DIOXADET AND CISPLATIN ANTI-TUMOR ACTIVITY INCREASE IN HYPERTHERMIC INTRAPERITONEAL CHEMOPERFUSION ON ADVANCED OVARIAN CARCINOMA MODEL

UDC 618.11.001.57–006.6:576.53
Received 14.05.2014

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The aim of the investigation was to study the antitumor activity of Dioxadet and Cisplatin in hyperthermic intraperitoneal chemoperfusion (HIPEC) on a rat model of peritoneal carcinomatosis with ascitic ovarian tumor.

Materials and Methods. 103 female Wistar rats were transfected ovarian carcinoma intraperitoneally, $1 \cdot 10^7$ tumor cells per an animal being injected. After tumor transfection the animals were randomized into 6 groups: group 1 (n=19) — control (0.5 ml normal saline, intraperitoneally); group 2 (n=14) — hyperthermic intraperitoneal perfusion with normal saline (HIPEP); group 3 (n=12) — Cisplatin in normal saline, 4 mg/kg intraperitoneally; group 4 (n=14) — HIPEC with Cisplatin, 20 mg/kg; group 5 (n=19) — Dioxadet in normal saline, 1.5 mg/kg intraperitoneally; group 6 (n=13) — HIPEC with Dioxadet, 15 mg/kg. Antitumor effects were assessed by extended life-span of the animals.

Results. Compared with control, HIPEP with normal saline increased the median survival of rats 2.5 times as much (p=0.008), intraperitoneal injection of Cisplatin and Dioxadet — by 2.2 (p=0.007) and 3.1 times (p=0.001), respectively, HIPEC with Cisplatin and Dioxadet — by 2.8 (p=0.002) and 5.5 times (p<0.001), respectively.

Conclusion. Hyperthermia itself has an antitumor effect on ovarian tumor growth in rats comparable to the effect of intraperitoneal administration of cytostatic agents. The administration of Cisplatin and Dioxadet in HIPEC significantly increases their antitumor activity, HIPEC with Dioxadet having the maximum effect.

Key words: Dioxadet; Cisplatin; hyperthermic intraperitoneal chemoperfusion; HIPEC; ovarian carcinoma.

Hyperthermia is a promising technique in current clinical oncology to improve the efficacy of cancer treatment, it being used in combination with surgical cytoreduction, chemo- and radiotherapy [1–4]. Hyperthermia itself is known to have an antitumor effect, as well as it increases cytotoxicity of anticancer agents and enhance their penetration in tissues; however, the technique has not been included in standard care of cancer patients yet [5–7]. One of the problems preventing the expansion of hyperthermic treatment in clinical practice is equipment deficiency and labor intensity. Public health safety systems of the Netherlands and France recommend hyperthermic intraperitoneal chemoperfusion (HIPEC) as a treatment standard for peritoneal carcinomatosis in colon cancer only; in other European countries and in Russia HIPEC is used as appropriate [2–4].

We consider HIPEC can be used intraoperatively in cytoreductive abdominal operations: as a self treatment method in primary tumors affecting an abdominal wall, as well as a preventive and treatment

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technique in peritoneal carcinomatosis. Anticancer agents used today in HIPEC are characterized by high local and systemic toxicity: pain after injection, abdominal adhesions; leucopenia and thrombocytopenia; nephro-, hepato- and neurotoxicity [8]. To improve the results of treatment and reduce toxicity it is necessary to search for novel anticancer drugs. In N.N. Petrov Research Institute of Oncology (Saint Petersburg, Russia) Dioxadet was developed — an antitumor agent of ethylenimine alkylating compounds. Dioxadet has a high contact anticancer effect and the more favorable profile of toxicity compared to other alkylating cytostatic agents [8, 9].

The aim of the investigation was to study the antitumor activity of Dioxadet and Cisplatin in hyperthermic intraperitoneal chemoperfusion on a rat model of peritoneal carcinomatosis with ascitic ovarian tumor.

