Research Article

Evaluation of Apoptotic Signals in Human Cancer Malignant Melanoma Me45 and Ovarian Carcinoma SKOV3 Cell Death after Chemotherapeutic Reaction with Cisplatin

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Conflict of interest: None.

Abstract：Objective: The aim of this study was to evaluate the influence of chemotherapy with cisplatin (CT-cisplatin) on cell death induction in human metastatic and resistant cancer cells. **Methods**: There were investigated the effects of chemotherapy with cisplatin (10μM). Two lines were used: human metastatic melanoma (Me45) and human ovarian carcinoma cell line resistance to cisplatin (SKOV-3). The cytotoxic effect of cisplatin by viability assay, trypan blue, apoptotic cell death by TUNEL assay and the expression of caspase 3 and 7 by immunocytochemistry were examined. **Results**: Cisplatin induced decreased cellular proliferation proportionally with increasing concentration and longer incubation time. The cytotoxic effect was more significant in melanoma cells. The necrosis was observed in 3% of ovarian carcinoma cells and in 8% of malignant melanoma cells, while apoptosis was observed in 1% of ovarian carcinoma cells and in 39% of malignant melanoma cells in comparison to control untreated cells. The increased expression of caspase 3 and 7 was detected after CT-cisplatin. **Conclusion:** The dose of cisplatin applied in the studies was sufficient to treatment of malignant melanoma. Our studies confirmed the resistance of human ovarian carcinoma to cisplatin.

Keywords: apoptosis; necrosis; cisplatin; ovarian carcinoma; malignant melanoma

Introduction

Each year about 200,000 women are affected by ovarian cancer, and similar number of people is died due to malignant melanoma. The both types of cancer are diagnosed at an advanced stage of the disease, characterized refractory to conventional chemotherapy (CT), and result poor prognosis[1–4]. The first line of therapy is cytoreductive surgery for ovarian cancer and combination of chemotherapy for malignant melanoma. Moreover, the application of chemotherapy is often connected with contrary side effects and the induction of drug resistance. The anticancer chemotherapy is one of the main and effective methods of cancer treatment, which induces apoptosis, necrosis or autophagy of cancer cells. Many chemotherapeutics are widely used and often their combinations, but still there is a need to clarify mechanisms of action in specific cancer types. Cisplatin is widely used anticancer chemotherapeutic in particular in ovarian cancer and also in malignant melanoma. Cis-diamminedichloroplatinum – platinum compound, is included to alkylating antineoplastic agents, interposed to health care in early 70', penetrates the cell membrane by diffusion and by active cooper transporter[5]. It is an inorganic platinum complex with cytostatic attribute; it inhibits DNA synthesis due to cross bonds forming between both single DNA strands, and it has an influence on RNA and protein synthesis in a lesser degree[6]. The actual active form of cisplatin is an electrophilic water complex which is formed intracellular due to replacement chlorine atoms by water; it reacts with DNA unspecific. The selective adsorption place is the guanine nitrogen N-7[5, 7]. However resistance to cisplatin is one of the main problems with chemotherapy in various types of cancer because of cell death process inhibition. The aim of apoptotic program is activation of apoptotic caspases. There are specific proteases that cause cell death by hydrolysis of different structural and functional proteins[8–9]. Caspases are synthesized in the cell as inactive zymogens (Procaspase) and activated by the autoproteolysis or activity of caspases or other proteases. Initiator caspases (caspase-2, 8, 9, 10) which are activated firstly, initiate a cascade effect of caspases (caspase 3, 6, 7) leading to cell death. Their activation requires, however, protein complex, which provides a close arrangement zymogens necessary to autocatalysis or allosterically activates the zymogen without processing caspases[10–11]. The activation of caspases in mammalian cells leads to two well-described routes: route associated with the cell membrane (extrinsic pathway), the mitochondria-dependent pathway (intrinsic pathway) pseudo-receptor pathway, induced by stress and sphingomyelin-ceramide signaling pathway[12–13]. All pathways proceed with different mechanisms but as a result, suicidal cell death is observed. In this multistage process, caspases – relatively lately discovered enzymes (14 enzymes identified in human so far) - play inestimable role[9,14–15]. In chemotherapy the possibility of influence on type of cell death, especially apoptosis induction is very important. It is one of the main research problems of oncologists and biochemists struggling with carcinomas. The cell death type induced by therapy is connected not only to oncological changes removal but also exerts general effects.

