Efficacy of Gemcitabine on Intracranial Erlich Tumor and its Determinants

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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 to 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, carmustine, cyclophosphamide, cisplatin, blood-brain barrier.
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).

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 × 10^6 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$^3$.

**Statistical 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).

**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

<table>
<thead>
<tr>
<th>No. series of experiment</th>
<th>Treatment</th>
<th>Number of mice</th>
<th>MLS, days</th>
<th>95 % CI</th>
<th>ILS, %</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>17</td>
<td>9</td>
<td>8.0–10.0</td>
<td>89</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine, 25 mg/kg</td>
<td>13</td>
<td>17</td>
<td>13.5–20.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Control</td>
<td>10</td>
<td>11</td>
<td>9.3–11.0</td>
<td>72</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine, 25 mg/kg</td>
<td>10</td>
<td>19</td>
<td>11.8–26.3</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Control</td>
<td>12</td>
<td>10</td>
<td>8.5–11.5</td>
<td>60</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine, 25 mg/kg</td>
<td>11</td>
<td>16</td>
<td>12.9–19.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

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

### Table 2

<table>
<thead>
<tr>
<th>No. group, treatment</th>
<th>Number of mice</th>
<th>MLS, days</th>
<th>95 % CI</th>
<th>ILS, %</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>14</td>
<td>9</td>
<td>7.5–10.5</td>
<td>78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II. Gemcitabine, 25 mg/kg</td>
<td>14</td>
<td>16</td>
<td>15.1–16.9</td>
<td>87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III. Carmustine, 25 mg/kg</td>
<td>10</td>
<td>13</td>
<td>12.0–14.0</td>
<td>44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IV. Cyclophosphamide, 150 mg/kg</td>
<td>12</td>
<td>11</td>
<td>10.8–11.8</td>
<td>22</td>
<td>0.010</td>
</tr>
<tr>
<td>V. Cisplatin, 9 mg/kg</td>
<td>14</td>
<td>10</td>
<td>8.2–11.8</td>
<td>11</td>
<td>0.404</td>
</tr>
</tbody>
</table>

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.
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 addi-
tion, in these experiments, the possibility of a higher accumula-
tion (by 2.2 times) in the tumor than the surrounding normal brain
tissue was established [9]. This can possibly explain higher cyto-
toxical 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 %, < 0.001),

which corresponds to the results of our previously performed pilot
experiments [10]. Other drugs studied had a significantly lower ef-
cicacy indicators; carmustine (44 %, < 0.001), cyclophosphamide
(22 %, p = 0.010), cisplatin (11 %, p = 0.404). We compared therapeu-
tic 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 metab-
olites 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 11N-la-
beled cisplatin. Its concentration in brain tissue 10–40 min after i.
v. administration was no more than 0.082 [13]. Therefore, cyclo-
phosphamide and especially cisplatin can be attributed to antitu-
mor 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 pa-

rameters 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 tis-

sue. 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 pro-
spect 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 therapeu-
tic 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 therapeu-
tic 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 of
extracranially implanted tumor was dose-dependent.

The results of clinical studies indicate the prospects of gemcit-
abine in the chemotherapy of CNS tumors, despite its relatively low
permeability through BBB. Therefore, in order to improve its pass-
ing of BBB and delivery to the tumor, it is important to develop var-
ious methods related to both the technique of gemcitabine admin-
istration and the modification of the drug itself.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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