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Action of 1,2,4-triazole and 1,2,4-triazine derivatives on cells of green monkey kidney in in vitro culture

Działanie pochodnych 1,2,4-triazolu i 1,2,4-triazyny na komórki nerki małpy zielonej w hodowli *in vitro*

The influence of 1,2,4-triazole, 1,2,4-triazine and 5-arylsulfonylamino-1,2,4-triazole derivatives on morphotic elements of green monkey cells in *in vitro* culture was investigated. The compounds under study were added into the culture at the concentrations of 10-400 mcg/ml. The action of chemicals (1-7) was evaluated after 20 and 40 hours. It was found that apart from dilution and cytoplasm vacuolization, they did not show other changes in the studied cells.

From the literature data it follows that triazole and triazine derivatives have pharmacological activity: antiviral, antibacterial, antifungal and anti-inflammatory (1-3, 8, 9). The following compounds were obtained due to the reaction of N^3-derived amidrazones with dimethyl dimethylacetylenedicarboxylate and dimethyl ester of N-arylsulfonyliminodithiocarbonic acid (4-7):

(1) 3-(2-pyridyl)-4-phenyl-5-carboxymethyl-1,2,4-triazole;
(2) 3,4-diphenyl-5-carboxymethyl-1,2,4-triazole;
(3) methyl-2-(5-oxo-3-(2-pyridyl)-4-phenyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-yliden ester of acetic acid;
(4) methyl-2-(5-oxo-3,4-diphenyl-1,4,5,6-tetrahydro-1,2,4-triazine-6-yliden ester of acetic acid;

(5) 3-(2-pyridyl)-4-phenyl-5-4-chlorophenylsulfonamide-1,2,4-triazole;
(6) 3-(2-pyridyl)-4-phenyl-5-4-tollilsulfonamide-1,2,4-triazole;
(7) 3,4-diphenyl-5-4-tollilsulfonamide-1,2,4-triazole.

The structure of the above compounds was confirmed by elemental analysis of infra-red spectrum, nuclear magnetic resonance and mass spectrum. Their purity was tested by means of chromatography. They were also subjected to various tests due to their potential ability to apply as medicines.

The compounds (1-7) were investigated as regards to their antibacterial and antiviral action as well as their influence on central nervous system (CNS). There was found strong antiviral action to VSV virus and the lack of action to CNS and flora of digestive tract in humans (4-7).

Due to positive pharmacological results, their action to cell morphotical elements and the level of their toxicity *in vitro* was evaluated.

MATERIAL AND METHODS

Cultures of green monkey cells were applied in the research. Cells were obtained from Sera and Vaccines Factory in Lublin. Parker's fluid (199) and Eagle's fluid (1959) (MEM) at the ratio 1:1 with the addition of 10% of bovine serum was used as growing medium. After the cells reproduction, growing medium was removed and replaced with medium consisting of Parker's and Eagle's fluids (1:1) without bovine serum. In order to investigate the compounds (1-7), cultures were set in "Plastic Leighton Tubes" by "Costar". After cells reproduction, the medium was removed and replaced with the medium without bovine serum and with addition of proper doses of the particular compounds.

The compounds were dissolved in 2% Tween 80. Dilutions of 10-400 mcg/ml were applied. Cell cultures have been treated with the studied compounds for 20 and 40 hours. Cells were colored with Giemsa's dye. Dried cultures were placed in Canadian balsam. Cells were evaluated using light microscope Jenamed and photographed using MF device (Zeiss Jena).

DISCUSSION

EFFECT OF 1,2,4-TRIAZOLE DERIVATIVES (1, 2)

Substances dissolved in 2% Tween 80 were added into the culture in such a dose as to obtain final concentrations of 200 and 400 mg/cm^3 of

triazole derivatives (1), and 100 and 200 mg/cm^3 of triazole derivatives (2). Cells, after coloring with Giemsa's dye, have been observed in light microscope for 20 and 40 hours because changes were slight after 12 hours as compared with control culture.

Gradual cytoplasm vacuolization was observed 20 hours after substance (1) addition at the dose of 200 mg/cm^3. No other changes in nucleus, cytoplasm, nucleoli nor other morphological elements of cells under study were found. After 40-hour action of the above substance at the dose of 200 mg/cm^3, only further cells vacuolization was observed.

Addition of substance (1) at a larger dose (400 mg/cm^3) into green monkey kidney culture did not cause any other cellular changes than further vacuolization during the following 20 and then 40 hours.

Substance (2) was applied at the doses of 100 and 200 mg/cm^3. Slight dilution of cytoplasm was noted in 20 hours after its addition at the dose of 100 mg/cm^3, and little vacuolization after 40 hours (Fig.1). Larger dose (200 mg/cm^3) did not cause further vacuolization. Other morphotical elements of cells were normal as compared with the control.

