Exp Oncol 2014
36, 3, 184–190

Experimental ONCOLOGY

INCREASE OF ANTITUMOR ACTIVITY OF CISPLATIN USING AGONIST OF GONADOTROPIN-REALISING HORMONE AND INHIBITOR OF AROMATASE ON THE MODEL OF ASCITES OVARIAN TUMOR

I.G. Tkalia[1], L.I. Vorobyova[1], A.N. Grabovoy[1], V.S. Svintsitsky[1], T.O. Tarasova[1], N.Y. Lukyanova[2], I.N. Todor[2], V.F. Chekhun[2]*

[1]*National Cancer Institute, Kyiv 03022, Ukraine*

[2]*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine*

Aim: **To study antitumor activity of triptorelin — agonist of gonadotropin-releasing hormone and exemestane — inhibitor of aromatase in monotherapy and in combination with cisplatin on the model of receptor-positive for estrogens and progesterone malignant ascites transplantable ovarian tumor (TOT), to assess therapeutic pathomorphosis and level of VEGF expression in tumor cells using different combinations of cytostatics and hormonal drugs.** ***Materials and methods.*** **72 female Wistar rats, which underwent intraperitoneal transplantation of ascitic TOT, by $5 \cdot 10^6$ cells per animal, have been involved in the study. Rats were divided into 8 groups, 9 rats in each group. Histological study with assessment of therapeutic pathomorphosis in TOT and immunohistochemical study has been carried out. Survival of animals in the studied groups has been evaluated.** ***Results.*** **Among animals treated in regimen of monotherapy, the most pronounced antiangiogenic activity in TOT has been observed on application of hormonal drugs (triptorelin — 39.4 ± 1.9 and exemestane — 33.9 ± 1.4%; p = 0.003), the highest grade of treatment pathomorphosis in TOT has been observed at treatment with cisplatin (11.7%; p = 0.001). Combination of triptorelin and exemestane has amplified antiangiogenic activity in TOT (12.2 ± 0.9%; p = 0.001), but has not significantly changed rates of pathomorphosis (22.1 ± 0.4%; p=0.005) and survival of animals (32.2%; p = 0.007) as compared with the same rates in rats treated with hormonal drugs in monotherapy. Significant correlation between VEGF expression and pathomorphosis has been established (relative part of viable tumor tissue (RPVTT)) in TOT (r = 0.712; p = 0.001), as well as between RPVTT and life-span of animals (r = −0.320; p = 0.007). However, lack of correlation between VEGF expression in cells of TOT and survival of rats has been determined (r = −0.194; p = 0.11). Combination of cytostatic agent with triptorelin or exemestane has demonstrated significantly high rates of therapeutic pathomorphosis (10.1 ± 0.1% and 16.2 ± 0.3%, respectively) and antiangiogenic activity in TOT (21.4 ± 1.4% and 15.0 ± 1.3%, respectively) as well as the highest survival of animals (100.0%, increase of life-span (ILS) = 231.9% and 85.7%, ILS = 205.8%, respectively) as compared with the same one in rats treated in regimen of monotherapy with cisplatin, triptorelin, exemestane or by combination of hormonal drugs. Among animals treated by combination of cytostatic drug with triptorelin, two were cured, and among rats, which received cisplatin and exemestane, one animal was cured.** ***Conclusions.*** **Triptorelin and exemestane increase antitumor activity of cisplatin in respect to the malignant ascitic TOT and significantly increase survival of animals, especially when triptorelin and cisplatin are used in combination.**

Key Words: **ascites transplantable ovarian tumor, rat, cytostatic, agonist of gonadotropin-releasing hormone triptorelin, inhibitor of aromatase exemestane, pathomorphosis, VEGF, survival.**

INTRODUCTION

The main reason of ineffectiveness of numerous efforts to improve the results of treatment of patients with ovarian cancer (OC) lies in the late diagnostics. Lack of pathognomic symptoms for the early stages of disease complicates this task. The principal difficulty consists in the fact that there is no scientifically substantiated understanding of pathogenesis of OC till present time. The understanding of OC pathogenesis would solve the problem of early diagnostics and opportunity to carry out more effective treatment. Late diagnostics, in turn, and aggressive clinical course of OC, as well as wide variety of histogenetic variants, complicate the study of pathogenesis. However, till present time, significant amount of experimental, epidemiological and clinical facts have been accumulated, which have shown that the leading role in OC pathogenesis is played by the interaction between complicated mechanisms of endocrine-metabolic and molecular-genetic disorders [1–4].

Hormone-dependence of OC is demonstrated by the results of different studies, which have determined that ovaries are not only producing sex steroid hormones, but also are tissue-target for gonadotropin classical and nonclassical steroid hormones, as well as gonadotropin-releasing hormone [5, 6]. Moreover, OC is characterized by the pronounced heterogeneity of molecular-biological markers of tumor cells and their complicated interactions with environmental factors that in combination form significantly variable tumor phenotype [7–9]. It has been proved that OC has high frequency of positive expression of all steroid hormone receptors [10–12]. Upon the impact of the last ones, rate of particular proteins synthesis in target-cells changes that causes the modulation of cellular signal pathways, change of metabolic processes in the cell and, finally, determines the capability of its proliferation, differentiation, apoptosis, adhesion and angiogenesis [2]. Interaction between

Submitted: August 01, 2014
*Correspondence: E-mail: j.tkalya@gmail.com

Abbreviations used: aGn-RH – agonist of gonadotropin-releasing hormone; BC – breast cancer; ERs – estrogen receptors; ICC – immunocytochemical test; IHC – immunohistochemical analysis; ILS – increase of life-span; MLS – mean of life-span; OC – ovarian cancer; PRs – progesterone receptors; RPVTT – relative part of viable tumor tissue; TOT – transplantable ovarian tumor; VEGF – vascular endothelial growth factor.

steroid hormones and many other molecular markers with tumor cell receptors as well as the mechanisms for receptor signals implementation in the cellular nucleus are insufficiently studied at breast cancer (BC) [13–15]. Nowadays, standard diagnostic and therapeutic criteria of hormonal, cytotoxic and target therapy have been determined for this disease [16, 17].

