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Characteristics of cytotoxic effect of decamethoxine in different cell cultures

Objective. *To* study parameters of cytotoxic effect of decametoxine in cell cultures.

Materials and Methods. Cytotoxicity of 0.02 % solution of decamethoxine was evaluated by microtechnique in cultivated cells of human adenocarcinoma of human larynx (HEP-2; Cincinnati strain) and dog kidney (MDCK).

Results and Discussion. Mean cytotoxic dose of decametoxine CD_{50} in HEP-2 cell culture makes up 3.213 and ranges from 2.627 to 3.716 mcg/ml. Therefore, mean MTC value in this culture is 1.563 and ranges from 1.314 to 1.858.

Estimation of cytotoxic action of decametoxine in MDCK cell culture revealed that CD_{50} makes up 12.5 mcg/ml and ranges from 10.51 to 14.87 mcg/ml, MTC ranges from 5.26 to 7.43 mcg/ml. In terms of indices of cytotoxic effect (CD_{50} and MTC), decametoxine is 4.0 times less toxic in MDCK cell culture than in HEP-2 cell culture.

Conclusions. Cytotoxic effect of decametoxine depends on type of the culture, concentration of the drug substance and duration of the drug exposure of the cells.

Key words
decametoxine, quaternary ammonium compounds, cell cultures, cytotoxic action, parameters of cytotoxicity.

Today as well as decades ago, viruses take one of the leading positions in the human pathology causing over 80 % of infectious diseases, which could develop as acute processes with epidemic spreading. Virus uniqueness is determined by their ubiquitarity, pathogenicity, peculiarities of the structure and close connection with metabolism of the infected cells in the course of its reproduction. The role of viruses in the human pathology is growing unceasingly. New viruses are emerging etiologies of which are to be established. There are two approaches to prevention of infectious diseases spreading, in particular virus infections: one of them provides for therapeutic measures. and the other one – prophylaxis. Therapy means, first of all, usage of antiviral medications of etiotropic and pathogenetic action; prophylaxis envisages a set of antiepidemic measures consisting of vaccination, quarantine and disinfection.

Usage of quaternary ammonium compounds (QACs) and disinfectants based on such compounds is one of the prospective approaches in the medical practice to prevent infectious agents spreading [3, 6, 11]. QACs belong to the group of surface-active compounds, they exhibit detergent action, they are freely soluble in water and can reduce surface tension of the cell membranes, and all this determines their potential bactericidal and virulicidal properties. Decametoxine is a typical representative of this group. It is a bis-quaternary ammonium compound (see Fig. 1).

Decametoxine is a highly active semisynthetic agent of fast action that is compounded of a synthetic decamethyl part of a molecule and menthol ether (L-menthol) of peppermint oil [3, 5]. One of the pharmaceutical forms of decametoxine is a 0.02 % sterile aqueous solution of topical administration known under the brand name "Decasan".

Topical administration of decametoxine as inhalation purposed for inhibition of infectious factors, in particular respiratory viruses that provoke exacerbations of bronchial asthma is considered to be promising. However, systematized studies of cytotoxic and virulicidal effects of decametoxine on different viruses have not yet been conducted. That is why

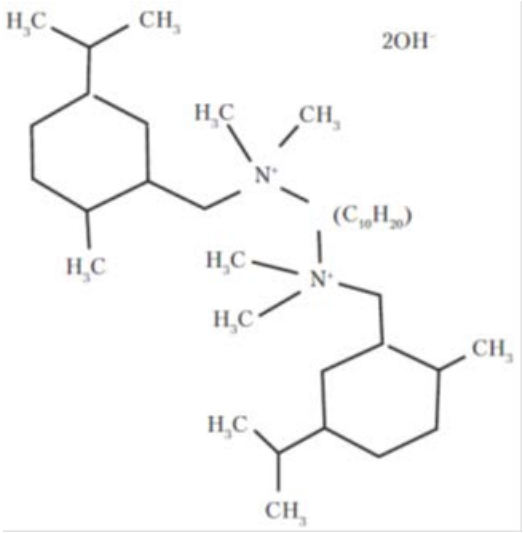

Figure 1. **Structural formula of bis-quaternary ammonium compound**

determination of parameters of decametoxine cytotoxicity in cell cultures is extremely relevant.

Objective — determination of parameters of cytotoxic effect of decametoxine in cell cultures.

