

Pharmacological exercise using a computer simulation of isolated preparation of guinea-pig ileum

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Abstract

A computer simulation of the isolated preparation of guinea-pig ileum is a useful educational tool for medical students to learn the actions of various drugs on intestinal smooth muscle *in vitro*. A pharmacological exercise using a computer simulation program is conducted by approximately 120 second-year medical students with five staff members of the Faculty of Medicine at Fukuoka University. The students are divided into 10 small groups, and each group was assigned a laptop PC on which the software program had been loaded. This program simulates various drug responses in isolated preparation of guinea-pig ileum. In the simulated experiments, students first select a variety of agonists (*e.g.*, muscarinic, nicotinic and histamine receptors), or their competitive or non-competitive antagonists at a range of doses, and observe their intestinal responses on the PC monitor. Then, the students draw the dose-response curves for various drugs and calculate their pA_2 , pD_2 and pD'_2 values. Using this simulation program, the students can learn the pharmacological basis of how intestinal motility is controlled in the enteric nervous system. This program may enable some of the learning aims for which such preparations are used to be achieved without the use of real animals. Thus, the pharmacological exercise using the computer simulation program is considered to show one direction in the current trend of pharmacological education.

Key words : Pharmacological exercise, Computer simulation program, Dose-response curves, Isolated guinea-pig ileum, Animal welfare

Introduction

The Fukuoka University Faculty of Medicine promotes active learning based on small group work for the purpose of cultivating the high-level problem-solving skills required of medical students. Active learning-type medical exercises are carried out in order for students to learn the basics of pharmacotherapy. Pharmacological exercises to observe the actions of drugs *in vivo* or *in vitro* are an important component of the educational curriculum for medical students, as they teach

the basics of drug treatment. In our department of pharmacology, a pharmacological exercise utilizing a computer simulation program for the isolated preparation of guinea-pig ileum has been actively applied in the teaching of second-year medical students. Based on a huge amount of raw data, this program simulates the intestinal responses caused by various drugs. The students can observe the responses to various drugs on a PC monitor simply by inputting the dose of the drug, and then draw a dose-response curve for the drug and then work on any assignments given in advance.

In recent years, the moral responsibility imposed on

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academic staff with regard to animal welfare (e.g., reducing pain of experimental animals and actively applying alternative means of learning that do not involve experimental animals) and ethical norms in relation to animal experiments have been tightened. It is becoming increasingly difficult to sacrifice experimental animals for the sole purpose of observing known drug reactions, even for educational and research purposes¹⁾²⁾³⁾. In addition, academic staff are forced to bear a heavy burden regarding the preparation of instruments and reagents for animal experiments. This pharmacological exercise, which utilizes a computer simulation program, is beneficial both as an alternative to animal experiments and for reducing the staff burden. Furthermore, it is used as an opportunity for students to apply pharmacological knowledge quickly and efficiently, while keeping up with the current educational trends.

Materials and Methods

1. Computer simulation program

The Department of Pharmacology (Faculty of Medicine, Fukuoka University) has adopted a computer simulation program named “SIMULATED PHARMACOLOGICAL PREPARATION,” which was provided by the British Pharmacological Society, for one of its pharmacological exercises since 2007. This program simulates the response isolated preparation of guinea-pig ileum 19 types of drugs. Real preparations of guinea-pig ileum from experimental animals are frequently used to demonstrate and quantitate the actions of drugs on intestinal smooth muscle. It shows little spontaneous activity, thus giving steady baseline results, and contains a considerable variety of receptor types, which allows the actions of many drugs to be demonstrated.

2. Pharmacological exercise contents

The pharmacological exercise is conducted by approximately 120 second-year medical students with five staff members from the Department of Pharmacology, using a PC room in which 140 desktop PCs are installed. The students are divided into ten small groups, and each group is assigned a laptop PC onto which the software program has been loaded. Each student can also use one desktop PC assigned for the analysis of the simulated results and the creation of presentation slides.

