SECTION 2

MEDICAL ECOLOGY

SENSITIVITY OF M.HOMINIS AND U.UREALITICUM TO ANTIBIOTICS

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In this work diagnostics of biomaterial on existence miko-and ureaplasmas by means of the standard test system "Mycoplasma IST 2" through of selective hydrolysis of an arginin and urea has been carried out. The sensitivity miko-and ureaplasmas to nine antibiotics of three classes is defined.

Keywords: mycoplasma, ureaplasma, mycoplasmosis, antibiotic resistance, tetracyclines, macrolides, fluoroquinolones.

Introduction. The causative agent of mycoplasmosis is mycoplasma, a microorganism taking an intermediate position between bacteria and viruses. A peculiarity of mycoplasmas is the absence of a cell wall (due to the lack of intrinsic enzymes that synthesize its components (muram and a-E-diaminopimelic acid)). When detecting mycoplasmas in genital scrapings in amounts exceeding 10^4 cells / ml, antibiotic therapy is necessary. Mycoplasmas are not sensitive to all antibiotics; moreover, individual resistance to certain antibiotics can develop. Mycoplasmas are known to be resistant to drugs, which action is associated with biosynthesis of cell wall proteins. Most mycoplasmas are sensitive to drugs that inhibit the synthesis of membrane and intracytoplasmic proteins. Therefore, conducting a sensitivity test is relevant in the selection of treatment with frequently used antibiotics.

Materials and Methods. As a test material, scrapings from the vagina of 100 female patients of reproductive age in Minsk (19–44 years old) were used. Standard test systems "Mycoplasma IST 2" (BioMerieux, France) were used. They include Urea-arginine broth and a 22-well panel that allows to identify a microorganism (M. hominis or U. urealyticum), the number of detected microorganisms (> 10^4 cells / ml or < 10^4 cells / ml) and their sensitivity to nine antibiotics of three classes. The strips with added components were incubated at a temperature of 36 °C ± 2 °C for 24 hours (growth of U. Urealyticum) and 48 hours (M. Hominis growth). In the presence of growth in the medium, specific substrates (urea for U. Urealyticum and arginine for M. hominis) and an indicator (phenol red) with microorganisms changed their color as a result of a pH change from yellow to crimson red. The obtained data was processed using Microsoft Office Excel 2012 spreadsheets and the Statistica 8.0 software package. Nonparametric methods were used for data processing. To determine the reliability of differences in the groups with mono- and mixed mycoplasmal infection, the $\chi 2$ criterion with the Yates correction was used.

Results. Of the 100 patients examined, 47 had urea- and mycoplasmas. In 24 (51,06 %) cases of 47, U. urealitycum was identified as a monoinfection (group I). In the remaining 23 (48,94 %) samples of both U. Urealyticum and M. Hominis (group II) were detected. Simultaneously, the sensitivity to antibiotics was determined. In group I, sensitivity to tetracyclines (tetracycline and doxycycline) was 100 %, to macrolides (josamycin, clarithromycin and pristinamycin) was also 100 %. Ureaplasma infection was resistant to fluoroquinolones: resistance to ofloxacin and ciprofloxacin was 74 % and 91,3 %, respectively. In group II, sensitivity to tetracycline and doxycycline was 95,7 % and 87 %, respectively. The effectiveness of macrolides is significantly reduced in mixed mycoplasmal infection; the sensitivity was the following: to josamycin – 39 %, to erythromycin and azithromycin – 4,3 %, to clarithromycin – 13 %. Patients of both groups were resistant to fluoroquinolones; resistance to ciprofloxacin was 95,7 % and to ofloxacin 87 %.

Conclusion. In general, ureaplasma and mycoplasma were sensitive to antibiotics of the tetracycline series, since the antibacterial effect of tetracyclines is to suppress the biosynthesis of the bacterial cell protein at the ribosome level inhibiting the initial stage of protein synthesis. High sensitivity to macrolides in monoinfection can be explained by their reversible binding to the 50S subunit of ribosomes, which leads to multiple disturbances of its functions. The development of the resistance of mycoplasmal infection to fluoroquinolones can be associated with mutations in the areas determining the resistance to quinolones.