Strategy of treatment of OC patients includes surgical component and chemotherapy. Despite the improvement of surgical treatment strategies and application of modern schemes of chemotherapy [17–21], remote results of treatment of patients with disseminated OC remain unsatisfactory [22]. The matter of hormonal therapy application in this category of patients remains discussable today. Over many years, hormonal treatment has been prescribed empirically as "therapy of despair" for the patients with chemoresistant and relapsing OC, when the other therapeutic methods have sputtered themselves out having low index of effectiveness [3, 5]. National USA standards recommend prescription of agonists of gonadotropin-releasing hormone (aGn-RH) and inhibitors of aromatase for the patients with chemoresistant and relapsing OC as well as debilitated patients [23].

Many studies on OC show that estrogens and androgens cause proliferation of ovarian tumor cells *in vivo* and *in vitro* [24, 25]. Moreover, some authors have determined that positive hormonal receptor status of OC, in particular strong expression of estrogen receptors (ER) and vascular endothelial growth factor (VEGF) are the factors of unfavorable clinical outcome [11, 26–29]. Nowadays, it has been proved that estradiol induces expression of VEGF and its receptors via ER-signal pathways in the cells of reproductive system organs at physiological and tumor angiogenesis [30]. Results of some studies have shown anti-proliferative and apoptotic effect of aGn-RH in ovarian tumor cells *in vivo* and *in vitro* [31]. Moreover, some authors have observed decrease of the level of VEGF and ER expression at treatment of ER-positive OC with inhibitors of aromatase, having noticed decrease of proliferation and apoptosis of tumor cells [32]. However, there are also contradictory scientific data [33–35].

Since no full understanding of mechanisms of realization of hormone-receptor signal in ovarian tumor cells, which launch cascade processes of proliferation, invasion and metastasis has been obtained, specification of these mechanisms will have significance for the substantiation of indication to hormonal therapy not at relapses of OC, but as a component of the primary complex treatment of patients.

Aim of study — to investigate antitumor activity of aGn-RH — triptorelin and inhibitor of aromatase — exemestane in monotherapy and in combination with cytostatic cisplatin on the model of receptor-positive for the estrogens and progesterone malignant ascites transplantable ovarian tumor (TOT), to evaluate pathomorphosis and expression of VEGF in tumor cells using different combinations of cytostatic and hormonal drugs.

MATERIALS AND METHODS

Animals. 72 female Wistar rats (130–150 g b.w.) bred in the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine (Kyiv, Ukraine) have been involved in the experiment. All care procedures have been provided in accordance with the recommendations for conducting the medical-biological studies with use of laboratory animals.

Tumor. Strain of TOT created in 1958 through the transplantation of ovarian tumor from rat, which underwent diaplacental impact of 7.12-dimethylbenz(a)anthracene, has been used. Strain of TOT was obtained from N.N. Blokhin Cancer Research Center (RAMS, Moscow, Russia). After preliminary passage on the 9th day, after intraperitoneal transplantation of TOT, ascitic liquid was taken from one rat and transplanted to 72 rats intraperitoneally by 0.5 ml, diluted with physiological solution and containing by $5 \cdot 10^6$ cells per animal. Transplantable ascitic liquid was immunocytochemically tested for the determination of expression of steroid hormone receptors.

Immunocytochemical test (ICC) has been carried out using monoclonal antibodies specific to the ER — anti-Rat Estrogen Receptor alfa (Clone 6F11, Thermo Scientific, USA), progesterone receptor (PR) — anti-Rat Progesterone Receptor (Clone PR-AT 4.14, Thermo Scientific, USA). For visualization of the results of reaction, Poly Vue system (Thermo Scientific, USA) conjugated with peroxidase has been applied and activity of enzyme has been developed using 3,3-diaminobenzidine as a substrate (Thermo Scientific, USA). After ICC reaction, specimens were stained with Mayer hematoxylin (Sigma, USA). For evaluation of ICC expression of ERs and PRs, semi-quantitative method was applied. When specific nuclear staining for steroid receptors was present, number of immune-positive and immune-negative cells was determined in percentage. Expression of steroid receptors per 1000 tumor cells has been defined in each cytological specimen. For the total assessment of ERs, PRs expression in TOT, method applied by us earlier [10] has been used. Grade of expression of steroid receptors was represented in scores: 0 score — lack of staining of tumor cell nuclei; 1 score — poor staining, $\leqslant$ 10%; 2 scores — moderate grade, 11–50%; 3 scores — high grade, 51–80%; 4 scores — hyperexpression, > 81% of cells. Quantity of stained nuclei of tumor cells more than 10% with moderate and high grade of staining was taken as positive expression of steroid receptors.

Therapy. Cisplatin has been used as solution for injection (0.5 mg/ml in 100 ml). Triptorelin was used in the form of dry substance of triptorelin acetate 0.1 mg+ mannite 10 mg and solvent in the form of sodium chloride 0.9% 1 ml. Exemestane was applied in the form of pills, 1 pill contains 25 mg of exemestane.

