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In Vitro Study of Cytostatic Activity of Baicalin, Baicalein, and Chlorophyllipt on HeLa-v Cell Line

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Cytostatic activity of baicalin, baicalein, and neogalenical drug Chlorophyllipt was studied *in vitro* on HeLa-v cells. Standard samples of Eucalimin, baicalin, and baicalein, as well as Chlorophyllipt and paclitaxel (reference drug Taxacad) were used. The cell deaths were determined by MTT assay in a Multiskan FC microplate reader with incubator. The effective inhibition concentration (IC_{50}) of the tested substances were: paclitaxel (4.0±0.4 μM)—baicalein (10.5±1.1 μM)—baicalin (16.5±1.7 μM)—sum of euglobals in Chlorophyllipt (24.1±2.5 μM). Chlorophyllipt was found to exhibit cytostatic activity. Cytostatic activity of baicalein, baicalin, and Chlorophyllipt was lower than cytostatic activity of the reference drug by 2.6, 4.1, and 6 times, respectively. The prospects of further evaluation of the synergetic effect of baicalin, baicalein, and chlorophyllipt used in combinations with different cytostatic agents for finding the most effective combination have been shown.

Key Words: *cytostatic activity; baicalin; baicalein; Chlorophyllipt; Taxacad*

Cancer is the one of major causes of death around the world [1]. In oncology, the most frequent types are malignant neo3plasms of the skin, breast, uterus, prostate, and lungs [2].

Chemotherapy is an expensive and long-term process and as a rule, it causes serious side effects [3]. Therefore, many efforts are now focused on the search and development of new drugs with fewer side effects and improvements of the existing treatment protocols [4].

Biologically active substances (BAS) obtained from plants can also be used in chemotherapy of various tumors [5]. Some BAS of plant origin can significantly reduce side effects of chemotherapy, moreover, in case of their simultaneous use with chemotherapeutical agents, the dose of latter can be reduced without any loss of their efficacy [4,6]. Among plant-derived BAS, flavonoids of *Scutellaria baicalensis* roots and euglobals from the plants of *Eucalyptus* genus attracted our attention. Flavonoids of *Scutellaria baicalensis* roots (aglycone baicalein and its glycoside baicalin) exhibit anti-ischemic, antiviral, antibacterial, antileukemic, and cytostatic activities [7]. Furthermore, some foreign authors show synergetic effect of these flavonoids with some cytostatic agents [8]. Euglobals also exhibit antiviral, antibacterial, and cytostatic properties [9-12]. These substances are the components of the following drugs: *Eucalypti* tinctura, neogalenicals Chlorophyllipt and Eucalimin, as well as in pharmacopeial plant raw material *Eucalypti viminalis* folia. It should be noted that cytostatic activity of these drugs is not studied yet.

The aim of this work was to study the cytostatic activity of baicalin, baicalein, and Chlorophyllipt on HeLa-v cells as an *in vitro* model of cervical carcinoma.

MATERIALS AND METHODS

We used Ukrainian pharmacopoeial standards of baicalein and baicalin (content of the main substance

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⩾98%), neogalenical drug Chlorophyllipt 1% ethanol solution for oral and local use (VIFITEKH), Eucalimin standard (pharmacopoeial monograph 42-3606-98, obtained from All-Russian Research Institute of Medicinal and Aromatic Plants), paclitaxel (drug Taxacad; Biokad), a concentrate for preparation of the solution for infusion (6 mg/ml, Biokad).

HeLa-v cells (BioloT) were cultured in DMEM/F-12 (1:1) with L-glutamine and HEPES (15 mM; BioloT), 10% fetal bovine serum (Biosera), and a mixture of penicillin (50-100 U/ml; Sigma-Aldrich) and streptomycin (50-100 μg/ml; Sigma-Aldrich). HeLa-v cells were seeded into 96-well plates (100 μl of cell suspension/well; 50,000 cells/ml) and incubated for 24 h (37°C; 5% CO_2). Then, 100 μl of the tested substance or drug solution at certain dilution were added to the wells for 72 h. After that, the medium was changed for a fresh portion and MTT solution (Sigma-Aldrich) was added to the wells for 2 h. After incubation, the medium was removed, and DMSO solution was added to the wells. Cytostatic activity was evaluated by the percentage of dead cells (Y, %):

$$Y=100-(A_x-A_0)/(A_c-A_0)\times 100,$$

where A_0 is optical density in wells with medium at 540 and 620 nm; A_x is optical density in wells with the test solution at 540 and 620 nm; A_c is optical density in wells with the control solution at 540 and 620 nm.

Effective concentration (IC_{50}, μM) inducing death of 50% cells was determined. Each dilution of the substances studied and control solution was performed in 6 replicates.