Prior to the exercise, the staff members use the Wingnet Education Support System, which is a high-speed

image transfer unit to give a lecture on the mode of autonomic nerve (parasympathetic nerve) control and teach students the basic knowledge—including specific concepts and terms—necessary for the completion of the exercise. In addition, the staff explain the types of animal experiment the program is used to simulate, and teach students about the contractile response of intestinal smooth muscle using an actual device that cannot be grasped visually (Fig. 1)⁴⁾. After confirming the student’s understanding, the staff assign the laptop PCs and explain the details of operating the simulation program and the exercises. Students create dose-response curves from the simulated results obtained from the drug response and calculate specified values (e.g., pA_2 , pD_2 and pD'_2) according to the analysis items, which are presented in advance. The students consider and discuss the results based on the obtained data and the calculated values. During this time, the staff support the exercise by confirming that the calculated values are obtained correctly while giving appropriate answers to questions from the students. At the end of the exercise, each

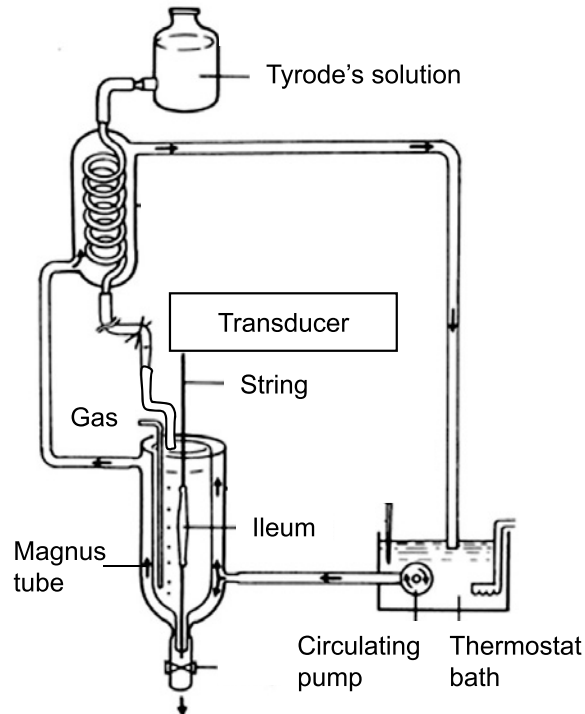


Figure 1. The Magnus device modeled in the simulation program.

Since Tyrode’s solution is kept at a constant temperature by a circulation pump, the environment of the specimen replicates the *in vivo* environment. This figure is reprinted and modified from the graphic in Reference No. 4.

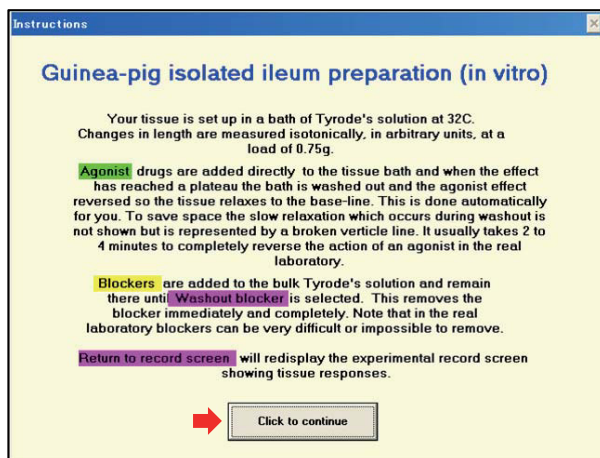
group presents the results of the exercise, and discussions are held between students and staff.