Treatment with exemestane was started in the day of transplantation and has been carried out during 10 days, with cisplatin and triptorelin — 48 hours after transplantation during 5 days. Therapeutic drugs were applied to animals in therapeutic doses according

to the current method recommendations and data of previously conducted studies [36–40].

Rats were divided into 8 groups, 9 rats in each group (Table 1).

Table 1. Groups of animals according to treatment regimen

Animals, n = 72	Treatment
Group 1 (C), n = 9	Physiological solution intraperitoneally 0.5 ml during 5 days and orally 0.5 ml for 10 days
Group 2 (P), n = 9	Cisplatin intraperitoneally 1.0 mg/kg – 0.15 mg/rat/per day or 0.3 ml + 0.3 ml of physiological solution/rat/per day during 5 days
Group 3 (T), n = 9	Triptorelin intramuscularly 0.1 mg/rat/per day for 5 days
Group 4 (E), n = 9	Exemestane orally 12.5 mg + 0.5 ml physiological solution/rat/per day during 10 days
Group 5 (P + T), n = 9	Combination of cisplatin and triptorelin in mentioned above doses for 5 days
Group 6 (P + E), n = 9	Combination of cisplatin and exemestane in mentioned above doses
Group 7 (P + T + E), n = 9	Combination of cisplatin, triptorelin and exemestane
Group 8 (T + E), n = 9	Combination of triptorelin and exemestane

Observation has been started in 24 hours after the last injection of exemestane. Died or euthanized animals underwent autopsy for macroscopic evaluation of dissemination of tumor process and pathological changes of internal organs. Cancer-changed organs of peritoneal cavity and pelvis of rats were removed for histological and immunohistochemical studies. In order to evaluate dynamics of tumor process objectively, studied animals were euthanized by one rat from each group on 14th, 17th and 19th day of observation. Moreover, macroscopic study of internal organs in intact rats was carried out for the objective analysis of pathological changes in organs of peritoneal cavity and pelvis of the studied animals.

We judged about antitumor effectiveness of treatment drugs by survival of animals, morphological assessment of treatment pathomorphosis (RPVTT) and the level of expression of VEGF in TOT. Rats' life span was evaluated by a mean of life-span (MLS) and an increase of life-span (ILS) as compared with control. ILS in percentage was calculated by formula:

$$ILS = \frac{MLS_0 - MLS_r}{MLS_r} \times 100\%,$$

where MLS_0 and MLS_r — mean of life-span rat's of trial and control groups, respectively.

Histological analysis. Obtained material was fixed in buffered 10% formalin with pH 7.4 and condensed in paraffin using histoprocessor Histos-5 (Milestone, Italy). From paraffin blocks, histological sections 5 mic thickness were prepared with the help of microtome Microm HM325 (Thermo Scientific, Germany). Sections were stained with hematoxylin-eosin. Analysis of pathomorphosis in tumor tissues was carried out according to K.A. Galakhin [41] determining a relative part of viable tumor tissue (RPVTT) in percentage.

Immunohistochemical analysis (IHC) has been carried out, with use of monoclonal rat antibody specific to VEGF — anti-VEGF Ab-1 RB-222 Polyclonal (Thermo Scientific, USA) as presented early [28]. For visualization of the results of reaction, kit of reagents EnVision system (DakoCytomation, Denmark) was used according to the recommendations of manufacturer. Sections were stained with Mayer hematoxylin. Grade of VEGF expression was evaluated in scores: 0 score — lack of staining of cytoplasm and membrane of tumor cells; 1 score — poor staining, 1–25% of tumor cells (VEGF+); 2 scores — moderate grade of proportional staining, 26–50% of tumor cells (VEGF++); 3 scores — high grade of staining or hyperexpression, more than 50% of tumor cells (VEGF+++) [17]. As positive expression of VEGF (VEGF+) was taken more than 25% of tumor cells with moderate and high immunohistochemical expression. As positive control, monoclonal antibodies against pan-cytokeratines were applied, as negative control — buffered physiological solution, which was placed onto histological sections instead of monoclonal antibodies.

Obtained results were studied and photographed using microscope Nikon Eclipse 80i with camera DS-5SMc/L2 and optical magnification ×400.

Toxic effect of antitumor drugs has been assessed by control of spleen weight and by macroscopic study of pathological changes in liver and kidneys of animals.

Statistical analysis. Processing of the results of study has been carried out using program package STATISTICA 6.0. Parametric statistics including method of Student's *t*-criterion was used. Correlation has been assessed using Pearson pair correlations. Survival of animals has been analyzed by Kaplan — Meier, log-rank criterion was used for paired comparisons, criterion χ^2 — for multiple comparisons between groups. Data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In this article, we will analyze results of study of control animals and rats with TOT of 2–4 groups.

In accordance with IHC study, grade of expression of ERs and PRs in ovarian tumor cells was high — 60% each.