Materials and Methods

Animals and tumor strain. The survey was carried out on 103 outbred female Wistar rats weighing 200–260 g cultured in Rappolovo, Russian Academy of Medical Sciences. The animals lived in standard animal facility and were given complete cubed feeding stuff (feed formula PK-120) manufactured by Laboratorkorm (Russia) and tap drinking water without limitation. All experimental animals were cared according to the recommendations of the Ethics Committee of N.N. Petrov Research Institute of Oncology, Ministry of Health of the Russian Federation on animal protection, and in compliance with National guidelines. There was used ovarian carcinoma (OC) strain from N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences. The rats were OC strain transfected by injecting $1 \cdot 10^7$ tumor cells.

The technique of chemoperfusion treatment. For chemoperfusion treatment we used the equipment manufactured by Russian State Scientific Center for Robotics and Technical Cybernetics: Mars — a mechatronic perfusion peristaltic pump; LOIP LB-200 — a thermostating precision water bath; Telets — a universal cybernetic complex for registration and analysis of vital parameters. In addition, we used a warming pan (Microlife FH 80, 30.5×34.5 cm, Germany), digital thermometers CheckTemp (Hanna, Germany), catheters (KD Medical GmbH Hospital Products, Germany), absorbable suture material (Safil 3/0 B/Braun, Germany), non-absorbable suture material (Ethibond Excel 2.0, Johnson & Johnson, USA), pharmaceutical drugs: hemobalance (Nature Vet, Australia), ketoprofen (Flamax®, PharmFirma Sotex, Russia), thiopental sodium (Joint-Stock Company Kurgan Medicines and Products Synthesis, Russia), ceftriaxone (Medochemie Ltd., Ciprus), Cisplatin (Pharmachemie B.V., Netherlands). Dioxadet was synthesized by Chemconsult Company (Russia) in accordance with laboratory standard operating procedures, originality identified by 1H Nuclear Magnetic Resonance (1H-NMR) spectroscopy, infrared spectroscopy, high performance liquid chromatography on Zorbax Eclipse XDB-C 18 4.6×150 mm, 5um (Agilent, USA); Dioxadet content being at least 95%.

The animals were anesthetized with ketoprofen, 5 mg/kg subcutaneously, and intraperitoneal thiopental sodium, 60 mg/kg followed by laparotomy and catheterization, digital thermometers being used as well. An abdominal wall was two-layer sutured using sterile absorbable suture material for muscular tissue, and non-absorbable — for skin. By means of a peristaltic perfusion pump the solution was pumped along the supply line into the thermostatic water bath, where it was heated to 45–46°C to enter the abdominal cavity. Perfusate temperature in the abdominal cavity was 40.5–41.5°C; perfusion taking 45 min, and perfusate volume being 200 ml. During the operation an animal was placed on a warming pan to prevent hypothermia. Abdominal cavity temperature, heart rate and cardiac rhythm were controlled using a digit thermometer, electrode sensors of a universal cybernetic complex for registration and analysis of vital function parameters. Rectal temperature was measured by a digital thermometer. After chemoperfusion, the abdominal cavity was washed with saline solution for 20 min, catheters and thermometers being removed, and the abdominal cavity being two-layer sutured. Immediately after the surgery and 24 h later, 93 mg/kg ceftriaxone was injected intramuscularly, hemobalance 0.1 ml and 10 ml normal saline — subcutaneously.

Experimental groups and antitumor effect assessment. After OC transfection the rats were randomized into 6 groups: group 1 (n=19) — control (intraperitoneal injection of normal saline, 0.5 ml); group 2 (n=14) — hyperthermic intraperitoneal perfusion with normal saline (HIPEP); group 3 (n=12) — Cisplatin in normal saline, 4 mg/kg intraperitoneally; group 4 (n=14) — Cisplatin HIPEC, 20 mg/kg; group 5 (n=19) — Dioxadet in normal saline, 1.5 mg/kg intraperitoneally; group 6 (n=13) — Dioxadet HIPEC, 15 mg/kg. Intraperitoneal injection and HIPEC with anticancer agents were performed using previously established maximum tolerated doses. All procedures were single, and carried out 48 h after OC transfection. The animals were followed up for 90 days. Antitumor effects of the treatment were assessed by the increase in survival of the animals comparing medians survival of rats from different groups.

Data processing. The experimental findings were statistically processed using GraphPad Prism 6, SPSS Statistics 17.0. The survival rate was statistically analyzed using Lilliefors test, the medians survival in the groups — using a nonparametric test (Mann–Whitney U test). P-values of <0.05 were considered significant.

