Efficiency and Tolerance of Aranose Combinations with Cisplatin and Gemcitabine in Experimental Lung Cancer

V. S. Pokrovsky, N. A. Lesnaya, V. I. Romanenko, and E. M. Treshalina

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The efficiency of aranose combinations with gemcitabine and cisplatin was studied in experimental Lewis lung epidermoid carcinoma. Significant synergism of all the studied combinations and their satisfactory tolerance was demonstrated. The gemcitabine+cisplatin+aranose combination was significantly more effective than gemcitabine+cisplatin.

Key Words: *chemotherapy; Lewis lung carcinoma; gemcitabine; cisplatin; aranose*

The search for new drug combinations is one of approaches to improving the efficiency of chemotherapy of small-cell and non-small-cell lung cancer. Effective drugs for lung cancer are platinum compounds, gemcitabine (*G*), and nitrosourea derivatives. The efficiency of gemcitabine+cisplatin (*GP*) combination characterized by a synergistic effect was demonstrated for experimental lung cancer [5,6].

We studied the efficiency of combinations of *P* and/or *G* with a Russian nitrosourea derivative aranose (*Ar*) characterized by a wide range of therapeutic doses and exhibiting synergism in combination with *P* [1,2]. Lewis transplanted epidermoid lung carcinoma (LLC) was chosen for evaluating the efficiency of polychemotherapy. This tumor metastasizes in the lungs, is sensitive to all the studied drugs, and is widely used in preclinical studies of antitumor activity of various drugs and their combinations [3,4].

MATERIALS AND METHODS

The study was carried out on male BDF_1 and C57Bl mice (18-24 g) bred at N. N. Blokhin Cancer Research Center. The animals were kept under conditions of natural light with standard fodder and had free access to water. Before therapy, the animals were divided into groups 8-14 animals each. Control group (n=10-12) received no therapy.

The LLC strain was obtained from Tumor Strain Bank of N. N. Blokhin Cancer Research Center. Passages 4-15 were used *in vivo*. The tumor was transplanted by the standard method [3,4].

The drugs were injected intraperitoneally. *G* was injected once on day 2 or 3 times on days 2, 5, and 8. *P* and *Ar* were injected daily 3 times on days 2-4 after tumor transplantation. *G* and *P* were dissolved in 0.9% NaCl, *Ar* in 5% glucose solution.

Lyophilized *G* (Eli Lilly) was used in 1% concentration for single injections in doses of 100, 200, or 300 mg/kg and in a dose of 60 mg/kg for a course of 3 injections (total dose of 180 mg/kg). *P* (Ebeve) was injected in a concentration of 0.025% in single doses of 2.5 or 3.0 mg/kg (total doses of 7.5 or 9.0 mg/kg, respectively). Lyophilized *Ar* (GLES) was injected in a 1% concentration in single doses of 100, 150, or 200 mg/kg (total doses of 300, 450, and 600 mg/kg, respectively). The drugs were injected in the following order: *G–P–Ar*, at 10-20-min intervals.

The treatment efficiency was evaluated by standard criteria: tumor growth inhibition (TGI), lifespan prolongation (LSP), and cure. The TGI≥50% and

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** vadimpokrovsky@yandex.ru. V. S. Pokrovsky

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LSP≥25% were considered significant. The animals were considered cured, if the tumor was not detected during 90 days [3,4].

Drug toxicity was evaluated by animal status and behavior, significant loss of body weight (≥30%) and spleen weight (indirect signs of total and hematological toxicities). Mortality and results of autopsies of dead animals or animals sacrificed by ether overdosage were recorded.

The data were statistically processed using Student's test modified by R. B. Strelkov by calculating the confidence intervals for the compared means. The differences were considered significant at $p<0.05$.

RESULTS

The effective and tolerated doses of each drug at optimal protocols of treatment were determined in preliminary tests (Table 1).

In case of *G* monotherapy, the maximum effect was observed after single dose of 300 mg/kg. Three doses of *G* (60 mg/kg) were also highly effective for TGI, but did not lead to LSP. The efficiencies of *P* and *Ar* were close to that of *G*; *P* treatment led to LSP=37%, while *Ar* therapy virtually did not change the lifespan. No cases of cure were observed in these groups. Monotherapy was sufficiently well tolerated: nothing special was noted in the status and behavior of mice, body weight loss on day 10 was ≤10%. A significant loss of the mean spleen weight was observed after *G* and *Ar* in comparison with the spleen weight in control animals (227 mg).

Hence, *Ar*, *P*, or *G* monotherapies even in doses close to MTD did not lead to appreciable LSP or cure of mice. Since the expected hematological toxicity of the combinations was high, low single doses were chosen for polychemotherapy: 2.5 mg/kg *P*, 100 or 150 mg/kg *Ar*, and 100 mg/kg *G*.

In order to predict and quantitatively evaluate the synergism of the triple combination, we studied combinations of two drugs and revealed their high efficiency (Table 2). The use of *GP* combination led to TGI of 92-93% ($p<0.05$) throughout the entire period of observation and cure of 38% animals, which is in agreement with the opinions of other authors on high efficiency of this combination [5,6]. Metastases were detected in the lungs of mice dead from the tumor. The tolerance of *GP* was satisfactory, body weight loss ≤20%. Autopsy showed a 50% reduction of the mean weight of the spleen ($p<0.05$) in comparison with the control group (84 and 169 mg, respectively).