The aim of this study was to evaluate the influence of cisplatin (cis-diamminedichloroplatinum) on cell death type (apoptosis/necrosis) and caspases expression induced in human ovarian cancer and malignant melanoma cell lines.

Material and Methods

Cell Culture: Two human malignant cell lines: SKOV-3 and Me45 were used. SKOV-3 is a human ovarian cancer cell line with epithelial morphology. This line is resistant to TNF and many cytostatic drugs including cisplatin, Adriamycin and diphtheria toxin. Me45 was derived from lymphatic node of a 35-years old patient (malignant melanoma metastasis) in Oncology Centre in Gliwice, Poland in 1997. The cells were grown in 25 cm^2 flasks in DMEM (Dulbecco's Modified Eagle Medium, Sigma) with addition of 10% fetal bovine serum (FBS, Lonza) and supplemented by antibiotics (antibiotic/antimycotic solution, Lonza). The cell culture was maintained in a humidified atmosphere at 37 ℃ and 5% CO_2. The medium was changed twice a week. The cells were treated with10 μM cisplatin solution.

Viability Assay: Cells were seeded in 96-well plates at 1×10^4 cells per well and incubated for 24 hours. Then cells were incubated with 10 μM cisplatin (Sigma Aldrich, Poland) solution for 24 and 72 hours.

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After incubation, medium was removed from cells and 0.5 mg/mL MTT (Sigma Chemical Co. St. Louis, MO, USA) was added per well for 2 h. Then formazan crystals were dissolved in 2-propanol and absorbance was measured at 570 nm with a microplate reader (EnSpire, Perkin Elmer. Poland). Each condition was repeated 4 times.

Trypan Blue Staining: The necrosis induction of chemotherapeutic was evaluated by trypan blue (BioRad, Poland) intravitally staining. The test makes use of natural cell membrane attribute which is, as a barrier for anions, cell membrane polarity. After cell death, due to permanent cell membrane damage and gradient charge loss between its inside and outside layer, stain particles, anions in physiological pH, can penetrate the cell interior and color the cytoplasm and/or the nucleus. The cells were seeded on the superforst basic microscopic slides and left overnight. Both cell lines were incubated for 24-hours with 10μM cisplatin (Sigma Aldrich, Poland) solution. Cells were stained after the experiment with trypan blue in PBS (1:1) solution. The reaction lasts 1–2 minutes and was followed by microscopic observations by BX51 microscope (Olympus, Poland).

Apoptosis Detection-TUNEL Assay: This method enables apoptotic cells detection due to its DNA fragmentation. In the TUNEL assay the terminal enzyme deoxynucleotidyl transferase labels the 3–OH ends of DNA activated during apoptosis with biotinylated nucleotides. These fragments are detected by immunoperoxidase staining. The apoptosis detection kit separates apoptosis from necrosis by specifically detecting DNA cleavage and chromatin condensation associated with apoptosis. Experiments were conducted in 8-well slides. Cells with stained nuclei were determined by counting 100 cells in 3 randomly selected fields. The counting was performed twice by two independent investigators. Samples were examined with a light simple microscope (Olympus BX51).

Immunocytochemical Detection of Caspase 3 and 7: Immunocytochemistry was performed using the ABC method. The cultures were fixed and dehydrated using 4% paraformaldehyde during 10 min. The samples were then permeabilized and blocked by incubation with 0.1% Triton X-100 in PBS. The enzymes expression was visualized with polyclonal antibody (1:100, anti-caspase 3 and 7, Santa Cruz, USA). For conventional bright-field microscopy (peroxidase-ABC labelling), the samples were incubated with diaminobenzidine-H_2O_2 mixture to visualize the peroxidase label and counterstained with haematoxylin for 30 sec. The samples were examined with the upright microscope (Olympus BX51). Stained cells were determined by counting 100 cells in randomly selected fields for twice. The result was judged positive if staining was observed in more than 5% of cells. The intensity of immunocytochemical staining was estimated as: (–) negative, (+) weak, (++) moderate and (+++) strong. All experiments were repeated three times.

