Efficacy of Gemcitabine on Intracranial Erlich Tumor and its Determinants

Authors

Alexander N. Stukov[1], Vladimir G. Bespalov[2,4], Valerij A. Alexandrov[2,4], Alexander L. Semenov[2,4], Galina S. Kireeva[3], Tatjana Y. Semiglazova[1], Larisa V. Filatova[1], Denis A. Baranenko[4]

Affiliations

1 Department of Innovative Methods of Therapeutic Oncology and Rehabilitation, N.N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russia
2 Laboratory of Cancer Chemoprevention and Oncopharmacology, N.N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russia
3 Laboratory of Carcinogenesis and Aging, N.N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russia
4 International Research Centre "Biotechnologies of the Third Millennium", ITMO University, St. Petersburg, Russia

Key words

ehrlich tumor, brain, gemcitabine, carmustine, cyclophosphamide, cisplatin, blood-brain barrier

received 16.07.2018
accepted 13.12.2018

Bibliography

DOI https://doi.org/10.1055/a-0824-6325
Published online: 25.9.2019
Drug Res 2020; 70: 86–90

ISSN 2194-9379

Correspondence

Vladimir Bespalov
Laboratory of Cancer Chemoprevention and Oncopharmacology
N.N. Petrov National Medical Research Center of Oncology
197758 St. Petersburg
Russia
Tel.: + 7/921/930 6429
bespalov_niio@mail.ru

ABSTRACT

Gemcitabine is quite effective in the treatment of brain tumors, although this drug has a limited ability to overcome the blood-brain barrier (BBB). Aim of study is to assess the therapeutic efficacy of gemcitabine and other drugs with different permeability of BBB in the model of intracranial tumor. The therapeutic activity of gemcitabine, carmustine, cyclophosphamide and cisplatin was studied in mice with intracranially implanted Ehrlich tumor, and also gemcitabine in various doses - with intramuscularly implanted tumor. On intracranial tumor model gemcitabine (25 mg/kg) increased the life span (ILS) by 60 – 89 % ($p < 0.001$), despite the fact that its permeability of the BBB is about 10 %. Therapeutic activity of carmustine, cyclophosphamide and cisplatin (ILS were 44, 22 and 11 %, respectively) corresponds with the BBB permeability for these drugs (90, 20 and 8 %, respectively). On intramuscular tumor model, gemcitabine showed significant antitumor effect at both 25 and 2.5 mg/kg, indicating a wide range of therapeutic doses of this drug. Pronounced therapeutic effect of gemcitabine on intracranial tumor most likely is due to the small but sufficient concentration of the drug that overcomes the BBB.

Introduction

Gemcitabine (2',2'-difluoro 2'-deoxycytidine) is anticancer agent from the group of pyrimidine antagonists (deoxycytidine analogue). The prospects for the clinical use of gemcitabine are primarily determined by the wide spectrum of its effectiveness with many solid tumors [1]. An important feature of gemcitabine as a chemotherapeutic drug is its activity on brain tumors both on primary tumors and cerebral metastases of solid tumors [2]. To this end, gemcitabine was most commonly used in combination with other drugs or together with radiation therapy, when gemcitabine was as radiosensitizer [3, 4].

The effectiveness of gemcitabine on brain tumors in the clinic is difficult to explain in terms of its low ability to overcome the blood-brain barrier (BBB) [4], due its low lipophilicity and possible other factors [5].

The aim of this study was to investigate the therapeutic activity of gemcitabine on intracranially implanted Ehrlich tumor in mice in comparison with some other anticancer drugs (carmustine,

cyclophosphamide and cisplatin), which have different permeability through BBB. In addition, the efficacy of gemcitabine has been studied on the growth of an intramuscularly implanted Ehrlich tumor using different doses.

Materials and Methods

Animals

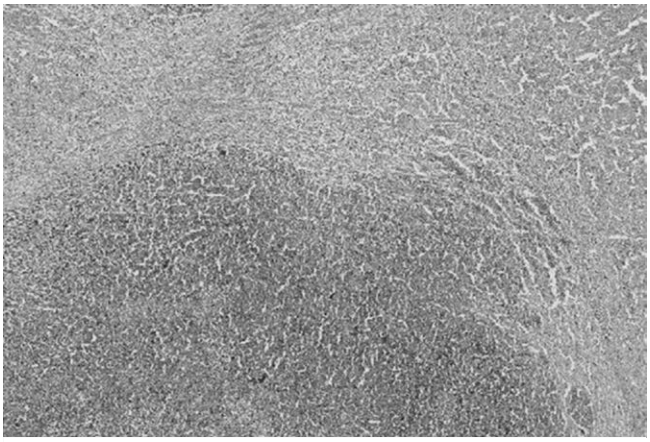
The study was carried out in 175 adult male BALB/c mice (25 – 30 g of body weight). All animals were kept under standard conditions (12/12 h light/dark regimen and at 21–23 °C). They received standard pellet laboratory diet (PK-120; Laboratorkorm, Moscow, Russia) and tap water *ad libitum*.

