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Antitumor efficacy of extracellular complexes with gadolinium in Binary Radiotherapy

A.A. Lipengolts [a,b,c,*], A.A. Cherepanov [a,b,c], V.N. Kulakov [b], E.Yu. Grigorieva [a], I.N. Sheino [b], V.A. Klimanov [c]

[a] Blokhin Russian Cancer Scientific Centre, Kashirskoe sh. 24, Moscow, Russian Federation
[b] Burnasyan Federal Medical Biophysical Centre, Zhivopisnaya 46, Moscow, Russian Federation
[c] National Research Nuclear University "MEPhI", Kashirskoe sh. 31, Moscow, Russian Federation

HIGHLIGHTS

- A new approach of using extracellular drugs in Binary Radiotherapy was proposed.
- Vascularization of three different transplanted murine tumors was studied.
- Significant efficacy of CERT with Gd-DTPA in curing adenocarcinoma Ca755 was shown.

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ABSTRACT

In this report the efficacy of extracellular pharmaceutical Gd-DTPA in Binary Radiotherapy was studied. The study was carried out in mice bearing transplantable adenocarcinoma Ca755 using X-ray based contrast enhanced radiotherapy as a practical implementation of Binary Radiotherapy. It was shown that intravenous administration of 0.3 ml of 0.5 M water solution of Gd-DTPA followed by X-irradiation at a dose of 10 Gy provides T/C% = 10 ± 3% and leads to complete tumor regression in 25% of mice.

1. Introduction

Binary Radiotherapy is a developing method of radiotherapy which uses special pharmaceuticals (let us call them BRT-drugs) to form absorbed dose field of desirable shape (Kulakov et al., 2013). Formation of absorbed dose field is caused by preferential interaction of external radiation with specific element (absorbing element) contained in a BRT-drug (Sheino, 2006). For example, thermal neutrons are more likely to interact with Gd and ^{10}B than with elements comprising soft tissues (H, N, C, O etc.), while X-rays with Gd, Au, Pt and other heavy elements as the result of photoabsorption. (Butterworth et al., 2012). Thus, saturation of a tumor with a pharmaceutical, which contains such absorbing elements and irradiation of it with certain type of radiation (neutrons or X-rays) leads to significant increase of absorbed dose in a tumor region only (Verhaegen et al., 2005; Robar et al., 2002). A dose increase is proportional to the concentration of an absorbing element. Practical implementations of this approach are Neutron Capture Therapy (NCT), utilizing thermal neutrons and ^{10}B or Gd as the target element, and Contrast Enhanced Radiotherapy (CERT)–utilizing X-rays and elements with $Z > 52$ (I, Gd, Au etc.). The efficacy of Binary Radiotherapy (both NCT and CERT) is highly dependent on the ratio of particular BRT-drug concentration in the tumor and the surrounding normal tissues (T/N ratio). Binary Radiotherapy has already been shown to be an effective tumor treating modality with intratumoral administration of iodine and

* Corresponding author at: Blokhin Russian Cancer Scientific Centre, Kashirskoe sh. 24, Moscow, Russian Federation.
E-mail address: lipengolts@mail.ru (A.A. Lipengolts).

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gadolinium extracellular contrast drugs (Santos et al., 1983; Mitin et al., 2009). However, intratumoral injection clinically has not been widely used. Any attempts to develop universal tumor-seeking BRT-drugs with systemic administration, which could provide desirable for BRT T/N ratio were unsuccessful by now. Some morphological and physiological properties of particular tumors can be used for that purpose. It is known that certain types of tumors (for example mammary tumors) contain highly vascularized necrotic regions. In such regions, thin layers of viable tissue can only be found around numerous blood vessels (Tannock, 1968). Thus for these types of tumors it would not be necessary to achieve a uniform distribution of a drug within the whole volume of tumor. However, it would be enough to create necessary concentration of BRT-drug just in vital regions of tumor. Those areas usually contain many blood vessels capable to deliver BRT-drug with a blood flow. Usually blood vessels in a tumor are very perforated because of the very high rate of angiogenesis. That also helps extracellular non-tumor specific compounds penetrate into the tumor volume. Thereby for highly vascularized types of tumor extracellular compounds with systemic route of administration could show high antitumor effect within CERT or Gd-NCT. Intravenous administration of gold nanoparticles has already been proved to be very effective in curing transplanted mammary tumor with CERT (Hainfeld et al., 2004). However gold nanoparticles are not exactly extracellular compounds, because of their partial penetration into tumor cells (Rahman et al., 2009). Also gold nanoparticles can't be used in NCT and are not approved for clinical use. The goal of this research was to study antitumor efficacy of BRT with a clinically approved extracellular contrast drug suitable both for NCT and CERT. Thus study of CERT efficacy with Gd-DTPA in curing transplanted mammary tumor (adenocarcinoma Ca755) in mice was performed. CERT was used as a method of treating cancer that is much simpler and cheaper than NCT, but at the same time utilizing the same binary approach. Gd-DTPA was chosen as approved for clinical use contrast drug that is suitable for application as BRT-drug both in NCT and CERT. Adenocarcinoma Ca755 was chosen as the result of comparative MRI studies of three murine transplanted tumors as the most vascularized one.