Results

Viability Assay: Fig. 1 showed the results of Me45 and SKOV-3 cells incubation with cisplatin for 24 and 72 hours. The decrease of cellular viability was observed with increasing cisplatin concentration. The effect was stronger after 72 hour-incubation in particular in metastatic melanoma cells.

Cell Death: After CT-cisplatin the cells were stained by trypan blue. In the Fig. 2 and 3 the results from necrosis detection were presented. The necrosis was observed in 3% of ovarian carcinoma cells and 8% of malignant melanoma cells in comparison to control untreated cells.

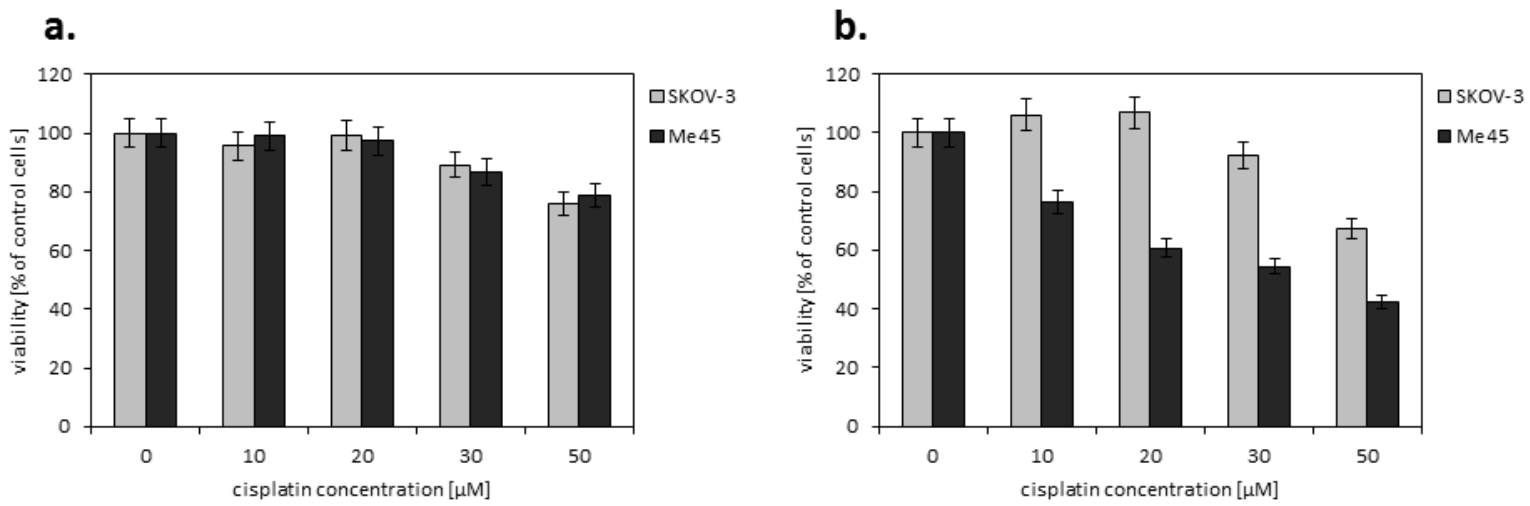

Fig. 1. The viability assay in Me45 and SKOV-3 cells after (**a**) 24-hours and (**b**) 72 hours incubation with 10 μM of cisplatin solution.

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The result from apoptosis detection is presented in the Table 1 and Fig. 3 for both investigated cell lines SKOV3 and ME-45 from TUNEL assay. In the Fig. 3 summary results from TUNEL assay and trypan blue are presented. The SKOV-3 incubated with cisplatin revealed the slightly TUNEL positively (1%) in comparison to untreated control cells. Me45 cell line exposed to cisplatin showed significant positively reaction (control cells – 1%, after incubation with cisplatin – 39% of apoptotic cells) (Fig. 3 and 4).