In all animals, which were administered suspension of cells of TOT and which received physiological solution (Group 1, C), strong hemorrhagic ascites was observed (volume of ascitic liquid 18 ± 2.5 ml). Macroscopically, significant tumor changes of parietal and visceral peritoneum, organs of peritoneal cavity and pelvis with invasion of omentum were observed in all rats. Many animals had tumor-like excrescences in serous tissue of intestine. Everting papillary tumor, which 2–5 times exceeded dimensions of normal ovaries of intact rats, with dissemination in corpus uteri and uterine tubes has been discovered in ovaries. Some animals had tumor in anterior abdominal wall. At macroscopic assessment of spleen, liver and kidneys, no significant pathologic changes were determined. Spleen weight was 1119.4 ± 10.1 mg. Histologically, excrescences of adenocarcinoma with broad necrosis, angiomatosis, hemorrhages (Fig. 1) were observed in ovaries and omentum. In most of rats, carcinomatosis of uterus, uterine tubes, mesentery and serous tissue of intestine was observed, in peritoneal wall — single metastases grown through the muscles. Viable parts of tumor are represented in some focuses

by quite large cells with big, moderately dense nuclei and cytoplasm of different grade of basophilia, and, in the other — cells with vacuoles in cytoplasm prevailed, which number significantly varied. RPVTT constituted 50.1 ± 4.7% in average (see Fig. 1; Table 2). At IHC study, irregular expression of VEGF in cells of TOT was determined (see Fig. 1). In parts of tumor consisting of cells with dense basophilic cytoplasm, high expression of VEGF (3+) was observed, among the vacuolated cells — moderate grade of expression of this marker, and in parts of degeneration — low. However, on the whole, grade of expression of VEGF in TOT of rats of the control group was high (77.8 ± 8.3%) (see Fig. 1, Table 2). MLS was 13.7 ± 1.5 days. Since maximal lifespan of rats of the control group was 20 days, in order to compare survival of animals from the other groups in respect to the control group, 20 days were taken as final point of observation interval. For rats of the 1st group, 20-day survival was 17.6% (1 rat), MLS — 13.7 ± 1.5 days (Table 3, Fig. 2, 3). Thus, represented model of TOT is characterized by aggressive clinical course of tumor process.

In animals, which received cisplatin (Group 2, P), less pronounced hemorrhagic ascites was observed as compared with rats of the control group (volume of ascitic liquid was 12.9 ± 5.8 ml). Dissemination of tumor process in organs of peritoneal cavity and pelvis in rats of this group did not differ from the same one as compared with control animals, but grade of invasion, number of tumor focuses and their dimensions were significantly lesser. In some animals, lysis of omentum was observed. In one rat, euthanized on day 17, there was no ascites and apparent involvement of peritoneal cavity, but ovaries were enlarged with everting papillary tumor. Insignificant icteritiousness of liver and kidneys was detected in two animals. Mean spleen weight was decreased by 26.4% (823.9 ± 10.0 mg) and as compared with the same one in control animals had significant difference ($p < 0.05$). Histologically, excrescences of adenocarcinoma had disseminated necrosis, hemorrhages, and strong angiomatosis. Parts with necrosis had abundant leucocytic and lymphocytic infiltration (see Fig. 1). In TOT, cells with vacuolated cytoplasm and nuclear polymorphism prevailed as well as cells with pyknotic nuclei. Sometimes, tumor cells with excrescent forms were observed and they looked like mesenchymal cells. Mean RPVTT in animals of this group was 11.7 ± 0.6% that significantly differed from the same index in control rats ($p = 0.01$). In parts with viable tumor cells, variability of VEGF expression was observed, in some cells, high expression was noted, in the other — poor expression being 58.3 ± 2.6% on average that was significantly lower than the same index of TOT in rats of the control group ($p = 0.01$) (see Table 2). As seen from data of Table 3 and Fig. 2, 3, survival rates of animals of the second group were significantly higher as compared with the same ones of control rats ($p = 0.016$). ILS was 104.4%.

Rats treated with triptorelin (Group 3, T) had volume of ascitic liquid 15.7 ± 6.1 ml, which in most animals was serous-"lactic". Distinctive feature of tumor process in most of the rats was insignificant "adhesive" process, lack of tumor involvement of parietal peritoneum, peritoneal cavity and pelvis, uterus and uterine tubes, atrophy of ovaries with insignificant papillary excrescences. In visual assessment of liver and kidneys, no pathological changes were demonstrable, in one of the animals moderate icteritiousness and flabbiness of liver were noticed. Mean weight of spleen was decreased insignificantly by 9.8% (1009.7 ± 10.0 mg) that had no significant difference in respect to the same one in control animals ($p > 0.05$).

Histologically, multiple necroses and hemorrhages (see Fig. 1) were detected in tumor focuses. Viable tumor tissue was represented in the form of islets. Cells with vacuolated cytoplasm prevailed in tumor tissue, though significant part of cells with proportionally stained basophilic cytoplasm was observed in it, and only small part of them had signs of dystrophic changes with pyknotic nuclei. Mean RPVTT constituted 30.3 ± 0.4% that is significantly lesser as compared with the same one rate in rats of the control group ($p = 0.001$) and significantly higher than in animals of group 2 ($p = 0.01$). Angiomatosis in tumor was observed more rarely in contrast to the TOT of animals in groups 1 and 2. Moderate grade of expression of VEGF in tumor cells was observed (39.4 ± 1.9%) that is significantly lower as compared with the same one in TOT cells of control group and group 2 ($p = 0.001$ and $p = 0.003$, respectively) (see Table 2). Survival of rats in this group was higher, than in control, and lower, than in animals of group 2 (see Table 3, Fig. 2, 3), but statistical analysis has determined lack of significant differences between rates ($p=0.22$ and $p=0.12$, respectively). ILS was 23.4%.

