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Modeling and Simulation in Molecular Pharmacology

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Abstract

A stochastic simulation program of drug-receptor interaction is presented. The user can select a set of conditions concerning the processes of response release (type of cellular response, drug distribution and metabolism etc.) and the program plots a family of dose-response curves. A comparison between experimental and simulated dose-response curves shows the validity of the model can be used either for testing hypotheses concerning drug-receptor interaction, for experimental design or for estimation of some specific parameters. The program can also be used for educational purposes.

Keywords

Stochastic Simulation; Drug-receptor Interaction; Pharmacodynamics.

Introduction

The hypothesis of pharmacological receptors existence (Clark, 1937) and the extensive studies of enzymatic reactions, led to a sound theoretical basis of molecular pharmacology [1]. Several processing methods for dose-response curves have been proposed, starting with linear transformations [2] and followed by direct methods [3].

Receptor theory is supported by a large amount of experimental evidence [4] and is now regarded as a "classical" theory, becoming a well-defined chapter of pharmacodynamics [5].

However, the mathematical models of drug-receptor interaction were build under several simplifying assumptions, which cannot be always fulfilled experimentally, and the discrepancies between theory and experiment might become quite large [6].

Our objective was to approach drug receptor interaction in a stochastic way, following the intimate phenomena and simulate these phenomena up to a level, which can be experimentally tested, i.e. up to generating dose-response curves.

Theoretical background

Classical model of drug-receptor interaction. [7]

The drug molecule of substance A interacts with its specific receptor **R** in a reversible way, forming the pharmacon-receptor (drug-receptor) complex **AR**:

$$A + R \underset{k_2}{\overset{k_1}{\Leftrightarrow}} AR \qquad (1)$$

The effect $\mathbf{E_A}$ produced by a dose **[A]** of the pharmacon is supposed to be proportional to the concentration of occupied receptors:

$$E_A = \alpha\,[AR] = E_{Amax} / (1 + K_A / [A]) \qquad (2)$$

where:

$\mathbf{K_A = k_2 / k_1}$ is the dissociation of the pharmacon - receptor complex **AR**

$\mathbf{E_{Amax}}$ = the maximal value of $\mathbf{E_A}$, reached when **[A]** is high.

A graphical plot of $E_A / E_{Amax} = f(\log [A])$ yields the well-known S - shaped dose- response curve.

Higher order stoechiometry **(m)** has also been found for various drugs, so that a more general equation of dose - response curves would be:

$$E_A = E_{Amax} / (1 + K_A / [A]^m) \qquad (3)$$

It is beyond the scope of this paper to present this model more extensively.

Probabilistic approach of drug - receptor interaction

The occupation of a receptor by a drug molecule can be regarded as a random event [8]. Let us consider a tissue with $\mathbf{N_C}$ cells, each cell exposing **n** specific receptors, hence the total number of receptors on the tissue is $\mathbf{N_t = N_C \cdot n}$. A certain dose of drug **A** is represented by $\mathbf{N_A}$ molecules which randomly occupy the receptors.

For a given (chosen) $\mathbf{K_A}$, the number $\mathbf{n_A}$ of drug molecules which would occupy receptors forming the complexes **AR**, is the solution **x** of:

$$K_A = (N_t - x)(N_A - x) / x \qquad (4)$$

With the simplest assumption, that all receptors have the same probability to by occupied, we can simulate this interaction by generating $\mathbf{n_A}$ random numbers between **1** and $\mathbf{N_t}$, each representing the receptor occupied by the drug molecule.

We look now to each cell and count the number **o(i)** of occupied receptors. We can compute the cellular response which can be either gradual (proportional to the fraction of occupied receptors **o(i)/n**) or of a all-or-none type (**0** if the number **o(i)** is below a pre-set threshold **t**, and **1** if $\mathbf{o(i) \geq t}$). Next step yields the tissue response $\mathbf{E_A}$ computed as sum of cellular responses.