Results

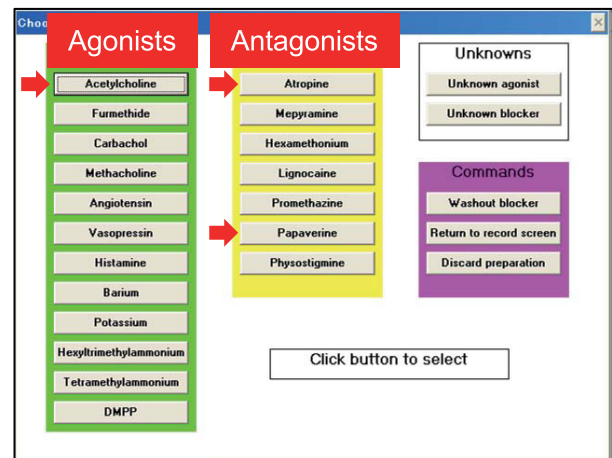
1. Utilization of the computer simulation program : Actions of agonists and antagonists on isolated preparation of guinea-pig ileum

The purpose of this pharmacological exercise is to set up an intestinal contraction model and to use the computer simulation program to teach students to understand the mode of autonomic innervation of the intestine. After opening and progressing through the pages of the simulation program, the students select an agonist (acetylcholine) that promotes intestinal contraction and

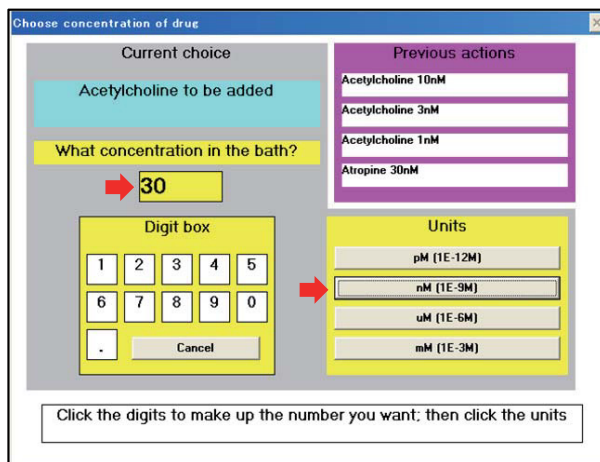
an antagonist (atropine or papaverine) that has an agonist inhibitory action (Fig. 2A, B, Table 1). When students set the concentration of acetylcholine (from 1×10^{-9} M to 1×10^{-4} M) and perform the 'treatment' operation (click on Units), the intestinal contraction reaction curve appears on the PC monitor (Fig. 2C, D, Fig. 3A–C). Surprisingly, even if the same numerical value is input and the reaction is observed repeatedly, the reaction shows the same tendency, but the same measured value is not always obtained. In other words, this simulation program incorporates individual differences in the drug response.



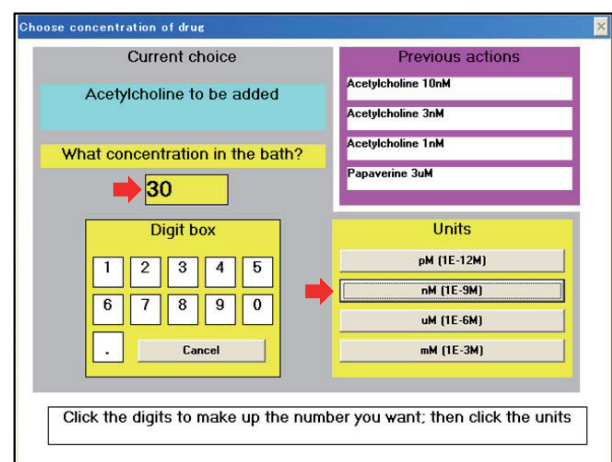
A



B



C



D

Figure 2. Computer screen pages for starting the program and drug administration.

Opening page of the simulation software program (A). The drug selection page includes twelve agonists and seven antagonists (B). Selection of drug dosage (C, D). Arrows show the place where the operator writes a number then clicks to administer the drug.

