A Method for Evaluation of Therapeutic Dose of Doxorubicin Hydrochloride Using Breast Tumor Cell Culture MCF-7

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Growth kinetics of human breast tumor cell in the presence of doxorubicin was studied using the method of UV spectrophotometery. Parameters of ecotoxicological cell growth model were established. Cell number predicted by the model equation corresponded to the observed cell count within the experimental error. The therapeutic dose of doxorubicin hydrochloride was determined.

Key Words: *growth kinetics; ecotoxicological model; doxorubicin*

Statistical data suggest that the use of anticancer drug does not significantly increase the total survival of patient [2]. This is largely associated with difficulties in rational regimen of drug usage [1,12,13]. The choice of the dose of anticancer drugs is mainly empirical. This is also true for doxorubicin hydrochloride, antitumor antibiotic of the anthracycline family used for breast cancer management [8-11,14,15].

Knowledge of tumor kinetics peculiarities allows optimizing drug treatment of tumor diseases. Various mathematical models were proposed for the depiction of tumor growth. However, most of them represent just a regression approximation of experimental data and have no biological sense [3,4,13].

Ecotoxicological (quasi-chemical) model of cell population growth was created taking into account the cell cycle mechanism. The exposure of a cell population to chemical compounds is qualitatively characterized by model kinetic coefficients. The use of these coefficients allows calculation of the therapeutic dose for certain tumor and certain drug [3,4,13].

The objective of this work was to develop a method for kinetic determination of doxorubicin therapeutic dose for further employment in clinical practice using the ecotoxicological model.

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MATERIALS AND METHODS

Growth kinetics of human breast tumor cells MCF-7 in the presence of doxorubicin (Doxorubicine-Ferein, 10 mg; lyophilizate for preparing a solution for intravascular and intracystic infusions; Bryntcalov-A Ltd.) was studied. Before the experiment, doxorubicin (10 mg) was dissolved in 10 ml physiological solution to obtain stock solution with 1 mg/ml concentration.

Cell viability evaluated visually in a Goriaev chamber after trypan blue staining was 95% (minimum required value 94% [9]).

Complete growth medium (CGM) contained 450 ml DMEM (PanEco), 146 mg L-glutamine (in 2 vials, PanEco), 45 ml fetal calf serum (10% of growth medium volume, Eurobio), and 125 μg/ml CGM gentamicin.

The effects of doxorubicin on MCF-7 cells was studied using MTT-test [3,5].

Doxorubicin solutions (2.5×10^{-4}, 2.5×10^{-3}, 0.025, 0.25, and 2.5 μg/ml) were prepared. Samples of cell suspension (180 μl) containing 10^4 cells and 20 μl doxorubicin (3 wells for each doxorubicin concentration) were placed in five 96-well plates (one plate for each experimental term). The plates were incubated in a thermostat (Geraeus) at 37°C and 5% CO_2. On days 1, 2, 3, 6, and 7, 50 μl medium was removed from each well of one plate and 25 μl MTT-reagent (5 mg/ml, Sigma) was added. Then the plate was incubated

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at 37°C and 5% CO_2 for 3 h, 50 µl medium was removed, 150 µl isopropranolol (LabTech) was added, then content was pipetted to complete dissolving of formazan crystals formed in cells (under microscopic control). The fluid was separated from cell fragments by centrifugation at 3000 rpm for 10 min using a Jouan device, 100 µl formazan solution was sampled from each well, transferred to a new plate, and optical density was measured at 540 nm on a Labsystems Multiscan MCC/340 spectrophotometer.

In parallel, a calibration curve for optical density as a function of cell concentration was constructed (Fig. 1). Five dilutions of the cell culture were prepared: 2.5×10^5, 1.25×10^5, 6.250×10^4, 3.125×10^4, and 1.563×10^4 cell/ml. The cell concentration was determined in a Goryaev chamber. The concentration of tumor cell in the samples was determined using the calibration curves (Fig. 1) and kinetic curves were constructed in coordinates cell concentration (ml^{-1}) as a function of time (days, Fig. 2).