By repeating this procedure for different doses $\mathbf{N_A}$, a graphical representation can be obtained plotting

$\mathbf{E_A = f(\log N_A)}$.

We can represent on the same plot several dose-response curves, corresponding to different chosen features: classical approach for various stoechiometric coefficients or stochastic approach for various thresholds.

Advanced features [8]

The form of our stochastic model described above has used the same set of simplifying assumptions used by classical receptor theory; adding a new feature: the possibility to generate dose-response curves under the hypothesis of a all-or-none cellular response.

We have developed the model introducing actually several new features, listed below.

a) Type of cellular response - this feature has already been mentioned. The tissue response, seen at a macroscopic scale is gradual but it can be obtained also as a summation of "all-or-none" cellular responses involving a variable number of responding cells, depending on the dose.

b) Type of cell gradual response (this frame becomes active if option "gradual" is selected in 'Type of cellular response'). The relationship between the cellular response and the number of occupied receptors might be linear, logistic or of another type.

c) Type of cell threshold (this frame becomes active if option "all-or-none" is selected in 'Type of cellular response'). We may consider all cells having the same threshold number of receptors to be occupied in order to trigger the response, or different thresholds, which would be expected to have a normal distribution.

d) Relationship between cell and tissue response. In order to compute the tissue response from cellular responses one must assume a certain relationship between the response yielded by the tissue and the response produced by the component cells. The most common case expected is to consider the global response as a sum of the individual responses (a plausible situation when the response is a secretion). It may obey other laws (muscular contraction); we have introduced only the logistic function as a non-linear relationship.

e) Drug distribution over the tissue. One of the assumptions we may doubt about is that we considered all receptors as having the same probability to be occupied, i.e. the drug molecules are uniformly spread over the tissue. This might be close to reality for rapidly diffusing drugs. In most cases we would rather expect a distribution imposed by a certain diffusion rate. Our model starts from the hypothesis of an exponential decrease of the drug concentration with the distance from the capillary, which would imply an exponential decrease of the probability of drug concentration for some cells.

The present version of the program offers only the options listed above. Further developments are still under construction and will be added to the list.

An important improvement would include temporal features. The present version takes into account only the equilibrium state (which is actually used for most dose-response studies). However, the simulation programs might be developed to trace on a temporal scale all the events for each drug molecule and receptor, respectively. This will open the possibility to introduce further advanced features like:

f) *Drug administration:* unique dose, repeated doses, and perfusion.

g) Drug metabolism and elimination: one can even set certain limits for liver and / or kidney functional capacities.

h) Competitive synergism or antagonism: we introduce also another type of molecules, (B), with affinity for the same receptors, characterised by their intrinsic activity, β. When $\beta = 0$, the competition process is called antagonism, when $\beta > 0$ - synergism. The classical receptor theory treats both with the same formulae. The probabilistic approach of the competition between two molecular species, A and B, to occupy the receptors is much more complicated, requiring several assumptions concerning the intimate processes of drug-receptor interaction.

i) Non-competitive processes. The same response as that produced by the drug A can be produced, inhibited or influenced (augmented or decreased) also by other mechanisms, not by occupying the specific receptors on which our drug acts. This case can be introduced either by modifying the maximal response achievable for high doses of the drug, or by adding a specific simulation of the parallel process and compute the global response.

j) Initial state of the cell or tissue. It is today accepted that the reactivity of a cell depends on several factors, which actually determine the cell "state". There are different cell states and they might be due to:

- different developmental stages of the cell,
- biological rhythms,
- age,
- previous state of the cell, etc.

An appropriate use of these factors could account for 'fade' or 'tachyphilaxis' phenomena, noticed in several experiments. The initial state of the cell would become a compulsory parameter in time-response studies.

Program description

The program is a Visual Basic 4.0 application, running under Microsoft Windows 95, designed to run on an IBM compatible PC.