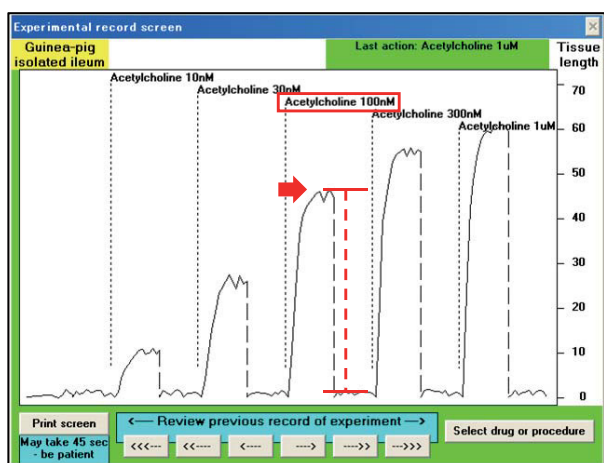
2. Evaluation of the pharmacological properties of agonists and antagonists

The students calculate the rates of contraction response stimulated with all concentrations of acetylcholine that appear on the PC monitor (Fig. 3A–C). Furthermore, after fully understanding the intestinal contraction response to acetylcholine, the acetylcholine-induced action is observed once more in the presence of

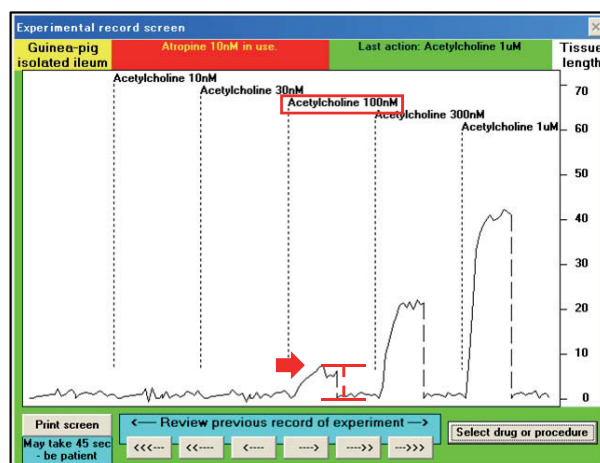
atropine or papaverine. Specifically, after the students perform the operation shown in Table 1, the students create dose–response curves for drugs to calculate the pA_2 , pD_2 or pD'_2 value. For the pA_2 value, the students create a Schild plot to learn how to evaluate the inhibitory properties of an antagonist (Fig. 4). After calculating each value and creating a Schild plot, the students evaluate the results and summarize them on presenta-

Table 1 : Operation of simulation program

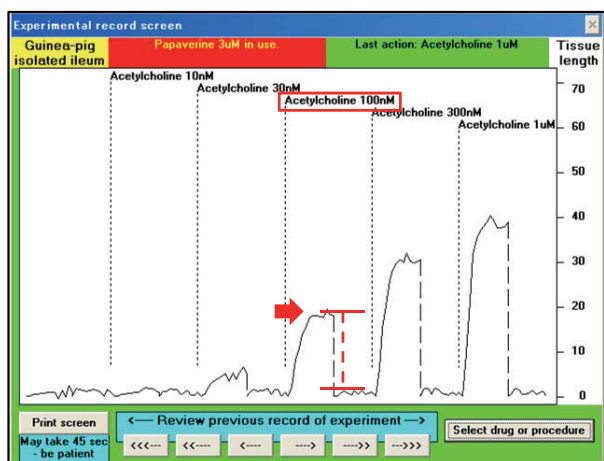
1. Select “Intestinal contraction” on the screen and click twice to start the program.
2. After understanding the explanatory text displayed on the screen, click “Click to continue” to proceed.
3. When you understand the explanation displayed next, click “Click to continue” again to proceed.
4. Click “Select drug or procedure”.
5. Select the agonist and inhibitor (antagonist) to be administered from the options.
6. When acetylcholine is treated at low to high dose, each contraction response curve appears. When the screen is full in the experiment log, click “Print Screen” to print on the printer. Students measure the magnitude of each reaction using a ruler and calculate the contraction rate.
7. Treat the same specimen with atropine followed by low to high acetylcholine.
8. After washing the specimen, treat with papaverine and treat with low to high acetylcholine.



A



B



C

Figure 3. Computer screen pages for the observation of response curves.

The response curve of acetylcholine (A). The response curve of acetylcholine in the presence of 10 nM atropine (B). The response curve of acetylcholine in the presence of 3μM papaverine (C). Arrows show the place of maximum response with 100 nM acetylcholine in the presence or absence of antagonists.

tion slides using the PowerPoint software program.

