BIOSENSOR FOR THE EXPRESS CONTROL OF GENOTOXICITY OF SOME PHARMACEUTICAL SUBSTANCES

[1,2]Shuliak L. M., [3]Fedelesh-Morenetz M. I., [3]Starodub M. F.

[1]National University of Pharmacy, Kharkiv, Ukraine

[2]Center for Laboratory Medicine "Alpha Labservice", Kharkiv, Ukraine, a-lab@meta.ua

[3]National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine, nfstarodub@gmail.com

Genotoxicity assessment is a key component in the safety assessment of any substances. They may exert general toxicity at corresponding concentrations (at levels that as a rule result in high and/or long exposures), endocrine disruption or genotoxicity even at low concentrations. Today are more than 100 different methods to assess genotoxicity but really no more than 20 test systems are practically used. The most of them based on the traditional approaches including living organisms of animals and even Genotoxicity assessment is a key component in the safety assessment of any substance. They may exert general toxicity at corresponding concentrations (at levels that as a rule result in high and/or long exposures), endocrine disruption or genotoxicity even at low concentrations. Today are more than 100 different methods to assess genotoxicity but really no more than 20 test systems are practically used. The most of them based on the traditional approaches including living organisms of animals and even plants. Among of the molecular approaches widely applied method of DNA comets. Among of the molecular approaches widely applied method of DNA comets.

In last time according to practice demands there is needed to have full information not on total toxicity only and above genotoxic effect of the environmental factors. Moreover, there is required the obtaining test results in an on-line regime. It is possible only at the realization of the application of the new generation of the instrumental analytical approaches based on the biosensor technology. The start in the development of such approaches intended for the determination of genotoxicity was done not long ago. Today we have the panel of the bacterial tests based on the DNA damage depending induction of the SOS repair system: SOS-Chromo, Umu, Lux-Fluoro, VitoTOX® and some others biosensors variants [1]. The Lux-Fluoro test is a unique combination of two bioassays, which coincidentally measure genotoxicity (SOS-Lux test) and cytotoxicity (Lac-Fluoro test) of single substances and mixtures of substances.

Earlier we have developed rapid recombinant bacterial biosensors for detection of genotoxicity of some nanoparticles of metal oxide [2] and mycotoxins [3] and analyzed their efficiency in genotoxicity screening. In the present report we present detected levels of genotoxicity of selected some pharmaceutical substances to demonstrate their activity in affecting genome mutation processes.

The method of detection was fiber optical SOS-biosensing with the application of recombinant C600 (pPLS-1) *Escherichia coli* cells on a cellophane membrane for the contact of the analytical unit with the transducer surface. All chemical reagents were obtained from Sigma-Aldrich (USA). The fiber optics biosensor on the basis of *E. coli* cells combining the SOS system, indicative of DNA-damaging agents, as a receptor component and a bioluminescence (lux) system as a rapid reporter technique was constructed as described earlier [2-4]. This device works in a differential regime, which allows registering comparative levels of fluorescence between the presence of the analyzed substance in the measuring cell and in its physiological solution (control sample).

Cellophane film was preliminary boiled in the distillate water during 15 min. Then, the small cylinders with the diameter about 5-6 mm were formed from the cellophane films. These cylinders were filled by the prepared suspension in LB medium at the concentration of 10^7-10^8 cell/mL

and equipped with optrodes. Such complex of the optrode with cell suspension was introduced into the measuring cell of the biosensor system. In the preliminary experiments [4] it was demonstrated that It was precisely cellophane that turned out to be the most suitable for the formation of a sensitive layer of indicator cells in comparison with their inclusion in the gel or photopolymerization on the surface of the optical fibers.

After incubation of the optrodes or the complex of the optrode with cell suspension in the measuring cell filled by the solution to be analysed during some time (from 10 to 90 min) at the room temperature the light emission was measured. The signal was presented in the units relative to the control value.

Among the pharmaceutical substances we chosen dioxidine, metronidazole and furatsilin which were analyzed by other investigators [5] by a similar lux-method (E.coli MG1655-pRecA-lux) in respect of their genotoxicity. It was necessary to compare the effectiveness and sensitivity of different types of analytical methods. Despite their fundamental similarity in sensitive elements, although they have a different composition, on the one side, but on the other one, they differ dramatically, since in our case the analysis is fulfilled technically not traditional way, but based on the design of the optical biosensor using optical fibers. It is compact device content two blocks with the optrodes and the special system for the introducing analysed samples. Both blocks are able to work in the differential regime according to which it is possible to control the relative luminescence level when one of the above mentioned substances was added to the measuring cell of one block and aliquot of the control probe was introduced into the other one.