Materials and Methods

Parameters of cytotoxic effects of decametoxine on cell monolayer and calculation of CD_{50} and MTC were determined in reinoculated cell cultures by microtechnique in accordance with the requirements of guidelines [10, 11].

Cell cultures. Reinoculated substrate-dependent cell lines of human adenocarcinoma of human larynx (HEP-2; Cincinnati strain) and dog kidney (MDCK) in the form of cryopreserved suspensions were taken from the Bank of Cell Cultures of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR), National Academy of Sciences of Ukraine. Prior to study initiation, cell cultures were adapted to cultivation conditions in 4 passages.

Nutritional media. Growth medium for preparation of cell monolayers of both cell cultures was based on conventional medium - RPMI-1640 - supplemented with fetal bovine serum (FBS) manufactured by Sigma (USA) made up to the final concentration of 5% and antibiotics: penicillin 100 U/ml and streptomycin 100 mcg/ml manufactured by Arterium (Ukraine). Maintenance medium (MM) of the same composition without serum was used for washing out of cell monolayers and virus cultivation.

Substrate-dependent cell cultures were cultivated by the standard method; the cells were removed from the growth surface using Versene solution. Suspensions of HEP-2 and MDCK cell cultures of the following inoculum concentrations: 0.4 · 106 and 0.6 · 106 kl/ml, respectively, in the growth nutritional medium were inoculated in the wells of the culture 96-well plate and incubated for 48 hours at 37 °C under 5 % CO_2 atmosphere until confluent monolayer was established. Its quality was controlled with the use of the inverted microscope. The growth medium was completely removed from the plate's wells and the cell monolayers were washed out two times using MM without serum, after which MM was removed from all the wells.

Cytotoxic effects of decametoxine were examined with the use of its finished 0.02 % solution (Decasan).

In the course of determination of the cytotoxic activity of decametoxine in HEP-2 and MDCK cell cultures, twofold serial dilutions of decametoxine officinal solution from 2^{-1} to 2^{-10} in the maintenance medium were prepared, in which active substance concentrations corresponded to the expected concentrations, namely: 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.780; 0.390; 0 and195 mcg/ml. The obtained solutions in each dilution were applied to four established cell monolayers. Treated cells were incubated for 72 hours at 37 °C under 5 % CO_2 atmosphere. Daily monitoring for the presence of non-specific degradation of cell monolayers was conducted under the inverted microscope. Final results were obtained 72 hours after sample application. Product dilution producing cytotoxic effect in the half of cell monolayers was determined by Karber's method and cytotoxic concentrations were determined in accordance with the guidelines [10].

Results and Discussion

Determination of antiviral activity of the drug products envisages studying of their impact on the virus that interacts with a cell, i.e. is in a certain phase of reproduction (from early to late). At that, a drug product for a long time (on the average from 24 to 72 hours) impacts an infected cell being in low concentrations limited to the maximum tolerable concentration (MTC), which could differ depending on the type of cell cultures. In contrast to antiviral activity, determination of virulicidal activity of disinfectants envisages detection of their influence on a cell-free virus, i.e. completed viral particles located beyond a cell (on

environmental samples, hand skin surface, household items, in biologic fluids etc.). At that, a disinfectant is used in high concentration but the duration of its impact on a cell-free virus is short (on the average from 1 minute to 1 hour).

Cytotoxic concentration (CD_{50}) of decametoxine was defined as a concentration of the active substance (mcg/ml) that causes cytotoxic effect in a half of processed cell monolayers after 72 hours of observation. MTC of decametoxine was determined as the highest concentration of the active substance (mcg/ml), which did not produce cytotoxic effect in any of decametoxine-treated cell monolayers within 72 hours of exposure (according to the data of the vital microscopy).

It has been established that mean cytotoxic dose of decametoxine CD_{50} in HEP-2 cell culture makes up 3.213 and ranges from 2.627 to 3.716 mcg/ml. Therefore, mean MTC value in this culture makes up 1.563 and ranges from 1.314 to 1.858.

Estimation of cytotoxic action of decametoxine in MDCK cell culture revealed that CD_{50} make up 12.5 mcg/ml and ranges from 10.51 to 14.87 mcg/ml. MTC of decametoxine in this cell culture makes up 6.25 respectively and ranges from 5.26 to 7.43 mcg/ml.