1) Dose-response curve

The contraction height at each concentration of acetylcholine is obtained as a percentage in comparison to the contraction at the highest response at each cumulative dose as the maximum response (100%). The horizontal axis represents the logarithmic value of the acetylcholine concentration, and then the vertical axis is plotted as the contraction rate at each dose (Fig. 3A). Acetylcholine is cumulatively administered so that it becomes 1 time, 3 times, 10 times and 30 times the standard amount. Log3 is 0.48, which is rounded up to 0.5.

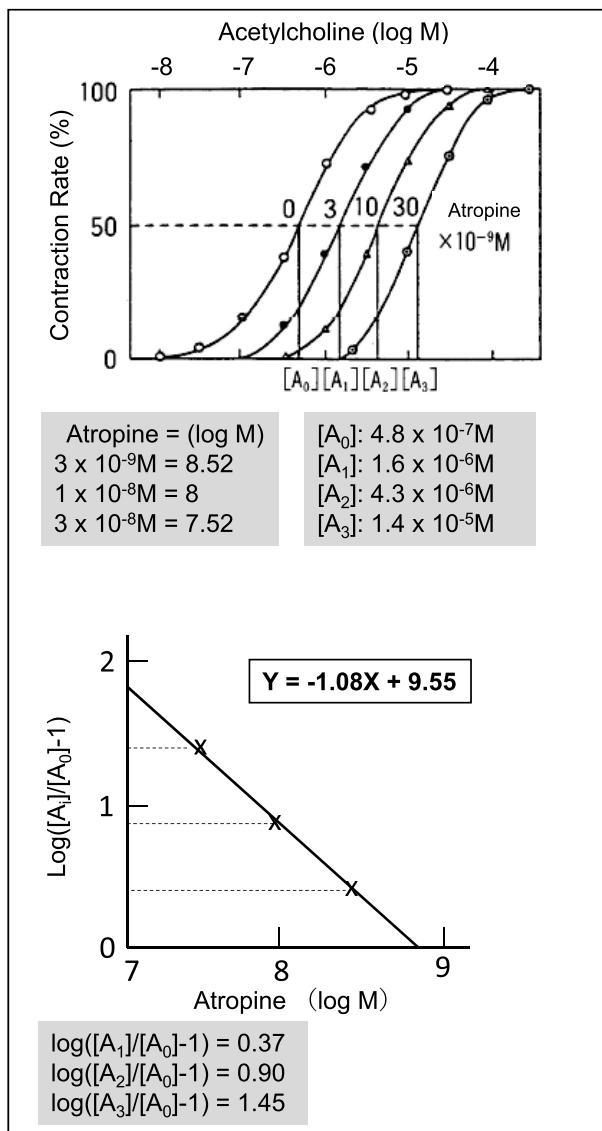


Figure 4. Calculation of pA₂ by the Schild plot method.

This figure is reprinted and modified from the graphic in Reference No. 4.

Dose-response curves in the presence of atropine or pappaverine are determined at each dose, with the maximum response of these controls as 100%.

2) pD₂

The pD₂ value is expressed as “the -log value of the molar dose of an agonist necessary to cause a response equivalent to 50% of the maximum response (E_{max})”. Since it is difficult to actually measure the 50% response dose in the experiment, a perpendicular line is drawn from the point with a reaction rate of 50% using a dose-response curve and the intersection with the horizontal axis is read.

3) pA₂

The pA₂ value is expressed as “the -log value of the molar dose of a competitive antagonist necessary for shifting the agonist dose-response curve to the higher dose side by 2 times”. It is difficult to obtain this value directly in experiments. When a certain dose of antagonist coexists, “how much the dose-response curve of the agonist is translated to the higher side” is determined from a formula. The mobility of the dose-response curve coexisting with a certain dose of antagonist can be shown as the ratio of the dose required to cause a certain response (usually a 50% response). Details of the calculation procedure are shown in Table 2.

