COMPARISON OF THE CYTOSTATIC EFFECTS OF THE GEMCITABINE PREPARATIONS GEMCITERA AND GEMZAR

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Cytostatic effects of Gemzar and Gemcitera, drugs containing gemcitabine as the active substance, have been studied using cell cultures of normal rat fibroblasts, MCF-7 (human breast cancer), and HeLa (cervical cancer) as alternative models. Both drugs exhibited high cytostatic activity. With respect to their sensitivity to Gemzar and Gemcitera, the model cells can be ordered as follows: HeLa < MCF-7 < fibroblasts. Gemcitera produced significant dose-dependent cytostatic effects on MCF-7 and HeLa cells, being comparable in this respect to the antitumor action of the reference drug Gemzar.

Key words: Gemzar, Gemcitera, gemcitabine, fibroblasts, cytotoxic activity, cell culture MCF-7, HeLa, breast cancer, cervical cancer.

Broadening the spectrum of chemotherapeutic agents and incorporating new drugs require a preliminary determination of their effectiveness and safety through the organization of adequate preclinical trials.

Preclinical trials were previously carried out most often in animal experiments, work that has several serious moral and ethical demands and limitations aimed at eliminating excessive cruelty and providing the humanitarian outlook required for improving medicine. Recent progress in medical and biological research and biotechnology has led to the creation of novel and more accessible methods for preclinical trials that provide alternative biomodels to animal experiments.

The alternative biomodels are test subjects that provide reliable medical and biological information and predict the biological activity of compounds and therapeutic effects without using experimental animals.

Animate and inanimate subjects such as single-cell organisms; chicken embryos; cultures of cells, tissue, and organs; and physicochemical and computer models can be used as an alternative biological model to animals. The most common among these are cell cultures, which can estimate both the specific pharmacological activity and the toxicity of drugs. Such research is especially important for antitumor cytostatic agents, which often are mutagenic and toxic. This limits research on their pharmacological bioequivalency in clinical trials. Therefore, cell cultures are used more broadly to study novel and more effective combinations of antitumor therapy, to discover new drugs, and to study comparatively the effectiveness and safety of various types of chemotherapy [1 – 6].

The goal of our work was to study cytostatic effects of Gemzar (**I**, Eli Lilly France S.a. C., France) and Gemcitera (**II**, TUTORS Laboratory, A. S. I. F. I. A., Argentina, produced by Laboratoria IMA S. A. I. S., Argentina), which contain gemcitabine as the active substance, using alternative models of cell cultures of normal rat fibroblasts, human breast cancer MCF-7, and cervical cancer HeLa. Gemcitabine (**III**, 2-deoxy-2′,2-difluorocytidine monochloride) is a broadly used modern antitumor drug that entered clinical practice in the last decade. Therefore, the creation of new drug forms of gemcitabine will make this drug more available for patients with various cancerous diseases such as non-small-cell lung cancer and cancer of the pancreas, bladder, breast, ovaries, cervix, etc.

EXPERIMENTAL PART

Cell culture of MCF-7 (human breast cancer) and HeLa (cervical cancer) were prepared from cell stocks of Ivanovskii Research Institute of Virology. They were cul-

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TABLE 1. Viability of MCF-7 Cells in the Presence of Gemzar and Gemcitera (% of Control)

Sample	Sample concentration					
	0.01 mg/mL	0.1 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL
Gemzar	(0.57 ± 0.04) 92.3	(0.47 ± 0.03) 88.6	(0.535 ± 0.07) 100.9	(0.91 ± 0.06) 74.0*	(0.74 ± 0.18) 60.1*	(0.64 ± 0.08) 52.0*
Gemcitera	(0.63 ± 0.13) 118.8*	(0.56 ± 0.03) 105.6	(0.51 ± 0.03) 96.2	(0.88 ± 0.1) 71.5*	(0.61 ± 0.2) 49.6*	(0.63 ± 0.05) 51.2*

Note. Here and in Tables 2 and 3: control = 100% vitality; *, reliable differences from control for $p = 0.05$ with optical density in parentheses.

tured in DMEM medium containing gentamycin (40 μg/mL) and fetal calf serum (10%) at 37°C and 100% humidity. Cells were formed into a monolayer, trypsinated, and placed into 96-well planchettes (Costar) as suspensions of half of the cells cultivated in a 50-cm^3 vial (200 μL/well).