Table 2. Antitumor effectiveness and toxic effect of therapeutic drugs in monotherapy by RPVTT, level of VEGF expression in cells of TOT and change of spleen weight in rats (n = 36)

Group of animals	RPVTT, %	VEGF, %	Spleen weight decrease, %
Group 1 (K), n=9	50.1 ± 1.6	77.8 ± 2.8	–
Group 2 (P), n=9	11.7 ± 0.2*	58.3 ± 2.6*	26.4*
Group 3 (T), n=9	30.3 ± 0.4*	39.4 ± 1.9*	9.8
Group 4 (E), n=9	31.4 ± 0.3*	33.9 ± 1.4*	8.7

Note: *$p < 0.05$ in comparison with the control group.

Animals treated in monotherapy regimen with exemestane (Group 4, E) had volume of ascitic liquid 13.3 ± 5.3 ml, which in most of them was serous-"lactic". The fact was interesting that at macroscopic assessment for organs of peritoneal cavity and pelvis in 6 rats, which died in earlier terms of observation and euthanized on days 14, 17, signs of tumor involvement were absent, ovaries were atrophic. In rat, which was euthanized on day 19, pronounced tumor process in organs of peritoneal cavity and pelvis was observed, but in two animals, which died on day 23 and 24, moderately pronounced tumor involvement was observed. Spleen weight decreased by 8.7% (1022.0 ± 8.4 mg) that differed insignificantly from the same one in intact animals ($p > 0.05$), liver and kidneys had no visual pathological changes. Histologically, in TOT, the same as in previous groups, multiple necrosis and hemorrhages, less pronounced angiomatosis were observed as compared with the same one in TOT of animals in control group and

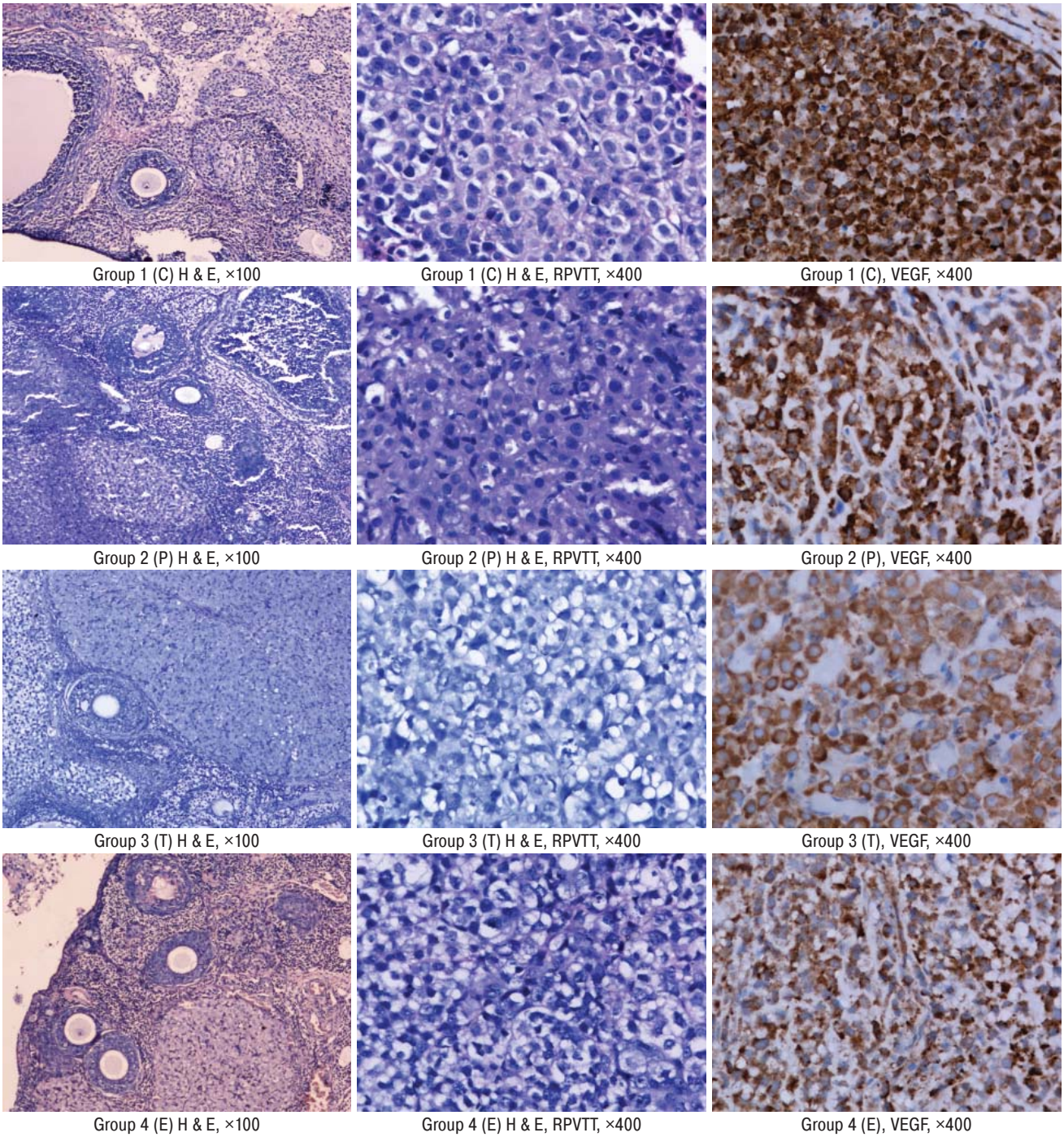

Fig. 1. Microphotography of transplantable tumor in ovaries of animals in the groups 1–4: Group 1 (C) — rats received physiological solution; Group 2 (P) — rats treated with cisplatin; Group 3 (T) — rats received triptorelin; Group 4 (E) — rats received exemestane. RPVTT — relative part of viable tumor tissue, VEGF — expression of VEGF in tumor cells. H & E — hematoxylin-eosin

