NEW BIOMEDICAL TECHNOLOGIES

Cell Culture Test System for Express Analysis of Cytotoxic and Growth-Stimulating Effects of Bioactive Compounds

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Cultures of human and mammalian cells presenting 4 types of differentiation (normal human fibroblasts and myoblasts, human and Syrian hamster hepatoma cells, and mouse/mouse hybridoma cells) were used in a panel biotest system. This system allowed to evaluate the cytotoxic and stimulatory effect of bioactive compounds by determining the dose-effect relationships and some quantitative parameters including LD_{50}. Examination of some biolactive compounds of different nature (sangviritrin, escin, deltostim, cycloheximide, dexamethasone) confirmed high efficacy of this biotest system.

Key Words: *human cell cultures; biotest system; cytotoxicity; growth-stimulating effects; biologically active compounds*

Cell biotest systems are widely used for preclinical testing of bioactive compounds (BAC) [4-6,7,9]. These systems allow to evaluate the effects of BAC on metabolic processes, parameters of cell growth, optimal doses of potential drugs for humans, and possible side effects. However, extrapolation of these results is difficult because of differences in cell differentiation determining some deviations in responses of various cell type (test objects) and, consequently, body reactions [8].

Here we describe a complex biotest system consisting of a special multiwell plate with human and mammalian cell cultures presenting 4 differentiation types (connective tissue, muscular, hepatocyte, and lymphoid), which allow to evaluate the effects of BAC on different human tissues. Moreover, this system allows quantitative evaluation of the toxic and stimulating effects of BAC, which extends the area of its application.

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MATERIALS AND METHODS

Normal (diploid) human fibroblasts and myoblasts, human HEP G2 or Syrian hamster HT-11B hepatoma cell, and mouse/mouse 12D5 or MLC-1 hybridoma cells were used in the study. Most cultures were obtained from the Medical Genetic Center, hepatoma lines were obtained from the Institute of Cytology and Oncology Research Center [1], and myoblast were cultured as described earlier [2].

The cells were cultured on DMEM supplemented with 5% bovine serum and 5% human placental serum or with 10% fetal calf serum, other reagents were described earlier [2,5,6]. Crystal violet and thiazolyl blue (MTT; Merck) were used.

The cells were cultured either in 96-well plates, or in Carrel glass and plastic flasks (Costar). BAC cytotoxicity was evaluated by incorporation of methylene blue and Giemsa dye as described elsewhere [5,6]. Crystal violet was used in the same way as methylene blue. For MTT staining, the plates were incu-

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TABLE 1. Cytotoxic Effect of BAC Tested on Cultured Cells with Different Types of Differentiation

Preparation	Postnatal human fibroblasts		Human myoblasts		Hamster hepatoma (HT-115) cells	
	LD_{50}	LD_{100}	LD_{50}	LD_{100}	LD_{50}	LD_{100}
Escin	100	170	275	>350	70	120
Deltostim	5.7	10-14	2.8	>200	6.3	14
Sangviritrin	0.5-1	4	4	>25	2.5	8
Cycloheximide	15	>200	120	>400	—	—

Note. Doses are given in μg/ml.

bated with the dye (20 μl/well, 5 mg/ml) for 4 h, centrifuged 15 min at 1500 rpm, and the supernatant was removed. Optical density was measured after 15-min incubation with dimethyl sulfoxide (100 μl/well) on an EFOS 9305 electrophotometer at 492 nm.

For evaluation of growth-stimulating activity of BAC the cells were seeded to 96-well plates. One day after seeding the nutrient medium was replaced with fresh serum-free medium, and the cells were maintained for subsequent 2 days. Thereafter, the medium was replaced with fresh medium supplemented with 3% serum and containing test BAC in various concentrations (for evaluation of the dose-effect relationship). The first two vertical rows without BAC served as the control. After 96-h culturing the cells were stained as described above and optical density was measured on an EFOS 9305 spectrophotometer at 549 nm (methylene blue and crystal violet) or 620 nm (Giemsa dye). Thus obtained dose-effect curves were used for calculation of LD_{50} and LD_{100}.

Morphological state of the cells was evaluated by light microscopy using an MBI-3 (LOMO) microscope adapted as an inverted microscope with the help of a special packing [4].

Calibration curves (staining intensity as a function of seeding density) and the dose-effect relationships were processed by standard statistical methods using Sigma Plot software.

RESULTS

The majority of selected cultures were characterized by orientated growth associated with the formation of confluent monolayer within at least 30 passages. They showed typical direct dependency between cell number in a well and optical density measured after staining with methylene blue, crystal violet, or Giemsa dye. Hybridoma cultures growing as a suspension were examined with MTT providing calibration curve.

In cells with various differentiation types LD_{50} and LD_{100} differed 2-8-fold in the same test (methylene blue staining; Table 1). This confirmed the correctness of the proposed approach: a panel of cell cultures as a biotest system.

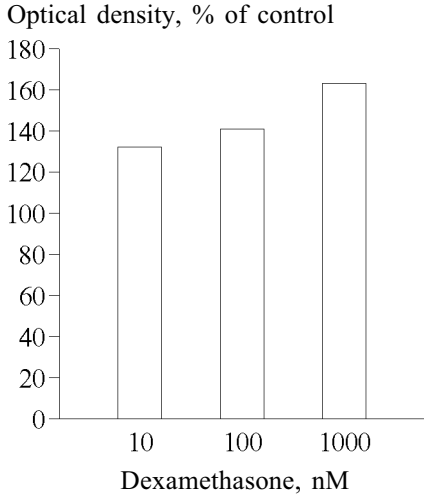

Fig. 1. Growth stimulating effect of dexamethasone on fibroblasts cultured in a medium with 3% serum. Control: without dexamethasone.

Experiments revealed the necessity of microscopic evaluation of cell morphology in each culture. High content of dead cells actively sorbing BAC and dye can distort the results.

In a special experimental series we showed that growth-stimulating activity of BAC should be evaluated in low-serum (3%) medium after preliminary incubation of cell culture panels with a serum-free medium. This procedure allows to minimize the effect of serum growth factors. Figure 1 illustrates the results of testing of dexamethasone growth-stimulating activity.

It should be also noted, that other mammalian cell cultures with similar growth and morphological properties can be used in the proposed biotest system.

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