Introduction

Gemcitabine (2',2'-difluoro 2'-deoxycytidine) is an 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, ...
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 similar: 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 calculation 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 × 10^5 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 Liliefors 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).

**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)
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 extra-cellular 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 cytotoxic 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 14C-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 14N-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 antitumour 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.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References