Since some chemical and, of course, pharmaceutical substances have a hydrophobic abilities we demonstrated that they may be dissolved in acetonitrile. In contrast, other organic solvents (e.g., ethanol) occasionally affected the detected genotoxicity index at a concentration of 3.0% and above. In case using acetonitrile at first the chemical substance should dissolved in 20.0% of acetonitrile at the concentrations of 1.0 and 2.0 μg/ml. These stock solutions were then diluted 1:100 times by the physiological solution and were used for assessing genotoxicity levels. The specific signal increased with increasing concentrations of acetonitrile in the samples analyzed. Not only the intensity of fluorescence of the referent cells was observed as the result of the inclusion of their operon with the complex of the reparative genes and activation of their intensity of work, but this process was also accompanied by a shift of the fluorescence maximum in a short period of time, as simultaneously with the reparative genes, expression of the fluorescent protein gene introduced in the above mentioned operon was activated. The appropriate chemical substance may more induce the increasing the fluorescence level. There is more convenient if the analyzed substance is hydrophilic and in this case as control probe should be used physiological solution buffered at Ph in neutral zone.

As a standard toxic element for the testing system it was used dimethylsulfate (DMS), mitomycin C (MC) and ethanol (Et) at the concentrations in range of 5 μM to 1.0 mM, 1 nM to 10 μM and 0,5-4%, respectively. It was demonstrated that for the DMS and MC the registered luminescence was appeared trough about 15-30 min and achieved maximal level during 150-180 min. After that time the level of the luminescent signal was stay on the some level or become to decrease though some time (no faster then 180-240 min and its depended on analysed substance. In spite of the relatively long time of the achievement of the maximal level of the signal, there is possible to currying express control at the exposition during 20 min. In case of the application of Et the appearance of the luminescence was revealed through 30 min and its level was decreased after the exposition about 200 min. It was analysed the dependence of the fluorescence level on the concentration of the used substances. As result of the investigation it was stated that Et aroused maximal level of the fluorescence at the concentration in 3%. In case of the using DMS and MC such effect was appeared at their concentration of 0.5 mM and 1 μM, respectively. The minimum of the concentrations which could be able to stimulate a marked increase in the level of the fluorescence are: 10 μM,

20 nM and 1% for DMS, MC and Et, respectively. In all cases the linear responses were between the minimal and maximal levels.

The above presented results testify that the development of the fibre optic SOS biosensor is able to control of the genotoxity of the number of chemical substances. There is necessary to underline that the obtained results are in good agreement with that received by others authors with the using analogues SOS systems but at the registration of different traditional registration approaches, namely, Luminescence Spectrometer (model LS 50 B of Perkin Elmer, UK), LKB Luminometer 1250; Pharmacia Biotech, Uppsala, Sweden) and others which are very complicate and expensive. The proposed SOS biosensor is very simple, may be modified for the application in field condition even. As it was demonstrated above this biosensor gives possibility to reveal genotoxicity of the chemical substances in on line regime, in particular during 20 min only if the optrodes with the immobilised appropriate cells will be prepared previously. In our preliminary studies it was shown that the optrodes with the immobilised SOS system may preserve functional activity during 1 day at the preservation of them in the LB medium.

As for dioxidine, metronidazole and furatsilina, it turned out that both of them are genotoxic and the registration of the increasing fluorescent signals begins from the concentrations eproximately in 10^{-6}, 10^{-5} and 10^{-6} M, respectively. These data testify that the developed by us biosensor allows not only simple fulfill analysis but it is more sensitive approximately on the one order then method proposed by other authors [5] with the application similar sensitive system.

Conclusion. The presented results allow to do a very important conclusion, namely, it is proposed the quite simple experimental approach allows rapid and easy determination of the genotoxic effects exerted by chemical substances including pharmaceutical too.

Reference

1. Starodub NF. Genotoxiticy: modern instrumental approaches for its control in environmental objects // J. Biosens Bioelectron. – 2015. – 6. – P. 2, http://dx.doi.org/10.4172/2155-6210.1000169
2. Starodub N.F, Savchuk M.V, Lukin V.E. Investigation of the genotoxicity of some nano-metal oxide particles by the fiber optic SOS-type biosensor // Int. J. Nanotechnol. Nanomed. Res. – 2017. P. 1-4. https://www.clytojournals.com/investigation-genotoxicity-some-nano-metal-oxide-particles-fiber-optic-sos-type-biosensor
3. Starodub N. F., Fedelesh-Morenetz M. I., Savchuk M. V., Taran1 O. P., Naboka O. I., Shuliak L. N. Control of the genotoxicity level of certain mycotoxins by a fiber optical SOS-type biosensor // World J. of Eng. Res. and Technol. – 2018. – V. 4, Issue 6.
4. Starodub N.F., Taran M.V. Analsis of the Efficiency of Fiber Optic Sos-Type Biosensor Work at the Different Ways of the Sensitive Layer Formation // Austin J. Biosens & Bioelectron. – 2016. – V. 2 Issue 2. –ISSN : 2473-0629 | www.austinpublishinggroup.com
5. Гуноев М.Б., Юсупова Х.Р., Кунтаева Т.М. Исследование генотоксичности лекарственных препаратов с использованием люминесцентных бактерий // XXXVI Международной научно-практической конференции: «Междисциплинарные исследования», Россия, г. Новосибирск, 09 января 2018, № 1(36). URL: https://sibac.info/archive/meghdis/1(36).