Prospects for the use of animal cell cultures in screening of pharmaceutical substances

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Abstract. Currently, there is a tendency to reduce the use of animals in conducting safety tests of chemical substances. Therefore, \textit{in vitro} methods are a good alternative or adjunct to \textit{in vivo} safety tests. This is especially important at the stage of pre-clinical drug trial. In 2004, the international standard for the principles of good laboratory practice (GLP) \cite{1} was adopted which regulates chemicals trials in cell cultures. However, in Russia, until recently, this issue has been neglected. Research works have been scarce. In 2013, the standard for GLP principles and compliance monitoring was adopted in Russia \cite{2}. The feasibility of using animal cell cultures as drug testing system has been proved by the experimental base and is now being introduced into practice \cite{3}.

1. Introduction
It should be understood that the safety of drug therapy is one of the most important tasks of modern health care. Adverse drug reactions rank No. 4–6 place among the causes of mortality in advanced countries. And of course the system for testing drugs before they are introduced into clinical practice is constantly being improved.

Any substance with biological activity intended to be subsequently used in touch with living systems, as a rule, is tested for its proper toxicity at the first stage. Certainly, the classical methods for evaluating the toxicity of biologically active substances, including pharmaceutical substances, still dominate in experiments. However, methods alternative to the classical experimental animal tests, namely models using animal cell cultures are increasingly found in toxicological studies \cite{4, 5, 6}. Despite the extensive development and acceptance of alternative methods in the world, they have not aroused widespread in our country. In addition to solving the ethical problems associated with the mass use and death of experimental animals, these methods can significantly reduce the cost and time of preliminary studies of new chemical drugs mainly at the stage of pre-clinical trials. Another indubitable advantage of \textit{in vitro} models is the ability to work with test substances directly on human or animal cell cultures which makes the obtained data more adequate when they are projected on a macroorganism. Furthermore, the use of cell cultures makes it possible to establish the nature of...
biological activity and the mechanism of realizing the effects of tested compounds directly at the cellular level and consider complex synergistic and/or multidirectional effects of test compound mixtures [7, 8]. Test cultures of animal cells derived from different tissues and organs exhibit different sensitivities to chemicals; and in vitro tests can thereby detect negative unpredictable reactions of individual tissues’ possible hypersensitivity in a macroorganism [9]. Test systems based on cell cultures are handy and can be used to solve a variety of tasks in the field of toxicology and cell biology, namely, to study a number of pharmaceuticals (potential or already in use) of practical interest.

Currently, introduced into practice and prescribed by the General Monograph (OFS.1.7.2.0002.15) are biological test methods for interferon preparations using cell cultures. This applies to substances and dosage forms of human interferon of all types of natural and genetic engineering origin [3].

Improving the pre-clinical trial stage of pharmaceutical substances is also particularly relevant in the treatment of heart arrhythmias where side effects are up to 30% of cases. Most of the traditional antiarrhythmics are known to have a cardiotoxic effect [10, 11, 12, 13, 14]. In this context, it is necessary to expand the arsenal of pre-clinical drug testing methods for an objective evaluation of their safety. It is essential to intensify the search for new drugs nonspecifically exhibiting antiarrhythmic activity, while remaining as safe as possible, for example, of the metabolic action type (dimephosphon, mexidol, emoxypine, cytochrome c) [15, 16, 17].

2. Authors’ research

2.1. Materials and methods

The in vitro studies were performed on passaged MDBK cell cultures (calf kidney cell culture) and KST (calf heart vessel cell culture). Standard culture media and solutions were used in the experiment. Culturing was performed on a steady-state monolayer basis at a temperature of 37°C based on the Eagle’s, Eagle’s MEM and Eagle’s DMEM culture media (figure 1). 5–10% of bovine serum and antibiotics (penicillin and streptomycin up to 100 μg/ml) were added to the culture medium. The cell lines were maintained by periodic passaging. Isolate passaging was performed as the monolayer was formed, usually after 4–5 days of culture. The cells were removed without a centrifuge, using Versene solution (0.02%) and trypsin solution (0.25%) at the ratio of 9 : 1. The seed concentration was within 70,000–100,000 c/ml. Etacisin and mexidol were tested at concentrations equivalent to therapeutic ones (0.5; 1.0 μg/ml and 5.0; 10.0 μg/ml, respectively). The combined use showed the concentration value of 2.5 μg/ml for mexidol and 1.0 μg/ml for etacisin.

2.2. Results

It was established that etacisin (1.0 μg/ml) caused a developmental delay of both cultures after the drug was added with the cell suspension. When etacisin was added on the formed monolayer, changes were observed corresponding to a pronounced cytopathic effect. Gradually, the cells were losing turgor and adhesive properties due to which the monolayer was quickly degraded (figure 2). There were multiple cell anomalies. There were cases of multinucleate MDBK and KST cell cultures, the formation of blade nuclei; the drug contributed to the formation of micronuclei (figure 3). The MDBK culture was found to be the most sensitive to the antiarrhythmic. The number of cells with two or more nuclei exceeded the benchmark by more than three times. Pyknoses of nuclei were observed. There appeared pathological mitosis forms; their number was five times larger than the normal range. Prevalent among them were pluripolar mitoses, mitoses with agglomeration, delay and fragmentation of highly condensed chromosomes (figure 4). The cell cytoplasm acquired the foamy form due to the large number of vacuoles (figure 5). Etacisin in a dose of 20 μg/ml caused a mitotic arrest at the prophase stage with the chromosome being significantly altered and did not match in shape and appearance the prophase chromosomes in the control. 3-hydroxymethyl-ethylpyridine succinate (mexidol) had no negative cytomorphological effects in both cell lines, nor did it affect the ratio of multinucleate cells as compared to the control.