The research was carried out in accordance with the ethical principles established by European Convention for the protection of vertebrata used for experimental and other scientific purposes (the Convention was passed in Strasburg, 18.03.1986, and adopted in Strasburg,

15.06.2006) and approved by Ethics Committee of N.N. Petrov Research Institute of Oncology, Ministry of Health of the Russian Federation.

Results and Discussion. Lilliefors test used to analyze the survival rate showed the distribution not to be normal, therefore rat survival rate in the groups was compared only by median survival.

The effect of antitumor agents injected intraperitoneally and HIPEC on animal survival rate. The increase in median survival in the group of HIPEP with normal saline (group 2) compared to the control (group 1) was 150% (p=0.008). The increase in median survival in groups with intraperitoneal Cisplatin (group 3) and Dioxadet (group 5) was respectively 117% (p=0.007) and 211% (p=0.001) compared to the control (group 1) (See the Table, Fig. 1). The findings strongly indicate hyperthermia itself to have an anticancer effect on OC growth in rats comparable with the effect of intraperitoneal administration of cytostatics.

The mechanisms of tumor cell death in hyperthermia are multifaceted [3, 4, 10, 11]:

1) the increase of vessel cell membrane permeability results in improved medication delivery to tumor cells and impaired transport of ions (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}), free radical oxidation. Ca^{2+} can be involved in hyperthermic cell death. Influxes of extracellular Ca^{2+} stimulate the activity of calmodulin-dependent protein kinases, the formation of inositol triphosphate and other signal cascades;

2) the induction of cytoskeleton disassembly, the increase of tumor pores, the changes of cleavage spindle centrosome organization and protein denaturation result in the formation of multinuclear nonclonal cells, promote anoikis (separation from neighbor cells) and apoptosis;

3) the inhibition of DNA, RNA and protein synthesis. Hyperthermia causes duplex DNA rupture resulted from denaturation and dysfunction of proteins recovering temperature damages such as DNA-polymerase, or due to the sedimentation of denaturated proteins on nuclear chromatin structures raising a barrier preventing enzyme recovery on a damaged area;

4) the induction of the synthesis of heat shock proteins (HSPs), which protect life-saving proteins in healthy cells. In hyperthermic conditions heat shock proteins can activate immune system. The importance of heat shock proteins including HSPs70 and HSPs90 in immune reactions have been demonstrated by different researchers, who showed that tumor cell heating therapy can fulfill a vaccine-like role and elicit anticancer immunity [12].

Cell death intensity from hyperthermia depends on a cell cycle. The cells in S- and M-phases are subjected to slow death, while G1-phase cells undergo prompt death immediately after exposure to hyperthermia. Microscopic analysis of M-phase cells subjected to hyperthermia showed the damage of their mitotic apparatus resulting in failure of mitosis and polyploidy. S-phase cells are also hyperthermia sensitive: after exposure to hyperthermia they appear to have chromosome damage [11].

The effect of Dioxadet and Cisplatin in intraperitoneal and chemoperfusion administration on survival of female rats with ovarian carcinoma

Group	Number of rats in a group	Mean lifespan, days	Median survival, days	95% Confidence interval		Increase of survival compared to control, %
				Lower limit	Upper limit	
Control (group 1)	19	14.40±2.28	9.0	9.6	19.2	—
HIPEP (group 2)	14	29.60±6.13	22.5 p_1=0.005	16.4	42.9	150
i.p. Cisplatin (group 3)	12	22.80±2.41	19.5 p_1=0.007 p_2=0.968	17.5	28.1	117
Cisplatin HIPEC (group 4)	14	34.60±6.48	25.5 p_1=0.002 p_2=0.635 p_3=0.374	20.6	48.6	183
i.p. Dioxadet (group 5)	19	28.50±3.24	28.0 p_1=0.001 p_2=0.511	21.7	35.3	211
Dioxadet HIPEC (group 6)	13	46.20±6.44	49.0 p_1<0.001 p_2=0.038 p_3=0.003 p_4=0.002 p_5=0.020	32.2	60.3	444

N o t e s: p_1 — differences of values are statistically significant compared to group 1; p_2 — compared to group 2; p_3 — compared to group 3; p_4 — compared to group 4; p_5 — compared to group 5.