BIBLIOGRAPHY

1. *Donetskaya, E. G.* Clinical microbiology: manual / E. G. Donetskaya. – Moscow: GEOTAR-Media, 2013. – 236–239 p.

2. *Borkhsenius, S. N.* Mycoplasma / S. N. Borkhsenius, O. A. Chernova, ed. V. M. Chernov. – SPb. : Science, 2012. – 320 p.

3. *Collins, B.* Centres for Disease Control and Prevention. Guidelines for treatment of sexually transmitted diseases. B. Collins // MMWR – Atlanta, 2015. – 34 p.

ISOLATION AND CHARACTERIZATION OF RODENT NERVOUS TISSUE-DERIVED CELL CULTURE

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Tissue-derived cell culture has been widely used in medical research. The ability to produce *in vitro* cultures of neuronal cells has been fundamental to advancing our understanding of nervous system functions. The perspective direction in the treatment of socially significant diseases is the application of cell replacement therapy. Therefore the development of methodological approaches to establishment of viable cell cultures is currently important.

Keywords: cell culture, nervous tissue, morphology, phenotype.

The development of methods of obtaining viable cell cultures of nervous tissue, which can be further used for modeling of pathogenic mechanisms of neurodegenerative diseases, new therapeutic protocol testing and the evaluation of treatment effectiveness are very important and actual up to date. The complexity of neuronal cell culture isolation is determined by the heterogeneity of nervous tissue (neuronal cells, astrocytes, oligodendrocytes, radial glia, ependymal cells, and microglia) and the lack of the standardized protocol of the establishment of cell culture. Therefore the development of methodological approaches to produce the cultures of neuronal cells *in vitro* is currently important and has been fundamental to advancing our understanding of nervous system functions.

Aim. The optimization of the neuronal cell culture isolation method and the estimation of its morpho-phenotypic specific features *in vitro*.

Materials and methods. Neuronal cells were isolated from the rodent brain (n=6) using three protocols: only the mechanical disaggregation of the tissue with the subsequent cultivation of the explants (1) and the combination of mechanical disaggregation with the treatment of the tissue with collagenase I type (2) or trypsin (3). The cell suspension was plated in the concentration of 10^6 cells per well of a 24-well plate, covered partly with fibronectin for improvement of cellular adhesiveness. The cells were cultivated in DMEM-F12 supplemented by 10 % fetal bovine serum, 1 % antibiotic-antimycotic and 1 % L-glutamine at 37 °C under 5 % CO_2 condition. The monitoring of cell cultures morphology and growth *in vitro* was done by a phase contrast microscopy method. The phenotype of the cell cultures was determined using the immunocytochemistry with the immunoperoxidase visualization of nestin, vimentin and nerve growth factor receptor (NGFR).

Results. It was established that the neuronal cell cultures isolated using the mechanical tissue disaggregation protocol formed the multicellular aggregates attached to a substrate on the 4–6th day, with the proceeded cellular growth. The majority of the nerve tissue cells (95 (90÷96) %) prone to the formation of sprouts and further intercellular contacts were observed after a mechanical tissue disaggregation combined with enzymatic treatment with trypsin solution. However, the neuronal cell cultures cultivated in the presence or absence of the special adhesive fibronectin cover did not significantly differ in the adhesive capacity.

It was shown, that the cell cultures obtained from the rodent brain via the mechanical and enzymatic treatment of nervous tissue had higher proliferative activity and colonial growth compared to the cell culture obtained via only the mechanical method. On the 10–14th day of cultivation, the cells acquired the typical morphology of nerve cells, characterized by the formation of neuritis, the tendency to form intercellular contacts, and also the expression of specific markers (vimentin, nestin, and NGFR) after a mechanical tissue disaggregation combined with enzymatic treatment with trypsin solution. The cell cultures were characterized by certain heterogeneity and can include stem / progenitor nerve cells, mature neuronal and glial cells. It was established that the cells isolated from the brain tissue of rodents via mechanical and enzymatic disaggregation revealed the expression of vimentin (93(89÷95) %), nestin (90(87÷91) %) and NGFR (91(89÷93) %) and there were no statistically significant differences in their expression in different isolation protocols.