Parameters of ecotoxicological equation were determined by nonlinear optimization approach:

$$t(C)=\frac{1}{np_x}\ln\left[\left(\frac{C}{C_0}\right)\times\left(\frac{K_1-C_0}{K_1-C}\right)^{1+n}\right], \qquad (1)$$

where t is time (days), C is tumor cell concentration (ml^{-1}), K_1 is limiting cell concentration, (ml^{-1}), $p_x=p+g+d_{11}C_{dox}$ is a coefficient characterizing the growth of cell population in the presence of doxorubicin (day^{-1}), g is natural cell death coefficient (day^{-1}), d_{11} is a coefficient of doxorubicin toxic effect on growing cells ($ml\times\mu g^{-1}\times day^{-1}$), $n=K_1/K_2$, $K_2=b/a$, where b is a coefficient, characterizing proliferation in intact population, day^{-1}; a is an autoinhibition coefficient, characterizing mature cell proliferation inhibition by yang ones, $day^{-1}\times ml^{-1}$. K_1 value is tied with p_x by ratio:

$$K_1=\frac{fbp-b_xp_x}{ap_x}, \qquad (2)$$

where f is a proliferation factor (f=2 for mitotic proliferation type and $b_x=b+d_{21}X_1$ is a coefficient of cell proliferation in the presence of doxorubicin.

Since doxorubicin does not affect dividing cells, *i. e.* $d_{21}=0$, $b_x=b$, p_x^{cid} value corresponding to complete cell death ($K_1=0$) can be defined by the formula:

$$p_x^{cid}=fp=2p \qquad (3)$$

so, cytocidic doxorubicin concentration is:

$$C_{dox}^{cid}=\frac{p_x^{cid}-p-g}{d_{11}}=\frac{p-g}{d_{11}} \qquad (4)$$

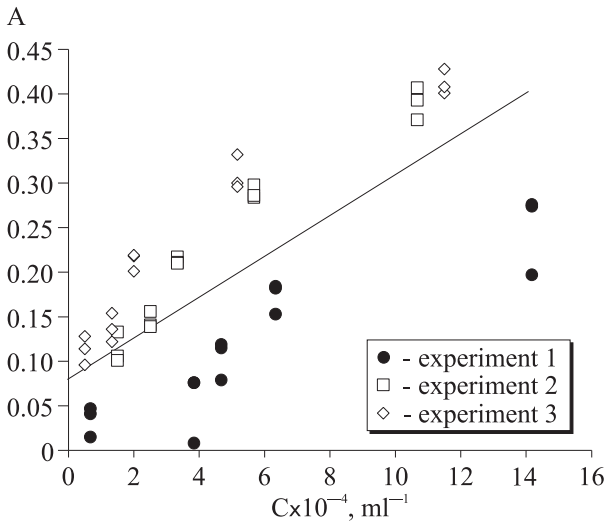

Fig. 1. Calibrating curve of optical density of formazan solution (*A*) as a function of cell concentration (*C*). Data from 3 parallel measurements and a common straight line *ax+b* (when *a*=0.023 and *b*=0.080) are provided.

Therapeutic doxorubicin dose (D), mg/m^2 body surface, was determined using the formula:

$$D=C_{dox}^{cid}\times V\times N \qquad (5)$$

where V=200 µl is cell suspension sample volume, N is the number of cells in 1 m^2. Taking into account that body surface area is equal to half-sum of cell area and considering cell shape to be spherical, one can calculate:

$$N=\frac{1\ m^2}{2\times\pi\times r^2}, \qquad (6)$$

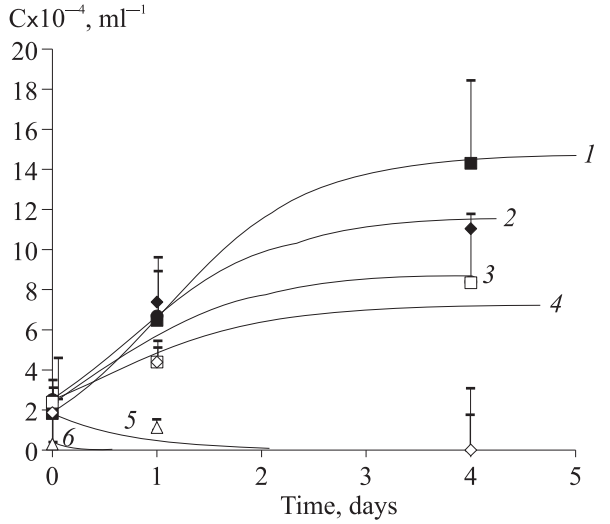

Fig. 2. Growth kinetics of human breast tumor cells MCF-7 in the presence of doxorubicin (dots correspond to experimental data and lines correspond to theoretical curves). *1*) control; *2*) doxorubicin 2.5×10^{-4} µg/ml; *3*) doxorubicin 2.5×10^{-3} µg/ml; *4*) doxorubicin 0.025 µg/ml; *5*) doxorubicin 0.25 µg/ml; *6*) doxorubicin 2.5 µg/ml.

where r is cell radius, m (3.016×10^{-5} according to literature).