2
Figure 1. MDBK cell line. Normal monolayer.

Figure 2. MDBK cell line monolayer degeneration.
Figure 3. MDBK cell line. Pyknosis and nuclear fragmentation.

Figure 4. Pathological multiipolar mitosis.
The karyological control showed that the modal number of chromosomes affected by etacisin corresponded to the normal values both for MDBK and KST cultures and was 48–50 (46–60 in the control); however, the drug significantly extended the variability range in the number of chromosomes within 35–85. The metaphase plates were significantly modified. Often, there appeared highly condensed chromosomes in the form of globules with unexpressed shoulders. The modal class of chromosomes affected by 3-hydroxymethyl-ethylpyridine succinate was 48–50; the variability range in the number of chromosomes was within 40–60, which also corresponded to the norm.

It was also shown that 3-hydroxymethyl-ethylpyridine succinate (mexidol) is able to partly neutralize the negative effects of etacisin when they are used together.

3. Conclusion
The results of the studies are indicative of high etacisin cytotoxicity in a dose of 1.0 μg/ml, which is equivalent to a therapeutic dose, as well as the ability of mexidol (2.5 μg/ml) to weaken the severity of the adverse effects of the antiarrhythmic agent etacisin. These studies confirm the retrospectively negative cardiotoxic effects of etacisin shown in clinical practice. The introduction of in vitro test-systems on a permanent regulated basis into the pre-clinical drug testing stage would make it possible to early detect their possible cytotoxic effects and the molecular-cellular “targets”.

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References
[1] OECD series on Principles of Good Laboratory Practice and Compliance Monitoring - No 14 - Advisory document of the working group on Good Laboratory Practice. The application of the Principles of GLP to in vitro studies (2004)
[2] GOST 31891-2012 Principles of Good Laboratory Practice (GLP). Application of GLP
[3] The biological test methods for interferon preparations using cell cultures (General Monograph) OFS.1.7.2.0002.15. (reference date: 10.29.2015)
[4] Anders M 2000 S-conjugate-dependent toxicity; alternatives to animal studies. Altern. Animal Testing & Experiment. AATEX 1(7) 30–36
[5] Eropkin M Yu 2004 The cell cultures as a model system in the biochemical-toxicological studies. Doctor Biol. Sci. Diss. St.Petersburg 354 p
[6] Chiba K, Makino J, Ohuchi J. et al. 1999 Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. Evaluation of cytotoxicity test on HeLa cells. Toxicol. In Vitro. 1(13) 189–198
[7] Dyakonov L P, Galnbek T V, Kulikova I L et al 2000 Animal cell culture: (Methods and Application in biotechnology) Moscow: Sputnik+ Co 398 p
[8] Zhukova O S, Abdrahmanov I K, Gerasimova G K, Dyakonov L P 2006 Cytotoxic effect of catalytic system containing phthalocyanine and naphtalocyanine complexes and ascorbic acid on normal and tumor cells Abstracts 46th ETCS International Meeting “In vitro cytotoxicity mechanisms”. Verona p 46
[9] Galnbek T V, Akinshina G T 2013 Modern trends and development of cell biotechnology in veterinary medicine. Proc. of the All-Russian Research Institute of Experimental Veterinary Medicine n. a. Ya R Kovalenko 77 219–227
[10] Alpert J and Francis G 1994 Treatment of myocardial infarction. Trans. from Engl. Moscow: Praktika Publ. 255 p
[11] Onopriev V V, Uvarov A V, Reznikov A Y 1994 Antiarrhythmic effects of richjocaine European students conference at the Charité for students and young doctors, Berlin, Germany p 7
[12] Khankoava A I 1998 Cardioprotector and cardiotoxic effects of antiarrhythmic compounds: ways of pharmacological correction, molecular mechanisms of action. Extended abstract of M. D. diss.: Volgograd 47 p
[13] Arutyunov G P and Rozanov A V 2003 Place of thrombolytic therapy in the treatment of cardiogenic shock Heart 1(2) 18–19
[14] Galenko-Yaroshevskiy P A, Skibitskiy V V, Boddi V B et al 1997 Cardiotoxic effects of antiarrhythmic drug ritmidazol and their correction with sufan, befol and their combinations Bulletin of Experimental Biology and Medicine 12(124) 640–644
[15] Balykova L A, Balashov V P, Shkolnikova M A and Kemaeva N N 1999 Effectiveness of dimephosphon in treating heart rhythm disturbances in children Russian Bulletin of Perinatolgy and Pediatrics 2 125–128
[16] Gurevich M A 2002 Pathogenesis and treatment of heart failure, myocardial infarction. Clin. Med. 4(74) 15–20
[17] Popov D A 2004 Pharmacological efficacy of antioxidant (emoxypine, dimephosphon), antiarrhythmic (trimecain) and cardiac (strophanthin) drugs in experimental anaphylaxis. Extended abstract of M. D. diss. Staraya Kupavna 20 p