Two pharmacopoeic samples (**I**, Serial No. A305461; **II**, Serial No. Gem3008) were studied. Samples were placed into planchette wells to give final concentrations 0.01 – 4 mg/mL in DMEM nutrient medium. Planchettes were incubated for 48 h. The effect of the drugs on cell growth was determined after incubation by the MTT microcolorimetric test [7] that is based on reduction of the tetrazole ring of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (**IV**) by mitochondrial dehydrogenases of living proliferating cells to form insoluble violet crystals of formazan and is a standard for estimating the toxicity of compounds in culture [1]. A solution of **IV** (10 mg/mL, 10 μL) in nutrient medium was placed into planchette wells with cells. The medium was removed after incubation for 4 h. Violet crystals of formazan precipitating on the bottom of the wells were dissolved in the planchette wells by DMSO. The optical density was measured on a Uniplan planchette photometer (Pikon, Russia) at 530 nm. Results were calculated as averages of four measurements of optical density (in arbitrary units) using standard EXCEL mathematical software. The vitality of cultures (% of corresponding control, intact cells from nutrient medium taken as 100%) was also calculated.

Fibroblasts were obtained from skin biopsies of newborn rats. Tissue was ground with scissors in medium 199 containing gentamycin (80 μg/mL) to fragments (0.5 – 1 mm^3) that were placed in culture vials and cultivated in medium 199 containing gentamycin (40 μg/mL) and fetal calf serum (20%) at 37°C and 100% humidity. Cells were formed into a monolayer, trypsinated, and placed into 96-well planchettes (Costar) as suspensions of half of the cells cultivated in a 50-cm^3 vial (200 μL/well).

Two samples (solutions of **I** and **II** in DMEM nutrient medium) were studied. Samples were placed into planchette wells to give final concentrations 0.01 – 4 mg/mL and 0.1 – 8 mg/mL. Results were calculated using the MTT test.

Statistical evaluation was performed using the nonparametric Wilcoxon-Mann-Whitney test, $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Gemzar and Gemcitera on MCF-7 cells

It was reasonable to use a culture of breast cancer cells, MCF-7, to estimate the comparative cytostatic activity of **I** and **II** because gemcitabine is indicated for therapy of this cancerous disease. Table 1 lists the results in both units of optical density and in percent of the appropriate control.

It was found that Gemzar and Gemcitera at concentrations of 1 – 4 mg/mL after 48-h incubation reduced reliably the vitality of MCF-7 cells by 51.2 – 52.0%, respectively, and did not affect their vitality at concentrations less than 1 mg/mL.

Differences in the cytostatic activity of the preparations were not reliable at the $p = 0.05$ level. The concentration of 4.0 mg/mL was probably close to the limiting one for these experiments because a concentration of 40 mg/mL (solubility limit of **I** and **II** according to drug instructions), from which the concentration of 4 mg/mL was prepared by dilution in cell culture, changes the acidity of the incubation medium, which can affect the vitality of the culture.

According to the literature, the mechanism of action of **III** on breast cancer cells includes activation of apoptosis, which is certainly an advantage of this drug. Furthermore, **III** can be used to overcome drug resistance to antitumor agents [2].