The optical density of the test and control samples was measured on a Multiskan FC microplate reader with incubator (Thermo Fisher Scientific) using SkanIt software for a personal computer.

Euglobals in Chlorophyllipt were assayed by reverse phase HPLC on an Agilent 1200 infinity chromatograph (Agilent Technologies), the concentration of euglobals was expressed in mg/ml that recalculated to μM. The chromatography conditions are described in detail in our works [13-15].

Statistical analysis of the results was carried out using Statistica 13.3 (StatSoft, Inc.). Significance of differences between the samples was evaluated by the nonparametric Mann–Whitney test. The differences between the control and test samples were significant at $p\leqslant 0.01$, and between the test samples at $p\leqslant 0.05$.

RESULTS

To determine the initial concentration of the sum of euglobals in chlorophyllipt, we performed reverse-phase HPLC analysis of this drug and Eucalimin standard at 275 nm. The content of euglobals in Chlorophyllipt was 5.3±0.2 mg/ml (equivalent to 0.0121±0.0003 mol/liter) and in Eucalimin standard 3.9±0.1 mg/ml; the sum of peak areas for main euglobals in Chlorophyllipt was greater than in Eucalimin standard (18,890±470 and 13,850±350 mAU×sec, respectively) (n=6; $p<0.05$).

At the next stage, we studied cytostatic activity of the test substances and drugs on HeLa-v cell line. The effective concentrations of the test substances and drugs were: paclitaxel (4.0±0.4 μM)–baicalein (10.5±1.1 μM)–baicalin (16.5±1.7 μM)–sum of euglobals in drug Chlorophyllipt (24.1±2.5 μM) (Fig. 1). The obtained IC_{50} values for the test substances correlate with the data for other cell lines [7-12]. The cytostatic activity of Chlorophyllipt was demonstrated for the first time.

Therefore, further studies of cytostatic activity *in vivo* of baicalin and/or baicalein, extracts from *Scutellaria baicalensis* root, and medicines from *Eucalyptus viminalis* leaves have some prospects as well as the development of drug forms on the basic of these substances. The most promising field is the study of the

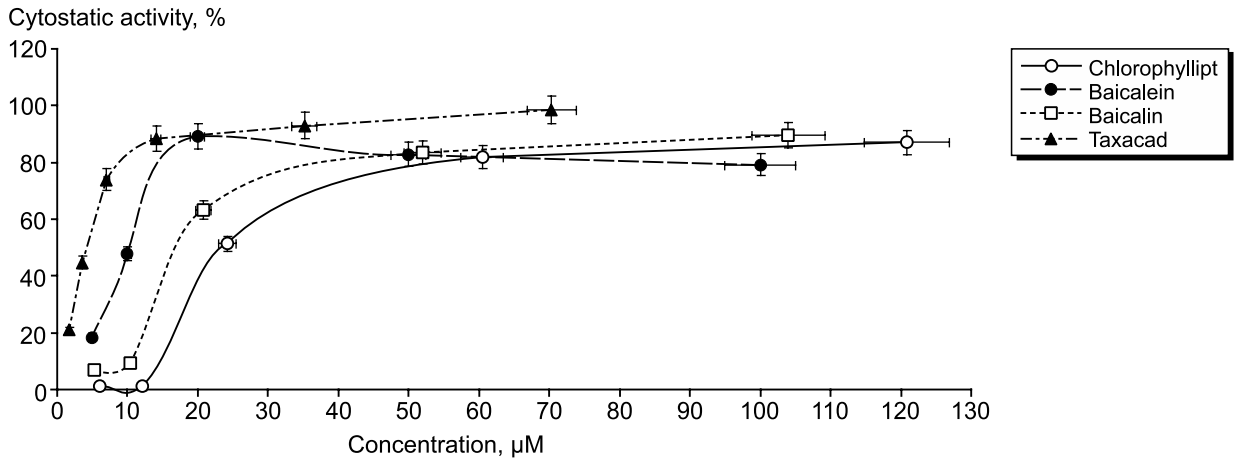

Fig. 1. Cytostatic activity *in vitro* of baicalin, baicalein, Chlorophyllipt, and Taxacad (paclitaxel) on HeLa-v cell line.

synergetic effect of baicalin, baicalein, and euglobals in combinations with paclitaxel or other cytostatic agents for finding the most effective mixture. It can be expected that the use of these substances and appropriate drug forms on the basis of these substances in combinations with common chemotherapeutic agents can reduce the intensity of side effects of chemotherapy (in particular, on liver, kidney, heart, and hematopoietic system) or increase the efficiency of chemotherapy. From the biopharmaceutical point of view, the most reasonable is to develop and study the cytostatic activity on *in vivo* model (tablets, capsules, suppositories, and solutions that contain euglobals, baicalin, and/or baicalein). These drug forms will be the most helpful for the treatment of malignant neoplasms in gynecology, proctology, urology, and gastroenterology.