Thus, in terms of indices of cytotoxic activity, decametoxine is a rather toxic substance for cell cultures. Similar studies of other authors established that MTC of decametoxine in a reinoculated cell culture of chorioallantoic membrane (CAM) of chick embryo when exposed during 24 hours amounts to 25 mcg/ml; and MTC of decametoxine in HEP-2 proliferous cell culture amounts to 4.0 mcg/ml [2, 7, 8]. Compared to that, MTC of acyclovir – a well-known anti-herpetic product – under the same conditions of exposure of HEP-2 cell culture monolayer amounts on the average to 125 mcg/ml, and MTC of ribavirin – 62,5 mcg/ml.

However, CD_{50} and MTC of decametoxine in MDCK cell culture is 4.0 times higher than in HEP-2 cell culture, which evidences a different sensitivity of the specified cultures to decametoxine: cell culture of human adenocarcinoma of human larynx is 4 times more sensitive to this drug product than the cell culture of dog kidney (see Fig. 2).

So, in both cell cultures, cytotoxic effect of decametoxine was realized completely after 24 hours of exposure and remained unchanged within 48 and 72 hours of observation. Mechanism of cytotoxic activity of decametoxine is related to surface-active properties of its active substance – bis-quaternary ammonium compound that modifies surface tension at interfacial 'cell membrane - culture fluid' and thus, breaks down the lipid layer of cell membranes at long-term cell monolayer exposure to the compound.

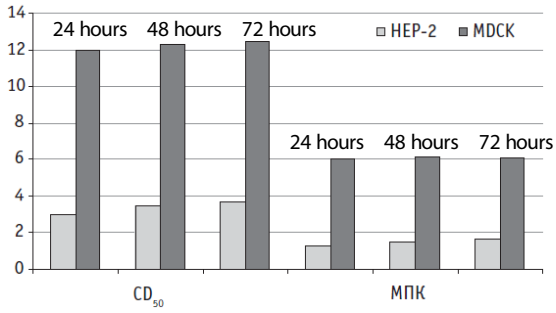

Figure 2. **Cytotoxic activity of decametoxine in HEP-2 and MDCK cell cultures depending on the duration of cell monolayer exposure to it.**

When using decametoxine as a disinfectant or a detergent, it has been confirmed that it as well as other QACs has a variety of advantages. QACs do not irritate the upper respiratory airways, have no strong odor, do not damage structural materials and tissues as well as not discolour them, and have disinfectant and detergent properties. Thus, they can be used in the presence of sick persons and medical staff; they are suitable for preventive and ordinary disinfection [3, 6, 11].

In case of medical application, decametoxine exhibits no local adverse effects, does not irritate mucous membranes, is not absorbed from their surfaces, i.e. there is no risk of systemic adverse reactions. Beside bactericidal activity, decametoxine demonstrates anti-inflammatory activity due to inhibition of serotonin production and exudation reduction [7, 11]; it exhibits desensitizing and antispasmodic effects and has an ability to increase sensitivity of microorganisms to antibiotics. The drug product was efficiently applied in a combined therapy of patients with infectious exacerbation of chronic obstructive pulmonary disease as well as for treatment of pneumonia and bronchial asthma [1, 4, 5, 9]. Difference in results of experimentally determined rather high cytotoxic activity of decametoxine in cell cultures and its harmlessness in case of local clinical application can be explained by different sensitivity of tissues to the drug product and the duration of cell exposure to it. Thus, in case of irrigation of cavities, mucous membranes or application as an inhalation, decametoxine exhibits a short-term effect on tissue cells, i.e. much less than 24

hours, which is insufficient for a cytotoxic activity manifestation.

So, results of the experimental studies on parameters of cytotoxic effects of decametoxine coincide with the literature data and confirm that it exhibits cytotoxic effect in cell cultures that depends on the type of the culture, concentration of the drug substance and duration of cell exposure to it.

Conclusions

1. Mean cytotoxic dose of decametoxine CD_{50} in HEP-2 cell culture makes up 3.213 and ranges from 2.627 to 3.716 mcg/ml. Respectively, mean MTC value in this culture makes up 1.563 and ranges from 1.314 to 1.858.
2. In MDCK cell culture, CD_{50} of decametoxine makes up 12.5 mcg/ml and ranges from 10.51 to 14.87 mcg/ml, MTC makes up 6.25 and ranges from 5.26 to 7.43 mcg/ml.
3. In terms of indices of cytotoxic effect, decametoxine is 4 times less toxic in MDCK cell culture than in HEP-2 cell culture.

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