Comparison of Cytotoxicity of Aminoglycoside Antibiotics Using a Panel Cellular Biotest System

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The cytotoxicity of four aminoglycoside antibiotics was studied by estimation of the dose-effect relationship using a panel cellular biotest system including cell cultures for test objects. The cultures represented 4 differentiation types: normal human fibroblasts and myoblasts, human or Syrian hamster hepatoma cells, and mouse/mouse hybridoma cells. It was found that three widely used antibiotics gentamicin, kanamycin, and neomycin exhibit similar, but not identical cytotoxicity parameters and differ distinctly from geneticin. Hence, the proposed panel biotest system helps to quantitatively evaluate and differentiate the effects of bioactive substances with similar chemical structure.

Key Words: *human cell cultures; biotest system; aminoglycoside antibiotics; cytotoxicity*

Aminoglycoside antibiotics possessing a wide spectrum of bactericidal effects (on gram-negative and gram-positive bacteria) were intensely studied for a long time, and some of them are traditionally used for the treatment of various infections. Indications to their use are constantly specified and widened [5,9-12,14]. These drugs have similar structure and show cytotoxicity when used in clinical practice (typically ototoxicity and nephrotoxicity). A set of bioactive substances (BAS) including aminoglycoside antibiotics gentamicin, neomycin, kanamycin, and geneticin was used in comparative study and evaluation of the potentialities of a previously designed panel cellular biotest system [6,7]. This test system detects the differences in the effects of drugs with similar chemical structure, differentiates their effects, and detects possible negative effects on human organism, and hence, can be used in preclinical trials of closely related compounds investigated as potential drugs.

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MATERIALS AND METHODS

Eight normal human cell strains (3 embryonal fibroblast and 3 postnatal fibroblast cultures, 2 myoblast cultures) and 3 transformed cell cultures (HEP G2 human hepatoma cells, GT-11B Syrian hamster hepatoma cells, 12D5 mouse/mouse hybridoma cells) were used in the study. The majority of these cell cultures were obtained from cell culture collection of Medical Genetic Research Center, hepatoma strains from the collection of Institute of Cytology, Russian Academy of Sciences, and Cancer Research Center, Russian Academy of Medical Sciences [1], and myoblast strains were derived as described previously [3].

Aminoglycoside antibiotics gentamicin, kanamycin, neomycin, and geneticin (Sigma) served as BAS and were added in doses 30 μg/ml-30 mg/ml culture medium.

The cells (except hybridomas) were cultured in DMEM with 5% bovine serum and 5% human umbilical serum or 10% fetal calf serum. Versene, trypsin, Hanks solution, methylene blue, and some other che-

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mically pure or extra pure Russian-made reagents were used in the study. Crystal violet and thiasolyl blue (MTT, both from Merck) were used for cell staining.

The cells were cultured in 96-well plates or precultured in glass Carrel flasks and disposable Costar and Nunk flasks, or Russian-made flasks. Twenty-four hours after cell inoculation the medium in wells was replaced with culture medium containing tested antibiotic. The cells were incubated with antibiotics for 3 days. Cytotoxicity of the test BAS was evaluated by methylene blue, crystal violet, or MTT binding as described previously [6,7]. Optical density of the samples containing stained cells was evaluated on an EFOS 9305 electrophotometer at λ=594 (for methylene blue or crystal violet) or at λ=492 (for MTT). The morphology of growing cells was evaluated under an MBI-3 microscope (LOMO) modified as inverted microscope with a special piece.

Calibration curves (relationship between cell staining intensity and number of inoculated cells in wells) and dose-effect curves were plotted on the basis of data statistically processed using Sigma-Plot software.

RESULTS

The dose-effect curves reflecting the effect of test drugs on cells were plotted for each antibiotic and each test object (4 cell cultures) using 2 test systems (crystal violet and thiasole blue). The effects of 4 antibiotics on human fibroblasts are presented in Fig. 1. The data indicate that the cytotoxicity of geneticin (drug widely used in clinical biological studies) far surpassed that of three other BAS, which however were also characterized by specific dose-effect curves. Similar, but not identical relationships were obtained for other test objects. It is noteworthy that 3 clinically used antibiotics (gentamicin, neomycin, and kanamycin) showed no cytotoxicity in concentrations below 250 μg/ml and minimum cytotoxicity in concentrations of 0.5-1.0 mg/ml (90-95% positively stained cells compared to the control). Geneticin in concentrations lower by one order of magnitude caused death of 90% myoblasts and 100% cells of three differentiation types. Moreover, even in a dose of 0.125 mg/ml geneticin caused death of about 50% myoblasts, 80% hepatoma and hybridoma cells, and almost 95% embryonic fibroblasts (only 6% positively stained cells in comparison with the control). Increasing the dose of geneticin to 250 μg/ml led to 100% fibroblast death; as for other cells, only 10% hybridoma and hepatoma cells and about 20% myoblasts survived. In other words, the toxic effects of geneticin were characterized by common regularities for the studied test objects, but qualitatively the effects differed significantly.

