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Comparative Study of Antitumor Efficiency of Intraperitoneal and Intravenous Cytostatics in Experimental Rats with Disseminated Ovarian Cancer

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Antitumor efficiencies of cytostatics dioxadet, cisplatin, mitomycin C, melphalan, and paclitaxel after a single intraperitoneal or intravenous injection in doses of 1.5, 4, 1.5, 2, and 5 mg/kg, respectively, were studied on the model of transplanted ovarian tumor in 124 rats. The antitumor effects were evaluated by the increase in median survival. Dioxadet, cisplatin, and melphalan injected intraperitoneally significantly prolonged the lifespan median – by 79, 88, and 114%, respectively, and were in fact ineffective, when injected intravenously. Intraperitoneal mitomycin C prolonged lifespan median by just 35%, intravenous – by 152%. Paclitaxel injected intraperitoneally and intravenously prolonged the lifespan median by 45 and 81%, respectively.

Key Words: *ovarian cancer; intraperitoneal chemotherapy; antitumor drugs*

More than 225,000 of new cases of ovarian cancer (OC) and 140,000 lethal outcomes of this disease are registered annually all over the world [10]. Standard treatment for disseminated OC is cytoreductive surgery followed by systemic intravenous (i/v) chemotherapy [12]. The results are not enough satisfactory, which necessitates the search for new approaches to treatment. A promising approach to improvement of the efficiency of therapy for OC is intraperitoneal chemotherapy, as OC cells metastasize mainly by the visceral and parietal peritoneum, and intraperitoneal (i/p) injections create several-fold higher drug concentrations in the abdominal cavity than i/v injections [5]. Meta-analysis of the results of treatment of 1819 patients with disseminated OC in 8 independent studies has shown that addition of i/p drugs to the therapeutic protocols improves survival [6]. Intraperitoneal chemotherapy is not yet included in standard treatment protocols for OC, and hence, in clinical studies, i/p injections of antitumor drugs are combined with i/v injections. The advantages of i/p chemotherapy can be proven in experimental studies by direct comparison of antitumor activity of cytostatics injected intraperitoneally or intravenously in the same doses.

We compare the antitumor activity of i/p and i/v cytostatics dioxadet, cisplatin, mitomycin C, melphalan, and paclitaxel on the model of transplanted ascitic OC in female rats.

MATERIALS AND METHODS

The study was carried out on 124 female Wistar rats. The rats were kept and all manipulations on them were carried out in accordance with the standards for handling and use of laboratory animals. The following cytostatics were used: dioxadet (Chemconsult), cisplatin (Teva), mitomycin C (Vero-mitomycin; Lance-Pharm), melphalan (Alkeran; GlaxoSmithKline), and paclitaxel (Taxacad; Biocad). Ascitic OC strain (N. N. Blokhin Russian Cancer Research Center) was used.

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Ascitic ovarian tumor strain served as the disseminated OC model was created by transplantation of OC from a rat exposed to a carcinogen transplacentally. The original histological type of the tumor was metastatic papillary adenocarcinoma; ascitic tumor was used in the experiment [1]. An adequate model of transplanted OC was used, developing in the abdominal cavity similarly as OC at late stages in humans.

On day 7 after i/p transplantation of OC to 4 rats, ascitic fluid was collected from one rat and transplanted i/p to 120 rats (0.5 ml ascitic fluid diluted in saline and containing 1×10^7 cells). The day of tumor transplantation was taken for day 0. After OC transplantation, the rats were divided at random into 12 groups, 10 per group. Two days (48 h) after OC transplantation, the animals were narcotized with ether and received a single i/p or i/v (into the caudal vein) injection of 2 ml saline (control) and antitumor drugs in previously determined MTD (experimental groups): 1.5 mg/kg dioxadet in saline, 4 mg/kg cisplatin as the initial solution, 1.5 mg/kg mitomycin C in saline, 2 mg/kg melphalan in the solvent attached to the drug, and 5 mg/kg paclitaxel in saline.

Antitumor effects of the drugs were evaluated by the increase in median survival (MS) [1].

The data were statistically processed by nonparametric Mann—Whitney *U* test using GraphPad Prism 6 and SPSS Statistics 17.0 software. The differences were significant at $p<0.05$.

RESULTS

In all rats, OC took in and rapidly progressed causing ascites and death. Some animals developed hemorrhagic ascites. In controls, autopsy revealed carcinomatosis of the visceral and parietal peritoneum, greater and lesser omentum, intestinal mesentery, and metastatic involvement of the lymph nodes in the abdominal cavity. The tumor looked like caseous mass in the space between the diaphragm and liver, stomach and liver. Histological studies of carcinomatous nodes in the peritoneum and involved lymph nodes detected OC metastases.

