Review
Quantitative Measurements of Pharmacological and Toxicological Activity of Molecules

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Abstract: Toxicity and pharmacological activity scales of molecules, in particular toxicants, xenobiotics, drugs, nutraceuticals, etc., are described by multiples indicators, and the most popular is the median lethal dose (LD$_{50}$). At the molecular level, reversible inhibition or binding constants provide unique information on the potential activity of molecules. The important problem concerning the meaningfulness of IC$_{50}$ for irreversible ligands/inhibitors is emphasized. Definitions and principles for determination of these quantitative parameters are briefly introduced in this article. Special attention is devoted to the relationships between these indicators. Finally, different approaches making it possible to link pharmacological and toxicological properties of molecules in terms of molecular interactions (or chemical reactions) with their biological targets are briefly examined. Experimental trends for future high-throughput screening of active molecules are pointed out.

Keywords: half-maximal inhibitory concentration; median lethal dose; toxicological and pharmacological indexes; predictive toxicology; structure–activity relationships

1. Introduction
Quantification of dose–response is a major issue in toxicology and pharmacology. The frontier between beneficial effects of a drug and toxicity is fuzzy, but it has been recognized for centuries that the effect in general depends on the dose. All over the world, fresh students in pharmacology and medicine have learnt the famous aphorism of Paracelsus (1493–1541): “dosis sola facit venenum”. Though hormesis [1] and hysteresis [2] are uncommon but important phenomena in pharmacology and toxicology, we will focus exclusively on concentration-dependent actions/effects of drugs. Indeed, in this article, we would like to clarify basic concepts used in pharmacology and toxicology that relate the general action of chemicals to physiological, pharmacological, and toxic effects. Working in a reductionist perspective, the effects of chemicals can be investigated first on biological targets, e.g., enzymes, receptors, nucleic acids, etc., and addressed in terms of molecular interactions. Then, chemical dose–response can be studied on whole organisms and cell cultures to quantify chemical actions at physiological level. Though with the fantastic developments of microfluidic and single-cell techniques, quantitative structure–activity relationships (QSAR), in silico predictive methods, artificial intelligence, and deep learning, we are at the eve of a revolution in predictive drug–response approaches [3], our presentation is focused on fundamental concepts rather than on innovative methodologies.

2. Indicators of Toxicity and Pharmacological Activity
2.1. The Half-Maximal Inhibitory Concentration (IC$_{50}$)

At the target level, IC$_{50}$ corresponds to the inhibitor concentration causing 50% inhibition of enzyme or receptor activity. Dose–response curves for enzyme inhibition or receptor binding can be determined graphically by plotting the remaining fractional activity of an enzyme ($v_i/v_0$) or the fractional occupancy (B) of a receptor as a function of inhibitor (or ligand) concentration [1].
Cheng and Prusoff [4] derived all equations describing the relationships between enzyme catalytic parameters, inhibition constants, and $IC_{50}$ for fast reversible inhibitors (Scheme 1, Table 1).

**Scheme 1.** Minimum mechanisms for reversible inhibition of enzyme catalysis. Inhibition can be purely competitive, inhibitor I competes with substrate S ($α → ∞$, orange box), non-competitive ($α = 1$, purple box), mixed ($α ≠ 1$, purple box), or uncompetitive, I binds to complex ES (Ki → ∞; $αKi ≪ Ki$, cyan box). If inhibition is partial, $β < 1$ (pink triangle).