Drugs

Several antitumor drugs were used in the experiments: gemcitabine (Gemzar, Lilly France, France), carmustine (BiCNU, Bristol-Myers Squibb S.r.L., Italy), cyclophosphamide (endoxane, Baxter, Germany), cisplatin (Cisplatin-Teva, Israel). All drugs taken injected intraperitoneally (i.p.) in a volume of 0.2 ml per 20 g of body weight of the animal, once. In the control groups, mice were administered with appropriate volume of saline solution.

Intracranial implantation of Ehrlich carcinoma

Intracranial tumors in mice were induced by implantation of 1×10^5 Ehrlich carcinoma cells in a sterile saline (0.025 ml) to a depth of 2 mm into the right forebrain tissue by described procedure [6]. Signs of intracranial tumor growth in mice were noted in 5 – 7 days after the tumor implantation and were manifested in cranial deformation, periodic tonic-clonic convulsions, decreased body weight. Tumor-bearing mice not receiving any treatment died in 7 – 14 days after the tumor implantation. Autopsy revealed asymmetry of the cerebral hemispheres and after microscopic examination of these zones showed brain tissue infiltration with tumor cells (▶ **Fig. 1**).



▶ **Fig. 1** Area of brain tissue with an infiltrative growing tumor (bottom) after intracranial implantation of Ehrlich carcinoma in a mouse. Histological preparation. Hematoxylin/eosin; x 50.

Study of therapeutic activity of gemcitabine and some other drugs on intracranially implanted Ehrlich tumor

The main criterion for evaluating the therapeutic effect of the drugs used in mice with intracranial tumors was the survival of animals. Increase of life span (ILS) of mice with intracranial Ehrlich tumor was calculated using formula:

$$ILS = \frac{MLS_T - MLS_C}{MLS_C} \times 100$$

where ILS–increase of life span (%); MLS_T–median life span of treated mice, days; MLS_C–median life span of control mice, days.

Two experiments on the model of intracranial Ehrlich carcinoma in mice were carried out.

Experiment №1. The study consisted of 3 repeated series conducted on the same design: 73 tumor-bearing mice were randomized into control (administration of saline solution) and experimental (treatment with gemcitabine, 25 mg/kg) groups. Saline solution and gemcitabine were administered i.p. once 24 h after the tumor implantation.

Experiment №2. Its purpose was to evaluate the therapeutic activity of gemcitabine in comparison with carmustine, cyclophosphamide and cisplatin on the model of Ehrlich carcinoma implanted intracranially in mice. In this experiment mice after the tumor implantation were randomized into 5 groups: I–control group, saline solution (n = 14); II–gemcitabine, 25 mg/kg (n = 14); III–carmustine, 25 mg/kg (n = 10); IV–cyclophosphamide, 150 mg/kg (n = 12); V–cisplatin, 9 mg/kg (n = 14). Based on our many years of experience in experimental chemotherapy, the selected doses of all drugs used were optimal therapeutic for Ehrlich tumor implanted in mice by different ways and adequate to compare their effects. The molecular weight of these substances was similiar: gemcitabine–263, carmustine–214; cyclophosphamide–279, cisplatin–300. Saline solution and all drugs were administered i.p. once in 24 h after the tumor implantation.

The therapeutic efficacy of drugs was evaluated after the calculations of MLS and ILS in tumor-bearing mice in the control and treated groups.