The efficiency of *PAr* combination was comparable to that of *GP*. No tumors were detected in mice on day 10 after transplantation. By day 26, no tumors were detected in 10-30% mice, while in the rest animals tumor growth was significantly inhibited at all doses of *Ar*, TGI being 89-93% ($p<0.05$).

The absence of tumors was observed until day 90; hence, 10-30% cure was observed. The status of mice was satisfactory. Autopsy of dead or sacrificed (on day 93 of the experiment) animals showed a significant (51-57%; $p<0.05$) decrease in the spleen weight in comparison with the control group. On the whole, the efficiency of *PAr* conformed to the known experimental data, which served as the basis for clinical use of this combination [1,2].

The efficiency of *GAr* combination was similar to that of *PAr*. Single injection of *G* in a dose of 200 mg/kg on days 10 and 26 of the experiment resulted in TGI of 100 and 63%, respectively, LSP 41% ($p<0.05$); no cases of cure were recorded. Reduction of the *G* dose in the combination to 100 mg/kg on days 10 and 26 led to TGI of 98 and 92%, respectively, with 33% of mice cured. Improvement of the direct effect and good delayed results after low dose of *G* can be due to a lesser immunotoxicity. Metastases were detected

TABLE 1. Efficiency and Tolerance of *G*, *P*, or *Ar* Monotherapy

Drug	Single dose, mg/kg	Day of injection	TGI after transplantation, %			LSP, %	Mean splenic weight loss, %
			on day 10	on day 18	on day 26		
G	60	2, 5, 8	98*	81*	67*	3	0
	200	2	83*	53*	42	11	29*
	300	2	97*	86*	77*	43*	23*
P	3	2-4	85*	61*	51*	37*	15
Ar	100	2-4	83*	58*	46*	16	52*
	200	2-4	74*	53*	38*	6	23

Note. Here and in Tables 2, 3: *$p<0.05$ compared to the control.

TABLE 2. Sensitivity of Subcutaneously Transplanted LLC to *GP*, *GAr*, and *PAr* Combinations

Treatment protocol	Single dose, mg/kg	Day of therapy	TGI after transplantation, %			Cure, %	LSP, %	Death from toxicity
			on day 10	on day 18	on day 26			
G	100	2	92*	92*	93*	3/8	42*	0/8
P	2.5	2-4						
G	60	2, 5, 8	—	—	—	0/8	—	7/8
Ar	100	2-4						
	200	2	100*	77*	63*	0/9	41*	0/9
	100	2-4						
	100	2	98*	91*	92*	3/9	18	0/9
	100	2-4						
P	2.5	2-4	100*	99*	93*	3/10	23	0/10
	100							
Ar	2.5	2-4	100*	98*	89*	1/10	29	0/10
	150							

in the lungs of mice dead from the tumors. No differences in the degree of lung involvement were visually detected.

The tolerance of *GAr* therapy depended on the protocol and *G* dose. The tolerance of single *G* doses of ≤200 mg/kg was satisfactory. No mortality because of toxicity or significant body weight loss was observed. The *G* dose of 100 mg/kg caused no changes in the spleen weight in comparison with the control group, while after the dose of 200 mg/kg the spleen weight decreased by 29%. The combination was highly toxic if *G* was injected in 3 doses of 60 mg/kg (total dose of 180 mg/kg). The mortality in this group reached 88% on days 9-13, the spleen weight decreasing by 79%. The time of death, spleen weight loss, and results of observation suggest that *G* in high single doses or its repeated injections in combination with *Ar* are fraught with the risk of cumulation of hematological toxicity.

Hence, all combinations proved to be significantly more effective than any of the monotherapies by all the criteria used. These data indicate pronounced synergism of the preparation in combinations and suggest high efficiency of the *GPAr* protocol. Because of possible increase in toxicity, *G* was used in low single doses of 100 or 50 mg/kg.

The *GPAr* therapy led to cure of 100% mice (Table 3). This effect was attained with a high dose of *P* and low doses of *G* and *Ar* ($^3/_4$, $^1/_6$-$^1/_3$, or $^1/_3$ MTD, respectively). The tolerance of *GPAr* was satisfactory and comparable to that of bi- or monotherapy. Body weight decreased by no more than 25% by days 3-7 after the end of therapy. This parameter normalized by

TABLE 3. Comparative Efficiencies of *GPAr* and *GP*

Drug	Single dose, mg/kg	Day of injection	LSP, %	Cure
G	100	2	42*	3/8
P	2.5	2-4		
G	100	2	—	14/14
P	2.5	2-4		
Ar	100	2-4		
	50	2	—	10/10
	2.5	2-4		
	100	2-4		

day 20 after the end of treatment. Autopsy showed no apparent pathological changes in the viscera.

Hence, *GPAr* proved to be highly effective and was well tolerated, the drug doses in the combination being very low. The expected side effects can be corrected by decreasing *G* or *Ar* doses. These data suggest combinations including *Ar* (*PAr*, *GAr*, *GPAr*) for further studies.

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