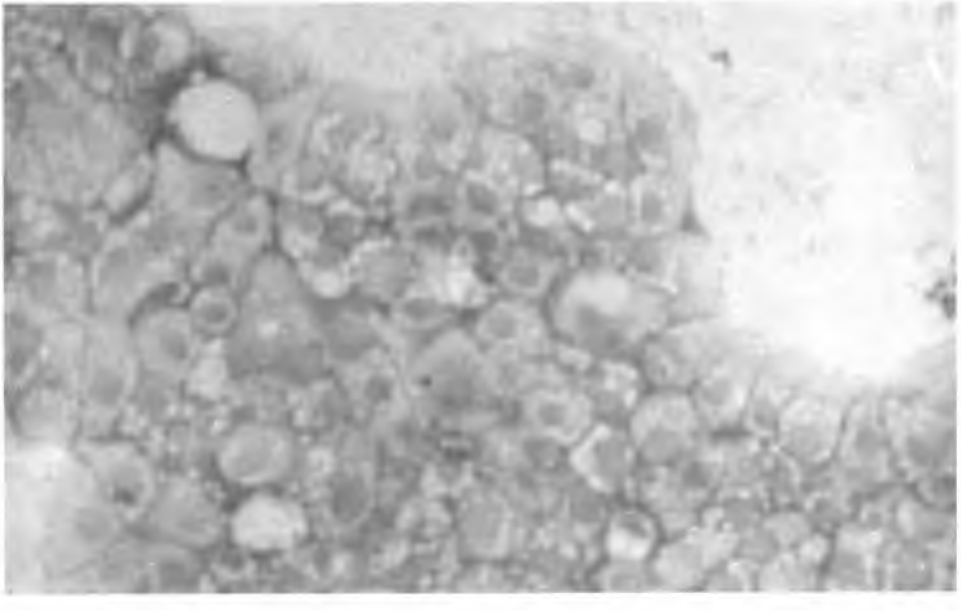

Fig. 1. The cells picture of green monkey kidney in *in vitro* culture after 40 hours' action 1,2,4-triazole derivatives (2) at the dose 400 mcg/cm^3

EFFECT OF 1,2,4-TRIAZINE DERIVATIVES (3, 4)

1,2,4-triazine derivatives (3, 4) were applied at the doses: 10-300 mg/cm^3 of substance (3) and 10-100 mg/cm^3 of substance (4).

Substance (3) used at the concentration of 10 mg/cm^3 caused only little changes in cells during 20 hours. Less coloring of nuclei with 1 or 2 nucleoli was observed as compared with control culture. Also slight vacuolization was visible. Vacuoles were most often fine and not always in the cell nucleus neighborhood.

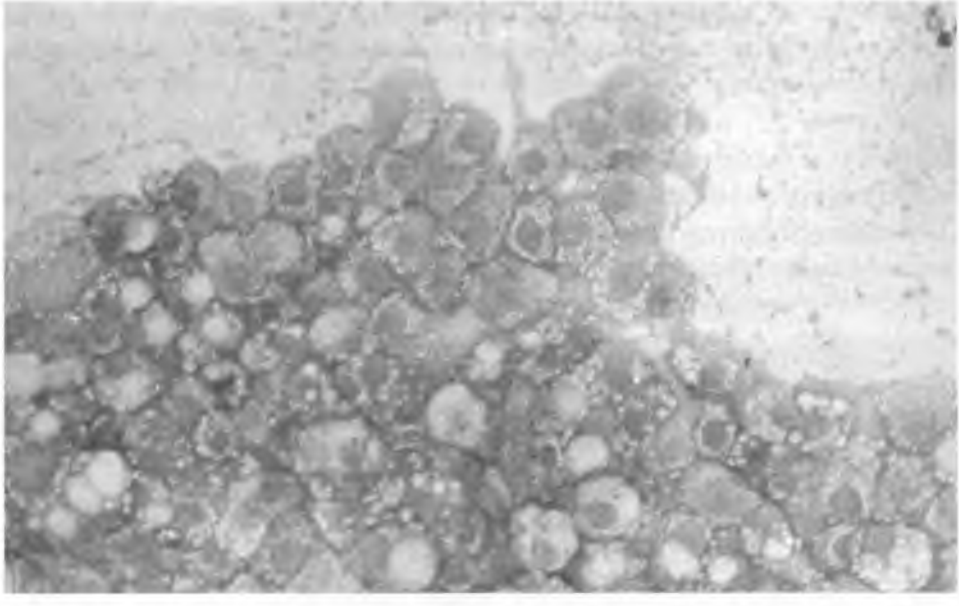

Fig. 2. The cells picture of green monkey kidney in *in vitro* culture after 40 hours' action 1,2,4-triazine derivatives (2) at the dose 400 mcg/cm^3

Substance (4) applied at the concentration of 10 mg/cm^3 only to small extent influenced culture cells (Fig. 2) and caused changes similar to those due to substance (3) at the same dose. It was observed that part of cells possessed grain-like intensively colored cytoplasm, especially in central regions of cell colonies. Larger cells showed cytoplasm vacuolization of different size and different intensity.