Immunocytochemical Detection of Caspase-3 and 7: Table 2 and Fig. 5 showed the results from immunocytochemical caspases detection. The expression of caspases 3 and 7 in both cell lines were observed. In the Me45 the expression of caspase-3 is higher (Fig. 5) than in SKOV-3. The similar expression of caspase-7 in both cell lines was revealed (Table 2). It can suggest that in both cell lines the extrinsic and intrinsic pathways were activated. The results evidence, that cisplatin is a chemotherapeutic drug which indicates cell death due to both mechanisms with apoptosis predominance in malignant melanoma cell line and necrosis predominance in ovarian carcinoma cell line (ryc.3, ryc.4).

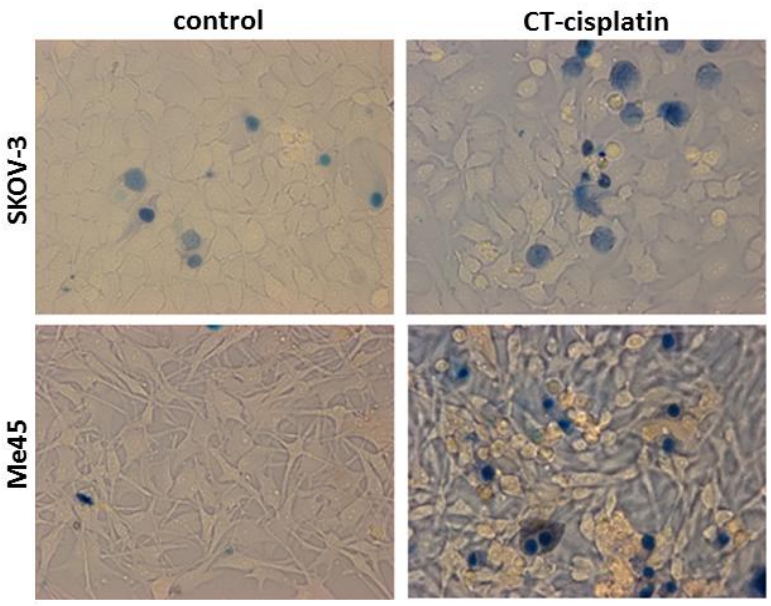

Fig. 2 Trypan blue staining of Me45 and SKOV-3 cells after chemotherapeutic reaction with cisplatin (Magnification ×400).

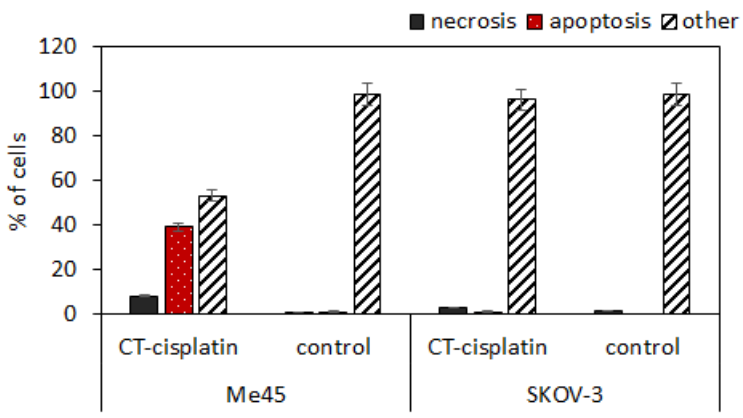

Fig.3 The confrontation of cell death type induced by cisplatin in human cancer cells: malignant melanoma and ovarian cancer. The results were obtained from TUNEL assay and trypan blue staining.

Table 1. Apoptosis evaluation by TUNEL assay in Me45 and SKOV-3 cells after and without 24-hours incubation with 10 μM of cisplatin solution