Study of therapeutic activity of gemcitabine on intramuscularly implanted Ehrlich tumor

The purpose of this experiments on mice (experiment №3) was to compare therapeutic efficacy of gemcitabine, used at a single dose of 25 (maximal) and 2.5 mg/kg (minimal) on growth of Ehrlich tumor implanted intramuscularly. 1×10^6 of tumor cells diluted in 0.2 ml of saline solution were inoculated into the femoral muscle of the right hind leg. Then all mice were randomized into 3 groups: I–control, saline solution (n = 18); II–gemcitabine, 25 mg/kg (n = 10); III–gemcitabine, 2.5 mg/kg (n = 10). Saline solution and gemcitabine were administered i.p. once in 72 h after the tumor implantation. In this experiment we had been evaluating tumor volume and tumor growth inhibition for 3 weeks after implantation. The tumor volume was calculated using the modified ellipsoidal formula [7]:

$$V = \frac{A \times B^2}{2}$$

where V–tumor volume, mm^3; A–the greatest longitudinal diameter (length), mm; B–the greatest transverse diameter (width), mm.

Tumor growth inhibition (TI) was calculated using the formula:

$$TI = \frac{V_C - V_T}{V_C} \times 100,$$

where TI–tumor growth inhibition (%); V_C–mean tumor volume in mice in the control group, mm^3; V_T–mean tumor volume in treated mice, mm^3.

Statistic analysis

Statistical analysis was performed using programs GraphPad® Prism 6, SPSS® Statistics version 17.0. The statistical analysis Lilliefors test, median life spans–Mann-Whitney U test, Student's t-test was used. $P < 0.05$ was considered statistically significant.

Ethical approval

All experimental procedure and also the design of these experiments was approved by the Ethics Committee of the N.N. Petrov National Medical Research Center of Oncology (St. Petersburg, Russia), following international guidelines for the care and use of animals.

Results

Effectiveness of a single treatment of gemcitabine against intracranial Ehrlich tumor in mice (Experiment № 1)

After intracranial implantation of Ehrlich tumor, the majority of mice in the control died by the 12th day, whereas in the treatment with gemcitabine 78–100 % of animals survived by this time. The ILS index in all 3 series was from 60–89 % in comparison with the control ($p < 0.001$) did not have significant differences (▶ **Table 1**).

From the beginning of the experiment to the 10th day after tumor implantation the body weight of mice in the control groups decreased from 25.6 ± 0.36 to 17.9 ± 0.33 g (by 30 %, $p < 0.001$), while in the gemcitabine-treated mice did not change (about 25.8 ± 0.61, $p = 0.345$).

Comparative therapeutic activity of gemcitabine, carmustine, cyclophosphamide and cisplatin on intracranial Ehrlich tumor in mice (Experiment № 2)

The results showed (▶ **Table 2**) that the greatest increase of life span (ILS) in mice was after administration of gemcitabine – by 78 % compared with the control, $p < 0.001$. Carmustine had a fairly high therapeutic activity (ILS - 44 %, $p < 0.001$).). A significantly less pronounced therapeutic effect was observed with the use of cyclophosphamide (ILS - 22 %, $p = 0.01$). Cisplatin with a single dose of 9 mg/kg had no therapeutic effect.

The therapeutic activity of gemcitabine on intramuscular Ehrlich tumor in mice (Experiment № 3)

Significant statistical inhibition of tumor growth (TI) was registered with a single administration of gemcitabine at both doses used (▶ **Table 3**), although these doses differed by a factor of 10.

Discussion

The high therapeutic activity of gemcitabine in an experiment with an intracranially implanted tumors, as well as its effectiveness in treating patients with brain tumors, is difficult to relate to the relatively low permeability of gemcitabine through BBB. Sigmond et al. [4] studied the penetration of gemcitabine into the tumor in 10 patients with a multiforme glioblastoma. Concentrations of gemcitabine in the plasma and tumor tissue were highly variable, so the passage of gemcitabine into the brain tumor in patients with a multiforme glioblastoma could be from 6–39 % [4]. In experiments on rats after a single i. v. administration of ^{14}C-gemcitabine (10 mg/kg)

▶ **Table 1** Survival of mice with intracranially implanted Ehrlich tumor after single i.p. treatment of gemcitabine (25 mg/kg).

No. series of experiment	Treatment	Number of mice	MLS, days	95 % CI	ILS, %	p
I	Control	17	9	8.0 – 10.0	89	–
	Gemcitabine, 25 mg/kg	13	17	13.5 – 20.5		< 0.001
II	Control	10	11	9.3 – 11.0	72	–
	Gemcitabine, 25 mg/kg	10	19	11.8 – 26.3		< 0.001
III	Control	12	10	8.5 – 11.5	60	–
	Gemcitabine, 25 mg/kg	11	16	12.9 – 19.2		< 0.001

MLS–median life span of mice; CI–confidence interval; ILS–increase of life span

▶ **Table 2** The effectiveness of a single i.p. administration of gemcitabine, carmustine, cyclophosphamide and cisplatin against intracranially implanted Ehrlich tumor in mice.