**Table 1.** Relations between reversible inhibitors and $IC_{50}$.

| Type of Inhibitor | $IC_{50}$ | $IC_{50}$ vs. [S]/$K_M$ |
|-------------------|-----------|-------------------------|
| Competitive       | $K_i\left(1 + \frac{[S]}{K_{cat}}\right)$ | if $[S] < < K_M$, $K_i = IC_{50}$ | Linear ascending plot |
| Non-competitive   | $K_i$     | $α = 1$                 | Linear horizontal plot |
| Mixed type        | $\frac{[S] + K_{cat}}{\left(\frac{K_i}{[S]} + \frac{[S]}{K_{cat}}\right)}$ | if $α = 1$, $K_i = IC_{50}$ | $α > 1$: curvilinear ascending plot $α < 1$: curvilinear descending plot |
| Uncompetitive     | $αK_i\left(1 + \frac{K_{cat}}{[S]}\right)$ | if $[S] >> K_M$, $αK_i ≈ IC_{50}$ | Curvilinear descending plot |

In the case of fast reversible inhibition, $IC_{50}$ depends on the type of substrate and its concentration, except for non-competitive inhibitors (Table 1). However, if reversible inhibitors are partial inhibitors, i.e., inhibitors that do not fully inhibit the enzyme—$β < 1$ in Scheme 1—even at the highest concentrations (for a recent example see [3]), dose–response curves do not reach zero at high inhibitor concentration. The height of asymptotic limit corresponds to the residual fractional activity. Moreover, for certain reversible inhibitors, called “slow-binding inhibitors”, equilibrium is established after a lag time that could be several minutes [6]. Lag times are related to the dissociation rate constant of ligands from a target and measure the residence time on the target. As pointed out, long residence times on targets cause temporal discordances between pharmacokinetics and pharmacodynamics [7]. Slow-binding ligands with in vivo long residence times on targets inhibit targets at doses far below $IC_{50}$. A recent work about a papain-like protease inhibitor illustrates this statement [8]. Thus, in all cases, $IC_{50}$ must be determined at equilibrium. Another complication results from possible allosteric effects. When there is a non-michaelian saturation, the Hill coefficients ($n_H$), derived from dose–response curves, provide evidence for multiple ligand binding when it is different from one [9]. This parameter is important in receptology.

Thus, as seen from Table 1, $IC_{50}$ is largely dependent on the ratio $[S]/K_M$. A replot of $IC_{50}$ as a function of $[S]/K_M$ was recently proposed [10]. This leads to clear inhibition patterns, depending on the value of $α$ (Table 1, right column).

Previous relationships are valid for reversible ligands/inhibitors. However, in the case of drugs that are irreversible, covalent inhibitors (R) of enzymes, or irreversible ligands of proteins, e.g., alkylating agents (Scheme 2), the value of $IC_{50}$ is not meaningful because the inhibition process is time-dependent. The following formalism is for irreversible enzyme...
in which proposed by Trevan in 1927 from studies on lethal doses of cocaine, digitalis, echitamine, phates and carbamates used as pesticides, drugs for glaucoma, and palliative drugs for different initial enzyme concentrations, using the sampling method of Aldridge [12].

![Scheme 2](image)

This is the case of irreversible inhibitors of acetylcholinesterase, such as organophosphates and carbamates used as pesticides, drugs for glaucoma, and palliative drugs for Alzheimer disease treatment [11].

In fact, for irreversible inhibition, \( IC_{50} \) must be regarded as the inhibitor concentration leading to 50% of inhibited enzyme after time \( t \), i.e., \( [E]_0/2 \). For most in vivo irreversible inhibition of enzymes where \( [E] \ll [I] \), the inhibition process obeys first-order kinetics (Equation (1)):

\[
[E]_t = [E]_0 \exp(-k_i \cdot t)
\]

in which \( k_i \) is the second-order rate constant (=\( k_f / K_i \)) with the reaction half-time, leading to \( [E]_0/2 \):

\[
t_{1/2} = \frac{\ln 2}{k_i}
\]

In that case, apparent \( IC_{50} \) values may be calculated for different incubation times (\( t \)) and different initial enzyme concentrations, using the sampling method of Aldridge [12].

\[
IC_{50(t)} = \frac{\ln 2}{k_i} \cdot t
\]