	SKOV-3		Me45	
	control	cisplatin	control	cisplatin
Apoptotic cells	1%	39%	0	1%

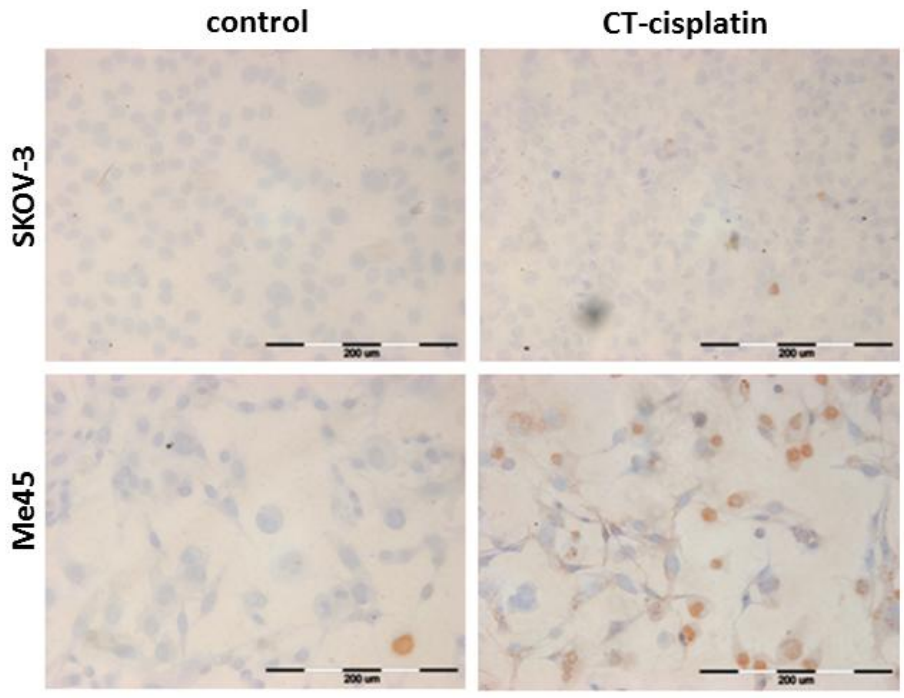

Fig. 4 TUNEL assay in Me45 and SKOV-3 cells with 10 μM of cisplatin solution (Magnification ×400).

Table 2. Immunocytochemistry ABC results in Me45 and SKOV-3 cells – expression of caspases-3 and -7 after and without 24-hours incubation with 10 μM of cisplatin solution

	SKOV-3		Me45	
	control	cisplatin treatment	control	cisplatin treatment
caspase-3	0	16.67% ++	1.15% +	49.75% ++
caspase-7	16% +	30.16 % ++	29.6 ++	33% ++/+++

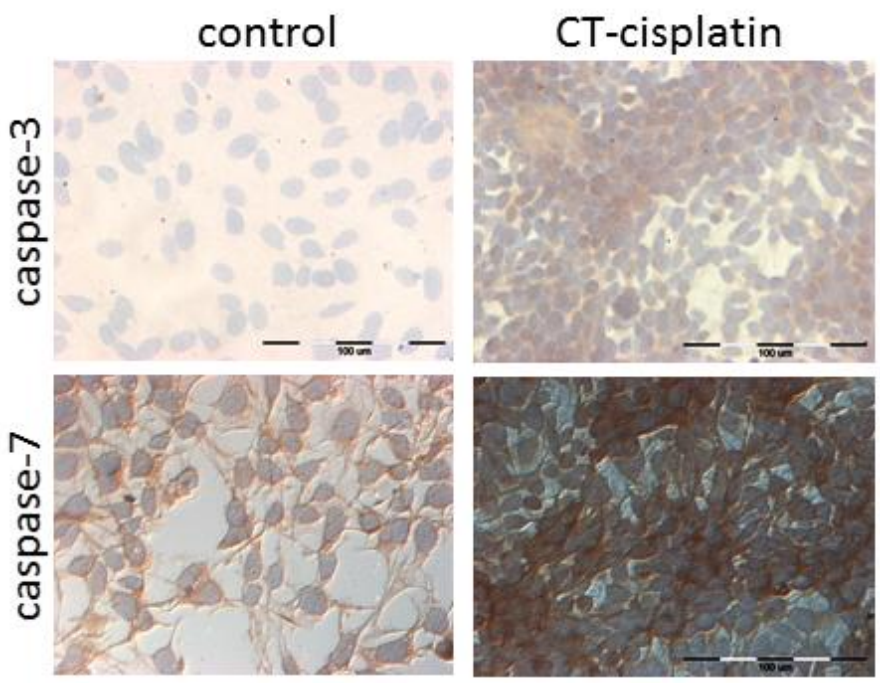

Fig. 5 Immunocytochemical staining of caspase-3 and -7 in Me45 incubation with 10 μM of cisplatin (Magnification ×400).