2. Materials and methods

C57Bl/6 female mice 20–22 g weight were used in all the studies. MRI of mice with transplanted tumors was performed on 7 T MRI scanner for animals Bruker BioSpin ClinScan. Abdominal coil for mice was used in all studies. Gadolinium extracellular contrast drug Magnevist (0.5 M solution of gadopentetate dimeglumine, Bayer, Germany) was used as the contrast drug in MRI studies and drug Dipentast® (0.5 M water solution of disodium gadopentetate, Epidbiomed Ltd., Russia) as BRT-drug. T2-weighted MR images were obtained first to clearly visualize tumor volume and then T1-weighted images were acquired – one before Gd-DTPA administration and another 5 min after administration. Three murine transplanted tumors: melanoma B16F10, Lewis lung carcinoma LLC and adenocarcinoma Ca755, were visualized. All tumors were transplanted by subcutaneous injection of 2×10^6 tumor cells to thigh (for MRI studies) or leg (for radiotherapy efficacy studies). Tumor visualization and CERT irradiation were performed on the 9th day after transplantation when tumors average size reached 6–7 mm.

Adenocarcenoma Ca755 was chosen for CERT with Gd-DTPA as a result of MRI studies. Animals with transplanted tumor were divided into five groups with 16 animals in each group. The 1st group underwent no treatment (untreated control group). Tumors of animals in the 2nd group were locally irradiated with X-rays to provide tumor absorbed dose of 10 Gy (irradiated control group 1). Animals in the 3rd group (test group) were administered intraperitoneally with 0.3 ml of Dipentast®, containing 23 mg of gadolinium and irradiated immediately after injection in the same conditions and at the same dose of X-rays as the 2nd group. Administration of Gd-DTPA solution was made with a single intraperitoneal injection. LD_{50} of Dipentast for intraperitoneal route of administration is 15 mmol/kg (0.6 ml per mouse) so administered dose of drug is half of LD_{50}. 4th and 5th groups of mice (irradiated control group 2 and 3) were irradiated at higher doses of X-rays – i.e. 15 Gy and 20 Gy respectively. 4th and 5th groups were added into the study for evaluation of dose enhancement in the 3rd group of mice. Irradiation was performed with a single fraction with X-ray machine having the voltage of 200 kV and dose rate of 1.3 Gy/min. During irradiation every mouse was covered with lead shielding 2 mm thick leaving just rear leg with tumor uncovered. Antineoplastic efficacy of the treatment was estimated by measuring tumor volume and survival time of mice. Tumor volume was determined by measuring tumor size with calipers in three perpendicular planes and then calculated from the formula of ellipse volume, considering the tumors to be of elliptic shape. Endpoints for evaluation of antitumor efficacy were: percent test/control (%T/C) tumor volume, tumor growth delay (TGD) and Log10 tumor cell kill. TGD is determined as

$$TGD=T–C$$

where T is the median time (in days) required for the treatment group tumors to reach a size of 1000 mm^3, C is the median time (in days) for the control group tumors to reach the same size.

log 10 tumor cell kill was calculated from the following formula:

$$\text{The log10 cell kill total (gross)}=[\text{T-C value in days}/(3.32)(\text{Td})]$$

where T is the median time (in days) required for the treatment group tumors to reach a size of 1000 mm^3, C is the median time (in days) for the control group tumors to reach the same size, Td is tumor volume doubling time.

Animals with complete tumor regression were excluded from final endpoints calculation. Statistical analysis of tumor volume and life span within each group was performed using Student distribution and Student's *t*-test.

3. Results and discussion

Magnetic resonance (MR) images of the tumors obtained during the study are presented in Fig. 1. One can see that adenocarcinoma Ca755 has the highest Gd-DTPA uptake in comparison with melanoma B16F10 and carcinoma LLC. Vascularization of adenocarcinoma Ca755 provides quite uniform distribution of extracellular Gd-DTPA within the tumor volume. This data allows to suggest that the distribution pattern could be sufficient for effective Binary Radiotherapy. To test this hypothesis CERT with Gd-DTPA of adenocarcinoma Ca755 murine tumor was carried out.

Tumor growth rate plots for 1st–3rd groups of mice are shown in Fig. 2. Endpoints of antitumor evaluation are represented in Table 1.

Fig. 3 shows Kaplan–Meier survival plots for 1st–3rd groups of mice. Life span median of mice in untreated, only irradiated and test groups are represented in Table 2.

In test group and irradiated control group 3 25% and 50% of animals respectively have full tumor regression and their life span was more than 180 days (mice were sacrificed on 180th day after tumor inoculation with no evidence of tumor). In contrast, in untreated control group, irradiated control group 1 and irradiated control group 2 no tumor regression was observed and life span

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