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THE INFLUENCE OF PLANT RAW MATERIALS, CONTAINING ELLAGIC ACID AND SELECTED ANTIBIOTICS ON IMMUNOLOGICAL RESPONSE IN MICE

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Abstract: It is demonstrated that the combined addition to a culture of aqueous extracts from tannin pharmacopoeial raw materials, containing elladic acid (Cortex Quercus, Folium Uvae ursi and Rhizoma Tormentillae) and the antibiotics: cefuroxime, cefoperazone and doxycycline influences the enhancement of survival of mouse thymocytes in cultures with supplementation of hydrocortisone. A cytotoxic test was used for the evaluation. The presented results confirmed that each of the applied aqueous extracts shows a better survival of mouse thymocytes after combined addition of an antibiotic to the culture. The extract from Cortex Quercus showed the highest activation when added to the culture with cefuroxime, whereas cefoperazone best stimulation demonstrated when combined with the extract from Rhizoma Tormentillae, and doxycycline with the extract from Folium Uvae ursi.

Keywords: Cortex Quercus; Folium Uvae ursi; Rhizoma Tormentillae; cefuroxime; cefoperazone and doxycycline

Physiological bacterial flora present in the alimentary tract, upper section of respiratory system and lower section of urinary system are a source of antigens which influence the proper development of immunity (1). Antibiotic therapy may, therefore, essentialy effect on the immunological strengths of the organism.

Accepting the decrease of immunology as a cause of a disease, which opens the infection way, the application of an antibiotic as a compound of antimicroorganism action and selectively toxic, is coming down to the level of symptomatic treatment which is charged with negative effects on immunology of the organism (2).

Doxycycline exerts inhibiting influence on both the antibacterial activity of serum and reaction of delayed oversensitivity (3), and also inhibits the production of peroxides through leucocytes and their degranulation *in vitro* (4).

Immunosuppressive activity of cefalosporins consists in the action on the early phases of proliferation response in the course of immunological reactions, both cell and humoral (5).

Cefoperazone significantly influences the early phase of humoral response, stimulating production of the antibodies of IgM class (6). Another cefalosporin – cefuroxime acts suppresively on the lymphocyte respons, enhancing the spontaneous activation of leucocytes and their chemotaxis, but exerts no significant effect on their fagocytosis and fungicidal activity (7).

Development of research on the immunological mechanisms of the organism and their significance in pathogenesis of numerous diseases has created possibilities of more effective therapy with immunomodulating medicines. The growing number of patients with disturbed immunity and the occurrence of pathogenic agents resistant to the commonly applied antibacterial medicines offer the possibility to apply combined therapy with antibiotics and immunomodulators. Such therapy could counteract, or partly mitigate the disturbances of immunological system caused by antibiotics.

The last years have shown that some naturally occurring substances demonstrate the capability of exerting immunotropic effects. Ellagic acid belongs to such substances. It was found that this acid exerts, depending on dose, an antimutagenic and antitumor action (8) whose mechanism is connected with antioxidative properties and inhibition of microsomal enzymes which metabolize xenobiotics and endobiotics (9, 10).

Ellagic acid demonstrates inhibitory action towards the development of skin and lung tumors in mice (11) and decreases the number of lung tubers in a similar experimental model (12).

In the cytotoxic test carried out in recent years in this Department, it was found that ellagic acid in

a combined action with antibiotics considerably enhanced the survival of thymocytes in mice (13, 14). The aim of this work was to demonstrate that the combined addition to a culture of aqueous extracts from tannin pharmacopoeial raw materials containing ellagic acid (Cortex Quercus, Folium Uvae ursi, Rhizoma Tormentillae) and the antibiotics: cefuroxime, cefoperazone and doxycycline influences the survival of thymocytes in mice in cultures with supplementation of hydrocortisone.

EXPERIMENTAL

Materials

Raw plant materials

Rhizoma Tormentillae, Cortex Quercus and Folium Uvae ursi derived from Zakłady Zielarskie „Kawon" at Gostyń, Poland. From these raw materials, decotions were prepared according to the Polish Ph. VI. Determinations were performed with undilute extracts and those diluted with RPMI. The dilutions used were 10x and 100x.

Reagents

Hydrocortisone (hydrocortisonum hemisuccinatum) (HC), 500 mg ampoules, Jelfa, Poland.

Fetal calf serum, Bioproduct.

Trypan blue, substance, Merck.

Antibiotics

Cefuroxime (Biofuroxim), Institute of Biotechnology and Antibiotics, Warsaw.

Cefoperazone (Cefobid), Institute of Biotechnology and Antibiotics, Warsaw.

Doxycycline (Doxycyclinum), Polfa, Warsaw.

Animals

Experiments were carried out with female mice of Balb-C strain, 4 weeks old, weighing 16 g (accordance of the Ethic Commission number 18/2003).

Methods

Cytotoxic test

In this test, the influence of a given agent on survival of mouse thymocytes in 18-20 h cultures with supplementation of hydrocortisone was evaluated (15). Immature thymocytes possess receptors for steroids on their surface which make them sensitive to the lysis action of hydrocotisone that induces the active process of autodestruction causing apoptosis (16, 17). During maturation and acquiring the immunity, cells lose these receptors and become steroid resistant. Thymocyte cultures are a good experimental model for a fast testing of various agents which can accelerate the maturation of lymphocytes T, thus exerting a thymomimetic action similar to that of thymus hormones.