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Discussion

It is commonly known, that cisplatin-DNA adducts bring outcytotoxic effect due to apoptosis induction[16,17]. Our results showed that the SKOV-3 cisplatin resistant cells underwent necrotic cell death after incubation with cisplatin similarly nonresistant Me45 cells, which was confirmed by other studies[17]. Moreover, it was demonstrated that cell death can occur as apoptosis and necrosis simultaneously after exposition to cisplatin[18,19]. The same effect was observed in our study. Low cisplatin doses used in the chemotherapeutic reaction induced apoptosis, high doses led to necrosis, and intermediate doses can induce both types of cell death[19–21]. We can suppose that the cisplatin dose used in our study was in the intermediate concentration. The number of necrotic cells was examined by trypan blue. Our results showed that low percent of cells died by necrosis (3% for SKOV-3 and 8% for Me45 cells). There was also examined apoptosis by TUNEL assay. We observed that SKOV-3 ovarian cell line is resistant to apoptotic cell death in contrary to malignant melanoma Me45 cells. It is common known that SKOV-3 cells are resistant to cisplatin and do not go apoptosis. Other studies showed that it is possible to activate apoptosis in these cells by using isoliquiritigenin (ISL), a licorice chalconoid. It is a bioactive agent with chemopreventive potential that has been patented for tumor treatment in China. Yuan and all investigated the mechanisms of ISL-induced apoptosis in ovarian carcinoma SKOV-3 cells. Those data indicate that ISL significantly inhibits SKOV-3 cell proliferation through increasing intracellular ROS levels, and causes apoptosis in SKOV-3 cell. ISL induces apoptosis via ER stress-triggered signaling pathways in SKOV-3 cells[22]. Rabik *et al*.[23] investigated the enhanced cytotoxicity in free cell lines: SQ20b head and neck squamous cell carcinoma cell line, SKOV-3 ovarian cancer and A549 non-small cell lung by O6-Benzylguanine (BG). All three lines had an increased DNA damage when BG was added to cisplatin treatment, as an evidence by increased platination and phosphorylated histone H2AX formation. The increase in cisplatin induced DNA damage after treatment with BG plus cisplatin is not sufficient to increase cytotoxicity or apoptosis in A549 cells. Additionally they observed ER-stress response in SKOV-3 cell lines. Apoptosis protease-activating factor-1 (Apaf-1), which plays a crucial role in the formation of the apoptosome, is absent or poorly expressed in a substantial percentage of metastatic melanomas and melanoma cell lines, which are unable to activate caspase-9 and execute the mitochondrial pathway of apoptosis[24]. In our results the malignant melanoma Me45 cells the increasing number of apoptotic nuclear were observed which suggest that this line is nonresistant to cisplatin. The investigations of other researches confirmed our results in metastatic melanoma and also in melanoma cell lines[25]. The authors investigated cisplatin-induced apoptosis in the Apaf-1-positive human metastatic Me665/2/21 melanoma cells. They additionally studied the role of caspase 9, 3 and 7 in apoptotic pathway. These results suggest that caspase-3 and caspase-7 are unessential for apoptosis and, in their cellular model; the point of no return could be out of the mitochondrial cascade[25]. Other studies showed that modulation of apoptotic pathways in human melanoma MeWo cells is drug dependent and highly associated with the drug-resistant phenotype[26].

We have observed in our study the similar expression of caspase 7 in both cell lines. In the Me45 the expression of caspase-3 is higher than that in SKOV-3. It suggests that in both cell lines the extrinsic and intrinsic pathways were activated. Caspases play an important role in the initiation and execution of apoptosis. Devarajan *et al*. observed that caspases-3 mRNA levels in commercially presented total RNA samples from breast, ovarian, and cervical tumors were either invisible (breast and cervical) or substantially decreased (ovarian) and that the sensitivity of caspase-3-deficient breast cancer (MCF-7) cells to undergo apoptosis in response to anticancer drug or other stimuli of apoptosis could be enhanced by restoring caspase-3 expression, suggesting that the loss of caspases-3 expression and function could contribute to breast cancer cell survival[27]. In some cases more than one caspase can be down regulated, contributing to tumor cell promotion and development[28–29]. Caspase-3 seems to be the most important among executioner caspases; it causes chromatin condensation, DNA degradation, decay of many substrates (as vimentin, topoisomerase I, STAT-1 protein, gelsolin). Measurement of its activity enables to evaluate the apoptosis intensity[30]. Other studies showed that cisplatin can activate independent on caspases apoptotic pathway in melanoma cells[4]. Our results can suggest that