No. group, treatment	Number of mice	MLS, days	95 % CI	ILS, %	p
I. Control	14	9	7.5 – 10.5		–
II. Gemcitabine, 25 mg/kg	14	16	15.1 – 16.9	78 %	< 0.001
III. Carmustine, 25 mg/kg	10	13	12.0 – 14.0	44 %	< 0.001
IV. Cyclophosphamide, 150 mg/kg	12	11	10.8 – 11.8	22 %	0.010
V. Cisplatin, 9 mg/kg	14	10	8.2 – 11.8	11 %	0.404

MLS–median life span of mice; CI–confidence interval; ILS–increase of life span in treated mice compared with control; *p* - in comparison with group I

▶ **Table 3** Therapeutic effect of gemcitabine administered at a single dose of 25 and 2.5 mg/kg on growth of intramuscularly implanted Ehrlich tumor in mice.

No. group, treatment	Number of mice	Tumor volume, mm³ (M ± m); TI %		
		Days after tumor implantation		
		6	10	14
I. Control	18	296 ± 4.5	983 ± 88	2414 ± 62
II. Gemcitabine, 25 mg/kg	10	106 ± 3.4; 64 % $p < 0.001$	235 ± 50; 76 % $p < 0.001$	763 ± 92; 68 % $p < 0.001$
III. Gemcitabine, 2.5 mg/kg	10	92 ± 4.7; 69 % $p < 0.001$	635 ± 73; 35 % $p = 0.008$	1612 ± 53; 33 % $p < 0.001$

TI–tumor growth inhibition, %; p - in comparison with group I

the accumulation of radioactivity in the cerebrum in 5 min–4 h was from 3–34 % in relation to the plasma level [8].

A study of the pharmacokinetics of gemcitabine in brain extracellular fluid and plasma showed [9] that the relative coefficient of distribution in normal rats ranged from 0.07–0.09 (i. e., 7–9 % in the brain tissue). However, in C6 glioma-bearing rats the uptake of gemcitabine noticeably increased and after the administration of a drug at a dose of 25 mg/kg, this coefficient reached 18 %. In addition, in these experiments, the possibility of a higher accumulation (by 2.2 times) in the tumor than the surrounding normal brain tissue was established [9]. This can possibly explain higher cytotoxical activity of gemcitabine for brain tumor cells.

This study showed a high therapeutic activity of gemcitabine on the intracranial Ehrlich tumor in mice (ILS was 78 %, $p < 0.001$), which corresponds to the results of our previously performed pilot experiments [10]. Other drugs studied had a significantly lower efficacy indicators; carmustine (44 %, $p < 0.001$), cyclophosphamide (22 %, $p = 0.010$), cisplatin (11 %, $p = 0.404$). We compared therapeutic activity of these drugs with their BBB permeability data found in the literature. In experiments on rats with constant i. v. administration of ^{14}C-labeled carmustine, the tissue/plasma ratio for the brain was 0.9 after 95–120 min [11]. Carmustine can be attributed to drugs with an extremely high BBB permeability (up to 90 %). In rats the brain/plasma concentration ratio of total active alkylating metabolites generated from the i. v. administration of cyclophosphamide and measured between 5 and 240 min was 0.20 (i. e., 20 %) [12]. The pharmacokinetics of cisplatin was studied in rats using ^{13}N-labeled cisplatin. Its concentration in brain tissue 10–40 min after i. v. administration was no more than 0.082 [13]. Therefore, cyclophosphamide and especially cisplatin can be attributed to antitumor drugs with a low BBB permeability. Our results on therapeutic activity of carmustine, cyclophosphamide and cisplatin (ILS 44, 22 and 11 %, respectively) on the intracranial tumor model evidently correspond to their BBB permeability (90, 20 and 8 %, respectively) and it is likely that there is a direct relationship between these parameters for the mentioned drugs.

One possible explanation for gemcitabine activity in intracerebral tumors is that this drug accumulates in the brain tumor [9] and is slower excreted from the tumor tissue compared to normal brain tissue. A low permeability through the BBB can contribute to a longer-term persistence of gemcitabine in the brain due to the difficulty of its passage back into the bloodstream. This, on the one hand, will provide an increase in the effect on the intracerebral tumor of the small concentration that overcomes the BBB after the systemic administration of gemcitabine, and, on the other hand, opens the prospect of its use for intrathecal therapy. It can not be ruled out that with the growth of a tumor in the brain, damage to the BBB occurs and it becomes more permeable for the cytotoxic drugs [14].