The test was performed under antiseptic conditions. The thymocytes obtained from fragmental thymuses were three times washed with RPMI-1640 and centrifuged (1800 rpm) for 7-8 min. After the last centrifugation, the thymocytes (4×10^6 cells/mL) were suspended in RPMI supplemented with 10% fetal calf serum. The thymocyte suspension was poured as 1 mL aliquots into antiseptic test-tubes with the addition of 10 μL of the obtained extracts from the raw plant materials. After 1 h, hydrocotisone HC was added in a dose of 50 μg/mL of thymocyte control. The prepared samples were incubated for 18-20 h at 37°C in a CO_2 atmosphere. Afterwards, the number of live and dead cells in each culture was calculated using colouring with 0.4% trypan blue. The results were calculated as the percentage of growth (or decrease) of the live cells in respect to control.

All the obtained results were statistically evaluated with the use of a Medisat (18) computer program, calculating SE – standard error of mean and the significance of differences for two connected samples (% of the relative survival in control is taken as 100%).

RESULTS

In order to get the answer whether the extracts from the raw materials containing ellagic acid in the combined action with antibiotics will act similarly as the acid alone, to the thymocyte culture the following antibiotics were added:

– cefuroxime in a dose of 30.0 μg per mL of culture,

– cefoperazone in a dose of 60.0 μg per mL of culture,

– doxycycline in a dose of 4.0 μg per mL of culture

while selecting the concentration of antibiotics, the literature data were taken into account concerning the cited therapeutic concentration in human serum (19).

The results from Table 1 indicate that the addition of aqueous extracts from Cortex Quercus and Folium Uvae ursi to the thymocytes culture statistically significantly increases the survival of mouse thymocytes.

No essential differences were found concerning the survival of mouse thymocytes in the combined action of cefuroxime with the extract from Rhizoma Tormentillae.

The results given in Table 2 concern the combined action of cefoperazone and the studied aqueous extracts. The best effects of thymocytes was obta-

Table 1. Influence of aqueous extracts from Cortex Quercus, Folium Uvae ursi and Rhizoma Tormentillae and cefuroxime on the viability of mouse thymocytes (% of thymocyte viability in cultures with cefuroxime is 105.7 ± 0.84, n=9)

Dilution of extract	Cortex Quercus	Folium Uvae ursi	Rhizoma Tormentillae
Undiluted	131.3±4.66 n=8 ***	128.4±3.12 n=14 ***	103.9±4.02 n=8
Diluted 10x	119.4±5.06 n=15 **	111.9±3.61 n=8 *	114.0±3.56 n=15 **

Table 2. Influence of aqueous extracts from Cortex Quercus, Folium Uvae ursi and Rhizoma Tormentillae and the medicinal product Cefobid (cefoperazone) on the viability of mouse thymocytes (% of thymocyte viability in cultures with Cefobid is 108.7±6.41, n=8)

Dilution of extract	Cortex Quercus	Folium Uvae ursi	Rhizoma Tormentillae
Undiluted	111.1±6.23 n=10	110.0±2.47 n=16 **	119.4±4.17 n=8 **
Diluted 10x	115.1±4.27 n=10 **	105.0±5.06 n=8	114.1±4.83 n=15 *

Table 3. Influence of aqueous extracts from Cortex Quercus, Folium Uvae ursi and Rhizoma Tormentillae and doxycyline on the viability of mouse thymocytes (% of thymocyte viability in cultures with doxycycline is 99.0±1.53, n=12)

Dilution of extract	Cortex Quercus	Folium Uvae ursi	Rhizoma Tormentillae
Undiluted	112.9±3.78 n=14 **	121.3±3.92 n=16 ***	119.4±4.33 n=8 **
Diluted 10x	107.7±4.34 n=15	116.5±4.37 n=8 **	115.7±4.19 n=23 **

ined in the cultures with the antibiotic and the aqueous extract from Rhizoma Tormentillae.
From the remaining raw materials, Cortex Quercus and Folium Uvae ursi, the survival of thymocytes in the cultures with hydrocortisone did not change.

The results given in Table 3 concern the combined action of doxycycline and the studied aqueous extracts. The best immunological effect for thymocytes was obtained when the extracts of Folium Uvae ursi and Rhizoma Tormentillae were added to the cultures.

The addition of the aqueous extracts of Cortex Quercus to the culture only insignificantly influence the growth of survival of mouse thymocytes in cultures with hydrocortisone.

CONCLUSIONS

The combined addition of cefuroxime and aqueous extracts from raw materials, containing ellagic acid and hydrocortisone to the thymocyte cultures increased the cell survival in case of extracts of Cortex Quercus and Folium Uvae ursi. The addition of extract of Rhizoma Tormentillae did not change the immunotropic activity.

Each aqueous extract, depending on the kind of antibiotic added to the culture, demonstrated the highest survival of mouse thymocytes. For example, the extract from Cortex Quercus showed the highest activation effect after combined addition to the culture with cefuroxime; cefoperazone demonstrated the best action together with the extract from Rhizoma Tormentillae, and doxycycline with the extract from Folium Uvae ursi.

Based on the results obtained, it can be judged that the application of supporting therapy with selected tannin raw materials, beside the appropriate antibiotic therapy, should be advanageous for the immunological system of patients.

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