A single i/p injection of any of the cytostatics led to a significant improvement of rat survival in comparison with controls, while i/v injections of dioxadet, cisplatin, and melphalan caused in fact no changes in rat survival in comparison with the control; only i/v mitomycin C and paclitaxel significantly improved survival. Intraperitoneal dioxadet, cisplatin, and melphalan significantly prolonged MS (by 79, 88, and 114%, respectively), while i/v injections of these drugs caused virtually no changes in the MS. Intraperitoneal mitomycin C prolonged significantly increased MS (by 35%), i/v – by 152% (the difference between i/p and i/v injections was significant). Paclitaxel prolonged MS by 45 and 81% in response to i/p and i/v injections (the difference between the two routes of administration was negligible) (Table 1). Hence, i/p injections of dioxadet, cisplatin, and melphalan were more effective then i/v injections of these drugs. Antitumor effects of i/p and i/v paclitaxel were similar. Mitomycin C was the only drug that proved to be more effective intravenously.

Predominant efficiency of the studied drugs in i/p injections can be presented as MS proportion in response to i/p injection ($MS_{i/p}$) to MS in response to i/v injection ($MS_{i/v}$). The $MS_{i/p}/MS_{i/v}$ on the transplanted OC model in rats is 2.7-3.0 for dioxadet, cisplatin, and melphalan, 0.8 for mitomycin C, and 1.3 for paclitaxel (Table 2). Differences in antitumor efficiencies of drugs in response to i/p or i/v injections thereof determine the molecular weight, area under the concentration—time curve (AUC), lipophilic or hydrophilic characteristics, need in metabolic activation, and mechanism of action. At lesser molecular weight the drug more rapidly penetrates into tumor cells, at larger AUC it stays longer in the abdominal cavity,

TABLE 1. Effects of i/p and i/v Drugs on Rat MS after OC Transplantation

Drug	i/p administration		i/i administration	
	MS, days	prolongation, %	MS, days	prolongation, %
Control	17.0		10.5	
Dioxadet	30.0*	79	11.0^{++}	5
Cisplatin	32.5*	88	12.0^{+}	14
Mitomycin C	22.0*	35	26.5*$^{+}$	152
Melphalan	37.0*	114	12.5^{++}	19
Paclitaxel	24.0*	45	19.0*	81

Note. *$p<0.05$-0.001 in comparison with respective control; $^{+}p<0.05$, $^{++}p<0.001$ in comparison with i/p injection.

TABLE 2. Predominant Drug Efficiency in i/p Injection and Their Pharmacological and Pharmacokinetic Characteristics [2,3,8,11,13]

Drug	$MS_{i/p}/MS_{i/i}$	Molecular weight	AUC	Hydrophilia/ lipophilia	Metabolic activation
Dioxadet	2.7	322	15	+/+	-
Cisplatin	2.7	300	12	+/-	-
Mitomycin C	0.8	334	30	+/-	+
Melphalan	3.0	334	65	-/+	-
Paclitaxel	1.3	853	1000	-/+	-

lipophilic drugs easier penetrate inside the cells, and the i/p drug is more effective if there is no need in metabolic activation [8].

Low molecular weights of dioxadet, cisplatin, and melphalan are associated with the predominant antitumor activity in response to i/p injections; no association of this kind is detected for mitomycin C; and high molecular weight of paclitaxel seems to attenuate its effect in i/p injection. AUC for dioxadet, cisplatin, melphalan, and mitomycin C is rather small, but enough for manifestation of antitumor effect after i/p injection. AUC for paclitaxel is very high, which presumably determines the antitumor effect of this drug in i/v injections.

Our data do not demonstrate a clear-cut relationship between the hydrophilic/lipophilic characteristics of the drugs and their antitumor activities in response to i/p injection. Preliminary metabolic activation by reductases with dithiol active centers is needed for only mitomycin C [3]. Mitomycin C is activated mainly after systemic administration after it passes through the liver, which explains the higher antitumor activity of the i/v drug administration.

The three most active i/p drugs in our experiment have mainly the alkylating mechanism of action. High efficiency of i/p dioxadet, cisplatin, and melphalan seems to be explained by their direct destructive effect towards the cellular DNA by alkylation, irrespective of the cell cycle, with the formation of intra- and inter-spiral sutures, and accumulation of these sutures causes tumor cell death [7,13]. The main mechanism of mitomycin C action also consists in the formation of transverse sutures between two DNA strands or within one strand [3] by guanine alkylation, but the need in metabolic activation in i/p injection seems to cancel this advantage. The mechanism of paclitaxel activity is basically different: the drug prevents mitosis by stimulating the microtubule assembly from tubulin dimers and stabilizes the microtubules by suppressing depolimerization [11]. In addition, paclitaxel forms micellas, preventing fixation to tumor cell surface [6], which reduces its effect in i/p injection.

Hence, our results directly prove higher efficiency of i/p injections of antitumor drugs in comparison with i/v injections of these drugs in the treatment of disseminated OC. The data explain the results of clinical trials [4,9] demonstrating lifespan prolongation in patients with disseminated OC as a result of addition of i/p injections of antitumor drugs to therapeutic protocols. However, our results indicate that not all antitumor drugs are more effective intraperitoneally. It seems that drugs with alkylating mechanism of action, low molecular weight, requiring no metabolic activation are best of all fit for i/p chemotherapy for disseminated OC. We recommend dioxadet, cisplatin, and melphalan for clinical trials of i/p chemotherapy for disseminated OC.

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