For assays in the presence of substrate, Maurer et al. [13] derived a simple algebraic relationship between \( IC_{50(t)} \) and the apparent second-order rate constant (\( k_i \)) (Equation (4)):

\[
IC_{50(t)} = \frac{\ln 2 \left( 1 + \frac{[S]}{K_m} \right)}{k_i \cdot t}
\]

The work of Gierse et al. [14], about inhibition of cyclo-oxygenases by non-steroidal anti-inflammatory drugs, perfectly demonstrates the difficulty to rely on \( IC_{50} \) values and compare drugs showing different time-dependent profiles. Richardson [15] also thoroughly discussed the meaning and limitations of \( IC_{50(t)} \) values for measuring the potency of irreversible inhibitors of acetylcholinesterase. Despite limitations, determination of \( IC_{50} \) at a fixed time point, \( IC_{50(0)} \), can be correlated with cell-based assays. It is, thus, regarded as a meaningful index for testing novel irreversible covalent inhibitors of enzymes, optimizing the structure–activity relationship and dose prediction of designed compounds [16,17].

2.2. Lethal Dose-50 (\( LD_{50} \))

When talking about effects on physiological processes in whole organisms, and not just inhibition/activation of enzymes and receptors, the concept of the median lethal dose (\( LD_{50} \)) is used. \( LD_{50} \) is the dose that kills 50 per cent of a group of animals (Figure 1). This index was proposed by Trevan in 1927 from studies on lethal doses of cocaine, digitalis, echitamine, insulin, dysentery, and diphtheria toxins [18]. Rodents are the most popular animal model for \( LD_{50} \) studies. Despite the fact that \( LD_{50} \) is criticized for low reproducibility [19], it is used for assigning substances to a toxicity class [20]. However, \( LD_{50} \) gives little information about the toxic effects of substances, yet is one of the most-known indicators of acute toxicity [21]. The dependence of toxicity on the substance dose (e.g., mg/kg) is a sigmoid dose–response curve on a semi-logarithmic scale. A typical curve is presented in Figure 1.
“resurgence of covalent drugs” [12]. This concerns numerous xenobiotics and also more and more potential drugs, given the prophylactic and post-exposure drugs against acute toxicity of chemicals. A recent example for these compounds, 3.1.1. Relationship between LD50 and IC50

3.1.2. Therapeutic Index (TI)

An important derived index is the LD50-shift, used to measure the effectiveness of prophylactic and post-exposure drugs against acute toxicity of chemicals. A recent example is provided by the effective action of an encapsulated phosphotriesterase intravenously administered to mice to hydrolyze, i.e., neutralize, the organophosphate paraoxon in blood of poisoned animals, and thus, to counteract the toxic action of this compound [24].

We should also point out that in the case of irreversible inhibitors, active chemicals may induce a cascade of irreversible events, so that LD50 varies with time. Thus, as for IC50 for these compounds, LD50 must be determined at a fixed time after exposure to a toxicant. This concerns numerous xenobiotics and also more and more potential drugs, given the “resurgence of covalent drugs” [12].

3. Relations between Indicators: From Practical Descriptors to Empirical Relationship

3.1. Relationships between IC50, the Median Effective Dose (ED50), and LD50

3.1.1. Relationship between LD50 and IC50

For minimization of the number of animals used for acute toxicity studies, empirical relationships between IC50 and LD50 have been established. It is possible to make an assumption about LD50 values based on in vitro IC50 data [25]. For example, the Interagency Coordinating Committee on the validation of alternative methods proposed an empirical formula for rats [26] (Equation (5)):

\[
LD_{50} \, (\text{mg/kg}) = 0.372 \log IC_{50} \, (\text{microg/mL}) + 2.024
\]  

3.1.2. Therapeutic Index (TI)

Despite LD50 being a useless endpoint because it cannot be extrapolated directly to humans [27], it is used in therapeutic index (TI) determination (Figure 2). TI is the ratio of the LD50 to the ED50 (Equation (6)).