TABLE 2. Vitality of HeLa Cells in the Presence of Gemzar and Gemcitera (% of Control)

Sample	Sample concentration					
	0.1 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL	8.0 mg/mL
Gemzar	(0.59 ± 0.16) 105.3	(1.45 ± 0.21) 109.8	(1.21 ± 0.09) 91.6	(1.02 ± 0.08) 77.2*	(0.95 ± 0.17) 71.9*	(0.49 ± 0.03) 37.1*
Gemcitera	(0.55 ± 0.11) 98.2	(1.37 ± 0.36) 103.7	(1.19 ± 0.22) 90.1	(0.97 ± 0.08) 73.4*	(0.93 ± 0.13) 70.4*	(0.53 ± 0.10) 40.1*

TABLE 3. Vitality of Rat Skin Fibroblast Cells in the Presence of Gemzar and Gemcitera

Sample	Sample concentration					
	0.1 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL	8.0 mg/mL
Gemzar	(0.54 ± 0.07) 94.7	(0.48 ± 0.09) 84.2	(0.49 ± 0.07) 85.9	(0.41 ± 0.08) 71.9*	(0.25 ± 0.03) 43.8*	(0.06 ± 0.05) 9.0*
Gemcitera	(0.58 ± 0.13) 101.7	(0.55 ± 0.19) 96.4	(0.61 ± 0.05) 107.0	(0.33 ± 0.08) 57.8*	(0.23 ± 0.03) 40.3*	(0.05 ± 0.01) 7.5*

Effect of Gemzar and Gemcitera on HeLa cells

Considering the broad use of antitumor agents, in particular **III**, for therapy of cervical cancer, HeLa cell culture, which is used to estimate the effectiveness of antitumor agents, was selected as the subject of further research [8].

Cells were incubated with the studied drugs for 48 h. Table 2 presents the results in both units of optical density and in percent of the appropriate control.

It was found that the vitality of HeLa cells was reduced reliably by the action of the studied drugs starting at a concentration of 2 mg/mL (by 27%). The maximum inhibition was observed at a concentration of 8 mg/mL of **I** and **II** with reduction of vitality averaging 60%. The drugs at a concentration of 4 mg/mL reduced the vitality of cells by 30% (vitality of MCF-7 cells was reduced by 50% at this concentration). It can be assumed that the IC_{50} for HeLa cells is close to 8 mg/mL. Thus, HeLa cells are less sensitive than MCF-7 cells to the action of the studied drugs. Differences in the action of Gemzar and gemcitabine on this cell culture were also not reliable.

The results confirmed indirectly the many investigations showing the greatest effectiveness of these compounds in combination with other antitumor agents, including beam therapy [9 – 12].

Vitality of normal rat skin fibroblasts in the presence of Gemzar and Gemcitera

A side effect of beam therapy of cancerous diseases, for example lung cancer, is the development of pneumosclerosis. The growth of connective tissue is an undesirable effect of therapy of many other neoplasms.

The effect of the gemcitabine drugs on the vitality of fibroblasts was also of definite interest. Furthermore, normal fibroblasts are a model for estimating the toxicity that is recommended by the RF MH [1].

TABLE 3 lists the results in both units of optical density and in percent of the appropriate control.

It was found that Gemzar and Gemcitera exhibit equally pronounced inhibition on rat skin fibroblasts. The cytostatic action was reliable at concentrations similar to those for MCF-7 breast cancer culture. The vitality of fibroblasts decreased by an average of 30% at a dose of 2 mg/mL. The vitality at concentrations of **I** and **II** of 4 mg/mL was reduced by 60%; at 8 mg/mL, by 90%. The resulting effect may be useful for reducing the degree of sclerosis, for example, after beam therapy [4].

Thus, the investigation of the effect of Gemzar and Gemcitera on the vitality of three cell cultures, breast and cervical cancer and normal fibroblasts, established that these drugs have identical cytostatic activity. The studied cultures can be placed in the following order of sensitivity to them: HeLa < MCF-7 < fibroblasts.

The results confirm the cytostatic action of gemcitabine that was found in other studies of cell cultures [2 – 4, 11, 12] and indicate that the two drugs Gemzar and Gemcitera are identical.

Our results confirm that cell cultures can be used as an alternative model for estimating the cytostatic activity of antitumor agents. The use of cell cultures as alternative models has great potential because this model is being improved (three-dimensional cell cultures have already been prepared [13]), enabling an approach to the *in vivo* tumor structure and carrying out rapid screening of new antitumor agents and their drugs.

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