From our point of view, the most likely explanation for activity in intracerebral tumors is that gemcitabine has such a wide range of therapeutic doses that even the small concentration passing the BBB (about 10 %) is enough to manifest a therapeutic effect. It can be assumed that in our experiments after i.p. administration of gemcitabine to mice at a dose of 25 mg/kg, a pronounced therapeutic effect is the result of the action of this drug directly on the intracranial tumor of a dose of 10-fold less, i. e., 2.5 mg/kg. In our experiments, using the model with intramuscular implantation of Ehrlich tumor in mice, the effect of gemcitabine was measured at a single dose of 25 and 2.5 mg/kg and a wide range of the therapeutic activity of this drug was confirmed. Gemcitabine showed a statistically significant antitumor effect not only with dose 25 mg/kg, but with dose 2.5 mg/kg, although the effect of inhibition on extracranially implanted tumor was dose-dependent.

The results of clinical studies indicate the prospects of gemcitabine in the chemotherapy of CNS tumors, despite its relatively low permeability through BBB. Therefore, in order to improve its passing of BBB and delivery to the tumor, it is important to develop various methods related to both the technique of gemcitabine administration and the modification of the drug itself.

Acknowledgement

This study was partially funded by Government of Russian Federation, Grant RFMEFI58117X0020.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] BC Cancer Agency BC Cancer Agency Cancer Drug Manual©. Gemcitabine, 2015 Available at http://www.bccancer.bc.ca/drug-database-site/Drug%20Index/Gemcitabine_monograph_1Jan2015.pdf Accessed 20 December 2017

[2] Bastiancich C, Bastiat G, Lagarce F. Gemcitabine and glioblastoma: challenges and current perspectives. Drug Discov Today 2018; 23: 416–423

[3] Maraveyas A, Sgouros J, Upadhyay S et al. Gemcitabine twice weekly as a radiosensitiser for the treatment of brain metastases in patients with carcinoma: a phase I study. Br J Cancer 2005; 92: 815–819

[4] Sigmond J, Honeywell RJ, Postma TJ et al. Gemcitabine uptake in glioblastoma multiforme: potential as a radiosensitizer. Ann Oncol 2009; 20: 182–187

[5] Degen JW, Walbridge S, Vortmeyer AO et al. Safety and efficacy of convection-enhanced delivery of gemcitabine or carboplatin in a malignant glioma model in rats. J Neurosurg 2003; 99: 893–898

[6] Chambers R, Gillespie GY, Soroceanu L et al. Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma. Proc Natl Acad Sci USA 1995; 92: 1411–1415

[7] Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol 1989; 24: 148–154. doi:10.1007/BF00300234

[8] Esumi Y, Mitsugi K, Takao A et al. Disposition of gemcitabine in rat and dog after single and multiple dosings. Xenobiotica 1994; 24: 805–817

[9] Apparaju SK, A Gudelsky GA, Desai PB. Pharmacokinetics of gemcitabine in tumor and non-tumor extracellular fluid of brain: An in vivo assessment in rats employing intracerebral microdialysis. Cancer Chemother Pharmacol 2008; 61: 223–229

[10] Stukov AN, Filatova LV, Latipova DKh et al. [Therapeutic activity of gemcitabine in intracranial tumors]. Vopr Onkol 2015; 61: 274–279 (in Russian)

[11] Levin VA, Kabra PA, Freeman-Dove MA. Relationship of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) pharmacokinetics of uptake, distribution, and tissue/plasma partitioning in rat organs and intracerebral tumors. Cancer Chemother Pharmacol 1978; 1: 233–242

[12] Genka S, Deutsch J, Stahle PL et al. Brain and plasma pharmacokinetics and anticancer activities of cyclophosphamide and phosphoramide mustard in the rat. Cancer Chemother Pharmacol 1990; 27: 1–7

[13] Ginos JZ, Cooper AJL, Dhawan V et al. [13N]cisplatin pet to assess pharmacokinetics of intra-arterial versus intravenous chemotherapy for malignant brain tumors. J Nucl Med 1987; 28: 1844–1852

[14] Lee SW, Kim WJ, Park JA et al. Blood-brain barrier interfaces and brain tumors. Arch Pharm Res 2006; 29: 265–275