\[
TI = LD_{50} / ED_{50}
\]
Despite LD50 being a useless endpoint because it cannot be extrapolated directly to humans [27], it is used in therapeutic index (TI) determination (Figure 2). TI is the ratio of the LD50 to the ED50 (Equation (6)).

\[
TI = \frac{LD_{50}}{ED_{50}} \quad (6)
\]

The half-maximal effective concentration of a compound, EC50, measures the potency of a compound to produce 50% of its maximum effect, either pharmacological efficacy or acute toxicity, and in that case, it coincides with IC50. On the other hand, the median effective dose, ED50, differs from EC50. It is the dose that produces an effect in half of the population. ED50 should be compared to the lowest dose that produces a significant toxic effect, rather than compared to LD50 [28]. Thus far, no observed adverse effect level (NOAEL) of drugs obtained in preclinical studies (with the help of a correction factor based on body surface area) is used for calculation of human equivalent dose (HED) in clinical studies [29].

In relation to humans, TI is defined as the ratio of the dose that produces toxicity in half the population (TD50) to the dose that produces a clinically desired or effective response (ED50) in half the population [30] (Equation (7)):

\[
TI = \frac{TD_{50}}{ED_{50}} \quad (7)
\]

At the same time, it is worth taking into account that TI comparison of different drugs is precarious since different desired or toxic effects can be chosen as endpoints [31].

3.1.3. Therapeutic Window

Therapeutic window represents the range of doses where a drug is effective without unwanted iatrogenic effects. While a pharmacology handbook writes that “TI is a measure of a drug’s safety, because a larger value indicates a wide margin between doses that are effective and doses that are toxic” [30], Yartsev [31] noticed that there are possibilities that TI remains the same but therapeutic window may change, so there could be a more rational notion than TI (Figure 3).
plex mixtures of chemicals where the resulting effects can be additive, antagonistic, or synergistic, risk assessment may be a challenge. Then, concentration additive models can...
be used for QSAR analysis of toxicity of chemical mixtures. The recent development of 2D molecular descriptors for the prediction of toxicity of chemical mixtures in the environment illustrates this methodological trend and the power of these new tools [41].

4. Conclusions

Taking into account the high cost, duration, and ethical aspects of conducting experiments on mammals, alternative animals have been introduced in toxicology and pharmacology trials, such as worms (Cenorhabditis elegans and planarians (Dugesia japonica, Schmidtea mediterranea) [42] or zebra fish (Danio rerio) [43]. With these models, instead of LD\(_{50}\), other measures have been used such as LC\(_{50}\), the median lethal concentration in water, and NOEC (no-observed-effect concentration). NOEC is a new toxicological parameter for risk assessment and quantification of pollutant toxicity in environments, in particular in water. Moreover, toxicity assays on different types of cell cultures have been extensively investigated. For example, cytotoxicity tests based on tetrazolium reduction [44] are an important tool in toxicity studies. They help to reduce the number of in vivo experiments with candidate molecules selected by in vitro screening studies. We should mention that specific tests have been developed to assess special cases of toxicity. A typical example is the photosensitivity to some xenobiotics, chemicals, cosmetics, and drugs. Organization for Economic Co-operation and Development experts developed in vitro phototoxicity tests on reconstructed human epidermis which use human-derived keratinocytes [45].

A century of research, animal studies, and alternative methods for investigating toxicity and pharmacological efficacy of chemicals have allowed definition of reliable parameters to quantify acute toxicity of xenobiotics and safe use of drugs. Novel approaches, still in development, combining QSAR, microfluidic devices using 2D and 3D cell cultures [46–48], new animal and cell models, and single-gene expression technologies allow high-throughput screening of huge libraries of chemicals, and mining new compounds of pharmacological interest or detecting unwanted toxic compounds in the environment.

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