4) Schild plot

A Schild plot is used in a method to obtain the log K_B value, and finally to calculate the pA₂ value. In this method, several doses of antagonist are used to determine the concentration of agonist in the presence of each concentration of antagonist. The logarithmic value (-log[B_i]) of the antagonist dose is plotted on the horizontal axis, and the agonist dose ratio (([A_i]/[A₀]-1)) is plotted on the vertical axis (Fig. 4). Then, the log K_B value is obtained from the intersection of the straight line connecting the point obtained by the least square method and the vertical axis. When the slope of the

Table 2 : Calculation procedure of pA₂ value

$$\begin{aligned} [A_i]/[A_0] &= 1 + [B]/K_B \quad (K_B : \text{antagonist dissociation constant}) \\ \log([A_i]/[A_0] - 1) &= \log[B] - \log K_B \\ -\log K_B &= pA_2 \text{ value} \end{aligned}$$

[A₀] : dose of the 50% reaction

[A_i] : dose of the 50% reaction existing antagonist

[B] : dose of antagonist

straight line is not approximately 1, $\log K_B$ is obtained using the intersection of the horizontal axis.

5) *van Rossum simplified chart*

Assuming that the dose–response curve of the agonist moves to x times higher in the presence of an Ax antagonist (see Fig. 4), “ $pA_2 = pAx + \log(x - 1)$ ” holds. According to van Rossum’s method, after determining the concentration that shows a certain reaction rate (50%) in the presence or absence of an antagonist, $\log(x - 1)$ is calculated by measuring the distance moved between the two curves and obtained from a simplified table (not shown here).

6) pD'_2

The pD'_2 value is expressed as the “ $-\log$ value of the molar dose of the non-competitive antagonist required to suppress the response of an agonist by half (suppress the maximum response of the dose–response curve to 50%)”. The maximum response in the absence of an antagonist is defined as 100%, and the response due to the highest dose of agonist is determined from the dose–response curve in the presence of the antagonist. When the value is plotted on the vertical axis and the antagonist dose is plotted on the horizontal axis, a straight line connecting the reaction rates in the presence of both high and low doses of antagonists is obtained. A vertical line is drawn from the point of the 50% reaction rate on this straight line, and the 50% inhibitory dose is determined from the intersection of the vertical line and the horizontal axis. When the horizontal axis is logarithmic, the pD'_2 value can be calculated as the pD_2 value. On the other hand, the pD'_2 value can be obtained with only one dose of antagonist, using the van Rossum simplified table (not shown here).

Discussion

If medical doctors fail to understand drugs’ mechanisms of action and their basic principles and respond to patients based solely on their test results only, it is not possible to make correct judgments or perform individualized treatment. It is necessary to fully understand the reaction of the target tissue according to the drug that is to be prescribed. It is difficult to gain an essential understanding of drug–induced biological reactions through passive learning in lectures. Thus, active learning pharmacological exercises using a computer simula-

tion program are indispensable for learning the basics of pharmacology.

On the other hand, the wave of educational reform that originated in the United States has spread to Japan, and the educational reform at medical universities is progressing⁵⁾. While the content of education has increased and advanced, the time required for lectures on basic medical subjects such as pharmacology has been decreasing year by year, and it has become difficult to secure professional staff⁶⁾. Shortages of time and professionals are also extremely serious problems behind the discussion about the ethical use of experimental animals. PC exercises incorporating simulation programs represent one of the best options for conducting efficient pharmacology exercises without experimental animals.

In pharmacological exercises using real guinea pig intestine, staff spend several days preparing the reagents, equipment and specimens. In addition to obviating the use of experimental animals, the performance of these exercises using a simulation relieves the burden of the staff. Thus, pharmacological exercises using this simulation software are considered to show one direction in the current trend of university medical education. Recently, the development of 3D simulation software that can observe changes in living tissue has been promoted. Thus, the use of advanced simulation software will make it possible to overcome ethical problems through animal experiments and make observations from more diverse viewpoints, such as anatomy, physiology and pharmacology.

Acknowledgments

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The authors declare no conflicts of interest in association with the present study.

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「The authors declare no conflict of interest.」