metastatic malignant melanoma Me45 is more sensitive to cisplatin than ovarian SKOV-3 cells. We can postulate that it is connected with multidrug resistance event in carcinoma cells and the mechanism of apoptosis. According to investigation of Aleksander *et al*.[31] cisplatin exposure induced in promyelocytic leukemia cells HL-60 extensive expression of BIK gene which is responsible for synthesis proapoptotic BIK protein. It is connected, together with BAD and BID proteins, with modulation of apoptosome functions and also, jointly with BCL-2/x_L through Apaf-1, it enables caspase-9 activation. This enzyme activates procaspase -3 and-7[32].

Summarizing, in Me45 the dominant type of cell death was apoptosis with moderate level of progression permits to using of cisplatin in preclinical and clinical experiments will achieve satisfactory effect, while ovarian cancer is resistant to cisplatin due to probably MDR mechanism. This indicate that cisplatin application in therapy of SKOV-3 is more complicated to very unfavorable general effects.

References

[1] Muggia FM, Braly PS, Brady MF, et al. Phase III randomized study of cisplatin versus paclitaxel versus cisplatin and paclitaxel in patients with suboptimal stage III or IV ovarian cancer: a gynecologic oncology group study[J]. J Clin Oncol, 2000, 18(1): 106–115.

[2] Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study[J]. J Clin Oncol, 2003, 21(17): 3194–3200.

[3] Mousavi-Shafaei P, Ziaee AA, Zangemeister-Wittke U. Increased cytotoxicity of cisplatin in SK-MEL 28 melanoma cells upon down-regulation of melanoma inhibitor of apoptosis protein[J]. Iran Biomed J, 2009, 13(1): 27–34.

[4] Del Bello B, Valentini MA, Comporti M, et al. Cisplatin-induced apoptosis in melanoma cells: role of caspase-3 and caspase-7 in Apaf-1 proteolytic cleavage and in execution of the degradative phases[J]. Ann N Y Acad Sci, 2003, 1010: 200–204.

[5] Brunton LL, Lazo JS., Parker KL. Farmakologia Goodmana&Gilmana (Pharmacology of Goodman & Gilman)[M]. Krzemiński TF, Buczko W, Czuczwar SJ. Lublin: Sientific Edition of Polisj Edition,2007, Vol. I: 1428–1431.

[6] Podlewski J, Chwalibogowska-Podlewska A, Leki współczesnej terapii (Pharmaceuticals of modern therapy), 2009 - Encyklopedia dla Lekarzy i Farmaceutów, (Guide for Physicians and Pharmacists)[M]. Warszawa: Medical Tribune Polska, 2009 Special edition: 185–186.

[7] Muschler E, Geisslinger G, Kroemer HK, Ruth P, Schäfer-Korting M. Farmakologia i Toksykologia (Pharmacology and Toxicology)[M]. Buczko W, Elsevier URBAN&PARTNER. Wrocław: Scientific ed. of Polish Edition, 2013,Vol. III,: 964–965.

[8] Jiang X, Wang X. Cytochrome C-mediated apoptosis[J]. Annu Rev Biochem, 2004, 73: 87–106.

[9] Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level[J]. Nat Rev Mol Cell Biol, 2008, 9(3): 231–241.

[10] Bao Q, Shi Y. Apoptosome: a platform for the activation of initiator caspases[J]. Cell Death Differ, 2007, 14(1): 56–65.

[11] Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death[J]. Nat Rev Mol Cell Biol, 2007, 8(5): 405–413.

[12] Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis[J]. Cell Death Differ, 1999, 6(2): 99–104.

[13] Green DR, Llambi F. Cell Death Signaling[J]. Cold Spring Harb Perspect Biol, 2015, 7(12): .