Thus, cytostatic activity of baicalein, baicalin, and neogalenical drug Chlorophyllipt in respect to cervical carcinoma on *in vitro* HeLa-v cell model is lower than cytostatic activity of the reference drug Taxacad (paclitaxel) by 2.6, 4.1 and 6 times, respectively. Further studies of the synergetic effect of baicalin, baicalein, and Chlorophyllipt in combinations with different cytostatic agents are required for choosing the most effective composition.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018;68(6):394-424. doi: 10.3322/caac.21492
2. The State Of Cancer Care in Russia in 2021. Kaprin AD, Starinsky VV, Shakhzadova AO, eds. Moscow, 2022. Russian.
3. Malhotra V, Perry MC. Classical chemotherapy: mechanisms, toxicities and the therapeutic window. Cancer Biol. Ther. 2003;2(4, Suppl. 1):S2-S4.
4. Zhang RX, Wong HL, Xue HY, Eoh JY, Wu XY. Nanomedicine of synergistic drug combinations for cancer therapy – Strategies and perspectives. J Control Release. 2016;240:489-503. doi: 10.1016/j.jconrel.2016.06.012
5. Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. Front. Pharmacol. 2020;10:1614. doi: 10.3389/fphar.2019.01614
6. Pezzani R, Salehi B, Vitalini S, Iriti M, Zuñiga FA, Sharifi-Rad J, Martorell M, Martins N. Synergistic effects of plant derivatives and conventional chemotherapeutic agents: an update on the cancer perspective. Medicina (Kaunas). 2019;55(4):110. doi: 10.3390/medicina55040110
7. Liu H, Dong Y, Gao Y, Du Z, Wang Y, Cheng P, Chen A, Huang H. The fascinating effects of baicalein on cancer: a review. Int. J. Mol. Sci. 2016;17(10):1681. doi: 10.3390/ijms17101681
8. Lu C, Wang H, Chen S, Yang R, Li H, Zhang G. Baicalein inhibits cell growth and increases cisplatin sensitivity of A549 and H460 cells via miR-424-3p and targeting PTEN/PI3K/Akt pathway. J. Cell Mol. Med. 2018;22(4):2478-2487. doi: 10.1111/jcmm.13556
9. Peng LY, He J, Xu G, Wu XD, Dong LB, Gao X, Cheng X, Su J, Li Y, Zhao QS. Euglobal-IIIa, a novel acylphloroglucinol-sesquiterpene derivative from Eucalyptus robusta: absolute structure and cytotoxicity. Nat. Prod. Bioprospect. 2011;1:101-103. doi: 10.1007/s13659-011-0021-9
10. Soliman FM, Fathy MM, Salama MM, Al-Abd AM, Saber FR, El-Halawany AM. Cytotoxic activity of acyl phloroglucinols isolated from the leaves of Eucalyptus cinerea F. Muell. ex Benth. cultivated in Egypt. Sci. Rep. 2014;4:5410. doi: 10.1038/srep05410
11. Takasaki M, Konoshima T, Etoh H, Pal Singh I, Tokuda H, Nishino H. Cancer chemopreventive activity of euglobal-G1 from leaves of Eucalyptus grandis. Cancer Lett. 2000;155(1):61-65. doi: 10.1016/s0304-3835(00)00406-7
12. Takasaki M, Konoshima T, Kozuka M, Tokuda H. Anti-tumor-promoting activities of euglobals from Eucalyptus plants. Biol. Pharm. Bull. 1995;18(3):435-438. doi: 10.1248/bpb.18.435
13. Boyko N, Pisarev D, Zhilyakova E, Pravlotskaya A, Novikov O, Makarevich N, Kuznietsova V, Sushchuk N. Study and modeling of the distribution process of some phenolic compounds between the solid and liquid phases. J. Adv. Pharm. Technol. Res. 2019;10(2):56-62. doi: 10.4103/japtr.JAPTR_392_18
14. Boyko NN, Zhilyakova ET, Pisarev DI, Novikov OO, Mizina PG, Saybel OL, Sidelnikova MK. A new method for selective extraction of euglobals with monoterpenoid structure from eucalyptus viminalis leaves. Voprosy Biol. Med. Farmatsevt. Khimii. 2019;22(12):12-22. Russian. doi: 10.29296/25877313-2019-12-02
15. Boyko NN, Pisarev DI, Zhilyakova ET, Novikov OO. The comparative analysis of the biologically active substances in tinctura Eucalypti and Chlorophyllipt medicines. Voprosy Biol. Med. Farmatsevt. Khimii. 2018;21(11):16-23. Russian. doi: 10.29296/25877313-2018-11-02