In Cisplatin (group 4) and Dioxadet (group 6) HIPEC compared to the control (group 1) increase in median survival was 183 (p=0.002) and 444% (p<0.001), respectively; Cisplatin had no significant differences of this value compared to its intraperitoneal administration (group 3) and HIPEP with normal saline (group 2). Dioxadet in HIPEC (group 4) compared to its intraperitoneal administration (group 5) and HIPEP with normal saline (group 2) raised median survival by 75 (p=0.002) and 118% (p=0.038), respectively. The increase in median survival in Dioxadet HIPEC was by 261% higher than in Cisplatin HIPEC (p=0.145) (See the Table, Fig. 1).

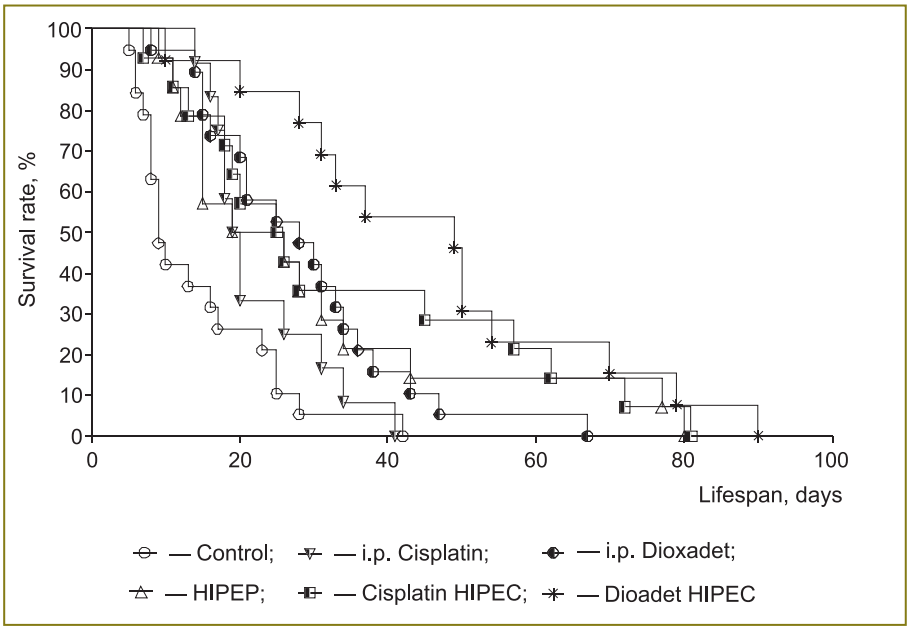

Fig. 1. The effect of Dioxadet and Cisplatin in hyperthermic intraperitoneal chemoperfusion on survival rate of animals with ovarian carcinoma (Kaplan–Meier curves)

The administration of Cisplatin and Dioxadet in HIPEC increases their antitumor activity that is consistent with the data reported in literature, i.e., hyperthermia exhibits synergism with anticancer agents, such as bleomycin, Adriamycin, platinum derivatives [4]. In our studies the highest efficiency of chemoperfusion treatment was found in Dioxadet HIPEC, when the antitumor effect potentiation of hyperthermia and the cytostatic was reached.

The efficiency of intraperitoneal administration of chemotherapeutic drug depends on molar weight of a compound, its lipid and water solubility, AUC value (area under the curve: concentration–time) and other parameters. Dioxadet and a comparative drug (Cisplatin) have a similar mechanism of action. They kill cells at all stages of a cell cycle, interact with nucleophilic groups of DNA, RNA and a protein, form inter- and intramolecular cross-links DNA–protein and DNA–DNA [9, 13]. Molar weight of Dioxadet (M=322.36 g/mol) is slightly higher than that of Cisplatin (M=300.05 g/mol). One of the main distinctions of physicochemical properties between the drugs is lipid and water solubility. The advantage of Dioxadet is its amphiphilic properties, while Cisplatin has nothing else but hydrophilic ones. Therefore, both agents enter a tumor cell in a similar way to make there a high concentration, while on the cell surface and in the space around the cell there is the high content of Dioxadet alone, due to its lipophilicity [13, 14]. AUC value for Cisplatin is 12, for Dioxadet — not applicable. By solubility and molar weight Dioxadet is similar to mitomycin C, which AUC value reaches 80. On this basis we may assume AUC value of Dioxadet to be about the same as that of mitomycin C that explains the higher antitumor effect of Dioxadet compared to Cisplatin if they are administered intraperitoneally.