Both substance (3) and (4) at the dose of 10 mg/cm^3 displayed only greater cytoplasm vacuolization in the time course after 40 hours. Substances (3) and (4) were also applied at higher concentrations: 300 mg/cm^3 (3) and 100 mg/cm^3 (4). Occurrence of large

number of vacuoles, sometimes forming tracery-like circle around nuclei, was observed in 20 hours after addition into the culture.

Between 20th and 40th hour after substance (3) and (4) addition at the above doses, the cell picture was not changed. Cells placed in outer layer of large colonies showed clear polarization sometimes. Cell nuclei situated in the basic part of cytoplasm adhering to inner cells were surrounded by great amount of fine vacuoles. Amorphous, weakly-dyed form of cytoplasm occurred in its part near free surface of cells.

EFFECT OF 5-ARYLSULFONAMIDE-1,2,4-TRIAZOLE DERIVATIVES (5-7)

Sulfonamide derivatives of triazole (5-7) were applied at the doses of 200 mg/cm^3 and 400 mg/cm^3.

Among the compounds under study, triazole sulfonamide derivatives (5-7) both at small and large doses manifested the weakest action. No changes, except for cytoplasm vacuolization, were observed in cells (Fig. 3). Similar changes were recorded both after 20 and 40 hours.

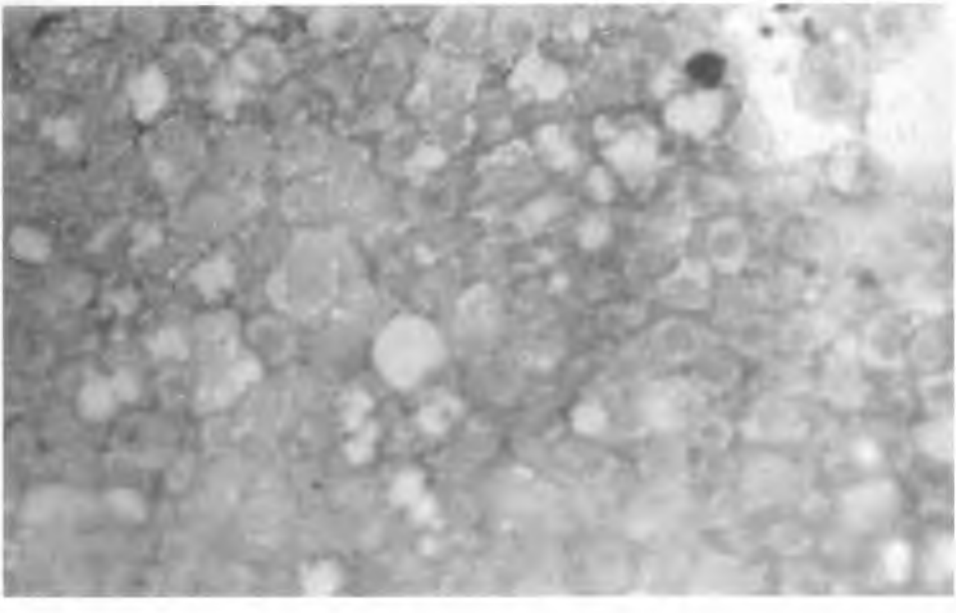

Fig. 3. The cells pictures of green monkey kidney in *in vitro* culture after 40 hours' action 5-arylsulfonamide-1,2,4-triazole derivatives (2) at the dose 400 mcg/cm^3

Cytopathic effect evaluations revealed that even at the dose of 1000 mcg/ml, lysis of the cell under study was not observed.

From the noted changes in the investigated cells due to 1,2,4-triazole, 1,2,4-triazine and sulfonamide-1,2,4-triazole derivatives action it follows that they are little toxic for the cells studied *in vitro* at applied doses.

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STRESZCZENIE

Nowo zsyntetyzowane pochodne 1,2,4-triazolu i 5-okso-1,2,4-triazyny, posiadające silne działanie antywirusowe, nie wykazujące działania na centralny układ nerwowy, zostały zbadane pod kątem działania antybakteryjnego i antygrzybiczego. Stwierdzono, że nie wykazują one wpływu na bakterie i grzyby wchodzące w skład flory fizjologicznej człowieka.