Referencia de Vectores (Ce.Re.Ve.), Ministerio de Salud de la Nación Argentina, Hospital Colonia Pabellón Rawson calle s/n (5164), Santa María de Punilla, Córdoba, Argentina
[b] Instituo de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín (3iA-UNSAM), 25 de Mayo y Francia (1650), San Martín, Buenos Aires, Argentina

HIGHLIGHTS

- The dose-response relations can be analyzed deterministically or statistically.
- The deterministic approach is based on the law of mass action.
- The statistical approach is based on the probabilities distribution of phenotype.
- Deterministic equations must be used to analyze dose-response in simple systems.
- Conversely, statistical models must be used in systems with quantal responses.

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ABSTRACT

Dose-response relations can be obtained from systems at any structural level of biological matter, from the molecular to the organismic level. There are two types of approaches for analyzing dose-response curves: a deterministic approach, based on the law of mass action, and a statistical approach, based on the assumed probabilities distribution of phenotypic characters. Models based on the law of mass action have been proposed to analyze dose-response relations across the entire range of biological systems. The purpose of this paper is to discuss the principles that determine the dose-response relations.

Dose-response curves of simple systems are the result of chemical interactions between reacting molecules, and therefore are supported by the law of mass action. In consequence, the shape of these curves is perfectly sustained by physicochemical features. However, dose-response curves of bioassays with quantal response are not explained by the simple collision of molecules but by phenotypic variations among individuals and can be interpreted as individual tolerances. The expression of tolerance is the result of many genetic and environmental factors and thus can be considered a random variable. In consequence, the shape of its associated dose-response curve has no physicochemical bearings; instead, they are originated from random biological variations. Due to the randomness of tolerance there is no reason to use deterministic equations for its analysis; on the contrary, statistical models are the appropriate tools for analyzing these dose-response relations.

1. Introduction

Dose-response relations and their associated parameters are crucial data in pharmacological and toxicological research. These relations are described by obtaining a dose-response curve from which parameters characterizing the molecule or the tissue-molecule system are estimated, i.e. lethal doses, potency, efficacy, affinity, etc (Kenakin, 2004). Dose-response curves can be obtained from systems at any structural level of biological matter, from the molecular (e.g. drug-receptors interactions) to the organismic level (e.g dose of toxic-proportion of affected individuals).

There are two types of approaches for analyzing the dose-response relations and obtaining the corresponding dose-response curves: the mechanistic or deterministic approach, based on the law of mass action (e.g. Michaelis-Menten equations), and the

* Corresponding author at: Hospital Colonia, Pabellón Rawson calle s/n (5164), Santa María de Punilla, Córdoba, Argentina.
E-mail address: gmougabure@gmail.com (G. Mougabure-Cueto).

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probabilistic or statistical approach based on the assumed probabilities distribution of certain phenotypic characters (e.g. probit regression) (Finney, 1978; Greco et al., 1995; Chou, 2006). Some authors propose models based on the law of mass action for analyzing the dose-response relations obtained from studies carried out along the entire range of biological systems (Chou, 2006). The law of mass action explains molecular interactions based on the probability of collision of the reacting molecules and therefore can be used to describe the dose-response curve at a molecular level. However, can models based on this law explain the dose-response curve at higher biological level? In this paper we show that the deterministic models can not explain the dose-response relations along the entire range of biological systems. To achieve that goal we discuss the different principles that determine the dose-response relation at two levels of biological organization: molecular level and organismic level. We used the drug-receptor system as an example of the molecular level, and bioassays with organisms responding quantally (e.g. bacterial or animal lethality bioassay) as an example of the organismic level. The aim of the manuscript is not to propose a novel method of analysis, but to discuss in which cases the use of statistical models is required for the analysis of the data. In summary, we show that dose-response curve obtained from bioassay with quantal response is explained by biological variation occurring at random (not by the law of mass action). In consequence, those dose-response relations should not be analyzed by deterministic approaches but via statistical approaches.

2. Biological action of chemical agents

The biological effect of chemical agents results from the interaction between the active compound and specific molecules in the biological structure, i.e. the site of action or receptor (Ariëns et al., 1979). However, the action of drugs involves a sequence of processes that can be grouped into pharmacokinetic and pharmacodynamic phases. The pharmacokinetic phase includes the processes of absorption, distribution, biotransformation, and excretion, and therefore determines the concentration of the active agent in the target tissue. The pharmacodynamic phase comprises the molecular interaction between active molecules and their specific site of action. This initiates the sequence of biochemical processes that finally ends in the biological effect measured (Ariëns et al., 1979). The main steps of the pharmacodynamic phase are: 1) drug-receptor interaction, inducing an initial stimulus, 2) transduction and amplification processes, transmitting the initial stimulus to the molecular effector system, and 3) generation of an effect by the activity of the molecular effector (Ariëns et al., 1979).

The effect of a drug on an organism clearly results from a series of biochemical and physiological process, from the initial absorption to the interaction with the site of action, and involves many molecular interactions.

3. Law of mass action and the dose-response curve

Molecular interactions such as drug-receptor can be considered chemical reactions; hence, the classical tools of chemical kinetics, particularly the law of mass action, can be used to analyze them. Thus, the efforts to describe the dose-response curves at the molecular level require some form of application of the law of mass action, and a variety of partially overlapping theoretical models has been developed (Ariëns et al., 1979; Clarke and Bond, 1998). A.J. Clark was the first to apply mathematical principles to the action of drugs and proposed that the fractional response caused by a drug is equal to the fractional occupancy of the receptors by the drug (Clarke and Bond, 1998; Kenakin, 2004; Maehle, 2005). In that simple model, if drug D combines with receptor R to form a complex D-R that produces a response, then:

$$D + R \underset{k_{-1}}{\overset{k_1}{=}} DR \quad (1)$$

where k_1 and k_{-1} = velocity constants. From the laws of mass action and mass conservation the following equations were derived:

$$E_D/E_m = [DR]/[R_T] = [D]/[D] + K_D \quad (2)$$

where E_D = effect, E_m = maximal response, [DR] = drug-receptor complex concentration, $[R_T]$ = total concentration of receptors, [D] = drug concentration, K_D = affinity-related parameter (Hathway, 1984).

If

$$E_D = E_m[D]/[D] + K_D \quad (3)$$

then Eq. (3) is an identical function to the Michaelis-Menten equation,

$$V = V_{max}[S]/K_M + [S] \quad (4)$$

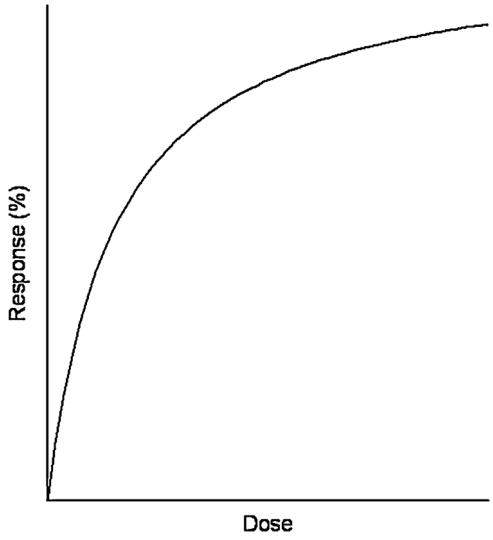

Fig. 1. Hyperbolic curve obtained when the response is plotted as a function of drug concentration.