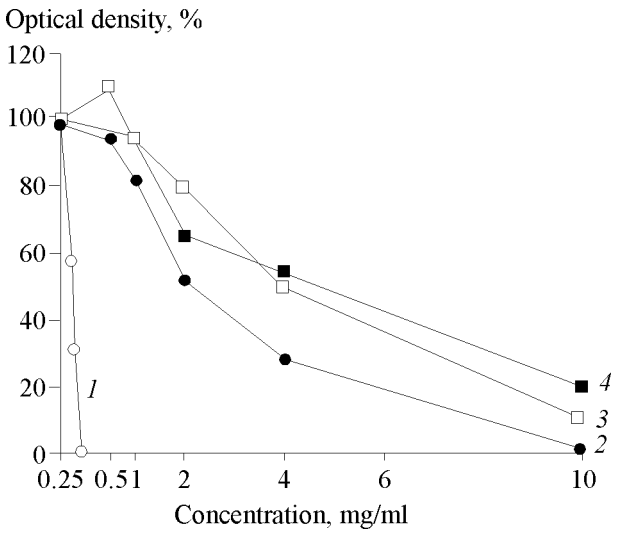

Fig. 1. Cytotoxic effect of aminoglycoside antibiotics on fibroblast cultures. Crystal violet staining. *1*) geneticin; *2*) gentamicin; *3*) neomycin; *4*) kanamycin. Optical density of cells cultured without antibiotics in medium with 10% serum is taken for 100%.

Similar but quantitatively different dose-effect relationships were observed for kanamycin (Fig. 2), the differences between myoblasts and hepatoma cultures were significant ($p<0.05$) and the differences between gentamicin and neomycin in different cultures were negligible.

TABLE 1. Cytotoxic Effect of Four Aminoglycoside Antibiotics on Cell Cultures in Panel Biotest System

Cell cultures		Gentamicin, mg/ml	Neomycin, mg/ml	Kanamycin, mg/ml	Geneticin, μg/ml
Human fibroblasts	LD_{50}	2.5	4.5	5.0	40
	LD_{100}	<10	>10	<20	<250
Human myoblasts	LD_{50}	3.5	3.0	7.0	100
	LD_{100}	<10	>10	<20	>500
Hybridoma cells	LD_{50}	3.5	4.5	6.0	40
	LD_{100}	>10	<10	>20	<250
Hep G-2 hepatoma cells	LD_{50}	2.0	4.0	4.0	50
	LD_{100}	<20	<10	<30	<500

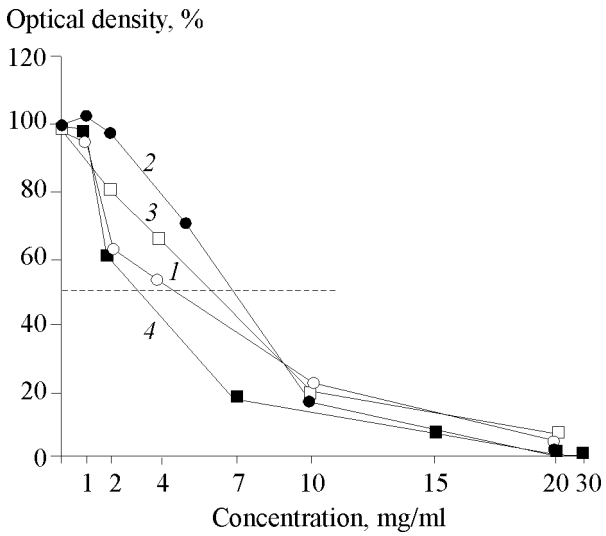

Fig. 2. Cytotoxic effect of kanamycin on cultured fibroblasts (*1*), myoblasts (*2*), hepatoma (*4*) and hybridoma (*3*) cells. Crystal violet and thiasolyl blue staining. Optical density of cells cultured without antibiotics is taken for 100%. Dashed line: LD_{50}.

Analysis of the dose-effect curves helped us to distinguish LD_{50} ranges (for the most sensitive and resistant cells): 2-3.5 mg/ml for gentamicin, 3-4.5 mg/ml for neomycin, and 4-7 mg/ml for kanamycin. Hence, clinically used antibiotics formed the following series: gentamicin-neomycin-kanamycin, while the cytotoxicity of geneticin was 50-100 times higher (LD_{50} = 40-100 μg/ml).

The findings (Table 1) indicate that the proposed panel cellular biotest system detects differences in the effects of BAS belonging to the same group of compounds. The data on cytotoxicity for 4 antibiotics (for example, higher toxicity of geneticin) are in line with published reports [2,5,10-13]. Importantly, that testing of cells with different differentiation types detects the specific effects of BAS on different human tissues. The proposed panel cellular biotest system detects and differentiates the effects of BAS with close chemical structure and hence, it can be used in preclinical trials of chemical drugs.

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