group 2 (see Fig. 1). Significant part of viable tumor cells had comparatively large sizes, proportionally stained basophilic cytoplasm, large light nuclei. Degenerating cells with enlightened cytoplasm and pyknotic nuclei were found relatively often. Rates of RPVTT and VEGF expression significantly did not differ from the same ones in TOT affected by triptorelin (31.4 ± 0.3% and 33.9 ± 1.4, respectively), but, at the same time, statistical analysis has determined significant differences between these rates and the same rates in rats of groups 1 and 2 ($p = 0.01$) (see Table 2). Survival of animals of this group (30.9 ± 13.9%) also did not differ significantly from the same one in rats, which received monotherapy with triptorelin. It was higher, than in control, and lower, than in animals of group 2, but differences between rates were insignificant ($p = 0.19$ and $p = 0.2$, respectively). ILS was 22.6% (see Table 3, Fig. 2, 3).

Table 3. Survival rates of rats in groups 1–4 (n = 36)

Group of animals	ILS, %	MLS, days	Min LL, days	Max LL, days	OS, %
Group 1 (C) n = 9	–	13.7 ± 1.5	6	20	17.6 ± 13.8
Group 2 (P) n = 9	104.4	28.0 ± 4.2*	16	54	54.8 ± 14.8*
Group 3 (T) n = 9	23.4	16.9 ± 2.1	11	27	37.1 ± 16.3
Group 4 (E) n = 9	22.6	16.8 ± 1.7	10	24	30.9 ± 13.9

Note: Min LL – minimal length of life; Max LL – maximal length of life; OS – 20-day survival; *$p < 0.05$ in comparison with the control group.

Obtained by us variable histological, IHC rates and the results of survival of animals treated with different therapeutic drugs in monotherapy served the basis for carrying out analysis of correlation between RPVTT in TOT, expression of VEGF in cells of tumor with life-

span of rats (Table 4). As seen from Table 4, there is an inverse correlation between survival of animals and rate of RPVTT in TOT (r = –0.320; p = 0.007), as well as direct correlation between RPVTT and expression of VEGF in tumor cells (r = 0.712; p = 0.001), no correlation between survival of animals and VEGF in TOT expression was detected (r = –0.194; p = 0.11).

Table 4. Correlation coefficients significant at $p < 0.05$ between life-span of rats, expression VEGF and RPVTT in TOT (n = 69)

Index	Correlation coefficient	p
Life-span (days) & RPVTT	–0.320	0.007
Life-span (days) & VEGF	–0.194	0.11
RPVTT & VEGF	0.712	0.001

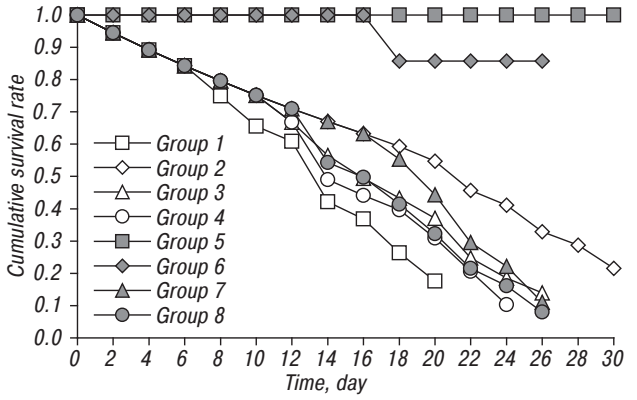

Fig. 2. Overall survival of rats in all groups. Kaplan — Meier curves, p = 0.005

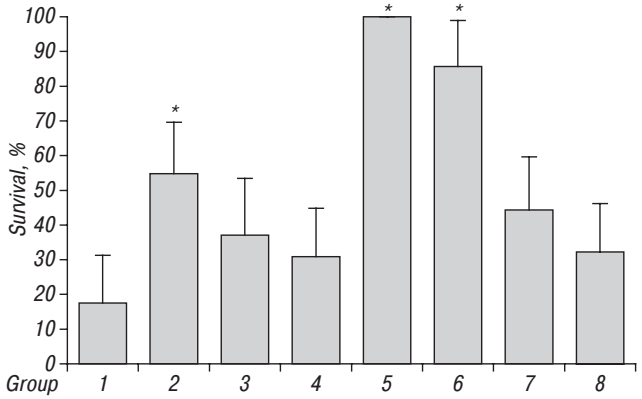

Fig. 3. Overall survival of rats in groups 1–8. *p < 0.05 compared to the control group

Thus, represented model of TOT, with positive hormonal receptor status and high expression of VEGF, had aggressive clinical course that was confirmed by macroscopic assessment of dissemination of tumor process, histological and IHC studies, as well as survival rates of animals. Obtained results have demonstrated effectiveness of cytostatic agents and hormonal drugs in monotherapy. We have shown that not only cisplatin, but also hormonal drugs triptorelin and exemestane in monotherapy caused therapeutic pathomorphosis in TOT that allows to judge indirectly about antiproliferative and apoptotic activity of these drugs [42–45]. However, grade of treatment pathomorphosis in TOT under the effect of cisplatin was significantly higher, than in monotherapy with triptorelin or exemestane. At the same time, hormonal drugs showed significantly higher antiangiogenic activity in TOT as compared with the same one in monotherapy with cytostatic drugs. Survival of rats, which received monotherapy with triptorelin or exemestane, was higher, than in the control group and lower, than in animals treated with cisplatin, but differences between rates had no significance. Represented results are confirmed by lack of correlation between VEGF expression in cells of TOT and survival of animals. At the same time, significant correlation between VEGF expression and treatment pathomorphosis (RPVTT) in TOT, as well as between RPVTT and life-span of rats has been determined. Moreover, we have observed that hormonal drugs had no significant toxic effect as compared with cisplatin.