RESULTS

Doxorubicin in concentration of 2.5×10^{-4}=0.025 μg/ml exhibits cytostatic activity against MCF-7 cell culture, and in concentration of 0.25-2.5 μg/ml produces a cytocidic action followed by cell population death.

Tumor cell concentration calculated using the ecotoxicologic model equation in most cases corresponds to experimental data within the experimental error. According to equation **(2)**, when doxorubicin concentration is above minimum cytocidic concentration, p_x value, and consequently, the product $b_x\times p_x$ is higher than product $2\times b\times p$. Therefore, doxorubicin concentrations of 0.25 and 2.5 μg/ml virtually correspond to negative cell population number (Table 1).

MCF-7 cell kinetic curves for the above doxorubicin concentrations are presented (Fig. 2).

The decrease in cell count observed during the first 2 days is described by exponential equation. This is probably associated with the absence of cell adaptation due to short exposure before addition of the serum. In this case, growth factors of the serum inducing cell transition from G_0 phase to cell division are destructed and the cell remains in the resting state after the end of adaptation period. Actually, medium replacement on day 2 led to an increase in cell concentration. Thus, p=0 in the absence of growth factors. Starting from day 2, the increase in cell concentration was described by complete equation.

Parameters of the model are presented in Table 2. For calculation of the therapeutic dose of doxorubicin, the mean value of d_{11} coefficient (16.3×1.8 ml×μg-1×day^{-1}) was taken. On the basis of these data, minimum doxorubicin concentration inducing the cytocidic effect was 0.127 μg/ml. Predicted doxorubicin therapeutic dose is 4.45 mg/m^2.

Model equation satisfactory described cell number in the culture within experimental error. Toxic action coefficients d_{11} and d_{22} are constant values characteristic of this cytostatic at constant substrate content in the medium.

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TABLE 1. Parameters of Ecotoxicologic Model for MCF-7 Cells in the Presence of Doxorubicin

Doxorubicin concentration μg/ml	p, day^{-1}	p_x, day^{-1}	b, day^{-1}	a, day^{-1}× ml^{-1}	g, day^{-1}	K_1	K_2	n	d_1, day^{-1}	d_{11}, ml× μg^{-1}× day^{-1}	C_0, ml^{-1}
0	2.500	2.924	13.308	1.1×10^{-6}	0.424	1.485×10^5	2.090×10^5	0.710	0	—	2.391
2.5×10^{-4}		2.928		1.4×10^{-6}	0.424	1.162×10^5	1.642×10^5	0.708	0.004	16.0	2.391
2.5×10^{-3}		2.965		1.8×10^{-6}	0.424	8.768×10^4	1.278×10^5	0.686	0.041	16.5	3.565
0.025		3.220		1.5×10^{-6}	0.320	7.248×10^4	1.533×10^5	0.553	0.400	16.0	3.174
0.25		6.423		1.4×10^{-6}	0.423	-3.462×10^4	1.643×10^5	-0.222	3.5	14.0	2.000
2.5		45.424		1.4×10^{-6}	0.424	-1.462×10^5	1.643×10^5	-0.890	42.5	17.0	0.304

TABLE 2. Concentration of MCF-7 cells

Time, days	Doxorubicin concentration μg/ml					
	0 (control)	2.5×10^{-4}	2.5×10^{-3}	0.025	0.25	2.5
0	4.75	4.75	4.75	4.75	4.75	4.75
1	2.957	3.391	4.043	3.739	3.435	0.565
2	1.826	2.391	2.522	2.391	1.870	0.304
3	6.478	7.391	6.696	4.391	4.391	1.13
6	14.304	11.043	8.391	8.348	0	0

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