From the main menu we select first the "parameter selection" menu where the values for all possible options are selected (fig.1). The set of parameters was presented and discussed above. Nevertheless, we should mention here that the deterministic model would yield "perfect" dose-response curves, which are useful for theoretical purposes. However, for exporting these data to the program of data processing, they can be superimposed with a *noise*, so that their final look would be closer to real (experimental) dose-response curves. In the present version of the program the "noise" option frame was not included in the parameter selection form but only when storing the results. For the didactic version it will be moved in the parameter selection form.

After parameter selection menu, the program shows the numerical settings screen, for eventual changes of default values of $\mathbf{N_C}$ (number of cells), **n** - [average] number of receptors on each cell), $\mathbf{K_A}$ - dissociation constant, **nd** - number of doses, the set of doses $\mathbf{N_A}$ (nd), **c** - number of curves to be plotted (max. 10), **t** - [average] threshold number of receptors to be occupied for a cell to "trigger". We can select the variable parameter - usually **t** or $\mathbf{K_A}$ that will be assigned **c** values. Now we can start the computation, first finding **x** of eq (4), and then the corresponding $\mathbf{E_A}$ - all for each dose for obtaining the dose-response matrix. The computations are repeated for each curve (**t** with a different **t** or $\mathbf{K_A}$).

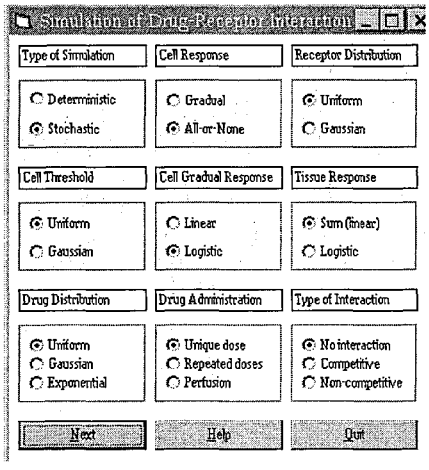

Figure 1 - Parameter selection menu

Then the results are presented as dose-response curves and the values of the parameters used are listed. A family of curves, corresponding to the variable numerical parameter is plotted on the same graphical representation, which makes an easier interpretation of the results. The results can be stored as dose-response pairs, either for a chosen (input) set of doses, or for a default set of doses (computed in a logarithmic scale of six log units symmetrically around half-maximal response). This option is very important for the connection of this program with another program dedicated to dose response curves analysis.

For deterministic dose-response curves, an option to add "noise" is presented, with uniform, proportional or gaussian noise.

The help module has still only short definitions but will be added with a tutorial format text.

Discussions

The main objective of the program is to get a deeper insight into intimate processes of drug-receptor interaction, since a good understanding of these processes would represent a great leap towards a major aim in medicine: *individualising the treatment.*

In order to achieve a good simulation program one must also provide some data about which most of present theories and studies dealt too less, especially due to several difficulties in setting up appropriate experiments. Thus, we hardly can answer questions like: For how long a drug molecule stays bound to its receptor?

Has the receptor structure been changed after the drug molecule uncouples off it? If yes, is this process reversible? For how long is the receptor unavailable? When can it be occupied again? How long is the latency period between the drug coupling and the release of the cellular response? And the list of questions can continue.

Our simulation program can use a set of hypothetical data for these parameters, vary only one at a time and see the consequences on the dose-response curves. This might help in estimating some of these parameters or, at least, some relations between them. It would also be beneficial for experimental design in pharmacodynamics or even for estimation of some numerical parameters. Identifying these values for an individual - by "fitting" simulated to experimental data would open the door towards predicting the response to a dose or to combination of drugs etc.

Conclusions

The simulation program presented is based on an original stochastic approach of molecular events in drug-receptor interaction, yielding dose-response curves built without using any equation associated with the process, but based on the probabilities of various successive events in drug-receptor interaction. The large palette of options among several conditions offers the possibility to look deep into the intimate mechanisms of response release.

The program is useful for researchers and students in pharmacology but also for medical students and doctors. A didactic version is in preparation.

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