CONCLUSIONS

In animals with TOT, which received monotherapy with cisplatin, triptorelin or exemestane, most pronounced angiogenic activity in tumor was observed with use of hormonal drugs. However, the highest grade of treatment pathomorphosis in TOT was observed at treatment with cisplatin. Significant correlation between expression of VEGF and treatment pathomorphosis (RPVTT) in TOT, as well as between RPVTT and life-span of animals, has been determined. However, lack of correlation between expression of VEGF in cells of TOT and survival of rats was observed.

Hormonal drugs have no significant toxic effect as compared with cisplatin.

Survival of rats treated with monotherapy using triptorelin or exemestane was higher than in the control group and lower than in animals, which received cisplatin, but differences between rates were not significant.

REFERENCES

1. Disaia PJ, Creasman WT. Clinical Oncologic Gynecology (3) (transl. from Eng. ed. by *EG Novikova*) M.: Rid Elsiver, 2012. 346 p. (in Russian).

2. Vorobyova LI, Svintsitsky VS, Tkalya YG. Hormonal carcinogenesis and rationale for the use of hormonal therapy in the treatment of patients with ovarian cancer. Clin Oncol 2013; **1** (9): 56–64 (in Ukrainian).

3. Urmancheeva AF, Kutusheva GF, Ulrich EA. Ovarian tumors (clinics, diagnostics and treatment). St. Petersburg: N-L, 2012: 68 (in Russian).

4. Vorobyova LI, Tkalia YG. The clinical significance of related hyperplastic processes of endometrium of patients with ovary malignant tumors. Onkologiya 2013; **4** (58): 286–93 (in Ukrainian).

5. King ER, Wong KK. Steroid hormones and ovarian cancer. Steroids — Clinical Aspect edited by Prof. Hassan Abduljabbar, 2011. 166 p. (http://www.intechopen.com/books/steroids-clinical-aspect/steroid-hormones-and-ovarian-cancer).

6. Cunat S, Hoffmann P, Pujol P. Estrogens and epithelial ovarian cancer. Gynecol Oncol 2004; **94** (1): 25–32.

7. Osinsky SP, Vaupel P. Microphysiology tumors. K.: Naukova Dumka, 2009. 256 p. (in Ukrainian).

8. Osinsky SP, Gluzman DF, Cliff J, ***et al***. Molecular diagnosis of tumors: fundamentals and practical applications. Monograph. K.: DIA, 2007. 248 p. (in Ukrainian).

9. Chekhun VF, Sherban SD, Savtsova ZD. Tumor heterogeneity — dynamical state. Onkologiya 2012; **1** (14): 4–12 (in Ukrainian).

10. Tkalia YG, Vorobyova LI, Svintsitsky VS, ***et al.*** Clinical significance of hormonal receptor status of malignant ovarian tumors. Exp Oncol 2014; **36** (2): 125–33 (in Ukrainian).

11. Alonso L, Gallego E, Jesús González F, ***et al.*** Gonadotropin and steroid receptors as prognostic factors in advanced ovarian cancer: a retrospective study. Clin Transl Oncol 2009; **11**: 748–52.

12. García-Velasco A, Mendiola C, Sánchez-Muñoz A, ***et al.*** Prognostic value of hormonal receptors, p53, ki67 and HER2/neu expression in epithelial ovarian carcinoma. Clin Transl Oncol 2008; **10**: 367–71.

13. Ahmed HG, Al-Adhraei MA, Al-Thobhani AK. Correlations of Hormone Receptors (ER and PR), Her2/neu and p53 Expression in Breast Ductal Carcinoma Among Yemeni Women. Open Cancer Immunol J 2011; **4**: 1–9.

14. Faheem M, Mahmood H, Khurram M, ***et al.*** Estrogen receptor, progesterone receptor, and Her 2 Neu positivity and its association with tumour characteristics and menopausal status in a breast cancer cohort from northern Pakistan. Cancer 2012; **6** (283): 1–8.

15. Ayadi L, Khabir A, Amouri H, ***et al***. Correlation of HER2 overexpression with clinicopathological parameters in Tunisian breast carcinoma. World J Surgical Oncol 2008; **6**: 112.

16. Shponka IS, Bondareva VA, Gritsenko PA. The role of tissue markers in assessment of biological properties and prognosis of breast cancer. International Medical Journal. 2005; **2**: 97–104 (in Ukrainian).

17. Perevodchikova NI. Guidelines for chemotherapy of tumors. M.: Practical Medicine, 2011. 512 p. (in Russian).

18. Bookman MA, Brady MF, McGuire WP, ***et al***. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a phase III trial of the Gynecologic Cancer Inter Group. J Clin Oncol 2009; **27**: 1419–25.

19. Bristow RE, Puri I, Chi DS. Cytoreductive surgery for recurrent ovarian cancer: a meta-analysis. Gynecol Oncol 2009; **112**: 265–74.

20. Maksimov SY, Huseynov KD. Targeted therapy in ovarian cancer. Pract Oncol 2010; **11** (4): 267–73 (in Russian).

21. Svintsitsky VS, Vorobyova LI. Malignant ovarian tumors: optimization of complex treatment. Onkologiya 2010; **12** (1): 52–6 (in Ukrainian).