The analysis of body weight change of the experimental animals during the first 10 days after hyperthermic intraperitoneal perfusion (Fig. 2) showed the maximum decrease of body weight among the rats given Cisplatin HIPEC (till the day 6 after perfusion). Minimum values of relative body weight in groups with Dioxadet HIPEP and HIPEC were the same. The findings can be due to the fact that Cisplatin has the more toxic effect than Dioxadet, therefore a postoperative period in rats with Cisplatin HIPEC was severer.

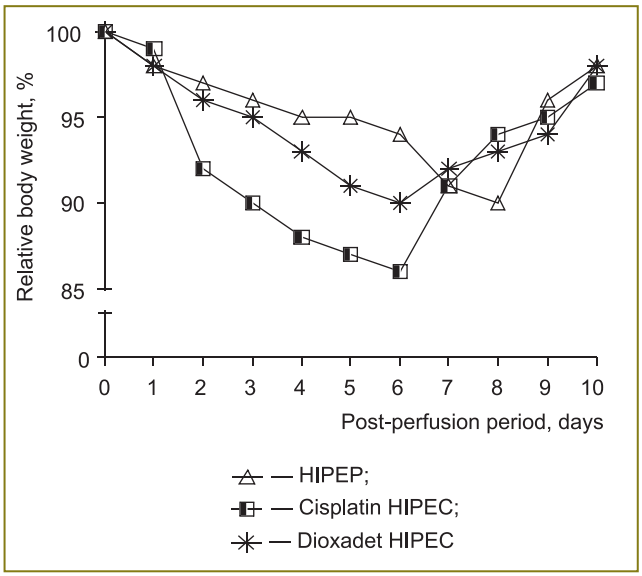

Fig. 2. The change of mean relative body weight of female rats with ovarian carcinoma within 10 days after hyperthermic intraperitoneal perfusion

HIPEC prospects for peritoneal carcinomatosis treatment. The obtained results enable to suggest that Dioxadet HIPEC can significantly improve the efficacy of peritoneal carcinomatosis treatment in patients with ovarian carcinoma. The advantage of Dioxadet administration for intraperitoneal chemotherapy of ovarian carcinoma is its higher efficiency compared to Cisplatin; moreover, Dioxadet causes no abdominal adhesions, while the complication of intraperitoneal injection of platinum compounds is an adhesions formation. In addition, entering the blood flow Dioxadet has only hematotoxicity, while platinum compounds exert hematotoxic, ototoxic, neurotoxic and nephrotoxic effects [9]. Dioxadet HIPEC can be advised for further clinical trials to be introduced into the practice of peritoneal carcinomatosis treatment in patients with an advanced ovarian carcinoma. Based on a broad spectrum of Dioxadet antitumor activity [9] it should be suggested that its high-dose pharmaceutical form can also be successively used in chemoperfusion therapy of peritoneal carcinomatosis caused by colon cancer, gastric carcinoma and other tumors.

Conclusion. Hyperthermic intraperitoneal perfusion with normal saline on the model of peritoneal carcinomatosis in ovarian carcinoma has an antitumor effect comparable to the effect of intraperitoneal administration of alkylating cytostatics of Cisplatin and Dioxadet. In hyperthermic intraperitoneal chemoperfusion Dioxadet has the more significant effect compared to Cisplatin.

Study Funding. The work was supported by the grant of the Ministry of Education and Science of the Russian Federation “The development of innovative treatment technology of disseminated ovarian carcinoma”, Agreement No.8305; Grant of President of the Russian Federation for young researchers and postgraduates carrying out prospective scientific investigations on promising directions of modernization of Russian economy in 2012–2014 “The development of an innovative anticancer drug for chemoperfusion therapy of peritoneal carcinomatosis”; a member of Youth Scientific Innovative Competition (“UMNIK”) of the Ministry of Education and Science of the Russian Federation (Fund for the promotion of the Development of Small Forms of Enterprises in the sphere of Science and Technology; Agreement No.9556p/14193 dated 04.07.2011, No.10810p/16939 dated 13.08.2012, No.141 ГУ1/2013 от 07.10.2013).

Conflict of Interests. The authors have no conflict of interest related to the study.

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