[14] Martin MC, Allan LA, Lickrish M, et al. Protein kinase A regulates caspase-9 activation by Apaf-1 downstream of cytochrome c[J]. J Biol Chem, 2005, 280(15): 15449–15455.

[15] Schafer ZT, Kornbluth S. The apoptosome: physiological, developmental, and pathological modes of regulation[J]. Dev Cell, 2006, 10(5): 549–561.

[16] Gonzalez VM, Fuertes MA, Alonso C, et al. Is cisplatin–induced cell death always produced by apoptosis? [J]. Mol Pharmacol, 2001, 59(4): 657–663.

[17] Cepeda V, Fuertes MA, Castilla J, et al. Biochemical mechanisms of cisplatin cytotoxicity[J]. Anticancer Agents Med Chem, 2007, 7(1): 3–18.

[18] Montero EI, Pérez JM, Schwartz A, et al. Apoptosis induction

and DNA interstrand cross-link formation by cytotoxic trans-[PtCl2(NH(CH3)2)(NHCH(CH3)2) : cross-linking between d(G) and complementary d(C) within oligonucleotide duplexes[J]. Chembiochem, 2002, 3(1): 61–67.

[19] Sancho-Mart ínez SM, Piedrafita FJ, Cannata-And á JB, et al. Necrotic concentrations of cisplatin activate the apoptotic machinery but inhibit effector caspases and interfere with the execution of apoptosis[J]. Toxicol Sci, 2011, 122(1): 73–85.

[20] Ueda N, Shah SV. Tubular cell damage in acute renal failure-apoptosis, necrosis, or both[J]. Nephrol Dial Transplant, 2000, 15(3): 318–323.

[21] Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis[J]. Am J Physiol, 1996, 270(4 Pt 2): F700–F708.

[22] Yuan X, Yu B, Wang Y, et al. Involvement of endoplasmic reticulum stress in isoliquiritigenin-induced SKOV-3 cell apoptosis[J]. Recent Pat Anticancer Drug Discov, 2013, 8(2): 191–199.

[23] Rabik CA, Fishel ML, Holleran JL, et al. Enhancement of cisplatin [cis-diammine dichloroplatinum (II)] cytotoxicity by O6-benzylguanine involves endoplasmic reticulum stress[J]. J Pharmacol Exp Ther, 2008, 327(2): 442–452.

[24] Ricci MS, Zong WX. Chemotherapeutic approaches for targeting cell death pathways[J]. Oncologist, 2006, 11(4): 342–357.

[25] Del Bello B, Valentini MA, Comporti M, et al. Cisplatin-induced apoptosis in melanoma cells: role of caspase-3 and caspase-7 in Apaf-1 proteolytic cleavage and in execution of the degradative phases[J]. Ann N Y Acad Sci, 2003, 1010: 200–204.

[26] Helmbach H, Kern MA, Rossmann E, et al. Drug resistance towards etoposide and cisplatin in human melanoma cells is associated with drug-dependent apoptosis deficiency[J]. J Invest Dermatol, 2002, 118(6): 923–932.

[27] Devarajan E, Sahin AA, Chen JS, et al. Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance[J]. Oncogene, 2002, 21(57): 8843–8851.

[28] Wang J, Jin Y, Xu Z, et al. Involvement of caspase-3 activity and survivin downregulation in cinobufocini-induced apoptosis in A 549 cells[J]. Exp Biol Med (Maywood), 2009, 234(5): 566–572.

[29] Parrish AB, Freel CD, Kornbluth S. Cellular mechanisms controlling caspase activation and function[J]. Cold Spring Harb Perspect Biol, 2013, 5(6): .

[30] Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases[J]. J Biol Chem, 1999, 274(29): 20049–20052.

[31] Aleksander E, Hibner G, Zajdel A, Paul-Samojedny N, Wilczok A. Ekspresja genów związanych z kaskadą kaspaz w komórkach białaczki promielocytarnej HL-60 eksponowanych na cisplatynę[J]. Farm. Przegl. Nauk., 2010, 10:47–51.

[32] Korzeniewska-Dyl I. Caspases--structure and function[J]. Pol Merkur Lekarski, 2007, 23(138): 403–407.

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