22. Cancer in Ukraine, 2012–2013. Morbidity, mortality, oncology service statistic data. Bulletin of the Ukrainian National Cancer Register 2014; (15): 52–3 (in Ukrainian).

23. NCCN Clinical Practice Guidelines in Oncology Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer. Version 2.2011.

24. Wang PH, Chang C. Androgens and ovarian cancers. Eur J Gynaec Oncol 2004; **25** (2): 157–63.

25. Armaiz-Pena GN, Mangala LS, Spannuth WA, ***et al.*** Estrous cycle modulates ovarian carcinoma growth. Clin Cancer Res 2009; **15**: 2971–8.

26. Ayadia L, Chaabounia S, Khabira A, ***et al.*** Correlation between immunohistochemical biomarkers expression and prognosis of ovarian carcinomas in Tunisian patients. World J Oncol 2010; **1**(3): 118–28.

27. Moghaddam SM, Amini A, Morris DL, ***et al.*** Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer. Cancer Metastasis Rev 2012; **31**:143–62.

28. Tkalia IG, Vorobyova LI, Svintsitsky VS, ***et al.*** Clinical significance of VEGF expression in patients with serous ovarian cancer depending on the tumor hormonal receptor status. Zdorovya jenschiny 2014; **6** (92): 169–77 (in Ukrainian).

29. Chambers SK, Clouser MC, Baker AF, ***et al.*** Overexpression of tumor vascular endothelial growth factor a may portend an increased likelihood of progression in a phase II trial of bevacizumab and erlotinib in resistant ovarian cancer. Clin Cancer Res 2010; **16**: 5320–8.

30. Ferrara N. The role of VEGF in the regulation of physiological and pathological angiogenesis. EXS 2005; **94:** 209–31.

31. Gründker C, Emons G. Role of gonadotropin-releasing hormone (GnRH) in ovarian cancer. Reprod Biol Endocrinol 2003; **1**: 65.

32. Hirakawa H, Yokoyama Y, Yoshida H, ***et al.*** Inhibitory effects of aromatase inhibitor on estrogen receptor-alpha positive ovarian cancer in mice. J Ovarian Res 2014; **7**: 4.

33. Fister S, Schlotawa L, Günthert AR, ***et al.*** Increase of doxorubicin-induced apoptosis after knock-down of gonadotropin-releasing hormone receptor expression in human endometrial, ovarian and breast cancer cells. Gynecol Endocrinol 2008; **24** (1): 24–9.

34. Le Page C, Huntsman DG, Provencher DM, ***et al.*** Predictive and prognostic protein biomarkers in epithelial ovarian cancer: recommendation for future studies. Cancer 2010; **2**: 913–54.

35. Stimpfl M, Tong D, Fasching B, ***et al.*** Prognostic value in breast and ovarian cancer vascular endothelial growth factor splice variants and their. Clin Cancer Res 2002; **8**: 2253–9.

36. Habriev RU. Guide to the experimental (preclinical) study of new pharmacological substances. M.: Medicina, 2005. 832 p. (in Russian).

37. Han J, Zhang S, Liu W, ***et al.*** An analytical strategy to characterize the pharmacokinetics and pharmacodynamics of triptorelin in rats based on simultaneous LC–MS/MS analysis of triptorelin and endogenous testosterone in rat plasma. Analytical and Bioanalytical Chemistry 2014; **406** (9–10): 2457–65.

38. Horvath JE, Bajo AM, Schally AV, ***et al.*** Effects of long-term treatment with the luteinizing hormone-releasing hormone (LHRH) agonist Decapeptyl and the LHRH antagonist Cetrorelix on the levels of pituitary LHRH receptors and their mRNA expression in rats. Proc Natl Acad Sci USA 2002; **99** (23): 15048–53.

39. Zaccheo T, Giudici D, Lombardi P, ***et al.*** A new irreversible aromatase inhibitor, 6-methylenandrosta-1,4-diene-3,17-dione (FCE 24304): antitumor activity and endocrine effects in rats with DMBA-induced mammary tumors. Cancer Chemother Pharmacol 1989; **23** (1): 47–50.

40. Zaccheo T, Giudici D, Ornati G, ***et al.*** Comparison of the effects of the irreversible aromatase inhibitor rexemestane with atamestane and MDL 18962 in rats with DMBA-induced mammary tumors. Eur J Cance 1991; **27** (9): 1145–50.

41. Grabovoy AN, Tarasova TO, Koshubarova MV. Histological evaluation of tumor response to chemo-/radiotherapy. Clin Oncol 2012; **6** (2): 138–43 (in Ukrainian).

42. Kim KY, Choi KC, Auersperg N, ***et al.*** Mechanism of gonadotropin-releasing hormone (GnRH)-I and -II-induced cell growth inhibition in ovarian cancer cells: role of the GnRH-I receptor and protein kinase C pathway. Endocrine-Related Cancer 2006; **13**: 211–20.

43. So WK, Cheng JC, Poon SL, ***et al.*** Gonadotropin-releasing hormone and ovarian cancer: a functional and mechanistic overview. FEBS J 2008; **275** (22): 5496–511.

44. Walker G, MacLeod K, Williams AR, ***et al.*** Estrogen-regulated gene expression predicts response to endocrine therapy in patients with ovarian cancer. Gynecol Oncol 2007; **106** (3): 461–8.

45. Losordo DW, Isner JM. Estrogen and angiogenesis: a review. Arterioscler Thromb Vasc Biol 2001; **21**: 6–12.